Reproducibility of C-Reactive Protein Analyses

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The aim of this study was to measure the reliability of different nephelometric techniques for measuring C-reactive protein (CRP). One hundred and twenty samples were obtained from 40 patients. All 120 samples were divided in three parts to measure CRP using three different methods. Reliability was determined by the kappa index and intraclass correlation coefficient. The intraclass correlation coefficient ranged from 0.78 to 0.94. When CRP values were categorized in four groups, the kappa index reached 75-86% and percentage of agreement varied from 95% to 97%. When CRP values were divided into two groups, the kappa index was 73% to 78% and the percentage of agreement was 86% to 89%. We found that CRP determinations with different nephelometric methods were highly reproducible, even when different analysts were involved. Ultrasensitive techniques are needed only if the clinical objective is to obtain a CRP measurement under 0.3 mg/dl.

Key words: *Peripheral artery disease. C-reactive protein. Acute coronary syndrome.*

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Reproducibilidad de los análisis de proteína C reactiva

El objetivo de este estudio es medir la reproducibilidad de diferentes técnicas nefelométricas para la determinación de la proteína C reactiva (PCR). Se obtuvieron 120 muestras de 40 pacientes. Cada muestra fue dividida en tres alícuotas y se determinó la PCR por tres procedimientos diferentes. La reproducibilidad se midió mediante el índice kappa y el coeficiente de correlación intraclase. El coeficiente de correlación intraclase varió entre 0,78 y 0,94. El índice kappa ponderado obtuvo valores entre 75 y 86% y el porcentaje de acuerdo entre las técnicas varió entre 95 y 97%. Al dicotomizar la PCR, el índice kappa varió entre 73 y 78% y el porcentaje de acuerdo entre 86 y 89%. Se concluye que la determinación de la PCR es muy reproducible con diferentes técnicas nefelométricas. Sólo si el objetivo del clínico es medir la PCR por debajo del límite de 0,3 mg/dl, sería necesario emplear técnicas ultrasensibles.

Palabras clave: *Enfermedad vascular periférica. Proteína C reactiva. Síndrome coronario agudo.*

INTRODUCTION

Many studies have found a relation between high concentrations of C-reactive protein (CRP) and the risk of acute myocardial infarction or death due to cardiac causes,[1,2] cerebrovascular accident,[1] and prognosis in stable and unstable angina.[3,4] Other studies have shown that patients with elevated CRP concentrations have a greater prevalence of arteriosclerotic disease,[5] an increased risk of thrombosis of the left ventricle in patients with infarction,[6] and a greater degree of development of carotid arteriosclerosis.[7] For this reason, the use of CRP in the prognostic stratification of patients with acute coronary syndrome has been proposed.[8] The role of CRP and other markers of inflammation as indicators of cardiovascular risk has been reviewed by García-Moll and Kaski.[9]

The aim of this study is to measure the reproducibility of CRP measurements by comparing CRP determinations made by three procedures in the same samples obtained from a group of patients with peripheral arteriopathy.

METHODS

Forty patients who were followed-up in the day

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ABBREVIATIONS

CRP: C-reactive protein
AL1: aliquot 1 (determination in the Biochemistry Service with a Dade Behring NII instrument)
AL2: aliquot 2 (determination in the Immunology Laboratory with a Dade Behring NII instrument)
AL3: Aliquot 3 (determination in the Biochemistry Service with a Dade Behring DN instrument)

hospital for a diagnosis of peripheral arteriopathy were selected to undergo a controlled clinical trial of the effect of transdermal nitroglycerin. Patients who required surgery or interventionist radiological studies, had a diagnosis of diabetes mellitus, were being treated with anti-inflammatory nitrites, or had serious liver disorders, a history of acute myocardial infarction, or recent cerebrovascular accident were excluded. The mean age of patients was 63.3 years (standard deviation [SD], 9.4 years; range, 43-78). Thirty-eight patients were men.

Three blood samples were obtained on different days from each patient for the determinations established in the study protocol, one of which was CRP. Each blood sample was distributed into three aliquots that were processed as follows:

–Aliquot 1 (AL1): sent to the Biochemistry Service for the determination of CRP by nephelometry using a Dade Behring BNII instrument. This is the routine procedure for CRP determinations in our hospital. The minimum detection level of this instrument is 0.3 mg/dL.

–Aliquot 2 (AL2): sent to the Immunology Laboratory for the determination of CRP by nephelometry using a Dade Behring BNII instrument. The minimum detectable concentration of the instrument is 0.03 mg/dL.

–Aliquot 3 (AL3): sent to the Biochemistry Service for the determination of CRP by nephelometry using a Dade Behring BN instrument. The minimum detectable valor was 0.32 mg/dL.

Each aliquot was processed by a different analyst who did not know the aim of the study and was unaware that two more determinations had been made. Seven AL1 samples, six AL2 samples, and four AL3 samples could not be processed.

The reproducibility of CRP as a continuous variable was evaluated using the intraclass correlation coefficient obtained by double data entry.[10] The intraclass correlation coefficient had values between 0 and 1. Values close to 1 indicate greater reproducibility.

To determine the reproducibility of CRP as a discrete variable, it was categorized into four groups: <0.50; 0.50-0.99; 1.00-1.49; and ≥1.50 mg/dL. Next, the kappa coefficient weighted with quadratic weights was estimated.

The statistical analysis was made with the Stata Intercooled program, version 6 (Stata Corporation, College Station, Tx, U.S.).

RESULTS

The AL1s had a mean value of 0.745 mg/dL (SD, 0.558) and a median value of 0.6 mg/dL (range, 0.3-3.0 mg/dL). The AL2s had a mean value of 0.528 mg/dL (0.466) and a median value of 0.416 mg/dL (range, 0.045-2.608 mg/dL). The AL3s had a mean value of 0.648 mg/dL (0.464) and a median value of 0.44 mg/dL (range, 0.32-2.54 mg/dL).

The intraclass correlation coefficients range from 0.78 (AL1 and AL2) to 0.94 (AL1 and AL3) (Table 1). In the three comparisons the 95% confidence interval (CI) excludes 0 and the reproducibility at the midpoint is more than 86%. Figures 1 to 3 show the relation between paired techniques, presenting the average on the Y-axis and the difference on the X-axis (in all three figures, the vertical axis is oversized to show the differences between techniques more clearly.[11] AL1 slightly overestimated the determinations obtained by AL2 (Figure 1) and AL3 (Figure 2); this overestimation tended to increase with the CRP level.

Tables 2 to 4 show the reproducibility of CRP as a discrete variable. The percentage agreement varied from 95.20% to 97.05% and the weighted kappa index ranged from 0.7524 to 0.8610 (P<.0001). When CRP

TABLE 1. **Reproducibility of the CRP determinations as continuous variables: intraclass correlation coefficient and 95% CI**

Analytical procedure 1	Analytical procedure 2	Intraclass coefficient of correlation	95% CI	Reproducibility in the midpoint
AL1	AL2	0.77712	0.67041-0.88383	0.86136
AL1	AL3	0.93841	0.90033-0.97650	0.98020
AL2	AL3	0.83594	0.76141-0.91047	0.88212

CI indicates confidence interval.

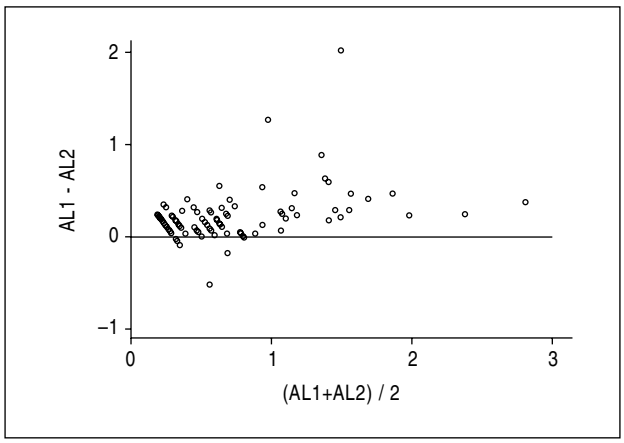

Fig. 1. Relation between the determinations of C-reactive protein in aliquots 1 (AL1) and 2 (AL2).

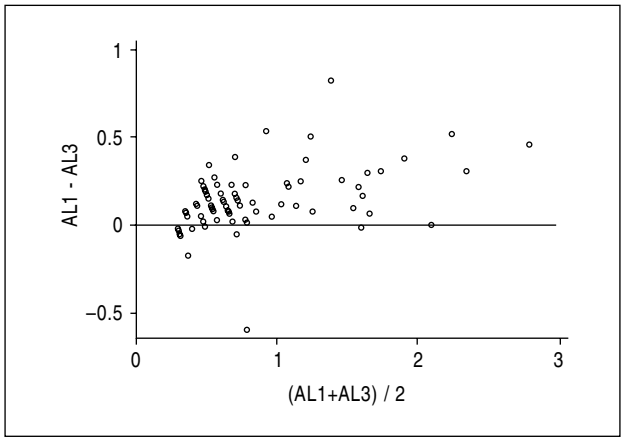

Fig. 2. Relation between the determinations of C-reactive protein in aliquots 1 (AL1) and 3 (AL3).

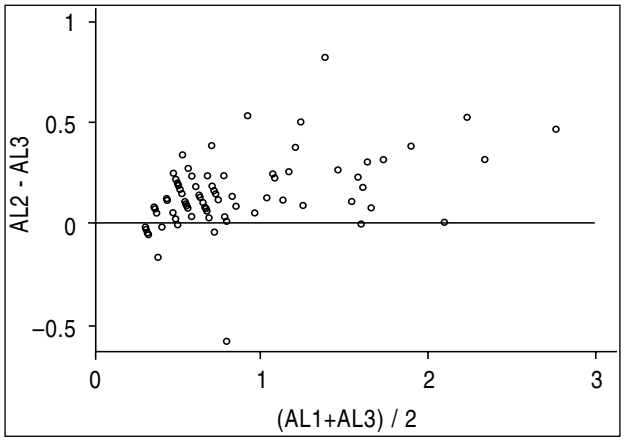

Fig. 3. Relation between the determinations of C-reactive protein in aliquots 2 (AL2) and 3 (AL3).

was classified into only two groups (<0.5 mg/dL or ≥0.5 mg/dL), the kappa index reached values of 0.7321 (between AL1 and AL2), 0.7697 (between AL1 and AL3), and 0.7849 (between AL2 and AL3) (P<.0001 in all three cases), and the percentage agreement ranged from 86.49 to 89.47.

TABLE 2. **Reproducibility between AL1 and AL2 as variables categorized by the weighted kappa coefficient**

	AL2				
	<0.50	**0.50-0.99**	**1.00-1.49**	**≥1.50**	**Total**
AL1					
<0.50	50	2	0	0	52
0.50-0.99	11	25	0	0	36
1.00-1.49	0	6	3	0	9
≥1.50	2	1	7	4	14
Total	63	34	10	4	111

Kappa index, 0.7524.

TABLE 3. **Reproducibility between AL1 and AL3 as variables categorized by the weighted kappa coefficient**

	AL2				
	<0.50	**0.50-0.99**	**1.00-1.49**	**≥1.50**	**Total**
AL2					
<0.50	51	1	1	0	53
0.50-0.99	12	25	0	0	37
1.00-1.49	0	5	4	0	9
≥1.50	0	1	4	9	14
Total	63	32	9	9	113

Kappa index, 0.8610.

TABLE 4. **Reproducibility between AL2 and AL3 as variables categorized by the weighted kappa coefficient**

	AL3				
	<0.50	**0.50-0.99**	**1.00-1.49**	**≥1.50**	**Total**
AL3					
<0.50	59	4	1	1	65
0.50-0.99	6	25	4	0	35
1.00-1.49	0	2	4	4	10
≥1.50	0	0	0	4	4
Total	65	31	9	9	114

Kappa index, 0.8055.

DISCUSSION

The reproducibility of CRP determination by nephelometry was very high, whether analyzed as a continuous or discrete variable. This reproducibility was independent of the analyst and nephelometric technique used.

The reproducibility of CRP is limited by the variability of biological levels: the intra-individual coefficient of variation can be as high as 30%, which is why it has been suggested that a single CRP determination should be used only to classify patients into two groups (high CRP/non-high CRP), but not for more detailed classifications (tertiles or quartiles).[12]

The only study that we found in which the reproducibility of CRP between two different techniques was analyzed resulted in a kappa index of 0.65 (lower than in this study) for classifications into two categories.[13]

Our result had two relevant consequences. One consequence was of a clinical nature, the reliability of the result was confirmed. The other consequence was economic, because it allows the choice of the technique to be performed to be based on non-clinical considerations (economic cost, time required for determination) because the result obtained with either technique will be similar.

The present study had three limitations: *1)* only clinical samples of unknown concentration were used, not standard patterns. Therefore, reproducibility could be estimated, but not validity (sensitivity and specificity); *2)* only one of the techniques had a very low detection level. If the purpose of the analysis is to classify patients with concentrations below 0.3 mg/dL, then the ultrasensitive technique is needed; *3)* the study was limited to nephelometric techniques; techniques like ELISA were excluded.

On the other hand, the selection of a sample of patients with vascular disease produced results that covered a broad range that could not have been covered only with subjects from the general population. This guarantees that the present results are applicable to real clinical situations. For example, in patients with unstable angina a greater risk of infarction has been identified with CRP levels over 0.36 (relative risk [RR]=2)[3] and a greater risk of death of death with CRP levels over 1 mg/dL (RR=3.4).[4] In both cases, any of the techniques used in this study would be suitable for adequately classifying patients. Special care was given to avoiding observer bias: the analysts did not know the purpose of the study and throughout its course they remained blind to the results of the other determinations. The distribution of the aliquots could not have influenced the final result because the three aliquots were obtained from the same blood extraction. Finally, statistical analysis was carried out without knowing what techniques were used.

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