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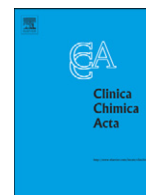

Effect of preanalytical variables on myeloperoxidase levels

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ABSTRACT

Background: Myeloperoxidase (MPO) levels have prognostic value in cardiovascular events, but information about preanalytical variables is scarce. This study evaluated the effect of different sample types and storage conditions on MPO measurements.

Methods: Plasma and serum samples [heparinized plasma (MPO-Hep), EDTA plasma (MPO-EDTA), and serum with (MPO-Gel) and without separator gel (MPO-Serum)] from 40 volunteers were assayed for MPO by ELISA (Bioxytech® MPO-EIA™ kit). To evaluate MPO stability, samples were stored at 18–25 °C, at 2–8 °C and at −20 °C and −80 °C for predetermined periods.

Results: MPO levels ranged from 16 to 69 ng/ml and were higher in patients with heart disease compared to healthy volunteers (35.0 vs. 24.9 ng/ml; $P=0.03$). There were no statistical differences between MPO-Hep, MPO-Gel and MPO-Serum ($P>0.05$), and MPO-Hep showed a good correlation with MPO-Gel and MPO-Serum ($r=0.775$ and $r=0.792$; $P<0.001$). No correlation between MPO-Hep and MPO-EDTA was found ($r=0.21$; $P=0.20$), and mean MPO-EDTA value was 1.8 times higher than MPO-Hep (51.4 vs. 28.7 ng/ml; $P<0.001$). Mean differences (ng/ml) between MPO-Hep and MPO-EDTA, MPO-Gel and MPO-Serum were 24.7 (19.5–30.0), 0.225 (−2.54–2.99), and 1.55 (−1.16–4.26), respectively.

Conclusions: EDTA had a significant effect on MPO results. MPO-Hep values correlate well with MPO-Gel and MPO-Serum. MPO levels seem to be stably frozen at −20 °C or −80 °C over 6 months depending on preanalytical handling.

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1. Introduction

Inflammation plays an important role in all stages of atherosclerotic plaque development, from initial dysfunction to formation of atheroma, plaque rupture and the resulting thrombotic complications [1–3]. Different inflammatory biomarkers have been studied [4]. Rupture and erosion of plaques with formation of an intramural thrombus are the most important morphological changes in the transformation of stable coronary lesions into clinically unstable ones. Lymphocytes and monocytes seem to make an important contribution to the pathophysiology of cardiovascular disease, in particular through the generation of proinflammatory cytokines. However, polymorphonuclear neutrophils (PMNs) may also be involved, and may modulate and signal inflammatory pathways by secreting enzymes, such as myeloperoxidase, that interact with target organs [5].

Myeloperoxidase (MPO), the most abundant component of azurophilic granules of leukocytes, is released after activation and degranulation of leukocytes, contributes to innate immune defenses, and has been shown to participate in the promotion and propagation of atherosclerosis [3]. MPO generates free radicals and diffusible oxidants, and can, therefore, oxidize LDL cholesterol and promote uptake by macrophages and foam cell formation [6,7]. It may also cause oxidative changes in HDL and impair reverse transport of cholesterol [8]. MPO activates metalloproteinases and promotes destabilization and rupture of the atherosclerotic plaque [9,10]; it catalytically consumes nitric oxide (NO) derived from endothelium, reduces its bioavailability and affects its vasodilator and anti-inflammatory functions [11,12]; and may also contribute to adverse ventricular remodeling after infarction [12,13].

Several studies have demonstrated the prognostic association of high levels of MPO and adverse cardiovascular events in healthy individuals [14], patients with chronic heart disease [15] or acute heart disease [16–18], patients being examined because of chest pain [19], and patients with heart failure [20,21]. However, its clinical use depends on further studies to define accurate and reproducible analytical methods. Moreover, preanalytical factors, such as sample type, handling and storage, must be fully evaluated [22]. The preanalytical sources of MPO

Abbreviations: PMNs, polymorphonuclear neutrophils; MPO, myeloperoxidase; NO, nitric oxide; CRP, C-reactive protein; hs, high sensitivity; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

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variability are still unknown, but the quality of assay results and correct clinical management depend on their accurate definition. This study evaluated the effect of different sample types and storage conditions on MPO measurements.

2. Materials and methods

2.1. Samples

The Clinical Laboratory Standard Institute (CLSI) protocol was used for method validation (CLSI EP9-A Protocol: Method comparison and bias estimation using patient samples. Approved Guideline, 1995. Clinical Laboratory Standard Institute, Wayne. PA). Blood samples were collected from 40 volunteers (17 men) aged 21 to 75 years. Twenty-five participants were healthy and 15 had cardiac disease. Eight patients had stable coronary artery disease, 3 had acute coronary syndrome, and 4 had heart failure. All participants answered a questionnaire about clinical information and signed an informed consent form. This study was conducted at the Hospital de Clínicas de Porto Alegre, RS, Brazil, and was approved by its Committee on Research Ethics.

2.2. Blood collection and sample storage

The study design is illustrated schematically in Fig. 1. Four blood samples were obtained from each study participant (Vacuette® tubes Greiner Bio-one), and one specimen was prepared from each sample: heparinized plasma (coated tube with sodium heparin; MPO-Hep), EDTA plasma (coated tube with EDTAK3; MPO-EDTA), serum (coated tube with micronized silica particles, to activate clotting; MPO-Serum), and serum with separator gel (coated tube with micronized silica particles and a barrier gel; MPO-Gel).

Immediately after sample collection, tubes were mixed gently to homogenize the material and were immediately kept on ice bath for no longer than 30 min until centrifugation at 2000 *g* for 10 min. After centrifugation, serum (with and without gel) and plasma (heparinized and with EDTA) specimens were divided into aliquots to be used in the study procedures.

To compare MPO results for the different tube preparations, the following specimens were taken from the samples from each volunteer: aliquots of plasma were obtained from the tubes with heparin and EDTA, and aliquots of serum, from tubes with and without gel. All these specimens were stored in a freezer at −20 °C for 36 h until measurement.

To evaluate the stability of MPO according to different storage periods and temperatures, aliquots of heparinized plasma, which is the sample of choice according to the manufacturer of the kit, were separated (n = 40) and stored at room temperature (18–25 °C) for 3 days; in a refrigerator (2–8 °C) for 3 and 7 days; in a freezer at −20 °C for 7, 30, 90 and 180 days and in a freezer at −80 °C for 30, 90 and 180 days. The measurement at 36 h after collection was considered the baseline for this stability study. The choice of this point was based on bench workflow feasibility and the manufacturer's instructions. For the analytical imprecision studies, 20 aliquots of MPO-Hep were obtained from a single sample stored at −20 °C and measured on the same day (intraassay CV) and 20 aliquots from a single sample were stored at −80 °C and measured on different days (interassay CV) throughout the assaying period,

2.3. Sample measurement and methodology

MPO level was measured using the ELISA method with a Bioxytech® MPO-EIA™ kit (OXIS Health Products, Inc. USA). A standard MPO solution (50 ng/ml) was poured on each plate to plot a 6-point standard curve by serial dilutions. All samples were diluted at a 1:10 ratio, with the sample diluting buffer, according to the manufacturer's instructions, so that all concentrations were within the method's range of linearity (1.56–25 ng/ml). Absorption was read at 405 nm using an ETI-Max 3000 DiaSorin analyzer.

2.4. Statistical analysis

Continuous variables are presented as mean ± SD, and categorical variables, as absolute numbers and percentages. The MPO levels had a normal distribution. Differences between means were evaluated using the Student *t* test for continuous variables and the χ^2 for categorical variables. Correlations between sample types were evaluated using the Pearson correlation coefficient, and mean differences were analyzed using the Bland–Altman method. Sample stability under different storage conditions was evaluated using repeated-measures ANOVA and intraclass correlation coefficients. Data were analyzed using the statistical program SPSS 14.0, and differences were classified as statistically significant at $P < 0.05$.

3. Results

Characteristics of individuals participating in the study are shown in Table 1. Mean age was 45 (21–75) years. MPO levels ranged from 16 to 69 ng/ml (mean: 28.7 ng/ml), with no difference between men and

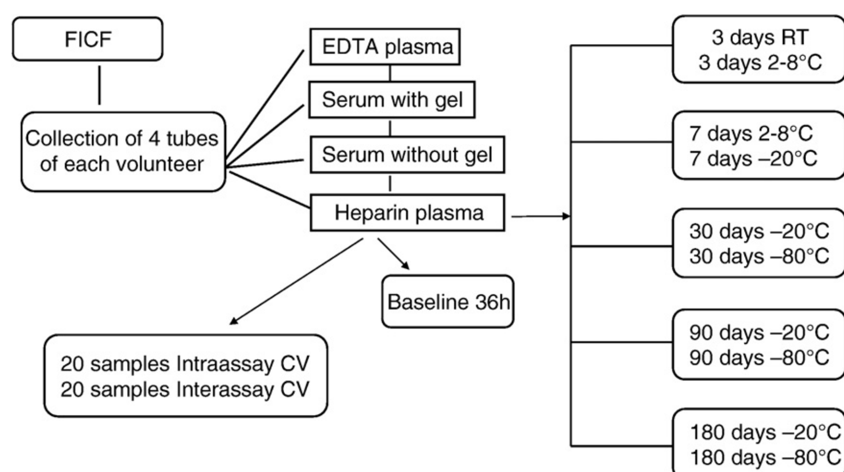

Fig. 1. Study design. FICF: free and informed consent form; RT: room temperature; CV: coefficient of variation; EDTA: ethylenediaminetetraacetic acid.

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