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Comparative study of human growth hormone measurements: impact on clinical interpretation

Abstract

Objectives: Human growth hormone (hGH) provocation test is an essential tool to assess growth hormone deficiency (GHD) in children and young adults. It is important to have a robust method to determine the hGH peak of stimulation. This work aimed to compare three common automated immunoassays for hGH quantification and to ascertain whether there are still result-related differences which can impact clinical decision.

Methods: We analyzed the GH provocation test for 39 young subjects from pediatric department of Montpellier hospital, admitted for suspicion of growth hormone deficiency. The full range of measurements as well as the peak level of serum GH were compared using three automated immunoassays on three different immunoanalyzers: IDS-hGH on iSYS, LIAISON-hGH on Liaison XL and Elecsys ROCHE-hGH, on COBAS 8000.

Results: A good correlation was obtained between methods for all measurements ($r^2 > 0.99$) by using Passing–Bablok regression analysis. Bland–Altman analysis showed the best agreement between IDS-hGH and LIAISON-hGH systems (bias = -14.5%) compared to Elecsys ROCHE-hGH (bias = 28.3%). When considering stratification of the study population and a unique cutoff, there were some discrepancies in interpretation of the results especially concerning the more recent Elecsys ROCHE-hGH assay. Nevertheless, when the adequate cutoff for each method was taken into account results were well correlated for all systems.

Conclusions: A cutoff for Elecsys Roche-hGH method was established to better explain the results. Clinician must be aware of the use of assay-specific cutoff to correctly integrate the results of GH tests in the GHD diagnosis.

Keywords: cutoff; growth hormone deficiency; human growth hormone (hGH) provocation test; immunoanalysis.

Introduction

Over the last decades, consensus guidelines for the diagnosis of growth hormone deficiency (GHD) deemed necessary to standardize assays for measuring human growth hormone (hGH). Considerable efforts have been made both in term of hGH reference preparation (recombinant 22 kDa hGH) as well as antibody specificity (the use of a monoclonal antibody recognizing the 22 kDa hGH being recommended) [1]. Whereas spontaneous hGH measurement is not informative, several dynamic stimulation tests are currently used for the diagnosis of GHD. These tests consist in administering molecules such as insulin, arginine, clonidine and L-dopa, capable of stimulating the pituitary gland, and thus the secretion of hGH. Diagnosis can be achieved by finding low serum hGH values which do not respond to stimulation tests. Assay variability, combined with the use of arbitrary cutoff values for the diagnosis of GHD, led to significant differences concerning hGH treatment decision [2–4]. In line with these findings, the French Society for Clinical Biology (SFBC) focused on the analytical discrepancies and the need of method harmonization in measuring

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serum hGH [5]. Lately, an international consensus statement concluded that major improvements were still necessary in the area of assay performance and comparability. The variability was attributed to several factors, including differences in the reference material in use, competitive vs. sandwich assay format, polyclonal vs. monoclonal antibodies, heterogeneity of circulating hGH isoforms (20 vs. 22 kDa) and binding to GH binding protein [6]. Wagner et al. [7] focused on the importance of the cutoff limits of GH stimulation tests to diagnose GHD and on the discrepancies in the performance of GH immunoassays. The aim of this work was to perform a comparative study of hGH measurements at different intervals of the test, using three widely used automated assays (IDS-hGH, LIAISON-hGH and Elecsys ROCHE-hGH). The hGH peak value was elected as the main variable for achieving clinical diagnosis and the assay related clinical interpretation was explored depending of the cutoff applied.

Materials and methods

Study population

From April to November 2019, 39 young subjects (24 boys and 15 girls) from the paediatric department of Montpellier hospital were included in this study. They were mainly admitted for GHD suspicion (short stature or growth retardation) in pre-and pubertal age (median age=13) and they were all treatment-naïve individuals. The study protocol was approved by the local Ethics Committee of Montpellier university hospital (IRB ID: 202000578) and registered to ClinicalTrials.gov (identifier: NCT04508673). Insulin-like growth factor I (IGF-1) and insulin-like growth factor binding protein 3 (IGFBP3) levels were measured at the same time as hGH in the provocation test. Characteristics of the study population are summarized in Table 1.

Testing protocol

All subjects underwent the insulin-induced hypoglycaemia stimulation test also called insulin tolerance test (ITT), considered the ‘gold

Table 1: Demographic and clinical features of the study population.

Study population, n=39 24 boys, 15 girls	Median (1°–3° quartile)
Age at stimulation test, years	13 (10–15.25)
BMI at the time of testing, %	17 (15–18)
IGF-1, ng/mL	175 (89–253)
SDS, at the time of testing	–2 (–2 to –0.5)
IGFBP3, ng/mL	3,451 (2,636–4,548.5)
SDS, at the time of testing	–1 (–2 to –0.3)

BMI, body mass index; IGF-1, insulin-like growth factor I; SDS, standard deviation score; IGFBP3, insulin-like growth factor binding protein 3.

standard’ test for GHD diagnosis. Following insulin administration by venipuncture (0.1 UI/kg/h), capillary glycaemia was monitored every 10 min. Simultaneously serum and fluoride tube plasma were taken for quantification of hGH and glucose starting from capillary glycaemia <0.45 g/L (T0) and also at 30, 45, 60 and 90 min (T30, T45, T60 and T90). Blood samples were centrifuged within 30 min after collection and used for measurements. The aliquots of residual serum were employed in the comparative analysis the same day and stored at –80 °C.

Assay methods

hGH levels were determined by the IDS-hGH assay (IDS Immunodiagnostic Systems Ltd, Boldon, UK), which is routinely used in our laboratory. Only values from IDS-hGH assay were considered for the clinical diagnosis. Serum was analyzed in parallel with the hGH assay of two other analyzers: LIAISON XL (Diasorin S.A., Antony, France), and COBAS 8000 e602 (ROCHE Diagnostics, Meylan, France). In the three systems, calibration test was carried on with the WHO International Standard for somatotropin (National Institute for Biological Standards and Control, code 98/574) from recombinant hGH. Isoform specificity of the antibody for the 22 kDa form of hGH was specified when reported by the manufacturers. For each assay, the degree of interferences as well as the main cross-reactivity were mentioned as reported by the manufacturers. Precision verification study was also presented for hGH assay on iSYS and on LIAISON XL analyzers whereas results for Elecsys ROCHE-hGH on COBAS 8000 e602 were as indicated by the manufacturer. Evaluation was performed in accordance with a modified protocol based on CLSI (Clinical and Laboratory Standards Institute) EP15-A3 [8]. To determine repeatability (within-run imprecision), two levels of internal quality controls (IQC) were measured in 30 replicates for each level within the same run. Within-laboratory imprecision was determined using two levels of IQC specific for each system five times a day during five following days (Table 2).

Statistical analysis and iSYS cutoff to Elecsys ROCHE-hGH assay

The results of the three assays were compared by Passing-Bablok regression analysis. The scatter of differences (%) was visualized by means of Bland–Altman plots [9]. The cutoff was calculated by using the linear regression equation as previously described [7, 10] for both correlation of iSYS-hGH vs. Liaison-hGH and vs. Elecsys ROCHE-hGH. Statistical analyses were performed using XLSTAT® software for Excel, version 2016.06.35661 (NY, USA).

Results

Comparison of hGH values

All samples from T0 to T90 of the GH test (total n=195) were measured for each patient on the three analyzers. Passing–Bablok regression analysis and Bland–Altman plot for assay comparability of hGH measurements are reported in Figure 1.

Table 2: Characteristic of the three automated assays for hGH.

Assay	IDS-hGH assay (ISYS, IDS immunodiagnostic systems)	LIAISON® hGH assay (LIAISON XL, Diasorin S.A.)	Elecsys ROCHE-hGH assay (COBAS 8000 e602, ROCHE Diagnostics)
Reference standards	WHO International Standard for somatotropin from NIBSC, code 98/574, >96% 22-kDa GH		
Assay type and anti hGH antibodies	CLIA (two steps sandwich); 2 mAb	CLIA (one step sandwich); 2 mAb	ECLIA (one step sandwich); 1 mAb and 1 pAb
Isoform specificity	100% hGH 22 kDa	hGH 22 and 20 kDa	hGH 22 and 20 kDa
Matrix proprieties	Human serum or plasma samples	Human serum only	Human serum or plasma samples
Interferences	Binding proteins ≤140 ng/mL Bilirubin ≤3.4 mmol/L Intralipids ≤80 mmol/L Hemoglobin ≤5 g/L Biotin ≤300 nmol/L Rheumatoid factor NA	Binding proteins NA Bilirubin ≤0.34 mmol/L Intralipids ≤130 mmol/L Hemoglobin ≤10 g/L Biotin NA Rheumatoid factor NA	Binding proteins NA Bilirubin ≤0.43 mmol/L Intralipids ≤130 mmol/L Hemoglobin ≤10 g/L Biotin ≤123 nmol/L Rheumatoid factor ≤600 UI/mL
Cross-reactivity	hGH 20 kDa undetectable up to 10 µg/L Placental hGH undetectable up to 200 µg/L Prolactin undetectable up to 40,000 µg/L Pegvisomant undetectable up to 50,000 µg/L	hGH 20 kDa Placental hGH (not adapted to pregnant women) Prolactin undetectable up to 1,000 µg/L Not adapted to patients treated with pegvisomant	hGH 20 kDa ≥75.4% up to 100 µg/L Placental hGH (not adapted to pregnant women) Prolactin ≤0.544% up to 470 µg/L Not adapted to patients treated with pegvisomant (not interfering with octreotide et cabergoline treatments)
Hook effect	Not observed up to 15,000 µg/L	Not observed up to 7,000 µg/L	Not observed up to 2,000 µg/L
Measuring range	0.05–100 µg/L	0.05–80 µg/L	0.030–50 µg/L
Controls	Bio-Rad Lyphochek Immunoassay Plus Control 1&2	LIAISON® Control hGH 1&2	PreciControl Multimarker 1&2
Within – run imprecision (% CV)			
High level IQC	1.29 (8.17 µg/L)	0.98 (18.35 µg/L)	1.7 (7.93 µg/L)
Low level IQC	0.97 (3.02 µg/L) (Laboratory results)	1.02 (3.63 µg/L) (Laboratory results)	1.8 (0.71 µg/L) (Manufacturer results)
Within – laboratory imprecision (% CV)			
High level IQC	3.93 (7.95 µg/L)	1.10 (18.45 µg/L)	2.7 (7.93 µg/L)
Low level IQC	3.10 (2.95 µg/L) (Laboratory results)	1.22 (3.65 µg/L) (Laboratory results)	2.8 (0.71 µg/L) (Manufacturer results)
LOQ	0.049 µg/L	0.052 µg/L	0.050 µg/L

hGH, human growth hormone; WHO, World Health Organisation; NIBSC, National Institute for Biological Standards and Control; CLIA, chemiluminescence immunoassay; ECLIA, electro-chemiluminescence immunoassay; mAb, monoclonal antibody; pAb, polyclonal antibody; LOQ, limit of quantification.

Passing–Bablok regression analysis was obtained comparing pairwise the three methods in a concentration range of 0.15–20 µg/L. The equations showed a slope of 0.889 (95% CI=0.890–0.906) for IDS-hGH vs. LIAISON-hGH and 1.160 (95% CI=1.148–1.174) vs. Elecsys ROCHE-hGH whereas the slope was 1.296 (95% CI=1.274–1.318) for LIAISON-hGH vs. Elecsys ROCHE-hGH (Figure 1A–C). The coefficient of correlation according to Spearman was calculated to be >0.99 for all three tests. Concerning the deviation from linearity, perfect linearity was obtained for the comparison between

IDS-hGH and LIAISON-hGH ($p=0.99$), whereas it was statistically significant ($p<0.005$) for both IDS-hGH and LIAISON-hGH assays vs. the Elecsys ROCHE-hGH system (see Supplementary material). The assay comparability was also assessed for all ranges of concentrations using the Bland-Altman plot. The best agreement was obtained between IDS-hGH and LIAISON-hGH assays with a negative bias of -14.5% (95% CI= -15.6 to -13.5), higher positives biases of 28.3% (95% CI= 26.2 to 30.4) and 42.2% (95% CI= 39.7 to 44.8) were found for IDS-hGH vs. Elecsys ROCHE-hGH and LIAISON-hGH vs. Elecsys

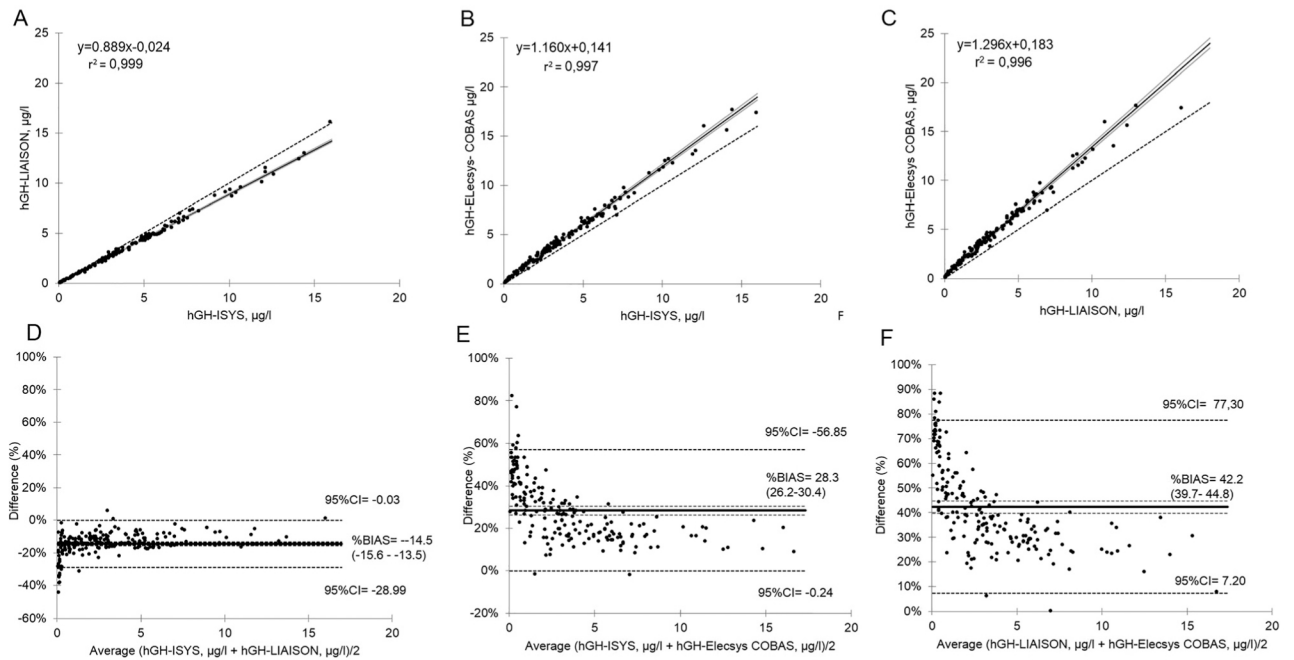


Figure 1: Pairwise comparisons of methods on hGH measurements by Passing–Bablok regression analysis. The dashed line is the line of identity, the thick line is the line of best fit and the grey lines are the 95% CI of the best fit line and Bland–Altman analysis, the dashed lines indicate the 95% confidence intervals (95% CI) and the grey lines the 95% CI of the bias: (A, D) IDS-hGH vs. LIAISON-hGH, (B, E) IDS-hGH vs. Elecsys ROCHE-hGH, and (C, F) LIAISON-hGH vs. Elecsys ROCHE-hGH.

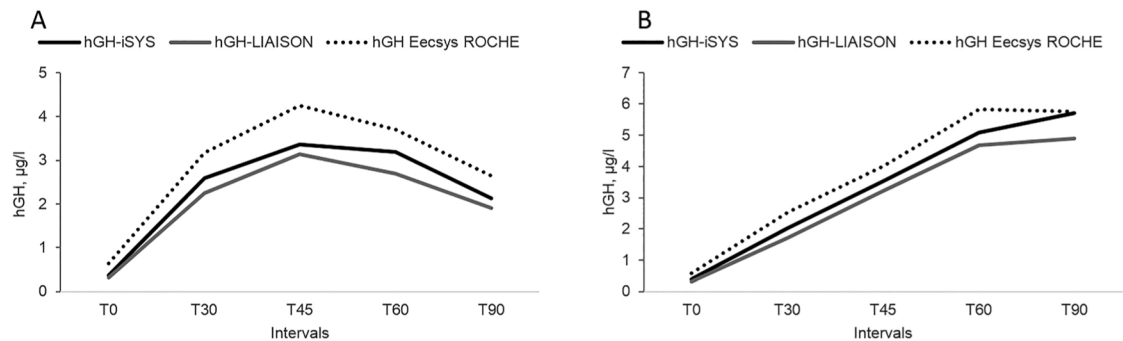
ROCHE-hGH, respectively (Figure 1D–F). In all cases, an important variability of the results was observed in the lower range of detection close to 0.15 µg/L.

Interpretation of hGH provocation test and cutoff limits

The study population was classified into two groups according to the diagnosis based on the hGH provocation test (insulin tolerance test) results together with auxological criteria and clinical features. The GH peak level was obtained from the IDS-hGH method considering the cutoff value of 7.09 µg/L. One group included subjects diagnosed with total or partial GHD and thus treated with recombinant GH (GHD/GHPD, n=29) and the second group was diagnosed as non-GH deficient, thus non-treated (GHND, n=10). Both groups included only treatment-naïve subjects (Figure 2).

The curve presented the median hGH peak value for the two groups of individuals and amongst them there was a large variety of response to the test. The results were presented as a median in order to compare the three methods and also to observe globally the provocation test curve in each group. The hGH concentration peak was obtained at T45 for the GHD/GHPD group (Figure 2A)

whereas the curve for the non-hGH-deficient group (GHND) presented a rather sharp increase of hGH level at T60 and a tendency to a plateau for the following interval indicating a prolonged secretion of hGH after stimulation (Figure 2B). The values measured with Elecsys ROCHE-hGH assay were overall higher despite differences were not statistically significant for any interval. When the unique cutoff of 7.09 µg/L was considered for interpretation of the test, 28% of the subjects resulted with a normal GH stimulation test by using both IDS-hGH and LIAISON-hGH methods despite 46% with Elecsys ROCHE-hGH assay. More precisely, at the intermediate values between 3.3 and 7.09 µg/L indicating a partial growth deficiency, results were still close between IDS-hGH and LIAISON-hGH (44%) and lower for the Elecsys ROCHE-hGH (approximately 36%). The percentages also changed for the subjects considered non-responder to the GH test (GH<3.3 µg/L) with equal 28% for both IDS-hGH and LIAISON-hGH despite 18% of the Elecsys ROCHE-hGH. Consequently, in the case of Elecsys ROCHE method quantification the risk would have been to consider some subjects as non-hGH deficient instead of partial deficient (18%) or as partial deficient subjects instead of deficient (10%). Although the deviations from linearity was statistically significant for the ROCHE method compared to the two others we tried to apply the calculation established by Muller and already described by Wagner for different



Naive to T, GHD/GHPD group, n= 29, intervals	T0	T30	T45	T60	T90
iSYS median, µg/l (1st-3rd quartile)	0.37 (0.20-1.20)	2.60 (0.53-4.33)	3.36 (2.10-5.93)	3.20 (2.10-5.06)	2.13 (1.16-3.30)
LIAISON median, µg/l (1st-3rd quartile)	0.30 (0.14-1.17)	2.25 (0.45-3.97)	3.14 (1.81-4.98)	2.70 (1.86-4.60)	1.90 (0.99-2.89)
Elecsys COBAS median, µg/l (1st-3rd quartile)	0.63 (0.32-1.75)	3.17 (0.74-5.07)	4.26 (2.40-7.20)	3.71 (2.36-6.21)	2.64 (1.51-4.06)

Naive to T, GHND group n=10, intervals	T0	T30	T45	T60	T90
iSYS median, µg/l (1st-3rd quartile)	0.38 (0.27-1.62)	2.01 (0.90-6.66)	3.53 (0.98-5.51)	5.09 (2.61-7)	5.71 (3.37-7.20)
LIAISON median, µg/l (1st-3rd quartile)	0.31 (0.22-1.60)	1.71 (0.78-6.23)	3.21 (0.87-4.88)	4.68 (2.41-6.76)	4.91 (3.04-6.23)
Elecsys COBAS median, µg/l (1st-3rd quartile)	0.57 (0.44-1.98)	2.52 (1.37-7.56)	4.00 (1.30-6.36)	5.82 (3.02-7.86)	5.77 (3.78-8.44)

Figure 2: Curve and median values of the three assays at each interval of hGH test for the two groups. (A) GHD/GHPD and (B) GHND.

commercially used immunoassays also for the Elecsys ROCHE-hGH method [9]. Since the correlation coefficient was high between the assays in the measurement range we considered the bias relatively low and we obtained a cutoff for Elecsys ROCHE-hGH at approximately 8.4 µg/mL. Similarly the cutoff was also calculated for the Liaison-hGH method as previously described and a similar value of 6.34 µg/L was obtained (Table 3). When the results were analyzed on the basis of the assay specific cutoff, the agreement was highly improved with only approximately 5% of discordance between the Elecsys ROCHE-hGH method and the two others.

Discussion

Three major automated immunoassay methods were compared for quantification of hGH in the context of stimulation dynamic test for GHD diagnosis in treatment-naïve individuals. Adequacy of the cutoff to the applied method allowed to smooth out all residual discrepancies in results and to reach full agreement among the different assays. The inadequate secretion of hGH is responsible for GHD in children and adolescents related to growth retardation, short stature and maturation bones delay. Diagnosis at an early stage is essential for successful therapy.

GH provocation tests are considered the foremost for GHD investigation, although combination of auxology, and imaging is needed to pose the diagnosis. Since the advent of immunometric techniques and automatization, much work was done to ensure uniformity and comparability of the hGH assays. An empirical approach to harmonize hGH measurements was suggested between methods whereas differences could be corrected to a common scale by using a commutable serum pool with a defined consensus value, drastically reducing the coefficient of variation (from 22 to 9%) [11]. The important work of Rakover et al. showed the high correlation between individual results by four different immunometric kits despite the poor agreement of hGH peak interpretation in response to provocation test. Interestingly, the recognition of different hGH isoforms had only a partial effect on inter-assay variability and a larger

Table 3: Cutoff limits for the three automated assays.

Assay	Regression equation (r ²)	Cutoff, µg/L
iSYS-hGH (IDS)	NA	7.09
Liaison-hGH (DiaSorin)	y=0.889x - 0.018 r ² =0.996	6.34
Elecsys COBAS-hGH (ROCHE)	y=1.177x + 0.127 r ² =0.996	8.36

contribution was probably due to the different standard preparations used in calibration for each assay [2, 3, 12]. Thus, a calibration against an international standard and the use of serum as the substrate of choice for all dilutions and measurements were highly recommended [5]. Nowadays, the problem of standardization of calibration is solved and most automated methods use standard from the National Institute for Biological Standards and Control (NIBSC). This standard (IS 98/574) is constituted of recombinant hGH isoform 22 kDa with a biological activity of 3 μ IU/ng. Besides, the majority of automated systems uses sandwich monoclonal immunoassay a part from Elecsys ROCHE-hGH using a polyclonal antibody. The issue of clonality is important since measurements are based on antibodies (polyclonal or monoclonal), recognizing several epitopes shared by different isoforms of the protein in the serum, some of which are not yet known [13]. In this work, although IDS-hGH and LIAISON-hGH methods used two different monoclonal antibodies, one recognizing specifically the 22 kDa form and the other both the 22 and 20 kDa forms respectively, they had very small differences in results. The problem regarding the variability and reproducibility of measurement methods has made it difficult to define a standard cutoff peak of hGH concentration between different methods. The cutoff of 7.09 μ g/L for GHD is often used when assays are calibrated with standard SI 98/574. In the work from Wagner et al. cutoff limits for hGH stimulation tests were established by using different immunoassays and isotope dilution mass spectrometry (ID-MS). This value varied between 4.32 and 7.77 μ g/L, depending of the method characteristics. A cutoff for GHD at 7.09 μ g/L (21.3 mIU/L) was found for the IDS-hGH system and at 6.25 μ g/L (18.8 mIU/L) for LIAISON-hGH, whereas Elecsys ROCHE-hGH system was not included in the study [7]. In this work, serum hGH from peak provocation test of 39 young persons with suspected GHD were analyzed by using three commonly used automated immunoassays. Our findings suggested that, although a good correlation was obtained between methods, when considering stratification of subjects with a unique cutoff of 7.09 μ g/L, there were still discrepancies between methods. Notably, the ROCHE-hGH system overestimating hGH secretion peak values might require the usage of complementary explorations to achieve a reliable diagnostic. An unreliable diagnosis could also deprive a false-negative patient of treatment, resulting in an unfavorable clinical outcome. The cutoff limit for each assay must be applied to assess whether small measurement discrepancies, conditioned by the method employed, could affect clinical decision-making. We obtained the cutoff for the new Roche method by transforming the iSYS cutoff value of 7.09 μ g/L into the

ROCHE system regression equation. The value of 8.4 μ g/L was used to reconsider patient's stratification and the correlation became very satisfying with nearly 100% agreement. Thus the impact of the analytical method on clinical interpretation can be a crucial springboard to further improve diagnosis and to tailor more accurately management of GHD subjects.

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Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: The study protocol was approved by the local Ethics Committee of Montpellier university hospital (IRB ID: 202000578 and registered to ClinicalTrials.gov (identifier: NCT04508673)).

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