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Comparison and Evaluation of Two C-Reactive Protein Assays Based on Particle-Enhanced Immunoturbidimetry

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The use of C-reactive protein (CRP) assays is increasing for a wide range of clinical conditions, and consequently the analytical performance requirements for CRP assays are also changing. For this reason, manufacturers have been developing CRP assays based on different methodologies to provide both high sensitivity and a wide measuring range. However, it is questionable whether these methods can meet the desired requirements for CRP assays. CRP Latex on the Cobas Integra 400 and CRP Tina-quant Latex on the Modular Analytics-P were evaluated in terms of detection limit, linearity, intra- and interassay precision, and comparability with 268 patient samples. The intra- and interassay precision of the two methods was $<4.1\%$ in the three pools with CRP concentrations ranging from 6.9 to 215 mg/L, and $>10\%$ in the pool with concentrations of $\sim$0.60 mg/L. The detection limits for CRP Latex and Tina-quant Latex were 0.20 and 0.22 mg/L, respectively. Both methods were linear up to 215 mg/L. There was a good agreement between the two assays, except for a scattering at concentrations near the detection limits. Deming regression analysis for CRP Latex (x-axis) and Tina-quant Latex (y-axis) yielded a slope of 1.067 ± 0.018, an intercept of –0.148 ± 0.358, and an $S_{y|x}$ of 5.10 ($r = 0.996$, $P < 0.0001$). The two assays gave comparable results. Low precision was determined for both assays, except for the low pool with a concentration of $\sim$0.60 mg/L. We concluded that both of these assays should be improved to meet high-sensitivity criteria. J. Clin. Lab. Anal. 21:71–76, 2007.

Key words: C-reactive protein; high-sensitivity C-reactive protein; method comparison; method validation; analytical performance characteristics

INTRODUCTION

C-reactive protein (CRP), the prototypical acute-phase protein, has a mass of 120 kDa and is composed of five identical polypeptide subunits held together by noncovalent interactions. This arrangement is very similar to that of another acute-phase protein known as serum amyloid P (1).

The most impressive biological characteristics of CRP are a rapid rise in serum concentration during the acute phase, the magnitude of the response (approaching a 1,000-fold increase within 24–48 hr), an equally quick return to very low concentrations, long-term stability during storage, lack of diurnal or seasonal variation, and lack of age and sex dependence. The plasma halflife of CRP is approximately 19 hr. CRP is predominantly synthesized by the liver and regulated by proinflammatory cytokines (primarily tumor necrosis factor alpha (TNF-α) and interleukin 6), but extrahepatic expression has also been documented (2–4).

It is generally accepted that mild inflammation and viral infections cause elevation of CRP in the 10–40 mg/L range, while active inflammation and bacterial infection produce levels of 40–200 mg/L. Levels over 200 mg/L are found in severe bacterial infections and burns (4).

Since atherosclerosis is now widely believed to represent a process of vascular inflammation (5),

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extensive research has focused on the utility of inflammatory biomarkers in clinical medicine. Accumulating evidence suggests that one such inflammatory biomarker, C-reactive protein (CRP), plays a critical role in the pathogenesis and prognosis of cardiometabolic risk (6–11).

The Centers for Disease Control and Prevention, and the American Heart Association have made recommendations regarding the application of high-sensitivity (hs) CRP to assess cardiovascular risk (12). Classifications of <1, 1–3, and >3 mg/L to differentiate low, moderate, and high risk have been generally adopted clinically (13).

Traditionally, CRP has been used clinically to monitor infection and autoimmune disorders. Automated methods with detection limits of 3–5 mg/L are routinely available in the clinical laboratory for this purpose (14). Although this detection limit is adequate for monitoring infection, it renders most of the current assays useless for assessing and predicting risk of coronary and cerebrovascular disease in apparently healthy populations (15).

To meet the requirements for various clinical conditions, manufacturers have been developing CRP assays based on different methodologies to provide both high sensitivity and a wide measuring range. However, it is questionable whether these methods can meet the desired requirements for a CRP assay. In our clinical laboratory, routine CRP analysis is performed on a Cobas Integra 400 Plus analyzer based on particle-enhanced turbidimetry. In addition, the Hitachi Modular Analytic-P is used for CRP analysis with a dedicated kit, the Tina-quant CRP Latex, based on a similar methodology to meet the requirements of emergency departments and intensive-care units for 24 hr. The objective of this study was to evaluate the analytical performance characteristics and comparability of these two assays.

MATERIALS AND METHODS

Serum samples for method comparison were collected from 268 patients for whom a CRP was requested for routine analysis. Blood left over from routine CRP tests was used, and since there was no additional blood was collected, no medical-records review or contact with the patients was performed, and no patient consent was obtained.

The CRP detection method on the Cobas Integra 400 Plus automated analyzer (Roche Diagnostics, Rotkreuz, Switzerland) is a particle-enhanced immunoturbidimetry (CRPLX, catalog no. 20764930; Roche Diagnostics, Mannheim, Germany) with a detection limit of 0.085 mg/L and an extended measuring range of 0.085–1600 mg/L (with auto rerun), according to the manufacturer. The CRP detection method on Hitachi Modular Analytics-P automated analyzer (Roche Diagnostics, Tokyo, Japan) is also a particle-enhanced immunoturbidimetry method (Tina-quant CRPLX, catalog no. 03002039; Roche Diagnostics, Mannheim, Germany) with a detection limit of 0.4 mg/L and extended measuring range of 0.4–560 mg/L (with auto rerun), according to the manufacturer.

The two assays are based on similar methodologies. Anti-CRP antibodies coupled to latex microparticles react with antigen in the sample to form an antigen/antibody complex. Following agglutination, this complex is measured turbidimetrically. The two assays are standardized with Certified Reference Material (CRM 470).

Method comparison tests were conducted on 10 different days with 268 patient samples. Serum CRP concentrations were measured on the Cobas Integra 400 Plus analyzer, and then on the Hitachi Modular Analytics-P within 2 hr.

The CRP concentrations of serum samples used for the method comparison study ranged from 0.15 mg/L to 290 mg/L and were selected according to the National Committee for Clinical Laboratory Standards (NCCLS) protocol (16): 43% of total samples was below 5 mg/L, 32% was 5–40 mg/L, 16% was 40–100 mg/L, 9% was >100 mg/L.

Four different serum pools with CRP levels of 0.6, 6.9, 61, and 215 mg/L were prepared to determine the intra- and interassay precision (coefficient of variation (CV)) of each method. We determined intra-assay CVs on the same day by measuring one sample 20 times in one analytical run. Interassay CVs were determined on 20 consecutive days on the basis of a single calibration.

We determined the limit of detection of each method by analyzing a zero calibrator 20 times and calculating a 3 SD limit.

Samples to test linearity were prepared from two serum pools. The low pool was prepared by mixing patient samples with CRP concentrations of $\sim$0.60 mg/L. The high pool was prepared by mixing patient samples with CRP concentrations of $\sim$215 mg/L. The high pool was diluted with the low pool to obtain the following final percentages of high pool: 100%, 75%, 50%, 30%, 20%, 10%, 5%, and 0%. Samples were assayed in duplicate in one analytical run.

During the working period the same quality control material (Precinorm Protein, catalog no. 10557897) and calibrator (Preciset Calibrator, catalog no. 11876406) provided by the manufacturer were used.

The SPSS statistical package (SSPS Inc., Chicago, IL) was used to perform a descriptive statistical analysis. The slope, intercept, $S_{y|x}$, and r were estimated using Deming regression analysis (17). Agreement between the

TABLE 1. Summary of intraassay precision data

		CRP latex (Cobas Integra 400 Plus)		Tina-quant CRP latex (Modular Analytics-P)	
Pool	N	Mean (mg/L)	CV (%)	Mean (mg/L)	CV (%)
1	20	0.63	13.8	0.61	11.7
2	20	6.94	2.4	7.41	1.8
3	20	61.90	0.7	64.84	0.7
4	20	215.84	1.1	214.66	0.5

TABLE 2. Summary of interassay precision data

		CRP latex (Cobas Integra 400 Plus)		Tina-quant CRP latex (Modular Analytics-P)	
Pool	N	Mean (mg/L)	CV (%)	Mean (mg/L)	CV (%)
1	20	0.66	12.6	0.63	11.9
2	20	ND*	ND[a]	6.77	4.1
3	20	61.24	0.9	63.51	2.1
4	20	210.06	1.9	210.61	1.6

[a]ND, not determined.

methods was assessed visually using a Bland-Altman plot (18), and the limits of agreement were also determined. The MedCalc (Mariaekerke, Belgium) statistical package was used for the Deming regression and Bland-Altman analyses.

RESULTS

To examine the precision of the two assays at different CRP concentrations, four serum pools were used. The intra- and interassay precision data are summarized in Tables 1 and 2. In both assays, all precisions, except for pool 1 (~0.60 mg/L), were under desirable levels.

The detection limits for Tina-quant CRP Latex on Modular Analytics-P and CRP Latex on Cobas Integra 400 Plus were 0.22 mg/L, and 0.20 mg/L, respectively.

We conducted linearity experiments by comparing the serial dilutions of the high-level serum pool with those of the low-level serum pool. Both methods were linear up to concentrations of 215 mg/L, as shown in Figs. 1 and 2.

The method comparison results are presented in Fig. 3. Deming regression analysis for CRP Latex on the Cobas Integra 400 Plus (x-axis) and Tina-quant CRP Latex on the Modular Analytics-P (y-axis) yielded a slope of 1.067 ± 0.018 and an intercept of -0.148 ± 0.358. The Bland-Altman analysis gave a mean difference of 5.8% between two methods (Fig. 4). Although the

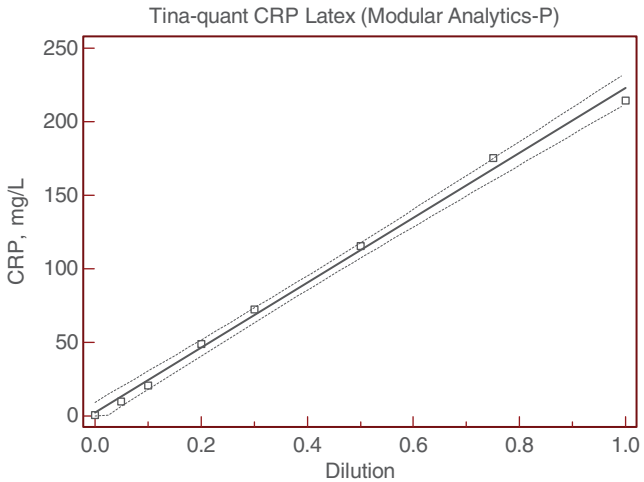

Fig. 1. Linearity of the Tina-quant CRP Latex assay at concentrations <215 mg/L. Samples were prepared as described in Materials and Methods and run in duplicate. The x-axis is the dilution of the high serum pool, and the y-axis is the concentration measured. The dashed lines indicate the 95% CI of the regression line. Linear regression analysis yielded the following results: slope = 220.376, intercept = 2.363, $S_{y|x} = 5.671$, and r = 0.998.

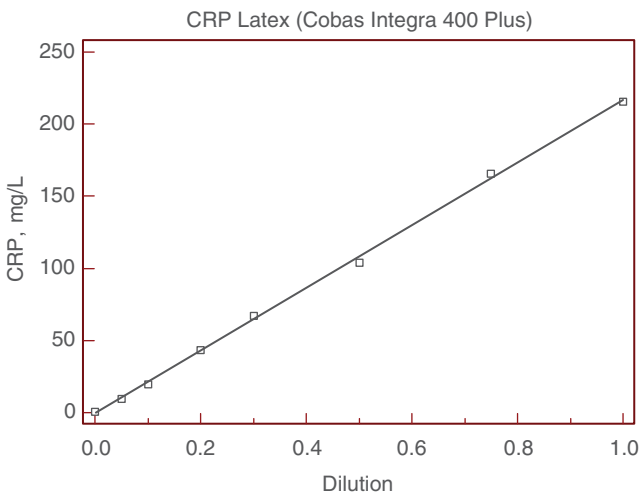

Fig. 2. Linearity of the CRP Latex assay at concentrations <215 mg/L. Samples were prepared as described in Materials and Methods and run in duplicate. The x-axis is the dilution of the high serum pool, and the y-axis is the concentration measured. Linear regression analysis yielded the following results: slope = 217.193, intercept = –0.467, $S_{y|x} = 2.554$, and r = 0.997.

absolute differences of the concentrations were small, we observed a relatively high scattering at the concentrations near the detection limits.

DISCUSSION

In clinical practice, CRP is used for a wide range of clinical conditions. Prospective studies have shown that

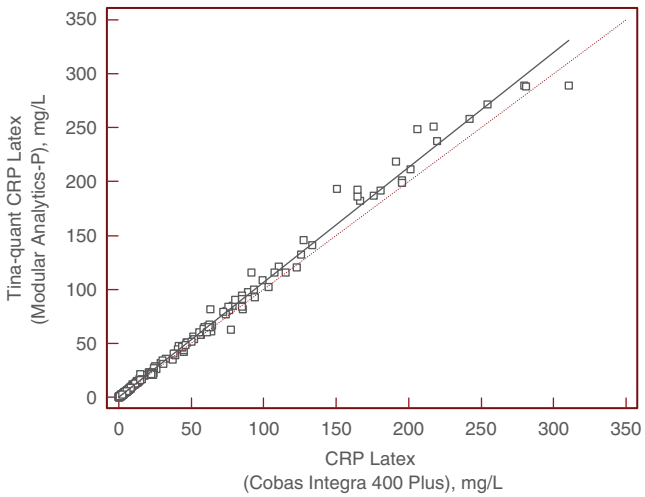

Fig. 3. Comparison of the methods. The solid line indicates the regression line, and the dashed line indicates the identity line. Deming regression analysis yielded the following results: slope = 1.067 (95% CI, 1.032–1.103), intercept = –0.148 (95% CI, −0.854–0.557), $S_{y|x} = 5.10$, and r = 0.996 (95% CI, 0.995–0.997).

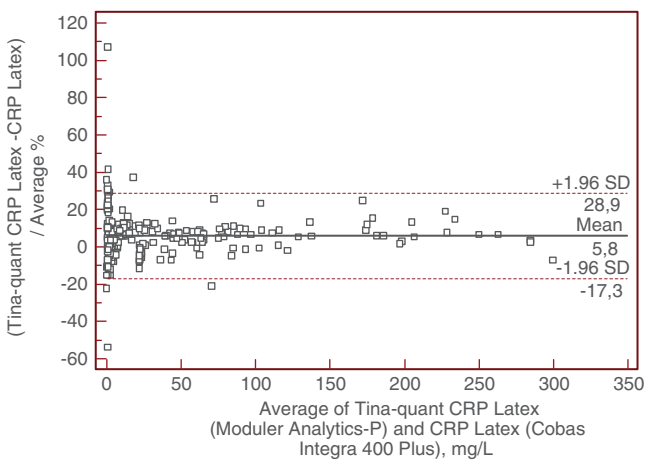

Fig. 4. Agreement between the methods by Bland-Altman analysis. The solid line indicates the mean percent difference between the methods, and the 95% CI for the percent difference is indicated by dashed lines.

hs-CRP is a powerful predictor of future first coronary events in apparently healthy men and women. However, traditional CRP assays do not have sufficient sensitivity in the range required to determine this cardiovascular risk (15).

Although the criteria for hs methods have not been exactly defined, it is generally accepted that they must be able to reliably measure CRP concentrations at least at the lowest cutoff point of 1 mg/L, and should have $<10\%$ of total imprecision across the linear range of the assay (19). In addition, it has been suggested that assays used in population-based studies and clinical research should be able to measure much lower CRP concentrations (e.g., 0.15 mg/L) (20). For this reason, manufacturers have been working intensively on marketing more-sensitive CRP methods. However, despite the manufacturers' claims, it has been reported that some commercially available hs-CRP methods do not meet the analytical and clinical requirements (14,19).

Most hs-CRP assays have a narrow linear range, and conventional CRP assays are incapable of measuring low concentrations. Therefore, there is a need for two different assays that can simultaneously and accurately determine a wide range of CRP concentrations (from 0.15 mg/L to 1,000 mg/L) in the clinical laboratory. If two different assays are used, clinicians should be informed about which CRP assay is the most highly sensitive. Otherwise, it may lead to misidentification of individuals at risk of future coronary events.

Because of the reasons mentioned above, we compared two methods and evaluated them in terms of analytical performance characteristics, such as the detection limit, linearity, and intra- and inter-assay precision. We found the detection limits for CRP Latex on the Cobas Integra 400 Pus and Tina-quant CRP Latex on the Modular Analytics-P to be 0.20 mg/L and 0.22 mg/L, respectively. Conversely, the limits of detection for CRP Latex on the Cobas Integra 400 Pus and Tina-quant CRP Latex on the Modular Analytics P were higher and lower, respectively, than the manufacturers' claims (0.085, and 0.40 mg/L, respectively). The determined detection limits were slightly higher than the desired level of 0.15 mg/L (according to data from population-based studies (14,20)).

Both methods were linear up to 215 mg/L. In the Tina-quant CRP Latex method, the 100% concentration measuring point is below the regession line and the method appears to be linear up to the 75% concentration of the pool. Since this point is within the 95% confidence interval (CI) of the regression line, we considered it linear up to 100% concentration (Fig. 1). When the auto-rerun capability of both analyzers is taken into consideration, the Cobas Integra 400 Plus has the advantage of measuring CRP concentrations up to 1600 mg/L, which is especially important for monitoring infectious and inflammatory diseases.

The intra- and interassay CVs of both assays were less than 4.1% at concentrations over 7 mg/L. However, the intra- and interassay CVs for CRP Latex on the Cobas Integra 400 Plus and Tina-quant CRP Latex on the Modular Analytics-P at concentration of ~0.60 mg/L were above 10%. In contrast to the satisfactory results at the relatively high CRP levels, these precision data

suggest that both methods have poor imprecision at low CRP levels. However, good precision is necessary to define the low level of inflammation present in apparently healthy subjects.

In the method comparison study with 268 patient samples, a strong correlation between the two assays was found. Despite this strong correlation, there was a scattering of results on the Bland-Altman plot near the detection limits. This scattering supports the relatively poor precision and sensitivity of both methods at low concentrations.

When all data are evaluated together, it is obvious that both assays need to be improved in terms of sensitivity and imprecision. In addition, even though it was lower than the total allowable error, which is the generally accepted goal in clinical laboratory practice, there was a proportional bias of 6.7% between the two assays. This indicates that there is still a need for standardization of CRP assays, even with the same manufacturer's assays, based on similar methodologies and calibrated with the same reference material. To address this issue, the Centers for Disease Control (CDC) has initiated a standardization program in which manufacturers of all hs-CRP reagents worldwide have been invited to participate (21). In a study evaluating the secondary reference materials for CRP assays, as a part of the CDC's effort to standardize hs-CRP assays, Kimberly et al. (22) reported that CRM 470 had slightly better characteristics than the other materials. Subsequently, the CDC standardization committee on hs-CRP confirmed this study, and reported that CRM 470 should be used in Phase II to harmonize various hs-CRP assays (21).

In conclusion, the two assays yielded similar results in the method comparison. The precision and sensitivity of both assays at low concentrations need to be improved. If this can be accomplished, these assays (especially the Tina-quant CRP Latex on the Modular Analytics) will increase the usefulness of CRP testing because they can provide a wide range of linearity, high throughput, and appropriateness for laboratory consolidation and automation. A method that is capable of measuring CRP concentrations accurately and precisely from 0.15 to many thousands of milligrams per liter is urgently needed. Such an assay could meet the demands of various clinical conditions, and enhance the cost-effectiveness and clinical utility of CRP testing.

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REFERENCES

1. Pepys MB. C-reactive protein fifty years on. Lancet 1981;1: 653–657.
2. Meier-Ewert HK, Ridker PM, Rifai N, Price N, Dinges DF, Mullington JM. Absence of diurnal variation of C-reactive protein concentrations in healthy human subjects. Clin Chem 2001;47: 426–430.
3. Frohlich M, Sund M, Thorand B, Hutchinson WL, Pepys MB, Koenig W. Lack of seasonal variation in C-reactive protein. Clin Chem 2002;48:575–577.
4. Clyne B, Olshaker JS. The C-reactive protein. J Emerg Med 1999;6:1019–1025.
5. Ross R. Atherosclerosis–an inflammatory disease. N Engl J Med 1999;340:115–126.
6. Verma S, Szmitko PE, Ridker PM. C-reactive protein comes of age. Nat Clin Pract Cardiovasc Med 2005;2:29–36.
7. Ridker PM, Rifai N, Pfeffer MA, et al. Inflammation, pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events (CARE) Investigators. Circulation 1998;98:839–844.
8. Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14719 initially healthy American women. Circulation 2003;107:391–397.
9. Albert CM, Ma J, Rifai N, Stampfer MJ, Ridker PM. Prospective study of C-reactive protein, homocysteine, and plasma lipid levels as predictors of sudden cardiac death. Circulation 2002;105: 2595–2599.
10. Ridker PM, Stampfer MJ, Rifai N. Novel risk factors for systemic atherosclerosis: a comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. JAMA 2001;285: 2481–2485.
11. Tice JA, Browner W, Tracy RP, Cummings SR. The relation of C-reactive protein levels to total and cardiovascular mortality in older U.S. women. Am J Med 2003;114:199–205.
12. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and prevention and the American Heart Association. Circulation 2003;107: 499–511.
13. Yeh ET, Willerson JT. Coming of age of C-reactive protein: using inflammation markers in cardiology. Circulation 2003;107: 370–371.
14. Roberts WL, Sedrick R, Moulton L, Spencer A, Rifai N. Evaluation of four automated high-sensitivity C-reactive protein methods: Implications for clinical and epidemiological applications. Clin Chem 2000;46:461–468.
15. Rifai N, Tracy RP, Ridker PM. Clinical efficacy of an automated high-sensitivity C-reactive protein assay. Clin Chem 1999;45: 2136–2141.
16. National Committee for Clinical Laboratory Standards. "Method comparison and bias estimation using patient samples. Approved guideline." NCCLS document EP9-A. Villanova, PA: NCCLS Publications; 2002.
17. Martin RF. General deming regression for estimating systematic bias and its confidence interval in method-comparison studies. Clin Chem 2000;46:100–104.
18. Bland JM, Altman DG. Comparing methods of measurements: why plotting difference against standard method is misleading. Lancet 1995;346:1085–1087.
19. Roberts WL, Moulton L, Law TC, et al. Evaluation of nine automated high-sensitivity C-reactive protein methods: implica-

tions for clinical and epidemiological applications. Part 2. Clin Chem. 2001;47:418–425.
20. Ledue T, Rifai N. Preanalytic and analytic sources of variations in C-reactive protein measurement: Implications for cardio vascular disease risk assessment. Clin Chem 2003; 49: 1258–1271.
21. Rifai N, Warnick GR. Quality specifications and the assessment of the biochemical risk of atherosclerosis. Clin Chim Acta 2004;346:55–64.
22. Kimberly MM, Vesper HW, Caudill SP, et al. Standardization of immunoassays for measurement of high-sensitivity C-reactive protein. Phase I: evaluation of secondary reference materials. Clin Chem 2003;49:611–616.