Comparison of Paraoxonase 1 Measurements in Serum and in Lithium-Heparin-Anticoagulated Plasma Samples

To the Editor:
Paraoxonase 1 (PON1) is a HDL-associated enzyme that catalyzes the hydrolysis of lipid peroxides in LDL and HDL and has been postulated as a member of the plasma antioxidant system. Decreased PON1 activity has been associated with atherosclerosis in persons with diabetes mellitus, familial hypercholesterolemia, and renal disease (1, 2).

Serum is the preferred sample for PON1 measurement because this enzyme requires calcium for both activity and stability. The presence of calcium chelators such as EDTA or citrate as anticoagulants inhibits PON1 activity (3). This is a serious limitation in retrospective studies, in which serum is not always available. Moreover, in studies on experimental animals, in which the amount of blood collected is often minimal, it is more convenient to use anticoagulants because the recovery of plasma is generally higher than that of serum and there is no interference by the clotting process.

Lithium heparin is an anticoagulant used extensively in laboratories around the world. Although it has been reported that lithium inhibits PON1 activity (4), several groups have reported studies on PON1 activity in lithium-heparin-treated samples, and the results obtained were consistent with those obtained in serum (5, 6). However, to the best of our knowledge, the reliability of lithium-heparin plasma samples has not been clearly demonstrated. The present study was designed to investigate the degree of agreement between measurements of PON1 activity and concentration in serum and in lithium-heparin-anticoagulated plasma samples.

We used samples from 100 consecutive patients attending the outpatient facility of Hospital Universitari de Sant Joan for routine biochemical analysis. Blood was collected into two different tubes: BD Vacutainer® (Becton Dickinson) with serum separator (SST™ II Plus, 13 × 75 mm), and BD Vacutainer with lithium heparin (LH 68 IU Plus, 13 × 75 mm). After the requested conventional tests were performed, the remaining portions were stored at −80 °C for PON1 measurements. The use of sample leftovers for methodologic assessments is in agreement with the European Law for Medical and Diagnostic Products. PON1 activity and concentration were measured as described previously (7, 8).

Because neither PON1 activity nor concentration followed a gaussian distribution, we analyzed differences between groups by Wilcoxon rank-sum test. The results are reported as medians and 95% confidence intervals (95% CIs). The associations between measurements in serum and plasma were analyzed by Deming regression (9). The degree of agreement between both types of samples was estimated by the Bland–Altman procedure (10).

The measured PON1 activity and concentration were slightly but significantly (P < 0.001) lower in lithium-heparin samples than in serum values [plasma PON1 activity, 211.9 U/L (95% CI, 85.1–716.6 U/L); serum PON1 activity, 221.3 U/L (95% CI, 102.6–721.7 U/L); plasma PON1 concentration, 72.6 mg/L (95% CI, 27.9–186.4 mg/L); serum PON1 concentration, 81.4 mg/L (95% CI, 31.6–188.0 mg/L)]. Deming regression analysis gave the following results for serum (x) vs plasma (y; values in parentheses are the SD): PON1 activity, \( y = 0.98 (0.014)x - 12.88 (3.62) \) U/L (\( r = 0.995 \)); PON1 concentrations, \( y = 0.86 (0.021)x + 0.27 (3.82) \) mg/L (\( r = 0.956 \)). Bland–Altman plots showing the degree of agreement between both measurements are shown in Fig. 1. The absolute mean (SD) differences (plasma vs serum) were −19.5 (18.7) U/L for PON1 activities (Fig. 1A), and −11.1 (17.2) mg/L for PON1 concentrations (Fig. 1B). The mean (SD) percentage variations were −7.8 (9.7)% for PON1

Fig. 1. Bland–Altman plots for PON1 measurements in serum and lithium-heparin plasma. The dashed lines represent 2 SD.
activities and −11.9 (16.1)% for PON1 concentrations.

We found a good association between PON1 measurements in serum and in lithium-heparin plasma, although there was some underestimation when plasma samples were used. Differences in analyte values for plasma and serum samples are common. Often, values measured in plasma are lower than those observed in serum because the fibrin clot retains some water and the serum becomes more concentrated (11). For most biochemical analytes, the variation ranges between 1% and 5% (12). We observed similar differences for PON1 activity and somewhat higher variations for PON1 concentration, but in both cases the variations were minor and, for practical purposes, negligible.

We conclude that lithium-heparin plasma samples may be an acceptable alternative for the study of PON1 because the effect of lithium heparin on PON1 measurements is relatively small. However, because the results are not completely equivalent, care should be taken when comparing data obtained for both types of samples, and serum and plasma should not be used together in the same study.

This study was funded by the Red de Centros de Metabolismo y Nutrición (RCMN C03/08) from the Instituto de Salud Carlos III, Madrid, Spain. J.M. is the recipient of a grant from the Generalitat de Catalunya (FI 05/00068). We thank Alberto Amelijide for help with the statistical analysis.

References

Natalia Ferré1 Jordi Camps1* Judith Marsillich1 Bharti Mackness2 Mike Mackness2 Blai Coll1 Mònica Tous1 Jorge Joven1

1 Centre de Recerca Biomèdica Hospital Universitari de Sant Joan Institut de Recerca en Ciències de la Salut Reus, Catalunya, Spain
2 University Department of Medicine Manchester Royal Infirmary Manchester, United Kingdom

Placental mRNA in Maternal Plasma and Its Clinical Application to the Evaluation of Placental Status in a Pregnant Woman with Placenta Previa-Percreta

To the Editor:
Fetal and/or placental mRNA in maternal plasma has been detected during pregnancy, and such mRNA tends to be stable against degradation (1). A quantitative study of plasma mRNA for γ-globin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) showed significantly higher concentrations in pregnant women than in nonpregnant women (2). These findings suggest that the quantitative analysis of placental mRNA in maternal plasma may be a useful method to monitor placental status. In this study, we measured placental mRNA in maternal plasma to evaluate residual placenta in a pregnant woman with placenta previa-percreta (PPP) and bladder invasion that was diagnosed by both magnetic resonance imaging and pathology examination. Although a supravaginal hysterectomy just after the cesarean section (the first surgery) was done at 37 weeks of gestation, a 16-cm placental mass close to the internal os of the uterus could not be removed. Therapy with methotrexate (MTX) was therefore initiated on days 1–4, days 14–17, and days 33–36 after the first surgery (on day 0) to aid resorption of the placental residue, and a second surgery was performed to complete the hysterectomy on day 75. The study protocol was approved by the Committee for the Ethical Issues on Human Genome and Gene Analysis in Nagasaki University, and written informed consent was obtained. Data regarding plasma mRNA concentrations did not influence clinical management.

Blood samples (10 mL) were collected at intervals and before and after the surgeries. Plasma mRNA was extracted as described by Ng et al. (1). Human chorionic gonadotropin (hCG) and human placental lactogen (hPL) were selected as representative placental mRNAs, and GAPDH mRNA was measured as a reference. The mRNA concentrations were determined using standard curves. The detection limit was 2.5 × 10−3 copies/μL for hCG, 1.5 × 10−2 copies/μL for hPL, and 1.2 × 10−3 copies/μL for GAPDH.

Placenta previa-percreta was confirmed at birth, and postpartum evaluation showed a severe placental lesion. The hCG and hPL mRNAs were not detected and the GAPDH mRNA level was 3% of the normal range (10%). Transvaginal ultrasonography was performed in the third trimester of pregnancy, and a significant increase in the placental volume was observed. Although there was a significant increase in the placental volume, the hCG and hPL mRNAs were not detected and the GAPDH mRNA concentration was within the normal range (10%). The postpartum evaluation showed a severe placental lesion and no evidence of placenta previa-percreta (PPP). The hCG and hPL mRNAs were not detected and the GAPDH mRNA concentration was within the normal range (10%). The postpartum evaluation showed a severe placental lesion and no evidence of placenta previa-percreta (PPP).

DOI: 10.1373/clinchem.2005.048231

*Address correspondence to this author at: Centre de Recerca Biomèdica, Hospital Universitari de Sant Joan, Institut de Recerca en Ciències de la Salut, C/. Sant Joan s/n, 43201-Reus, Catalunya, Spain. Fax 34-977-312569; e-mail jcampes@grupasagesa.com.