Comparison of 3 Third-Generation Assays for Bio-intact Parathyroid Hormone

To the Editor:

Current second-generation parathyroid hormone (PTH) assays suffer from interference by circulating PTH fragments. Newer assays that measure bio-intact PTH, i.e., the biologically active whole PTH molecule (1–84 PTH), have overcome this problem (1, 2). Our study was designed to evaluate and compare 3 commercial third-generation assays based on a chemiluminescence immunoassay, ELISA, and IRMA for bio-intact PTH and to compare these with a second-generation PTH assay.

EDTA-plasma samples were collected from 40 healthy blood donors (age range, 18–67 years; mean age, 39 years) and from 26 patients with predialysis chronic kidney disease (age range, 28–86 years; mean age, 65 years). Bio-intact PTH was measured by (a) a chemiluminescence immunoassay on the Nichols Advantage® platform (Nichols Institute Diagnostics) (2); (b) an IRMA using the Duo PTH reagents (Scantibodies Laboratory) (1); and (c) an ELISA using the Human BioActive Intact PTH reagents (Immutopics), which is a 2-site assay in which 2 goat polyclonal antibodies recognize epitopes within the midregion/carboxy terminus and at the initial amino terminus. For comparison, we performed

![Fig. 1. Regression analysis for comparison of the 3 bio-intact PTH assays [IRMA (A), chemiluminescence immunoassay (CLIA; B), and ELISA (C)] vs the second-generation Elecsys assay, and for the comparison between the IRMA and the chemiluminescence immunoassay (D).](image-url)

The data are for the healthy individuals (n=40). The regression analysis equations are as follows: (A), y = 0.40x + 3.4 ng/L (r = 0.90; P < 0.001); (B), y = 0.66x + 1.1 ng/L (r = 0.96; P < 0.001); (C), y = −0.44x + 66 ng/L (r = −0.10; P, not significant); (D), y = 0.62x + 2.5 ng/L (r = 0.95; P < 0.001).
a second-generation electrochemiluminescence PTH assay on the Elecsys 2010 platform (Roche Diagnostics). Ionized calcium and 25-hydroxyvitamin D (Nichols Advantage) were also measured in all samples.

Results for the healthy individuals are presented in Fig. 1. The bio-intact PTH values measured by the IRMA and chemiluminescence immunoassay were, on average, 50% and 30% lower, respectively, than the PTH measured by the Elecsys. Regression analysis demonstrated significantly different slopes (P < 0.001), but not intercepts, for both the IRMA and chemiluminescence immunoassay compared with the Elecsys assay. Most striking was the discordance between the ELISA and Elecsys, and the Elecsys assay. Comparison between the ELISA and Elecsys revealed a significantly different intercept (P < 0.001). The results obtained with the third-generation IRMA and the chemiluminescence immunoassay also differed, despite the good correlation, and regression analysis demonstrated a significant difference for the slope (P < 0.001) but not for the intercept. The finding of higher results with the chemiluminescence immunoassay concurs with the results reported by Boudou et al. (3), but immunoreactivity does not seem to be implicated in these differences (1–3). In addition to the apparent differences in assay design, other possible reasons for the lack of agreement between the assays investigated could be matrix effects and/or differences in standardization. The problems hampering immunoassay standardization are many and vary for different analytes, as has been reviewed by Stenman (4). Thus, the use of independent reference intervals for each of the bio-intact PTH assays is clinically important.

We observed no significant differences between healthy individuals and patients with predialysis chronic kidney disease for 25-hydroxyvitamin D, but mean concentrations of bio-intact PTH (by chemiluminescence immunoassay; P <0.001) and ionized calcium (P <0.05) were higher in the patients. PTH measured by Elecsys was, on average, 75% higher than the measured bio-intact PTH in patients, consistent with the expected presence of high concentrations of circulating PTH fragments in patients with impaired renal function. In addition, the distribution of bio-intact PTH analyzed by the chemiluminescence immunoassay (mean, 71 ng/L; range, 5–227 ng/L) was wider in the patient group than in the healthy individuals (23 [11–50] ng/L).

Despite our strict adherence to the ELISA protocol and 3 independent runs with the same set of samples, this assay generated inconsistent results, which require additional methodologic studies to fully explain. We conclude that although the third-generation bio-intact PTH ELISA requires further evaluation, the chemiluminescence immunoassay and IRMA are comparable.

References

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Adsorption of Mycophenolic Acid and Its Phenolic Glucuronide to Glass, Polystyrene, and Polypropylene Containers

To the Editor:

Drugs in solution may be adsorbed to the surface of containers and thus be removed from solution. We studied the loss of mycophenolic acid (MPA) and its major metabolite mycophenolic acid glucuronide (MPAG) from glass, polystyrene, and polypropylene containers based on solvent and time of contact.

MPA was from Hoffmann-La Roche and MPAG from Analytical Services International Ltd. Acetonitrile (Uvasol; purity >99.8%), methanol (Uvasol; purity >99.8%), and orthophosphoric acid (85% Suprapur; Merck) were HPLC grade. The NaCl solution (9 g/L, pH 7.4) was from Fresenius Kabi. Stock solutions of MPA, MPAG, and MPA/MPAG were prepared in acetonitrile–water (80:20 by volume) and stored in poly-styrene tubes at −80 °C. The chromatographic analysis was performed as described previously (1), with a minor gradient modification.

Studies were performed on ice, which stabilizes drugs for at least 8 h (2). The MPA/MPAG stock solution was diluted with acetonitrile, methanol, or isotonic NaCl in five 6-mL glass (VSM; 69% SiO2, 13% Na2O), polystyrene (Elvetroc), or polypropylene (Sarstedt) tubes to obtain MPA and MPAG concentrations of 0.5 and 5 mg/L, respectively. The solutions were vortex-mixed and then transferred to another tube of