Measurement of C-reactive Protein: Two High Sensitivity Methods Compared

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C-reactive protein (CRP) is an acute phase marker and a predictor of the risk of developing atherosclerotic complications. However, as a predictor of this risk, high sensitivity measurements are needed, and high sensitive CRP (hsCRP) assays have been developed. In this study, we experimentally compared two hsCRP assays, based on nephelometry and turbidimetry, both implemented on automated analyzers. Linearity, imprecision, turbidity interference, and results in the assay of 96 samples have been compared. Method comparison of the same two analytical systems in the assay of CRP was also performed on the basis of results in an

interlaboratory external quality assessment scheme (EQAS). The two systems were found to perform substantially equally, both in hsCRP and in CRP measurement, but in the hsCRP assay the precision of nephelometry (CV% in the interval 3.0–5.8) was lower than that of turbidimetry (CV% in the interval 1.8–2.3). The classification of results by the two methods into three predefined relative risk classes gave 18% rate of discordance, in any case by one class only. The two methods proved reliable and comparable in the measurement of hsCRP, but precision should be improved. *J. Clin. Lab. Anal.* 18:280–284, 2004. © 2004 Wiley-Liss, Inc.

Key words: atherosclerosis; C-reactive protein high-sensitivity; immunoassay; nephelometry; turbidimetry

INTRODUCTION

C-Reactive protein (CRP) is a pentameric acute phase protein synthesized by the liver (1). The serum CRP concentration may increase up to 1,000-fold in the presence of infections, trauma, surgery, and acute inflammatory events, since long time CRP has been considered a very sensitive although unspecific marker of acute phase (2). More recently, chronic subclinical inflammation has been shown to be an important component in the development and progression of atherosclerosis (3). CRP levels have been shown to be correlated to the risk of unfavorable events in acute coronary syndromes, even at concentrations lower than the accepted upper reference limit (<5 mg/L) (4). The risk assessment based on the detection of such chronic inflammation requires high-sensitivity methods, allowing precise and accurate measurements of CRP in the interval between 0 and 10 mg/L.

Most frequently used methods for the routine measurement of serum CRP are based on particle-enhanced immunonephelometry or immunoturbidimetry, and their detection limit is near 2 mg/L, with an analytical interval up to 200 mg/L. Many manufac-

turers, however, have made available modifications of their analytical systems (instruments plus reagents and calibrators), lowering their detection limits to ≤ 0.2 mg/L. CRP measurements with such modified systems are suitable for cardiovascular risk assessment and are generally referred to as high-sensitivity C-reactive protein (hsCRP). A number of such systems have been comparatively evaluated (5–11).

The aim of this study was the comparative evaluation of two widely used systems for hsCRP measurement, based on particle enhanced immunoturbidimetry or immunonephelometry, implemented on automatic analyzers. Data concerning the performance of the two systems in the measurement of CRP are also reported for comparison.

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MATERIALS AND METHODS

Assessment of hsCRP Assays

Automated hsCRP measurements were performed with particle-enhanced immunonephelometry and immunoturbidimetry with an Immage analyzer (Beckman Coulter, Milano, Italy) and a Modular clinical analyzer (Roche Diagnostics, Milano, Italy), respectively, following the manufacturers' instructions, and using reagents and calibrators specifically designed for high sensitivity measurements. The declared detection limits were 0.2 mg/L (immunonephelometry) and 0.03 mg/L (immunoturbidimetry).

For method comparison experiments, 96 fresh serum samples, selected from the routine laboratory work load to cover the 0.2–13.0 mg/L interval of CRP concentration, were assayed on the two systems in two different analytical series, at 1 month time interval. For linearity and imprecision assessment, two serum pools ("low" and "high," hsCRP 2.12 and 8.21 mg/L, respectively, by nephelometry) were mixed in seven different proportions (volume fractions of the high pool from 0 to 1), and each mixture was assayed in 20 replicates by the two methods. The possible effect of turbidity was evaluated by spiking 1-mL aliquots of two serum pools (hsCRP 4.40 and 11.3 mg/L) with 30 μ L and 60 μ L of Intralipid (20% emulsion; Pharmacia & Upjohn, Verona, Italy). The concentration in the spiked aliquots, measured by the two systems, was expressed as a percentage of that measured in unspiked aliquots.

Assessment of CRP Assays

The results obtained in one yearly cycle (2002–2003) of the EQAS of the Regione Lombardia were used for this evaluation. Lombardia is a region of northern Italy, whose laboratories participate in mandatory EQA schemes managed by the public health care administration. The EQAS is based on yearly cycles. During each cycle six pairs of lyophilized are distributed to the participants, who are asked to perform the measurements and to submit the results within a stated time interval. At the end of each exercise and of each cycle, cumulative reports are generated and distributed. A total of 186 laboratories participated in the CRP EQAS. For the required measurements, 45 participants (24%) used "Modular-family" analyzers, 33 participants (18%) used "Image-family" analyzers; the remaining 108 (58%) used analytical systems/methods classified into 10 additional systems or "system families." At the end of the cycle, 12 pairs of "robust" estimates of each system's performance (concentrations in the interval 7.30–86.2 mg/L) were available and compared. Each estimate was the mean of either 33 or 45 single

measurements in different laboratories with the same system.

Statistical Analysis

Ordinary parametric tests (mean and SD) were used. Method comparison results were analyzed by Deming's regression: intercept and slope values were considered significantly different from 0 and 1, respectively, when 0 and 1 were not included in the 95% confidence interval of the relevant statistic.

Assessment of the Clinical Relevance of Intermethod Differences

The samples ($n = 96$) from method comparison were split according to their nephelometry results into three class of risk: low (hsCRP < 1 mg/L), medium (hsCRP 1–3 mg/L), and high (hsCRP > 3 mg/L) (12). Then, within each class risk, the classification of the samples according to their turbidimetry results was considered, and the concordance/discordance of the turbidimetry vs. the nephelometry classification was computed.

RESULTS

Within-run imprecision, measured at seven concentration levels in the interval 2.12–8.21 mg/L (nephelometry) and 2.52–10.54 mg/L (turbidimetry) from 20 replicate measurements, is shown in Table 1. There was no evident relationship between concentration and imprecision, but at the 0.21 mg/L level (additional experiment) the imprecision (turbidimetry) raised to 13.9%. Linearity in the same concentration intervals, assessed as correlation coefficient of mean measured value against volume fraction, was $r = 0.9999$ (nephelometry) and $r = 1.000$ (turbidimetry). In the evaluation of turbidity effects, the concentration values measured in the presence of artificially induced turbidity (spiking with Intralipid) were expressed as percentages of the values measured in unspiked serum aliquots. Results are shown in Table 2.

Results of method comparison for the whole group ($n = 96$; interval: 0.2–13.2 mg/L, by nephelometry) are shown in Fig. 1. This graphic assessment confirms a rather different intermethod behavior in the two analytical series. Applied to the whole set of results, statistical analysis gave: slope = 0.971 (0.907–1.036), not significantly different from 1; intercept = -0.21 mg/L (-0.38 – 0.04), significantly different from 0; $r = 0.960$. Results of method comparison for the group of samples with hsCRP (nephelometry) ≤ 5 mg/L ($n = 70$) are shown in Fig. 2. Statistical analysis gave: slope = 0.969 (0.806–1.132), not significantly different

TABLE 1. Within-run imprecision of the two methods*

Sample no.	Nephelometry			Turbidimetry		
	Mean mg/L	SD mg/L	CV (%)	Mean mg/L	SD mg/L	CV (%)
1	2.12	0.09	4.27	2.52	0.06	2.29
2	3.58	0.20	5.52	4.46	0.08	1.78
3	5.16	0.15	2.96	6.42	0.13	1.98
4	5.86	0.29	5.01	7.39	0.16	2.19
5	6.45	0.37	5.74	8.47	0.18	2.16
6	7.25	0.42	5.75	9.38	0.19	2.02
7	8.21	0.38	4.68	10.5	0.22	2.04

*Seven concentration levels, 20 replicate measurements each.

TABLE 2. Effect of turbidity induced by spiking with Intralipid*

Sample no.	CRP measured		
	Intralipid added μL/mL	Nephelometry (%)	Turbidimetry (%)
1	0	100.0	100.0
2	30	100.4	100.0
3	60	100.7	100.0
1	0	100.0	100.0
2	30	100.9	100.0
3	60	99.7	100.0

*Values are expressed as percentage of the values measured in unspiked samples (nephelometry: sample 1 = 4.40 mg/L; sample 2 = 11.3 mg/L).

Fig. 1. Scatter plot of the hsCRP values measured with two different analytical systems in 96 individual serum samples. Individual data points are compared with the equivalence line (dotted); statistical assessment is in the text.

Fig. 2. Same as Fig. 1, referring to samples with hsCRP ≤ 5 mg/L (n = 70).

Fig. 3. Scatter plot of CRP assay results in a yearly cycle of EQAS. Single points are mean values from the results of 33 (nephelometry) and 45 (turbidimetry) laboratories using the same analytical systems as in the hsCRP assay in the experiment summarized in Figs. 1 and 2.

from 1; intercept = -0.20 mg/L ($-0.49-0.08$), not significantly different from 0; $r = 0.896$.

Figure 3 shows the turbidimetry/nephelometry comparison from the cumulative reports of a yearly cycle of EQAS for CRP. As mentioned before, results are the mean values of 33 nephelometric and 45 turbidimetric measurements performed in different laboratories with

TABLE 3. Classification of 96 single samples into three relative risk classes according to their hsCRP concentration measured by two methods*

Relative risk class	HsCRP mg/L	Immunonephelometry no. (% of total)	ImmunoTurbidimetry	
			Concordant no. (% of total)	Discordant no. (% of total)
Low	<1	14 (15%)	12 (12.5%)	2 (2,1%) ^a
Medium	1–3	39 (40%)	30 (31.2%)	4 (4.2%) ^a 5 (5.2%) ^b
High	>3	43 (45%)	37 (38.5%)	6 (6.3%) ^a
Total		96 (100%)	79 (82.3%)	17 (17.7%)

*Risk classes according to reference (12).

^aHigher by one relative risk class

^bLower by one relative risk class

the same instrument. Correlation/regression analysis gave: slope = 1.004 (0.916–1.093), not significantly different from 1; intercept = –1.33 mg/L (–6.18–3.51), not significantly different from 0; $r = 0.992$.

Table 3 shows the percentage of concordant and discordant classifications of single measurements with either nephelometry or turbidimetry into three relative risk classes. As a whole, 82% concordant and 18% discordant classifications were shown. In cases of discordant classification, this was only by one risk class difference.

DISCUSSION

CRP is emerging as a potential risk factor and predictor for future coronary events. In order to assess this, however, the analytic performance requirements for CRP assays have changed. In particular, higher sensitivity and precision than previously needed is now required (7,8). In this paper, we comparatively examined the performance characteristics of the two most widely used (in the Regione Lombardia) commercial analytical systems for CRP assay, for which specific adaptations for hsCRP measurement are available. The two systems appeared substantially equivalent as far as some basic characteristics like linearity in the useful concentration interval and lack of interference from sample turbidity. Imprecision was somewhat lower with the turbidimetry-based instrument. Absolute evaluation of acceptability of analytical imprecision, in view of the expected medical use and based on biological variation data, is presently difficult because of the extremely variable data reported about CRP biological variation (13–15).

Risk evaluation by reference to relative risk classes defined in a different environment requires analytical accuracy (or trueness) in addition to precision. Uniform accuracy levels are attained through traceability to a common reference (material or method) of higher metrological hierarchy. In the case of CRP, this has

been achieved internationally by the use of an internationally agreed common calibrator (16). Data from EQAS, as here reported, shows that such an endeavor was successful, and that existing interlaboratory or intermethods differences are attributable to imprecision when the two specific measurement systems are considered. From this point of view, nephelometry- or turbidity-based methods seem to work equally well. Traceability to international calibration is transferred to hsCRP measurement by dilution (17), and this also appears to have been successful (5,6). From the point of view of accuracy, nephelometry- or turbidity-based methods seem to also work equally well in hsCRP measurement. In our comparison experiments, we observed a statistically significant agreement between measurements results, even if the interseries sources of variation (including repeated calibration) were included, in order to better mimic the real routine laboratory setting. Individual differences are mainly due to imprecision. This should be improved especially for immunonephelometry. Different levels of agreement/disagreement have been reported in the literature comparing different analytical systems for hsCRP measurement in serum (5,6).

Comparing the capacity of the two systems in classifying single results into predefined relative risk classes, the overall misclassification-event rate was 18%. In this comparison, we have arbitrarily chosen the nephelometry as the reference method, but actually we do not know which of the two methods is more accurate. On the other hand, this is a comparative assessment. The same rate of concordant/discordant classification is observed if turbidimetry is taken as the reference for comparing nephelometric results.

In the evaluation of such a figure, it should be considered that the classification is only based on the result of a single measurement and that any observed classification difference is by only one relative risk class. These observations agree with the previous conclusion

that imprecision, rather than inaccuracy, is the prevailing analytical problem.

As a whole, we conclude that in the assay of hsCRP, to define the relative cardiovascular risk, the two analytical systems are nearly equivalent. Improvement of precision should lead to better comparability of individual measurements.

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