Etienne Cavalier*, Pierre Delanaye, Pierre Lukas, Agnes Carlisi, Romy Gadisseur and Jean-Claude Souberbielle

Standardization of DiaSorin and Roche automated third generation PTH assays with an International Standard: impact on clinical populations

Abstract

Background: Standardization of parathyroid hormone (PTH) assays is a major issue, especially in hemodialyzed (HD) patients. Two automated third generation PTH assays (Roche Elecsys and DiaSorin Liaison) are now available. These assays are specific for the (1-84) PTH and do not cross-react with the (7-84) fragment, contrary to second generation (intact) assays. We aimed to calibrate the two methods against the WHO International PTH Standard (IS) 95/646 to see if the two assays could provide comparable results in a population of healthy subjects, HD patients and patients suffering from primary hyperparathyroidism (PHP).

Methods: We selected 79 healthy subjects and two populations of patients presenting PTH disorders: 56 HD and 27 PHP patients. We reconstituted the IS in a pool of human serum containing undetectable levels of 1-84 PTH and prepared 13 serum standards ranging from 0 to 2000 pg/mL. The standards were run on the two instruments to calibrate the assays on the IS. The different populations were run before and after restandardization.

Results: As these kits were differently calibrated, the results obtained after restandarization were significantly different. Restandardization process improved concordance between assays and, taking the analytical variability of the two kits into account, the results could be considered to be similar.

Conclusions: Restandardization of automated third generation PTH assays with the WHO 1-84 PTH Standard significantly reduces inter-method variability. Reference ranges and raw values are totally transposable from one method to the other in healthy subjects, but also in diseased patients, e.g., with HD or those suffering from PHP.

Keywords: chronic kidney disease; International Standard; parathormone; standardization.

***Corresponding author: Dr. Etienne Cavalier,** Service de Chimie Médicale, CHU de Liège, Domaine Universitaire du Sart-Tilman, 4000, Liège, Belgium, Phone: +32 43667692, Fax: +32 43667691, E-mail: Etienne.cavalier@chu.ulg.ac.be

Etienne Cavalier: Department of Clinical Chemistry, University of Liege, CHU Sart-Tilman, Liege, Belgium

Pierre Delanaye, Pierre Lukas, Agnes Carlisi and Romy Gadisseur: Department of Nephrology, Dialysis and Transplantation, University of Liege, CHU Sart-Tilman, Liege, Belgium

Jean-Claude Souberbielle: Laboratoire d'Explorations Fonctionnelles, Hôpital Necker-Enfants malades, Paris, France

Introduction

Parathyroid hormone (PTH) determination is not an easy task [1]. Indeed, next to the active, (1-84) peptide, different fragments can be found at different concentrations in the circulation. Among these fragments, the "non-(1-84) PTH", commonly called (7-84) PTH, is a group of fragments truncated in the amino-terminal part of the peptide, that can either be obtained after cleavage of the (1-84) PTH or directly secreted by the parathyroid glands [2]. If the concentration of these fragments is quite low in normal healthy subjects, they accumulate in patients suffering from chronic kidney diseases (CKD) and can represent up to 40%–50% of the (1-84) PTH in hemodialyzed (HD) patients [3, 4]. PTH determination is routinely performed in clinical laboratories on most of the available automates. These assays are called "intact" or "second generation" PTH assays and recognize both the (1-84) peptide and the (7-84) PTH fragment, this latter being recognized differently by the different assays on the market according to the cross-reactivity of the antibodies used. The absolute values obtained with these kits are thus often different from one kit to another [5]. Recently, two automated third generation PTH assays (Roche Elecsys and DiaSorin Liaison) became available. These assays are specific for the (1-84) PTH and do not cross-react anymore with the (7-84) fragment, contrary to second generation assays. Third generation assays could thus theoretically provide results that could be interchangeable, even in HD patients. Unfortunately, a paper

recently shown that the two methods could not be used interchangeably due differences in calibration, matrix effects or avidity of the antibodies [6]. In the present study, we aimed to calibrate the two methods against the WHO International PTH Standard (IS) 95/646 to see if the two assays could provide comparable results in a reference population of healthy subjects, HD patients and patients suffering from primary hyperparathyroidism (PHP).

Materials and methods

Third generation parathyroid hormone (PTH) assays

The Cobas Elecsys PTH (1–84) immunoassay (Roche Diagnostics, Germany) is a one-step sandwich electro-chemiluminescence immunoassay (ECLIA). The first antibody is a monoclonal biotinylated antibody against the N-terminal region 1–5, and the second antibody is a monoclonal antibody labeled with a ruthenium complex that reacts with the C-terminal region 54–59. The assay is claimed to be calibrated against the 95/646 WHO IS PTH 1-84 (human, recombinant). The CV of the method is 9.4%, 4.7%, 4.1% and 3.1% at 13.2, 27.6, 339 and 1398 pg/mL, respectively.

The Liaison (1-84) PTH immunoassay (DiaSorin, Stillwater, MN, USA) is a two-step automated sandwich chemiluminescent immunoassay (CLIA) that uses two polyclonal antibodies: the first one, specific for the N-terminal part of the peptide is bound to isoluminol and the second one, specific for the C-terminal part of the peptide, is bound to paramagnetic beads. The manufacturer does not provide any information on the standardization of the assay. The CV of the method is 5.5%, 4.1%, 4.0% and 4.7% at 10.6, 33.5, 378 and 1662 pg/mL, respectively.

Both assays do not recognize the 7-84 PTH.

Patients

We selected a reference population of 79 healthy subjects (56.1±16.8 years old, 25 males) that presented normal calcium and phosphorus levels, eGFR >60 mL/min/1.73 m^2 and 25(OH) vitamin D levels >30 ng/mL. We also selected two populations of patients presenting PTH disorders, a HD population of 56 patients (66.8±16.8 years old, 33 males) and a population of 27 patients suffering from mild PHP (64.8±10.5 years old, 8 males).

All the samples were leftover serum samples that underwent one cycle of freeze/thawing and that had been kept frozen at −80°C until determination. Our previous reports on third generation PTH have shown that the peptide was very stable under these conditions of storage [7].

Preparation of the 95/646 WHO International PTH Standard and of the calibration curves

The 95/646 WHO IS PTH 1-84 is the first IS for parathyroid hormone made of recombinant, 1-84, human PTH established by the Expert Committee on Biological Standardization of the WHO in October 2009. Each ampoule contains 100 µg of PTH 1-84.

We reconstituted the WHO Standard with 10 mL of 0.1 N PBS acetic acid solution containing 0.1% of bovine serum albumin (BSA). All the further dilutions of this stock solution were then prepared at +4 °C in a pool of human serum containing undetectable levels of 1-84 PTH, constituted from remnant samples obtained in patients suffering from severe hypoparathyroidism or non-PTH-mediated hypercalcemia.

We thus constituted 13 serum standards containing 2000, 1667, 1000, 800, 500, 303, 200, 100, 80, 50, 25, 10 and 0 pg/mL of 1-84 PTH. These standards were run in duplicate on each instrument and the Relative Light Units (RLU) provided by the photomultiplicators were plotted against the PTH concentration of each standard to give a calibration curve. Next, the patients' samples were run in singlicate on the two instruments. The RLU obtained for each patient was then reported for both instruments on the calibration curve.

Results

There was an excellent correlation between the RLU of the standards obtained on the two instruments (r=0.9975). Passing-Bablok regressions observed before and after the recalibration of the kits with the WHO IS are presented in Table 1.

The Bland-Altman plot showed a mean±SD difference of (Elecsys-Liaison) 31.9%±20.8% vs. 2.3%±18.4% before and after recalibration. The plots for the specific populations are shown in Figure 1.

In the subpopulations, we observed a mean difference of 31.9%±20.8% vs. 2.3%±18.4% for the healthy normal

Table 1 Passing-Bablok regressions observed before and after recalibration of the DiaSorin Liaison and Roche Elecsys 3rd generation PTH assays with the WHO International Standard in three different populations.

Patients	Before recalibration	After recalibration
Whole population	Liaison=0.85 Elecsys −5.5	Liaison=1.08 Elecsys −3.5
Normal healthy	Liaison=0.69 Elecsys −1.8	Liaison=1.13 Elecsys −4.4
Hemodialyzed	Liaison=0.97 Elecsys −23.1	Liaison=0.98 Elecsys +9.0
Primary hyperparathyroidism	Liaison=0.86 Elecsys −9.1	Liaison=1.16 Elecsys −6.7

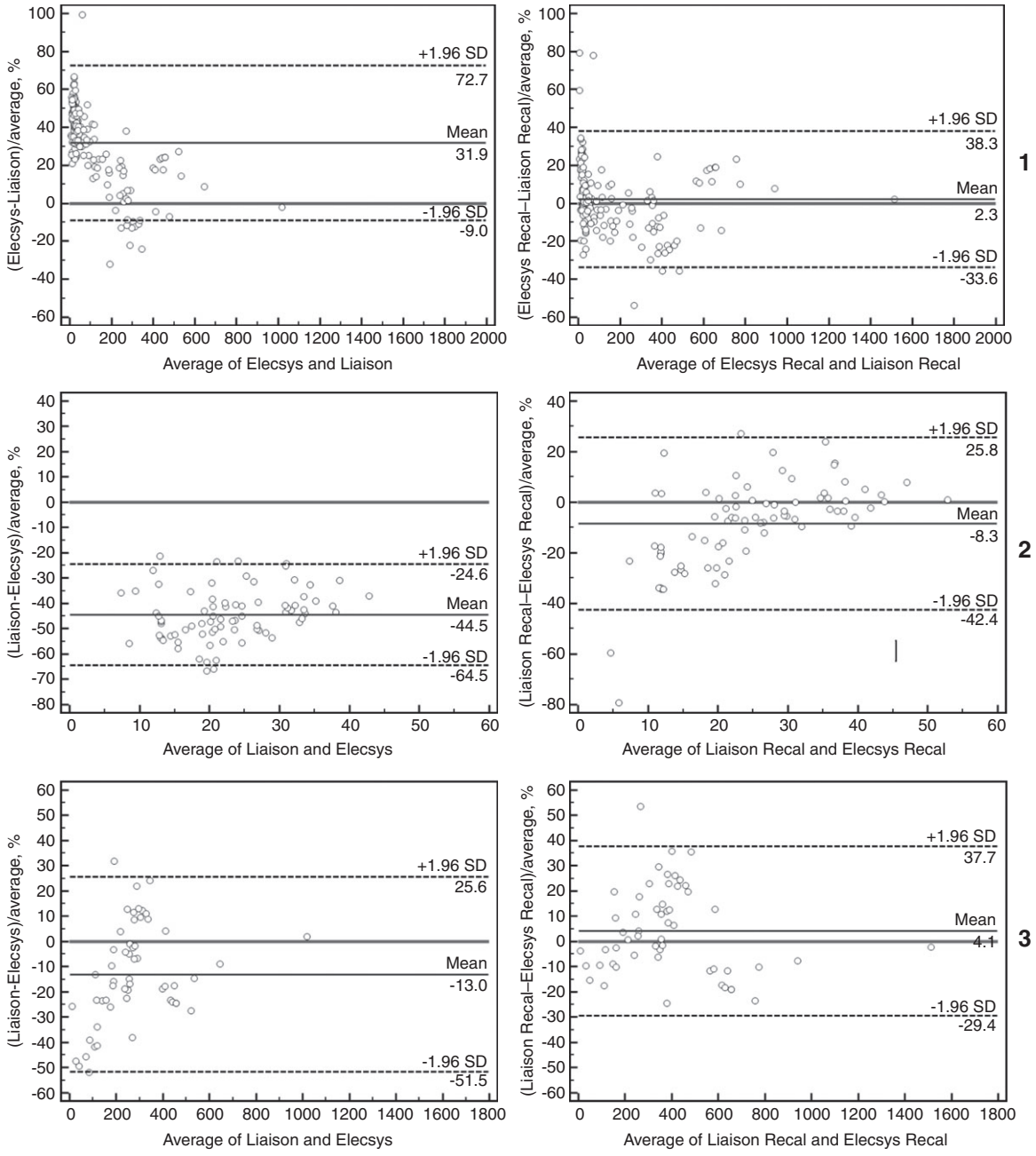

Figure 1 Results of Bland-Altman plots for DiaSorin Liaison and Roche Elecsys 3rd generation PTH assays obtained in three different populations (1: healthy individuals; 2: patients suffering from mild primary hyperparathyroidism and 3: hemodialyzed patients) before and after a restandardization of the assays with the WHO International PTH Standard 95/646. "Elecsys Recal" and "Liaison Recal" are the data obtained after standardization.

population, −44.5±10.2 vs. −8.3±17.4% for the HD patients and −13.0±19.6% vs. 4.1±17.1% for the PHP patients.

The reference range calculated with the robust method [8] on the normal healthy individuals was 6–33 pg/mL for Liaison and 4–49 pg/mL for Elecsys. After restandardization, the reference range was quite similar with both methods: 11–46 pg/mL for Liaison and 8–45 pg/mL for Elecsys. In this population, the medians (IQR) observed with the two instruments before calibration were significantly different ($p<0.0001$): 16.6 (12.5–22.1 pg/mL) for Liaison and 26.8 (20.4–37.7 pg/mL) for Elecsys. The impact of the recalibration on the medians was an increase of 39.3% for Liaison [23.4 (15.4–31.8 pg/mL)] whereas it was a decrease of 9.3% for Elecsys [26.9 (17.5–31.8 pg/mL)], and the significant difference between these two medians disappeared.

In HD patients, the Mann-Whitney test showed that the −6.8% difference observed between the medians obtained by Liaison and Elecsys before calibration [median (IQR): 248 (156–350) and 265 (177–318 pg/mL), respectively] was not significant. After recalibration, the median increased by 51% for Liaison and 29% for Elecsys but the difference of +8.6% [374 (239–517) and 342 (217–420) pg/mL] remained non-significant. Finally, the restandardization of the assays did not change the classification of the patients according to the KDIGO guidelines: nine patients were classified differently before restandardization, and nine patients remained classified differently after.

In patients suffering from mild PHP, the medians observed with two assays were significantly different (p=0.01) whereas this difference disappeared after standardization. For Liaison, median increased by 60.5% as it moved from 48.1 (32.7–75.2 pg/mL) to 77.2 (56.9–109.3 pg/mL). For Elecsys, we observed a 7.3% increase of the median: from 66.5 (53.6–91.2 pg/mL) to 71.7 (56.0–102.6 pg/mL). If the difference between the medians was of 38.3% before standardization, it decreased to 7.1% after.

Discussion

Restandardization of PTH assays is of importance to reduce inter-method variability [9], particularly in HD patients [10]. An IFCC workgroup, in which representatives of relevant clinical and scientific professional organization and manufacturers of most PTH immunoassays is thus working on the current status of PTH measurement and on the identification of priorities for improvement.

In this work, we aimed to evaluate the impact of the restandardization of two automated third generation PTH assays with the WHO IS 95/646 in three different groups of patients, as it is considered as a suitable candidate [9]. By moving very fast into a human serum matrix after reconstitution, we managed to make this IS as commutable as possible. As claimed by the manufacturer, our results showed that the DiaSorin Liaison was not calibrated against this standard. However, we were surprised to see that Roche Elecsys, claimed to be traceable to this standard, was not very well calibrated. Indeed, we observed differences as high as −9% and +25% in healthy individuals and HD patients after having restandardized the assay, compared to the values obtained with Roche calibration.

Calibrating the assays with our 13 points curve significantly reduced the bias between the results obtained from 31.9% to 2.3%. From a global perspective, this is very important because these results show that, if both kits were correctly calibrated against the traceable WHO IS, the variation between laboratories using these assays would decrease. The reference ranges could be the same with the two assays and raw values, instead of multiples of upper reference ranges, could be used in HD patients for clinical cut-offs. Finally, the traceability of the assays to the WHO IS could be achieved with the recently published liquid chromatography-tandem mass spectrometry (LC-MS/MS) method allowing the quantification of serum 1-84 PTH after trypsic digestion [11].

These conclusions, however, are only correct for third generation PTH assays. Indeed, for second generation ones, even if a standardization can be achieved in healthy populations, this will probably not be the case for CKD patients due to different cross-reactivity of the antibodies with the 7-84 PTH.

Restandardization of the assays with the WHO IS significantly reduced the systematic error as expected, but it did not logically reduce the random error of approximately ±18% around this bias, as shown by the Bland-Altman plots. This random error is due to different factors, but the imprecision of the methods remains the most important source of error. It is thus important to keep in mind that restandardization will not overcome all the problems linked to PTH assays variation. Manufacturers still need to improve their methods to reduce the coefficient of variation of PTH assays which should be, at least, lower than 7.5% (50% of the intra-individual coefficient of variation of PTH).

Our study has some limitations as it is a single center study on a limited number of patients. Using percent changes in the lower range of PTH may also be misleading. However, by directly diluting the standards in human serum after the WHO IS reconstitution, we have tried to overcome the very important commutability problem [12], which does not mean that our standard is completely commutable. Indeed, we did not demonstrate that the IS, as reconstituted by our method, had the same characteristics as PTH in patients' samples when measured with all the immunoassays [9]. Unfortunately, we have no means to assess its total commutability. Finally, it has been suggested that PTH secretion increases with age [13]. As mean ages of our studied groups were only moderately different (56 years in healthy subjects, 68 years in HD patients, and 64 years in patients with PHPT), we did not consider that they could have impacted the results.

In conclusion, our results show that restandardization of automated third generation PTH assays with the WHO 1-84 PTH Standard significantly reduces inter-method variability. Reference ranges and raw values are totally transposable from one method to the other in healthy subjects, but also in diseased patients, e.g., with HD or those suffering from PHP.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.
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References

1. Souberbielle JC, Friedlander G, Cormier C. Practical considerations in PTH testing. Clin Chim Acta 2006;366:81–9.
2. Murray TM, Rao LG, Divieti P, Bringhurst FR. Parathyroid hormone secretion and action: evidence for discrete receptors for the carboxyl-terminal region and related biological actions of carboxyl- terminal ligands. Endocr Rev 2005;26:78–113.
3. D'Amour P, Brossard JH. Carboxyl-terminal parathyroid hormone fragments: role in parathyroid hormone physiopathology. Curr Opin Nephrol Hypertens 2005;14:330–6.
4. Lepage R, Roy L, Brossard JH, Rousseau L, Dorais C, Lazure C, et al. A non-(1-84) circulating parathyroid hormone (PTH) fragment interferes significantly with intact PTH commercial assay measurements in uremic samples. Clin Chem 1998;44:805–9.
5. Souberbielle JC, Boutten A, Carlier MC, Chevenne D, Coumaros G, Lawson-Body E, et al. Inter-method variability in PTH measurement: implication for the care of CKD patients. Kidney Int 2006;70:345–50.
6. Hecking M, Kainz A, Bielesz B, Plischke M, Beilhack G, Hörl WH, et al. Clinical evaluation of two novel biointact PTH(1-84) assays in hemodialysis patients. Clin Biochem 2012;45:1645–51.
7. Cavalier E, Delanaye P, Hubert P, Krzesinski JM, Chapelle JP, Rozet E. Estimation of the stability of parathyroid hormone when stored at –80 degrees C for a long period. Clin J Am Soc Nephrol 2009;4:1988–92.
8. Horowitz GL. Defining, establishing and verifying reference intervals in clinical laboratory; approved guideline, 3rd ed. Wayne, PA: CLSI, 2008.
9. Sturgeon CM, Sprague SM, Metcalfe W. Variation in parathyroid hormone immunoassay results – a critical governance issue in the management of chronic kidney disease. Nephrol Dial Transplant 2011;26:3440–5.
10. Almond A, Ellis AR, Walker SW. Current parathyroid hormone immunoassays do not adequately meet the needs of patients with chronic kidney disease. Ann Clin Biochem 2012;49:63–7.
11. Kumar V, Barnidge DR, Chen LS, Twentyman JM, Cradic KW, Grebe SK, et al. Quantification of serum 1-84 parathyroid hormone in patients with hyperparathyroidism by immunocapture in situ digestion liquid chromatography-tandem mass spectrometry. Clin Chem 2010;56:306–13.
12. Miller WG, Myers GL, Rej R. Why commutability matters. Clin Chem 2006;52:553–4.
13. Marcus R, Madvig P, Young G. Age-related changes in parathyroid hormone and parathyroid hormone action in normal humans. J Clin Endocrinol Metab 1984;58:223–30.