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Evaluation of fluorescence hs-CRP immunoassay for point-of-care testing

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Abstract

Background: C-reactive protein (CRP) is one of acute phase respondents that has been used to monitor infection and inflammation episodes. Recent studies have shown that high-sensitivity C-reactive protein (hs-CRP) is a potential risk predictor for future atherosclerosis and cardiovascular diseases (CVD).

Methods: We previously developed a fluorescence-based immunochromatographic method for measuring hs-CRP concentrations (*i*-CHROMA™ hs-CRP assay) in blood. Whole blood was mixed with detector buffer, and then loaded onto a test cartridge. After 10 min of incubation, the test cartridge was inserted and scanned for acquisition of fluorescence intensity in a laser fluorescence reader (*i*-CHROMA™ reader). The fluorescence intensity was microprocessed and converted into the concentration of CRP in blood. The test result of 150 samples by the *i*-CHROMA™ hs-CRP assay method was compared and evaluated with those by TBA 200FR turbidimetry and BN II nephelometry method. The Deming regression and the Bland-Altman difference plot analysis were used for comparison of hs-CRP test result.

Results: The *i*-CHROMA™ hs-CRP assay system exhibited a good linearity with in the whole measuring range ($R=0.997$). The imprecision of intra- and the inter-assay CVs (coefficient of variation) of assay system were CVs< 3% and < 5% in the range of 0.5–20 mg/l, respectively. The *i*-CHROMA™ hs-CRP assay method correlated well with TBA 200FR turbidimetry and BN II nephelometry assay method ($R=0.988$, $N=143$ and $R=0.989$, $N=143$).

Abbreviations: hs-CRP, high-sensitive C-reactive protein; CVD, cardiovascular disease; mAb, monoclonal antibody; CV, coefficient of variation; POCT, point-of-care testing.

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Conclusion: The *i*-CHROMA™ hs-CRP assay system is comparable to those of other well-known fully automated hs-CRP assay and is suitable for point-of-care testing (POCT) in detection and quantification of hs-CRP.

Keywords: hs-CRP; Immunoassay; Anti-CRP-mAb; Cardiovascular diseases; POCT

1. Introduction

CRP has been well-known as one of acute phase reactants and used as a marker of inflammation/infection for clinical purposes. It is synthesized in liver by induction of interleukin-6 and secreted greatly into plasma during acute phase response to tissue injury, infection or other inflammatory episodes. The plasma CRP rises thus from a normal level of < 5 to 500 mg/l during the body's general, non-specific responses. CRP exhibits several functions associated with host defense mechanism: it opsonises bacteria, promotes agglutination, and increases phagocytes at the site of inflammation [1]. In addition, CRP takes part in scavenge process of DNA and cell debris by activating classical pathway of complement system through its calcium-dependent binding to phosphorylcholine of cell membrane [2].

CRP, along with cholesterol, has been suggested to be critical component in the development and progression of atherosclerosis [3]. A series of studies have recently shown that a chronic, low-level CRP of the vascular endothelium has a pivotal role in the development of CVD including coronary heart disease, ischemic stroke, and acute myocardial infarction [4–6]. Thus hs-CRP has emerged as a marker of the most independent predictive risk factor for future atherosclerosis and CVD [7,8]. Epidemiologic studies with hs-CRP on healthy individuals have shown a reference interval: median values, 0.6 to 1.1 mg/l; 75th percentile values, 1.4 to 2.5 mg/l; and 90th percentile values, 3 to 5.4 mg/l and these CRP levels can categorize the group into, respectively, low-, moderate-, and high-risk subgroups for future cardiovascular events [9,10]. The traditional CRP tests for monitoring infection and inflammation measure CRP level > 5–10 mg/l and are too insensitive for quantification of hs-CRP for predicting future CVD. CRP assay methods including immunoturbidimetry and immunonephelometry have thus been developed with sensitivity of detection of CRP < 1 mg/l.

We previously reported the development of fluorescence immunochromatographic assay system for hs-CRP in whole blood [11]. In this study, we wanted to show improvement of imprecision performance and to evaluate if the *i*-CHROMA™ hs-CRP assay system could provide fast, reliable, and reproducible result in comparison with the fully automated methods. Compared to the old version of assay system, two main renovations in the new assay version were a size reduction to 1/3 of the original laser fluorescence reader and an expansion of working range from 0.15–10 to 0.15–50 mg/l.

2. Methods

Whole blood samples were collected for quantification of hs-CRP from 150 random individuals who had visited for their annual health check-up at Ajou University Medical Center in Suwon, Korea. All adult females and males were in a range of the ages 30 to 80 years. After fully explaining the nature and possible consequences of study, we obtained informed consents from the study participants.

We measured CRP concentrations in side-by-side assays using fluorescence hs-CRP immunochromatographic method, TBA 200FR analyzer (Toshiba) of immunoturbidimetric method, and BN II analyzer (Dade-Behring) of nephelometric method, respectively. While whole blood was used for quantification of hs-CRP in the *i*-CHROMA™ reader, serum was run for determination of hs-CRP concentration in TBA 200FR and BN II analyzer. The test results were analyzed and compared using Medcalc ver. 7.6 software program (Mariaekerke, Belgium) for Bland-Altman difference plot and Derming regression analysis.

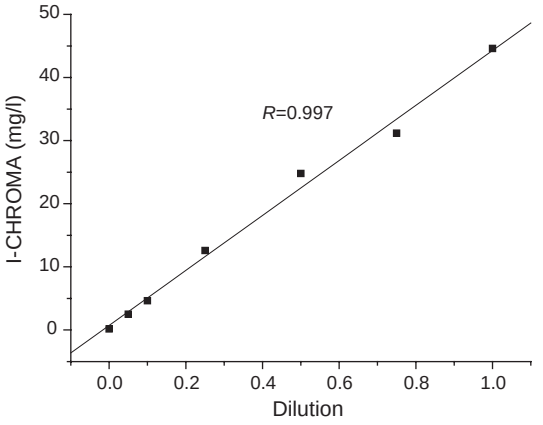

Fig. 1. Linearity of the *i*-CHROMA™ hs-CRP assay system at concentrations < 50 mg/l. The high pool (~ 44.5 mg/l) was diluted with the low pool (~ 0.15 mg/l) to the following final percentages: 100%, 75%, 50%, 25%, 10%, 5%, and 0%. Samples were assayed in triplicate in one analytical run at each CRP level. The correlation coefficient of linear regression was shown ($R = 0.997$).

The *i*-CHROMA™ hs-CRP Test consisted of a detector buffer, a disposable hs-CRP strip cartridge, and an *i*-CHROMA™ reader. For measurement of hs-CRP concentration in fluorescence immunoassay system, Ten microliters of whole blood was mixed well with 60 μl of detector buffer containing fluorescence labeled anti-CRP-mAb and anti-rabbit-IgG. The mixture was then loaded onto well of test cartridge. The cartridge was inserted after 10 min of immune reaction and scanned for acquisition of fluorescence intensity on test and control line, and the fluorescence intensity was converted into CRP concentration in the *i*-CHROMA™ reader.

Samples for linearity studies of the *i*-CHROMA™ hs-CRP assay system were prepared from 2 whole blood pools. The high pool was prepared using a combination of patient samples with adjustment to hs-CRP concentration of ~ 44.5 mg/l and the concentration of low pool was ~ 0.15 mg/l. The high pool was diluted with the low pool to the following final percentages: 100%, 75%, 50%, 25%, 10%, 5%, and 0%. Samples were assayed in triplicate in one analytical run at each CRP level. We also evaluated the imprecision of intra- (within day) and inter-assays (between days) variations to determine the accuracy of system. The intra-assays were performed on 10 replicate tests for each CRP concentration of 0.5, 5, 10, and 20 mg/l. The inter-assays were conducted on 5 sequential days, 2 runs per day, with triplicates at each CRP concentration.

3. Results

To examine the linearity of the *i*-CHROMA™ hs-CRP assay system, the whole blood pools were prepared. The correlation coefficient of linear regression was $R = 0.997$ as shown in Fig. 1, indicating that assay method exhibited a good linearity in the working range of 0–50 mg/l. The intra- and the inter-assay CVs of the *i*-CHROMA™ hs-CRP assay method showed, respectively, 2.9% and 4.18% at 0.5 mg/l, 2.3% and 4.8% at 5 mg/l, 2.5% and 4.9% at 10 mg/l, and 2.8% and 4.8% at 20 mg/l (Table 1). These results suggested that the new assay version with the *i*-CHROMA™ reader was much improved in imprecision performance in comparison to the old version of assay system [11].

The hs-CRP concentrations of 150 samples from healthy adult random population were measured in side-by-side assays by the *i*-CHROMA™ reader, TBA 200FR, and BN II analyzer. The whole blood was used for measurement of hs-CRP concentration in chromatographic assay on the *i*-CHROMA™ reader, and serum was run for determination of hs-CRP concentration in turbidimetric assay on TBA 200FR and in nephelometric assay on the BN II analyzer. Among 150 blood donors, 7 samples showing > 50 mg/l of hs-CRP concentration in the *i*-CHROMA™ reader were excluded from analysis and comparison for the Deming regression and the Bland-Altman plot.

The new version of hs-CRP assay system showed significant correlations with both the immunoturbidimetry method ($R = 0.988$, $p < 0.001$) and the immunonephelometry method ($R = 0.989$, $p < 0.001$).

Table 1
Imprecision of fluorescence hs-CRP immunoassay system

CRP (mg/l)	Intra-assay (within-run)			Inter-assay (between-run)[a]		
	Mean	S.D.	CV%	Mean	S.D.	CV%
0.5	0.53	0.02	2.9	0.53	0.02	4.1
5	5.22	0.12	2.3	5.20	0.25	4.8
10	9.80	0.25	2.5	10.07	0.49	4.9
20	19.91	0.55	2.8	20.07	0.97	4.8

[a] Inter-assay was conducted on 5 sequential days, 2 runs per day with triplicates at each CRP concentration.

As a reference, there was also a good correlation between 2 fully automated methods ($R = 0.994$, $p<0.001$). A regression analysis performed according to the Deming method between *i*-CHROMA™ (*y*-axis) and TBA 200FR (*x*-axis) yielded a slope of 0.98 (95% confidence interval, 0.93–1.04) and a *y*-intercept of 0.09 mg/l (95% confidence interval, 0.93–1.04 mg/l) (Fig. 2A). The Deming regression analysis for *i*-CHROMA™ (*y*-axis) vs BN II (*x*-axis) results yielded a slope of 1.07 (95% confidence interval, 1.01–1.12) and a *y*-intercept of 0.22 mg/l (95% confidence interval, 0.04–0.40 mg/l) (Fig. 2C). The Bland-Altman difference plots revealed little disagreement between the *i*-CHROMA™ and TBA 200FR

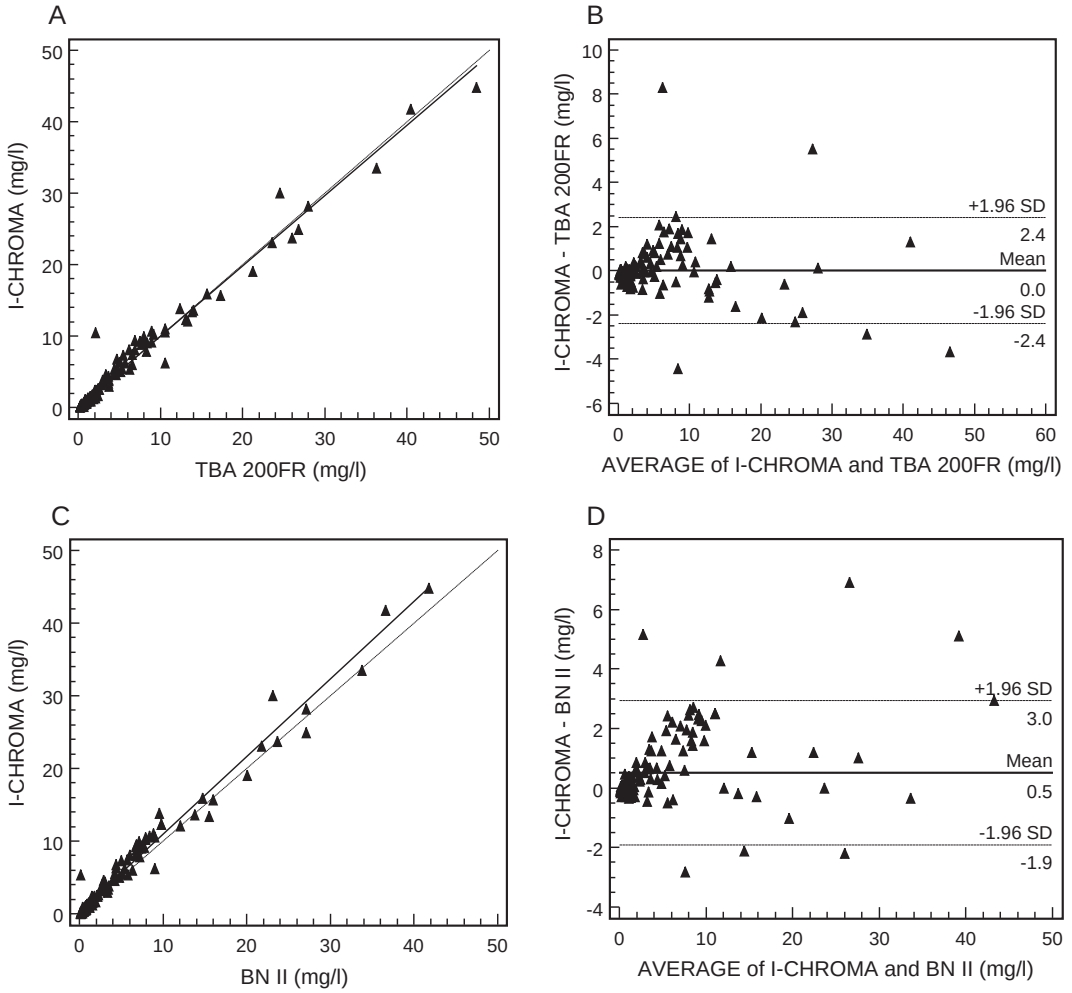

Fig. 2. Comparisons of hs-CRP test methods with 143 samples in side-by-side assays as performed by the *i*-CHROMA™ vs TBA 200FR methods (A and B) and by the *i*-CHROMA™ vs BN II methods (C and D). While whole blood was used for quantification of hs-CRP in the *i*-CHROMA™ reader, serum was run for determination of hs-CRP concentration in TBA 200FR and BN II analyzer. A and C show Deming regression analysis for the *i*-CHROMA™ vs TBA 200FR methods and the *i*-CHROMA™ vs BN II methods, respectively. The solid line indicates the regression line, and the dashed line indicates identity line. The slope, intercept, and correlation coefficient (R) of A and B are, respectively, 0.98 (95% CI, 0.93–1.04), 0.09 mg/l (95% CI, 0.93–1.04 mg/l), 0.988 and 1.07 (95% CI, 1.01–1.12), 0.22 mg/l (95% CI, 0.04–0.40 mg/l), 0.989. B and D show Bland-Altman plot analyses between compared methods. The difference between two values in the *y*-axis is plotted against the mean difference in the *x*-axis. The solid line represents the mean difference in measured hs-CRP concentration between the methods, and the dashed lines are ± 1.96S.D.

methods (Fig. 2B) or between the *i*-CHROMA™ and BN II methods (Fig. 2D). The maximum difference results between the *i*-CHROMA™ and TBA 200FR methods varied from −2.4 to 2.4 mg/l, with a mean difference of 0.0 mg/l. The maximum difference results between the *i*-CHROMA™ and BN II methods varied from −1.9 to 3.0 mg/l, with a mean difference of 0.5 mg/l. These observations indicated that the *i*-CHROMA™ hs-CRP assay method gave a confident performance compared to other well-known automated assay methods.

4. Discussion

The purposes of this study were to show the technical performance in terms of imprecision of the *i*-CHROMA™ hs-CRP method with new version of assay system and to evaluate if the *i*-CHROMA™ hs-CRP Test system could be a reliable POCT platform in comparison with other automated assay methods. The new version of the assay system has been renovated to have a wider working range and more portable size of laser fluorescence reader compared to the old version of the assay system. Whereas the working range and the size of reader in the old assay version were 0.15–10 mg/l and a dimension of 374L × 330W × 170H mm, respectively, those of the new assay version were changed to 0.15–50 mg/l and a portable dimension of 250L × 185W × 80H mm [11]. The *i*-CHROMA™ reader was developed by removing ellipsoidal mirror and PM tube from the original laser fluorescence reader. The *i*-CHROMA™ reader showed the same sensitivity as the old one even without them.

Despite the expansion of the working range of the assay system, the intra-and the inter-assay showed CVs < 3% and < 5%, respectively, at each tested concentration of hs-CRP as shown in Table 1. Specially CVs < 5% at 0.5 mg/l in both the intra- and the inter-assay indicated that the *i*-CHROMA™ hs-CRP Test system can be applied for long-term, risk assessment of CVD since assay imprecision with CVs < 10% at 0.2–0.5 mg/l was proposed as acceptable methods for risk stratification [12,13]. When the comparability of the *i*-CHROMA™ hs-CRP Test method was evaluated with hs-CRP results from 143 random samples, it demonstrated that the *i*-CHROMA™ hs-CRP Test method corresponded well to fully automated assay methods as shown in Fig. 2. A noticeable point was that correlations between *i*-CHROMA™ and each comparison method were as excellent as that of between two well-known automated assay methods. That whole blood as well as serum can be used in the *i*-CHROMA™ hs-CRP Test would be one more advantage over fully automated methods that use only serum.

While fully automated immunoassay formats as exemplified are available for quantification of hs-CRP and have an advantage of fast process in large numbers of samples, most of these methods are impractical or expensive for use of POCT. The criteria for POCT include affordable cost, a disposable device, and minimum maintenance/technical expertise required to perform tests [14]. The sample should be applied directly to the device, which should require only a small sample volume, and the assays should have a rapid turnaround time with good accuracy. Considering criteria for POCT, the *i*-CHROMA™ hs-CRP assay method goes well with them. There are some POC devices for the determination of CRP, such as the NycoCard™ CRP whole-blood test (Axis Shield), and QuickRead™ CRP whole-blood/serum/plasma assay (Orion Diagnostica). Analytical detection limits of QuickRead™ CRP whole-blood/serum/plasma assay is too high, over 5 mg/l, to be used for hs-CRP. Recently, there was a report that as increasing sample volume, the NycoCard™ CRP whole-blood test changed the detection range from 5–150 mg/l to 0.6–18.8 mg/l providing sufficient sensitivity for cardiovascular risk monitoring [15]. Both assay systems, however, require manual dispensing of the assay-specific reagents and the sample. The *i*-CHROMA™ hs-CRP Test is suitable not only for lower range between 0.15 and 10 mg/l but also for higher range between 10 and 50 mg/l. Thus the *i*-CHROMA™ hs-CRP assay method is comparable to other fully automated assay methods and appears to be well suited as a POCT platform for determination of hs-CRP concentration in whole blood.

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