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Reflection

C-Reactive Protein: Eighty Years from Discovery to Emergence as a Major Risk Marker for Cardiovascular Disease

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C-reactive protein (CRP)[2] was discovered in 1930 by William Tillett and Thomas Francis from the Rockefeller University. They described a third serologic fraction, or "fraction C," that could be isolated from patients infected with pneumococcus that was distinct from previously known capsular polysaccharide and nucleoprotein fractions detectable by specific antibody response *(1)*. A decade later, Oswald Avery and Maclyn McCarty—the research team who originally described the "transforming principle" and the concept that genes are made of DNA—also described CRP as an "acute-phase reactant" that was increased in serum of patients suffering from a spectrum of inflammatory stimuli, including myocarditis and the inflammation associated with rheumatic fever *(2–4)*. Early clues that this inflammatory biomarker might be linked to atherothrombosis are evident in 2 case reports presented by Gunnar Löfström from the State Bacteriologic Laboratory in Stockholm in 1943, in which increases in CRP following acute myocardial infarction are described *(5)*. In the mid 1950s, case series presented by Irving Kroop and others indicated that CRP concentrations consistently increase after coronary ischemia and myocardial necrosis, data that was clinically important, as diagnostic tools for acute coronary syndrome did not yet include creatinine kinase or troponin *(6)*. By the mid 1980s, the work of John Volanakis, Mark Pepys, Irving Kushner, and others had identified CRP as a hepatically derived, nonglycosylated, circulating pentraxin composed of 5 identical subunits arranged with pentameric symmetry that had characteristic calcium-dependent binding to specific ligands, including binding to LDL cholesterol *(7–13)*. They and other investigators further demonstrated that the bulk of circulating CRP is produced by hepatocytes largely under regulatory control of inflammatory cytokines including interleukin-6 (IL-6) and tumor necrosis factor-α; that the plasma half-life of CRP is approximately 19 h under basal and stress conditions; and thus that the plasma concentration is largely determined by synthetic rate *(14, 15)*.

Despite these data, cardiovascular interest in CRP did not reemerge until the publication of several confirmatory descriptions of CRP increase among patients with established or acute atherothrombotic syndromes. Notable among these reports were data from Frederick de Beer, Brad Berk, and Wayne Alexander *(16, 17)*, who described increased CRP concentrations among patients with "active" coronary disease, as well as highly influential studies from Attilio Maseri, Giovanna Liuzzo, Luigi Biasucci, and Frits Haverkate *(18, 19)*, in which increased concentrations of CRP were again observed among those with unstable angina or chronic atherothrombotic disease. Because concentrations of CRP increase after myocardial ischemia, however, these studies of individuals with known vascular disease were not informative regarding the key question of whether CRP concentrations are increased in advance of disease expression.

From a research perspective, the only way to address this issue was to perform prospective cohort studies in which initially healthy individuals underwent CRP measurement and then were followed over time to see if baseline CRP increases associate with future vascular events. In the first study to use this design, Lew Kuller, Russell Tracy, and the Multiple Risk Factor Intervention Study (MRFIT) investigators found such an association, but only for fatal events among high-risk populations, predominantly smokers *(20)*. Unfortunately, as smoking alone leads to secondary increases in CRP, these data could not distinguish whether CRP increases were simply a result of the disease process or

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[2] Nonstandard abbreviations: CRP, C-reactive protein; IL-6, interleukin-6; MRFIT, Multiple Risk Factor Intervention Study; PHS, Physicians Health Study; CARE, Cholesterol and Recurrent Events; AFCAPS/TexCAPS, Air Force/Texas Coronary Atherosclerosis Prevention Study; JUPITER, Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin; hsCRP, high-sensitivity CRP; NF, nuclear factor; MONICA, Monitoring of Trends and Determinants in Cardiovascular Disease; EPIC, European Prospective Investigation of Cancer; NHANES, National Health and Nutrition Examination Survey; PROVE IT–TIMI 22, Pravastatin or Atorvastatin Evaluation and Infection Therapy: Thrombolysis in Myocardial Infarction 22; REVERSAL, Reversing Atherosclerosis with Aggressive Lipid Lowering.

were a significant biomarker of risk preceding onset of disease itself.

This paradigm changed in 1997 with the publication of a prospective evaluation of CRP performed within the Physicians Health Study (PHS), a large-scale prospective cohort of initially healthy American men *(21)*. In that study, baseline CRP concentrations were significantly higher among those who subsequently went on to have myocardial infarction or stroke than among those who did not. Importantly, CRP concentrations predicted future vascular risk among nonsmokers as well as among those with no other major risk factors. In follow-up studies of these men, CRP concentrations were found to add prognostic information to that of total and HDL cholesterol, to be fully independent of both lipid and nonlipid risk factors, and to be predictive of incident peripheral arterial disease and sudden death *(22–25)*.

The 1997 PHS data also provided critical evidence that CRP might have utility in determining the efficacy of therapies commonly used for vascular risk reduction. All participants in the PHS had been randomly allocated to receive aspirin at a dose of 325 mg on alternate days, a therapy that reduced vascular event rates 44% in the cohort as a whole. However, when the results of the PHS were stratified by baseline CRP concentrations, the reduction in risk attributable to aspirin was greatest among those with the highest levels of inflammation *(21)*. As aspirin is an antiinflammatory as well as an antiplatelet agent, this observation suggested that inflammation might represent a modifiable risk marker for cardiovascular disease, susceptible to agents with putative antiinflammatory effects such as aspirin, statins, and modifiers of the renin-angiotensin-aldosterone system. The PHS data did not indicate that CRP itself was a causal agent, since other inflammatory biomarkers in these same men including fibrinogen, soluble intercellular adhesion molecule 1, and IL-6 also predicted future vascular events *(26, 27)*. Taken together, however, these prospective epidemiologic data provided a strong basis of support for the inflammatory hypothesis of atherosclerosis *(28–30)*.

The observation in the PHS and confirmation in the subsequent Women's Health Study *(31, 32)* that CRP concentrations predict incident thromboembolic stroke proved important for understanding interactions between lipid-lowering therapy and inflammation, particularly as statins lower the risk of stroke despite LDL cholesterol not being a major risk marker for stroke. In 1998, investigators working in the Cholesterol and Recurrent Events (CARE) trial reported that the clinical benefit of statins in terms of event reduction was greater among those with increased CRP, and that statin therapy reduces CRP concentrations in a largely LDL-independent manner *(33, 34)*. These observations were subsequently confirmed for all statins *(35–39)*, although more potent agents such as rosuvastatin appear to result in even greater LDL and CRP reductions. Thus, by 1999, evidence was rapidly accruing not only that CRP might represent a novel biomarker of vascular risk, but that CRP evaluation might also merit consideration as a method to monitor pharmacologic interventions used to prevent and treat cardiovascular disease.

Soon thereafter, in hypothesis-generating data from the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) published in 2001, it was observed that statin therapy reduced vascular event rates among those with increased CRP but low concentrations of LDL cholesterol *(40)*. In marked contrast, among AFCAPS/TexCAPS participants with low CRP and low LDL, no benefit of statin therapy was observed in terms of event reduction despite reductions in cholesterol. This observation had potential relevance for prevention, particularly as the large-scale Women's Health Study demonstrated conclusively in 2002 that those with low cholesterol but high CRP represent a high-risk group of patients outside current guidelines for statin therapy *(41)*. However, as the CRP analysis of AFCAPS/TexCAPS was performed on a post hoc basis, a large prospective trial of statin therapy among those with low LDL but increased CRP would be needed to directly test this hypothesis. That trial, the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER), was launched in 2003 *(42)*.

Because the range of CRP reported in clinical studies of vascular risk was often far below thresholds detectable by standard CRP assays that had lower detection limits of 5–10 mg/L, several investigators including Nader Rifai, Gary Myers, Francesco Dati, and William Roberts worked to develop and validate high-sensitivity methods for CRP measurement that over time became known as "hsCRP" *(43–46)*. This important work led to reproducibility standards for all commercial tests for hsCRP, a step that not only ensured a common analytic structure for investigators in the CRP field, but also provided the basis for Tom Pearson, George Mensah, Sid Smith, and their colleagues to draft the first set of clinical guidelines for use of hsCRP as an adjunct to global risk prediction, guidelines that were formally endorsed in early 2003 by the American Heart Association and the CDC *(47)*. Those guidelines formalized reporting standards for hsCRP whereby concentrations of <1, 1–3, and >3 mg/L are now used clinically to suggest lower, moderate, and higher relative risk of incident vascular disease within the context of global risk evaluation.

The availability of commercial assays for hsCRP quickly led to confirmations of CRP as an independent

predictor of future cardiovascular events in more than 30 diverse population cohorts, including those led by Wolfgang Koenig [the Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA)-Augsberg Cohort *(48, 49)*], Christie Ballantyne (the Atherosclerosis Risk In Communities study *(50)*), Matthijs Boekholdt [the European Prospective Investigation of Cancer (EPIC)-Norfolk Study *(51)*], Eric Rimm (the Health Professional Follow-Up Study and the Nurses Health Study *(52)*), John Danesh (the British General Practice Cohort and the Reykjavik Heart Study *(53, 54)*), David Curb (the Honolulu Heart Study *(55)*), Mary Cushman (the Cardiovascular Health Study *(56)*), Lyle Best (the Strong Heart Study *(57)*), Jukka Salonen (the Kuopio Ischemic Heart Disease Study *(58)*), and Peter Wilson and Chris O'Donnell (the Framingham Heart Study *(59)*). Using data from the National Health and Nutrition Examination Survey (NHANES), the Framingham cohort, and the Women's Health Study, respectively, Earl Ford, Natalia Rost, and Brendan Everett also confirmed the independent predictive value of hsCRP for stroke *(60–62)*.

Based on the consistency of these data, clinical risk algorithms known as the Reynolds risk scores for both women *(63)* and men *(64)* were developed and validated that, in addition to traditional risk factors, incorporate information on both inflammation (hsCRP) and genetics (parental history of myocardial infarction before age 60 years). Freely accessible to clinicians at www.reynoldsriskscore.org, these novel prediction algorithms allow a simple translation of data on inflammation and genetics into clinical practice, correctly reclassifying approximately 20% to 30% of those at intermediate risk into clinically relevant higher or lower risk categories. The statistical basis for reclassification and the need to move beyond simple C-statistics to evaluate novel risk markers was developed by Nancy Cook *(65, 66)* and then expanded to include formal indices of reclassification as described by Michael Pencina and Ralph D'Agostino *(67)*. Using these techniques, the utility of hsCRP for risk reclassification has recently been confirmed in the Framingham Heart Study *(59)*.

Following the observation by Aruna Pradhan in the Women's Health Study in 2001 that hsCRP concentrations also predict incident diabetes *(68)*, many investigators reported on relationships between CRP, insulin resistance, and metabolic syndrome. Prominent among these are data from David Laaksonen, Leo Niskanen, Naveed Sattar, Andreas Festa, Steve Haffner, and Paresh Dandona indicating that hsCRP predicts both metabolic syndrome and diabetes even after adjusting for insulin concentrations, and that insulin itself may regulate CRP expression *(69–71)*. These data are intriguing given work from Allison Goldfine and Steven Schoelson delineating the role of nuclear factor (NF)-κB inhibition in the genesis of diabetes as well as the ability the antiinflammatory agent salsalate to increase adiponectin, reduce CRP and IL-6, and improve glucose tolerance among type 2 diabetic patients *(72)*. Many investigators subsequently reported associations between diet, exercise, and CRP, including the work of Samia Mora relating both "fitness and fatness" to inflammatory parameters *(73, 74)*.

For many clinicians, the observation that measuring hsCRP could identify individuals at high risk for future vascular disease and diabetes provided necessary but not sufficient evidence to introduce hsCRP into routine clinical care, since targeted interventions for patients with increased hsCRP had not yet proven effective at reducing risk. Several recent studies have now addressed this second level of evidence. Among individuals with acute coronary ischemia, both the Pravastatin or Atorvastatin Evaluation and Infection Therapy: Thrombolysis in Myocardial Infarction 22 (PROVE IT–TIMI 22) trial published in 2005 *(75)* and the A to Z (Aggrastat to Zocor) trial published in 2006 *(76)* demonstrate that best clinical outcomes after initiating statin therapy accrue among those who not only reduce LDL cholesterol to <70 mg/dL (1.81 mmol/L), but also reduce hsCRP to <2 mg/L. Similarly, in the Reversing Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) trial, atherosclerotic regression with statin therapy as detected by intravascular ultrasound was only observed among those who reduced both hsCRP and LDL cholesterol after starting statin therapy *(77)*. These effects are particularly robust for the prevention of stroke, where LDL reduction alone following statin therapy has not been found sufficient, whereas those who additionally achieve low concentrations of hsCRP after initiating statin therapy appear to have substantially reduced stroke incidence *(78)*. On this basis, it has been proposed that physicians consider dual goals for statin-treated patients that include both low concentrations of LDL cholesterol and low concentrations of hsCRP *(79)*.

Finally, in May 2008, the JUPITER trial was stopped early by its Independent Data and Safety Monitoring board due to the emergence of a statistically extreme benefit of rosuvastatin among apparently healthy men and women with low concentrations of LDL cholesterol (<130 mg/dL) but increased concentrations of hsCRP (>2 mg/L). Specifically, among 17 802 participants in JUPITER followed for up to 5 years, random allocation to rosuvastatin resulted in a 54% reduction in myocardial infarction ($P = 0.0002$), a 48% reduction in stroke ($P = 0.002$), a 47% reduction in need for arterial revascularization procedures ($P < 0.00001$), and a 20% reduction in all-cause mortality

($P = 0.02$) compared with placebo *(80)*. In JUPITER, effects among patients with increased hsCRP were consistent in all subgroups evaluated, including those traditionally assumed to be low risk, such as women, those with low Framingham Scores, and those with native LDL concentrations <100 mg/dL (2.59 mmol/L). In this hard-endpoint trial conducted among those with optimal lipid concentrations according to current prevention guidelines [median LDL at study entry 108 mg/dL (2.80 mmol/L), median HDL 49 mg/L (1.27 mmol/L)], a 37% reduction in the primary trial endpoint was observed in the subgroup with increased hsCRP but no major risk factor other than increased age. The number-needed-to-treat for the primary 5-year projected endpoint in JUPITER for individuals with increased hsCRP was 25, a value if anything smaller than that observed in prior statin prevention trials targeting those with overt hyperlipidemia. All of the above risk reductions were evident within months of initiating therapy. In JUPITER, the strategy of screening for hsCRP to define a high-risk population for statin therapy was also effective among women and minority populations.

Despite this rapid accumulation of clinical evidence, it is important to remember that the function of CRP in human physiology remains uncertain. As CRP precipitates ligands and activates the classical complement pathway *(81)*, a putative role for CRP has often been hypothesized for pattern recognition, host defense, and enhancement of the innate immune response. This hypothesis is supported by evidence of both evolutionary and phylogenetic conservation of CRP *(7, 8)*. Further, based on work from the laboratories of Ishwarlal Jialal, Alexander Szalai, Ke Chen, Allan Zhao, James Willerson, Ed Yeh, Haim Danenberg, Radjesh Bisoendial, Erik Stroes, John Kastelein, Hans Sauerwein, Subodh Verma, and others, a range of potential effects of CRP on human physiology have been suggested, including inhibition of fibrinolysis, promotion of tissue factor, reduction of endothelial nitric oxide, increases in cellular adhesion, and induction of gluconeogenesis and leptin resistance *(82–89)*. However, as Mark Pepys has pointed out, no deficiency state in humans is known, data on lack of function do not currently exist, and thus careful studies with direct CRP inhibitors are needed to address issues of function and potential causal pathways *(90)*. The complexity of such studies should not be underestimated, as preparations of human CRP free of sodium azide and lipopolysaccharide are difficult to obtain and animal models are limited owing to wide interspecies differences in CRP ligand recognition and acute-phase response activity *(91, 92)*.

To date, genetic studies of CRP have been conflicting, and links between genotype, phenotype, and vascular risk as they associate with CRP remain inconclusive. For example, in the recent Copenhagen City Heart Study, Jeppe Zacho and Borge Nordestgaard provided cross-sectional data that strongly affirm the role of hsCRP as a potent biomarker of vascular risk but were unable to link specific polymorphism within the CRP gene with that risk despite associations between the polymorphisms evaluated and plasma hsCRP concentrations *(93)*. On this basis, some have concluded that CRP is thus only a biomarker of risk, and not a causal participant in the atherothrombotic process. It is important to recognize, however, that the Mendelian randomization technique used in that study is itself controversial, and that the ability of the approach to firmly establish or refute a biologically plausible pathway is limited. Scientifically, lack of support for a causal relationship in any one study does not prove noncausality, and issues of sample size and selection bias due to differential exposures among affected and unaffected individuals make defining an informative null in this setting difficult. Further, recent genome-wide association studies indicate that at least 7 distinct loci are involved in basal CRP expression, limiting interpretation of data from any 1 locus *(94, 95)*. Thus, only through direct experimentation will it be possible to ultimately clarify whether CRP is a direct participant in the atherothrombotic process. Similarly, only through carefully designed clinical trials using targeted antiinflammatory therapies will it be possible to directly test the inflammatory hypothesis of atherothrombosis *(96)*.

None of these issues mitigate the use of hsCRP as a robust biomarker for the development of cardiovascular disease among apparently healthy men and women, including those with low concentrations of cholesterol and those at intermediate risk. As tested prospectively in the JUPITER trial, use of hsCRP to identify a population that will greatly benefit from preventive therapy is now firmly established *(80)*. Robert Glynn, the biostatistician responsible for data analysis within JUPITER, has conservatively estimated that application of the strategy of hsCRP screening followed by high-dose statin therapy over a 5-year period could prevent more than 250 000 heart attacks, strokes, revascularization procedures, and premature vascular deaths in the US alone. Thus, almost 80 years after its discovery, CRP has emerged as a major new tool for the prevention of heart disease, stroke, and all-cause mortality.

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