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RESEARCH ARTICLE

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A comparison study between wide-range and high-sensitivity C-reactive protein assays (Roche Cobas c702) for low C-reactive protein concentration in patients with cardiovascular risk

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Abstract

Background: Low concentration C-reactive protein (CRP) has favorable prognostic significance in patients with cardiovascular risks.

Methods: We compared the wr-CRP method with the hs-CRP method both on Roche Cobas c702 analyzer for the determination of low CRP concentration (<20 mg/L) including 200 patients treated in Cardiology Department in Beijing Tsinghua Changgung Hospital (Beijing, China) from December 2018 to March 2019.

Results: The two methods were highly correlated (Spearman's rho = 0.995). Deming regression was used to fit the regression analysis model, giving a slope of 1.058 with an intercept of 0.008. The median method difference (wr-CRP − hr-CRP) was 0.120 mg/L (95% CI, 0.086-0.200 mg/L), and the median percent differences were 7.34% (95% CI, 4.27%-8.47%). The percent bias between both methods at the given cutoff CRP values of 1, 3, and 10 mg/L evaluated by Deming regression was 6.60%, 6.07%, and 5.88%, respectively, all of which were less than the acceptable standard (12.50%). The percentage of sample results concordant by both methods for the risk stratification was 96.0% (*kappa* = 0.937, $P < 0.001$).

Conclusions: Roche wr-CRP and hs-CRP assays are highly concordant in determining low concentration CRP. Wr-CRP may be used as an alternative to hs-CRP assay on Roche Cobas c702 analyzer to assess the cardiovascular risk, considering its convenience and lower costs.

KEYWORDS
cardiovascular disease risk, comparative study, C-reactive protein

1 | INTRODUCTION

In terms of clinical application, CRP seems to be a stronger predictor for early detection of asymptomatic individuals at risk for future vascular events.[1,2] Using widely available high-sensitivity assays, CRP levels of <1, 1-3, and >3 mg/L correspond to low-, moderate-, and high-risk groups for future cardiovascular events.[3-5] It has been shown that individuals with a CRP level of >3 mg/L have an adjusted

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10-year relative risk of 1.45 of coronary heart disease compared to individuals with <1 mg/L[3].

Ideally, physicians order one single test and obtain either a low CRP result predicting low atherosclerotic risk or a high CRP result indicating rather severe inflammation. This option might minimize confusion in ordering laboratory tests and decrease the patients' medical expenditure.[6,7] Nephelometry and immunoturbidimetry techniques have been developed to determine serum CRP in low concentration.[8,9] Over the last several years, wide-range C-reactive protein (wr-CRP) has been proposed as an alternative to high-sensitivity C-reactive protein (hs-CRP) in microinflammation detection and cardiovascular risk assessment. Ori Rogowski, et al[10] and Tomer Ziv-Baran et al[2] found that the Bayer wr-CRP assay performed presents a reasonable alternative to the Dade Behring hs-CRP assay in apparently healthy individuals. Diana C. Grootendorst et al[8] reported that there was good agreement between Roche CRP and Dade Behring hs-CRP in patients with end-stage renal disease, and Denis Monneret et al[11] have recently shown that Roche Cobas c501 wr-CRP has been proposed as an economical alternative to Roche Modular P800 hs-CRP for the evaluation of low-grade inflammation-associated cardiovascular risk. The Roche wr-CRP assay with a similar limit of quantitation is more use expedient with wider detecting linearity and lower costs. The objective of this study was to assess the concordance of wr-CRP and hs-CRP methods in the determination of low concentration of C-reactive protein in patients with cardiovascular risk and verify results from these studies.

2 | MATERIALS AND METHODS

2.1 | Samples

We enrolled patients with low CRP concentration with atherothrombosis risk treated in the Cardiology Department of Beijing Tsinghua Changgung Hospital from December 2018 to March 2019 in which either wr-CRP or hs-CRP was assayed, and excluded those with CRP ≥ 20 mg/L. Each participant was enrolled once and 200 cases met our criteria. Blood samples were collected in heparin-lithium anticoagulated tubes (Vacuette Greiner, ref#474084) and analyzed within 4 hours after blood is withdrawn. All tubes were centrifuged on Sorvall ST 16R centrifuge (Thermo Scientific) for 10 min at 2000 *g* (temperature 19.0 ± 0.4°C).

2.2 | Assay procedures

C-reactive protein values were analyzed by wr-CRP and hr-CRP methods on a Cobas c702 analyzer (Roche Diagnostics), using the latex-enhanced immunoturbidimetric assay (wr-CRP: C-Reactive Protein Gen. 3 reagent kit, ref#05172373190, turbidity measurement at 546 nm; hr-CRP: Cardiac C-Reactive Protein Latex High Sensitive reagent kit, ref#05950864190, turbidity measurement at 570 nm). The analytic measurement range of wr-CRP and hr-CRP was 0.3-350 and 0.15-20 mg/L, respectively. Both methods were calibrated and internal quality controls (IQC) were established before the determination, and accuracy was verified with satisfactory results of the external quality assessment programs organized by the National Center for Clinical Laboratories of China (NCCL). Roche hs-CRP assay was taken as the comparison method based on previous analytical and clinical validations.[12,13]

2.3 | Statistical analysis

Method comparison and bias estimation were performed referring to Clinical & Laboratory Standards Institute (CLSI) EP09C document.[14] Because the distribution of CRP results was skewed rightward, median concentrations were computed and method differences were assessed by the Wilcoxon signed rank test. Deming regression was used to evaluate the slope, intercept, and *r*. The percent bias at each cutoff value (1, 3, and 10 mg/L of hs-CRP) was determined using the regression equation, and the percent bias was compared to the acceptable standard of half of the allowable total error (12.5%, from the National Center for Clinical Laboratories of China).[15] According to the recommendations of the guideline, all participants were further classified into low, moderate, or high cardiovascular risk using the cutoff value of <1, 1-3, and >3 mg/L, respectively.[1,16] The agreement of the risk classification of the patients by both methods was observed by kappa statistic. Cohen's kappa coefficient (*kappa*) < 0.20, 0.21-0.39, 0.40-0.59, 0.60-0.79, 0.80-0.90, and >0.90 can be roughly interpreted as none, minimal, weak, moderate, strong, and almost perfect agreement.[17] All statistical tests were two-tailed, and a *P* value <0.05 was considered to be statistically significant. IBM SPSS Statistics for Windows, version 24 (IBM Corp.), and EP evaluator release 12.0 (Data Innovations LLC) were used for statistical analysis.

3 | RESULTS

We enrolled 200 participants with CRP level of <20 mg/L assayed with either wr-CRP or hs-CRP method in the study. The mean ± SD age of participants was 46.3 ± 6.7 years (84 women and 116 men, respective mean ± SD age being 46.7 ± 6.4 and 46.1 ± 7.0 years). Between-run precision (six consecutive months) showed coefficients of variation in the range of 2.78%-4.24% and 2.72%-4.51% for the wr-CRP and hs-CRP methods, respectively. The median of CRP results by wr-CRP method (2.800 mg/L) was significantly higher than that of hs-CRP method (2.680 mg/L; $Z = -6.901$, $P < 0.001$).

3.1 | Correlation and regression

C-reactive protein results were not normally distributed, so Spearman's rank correlation analysis was used to assess correlations between both methods. As shown in Table 1, the CRP results showed a significant correlation between both methods for the total (Spearman's rho = 0.995), low-risk (hs-CRP < 1 mg/L; Spearman's rho = 0.930), moderate-risk (1 ≤ hs-CRP ≤ 3 mg/L;

TABLE 1 Spearman's rank correlation and Deming regression of CRP results between wr-CRP and hs-CRP methods

Group	n	Spearman's rank correlation[a]		Deming regression				
		Spearman's rho	*P* value	Deming regression equation	Slope (95% CI)	Intercept (95% CI)	*r*	*P* value
Total group	200	0.995	*P* < 0.0001	*y* = 0.008 + 1.058*x*	1.058 (1.036-1.079	0.008 (−0.020 to −0.036)	0.9292	*P* < 0.0001
Subgroup								
Low-risk (hs-CRP < 1 mg/L)	49	0.930	*P* < 0.0001	*y* = −0.029 + 1.111*x*	1.111 (0.990-1.232)	0.029 (−0.099 to 0.040)	0.9292	*P* < 0.0001
Moderate-risk (1 ≤ hs-CRP ≤ 3 mg/L)	55	0.928	*P* < 0.0001	*y* = −0.148 + 0.985*x*	0.985 (0.897-1.073)	0.148 (−0.018 to 0.315)	0.9484	*P* < 0.0001
High-risk (hs-CRP > 3 mg/L)	96	0.983	*P* < 0.0001	*y* = −0.518 + 1.146*x*	1.146 (1.046-1.246)	−0.518 (−0.990 to 0.047)	0.9543	*P* < 0.0001

Note: Deming regression was used to evaluate the slope, intercept, and *r*. 95% CI—95% confidence interval. Spearman's rho—Spearman's coefficient of rank correlation.
[a]CRP results were not normally distributed, and Spearman's rank correlation analysis was used to assess correlations between both methods for the total, low-risk, moderate-risk, and high-risk groups.

FIGURE 1 Deming regression of CRP results between wr-CRP and hs-CRP methods. The dashed line represents the line of identity, whereas the solid line represents the Deming regression line. Slope = 1.058 (95% CI: 1.036-1.079); intercept = 0.008 (95% CI: −0.020 to 0.036); *r* = 0.9292; n = 200

Spearman's rho = 0.928), and high-risk (hs-CRP > 3 mg/L; Spearman's rho = 0.983) groups.

Deming regression analysis gave a slope of 1.058 (95% CI: 1.036-1.079) with an intercept of 0.008 (95% CI: −0.020 to 0.036) for the total group (Figure 1), a slope of 1.111 (95% CI: 0.990-1.232) with an intercept of 0.029 (95% CI: −0.099 to 0.040) for low-risk group, a slope of 0.985 (95% CI: 0.897-1.073) with an intercept of 0.148 (95% CI: −0.018 to 0.315) for moderate-risk group, and a slope of 1.146 (95% CI: 1.046-1.246) with an intercept of −0.518 (95% CI: −0.990 to 0.047) for high-risk group. The detailed Deming regression results are shown in Table 1.

3.2 | Method comparison and bias evaluation

Ranked order difference plots and ranked order percent difference plots between both methods were drawn referring to the CLSI EP09C protocol. The differences exhibited a constant coefficient of variation. The median method difference (wr-CRP − hr-CRP) was 0.120 mg/L (95% CI, 0.086-0.200 mg/L; Figure 2A), and the median percent difference [(wr-CRP − hr-CRP)/hr-CRP × 100%] was 7.34% (95% CI, 4.27%-8.47%; Figure 2B). Besides, the predicted bias was calculated using the equation from the Deming regression analysis. The percent bias between both methods at the given cutoff CRP values of 1, 3, and 10 mg/L evaluated by Deming regression analysis was 6.60%, 6.07%, and 5.88%, respectively. In addition, the percent biases evaluated by Passing-Bablok regression and ordinary linear regression were all less than the acceptable standard (12.50%; Table 2).

3.3 | Agreement assessment

We further assessed the agreement between both methods by kappa statistic. The percentages of low-, moderate-, and high-risk

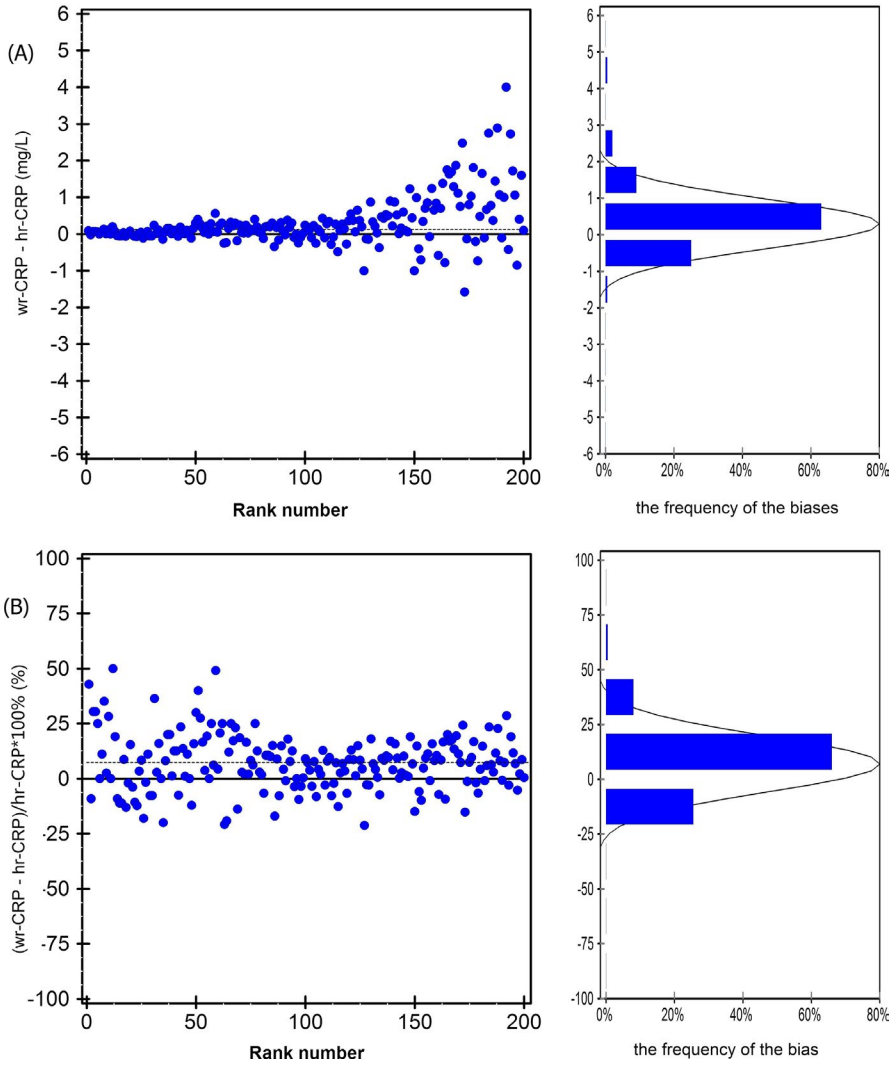

FIGURE 2 Bias evaluation of CRP results between wr-CRP and hs-CRP methods by EP evaluator analysis. (A) Left figure is the ranked order difference plot, the *x*-axis represents the rank number of the sample (1-200), and the *y*-axis represents the median method difference (wr-CRP − hr-CRP). The thin dashed line indicates the median difference (0.120 mg/L, 95% CI: 0.086-0.200 mg/L). The right figure is the distribution plot of the frequency of the bias. (B) Left figure is the ranked order percent difference plot, the *x*-axis represents the rank number of the sample (1-200), and the *y*-axis represents the median percent difference [(wr-CRP − hr-CRP)/hr-CRP × 100%]. The thin dashed line indicates the median percent difference (7.34%, 95% CI: 4.27%-8.47%). The right figure is the distribution plot of the frequency of the bias

subjects were 24.5% (49/200), 27.5% (55/200), and 48.0% (96/200) for high-sensitivity CRP method and 23.5% (47/200), 28.5% (57/200), and 48.0% (96/200) for wr-CRP method. A total of 96.0% (192/200) of the participants were classified into the same tertile by both methods (*kappa* = 0.937, *P* < 0.001). Compared to the hs-CRP method, the wr-CRP method reclassified 4.0% (8/200) of the participants: 1.5% (3/200) were reclassified to a lower risk group while the remaining 2.5% (5/200) to a higher risk group (Table 3).

4 | DISCUSSION

This study confirms that wr-CRP immunoturbidimetry assay is highly correlated with hs-CRP on Roche Cobas c702 analyzer at low-grade inflammation levels (Spearman's rho = 0.995), even below the threshold cutoff of moderate cardiovascular risk range at 1 mg/L. The wr-CRP method provides results increased about 0.12 mg/L compared to hs-CRP. This bias was in accordance with that from Itzhak Shapira et al's study,[10] wr-CRP results on Bayer Advia 1650 system were higher than hs-CRP results on Dade Behring BN II Nephelometer (0.039 ± 0.317 mg/L). Nitsan MAHARSHAK et al's study[18] reported that wr-CRP results on Bayer Advia 1650 system were higher than hs-CRP on Dade Behring BN II Nephelometer (0.64 mg/L). Tomer Ziv-Baran et al's study[2] demonstrated wr-CRP results on Bayer Advia 2400 were higher than hr-CRP on Dade Behring BN II Nephelometer (0.15 ± 0.29 mg/L) before judgment. However, other studies showed a negative bias for wr-CRP comparing to hs-CRP, and Monneret et al's study[11] reported that wr-CRP values on Roche Modular P800 were lower than hs-CRP on Roche Cobas c501 analyzer (−0.11 ± 0.17 mg/L). Yaron Arbel et al's study[19] showed that wr-CRP on Bayer Advia 1650 analyzer was lower than hs-CRP on Dade Behring BN II Nephelometer (−0.21 mg/L).

Both methods were calibrated by Roche multiply Cfas Proteins calibrator, which had been standardized against the IFCC Certified Reference Material (CRM) 470 standard[20,21]; however, their different calibration mode (wr-CRP: 6-point spline; hs-CRP: line graph), or assay type (wr-CRP: 2-point end; hs-CRP: rate A), or reagent ingredients (wr-CRP: mouse immunoglobulins in Reagent 3; hs-CRP: mouse immunoglobulins in Reagent 1), or even the main wavelength (wr-CRP: 570 nm; hs-CRP: 546 nm) may cause the mean discrepancies for determining the low concentration of CRP (data from instrument package insert: wr-CRP: version 7.0, 2014; hs-CRP: version 5.0, 2014).

TABLE 2 Bias results at the specific cutoff value of CRP values by different regression models

Regression model	Regression equation	Cutoff value (mg/L)	Predicted value (mg/L)	Bias (mg/L)	Percent bias (%)	Acceptable standard (%)
Deming regression	$y = 0.008 + 1.058x$	1.00	1.07	0.07	6.60%	±12.5
		3.00	3.18	0.18	6.07%	±12.5
		10.00	10.59	0.59	5.88%	±12.5
Passing-Bablok regression	$y = -0.002 + 1.074x$	1.00	1.07	0.07	7.20%	±12.5
		3.00	3.22	0.22	7.33%	±12.5
		10.00	10.74	0.74	7.38%	±12.5
Ordinary linear regression	$y = 0.024 + 1.052x$	1.00	1.08	0.08	7.60%	±12.5
		3.00	3.18	0.18	6.00%	±12.5
		10.00	10.54	0.54	5.44%	±12.5

Note: The percent bias between both methods at the given cutoff CRP values of 1, 3, and 10 mg/L was evaluated by three regression models. The percent bias was all less than the acceptable standard of half of the allowable total error (12.5%, provided by the National Center for Clinical Laboratories of China).

TABLE 3 Classification of the individuals into risk groups according to wr-CRP and hs-CRP assays

	hs-CRP (mg/L)			
wr-CRP (mg/L)	Low risk (<1)	Moderate risk (1-3)	High risk (>3)	Total
Low risk (<1)	45	2	0	47
Moderate risk (1-3)	4	52	1	57
High risk (>3)	0	1	95	96
Total	49	55	96	200

Note: The values presented as the number of concordant individuals in the same classification judged by both methods. This table showed that 96.0% (192/200) of the participants were classified into the same tertile (*kappa* = 0.937, $P < 0.001$). Compared to the hs-CRP method, the wr-CRP method reclassified 4.0% (8/200) of the participants: 1.5% (3/200) were reclassified to a lower risk group while the remaining 2.5% (5/200) to a higher risk group.

The cutoff value for low risk (<1 mg/L), moderate risk (1-3 mg/L), and high risk (>3 mg/L) of coronary heart disease was recommended in the consensus conference of the Centers for Disease Control and Prevention (CDC) and the American Heart Association (AHA).[16] The slope and intercept were not significantly different from 1 and 0 in low- and moderate-risk groups. The 95% CI of slope in high-risk group was 1.046-1.246, not including 1. It may be considered that there was a small proportional deviation between wr-CRP and hs-CRP in high-risk group. Considering that the different regression models may affect the results of bias evaluation, the percent bias was further evaluated by three regression models between both methods at the given cutoff CRP values of 1, 3, and 10 mg/L, and all the percent bias were acceptable in clinical practice. Agreement study showed that the classification concordance rate was 96.0%, indicating almost perfect agreement between both methods. The reclassification rate was 4.0%, mainly over-estimation of the risk using the Roche wr-CRP method. The currently used tertile cut points are derived from Caucasian population,[16] which may not be appropriate for Asian population groups. Further research is needed to determine the utility of hs-CRP measurements for cardiovascular risk prediction in Asian populations, and the appropriate cut point values derived from these populations are needed.[13] Moreover, additional clinical and biological data such as hypersensitive troponin are required for further study.

Overall, this study is close to those of previous studies, which showed a strong correlation between wr-CRP and hs-CRP at low concentrations <20 mg/L. In view of its advantages of convenience and low costs, the Roche wr-CRP assay may be used as an alternative to Roche hs-CRP method for routine evaluation of the cardiovascular risk for patients with low concentration of CRP.

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The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee of Beijing Tsinghua Changgung Hospital (approval number 18187-0-01).

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