standard addition method and prepare enriched serum calibrators. Another advantage of the serum-based calibrators is that the yields of derivatized MMA and d3-MMA, i.e., the peak heights, are consistently high. During method development, we chose a serum sample volume of 500 µL per SPE column, based on a previously published method (12). Preliminary experiments have shown that the quality of the MMA analysis, in terms of recovery, precision, and LOQ, would be unaffected or even improved by use of 150 µL of serum instead. With the smaller sample volume, the ion suppression almost disappears (signal, 91%–100% of the expected value).

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References

Unexpected Serum Parathyroid Hormone Profiles in Some Patients with Primary Hyperparathyroidism, Philippe Boudou, Fidaa Ibrahim, Catherine Cournier, Emile Sarfati, and Jean-Claude Souberbielle (Departments of Hormonal Biology and Endocrine Surgery, Hopital Saint-Louis, Paris, France; Department of Rheumatology, Hopital Cochin, Paris, France; Physiology Laboratory, Hopital Necker-Enfants Malades, Assistance Publique, Hopitaux de Paris, Paris, France; INSERM U 671, Paris, France; * address correspondence to this author at: Department of Hormonal Biology, Saint-Louis University Hospital and INSERM U 671, 1 avenue Claude Vellefaux, 75475 Paris cedex 10, France; fax 33-1-42-49-42-80, e-mail philippe.boudou@sls.aphp.fr)

Background: Third-generation parathyroid hormone (PTH) assays have been reported to measure only intact PTH(1–84), in contrast to second-generation assays, which also detect PTH(7–84) fragments. Higher PTH measurements were observed with third- than with second-generation PTH assays in a few patients with either severe primary hyperparathyroidism or parathyroid carcinoma.

Methods: We analyzed biological data [second- and third-generation PTH assays, 25-hydroxyvitamin D (25-OHD), calcium, and phosphate concentrations] obtained before and after surgery for 2 groups of patients selected from a large series of consecutive patients with primary hyperparathyroidism (PHPT): 7 female patients with surgically and histologically confirmed PHPT (group 1) and a matched group (group 2).

Results: For group 1 but not group 2, PTH concentrations measured by third-generation PTH assays before surgery were higher than those measured by the second-generation assays. Circulating 25-OHD, calcium, and phosphate concentrations were similar in both groups. In addition, PTH values measured with the third-generation PTH assays in group 1 decreased after surgery. Conclusions: Our results confirm that third-generation PTH assays do not measure only PTH(1–84). The frequency of this unexpected finding of markedly lower PTH concentrations than previously reported was ~5% in patients with PHPT without malignancy. We do not know whether the presence of this unexpected profile is predictive of malignancy.

Second-generation parathyroid hormone (PTH) assays, also termed intact PTH assays, measure not only PTH(1–84) but also a PTH fragment that resembles PTH(7–84) (1). To date, 2 new commercial third-generation assays have been developed: the Cyclase Activating PTH IRMA (Scantibodies Laboratory, Inc.) and the Nichols Advantage® chemiluminescent Bio-Intact PTH assay (Nichols Institute Diagnostics). These assays do not cross-react with N-terminally truncated PTH fragments, including
PTH(7–84) (2, 3). In accordance with these observations, PTH concentrations were lower when measured with third-generation than with second-generation assays in healthy individuals (4–6), in patients with primary hyperparathyroidism (PHPT) (4, 6, 7), and in dialysis patients (8). However, 2 recent reports described PTH concentrations that were higher when measured with third- than with second-generation assays in 3 patients with parathyroid carcinoma (9) and in 1 patient with severe PHPT (10). In this report, we present a similar PTH profile in 7 women with PHPT [group 1; mean (SD) age, 57.2 (13.5) years; range, 37–74 years] whose PTH concentrations were evaluated simultaneously with both third- and second-generation PTH assays. These patients were from a group of 145 consecutive PHPT patients [28 men and 117 women; mean age, 61.7 (13.2) years], living in Paris and its suburbs (4), who underwent surgery in our Endocrine Surgery Department during a 10-month period. A group of 74 healthy persons (14 men and 60 women) from the same area [age, 62.2 (4.6) years] served as the control group for determination of the reference interval for each PTH assay tested. All of the control individuals had a serum 25-hydroxyvitamin D (25-OHD) concentration >50 nmol/L. This investigation was approved by the local ethics committee and was conducted in accordance with the guidelines published in the Helsinki Declaration. Informed consent was obtained from all participants.

Blood samples were collected after an overnight fast, just before the patients entered the operating room, and after surgery. Samples were centrifuged at 4 °C, and serum aliquots were promptly frozen at −80 °C. The length of this procedure never exceeded 2 h because the stability of PTH measured with the third-generation assays is shorter than that measured with second-generation assays (2, 5). We used standard methods to measure calcium, phosphate, albumin, and creatinine and RIA (DiaSorin) to measure serum 25-OHD concentrations in PHPT patients and in the control group. Serum PTH was measured with 4 different assays, based on 2-site immunometric methods: the Duo PTH IRMA (Scantibodies Laboratory, Inc.), which includes 2 different assays—the total intact and the cyclase-activating PTH assays; and 2 immunochemiluminometric assays from Nichols Institute Diagnostics, the Nichols Advantage® chemiluminescent intact and biointact PTH assays. The total intact PTH (T-iPTH) and intact PTH (iPTH) assays are second-generation assays, whereas the cyclase-activating PTH (CA-PTH) and Bio-Intact PTH (Bio-iPTH) assays are third-generation assays. Briefly, both second- and third-generation PTH assays use purified goat polyclonal antibodies. Two types of antibodies were used. Capture antibodies, common in both generations of assays, were directed against the PTH(39–84) region and differed in their interaction with the solid phase: antibodies immobilized on polystyrene-coated beads (Scantibodies) or biotinylated antibodies reacting with streptavidin-coated particles (Nichols). Detection antibodies, specific to each generation, were directed either against the PTH(1–34) region in second-generation assays, or against the PTH(1–6) region in third-generation assays. These antibodies were labeled with either iodine (Scantibodies) or acridinium ester (Nichols). In each case, assays were run according to the manufacturer’s protocol, and analytical characteristics for these PTH assays have been reported previously (2, 5, 6, 11).

Our normative serum PTH concentrations [95% confidence interval (95% CI)] were 10–46, 11–60, 8.4–34, and 9–41 ng/L when measured by the Scantibodies T-iPTH, Nichols iPTH, Scantibodies CA-PTH, and Nichols Bio-iPTH assays, respectively. Otherwise, the upper limits of the reference interval (95% percentile of the reference population) proposed by Scantibodies were 66 and 39 ng/L for the T-iPTH and CA-PHT assays, respectively, whereas those suggested by Nichols were 66 and 50 ng/L for the iPTH and Bio-iPTH assays, respectively. Individual data on these 7 patients (group 1) are shown in Table 1. T-iPTH [median (range), 62.7 (56.0–164.8) ng/L] and iPTH [94.0 (64.0–188.0) ng/L] concentrations were associated with higher CA-PTH/T-iPTH ratios [median (range), 1.50 (0.85–2.18)] and Bio-iPT/iPTH ratios [1.25 (0.90–2.63)]. Serum PTH concentrations measured before surgery in group 1 by both the CA-PTH and Bio-iPTH assays (patients 1–5) or by only 1 assay (patients 6 and 7) were higher than those measured by the T-iPTH and iPTH assays. In 4 patients, blood samples were available either 2 days (patients 2, 3, and 5) or 1 month (patient 4) after surgery, and results showed a decrease in serum PTH concentrations measured by both generations of assays (see Table 1). At that time, PTH concentrations measured by third-generation assays were lower than those measured by the second-generation assays.

To better analyze these observations, we selected from the 138 PHPT patients a group of 7 PHPT patients matched by age [mean (SD), 57.1 (11.6) years], sex, T-iPTH value [median (range), 67.5 (44.0–159.0) ng/L], and iPTH value [median (range), 85.0 (68.0–186.0) ng/L], but with lower CA-PTH/T-iPTH ratios [median (range), 0.63 (0.50–0.72)] and Bio-iPTH/iPTH ratios [0.54 (0.50–0.74)]. We designated these patients as group 2.

The third-generation assays have been reported to measure only the full-length PTH(1–84) (2, 3), unlike the second-generation assays, which cross-react with PTH(7–84). In keeping with this characteristic, the mean PTH concentrations measured by the third-generation assays were between 62% and 73% of the mean PTH concentrations measured by the second-generation assays in healthy controls (4–6) as well as in PHPT patients (4, 6, 7), whereas the PTH concentrations measured by the CA-PTH and/or the Bio-iPTH assay in the 7 PHPT patients in group 1 before surgery were higher than those measured by the T-iPTH and/or the iPTH assay. Because this finding was noted with the assays from both companies, these unexpected data did not seem to be caused by an analytical pitfall, but instead they provide evidence that the third-generation assays recognize a molecule with similarities to PTH(1–84) that is not recognized by the
second-generation assays. Our observation is similar to those reported in 3 patients with parathyroid carcinoma and very high serum PTH concentrations (9) and in 1 patient with severe PHPT in whom a parathyroid carcinoma was not histologically excluded (10). In contrast to these patients, our patients were not affected with severe disease and had no histologic indications of malignancy, such as a palpable neck mass. In the group 1 patients, PTH values decreased after surgery and were then lower when measured with the third-generation than with the second-generation PTH assays regardless of the assay used, as described previously (10). Our data and those reported previously (9, 10) are consistent with the presence of a PTH molecule in some patients that is produced in excessive amounts by a parathyroid adenoma or carcinoma and regresses after parathyroidectomy, as shown in our study and by Räkel et al. (10), or persists for the duration of a 12-month treatment with a calcimimetic agent (9). D’Amour et al. (12) used HPLC to identify a new PTH form, amino PTH (N-PTH), which is distinct from PTH(1–84) and is overproduced in primary and secondary hyperparathyroidism. N-PTH is recognized by the CA-PTH assay, suggesting that amino acid residues 1–6 of PTH are present (12), but it is not recognized by the T-iPTH assay, suggesting that unlike PTH(1–84), N-PTH is modified in the region of amino acid residues 12–18 (10, 12). The authors hypothesized that N-PTH might be a PTH(1–84) variant that is phosphorylated on serine-17 (12). Whether N-PTH can bind to the PTH receptor and have biological actions is still unknown.

Analysis with the Mann–Whitney U-test indicated that circulating concentrations of 25-OHD [median (range), 30.5 (18.75–46.25) nmol/L in group 1 vs 31.25 (16.25–45.00) nmol/L in group 2 (P = 0.83)], calcium [2.91 (2.56–3.44) mmol/L in group 1 vs 2.75 (2.50–3.00) mmol/L in group 2 (P = 0.34)], and phosphate [0.81 (0.60–1.16) mmol/L in group 1 vs 0.97 (0.55–1.30) mmol/L in group 2 (P = 0.75)] were similar in both groups of patients already diagnosed with PHPT (4), suggesting that overproduction of N-PTH is not particularly associated with secondary HPT resulting from vitamin D deficiency and that N-PTH may have an activity similar to that of PTH(1–84).

More precise indexes of PTH action not evaluated in our study, such as ionized calcium, maximal renal tubular reabsorption of phosphate (TmPi)/glomerular filtration rate; 1,25-dihydroxyvitamin D, nephrogenic cAMP, or bone turnover markers, would likely provide better information on the biological activity of N-PTH. It should be noted that with all of the assay methods that we used, our 7 patients (group 1) had serum PTH values above the reference interval established in healthy persons with a serum 25-OHD concentration >50 nmol/L. In contrast, before surgery, T-iPTH and iPTH concentrations were below the upper limits of the reference intervals proposed by each company in 4 patients and 1 patient, respectively, whereas the third-generation assays measured increased PTH concentrations. According to the presurgery evaluation, 3 of these 4 patients were hypercalcemic; thus, their second-generation PTH results, although within the reference interval, were clearly not consistent with their measured calcium concentrations, a finding that supports the diagnosis of PHPT. In contrast, although patient 6 was repeatedly found to be hypercalcemic at diagnosis, our
presurgery evaluation showed that this patient had a calcium concentration within the reference interval. With these findings the diagnosis of PHPT might have been missed in a patient in whom the diagnosis was not yet established. The T-iPTH and iPTH concentrations were within the manufacturers’ reference intervals.

The present report confirms that third-generation PTH assays do not measure only PTH(1–84). In our study, although close to 5% of PTH concentrations were higher when measured with third- than with second-generation assays, this did not influence the diagnosis of PHPT in the large series of consecutive patients with surgically and histologically confirmed PHPT without any feature of malignancy and with markedly lower PTH concentrations than those reported previously. Further studies are needed to evaluate whether the presence of this unexpected PTH profile is predictive of malignancy.

References


Analytical Performance and Diagnostic Accuracy of Immunometric Assays for the Measurement of Circulating Oxidized LDL, Paul Holvoet, Elizabeth Macy, Michele Landeeloo, Dan Jones, Jenny S. Nancy, Frans Van de Werf, and Russell P. Tracy (1 Department of Cardiovascular Diseases, Katholieke Universiteit Leuven, Leuven, Belgium; 2 Laboratory for Clinical Biochemistry Research and Department of Pathology, University of Vermont College of Medicine, Burlington, VT; 3 address correspondence to this author at: Atherosclerosis and Metabolism Unit, Department of Cardiovascular Diseases, Katholieke Universiteit Leuven, Herestraat 49, PB 705, B-3000 Leuven, Belgium; fax 32-16-347114, e-mail paul.holvoet@med.kuleuven.be)

Background: Oxidized LDL (ox-LDL) plays an important role in the pathogenesis of coronary heart disease (CHD). Several tests for circulating ox-LDL have been published. We believe it is critical to carefully evaluate these assays because small differences in performance may have profound effects when results are compared; we therefore compared the analytical and clinical performances of 2 assays: one developed in our laboratory and a commercial assay (Mercodia) that uses the same monoclonal antibody (4E6).

Methods: We determined the variance of ox-LDL in both tests, including its longitudinal stability (n = 225; 3 time points per person) and its diagnostic accuracy, by ROC analysis of 95 consecutive CHD patients and 20 controls.

Results: The between-person variability was 77% for the in-house assay (with the remaining 23% being within-person and analytical variance) and 74% for the commercial assay. For comparison, previously reported values were 66% for high-sensitivity C-reactive protein and 82% for total cholesterol. The areas under the curves for CHD in the 2 assays were identical (0.85). The odds ratios (logistic regression) for CHD among persons with high ox-LDL (≥15 mg/L) compared with persons with low ox-LDL were not different: 4.3 (95% confidence interval, 1.4–12) for the in-house assay and 3.3 (1.1–10) for the commercial assay.

Conclusions: The longitudinal stability of ox-LDL, as assessed by multiple measures in people over time, is similar to that of total cholesterol and high-sensitivity C-reactive protein. Both assays tested similarly distinguish between healthy controls and CHD patients.

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Oxidized LDL (ox-LDL) plays an important role in the pathogenesis of atherosclerosis (1–3). We and others have demonstrated that subclinical atherosclerosis (4) and clinical coronary heart disease (CHD) (5–9) are associated with higher concentrations of circulating ox-LDL. Recently, we showed, in older adults in the Health, Aging, and Body Composition (Health ABC) cohort (10), that high CHD risk status (based on Framingham score) before

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