CDC/AHA Workshop on Markers of Inflammation and Cardiovascular Disease

Application to Clinical and Public Health Practice

Report From the Laboratory Science Discussion Group

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The Centers for Disease Control and Prevention (CDC) in collaboration with the American Heart Association (AHA) convened a workshop in Atlanta, Ga, on March 14 and 15, 2002, titled, “CDC/AHA Workshop on Inflammatory Markers and Cardiovascular Disease: Applications to Clinical and Public Health Practice,” which was intended to address issues about the appropriate selection and use of inflammatory markers to predict cardiovascular disease (CVD) risk.1 Three concurrent discussion groups on issues related to laboratory, clinical, and population science were held. This report details the discussions and findings of the laboratory science group.

Recommendations for Clinical Practice

1. Of the inflammatory markers identified, C-reactive protein (CRP) has the analyte and assay characteristics that are most conducive for use in practice.
2. To obtain a CRP concentration in metabolically stable patients, 2 measurements, fasting or nonfasting, should be made (optimally 2 weeks apart) and the results averaged. If the CRP level is >10 mg/L, then the test should be repeated and the patient examined for sources of infection or inflammation.
3. CRP results should be expressed only as milligrams per liter and expressed to 1 decimal point.
4. Risk assessment should be modeled after the lipids approach via 3 risk categories: low risk, average risk, and high risk. On the basis of the CRP population distributions, the following tertiles are recommended for categorizing patients: low risk, <1.0 mg/L; average risk, 1.0 to 3.0 mg/L; and high risk, >3.0 mg/L. It should be recognized that other acute inflammatory conditions may result in mildly to moderately increased CRP levels, such as inflammatory bowel disease,2 rheumatoid arthritis,3 and long-term alcoholism.4
5. Performance goals for CRP measurement, similar to those developed for total cholesterol, HDL and LDL cholesterol, and triglycerides, need to be developed with a view toward better characterization of the total allowable error required to measure CRP reliably.

Discussion

The Laboratory Science Discussion Group was charged with reviewing the available analytical data on inflammatory markers and recommending those, if any, that were ready to move from the research setting into the routine clinical laboratory. The markers were divided into 3 groups of molecules: cytokines and chemokines, soluble adhesion molecules, and acute-phase reactants. The laboratory group primarily focused on the availability of commercial assays, their limitations and strengths, ability to standardize assay results, assay performance, and how results should be interpreted for assessing the risk of future CVD.

As our knowledge of the atherosclerotic process has improved in recent years, evidence suggests that after initiation, inflammation plays a significant role in the development of this disease. A variety of markers associated with inflam-
mation in the arterial wall, which also are detectable in the circulation, have thus been evaluated as potential indicators for predicting risk of coronary events. Among these markers are nonspecific acute-phase reactants such as fibrinogen, CRP, and serum amyloid A (SAA), which, when present in low concentrations, are reflective of a low-grade chronic inflammatory disease; more specific primary inflammatory signals, including cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), and vascular adhesion molecules such as soluble intercellular adhesion molecule-1. Although the initial interest in these markers was confined to the basic research arena, there has been a significant and expanding interest in the clinical application of these markers, despite a lack of consensus about which markers are of clinical use and in what clinical context they should be measured. This interest has led to the development of a variety of commercial assays for these markers.

Cytokines and Chemokines

The cytokines and chemokines that have been investigated as possibly being related to atherosclerosis include several interleukins (IL-1β, IL-6, IL-8, IL-10, and IL-18), TNF-α, and monocyte chemotactic protein-1. Because cytokines are confined primarily to the research laboratory, they typically are assayed with ELISA techniques. Improvements in assay techniques, such as an automated microplate system, chemiluminescent assays, and multiplex assays, have resulted in reasonably acceptable within- and between-run precision for a number of assays, even at the low concentrations needed in the context of atherosclerosis. At present, these assays remain research tools for clinical and preanalytical reasons. Analyte stability for these molecules is a major limitation in routine clinical settings because serum and plasma must be rapidly separated from blood cells and assayed immediately or frozen to at least −70°C to prevent analyte degradation. Some of these cytokines, such as IL-1β, also have a short half-life. For inflammatory markers to be useful for predicting the risk for a coronary event, the assays must reliably quantify concentration levels in apparently healthy populations so that individuals can be divided into risk categories. Many of the commercial assays are designed to measure the higher concentrations of cytokines encountered in acute inflammatory processes and thus cannot quantify levels within the reference range of apparently healthy individuals without modification. Although international standards exist for several cytokines, these assays may be difficult to standardize because the different monoclonal antibody pairs employed for each assay do not permit comparable recognition in individual patient samples.

Soluble Adhesion Molecules

E-selectin, P-selectin, intracellular adhesion molecule-1 (sICAM-1), and vascular adhesion molecule-1 (sVCAM-1) are soluble adhesion molecules that have been examined as possible markers for atherosclerotic risk. The present discussion group concluded that these molecules, for reasons similarly stated for the cytokines, are precluded at this time for routine use in the clinical laboratory as markers of inflammation.

Acute-Phase Reactants

The acute-phase reactants that have been investigated as inflammatory markers of atherosclerosis are fibrinogen, SAA, and CRP.

Fibrinogen is a clotting factor produced by the liver that usually is measured in sodium citrate plasma or EDTA plasma. Depending on the method used for analysis, the stability of fibrinogen complicates its measurement. If a clotting-based activity assay is to be used, then fibrinogen is relatively unstable if not analyzed immediately; however, if immunoassay-based procedures, usually nephelometric or turbidometric, are used, then EDTA plasma is stable, even if stored at room temperature for 24 hours. Numerous commercial methods are available either as immunoassays or functional assays. The between-day precision of most of these assays ranges from 4% to 8% as coefficient of variation (CV). The immunoassays generally perform better than do the functional procedures. Although a World Health Organization (WHO) standard exists for fibrinogen, it is only for mass assays and not the functional assays that are most commonly used. Despite evidence that fibrinogen is a good marker for inflammation, the use of different methodologies for measurement makes standardization problematic and thus less than suitable for routine use as a clinical marker for atherosclerotic risk.

One commercial nephelometric assay for SAA with a CV of <9% is available in the United States for research use only. The standardization of SAA also may be problematic because of the existence of multiple SAA phenotypes; however, establishment of the first international standard for SAA should enhance efforts to standardize this analyte. The marker of inflammation that has received the most attention recently as a potential marker of atherosclerotic risk is CRP. CRP is an established marker of inflammation that rises several hundredfold in response to acute injury, infection, or other inflammatory stimuli. One of the most attractive features of CRP is its preanalytical stability in serum or plasma, at room temperature or frozen, and for extremely long periods. A commonly assigned cutoff value for CRP is <10 mg/L, with concentrations of 10 to 40 mg/L associated with mild inflammation and concentrations of 40 to 200 mg/L associated with acute inflammation and bacterial infection. Most procedures usually have used a lower reporting level of 3 mg/L. The concentrations of CRP measured to assess atherosclerotic risk caused by chronic inflammation are much lower than those measured in acute inflammation. As a result, assays were developed to measure CRP in the range below the detection limit of conventional assays. These new assays are referred to as high-sensitivity CRP (hsCRP) assays, and they can detect concentrations accurately and reproducibly down to 0.3 mg/L. Many commercial automated assays are available with adequate sensitivity to measure hsCRP in the concentration range necessary to evaluate atherosclerotic risk to apparently healthy individuals. The availability of so many methods greatly facilitates the widespread use of this marker in clinical laboratories.

Several studies have evaluated the performance of a number of the newer-generation commercial assays and found that all had a lower limit of sensitivity of ≤0.3...
Variability among the assays can lead to the misclassification of patients and can hamper the implementation of population-based medical decision points. The general conclusion from the studies was that further standardization efforts are needed to improve the clinical utility of hsCRP assays. Nearly all of the hsCRP assays are, according to vendor claims, calibrated to either the WHO 1st International Standard 85/506, introduced in 1986, or Certified Reference Material 470 (CRM 470), introduced in 1993. The value assigned to CRM 470 was derived from comparison with WHO 85/506 with the use of a very high-precision transfer protocol. As indicated above, several reports have documented bias-related problems attributed to standardization or to poor value transfer by the vendor. To address this issue, the CDC initiated an effort to assist manufacturers in standardizing their hsCRP assays. Because the current reference materials were developed for use in traditional applications, the relatively high levels of CRP in the 2 primary reference materials (49 mg/L for WHO 85/506 and 39.2 mg/L for CRM 470) were suggested as a reason for the variability among assays. Recent studies by the CDC and others have shown that CRM 470 performed similarly to patient samples with regard to imprecision, linearity, and parallelism when diluted to concentrations in the normal range for apparently healthy people. The next phase of the CDC effort will attempt to harmonize various hsCRP assays in conjunction with a standard value transfer protocol that will use CRM 470.

The College of American Pathologists (CAP) initiated a proficiency testing survey for hsCRP assays in 2002. Participant performance in the survey is evaluated on the basis of method peer group comparisons. The reported peer group CVs from the 2002 “B” mailing ranged from 9% to 57% at an hsCRP level of 1.0 mg/L and 4% to 60% at an hsCRP level of 2.0 mg/L. Although the large and variable CVs would indicate significant within- and among-peer group variation, a closer inspection of the individual laboratory results seems to indicate a potential problem in units reporting. The CAP survey reports hsCRP values as milligrams per liter, but because many of the assay systems express hsCRP results in milligrams per deciliter, or milligrams per liter, the error in result reporting appears to come from laboratories that may have obtained results in milligrams per deciliter and failed to multiply by 10 to convert and report results as milligrams per liter. This error clearly underscores the need for laboratories to verify in which units their hsCRP results are expressed and reported. When suspected erroneous results were deleted and performance was reevaluated, the CV ranges improved to 8% to 14% and 4% to 12%, respectively, for all of the method groups except one. Although these recalculated CVs indicate reasonable performance, for many of the assay systems there remains a need to further standardize and improve the overall performance of hsCRP measurements. By comparison, the variation for total cholesterol and HDL cholesterol on the CAP survey averages 3.5% and 6%, respectively.

After considering analyte stability, the commercial availability of assays, the standardization of those assays to allow comparison of results, and the precision of the assays, the present discussion group favored CRP from a clinical chemistry perspective as the best marker of inflammation to assess atherosclerotic risk. The improvement of assays for other markers or evidence for the benefits of combinations of assays may have advantages over CRP. Further research is required, however, and the laboratory discussion group recommended that measurement of alternative or additional analytes to assess atherosclerotic risk not be performed until such evidence is available.

Once these conclusions were reached, the deliberations of the laboratory discussion group focused entirely on CRP.

Sources of Variation

For CRP to be measured correctly, issues about preanalytical and analytical variation must be better understood, and guidance must be provided to minimize their effect on test results. Preanalytical factors include variation from physiological variables such as race, age, sex, season, and lifestyle, and from specimen collection variables such as fasting, collection time, specimen type, and storage.

Physiological Variables

A review of the findings from several population studies indicates that no sex- or ethnicity-specific decision points are warranted for assessing risk with CRP. Comparable distributions of CRP concentrations among women not receiving hormone replacement therapy and men have been reported in several US and European studies. For both sexes, the 50th percentile of CRP measured in the various study populations was about 1.5 mg/L. No significant differences were found in the distribution of CRP concentration among white, African American, and Mexican American men in data from the National Health and Nutrition Examination Survey III (NHANES III). A comparable CRP distribution was found in Japanese men, with slightly lower concentrations in Japanese women. Although the geometric mean for CRP concentration in Indian Asians was 17% higher than it was in whites, the difference was not significant when the results were adjusted for central obesity and insulin resistance.

Most studies have reported no relation between age and CRP concentration for individuals 20 to 70 years old. CRP concentration varied only slightly in a study that included 15 770 women: Median CRP concentrations for individuals 20 to 70 years old were 1.31, 1.89, 1.99, and 1.52 mg/L, respectively.

No consistent pattern of change in CRP values was reported from a study (Seasonal Variation of Blood Cholesterol Study [SEASON]) specifically designed to examine seasonal changes in cardiovascular risk. Within and between subjects, variation in several studies was as high as 63% and 92%, respectively. In a study to assess sources of variation of CRP in a healthy population, 3 measurements made at monthly intervals are recommended to define an individual’s steady-state CRP concentration, provided there is no intercurrent infection. In another study, 2 independent CRP or total cholesterol measurements taken 3 months apart enabled classification of up to 90% of subjects within one quartile of each other. Analysis of these data in the context of the recommended tertile cutpoints indicated that >95% of subjects would be classified in the exact tertile of risk or vary
by one tertile (see Figure 1). On the basis of this evidence, the laboratory group recommended that the average of 2 independent measurements (fasting or nonfasting) of CRP, taken at least 2 weeks apart, be used to establish a person’s risk for future coronary events.

**Specimen Collection**

In general, no significant difference has been observed between samples collected either fasting or nonfasting; however, in assays based on turbidimetry and nephelometry, in which optical clarity is important, collecting a fasting sample may be necessary. Time of day for sample collection is not significant because diurnal variation for CRP is negligible.

The numerous population distribution studies of CRP reported in the literature are based on CRP measurements in EDTA-containing plasma or serum. The difference in CRP values among heparinized or EDTA-containing plasma or serum is unlikely to cause a significant problem in risk-stratifying subjects for CVD risk. CRP concentration was stable at 4°C for 60 days, and when stored at ≤70°C remained unchanged for months or years.

**Interpretation of hsCRP Results**

Numerous studies have reported hsCRP concentration distributions relative to coronary event outcomes from which risk estimates in tertiles, quartiles, or quintiles have been proposed. Initial efforts to develop algorithms to estimate a person’s risk for future coronary events were based primarily on quintiles of hsCRP. After extensive discussion, the present discussion group concluded that for ease of clinical interpretation, a more simplified set of 3 cutpoints that are similar to those developed and used for cholesterol would be the best approach. On the basis of distributions of hsCRP from 12 populations involving >19,000 people (Figure 2) and the frequency distribution of hsCRP in 22,403 US adults, the present discussion group recommended a more simplified set of cutpoints based on approximate tertiles of the US population. The cutpoints recommended for risk assessment are <1.0 mg/L (low risk), 1.0 to 3.0 mg/L (average risk), and >3.0 mg/L (high risk). If the hsCRP concentration is ≥10 mg/L, then the test should be repeated and the person examined for sources of infection or inflammation.

The units of measure recommended by the present discussion group are milligrams per liter, and hsCRP results should be expressed to 1 decimal point. As stated earlier, these cutpoints appear to apply irrespective of sex and race.

**Summary**

The role of inflammation in atherogenesis has been extended to the measurement of a number of analytes or “markers” associated with inflammation in the circulation. Numerous epidemiological studies have been published that indicate that some of these markers provide additional and independent prediction of risk for atherosclerotic events. From a clinical chemistry and laboratory medicine perspective, hsCRP is the best candidate at this time for use as a marker of inflammation in clinical practice.

Despite the endorsement of hsCRP by the present discussion group as the inflammation marker of choice, the other analytes considered hold promise as markers of inflammation but are limited by their instability, lack of commercial assays applicable to the routine setting, inadequate performance, and lack of standardization. Until such improvements are made, hsCRP remains the best choice for assessing inflammation.

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