patient’s estimated function by 15 to give the mL of 200 g/L PAH diluted to a final volume of 200 mL with intravenous diluent. Patients were kept fasting for 4 h before the test (patients with diabetes mellitus for 2 h). In the hour preceding the test, patients were instructed to drink four glasses of water. Patients were also asked not to void within 45 min of their appointment time.

At the start of the test, patients were asked to void a pretest urine sample (U0), and an initial blood sample was drawn (P0), followed by intravenous administration of the primary solution over a 2-min period and initiation of the sustaining solution at 1 mL/min. After the priming dose was completed, a hydrating solution of 25 g/L dextrose was attached with a flow rate of 2–3 mL/min. After a 45-min equilibration period, patients were asked to void (U1). A blood sample was also drawn (P1) within 5 min of the urine collection from the arm opposite the one used to administer intravenous solutions. After 30 min, patients were asked to empty their bladders again (U2), and time and volume were recorded. Another blood sample was immediately taken (P2). Bladder voiding and blood draws were repeated every 30 min until samples U3 and P4 were obtained. Completeness of bladder emptying for all urine collections was monitored with a portable ultrasound bladder scanning device (Diagnostic Ultrasound). The urine volume (mL) was divided by the collection time (min) to give the flow in mL/min. The RPF was calculated for each period. The RPF (mL/min) for period 1 equaled the concentration of PAH in U1 (mg/L) multiplied by the flow (mL/min) divided by the average of the concentrations of PAH in P1 and P2 (mg/L). RPF corrected by body surface area was also calculated as: body surface area = weight (kg)0.425 × height (cm)0.725 × 0.007184.

The results of the method comparison for the split urine and plasma samples measured by CE and colorimetric methods are shown in Fig. 1, A and B. The RPF values obtained by CE and colorimetric methods for all study participants are compared in Fig. 1C. The overall mean RPF for CE was 322 mL·min⁻¹·1.73 m² and for colorimetric was 322 mL·min⁻³·1.73 m² with an R² of 0.979.

In conclusion, measurement of urine and plasma PAH by CE is an accurate alternative to the colorimetric assay for assessment of RPF. This new CE method reduces drug interferences, analysis time, and required volumes of reagents and blood. The method comparison results, recoveries, and assay variation are all acceptable for this new methodology. With the CE method, there is less reagent preparation than with the colorimetric assay, which required preparation of many calibrators. Finally, computer-controlled instrumentation for the CE assay also allows for automation and eliminates time-consuming calculations.

References

Population Distributions of C-reactive Protein in Apparently Healthy Men and Women in the United States: Implication for Clinical Interpretation, Nader Rifai1,2* and Paul M. Ridker2,3.1 Department of Laboratory Medicine, Children’s Hospital, Boston, MA 02115; 2 Center of Cardiovascular Disease Prevention and 3 Division of Cardiovascular Disease, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA 02115; 4 address correspondence to this author at: Children’s Hospital, Department of Laboratory Medicine, 300 Longwood Ave., Boston, MA 02115; fax 617-713-4347, e-mail nader.rifai@tch.harvard.edu

Measurement of the acute-phase reactant C-reactive protein (CRP) has been used historically in the diagnosis and monitoring of active infection or inflammation. Recent prospective epidemiologic studies have demonstrated that CRP, at concentrations within the reference interval, is a strong predictor of myocardial infarction (1–3), stroke (1, 2, 4), sudden cardiac death (5), and peripheral arterial disease (6) in apparently healthy adults. High-sensitivity methods that are capable of reliably measuring CRP concentrations ≤0.15 mg/L (approximately the first and second percentiles of CRP distribution in healthy adults) have, therefore, been developed (7, 8). Furthermore, an algorithm for risk assessment of future coronary events that combines both CRP concentration and total cholesterol:HDL-cholesterol ratio has been proposed (9). According to this algorithm, an individual’s risk can be estimated with use of quintiles of CRP and lipids derived from ongoing population-based surveys.

Because high-sensitivity methods have only recently become available, the frequency distributions of CRP in apparently healthy US adults have not been carefully examined. Such information is useful in determining the most appropriate CRP cutpoints for the risk assessment algorithm in men and women. In this report, we describe...
the frequency distribution of CRP in 22,403 US adults and propose new CRP cutpoints for clinical interpretation.

CRP measurement was performed in 22,403 apparently healthy individuals participating in the Physicians’ Health Study (PHS), the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS), the Women’s Health Initiative (WHI), and the Women’s Health Study (WHS). Details of these studies have been published elsewhere (10–13). To avoid the issue of confounding by disease outcome, we evaluated only those individuals who were free of cardiovascular disease at study entry and remained free from disease during follow-up. To avoid confounding by drugs, we eliminated those women taking hormone replacement therapy. In total, our study included 15,770 women not taking hormone replacement therapy from the WHS and 348 from the WHI, 5,742 men and women from AFCAPS/TexCAPS, and 543 men from the PHS. Participants’ ages ranged from 40 to 84 years. Plasma samples for the WHS were stored in liquid nitrogen, and those for the other three studies were stored at −70 to −80 °C until analysis.

In this study, CRP was measured in various cohorts by three different methods. In the PHS, CRP concentration was determined by an in-house competitive ELISA with use of polyclonal anti-CRP antibodies (Calbiochem-Novabiochem) and a calibrator that was traceable to WHO Reference Material 85-506 (14). The run-to-run imprecision, reflected by the CVs, at CRP concentrations of 1.05, 2.08, and 2.52 mg/L was 5.7%, 5.5%, and 6.8%, respectively. In the AFCAPS/TexCAPS and WHI studies, CRP was measured by a particle-enhanced assay performed on the BNII Nephelometer (Dade Behring) (8). The assay used monoclonal anti-CRP antibodies and a calibrator that was traceable to the WHO Reference Material. The run-to-run imprecision at CRP concentrations of 0.15, 0.49, and 1.90 mg/L was 4.9%, 6.4%, and 5.8%, respectively. In the WHS, CRP was measured by latex-enhanced reagents from Denka-Seiken on a Hitachi 911 automated analyzer (Roche Diagnostics) (18). The assay used monoclonal anti-CRP antibodies and a calibrator that was traceable to the WHO Reference Material. The run-to-run imprecision at CRP concentrations of 0.71, 0.41, and 1.88 mg/L was 5.1%, 3.3%, and 2.5%, respectively.

We have previously demonstrated that the three methods are analytically and clinically comparable. With the Dade Behring as the comparison method, the in-house ELISA gave a slope of 0.99 and an intercept of 0.36 mg/L (r = 0.95; S\textsubscript{yl} = 0.14; n = 288), and the Denka Seiken method gave a slope of 0.97 and an intercept of −0.05 mg/L (r = 0.995; S\textsubscript{yl} = 0.31; n = 388) (7,8).

The distributions of CRP for both men and women were highly skewed to the right, and <5% of men and women had CRP >10 mg/L, findings consistent with earlier reports (15–17). In our largest cohort (WHS), we demonstrated only a slight change in CRP concentration with age: median CRP concentrations for individuals 45–54, 55–64, 65–74, and ≥75 years of age were 1.31, 1.89, 1.99, 1.52, and 1.52 mg/L, respectively (18). In addition, the median CRP concentrations seen in our cohorts are comparable to those reported from other populations (15–17, 19). The frequency distribution of CRP values for these apparently healthy US men and women not taking hormone replacement therapy were comparable (Table 1A and 1B). This observation has a substantial clinical implication because it clearly demonstrates that a single set of cutpoints for risk assessment of future coronary events can be used for both genders. A similar observation regarding the similarity of CRP distribution among Scottish men and women has been reported (15). However, in a German cohort, women tended to have slightly, but significantly higher, CRP concentrations compared with men, which was attributed to estrogen use (15). Hormone replacement therapy, whether it is with estrogen alone or in combination with progestin, is known to significantly increase CRP concentration (20,21). On the basis of findings from the WHI (22) and the Heart and Estrogen/Progestin Replacement Study (23), hormone replacement therapy is not currently recommended for women with or without heart disease for the purpose of reducing future risk of coronary events. It therefore is imperative that future studies that examine the popula-

| Table 1. CRP distribution in apparently healthy US men and women. |
|---------------------|---------------------|---------------------|---------------------|
| **A. CRP distribution by quintiles in individual studies** |
| Quintile | PHS (10) (n = 543) | AFCAPS (11) (n = 5742) | WHI* (12) (n = 348) | WHS (13)* (n = 15,770) |
| 1 | <0.5 | <0.7 | <0.7 | <0.5 |
| 2 | 0.5–1.0 | 0.7–1.1 | 0.7–1.3 | 0.5–1.1 |
| 3 | 1.0–1.9 | 1.1–1.9 | 1.3–2.5 | 1.1–2.1 |
| 4 | 1.9–3.1 | 2.0–3.8 | 2.5–4.3 | 2.1–4.2 |
| 5 | >3.1 | >3.8 | >4.3 | >4.2 |
| **B. CRP distribution of the total population by percentiles** |
| Percentile | Women* | Men |
| 5th | 0.19 | 0.28 |
| 10th | 0.29 | 0.40 |
| 25th | 0.61 | 0.80 |
| 50th | 1.52 | 1.50 |
| 75th | 3.48 | 3.20 |
| 90th | 6.61 | 6.05 |
| 95th | 9.14 | 8.55 |

* Women were not taking hormone replacement therapy.
tion distribution of CRP exclude women receiving hormone replacement therapy.

As indicated earlier, the initially proposed algorithm for assessing future coronary risk used quintiles of CRP and total cholesterol:HDL-cholesterol ratio. Recently, it has been shown that quintiles and tertiles of CRP are similarly associated with future risk of coronary event (24). Furthermore, when combined with CRP, the ability of LDL-cholesterol to predict cardiovascular disease was comparable to that of the total cholesterol:HDL-cholesterol ratio (18). Therefore, for ease of clinical interpretation, a more simplified set of cutpoints that are based on approximate tertiles of the US population (<1.0, 1.0–3.0, >3.0 mg/L) could be used (25). These cutpoints have the advantage of being easy to remember and, when added to LDL-cholesterol cut points of <1300, 1300–1600, and >1600 mg/L, can be used in an algorithm for risk assessment of cardiovascular heart disease in primary prevention (Fig. 1). The LDL-cholesterol cutpoints used are those recommended by the National Cholesterol Education Program (NCEP).

The limitations of this study deserve mention. The CRP concentrations in the examined cohorts were measured by three different methods. We previously demonstrated that the three methods used were analytically and clinically comparable (7, 8). All three methods used calibrators that are traceable to the WHO Reference Materials and were able to classify individuals comparably into quartiles of risk. The consistency in the population distributions among the various cohorts despite the use of three different methods is a good indication of the comparability of these assays and further strengthens our findings.

Some of the study samples (PHS, AFCAPS/TexCAPS, WHI) were stored at −70 °C to −80 °C, and others (WHS) were stored in liquid nitrogen for several years before analysis. CRP has long been recognized as a very stable protein. In fact, no significant change was seen in CRP concentrations of samples stored at −70 °C for up to 20 years (26). In-house data show only a minor effect of storage in liquid nitrogen for 6 months on CRP values of samples collected from apparently healthy individuals [mean (SD) for fresh samples, 4.3 (4.9) mg/L vs 4.1 (4.5) mg/L for frozen samples; n = 23]. The comparable CRP distribution seen among the various examined groups attests to the stability of this protein when stored at different temperatures of −70 °C or lower.

The examined populations consisted predominantly of Caucasians, thus raising the question as to whether the recommended cutpoints and risk estimates may be expanded to other ethnic groups. The CRP distributions reported here were not only comparable to those reported from European populations but also to those for Japanese men (15, 17, 19). Japanese women, however, seem to have lower CRP values (17). No data currently exist regarding the distribution of CRP in African-American and Hispanic adult populations. Clearly, studies are urgently needed to determine whether ethnic-dependent CRP cutpoints are needed for clinical decision-making.

In conclusion, we have demonstrated a similar distribution of CRP concentrations among apparently healthy US men and women. In addition, we have proposed a new set of cutpoints to be used clinically in assessing the risk of future coronary events in both genders.

Dr. Ridker is named as a co-inventor on patents filed by Brigham and Women’s Hospital that pertain to inflammatory markers and cardiovascular disease.
References


Distributions of C-reactive Protein Measured by High-Sensitivity Assays in Apparently Healthy Men and Women from Different Populations in Europe, Armin Imhof,1 Margit Fröhlich,1 Hannelore Loewel,2 Nicole Helbecque,3 Mark Woodward,4 Phillippe Autour,5 Gordon D.O. Lowe,6 and Wolfgang Koenig7,* 1Department of Internal Medicine II, Cardiology, University of Ulm, D-89081 Ulm, Germany; 2GSF-National Research Center for Environment and Health, Institute for Epidemiology, 85764 Neuberg, Germany; 3Inserm U508, Institut Pasteur de Lille, 59079 Lille Cedex, France; 4Institute for International Health, University of Sydney, Sydney NSW 2042, Australia; 5Department of Medicine, Royal Infirmary, Glasgow G31 2ER, United Kingdom; *address correspondence to this author at: Department of Internal Medicine II, Cardiology University of Ulm, Robert-Koch-Strasse 8, D-89081 Ulm, Germany; fax 49-731-500-33872, e-mail wolfgang.koenig@medizin.uni-ulm.de)

C-reactive protein (CRP), the classic marker of acute-phase response, is an indicator of a variety of pathologic processes, including infections, tissue damage, and chronic inflammatory diseases (1,2). The majority of more than 15 well-conducted prospective studies in initially healthy individuals have shown a strong and independent association between concentrations of CRP within the reference interval (<5 mg/L) and future major cardiovascular events (3), although in some of them, no such association could be established (4–7). The summary estimate of the relative risk in formal metaanalysis was 2.0 (95% confidence interval, 1.6–2.5) (3). Furthermore, CRP has been shown to add to risk prediction beyond and above established cardiovascular risk factors (8). On the basis of data from the Physicians’ Health Study and the Nurses’ Health Study, an algorithm for risk assessment of future coronary events that combines both CRP concentration and the ratio of total cholesterol to HDL-cholesterol has recently been proposed (9).

Because atherosclerosis represents a low-grade inflammatory process in the vascular bed, high-sensitivity (hs) assays are needed when using circulating CRP concentrations for risk prediction in cardiovascular diseases. Such assays have been developed and are now commercially available (10,11). However, before screening of individuals at risk can be recommended, CRP distributions in apparently healthy adults in the general population must be known. Such information is scarce. Furthermore, in previous reports, women using oral contraceptives or receiving hormone replacement therapy (HRT), both of