Comparability of high-sensitivity CRP methods to detect maturity-onset diabetes of the young due to *HNF1A* mutations

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C-reactive protein (CRP) is an acute-phase protein synthesised in the liver that can rise rapidly in response to infection or tissue injury.[1] The CRP methods may be categorised as those optimised for performance between 5 mg/L and 200 mg/L for detection and monitoring of inflammation and infection,[1] and those applicable in the 1–20 mg/L range, so-called high-sensitivity assays, for application in cardiovascular risk assessment.[2]

An increased understanding of the role of genetic factors[3,4] in CRP expression have led to suggestions that plasma CRP may provide a screening tool for the identification of patients with maturity-onset diabetes of the young (MODY) due to mutations in the *HNF1A* gene.[5–7] Currently, *HNF1A*-MODY is under-diagnosed due to restricted awareness and costs of genetic testing, and consequently many patients are mislabelled as having more common forms of diabetes and may receive suboptimal treatment.[8]

HNF1A regulates expression of CRP, so haploinsufficiency due to inactivating mutations results in lower plasma CRP concentrations in patients with *HNF1A*-MODY compared with other diabetes subtypes, but the absolute values appear CRP method-dependent.[7] The present study clarifies method differences in the concentration range of relevance to this clinical application.

Three CRP methods were obtained from Siemens Healthcare Diagnostics (Frimley, UK) and applied using the manufacturer's recommendations. Two immunoturbidimetric assays, high-sensitivity CRP method (HCRP) and a wide-range CRP method (WCRP) were applied on an ADVIA 2400 general chemistry analyser. The third method (ICRP) was a chemiluminescence immunoassay applied on an Immulite 2000 analyser. Each method had kit-specific calibration material. The immunoturbidimetric methods used six-point calibration, HCRP covering the range 0.16–10 mg/L and WCRP 0.02–164 mg/L. The ICRP method utilised a master curve over the range 0.2–100 mg/L with an on-instrument two-point adjustment.

Lithium heparin plasma samples sent to the clinical biochemistry laboratory for CRP analysis were used for all studies. Intra-assay imprecision was assessed by analysing 11 samples in quadruplicate, inter-assay imprecision with three clinical samples measured in duplicate on 10 separate days. The limit of quantification (LOQ) was defined at an inter-assay imprecision of 20%.[9] The method comparison utilised 121 samples with values evenly distributed between 0.02 mg/L and 5 mg/L. Spiked samples were used to assess the effect of common interferences: haemolysis (haemoglobin up to 0.68 mmol/L), lipaemia (triglycerides, in the form of intralipid, up to 26.7 mmol/L) and icterus (bilirubin up to 334 µmol/L).

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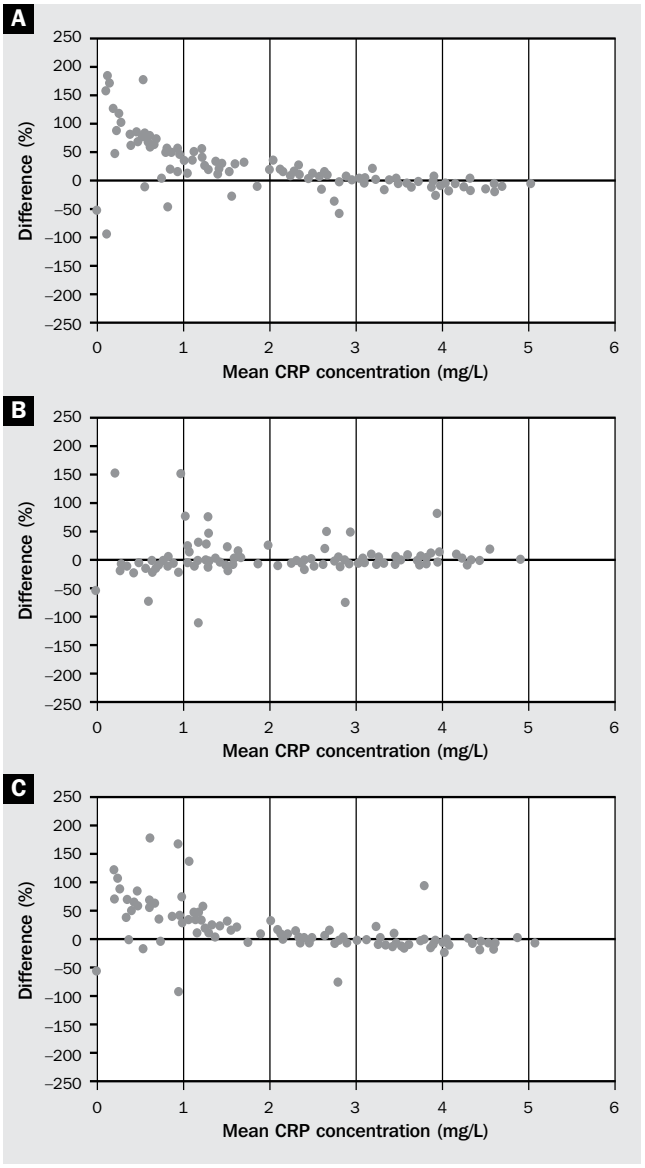

Fig. 1. Bland-Altman figures for differences between CRP methods: **A)** HCRP *vs.* WCRP; **B)** HCRP *vs.* ICRP; **C)** ICRP *vs.* WCRP.

Effects of the interfering substances were considered significant if an absolute difference of >5% between spiked and unspiked samples was noted. Assessment of imprecision utilised geometric mean, standard deviation and percentage coefficient of variation (%CV). Method comparisons are presented as Bland-Altman plots and evaluated using Passing-Bablock regression using Analyse-It software within Excel.

Intra-assay imprecision of clinical samples (n=11, measured in quadruplicate) between 0.05 mg/L and 2.5 mg/L was superior for HCRP (mean %CV: 1.5; range: 0.4–6.2%) compared to WCRP (mean %CV: 4.2; range: 0–13.3%) and ICRP (mean %CV: 3.9; range: 1.4–9.4%). Inter-batch imprecision for HCRP was 7.5% at 0.41 mg/L, 2.6% at

0.92 mg/L and 3.8% at 3.4 mg/L; for WCRP 10.5% at 0.11 mg/L, 6.6% at 0.48 mg/L and 4.9% at 3.38 mg/L; for ICRP 8.2% at 0.37 mg/L, 6.5% at 0.89 mg/L and 4.3% at 3.31 mg/L.

The limit of quantification for all assays based on extrapolation of imprecision profiles was below 0.01 mg/L for all three assays. None of the methods was significantly affected by haemolysis or bilirubin. Lipaemia appeared to reduce measured CRP values with all three methods although only for WCRP did this become >5%, and this was only at the highest triglyceride concentration assessed (26.7 mmol/L).

Comparative differences between each of the methods are presented as Bland-Altman plots in Figure 1. Passing and Bablock analysis, intercept (95% confidence interval [CI]) and slope (95%CI) showed HCRP = 0.457 (0.379–0.508) + 0.857 (0.830–0.889) (WCRP); HCRP = –0.05 (–0.10 to –0.01) + 1.00 (0.97–1.03); ICRP = 0.451 (0.394–0.514) + 0.851 (0.822–0.879) (WCRP). Significant constant bias was detected for all comparisons and significant proportional bias was detected between HCRP and WCRP and between ICRP and WCRP.

All three methods evaluated had acceptable imprecision characteristics, limit of quantification values and tolerance to commonly observed interferences. However, while the methods compared well at concentrations above 2 mg/L, there were large and significant differences below 2 mg/L and particularly below 1 mg/L. The detection both of significant proportional and constant bias confirms previous method comparability data.[8]

The WCRP method was selected for the original studies as its calibration strategy utilised the lowest-value calibration material (0.02 mg/L), 10-fold lower than those for HCRP (0.16 mg/L) and ICRP (0.20 mg/L). This calibration difference may account for some of the differences in bias observed between WCRP and both HCRP and ICRP.

A study of high-sensitivity methods in the context of cardiovascular disease risk[10] also concluded that significant differences existed, and it called for improved standardisation. Subsequent efforts to improve standardisation of CRP methods include a new formulation of the certified reference material, currently available as ERM-DA474/IFCC.[11] It would be hoped that this reference material coupled with consistent instrument protocols will lead to improved comparability between methods in the future. Until such standardisation is available, a pragmatic threshold such as <0.5 mg/L could be adopted to identify those at higher risk of *HNF1A*-MODY. Alternatively, results could be compared to locally derived CRP distributions of healthy age-matched individuals and/or patients with diabetes.

In conclusion, all three high-sensitivity CRP methods appear reproducible but demonstrate accuracy differences at concentrations below 2 mg/L, the concentration range of interest as a screening biomarker for patients with diabetes secondary to *HNF1A*–MODY.

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