

Simple and Reproducible ELISA for C-Reactive Protein

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Featured Article: Macy EM, Hayes TE, Tracy RP. Variability in the measurement of C-reactive protein in healthy subjects: Implications for reference intervals and epidemiological applications. *Clin Chem* 1997;43:52–8.²

The purpose of the work reported in this paper was 2-fold. First, we sought to develop an immunoassay for C-reactive protein (CRP)³ that could quantify CRP present at low concentrations in healthy people and that had characteristics such as ease of use, high throughput, standardization, and portability. In addition, because CRP was an acute-phase reactant, we wanted to demonstrate that although it had greater biovariability than other biomarkers such as cholesterol, CRP was suitable for use in epidemiological research.

Other investigators had previously worked with CRP, but our interest had its origins in a 1990 discussion I had with Lew Kuller, a colleague in the Cardiovascular Health Study (CHS). CHS was a multicenter longitudinal epidemiology study of elderly men and women, funded by NIH. I had received NIH funding to pursue biomarkers of coagulation as possible cardiovascular disease (CVD) risk factors. A few years earlier, the Northwick Park Heart Study had reported pioneering findings that fibrinogen and other coagulation factors were potential CVD risk factors, and we were eager to see if we could reproduce these results. We observed that in CHS fibrinogen and factor VIIIc were associated with CVD, but factor VIIc wasn't. Because fibrinogen and factor VIIIc were acute-phase reactants, this finding suggested to us, and others as well, that systemic inflammation, not coagulation per se, might be the culprit.

At this time Kuller and I were puzzling over a recent abstract reporting that low albumin values appeared to predict CVD events, results that also suggested the presence of inflammation because albumin

is a negative acute-phase reactant. We studied albumin concentrations in several projects but decided to use an acute-phase reactant with a greater dynamic range. Since its discovery in 1929, CRP had become a useful marker of acute inflammation (1). Over the years research assays for CRP had been developed in RIA and immunoradiometric assay formats. In particular, Mark Pepys' laboratory had done important research on CRP, demonstrating in 1982 an increased CRP in patients following the onset of acute myocardial infarction.

We decided on an ELISA format, and because we had developed numerous ELISAs, we found developing a CRP assay to be a relatively straightforward process. We chose to use commercially available reagents for generalized application. We evaluated CRP purity by gel filtration and SDS gel electrophoresis, and we characterized polyclonal antibodies by using Western blotting and establishing avidity. Settling on a reagent pair and a competitive format, we used the new assay to study biological variance, believing (correctly it turns out) that because CRP was an acute-phase protein, reviewers would question its utility in epidemiological and clinical research. Thus, data on variability would prove valuable. For this investigation we used the format of Fraser, in which analytical, within-subject, and between-subject variances are all established and compared (2).

We used the new CRP assay in CHS. The first preliminary results, based on data from healthy people, suggested that CRP was a CVD risk factor in otherwise healthy people. We presented our findings at the 1994 annual meeting of the American Heart Association Council on Epidemiology and Disease Prevention. At that meeting we organized an informal get-together of investigators interested in "inflammation and CVD". The attendees at that first meeting of the Pelican Club (named for the "Pelican Room," which we rented for the event) made up for their small number with enthusiasm. We continued to host the Pelican Club at the Epidemiology Council meetings, and the number of "members" attending increased. In all we had 5 meetings from 1994 to 1999, with more than 40 attendees at the later meetings. Early attendees included Paul Ridker from Boston, who had the insight to suggest collaborating on the Boston-based Physicians Health Study. We published the Physicians Health Study results in 1997 (3), along with results from CHS and Kuller's

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² This paper has been cited more than 340 times since publication. Address correspondence to the author at: University of Vermont College of Medicine, 208 South Park Drive, Suite 2, Colchester, VT 05446-5864. Fax 802-656-8965; e-mail russell.tracy@uvm.edu
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³ Nonstandard abbreviations: CRP, C-reactive protein; CHS, Cardiovascular Health Study; CVD, cardiovascular disease.

Rural Health Promotion Study (4). These studies and many others provided impetus to the field, resulting in a CDC/American Heart Association Consensus Panel recommendation for the clinical use of CRP (5). Commercial assays with greater reproducibility, standardization, and throughput, especially the Dade-Behring BN-II assay (6), have appropriately replaced our original ELISA.

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