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Journal of Immunological Methods 311 (2006) 87–95

JIM Journal of Immunological Methods

www.elsevier.com/locate/jim

Research paper

Determination of C-reactive protein with an ultra-sensitivity immunochemiluminometric assay

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Received 31 May 2005; received in revised form 17 January 2006; accepted 20 January 2006
Available online 6 March 2006

Abstract

C-reactive protein (CRP), the classic acute phase reactant, is strongly associated with increased risk of cardiovascular events. The demand for measuring serum CRP levels has been predicted to increase. We developed an ultra-sensitivity in-house immunometric assay on polystyrene beads for measuring CRP and studied its analytical and clinical performance. The assay used a pair of monoclonal anti-CRP antibodies and detected CRP in a 1-step immunometric assay with a chemiluminescence signal. The calibration was traceable to the World Health Organization reference material. The assay covered a linear range of 0.01 to 50.00 mg/L. The analytical detection limit calculated from the mean level plus 3 SD of the zero calibrator was 0.004 mg/L. The within-run imprecision was 7.0%, 5.2%, and 4.1% for mean CRP levels of 0.02 mg/L, 1.44 mg/L, and 11.04 mg/L, respectively. The between-run imprecision was 9.2%, 7.0%, and 6.0% for mean CRP levels of 0.02 mg/L, 1.49 mg/L, and 10.90 mg/L, respectively. The average recovery was 102.0% ($n=6$). The assay correlated well with a high-sensitivity latex-enhanced nephelometric assay (regression line $y=0.865x+1.333$, $r=0.974$, $S_{y/x}=3.415$, $n=47$ for 0–50.00 mg/L and $y=1.076x-0.080$, $r=0.985$, $S_{y/x}=0.989$, $n=29$ for 0–20.00 mg/L). The central 95 percentile reference interval for Han Chinese residing in Taiwan was 0.02–4.33 mg/L ($n=469$). There was no significant difference in serum CRP levels between healthy male and female subjects (median, 0.34 and 0.31 mg/L, respectively); however, CRP levels increased moderately with age ($r=0.276$, $P<.05$). The reference values for the Chinese population were about 5-fold lower than those for the United States population. This ultra-sensitivity immunochemiluminometric assay for CRP is rapid and accurate and can be used to assess cardiovascular risk.

Keywords: Chinese; Ultra-sensitivity C-reactive protein; Immunometric assay

1. Introduction

Evidence from epidemiologic studies has recognized inflammation as a pivotal contributor to the pathogenesis of atherosclerosis. C-reactive protein (CRP), the classic acute phase marker indicative of inflammatory reactions, consists of 5 identical polypeptide chains that form a ring and have a molecular weight of 120 kDa. CRP is synthesized mainly

Abbreviations: CRP, C-reactive protein; hs-CRP, high-sensitivity C-reactive protein; ICMA, immunochemiluminometric assay.

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doi:10.1016/j.jim.2006.01.020

by hepatocytes in response to cytokines and has a plasma half-life of 19 h (Kuller et al., 1996; Tracy et al., 1997; Ridker et al., 1997; Pepys and Hirschfield, 2003). When CRP is bound with ligands such as phosphatidylcholine, modified low-density lipoprotein, damaged cell membrane, or apoptotic cells, it is recognized by C1q or factor H (or both) and activates the complement pathway (Pepys and Hirschfield, 2003). Evidence is compelling that CRP has a role in mediating innate immunity, proinflammatory pathophysiologic effects, and prevention of autoimmunity (Pepys and Hirschfield, 2003; Verma et al., 2004). Recent studies have suggested that CRP has proatherogenic properties in neutrophil adhesion, endothelial activation, production of cytokines and nitric oxide, and the progression of atherosclerosis (Verma et al., 2002). Serum CRP is a useful biomarker for disease activity and therapeutic response in inflammatory, infectious, and neoplastic conditions.

With the development of high-sensitivity CRP (hs-CRP) assays, CRP has become a useful biomarker of inflammation for predicting future cardiovascular events in apparently healthy subjects (Ridker et al., 1998; Koenig et al., 1999; Danesh et al., 2000; Chew et al., 2001; Yeh and Willerson, 2003), in the diagnosis of osteoarthritis (Spector et al., 1997) and neonatal infection (Wasunna et al., 1990), and in the prognosis of acute coronary syndromes (Rifai and Ridker, 2001). Serum CRP levels may be used in conjunction with the lipid profile to identify apparently healthy men and women at risk for developing cardiovascular events and, in conjunction with cardiac troponin I or T, for predicting clinical outcomes in acute coronary syndromes (Yeh and Willerson, 2003).

The genetic background and its interaction with the environment may contribute to the susceptibility for and the progression and complications of atherosclerosis. Genetic effects on the baseline values of CRP have been documented, and the heritability of serum CRP values has been estimated at approximately 40% to 50% (MacGregor et al., 2004; Zee and Ridker, 2002). No substantial difference has been reported in the distribution of CRP across sex and ethnic groups (Ridker, 2003). However, few data are available for Asian populations, but the Japanese have a relatively low serum CRP level, with only 0.16 and 0.09 mg/L of the 50th percentile for men and women, respectively (Yamada et al., 2001; Lear et al., 2003).

Immunonephelometric or immunoturbidimetric assays using a single polyclonal antibody have been the main methods clinical laboratories have used to measure CRP for detecting active inflammation and infection. However, these methods have limited sensitivity and poor precision in the lower range and cannot detect quantities <5–10 mg/L. Several modifications have been made to increase the detectable signal, including the use of enzyme- or fluorescent-compound-labeled tracer antibodies. The minimum CRP level detectable is approximately 0.05 mg/L by the immunoradiometric assay (Hutchinson et al., 2000) and 0.007 mg/L by ELISA (Macy et al., 1997). Most of the original studies that examined the clinical use of hs-CRP in predicting future myocardial infarction and stroke used an in-house ELISA assay. The analytical and clinical performance of the assay was compared with a commercially available latex-enhanced turbidimetric N High Sensitivity CRP (Dade Behring, Newark, Delaware) (Rifai et al., 1999; Ledue et al., 1998), which has a detection limit of 0.18 mg/L and was approved by the US Food and Drug Administration for assessing cardiovascular risk. Most commercial hs-CRP methods are capable of detecting CRP concentrations >0.2 mg/L, which is approximately the first and second percentile, with a 50th percentile of the U.S. population about 1.50 mg/ L (Rifai and Ridker, 2003). These methods are sufficiently reliable to measure CRP levels in the U.S. population. However, the 50th percentile of the Japanese population is 10-fold lower than that of the Western population (Yamada et al., 2001), and an ultra-sensitive CRP assay may be needed for measuring CRP levels in Asian populations. Here, we report the development of an ultra-sensitive immunochemiluminometric assay (ICMA) using an acridinium ester label. The assay uses 2 commercially available monoclonal antibodies that recognize the different epitopes of CRP. The assay generates a luminescence-enhanced signal to improve the detection of low concentrations of CRP, reaching a sensitivity of 0.004 mg/L. The assay correlated well with the latex-enhanced immunoassay and was used to evaluate the reference values in healthy adult Chinese and to study the effects of age and sex on serum CRP levels.

2. Materials and methods

2.1. CRP calibrator, anti-CRP antibodies, and reagents

The CRP calibrator was from the European Community Bureau of Reference Certified Reference Material for proteins in human serum (IFCC CRM470) (Kimberly et al., 2003). Anti-CRP monoclonal antibodies were purchased from Research Diagnostics, Inc. (Flanders, New Jersey). Unless specified, all other reagents were from Sigma (St. Louis, Missouri).