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REVIEW

Controversies in cardiovascular medicine

C-reactive protein is a mediator of cardiovascular disease

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C-reactive protein is postulated to embody an index that can reflect cardiovascular risk and can be used to independently predict major cardiovascular events and mortality. On the other hand, credible experimental data have become available that demonstrate the abundant presence of C-reactive protein in atherosclerotic lesions and, moreover, identify C-reactive protein as an initiator of several pathogenic pathways that can cause atherogenic changes. Consequently, there has been a paradigm shift in which C-reactive protein is no longer regarded as merely an indicator of cardiovascular risk, but increasingly considered a direct partaker in the pathogenesis of atherosclerotic cardiovascular disease. These data underscore the need to explore risk-reducing interventions that selectively inhibit C-reactive protein activity as a novel strategy to prevent clinical manifestations of atherosclerosis.

Keywords C-reactive protein

Introduction

C-reactive protein, among other systemic inflammatory mediators, has been widely accepted as a potent risk indicator, independently predicting future cardiovascular events. The impact of C-reactive protein on cardiovascular outcome has been corroborated by a large number of observational studies and meta-analyses. These studies show that an elevated C-reactive protein has a clear prognostic value for major cardiovascular events and mortality, whereas the lowering of C-reactive protein is associated with a reduction in cardiovascular risk. Combining these findings with experimental observations has lead to a paradigm shift in which C-reactive protein is no longer merely a marker, but is increasingly considered as a mediator of cardiovascular disease. In the present issue of 'controversies in cardiovascular medicine', we will focus on the emerging evidence supporting a potentially causal role of C-reactive protein in cardiovascular disease (*Figure 1*).

Epidemiology

Evidence has cumulated to show that C-reactive protein levels are associated with different aspects of the cardiovascular risk spectrum. For instance, C-reactive protein levels were higher among people who were physically inactive,[1–4] had worse cardiorespiratory fitness,[5,6] and were more obese.[7] C-reactive protein levels were also associated with the presence and extent of the metabolic syndrome,[8–10] with the presence of subclinical atherosclerosis,[11] and with the progression of atherosclerosis.[12,13] Besides these cross-sectional associations, C-reactive protein levels were also associated prospectively with the risk of new-onset diabetes mellitus[14,15] and hypertension.[16] Moreover, plasma levels of C-reactive protein have been acknowledged to have a predictive value for the development of future cardiovascular events. Among the first studies to show this association was a study of patients hospitalized for unstable angina.[17] Those with elevated C-reactive protein levels at the time of hospital admission were more prone for revascularization, myocardial infarction, or mortality during hospitalization. Another study involved patients with either stable or unstable angina, revealing that patients in the highest quintile of the C-reactive protein distribution (>3.6 mg/L) had a two-fold increased risk of a coronary event compared with those in the lower quintiles.[18] These observations were soon followed by a range of prospective studies in different populations. Although heterogeneity exists in the predictive value

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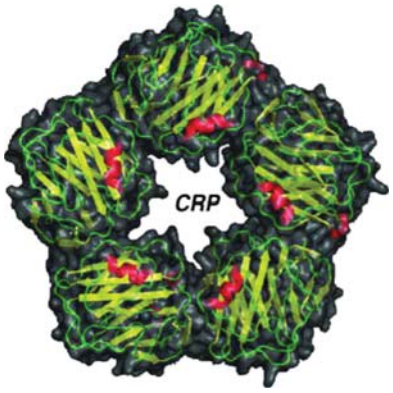

Figure 1 Molecular structure of C-reactive protein. C-reactive protein constitutes a pentameric molecule with a C1q-binding site located on the effector face, and on the opposite a 'recognition' face that binds calcium ions and phosphocholine.

of C-reactive protein between individual studies, general consensus has emerged acknowledging a relation between C-reactive protein and cardiovascular disease. Some studies were performed in patients with established cardiovascular disease including coronary heart disease[19] and peripheral artery disease.[20] In addition, a wide range of prospective studies showed that C-reactive protein levels predict future vascular events among people without clinically manifest cardiovascular disease at the time of C-reactive protein measurement. In the Physicians' Health Study, apparently healthy men in the highest quartile of the C-reactive protein distribution had a 2.9-fold increased risk of future myocardial infarction, compared with those in the lowest quartile.[21] This association was independent of established cardiovascular risk factors. Accordingly, asymptomatic women in the Women's Health Study who had baseline C-reactive protein levels in the top quartile had a 4.8-fold increased risk of vascular events compared with those in the bottom quartile.[22] After that, many prospective studies including the Women's Health Initiative,[23] the Framingham Heart Study,[24] the EPIC-Norfolk cohort,[25] the Cardiovascular Health Study,[26] the MONICA-Augsburg cohort,[27] and the Reykjavik Study[28] have reported similar findings. In addition, a meta-analysis estimated that the odds ratio for future coronary heart disease was 1.58 [95% confidence interval (CI), 1.48–1.68] for people in the top tertile vs. those in the bottom tertile of the C-reactive protein distribution. The study population in which the predictive value of C-reactive protein levels was consistently shown to be limited were the elderly.[29–31] Another set of studies have focused on the association between C-reactive protein levels and other vascular diseases including stroke and peripheral artery disease. The majority of these studies revealed that elevated C-reactive protein levels were associated with an increased risk of future stroke,[32–37] although not all were in agreement.[38] C-reactive protein levels were also shown to be associated with the risk of developing new-onset peripheral artery disease.[39–41]

On the basis of the strong evidence for a prospective association between C-reactive protein levels and cardiovascular risk, a statement from the Centers of Disease Control and the American Heart Association suggested that C-reactive protein measurement may be used to direct further evaluation and therapy for those categorized as intermediate risk by global risk assessment.[42] Evidence supporting the concept that C-reactive protein is useful for reclassification of people estimated to be at intermediate risk is based on studies which show that risk algorithms including C-reactive protein can more often classify people correctly into clinically useful risk categories, than models without C-reactive protein.[43–47]

Epidemiological evidence for a causal role of C-reactive protein?

Whereas the status of C-reactive protein as risk marker is beyond reasonable doubt, there is an ongoing debate whether C-reactive protein is an active participant in atherogenesis or merely an innocent bystander. Besides a range of experimental studies addressing the potentially causal role of C-reactive protein (see further), epidemiological studies have predominantly focused on the Mendelian randomization. This approach assumes that if causality indeed exists, genetic variants in the C-reactive protein gene that are associated with altered C-reactive protein plasma levels should also be associated with a consistently and proportionally altered cardiovascular risk. Several single-nucleotide polymorphisms in the C-reactive protein gene with a consistent impact on baseline C-reactive protein levels have been identified; for instance, rs18000947 and rs7553007 being associated with lower C-reactive protein levels and rs3093077 and rs3093059 with higher C-reactive protein levels.[10,48–66] Few studies have found consistent associations among genetic C-reactive protein variants, C-reactive protein levels, and cardiovascular risk;[51,59] however, the majority of studies using this design were negative.[52,54,55,57,62,63] Whether the latter implies a true lack of association or result from the lack of statistical power is unknown. Of note, it has been acknowledged that this type of studies requires at least 15 000 cases to have sufficient statistical power to detect such associations.[67] A recent large-scale analysis achieved this statistical power and showed that rs7553007 in the C-reactive protein locus was associated with 20.7% (95% CI, 17.9–23.4; $P = 1.3 \times 10^{-38}$) lower C-reactive protein levels, which yielded a predicted odds ratio for coronary heart disease of 0.94 (95% CI, 0.94–0.95).[66] The observed odds ratio was 1.00 (95% CI, 0.97–1.02), providing an argument against a causal role for C-reactive protein in the development of coronary heart disease.

Biology

There is convincing experimental evidence linking C-reactive protein to plaque disruption and the onset of cardiovascular events. C-reactive protein mRNA and protein is abundantly present in atherosclerotic lesions,[68–71] whereas the level of C-reactive protein correlates with the extent of atherosclerotic disease.[72–74] At the same time, C-reactive protein itself elicits a wide array of pro-atherogenic effects in a majority of cell types involved in atherogenesis, mostly mediated via Fcγ receptor-dependent pathways.[75–81] Following receptor engagement, various signalling cascades are activated, including NFκB, p38 MAPK, and Jun N-terminal kinase signalling pathways as well as the CD40/CD40 ligand signalling dyad.[82–86] Activation of these pathways elicit a series of pro-atherogenic changes.

Vascular endothelium

C-reactive protein stimulates leucocyte–endothelium interactions via the modulation of endothelium-derived molecules and chemokines in various cultured endothelium cells.[85,87–90] C-reactive protein decreases endothelial nitric oxide synthase (eNOS) mRNA protein as well as bioactivity.[90,91] Underlying mechanisms comprise decreased stability of eNOS mRNA,[90] blunted eNOS phosphorylation at Ser1179,[81] uncoupling of eNOS,[92] and superoxide anion release from NAD(P)H oxidase.[93,94] C-reactive protein also increases LOX-1 expression, crucial for oxidized (ox)LDL's detrimental effects on endothelial function[95] and enhances angiotensin II-induced pro-inflammatory effects.[96] C-reactive protein also impairs the number and function of endothelial progenitor cells[97] by promoting apoptosis[98] and attenuating their migration and adherence capacities.[99] More recently, C-reactive protein has been observed to damage the endothelial glycocalyx, further promoting the sensitivity of the endothelium towards pro-atherogenic insults.[100]

In experimental animal models overexpressing human C-reactive protein, data have been conflicting.[93,101] Since animals, however, do not express C-reactive protein, it is difficult to weigh the relevance of these observations. In contrast, we recently confirmed direct harmful effects of C-reactive protein on endothelium-derived vasomotor function in humans. Thus, systemic infusion of recombinant human C-reactive protein raising C-reactive protein levels to ≈24 mg/L activated the vascular endothelium as attested by significant elevation in circulating vWFAg and E-selectin. Concomitantly, endothelium-derived vasorelaxation was impaired, particularly under hypercholesterolaemic conditions.[102,103]

Coagulation pathways

C-reactive protein induces a prothrombotic state by stimulating tissue factor release from mononuclear,[104,105] endothelial, and smooth muscle cells.[106] In addition, C-reactive protein increases plasminogen activator inhibitor-1 activity in human aortic endothelial cells[107] with a concomitant reduction in tissue plasminogen activator activity,[108] resulting in an overall impaired fibrinolytic capacity. In line, human volunteers also exhibit increased thrombin generation and impaired fibrinolysis upon C-reactive protein challenge.[102]

Plaque remodelling

Recent reports have demonstrated C-reactive protein's ability to increase matrix metalloproteinase (MMP) synthesis with ensuing collagen-degrading activity in monocytes–macrophages as well as cultured endothelia.[84,86,109,110] In parallel, interleukin (IL)-8 is stimulated, which has been suggested to further enhance the disbalance between MMPs and tissue inhibitors of metalloproteinases within the atherosclerotic plaque in favour of an unstable plaque phenotype.[111,112] Recently, C-reactive protein was also shown to elicit the activation of peripheral leucocytes with ensuing secretion of plaque-destabilizing mediators including MMP-9, monocyte chemoattractant protein-1, and plasminogen activator urokinase.[113]

Complement

C-reactive protein activates the complement system,[114] which has been shown to result in a significant increase in infarct size in various myocardial infarction models.[115–118] In contrast, the inhibition of C-reactive protein 'binding' (*Figure 2*) using a small-molecule inhibitor was able to attenuate the increase in infarct size in rats.[119]

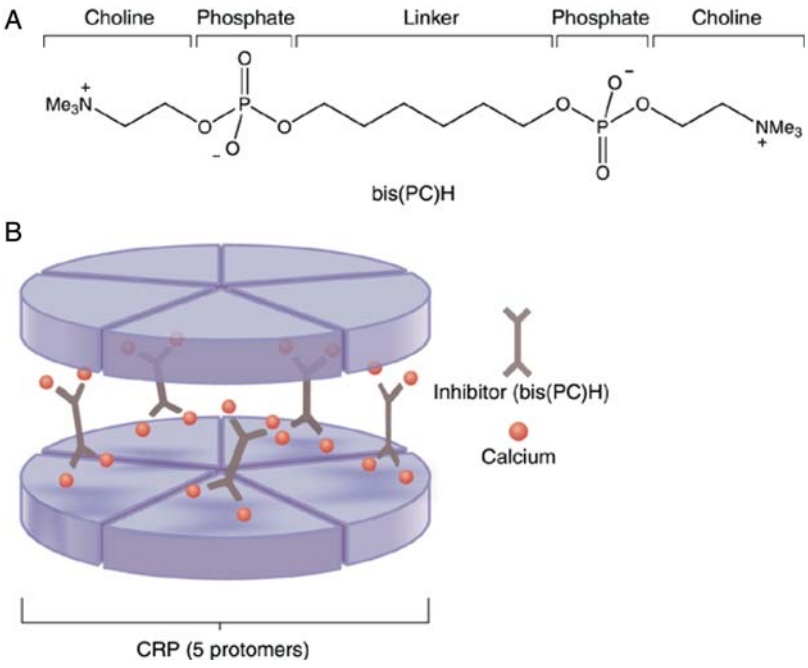

Figure 2 Specific inhibitor of C-reactive protein. This small molecule, termed bis(PC)H, is designed to interact with calcium ions bound to C-reactive protein, preventing C-reactive protein from interacting with ligands. Reprinted with permission from Macmillan Publishers Ltd, © 2010 Nature Publishing Group, a division of Macmillan Publishers Limited (2006).[131]

C-reactive protein or contamination?

Critics have claimed that 'all these convincing pro-atherogenic' effects should in fact be attributed to 'contaminating' endotoxins present in commercially available preparations.[120] However, using carefully designed control experiments, several groups have shown that C-reactive protein itself is a major contributor. Thus, Jialal and Devaraj[100,108] showed that heat-induced C-reactive protein denaturation, which should have no effect on endotoxins, abolished the pro-atherogenic effects. In line, Bisoendial *et al.*[121,122] showed that the trace amount of endotoxin present in recombinant human C-reactive protein (following additional purification steps) does not contribute to the pro-atherogenic effects. In summary, C-reactive protein itself exerts pro-atherogenic effects, both *in vitro* and *in vivo* in humans.

Intervention

With C-reactive protein being a strong risk factor for cardiovascular disease (*Figure 3*), and in light of evidence that C-reactive protein may play a biological role in atherosclerosis, the question arises as to whether an intervention to lower C-reactive protein levels may constitute a viable therapeutic strategy. In support of this hypothesis, several known anti-atherosclerotic interventions have been reported to reduce C-reactive protein.

Weight loss

An example of a lifestyle intervention that reduces C-reactive protein levels, as well as cardiovascular risk, is weight loss. In a randomized, controlled trial consisting of 120 obese women, half the participants received detailed advice about how to achieve 10% weight reduction using diet and exercise, whereas the other half were given general information regarding a healthy lifestyle. After 2 years, women in the intervention group had a stronger decrease in body mass index (-4.2, $P < 0.001$), as well as a stronger reduction in C-reactive protein levels (-1.6 mg/L, $P = 0.008$), than women in the control group.[123] Similarly, weight loss in morbidly obese patients has been shown to induce a significant decrease in C-reactive protein and IL-6 concentrations in association with an improvement of insulin resistance.[124] In another study, baseline measures of obesity were significantly associated with C-reactive protein levels and subsequent weight loss was shown to result in a proportionate reduction in C-reactive protein.[125]

Statin therapy

Statin therapy, besides lowering LDL cholesterol (LDL-C), represents a pharmacological intervention that reduces both C-reactive protein levels and cardiovascular risk. Pravastatin has prospectively been shown to reduce C-reactive protein levels by

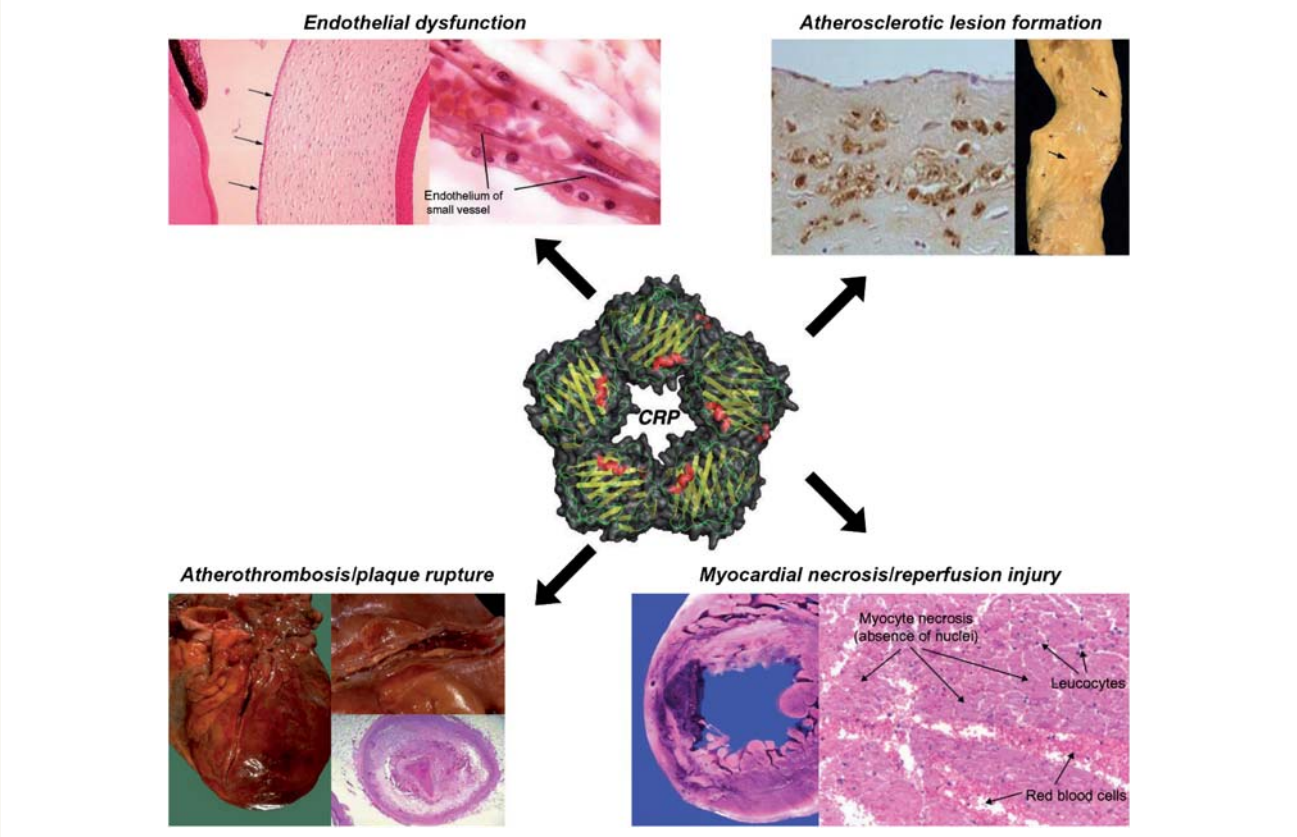

Figure 3 C-reactive protein can be considered a long-term risk factor for cardiovascular events throughout the entire spectrum of atherogenesis, ranging from fatty streak formation to atherothrombosis and tissue injury of ischaemic regions.

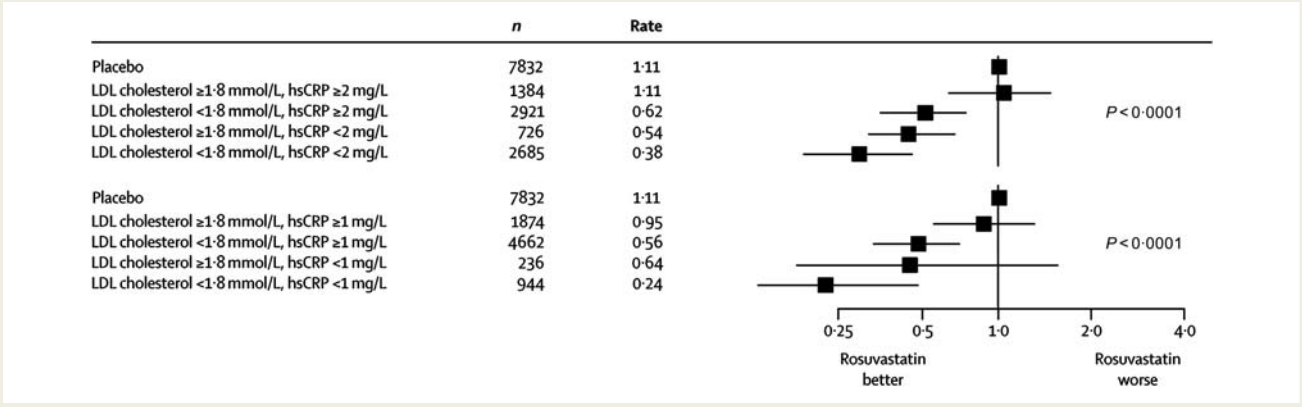

Figure 4 Hazard ratios for incident cardiovascular events in JUPITER according to achieved concentrations of low-density lipoprotein (LDL) cholesterol and high-sensitivity C-reactive protein (hsCRP) after initiation of rosuvastatin. Data are adjusted for age, baseline low- and high-density lipoprotein cholesterol, baseline hsCRP, blood pressure, sex, body mass index, smoking status, and parental history of premature coronary heart disease. Event rates are per 100 person-years. Reprinted with permission from Ridker *et al.*[132]

more than 15% within 12 weeks, in a largely LDL-C-independent manner.[126] A long-term study yielded even more pronounced results: after 5 years of pravastatin treatment, C-reactive protein levels were reduced by over 35% compared with placebo, again unrelated to the magnitude of the lipid alterations.[127] In addition, measurements of C-reactive protein in the AFCAPS/TEXCAPS suggested that patients with above-median baseline C-reactive protein levels still had cardiovascular benefit from lovastatin therapy, even in the absence of overt dyslipidaemia.[128] Also in this trial, lovastatin reduced C-reactive protein by almost 15%.[128] It was mostly this finding that prompted the design of the JUPITER trial: a prospective evaluation of the efficacy of rosuvastatin in the primary prevention of major cardiovascular events in persons with elevated C-reactive protein, but low LDL-C levels.[129] This trial thus employed a known C-reactive protein-lowering agent[130] to reduce cardiovascular risk in a group of people whose risk was considered to be mainly determined by their high C-reactive protein levels. Rosuvastatin reduced LDL-C by 50% and C-reactive protein levels by 37%, and very effectively decreased cardiovascular events vs. placebo. The hazard ratio for the primary endpoint (non-fatal myocardial infarction, non-fatal stroke, hospitalization for unstable angina, arterial revascularization procedure, or confirmed death from cardiovascular events) was 0.56 (95% CI, 0.46–0.69; $P < 0.00001$), after a median follow-up of 1.9 years. Pre-specified analyses showed that patients benefited particularly from this drug if low concentrations of both LDL-C and C-reactive protein were achieved.[131,132] In addition, lower-achieved values of C-reactive protein were associated with the largest risk reduction (*Figure 4*). Here too, the LDL-C decrease hardly correlated with the C-reactive protein decrease ($r = 0.15$), at least suggesting that the C-reactive protein reduction may have contributed to the reduction in cardiovascular risk.[131,132]

What can we learn from these two examples? Both weight loss and statin therapy apparently result in a reduction in low-grade inflammation, underlying a reduction in cardiovascular disease risk. The big question is: is this anti-atherogenic effect (partially) mediated by C-reactive protein reduction, or are the lower C-reactive protein levels a mere reflection of the reduction in inflammatory status? To answer this question, the impact of specific inhibitors of C-reactive protein should be tested. A first clue that specific C-reactive protein inhibition may be beneficial came in 2006, when Pepys *et al.* reported that a small-molecule inhibitor of C-reactive protein was able to reduce myocardial infarct size as well as cardiac dysfunction produced by injection of human C-reactive protein in rats.[119]

Conclusion

Currently available evidence indicates that C-reactive protein is a pro-atherogenic factor throughout the entire spectrum of cardiovascular disease, ranging from fatty streak formation to clinical events. Thus, C-reactive protein is to be considered as an established risk factor for cardiovascular risk, comparable to established cardiovascular risk factors such as hypertension and diabetes.

Besides its role as a 'bystander', C-reactive protein has been shown to exert a wide array of pro-atherogenic effects, thereby implying a causal role for C-reactive protein by driving inflammation as well as thrombosis. These data underscore the need to explore the impact of selectively targeting C-reactive protein as a novel approach to prevent clinical manifestations of atherosclerosis.

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