Introducing the wide range C-reactive protein (wr-CRP) into clinical use for the detection of microinflammation

Ori Rogowski\textsuperscript{a}, Yaffa Vered\textsuperscript{b}, Itzhak Shapira\textsuperscript{a,*}, Meirav Hirsh\textsuperscript{a}, Vera Zakut\textsuperscript{c}, Shlomo Berliner\textsuperscript{a}

\textsuperscript{a}Department of Medicine “D” and Institute for Special Medical Examinations (MALRAM), Tel Aviv Sourasky Medical Center, Tel Aviv, affiliated to Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, 6 Weizman Street, Tel Aviv 64239, Israel
\textsuperscript{b}The Central Laboratory, Tel Aviv Sourasky Medical Center, Tel Aviv, affiliated to Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, 6 Weizman Street, Tel Aviv 64239, Israel
\textsuperscript{c}The Laboratory of Clinical Immunology, Tel Aviv Sourasky Medical Center, Tel Aviv, affiliated to Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, 6 Weizman Street, Tel Aviv 64239, Israel

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Abstract

Background: The determination of low grade inflammation in apparently healthy individuals (microinflammation) has prognostic significance in terms of future vascular events and accelerated atherothrombotic disease.

Methods: We compared the Bayer wide range (wr)-C-reactive protein (CRP) immunoturbidometric assay on the ADVIA 1650 system to the Dade Behring high sensitivity (hs)-CRP on the BNII Nephelometer in 1446 apparently healthy individuals having a relatively low (<10 mg/l) concentration. The correlation between the 2 assays was also analyzed in relation to other commonly used microinflammatory biomarkers.

Results: A significant (p <0.0005) correlation was noted between the hs-CRP and the wr-CRP for the entire cohort (r = 0.99) as well as for both women (r = 0.99 n = 483) and men (r = 0.99 n = 963). The mean difference between the measures (hs-CRP minus wr-CRP) was 0.039 (SD 0.317). The Deming regression results for the entire cohort showed a slope of 1.112 ± 0.004 and an intercept of −0.263 ± 0.01.

Conclusions: The Bayer wr-CRP assay performed presents a reasonable alternative to the Behring Dade hs-CRP assay. The advantages of the wr-CRP assay are its online and real time availability as well as lower costs.

Keywords: Microinflammation; Wide range C-reactive protein; High sensitivity C-reactive protein

Abbreviations: hs-CRP, high sensitivity C-reactive protein; wr-CRP, wide range C-reactive protein; BMI, body mass index; CVA, cerebrovascular accident; PAOD, peripheral artery obstructive disease; ESR, erythrocyte sedimentation rate; WBCC, white blood cell count; PMN, polymorphonuclears; CAP, College of American Pathologists; FCRS, Framingham Coronary Heart Disease Risk Score.

* Corresponding author. Tel.: +972 3 6974254; fax: +972 3 6973635.
E-mail address: shapiraiz@tasmc.health.gov.il (I. Shapira).
1. Introduction

It has been repeatedly shown that atherothrombosis is associated with the presence of low grade, subclinical and smoldering internal inflammation (herewith denoted as microinflammation) [1–3]. High sensitivity C-reactive protein (hs-CRP) assays have emerged as promising laboratory methods for the determination of the presence of this microinflammation and for the assessment of its intensity [4]. In fact, the determination of CRP concentrations might help to single out individuals with an increased atherothrombotic risk [5,6]. In addition, it might have also a direct pathogenetic role as well [7].

2. Participants and methods

2.1. Study population

We prospectively determined the concentrations of CRP in a group of apparently healthy individuals and those with atherothrombotic risk factors who are routinely recruited into the Data Base of the Tel Aviv Medical Center Inflammation Survey (TAMCIS) [8–10]. This is a relatively large cohort to whom we invited apparently healthy employees of the Tel Aviv Medical Center and the Tel Aviv Municipality (Israel) in addition to individuals with atherothrombotic risk factors who are being followed-up in the medical center’s outpatient clinics. Members of the medical staff, retired employees of the medical center and the municipality as well as individuals being evaluated in our outpatient health screening program were also recruited. All the individuals included in the present survey gave their written consent according to the instructions of the Institutional Ethics Committee. Appeals to participate were distributed on bulletin boards and in notes that were included with the monthly paycheck of the medical personnel as well as personal invitations to the patients in various outpatient clinics. Members of the medical staff, retired employees of the medical center and the municipality as well as individuals being evaluated in our outpatient health screening program were also recruited. All the individuals included in the present survey gave their written consent according to the instructions of the Institutional Ethics Committee. Appeals to participate were distributed on bulletin boards and in notes that were included with the monthly paycheck of the medical personnel as well as personal invitations to the patients in various outpatient clinics. Excluded were any individuals with an underlying inflammatory disease (arthritis, inflammatory bowel disease, etc.) as well as those with any infections or other inflammatory conditions, including infarction, surgery or angiography during the 6 months preceding study enrollment. We also excluded individuals with anemia (hemoglobin 13.5 g/dl for men and 11.7 g/dl for women) and those treated with steroid or non-steroidal anti-inflammatory medication, except for aspirin (at doses <325 mg/day). They were examined following an overnight fast.

2.2. Definition of risk factors

Diabetes mellitus was defined as fasting blood glucose of >125 mg/dl, the use of insulin or oral hypoglycemic agents, hypertension as blood pressures of >140/90 mm Hg, the use of anti-hypertensive medications while hyperlipidemia was defined as cholesterol or triglyceride concentrations of >200 mg/dl, or the use of HMG-CoA reductase inhibitors or fibrates. We included smokers both present (≥5 cigarettes/day) and past (none for ≥30 days). For the definition of the metabolic syndrome we used The National Cholesterol Education Program (NCEP) Adult Treatment Panel-III guidelines [11] defining the metabolic syndrome as the presence of ≥3 of the following risk determinants: (1) increased waist circumference (>102 cm for men, >88 cm for women), (2) increased triglycerides of 1.70 mmol/l (≥150 mg/dl), (3) low HDL cholesterol (1.03 mmol/l for men [=40 mg/dl], 1.29 mmol/l for women [≤50 mg/dl]), (4) hypertension (systolic blood pressure ≥130 mm Hg or diastolic pressure ≥85 mm Hg) or antihypertensive medication use, and (5) impaired fasting glucose 6.1 mmol/l (≥110 mg/dl). The Framingham Coronary Heart Disease Risk Score (FCRS) was calculated based on the LDL points according to the formula of Wilson et al [12].

2.3. Laboratory methods

2.3.1. Analyses

2.3.1.1. High sensitivity C-reactive protein (hs-CRP). High-sensitivity C-reactive protein (hs-CRP) was analyzed by an immunonephelometric assay on a BN II analyzer (Dade Behring, Marburg, Germany) using Dade Behring kit for hs CRP assay. This assay is based on particle-enhanced immunonephelometry and it enables the measurement of CRP concentrations as low as 0.16 mg/l. The assay is based on the measurement of polystyrene particles coated with monoclonal antibodies specific to human CRP that aggregate when mixed with samples containing human CRP. These
aggregates scatter a beam of light passed through the sample. The intensity of the scattered light is proportional to the concentration of the relevant protein in the sample. In each run, known concentrations of CRP are examined by using the Dade Behring control sera as required by the manufacturer. The standard curve is automatically plotted by using a standard sample obtained from the manufacturer, as described [13]. The results of hs-CRP in our laboratory are routinely compared to the ones obtained by College of American Pathologists (CAP) as required by the ISO 9001 quality management systems requirements and are in good agreement.

2.3.1.2. Wide range CRP (wr-CRP). Wide range CRP (wr-CRP) in human serum was analyzed by an immunoturbidimetric assay on the ADVIA 1650 chemistry system using Bayer ADVIA kit for wr-CRP. It has a particularly wide sensitivity range (the lower limit of detection being 0.12 mg/l). The wr-CRP reagent is a suspension of uniform polystyrene latex particles coated with anti-CRP antibody. When serum-containing CRP is mixed with the latex reagent, agglutination takes place resulting in an increase in the turbidity. This turbidity was measured at 571 nm. The CRP concentration in serum was determined from a calibration curve that is generated with the calibrators.

2.3.1.3. The use of hs-CRP control. We have presently used a hs-CRP control Level II from Randox Laboratories Ltd. (Ardmore, Diamond Road, Crumlin, Co. Antrim, UK). This is a ready to use control in a stabilized protein base. Each batch of CRP control is evaluated at Randox by latex-enhanced immunoturbidimetry with reference to material standardized against an appropriate international reference Preparation (CRP 470).

2.4. Statistical analysis

All data was summarized and displayed as mean ± SD for the continuous variables (age, body mass index [BMI], all the inflammation markers, etc.), and as number of patients plus the percentage in each group for categorical variables (smoking and other cardiovascular risk factors, medications, etc.). The crosstabs and descriptive procedures were used to produce frequencies of categorical variables and means ± SD of continuous variables, respectively.
The hs-CRP has non-normal distribution, thus we used a logarithmic transformation which converts it to normal distribution for all the statistical procedures like correlations. For all categorical variables the Chi-Square Phi and Cramer’s $V$ statistics was used for assessing the statistical significance between the two genders. Pearson correlations for confounding variables were used to evaluate the association between the two CRP measurement methods and the different inflammatory variables including white blood cell count, log (hs-CRP) and the FCRS as well as the number of components of the metabolic syndrome. In order to compare between the two methods of measurement we used the Bland-Altman analysis, to evaluate the mean difference between the methods, and the Deming regression to evaluate the slope and

![Expected values (mg/l) vs. Measured values (mg/l)](image)

**Fig. 1.** Linearity of wr-CRP assay (dilution of patient sample).

![Percent of People in CRP Group](image)

**Fig. 2.** Percent of the individuals in the 3 categories of hs-CRP and wr-CRP.
the intercept of the regression line, best fitted between the methods. The level of significance used for all of the above analyses was two-tailed, \( p < 0.05 \). The SPSS statistical package was used to perform all statistical evaluation (SSPS Inc., Chicago, IL).

3. Results

We included 1446 individuals for whom we obtained results from both methods. The mean \( \pm \) SD age was 44.8 \( \pm \) 10.7 years (483 women and 963 men their respective mean \( \pm \) SD ages being 45.2 \( \pm \) 10.4 and 44.5 \( \pm \) 10.8 years). A list of atherothrombotic risk factors and vascular events in both women and men is shown in Table 1 while the intake of medication is reported in Table 2. We compared the results of 3 samples to those obtained by the hs-CRP and the CAP target value allowed. Table 3 shows that both assays are within the CAP allowed target. The results of the wr-CRP obtained in 10 samples agreed well to results by Randox (0.86 \( \pm \) 0.02 and 4.4 \( \pm \) 0.02 vs. 1.06 \( \pm \) 0.21 and 3.82 \( \pm \) 0.76, respectively). Within-run and between run precision (23 consecutive days) showed CVs of 0.5–2.4% and in the range of 4.4–5.3%, respectively. The assays tested on diluted patient and control serum (11 levels each) were linear in the range of 0.1–136 mg/l (Fig. 1).

The distribution of hs-CRP concentrations according to the cut offs of <1, 1–3 and 3–10 mg/l [7] is shown in Fig. 2 for both genders. We calculated the correlation between hs-CRP and wr-CRP for the entire cohort as well as for the above mentioned 3 groups of CRP concentrations. The results show significant correlation between both methods (Table 4). The mean difference between the measures (hs-CRP minus wr-CRP) was \( /C0 \) 0.039 with a SD of the bias of 0.317. Both CRP methods correlated to other inflammatory biomarkers including the erythrocyte sedimentation rate, fibrinogen concentrations, the white blood cell and polymorphonuclear leukocytes counts (Table 5). The correlation between the concentration of CRP and the age, BMI, components of the metabolic syndrome and the 10-year FCRS are shown in Table 6. Again, a similar correlation exists between both methods of CRP determination. The Deming regression line between the 2 methods, and the regression equation is shown in Fig. 3.

4. Discussion

C-reactive protein has recently emerged a useful biomarker for clinical purposes in asymptomatic
individuals [14]. This biomarker is accurate, reliable, and effective for the early detection of individuals at risk for future vascular events [15–17], and explains why most laboratories use high sensitivity assays (<5 mg/l CRP concentrations). In fact, these concentrations are not taken any more as “normal” values since it is clear that individuals with CRP concentrations <5 mg/l have an atherothrombotic disease [18] or are predisposed to future events [19].

The recent introduction of a wr-CRP assay that can be performed 24 h a day, 7 days a week at a relatively low cost, raises the question of how does it correlate with the widely used immunoassay that uses the BNII Nephelometer. We were especially interested in individuals with relatively low CRP concentrations. This interest stems from the following reasons:

1. The possibility to perform the test at any time period during the day without additional costs of laboratory personnel. In fact, the participants of our health screening program receive the results before leaving the clinic and can discuss them with their physician.
2. The possibility to use a sensitive assay that covers a wide range of concentrations for individuals who appear in the Emergency Room with an acute
ischemic event including myocardial infarction or stroke. This enables us to use this biomarker for a real-time risk stratification strategy.

3. The possibility to perform the test out of the primary tube at a relatively low cost (2$ value of reagents as opposed to 4$) on a unified platform for all chemistry and endocrinology tests.

The present results are significant in that they show that the wr-CRP assay correlates significantly with the hs-CRP. In fact, the similar correlation between these two assays and other biomarkers as well as individuals' risk factors further support the notion that the information obtained from both of them is almost interchangeable in this regard.

In the present study we did not limit ourselves to a correlation between the two assays solely but evaluated the correlation between the particular determination in a specific assay and the clinical data of the participants. In fact, we found that the degree of correlation between CRP concentrations and age, BMI, the calculated 10-year FCRS and the number of components of the metabolic syndrome is similar for both assays. The same is true for the correlation between CRP concentrations and other inflammatory biomarkers, including the Westergren erythrocyte sedimentation rate, white blood cell count and the number of polymorphonuclear leukocytes.

Finally, it should be mentioned that the Dade method is also available on routine platforms such as Dade RxL Dimention. In addition, it should be stressed that no financial support was obtained from either of the manufacturers or their representatives.

We conclude that the wr-CRP might present an alternative to hs-CRP in apparently healthy individuals with and without atherothrombotic risk factors. It looks as if both wr-CRP and hs-CRP have a similar yield of detection and quantitation of microinflammatory activity in this groups of individuals. Availability and low cost might present an advantage for the wr-CRP assay.

References


