Determination of C-Reactive Protein: Comparison of Three High-Sensitivity Immunoassays, Natalie Khuseyinova,1 Armin Imhof,2 Gerlinde Trischler,3 Dietrich Rotheneh- bacher,2 Winston L. Hutchinson,3 Mark B. Pepys,3 and Wolfgang Koenig1* 1Department of Internal Medicine II, Cardiology, University of Ulm Medical Center, Robert-Koch-Strasse 8, D-89081 Ulm, Germany; 2Department of Epidemiology, German Centre for Research on Ageing, University of Heidelberg, D-69115 Heidelberg, Germany; 3Centre for Amyloidosis and Acute Phase Proteins, Department of Medicine, Royal Free and University College Medical School, London NW3 2PF, United Kingdom; author for correspondence: fax 49-731-500-33872, e-mail wolfgang.koenig@medizin.uni-ulm.de

Atherosclerosis is an inflammatory condition (1), and progression to atherothrombotic events is associated with systemic evidence of low-grade inflammation. Circulating concentrations of C-reactive protein (CRP) within the range previously considered to be normal have been shown to predict future cardiovascular events independently in initially healthy individuals as well as in patients with manifest atherosclerotic disease, even in the absence of hyperlipidemia (2–4). The detection of such subclinical inflammation requires high-sensitivity methods for CRP measurement. Recently, we reported on the analytical performance of a new sensitive solid-phase monoclonal-polyclonal IRMA (5). This assay has a very low analytical detection limit of 0.05 mg/L, and in a cohort study, CRP determined by this method showed a strong linear association with future coronary endpoints (4). Here we compare the performance of the IRMA specifically designed to evaluate the low CRP range with that of an ultrasensitive latex-enhanced immunonephelometric test and an immunoturbidimetric assay in a large case–control study. Additionally, we estimated the risk for coronary artery disease (CAD) given an increased CRP concentration as determined by the three assays.

The cases consisted of 312 patients 40–68 years of age with clinically stable CAD, who underwent elective coronary angiography in the Department of Cardiology at the University of Ulm Medical Centre and who had one or more epicardial coronary stenoses of ≥50% in luminal...
diameter. Voluntary blood donors (n = 476) from the local blood bank served as controls. In these individuals, symptomatic CAD was excluded by the Rose questionnaire. Venous blood was obtained under standardized conditions in the morning before diagnostic coronary angiography in cases and after blood donation in controls. EDTA-plasma specimens were centrifuged at 3000g for 10 min, immediately aliquoted, and frozen at −70 °C until analysis. Cases and controls were “frequency matched” by age and gender, and a sampling ratio of ~1:1.5 was intended to ensure adequate power of the study. The protocol was approved by the ethics committee of the University of Ulm, and all participants gave informed consent on entry into the study.

CRP was measured by three different analytical assays; the methods have been described in detail elsewhere (5–7). Briefly, the first method, the IRMA, used a five-point calibration with WHO International Reference Standard 85/506. The assay range was 0.05–10 mg/L. Samples with concentrations >10 mg/L were remeasured at higher dilutions. This method was designed specifically to measure serum/plasma CRP in the previously established reference interval for healthy individuals (8), and although highly reproducible, its precision may not be optimal for higher concentrations in samples that required dilution. CRP concentrations were determined in triplicate, and the mean was used for analysis. The interassay CV for CRP over all ranges was 12%.

The second assay was immunonephometric (Dade Behring N Latex High Sensitivity CRP™ mono assay) on a Behring Nephelometer II analyzer. The detection limit for CRP was 0.17 mg/L, and the measuring range was 0.175–1100 mg/L, according to the manufacturer. The interassay CVs at 11.5 and 47.9 mg/L were 6.1% and 3.5%, respectively.

The third assay was immunoturbidimetric (Tina-quant CRP detection method; Roche Diagnostics) performed on a Hitachi 717 automated analyzer. The manufacturer claimed the detection limit to be 0.1 mg/L and the extended measuring range (with reruns) to be 0.1–240 mg/L. The between-assay CV was 2.6% at 4.65 mg/L CRP.

Because CRP distributions were highly skewed to the right, logarithmic transformation was performed, and CRP values are expressed as geometric means. Differences in the CRP concentrations among cases and controls were analyzed with the Wilcoxon rank-sum test. We also calculated percentiles of the CRP concentration for each method. In addition, CRP values were divided into quintiles according to the distribution in the control group for each assay. Multivariable logistic regression analysis was carried out to estimate the independent association of increased CRP values with presence of CAD, while simultaneously controlling for confounding factors (9). Odds ratios (ORs) were calculated and expressed with their 95% confidence intervals. All P values were two-tailed, and values <0.05 were considered statistically significant. Finally, Deming regression analysis and Bland–Altman analysis were performed to assess agreement between assays (10, 11). The IRMA was taken as the comparator method based on previous analytical and clinical validations (5) and assuming that analytical variation of the IRMA (i.e., the error of the measurement process itself) was small (12).

The baseline characteristics of all study participants have been published previously (13). In the overall sam-

<table>
<thead>
<tr>
<th>Quintiles (range) of CRP, mg/L</th>
<th>IRMA</th>
<th>Dade-Behring</th>
<th>Roche</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1*</td>
<td>&lt;0.47</td>
<td>&lt;0.49</td>
<td>&lt;0.81</td>
</tr>
<tr>
<td>Q2</td>
<td>0.48–0.99</td>
<td>0.5–0.85</td>
<td>0.82–1.32</td>
</tr>
<tr>
<td>Q3</td>
<td>1.00–1.82</td>
<td>0.86–1.39</td>
<td>1.33–1.97</td>
</tr>
<tr>
<td>Q4</td>
<td>1.83–3.70</td>
<td>1.40–2.69</td>
<td>1.99–3.53</td>
</tr>
<tr>
<td>Q5</td>
<td>&gt;3.71</td>
<td>&gt;2.7</td>
<td>&gt;3.54</td>
</tr>
</tbody>
</table>

Frequencies (%) according to proposed CRP cutpoints for cardiovascular risk assessment

| OR (95% CI)* for coronary artery disease according to CRP distribution |
|-----------------------------|-----------------------------|-----------------------------|
| Q1                          | 1 (Reference)               | 1 (Reference)               | 1 (Reference)               |
| Q2                          | 1.57 (0.92–2.68)            | 1.45 (0.85–2.48)            | 1.50 (0.88–2.56)            |
| Q3                          | 1.17 (0.68–2.03)            | 1.37 (0.79–2.35)            | 1.27 (0.73–2.21)            |
| Q4                          | 1.66 (0.97–2.85)            | 1.45 (0.84–2.50)            | 1.56 (0.91–2.69)            |
| Q5                          | 2.51 (1.44–4.37)            | 2.48 (1.42–4.31)            | 2.55 (1.46–4.44)            |

* Q, quintile; CI, confidence interval.
* Adjusted for age, gender, body mass index, cigarette smoking, statin use.
Fig. 1. Comparison of methods using case and control samples. 

(A and B), Deming regression analysis based on the original data. The solid line indicates the regression line, and the dashed line indicates the line of unity. Values >10 mg/L were excluded. (A), IRMA vs Dade-Behring: slope (SE), 0.86 (0.01); intercept (SE), −0.096 (0.03) mg/L; \( 95\% \text{ CI} = 0.11 \) mg/L; \( r = 0.94 \); (B), IRMA vs Roche: slope (SE), 0.96 (0.01); intercept (SE), 0.26 (0.04) mg/L; \( 95\% \text{ CI} = 0.18 \) mg/L; \( r = 0.94 \). (C and D), Bland-Altman analysis. The solid line indicates the mean difference between the assays, and the dashed lines indicate the 95% limits of agreement. (C), IRMA vs Dade-Behring: mean difference, −0.39 mg/L; 95% limits of agreement, −1.88 and 1.11 mg/L; (D), IRMA vs Roche: mean difference, 0.19 mg/L; 95% limits of agreement, −1.20 and 1.57 mg/L. (E and F), frequency distribution of log-transformed CRP concentrations in the study population for the IRMA and Dade-Behring (E) and the IRMA and Roche (F) methods.
ple (n = 788), CRP concentrations ranged from 0.055 to 95 mg/L as measured by the IRMA, from 0.16 to 86 mg/L as measured by the Dade-Behring assay, and from 0.29 to 50 mg/L as measured by the Roche method. Geometric means of CRP concentrations were similar for the IRMA and Dade-Behring methods, whereas the Roche assay showed slightly higher values (Table 1). Differences in CRP concentrations between cases and controls were similar when measured by the IRMA (1.71 vs 1.27 mg/L; \( P = 0.0006 \) after adjustment for age) and by the Dade-Behring method (1.49 vs 1.14 mg/L; \( P = 0.0003 \) after adjustment for age). The geometric means for CRP by the Roche assay (2.16 mg/L in cases vs 1.73 mg/L in controls; \( P = 0.0004 \) after adjustment for age) were higher than for the IRMA and Dade-Behring tests. Table 1 also presents the quintile cutpoints for CRP measured by the three assays. The evaluation of CRP within quintiles yielded comparable results for the IRMA and the two other methods. Frequency distribution of all study participants according to clinical cutpoints of CRP concentration, recently proposed for cardiovascular risk prediction, revealed that risk assessment using the Roche method for some individuals would lead to a shift from low to moderate risk (Table 1). However, all three assays showed almost identical adjusted ORs for the presence of CAD across CRP quintiles (Table 1). Distributions of CRP concentration by percentiles for all three assays are available as a Data Supplement accompanying the online version of this Technical Brief at http://www.clinchem.org/content/vol49/issue10/.

Agreement among the three immunoassays is presented graphically in Fig. 1. Deming regression analyses (range, 0–10 mg/L) for the IRMA \( x \) and Dade-Behring method \( y \) yielded a slope (SE) of 0.86 (0.01), intercept (SE) of \(-0.096 \) (0.03) mg/L, and \( S_{\text{yx}} = 0.11 \) mg/L \( (r = 0.94; \text{Fig. 1A}) \). Similar results were found for the IRMA and the Roche assay with a slope (SE) of 0.96 (0.01), intercept (SE) of 0.26 (0.04) mg/L, and \( S_{\text{yx}} = 0.18 \) mg/L \( (r = 0.94; \text{Fig. 1B}) \). We also found similar agreement between the IRMA and the Dade-Behring method (Fig. 1C), as well as between the IRMA and the Roche assay (Fig. 1D) in the low CRP range (0–10 mg/L; Fig. 1, legend).

At CRP concentrations >30 mg/L, however, we frequently obtained differences >2 SD by Bland–Altman analysis for all assay pairs (data not shown). For 0–100 mg/L CRP, Deming regression analyses yielded equations with a slope (SE) of 0.7 (0.01), intercept (SE) of 0.2 (0.09) mg/L, and \( S_{\text{yx}} = 1.9 \) mg/L \( (r = 0.89) \) for the IRMA and the Dade-Behring assay, and a slope (SE) of 0.67 (0.01), intercept (SE) of 0.83 (0.1) mg/L, and \( S_{\text{yx}} = 0.84 \) mg/L \( (r = 0.87) \) for the IRMA and the Roche assay. Comparison of the Bland–Altman plots across the whole range of CRP concentration gave a mean difference between the IRMA and the Dade-Behring assay of −0.67 mg/L (95% limits of agreement, −7.29 and 5.95 mg/L) and a mean difference of −0.12 mg/L (95% limits of agreement, −7.81 and 7.37 mg/L) between the IRMA and the Roche assay.

Finally, frequency distributions of log-transformed CRP concentrations revealed comparable results for the IRMA and the Dade-Behring assays (Fig. 1E). As seen before, comparison between the IRMA and the Roche assay showed slightly higher values at low concentrations with the latter method than with the IRMA (Fig. 1F).

The present study revealed that CRP concentrations between 0.05 and 10 mg/L measured by an IRMA are in good agreement with those determined by the Dade-Behring assay. The Roche assay showed less agreement with the two other methods at CRP concentrations <1 mg/L. At high CRP concentrations (>30 mg/L), large differences were seen among all CRP assays examined. Studies published to date, which included only small numbers of participants, have already reported such discrepancies among the various CRP assays at concentrations >50 mg/L (7, 14), and our large case–control study (n = 788) confirmed these previous findings. The precision for higher CRP concentrations of all immunoassays designed for high-sensitivity measurements may be affected by sample dilution, with consequent exaggeration of any imprecision by multiplication, as well as by important matrix effects reflecting the diluent used. Furthermore, different methods for calibration curve fitting used in the studied assays could offer another explanation for existing discrepancies.

In our population, the geometric means and percentiles of the CRP distribution were very similar to those published previously (15, 16). More importantly, quintile cutpoints in our study were almost identical to clinical quintile cutpoints used in the recently proposed algorithm for cardiovascular risk assessment (17, 18). With all assays tested, an independent association between higher CRP concentrations and the presence of CAD could be demonstrated. Although the Roche assay gave slightly higher CRP values at concentrations <1 mg/L than the IRMA and the Dade-Behring, all three methods showed good concordance at 1–3 mg/L, the critical concentration range for cardiovascular risk assessment (19, 20).

On the basis of the results in this large case–control study, we conclude that all three assays have comparable clinical sensitivity and are likely to have similar clinical efficacy. Thus, the Dade-Behring and the Roche assays, as fully automated methods and being commercially available, may offer reliable alternatives to the in-house IRMA. Moreover, these assays are also satisfactory and suitable for atherosclerotic risk assessment in clinical practice.

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References