Endocrinology **Edited by: M. Bidlingmaier/J. Kratzsch**

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Quality assurance in the analysis of growth hormone and insulin-like growth factor I in disorders of the somatotropic axis

DOI 10.1515/labmed-2015-0116
Received January 1, 2015; accepted February 2, 2015

Abstract

Background: Reliable laboratory analysis is fundamental to diagnostics, therapy, and follow-up of growth disturbance and secretory dysfunction of growth hormone (GH) and insulin-like growth factor I (IGF-I). Currently available commercial assays have their limitations, as they show large variations in hormone concentrations measured.
Methods: The recommendations of an expert workshop with practicing endocrinologists from the fields of pediatrics and internal medicine and with laboratory physicians, with reference to the outcome of the interdisciplinary consensus conference in Keswick (Virginia, USA) in 2009, were used.
Results: Among the quality criteria stipulated by the workshop participants are the use of uniform reference standards, documentation of analytical conditions (such as calibrators, binding epitopes, cross-reactivity, and methods for removal from the binding protein), batch-to-batch consistency, and low inter-assay variability. The participants recommended developing assay-specific thresholds and reference intervals based on large and well-defined reference populations. It is furthermore recommended to delineate the assay quality, particularly with reference to clinically important cutoffs.
Conclusions: The manufacturers of diagnostic assays should be obliged to regularly monitor and report the implementation of quality criteria. Only assays that are evaluated according to uniform quality standards and that are employed clinically permit informed diagnostic and therapy of patients with GH secretory dysfunction, preventing avoidable burden on both patients and paying authorities.

Keywords: analytics; assays; growth hormone; IGF-I; quality assurance.

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Introduction

The growth hormone (GH) is an anabolic proteohormone. It is produced in the anterior pituitary and secreted under the control of the hypothalamic hormones growth-hormone-releasing hormone (GHRH) and somatostatin [1]. Insulin-like growth factor I (IGF-I) is mainly formed under the control of GH in the liver and secreted. In plasma, IGF-I is bound to specific carrier proteins (IGF-binding protein IGFBP). GH and IGF-I primarily affect the growth and differentiation of cells. In childhood and

adolescence, they contribute significantly to longitudinal growth; in adulthood, they regulate, *inter alia*, the glucose and lipid metabolism [1, 2]. A reliable laboratory analysis and informed interpretation of measurement results are the cornerstones of an accurate diagnosis and the treatment to be derived from this for patients with disorders of growth and GH secretion.

However, assay-specific problems and insufficiently characterized reference ranges create doubts about the possibility of the clinical implementation of current consensus recommendations on the diagnosis and treatment of GH deficiency and acromegaly [3–5]. This leads to the need to achieve harmonization of GH and IGF-I measurements as well as consistent quality assurance. Such efforts were already made in the past. Among them, one should point out the results of the interdisciplinary consensus conference in Keswick (Virginia, USA) in 2009, which was attended by representatives of international professional associations and representatives of regulatory agencies, manufacturers of reagents and the pharmaceutical industry [6].

The following provides a summary of the results of a workshop attended by clinical endocrinologists and laboratory physicians in collaboration with the Academy for Education and Training of the German Society of Endocrinology and the Endocrinological Laboratory Diagnostics section of the German Society for Clinical Chemistry and Laboratory Medicine. The objective of the workshop was to determine the level of quality in the diagnosis of GH and IGF-I and to develop ways for improvement.

In short

A reliable laboratory analysis is a cornerstone of an accurate diagnosis and the treatment to be derived from this for patients with disorders of growth and GH secretion. A therapeutic decision, such as treatment of GH deficiency with daily injections of GH, must be well founded and based on reliable laboratory results.

Recommendations for the standardization of assays

The central problem of current GH and IGF-I assays is the poor comparability of measurement results obtained by different analytical methods. Currently, the use of different reference preparations to calibrate assays contributes significantly to the high variability of measurement results. The exclusive use of the recombinant international reference preparation 98/574 as a single calibrator has been demanded for GH for some time [6]. As for IGF-I assays, the Keswick consensus speaks clearly in favor of the recombinant WHO standard IS 02/254 [6]. In fact, it has been shown that the uniform use of a recombinant calibrator renders measurement results obtained by different methods more comparable [7].

In serum, GH occurs in different isoforms, which are detected variously by the current antibodies in commercial assays. A standardized GH assay should be specific to the 22-kDa form and provide accurate measurement results, especially in the lower concentration range [6]. The dissociation constants and binding epitopes of the antibodies used should also be specified, like the cross-reactivity of the antibodies with other GH isoforms. As a standard preparation and antibodies have the highest impact on the variability, it seems desirable over the long term to use only assays for routine tests that are identical in terms of the standard and antibodies.

The clinical relevance of analytical interference with GH binding proteins (GHBP) [8] is questionable. However, manufacturers should check the interference of their GH assays with GHBP and provide a physiological account. In regard to the interference of IGF binding proteins with IGF-I assays [9], the respective method used to separate IGF-I from its high-affinity binding proteins should be described and validated. The impact on the analytical method stemming from altered binding protein patterns in connection with comorbidities such as renal failure, liver dysfunction or diabetes mellitus should be known as well [6]. When studying the effect of the GH receptor antagonist pegvisomant, a structural analog of the human GH used for the treatment of acromegaly, GH assays should be used that do not interfere with pegvisomant [10].

Depending on the assay, measurement results of GH and IGF-I assays have so far been stated not only as mass concentrations (µg/L), but also in international units (U/L). But the units were defined arbitrarily, and there is no clear link to the mass of hormones. Concentrations should, therefore, be stated uniformly as mass concentrations [6].

The validation of immunoassays should be done using sera from healthy volunteers and patients with disorders of GH secretion. As part of the validation of IGF-I assays, particularly sera from patients with diabetes mellitus, chronic renal and hepatic impairment should be examined. It may help to establish a serum database that manufacturers can use to run longitudinal tests on, and adjust, the performance of their assays. The assay quality (accuracy and precision) should be described in the lower and upper limits of the reference ranges (IGF-I) or at clinically important decision limits (GH stimulation and suppression tests).

Manufacturers should regularly publish data on batch consistency and inter-assay variability, as well as reports on the quality control of modifications to essential components of assays.

In short

Quality criteria for GH and IGF-I assays involve the use of recombinant calibrators 98/574 for GH assays and the WHO standard IS 02/254 for IGF-I assays, the exact characterization of the antibodies used and, in the case of IGF-I, the documentation of interference with binding proteins and their interference effect in different patient populations. The assay quality should be described particularly in the lower and upper limits of the reference ranges or at clinically important decision limits.

Quality criteria for reference ranges and limits

Normative data must be based on large, clinically relevant reference populations and – depending on the parameters – stratified by age group, gender, or other important factors. Manufacturers should provide assay-specific normative data for IGF-I and for dynamic GH tests, while scientific societies and associations should act as technical advisors to relevant studies.

Given the significant method-based differences in assay results as well as in terms of the expenditure, which good reference range studies entail, a possible harmonization of assay results through mathematical-statistical methods of conversion has been discussed over and over again. The consensus conference [6] and the workshop participants agreed that this did not represent a satisfactory option for IGF-I measurements and the corresponding reference ranges. The participants considered the conversion of limits of dynamic tests justified only when ethical reasons made it impossible to conduct studies to determine method-specific decision limits.

Also needed is a better description and improved comparability of reference cohorts with respect to the subsequent patient group, e.g., in connection with phenotypes, drugs and stages of puberty. When using data from epidemiological approaches, the effect of possible influencing factors must be clarified. Gender-specific reference ranges for IGF-I are especially necessary in childhood and puberty after the age of 10 [6]. For the age range between 0 and 20 years, IGF-I reference ranges should be specified for 1-year age intervals. The number of samples should be sufficiently high (n>120 for individual age strata). Longitudinal cross-sectional data are necessary when it comes to puberty. Especially in the case of pubescent adolescents, it is crucial to carry out a differentiated evaluation of measured IGF-I levels. As age and pubertal development correlate only to a very limited degree, the IGF-I reference ranges during this period, if possible, should be adjusted according to the stages of puberty. For adults, increments of five, perhaps even 10, years are sufficient. Ethnicity and body mass index (BMI) do not affect IGF-I measurements significantly, which means that it does not appear to be necessary to adjust reference ranges for these factors [6].

In short

Quality criteria for reference ranges and limits include, in particular, clinically relevant and assay-specific normative data. Reference cohorts should be comparable with respect to the subsequent patient group. Depending on the parameters, reference ranges and limits need to be stratified by age group, gender, or other important factors. For children and adolescents, the IGF-I reference ranges should additionally be stratified by puberty stages.

Diagnosis of GH deficiency: decision limits and interpretation of results

The clinical context must be taken into account in connection with the diagnosis and progress-monitoring of treatment of growth and GH-secretion disorders. Diagnostic accuracy correlates with the clinical probability of the presence of the disease to be analyzed. Determination of individual GH levels is not sufficient to confirm GH deficiency, because the hormone is released from the pituitary gland in a pulsatile manner – generally, it cannot be detected between pulses physiologically speaking. In order to assess the functionality of the GH-IGF-I axis reliably, stimulation tests are required. The insulin tolerance test (ITT) is used to check the integrity of the entire hypothalamic-pituitary-adrenal axis. In healthy individuals, insulin-induced hypoglycemia produces a maximum stress response with secretion of GH. The secretion response does not occur in patients with GH deficiency. In Germany, the GHRH-arginine test is frequently used, in addition to the ITT, as a test for the GH stimulation at the central level. It checks the maximum secretory capacity of the hypothalamus and the pituitary gland [3].

Children and adolescents

There is no diagnostic gold standard to clarify GH deficiency. As an indication for initiating GH therapy in children, two independent tests (arginine, clonidine, glucagon, insulin and spontaneous secretion) are required, apart from growth-based criteria like growth rate and body height [5]. Traditionally, guidelines in pediatrics and adolescent medicine have fixed the decision limit for diagnosing GH deficiency as part of a stimulation test at a maximum GH level of 10 µg/L [11]. By using assays based on the calibrator 98/574, this level was reduced to a maximum of 8 µg/L GH [5], which represents at best only an approximate estimate.

The determination of IGF-I is used for screening, but a reduced reading alone is still not proof of GH deficiency. If the IGF-I concentration in the screening is above -1.0 standard deviations from the mean of the reference interval (SDS), GH deficiency is not likely. The determination of IGF-I should be included in the monitoring of GH therapy. In children, the individual fluctuations of IGF-I must be taken into account. Previous studies have described an IGF-I target range of 0 to +2 IGF-I-SDS [12].

Adults

At the start of a major GH deficiency in adults, replacement of GH is indicated for patients with a known failure of at least one other pituitary hormone (except for prolactin) in connection with a disease of the hypothalamic-pituitary system. In order to diagnose or exclude GH deficiency, a single dynamic test should be performed for adults. The severity of hypopituitarism should be factored into the assessment [13]. A more advanced age and a high BMI are associated with lower GH levels.

In an ITT context, the traditional GH decision limits for severe GH deficiency in adults are <3.0 µg/L. Since GHRH-arginine stimulates the somatotropic axis more than does insulin-induced hypoglycemia, the decision limit for this test is higher than that for the ITT. In the GHRH-arginine test, it is highly dependent on the BMI, and is 9.0 µg/L for a BMI between 25 and 30 kg/m^2 [14]. A diversification of the limits according to BMI and gender makes sense for patients with a pituitary disease.

In adults, too, the sole determination of IGF-I is only of limited use to screen for GH deficiency. The determination of IGF-I should be included in the monitoring of GH therapy.

In short

As an indication for initiating GH therapy in children, two independent stimulation tests are required, apart from growth-based criteria. The decision limit for diagnosing GH deficiency is a maximum of 8 µg/L for assays that use the calibrator 98/574. In adults, a single dynamic test should be performed for the diagnosis of GH deficiency. The traditional decision limits for severe GH deficiency are <3.0 µg/L (ITT) and/or <9.0 µg/L (GHRH-arginine, BMI 25–30 kg/m^2). The exclusive determination of IGF-I to screen for GH deficiency is only of limited use for all age groups, but the IGF-I analysis should be included in the monitoring of GH therapy.

Conclusions

The comparability of currently available commercial assays for the determination of GH and IGF-I is still insufficient today. Therefore, it is necessary to raise awareness about the quality of immunoassays, make rational use of diagnostic tools, and counteract an interpretation of measurement results that is often too uncritical. The primary objective should be to make the quality criteria for assays known and thus motivate the manufacturers of diagnostic tools to do more for their implementation. Quality criteria and guidelines should be evaluated regularly to ensure a sustainable quality assurance process. Manufacturers, as well as experts from relevant professional associations, should also plan and conduct interdisciplinary studies to ensure an independent validation of assays and the establishment of assay-specific decision limits and/or reference intervals for appropriate reference populations. Finally, physicians should know about the quality and assays of laboratories and take into account the clinical context of the individual patient when it comes to diagnostic strategy, treatment decisions and follow-ups.

Consequences for clinical and medical practice

Everyone has a role to play in the quality assurance of GH and IGF-I analyses: Manufacturers should check and document regularly the quality criteria for their assays and their compliance with the recommendations of the Keswick consensus. Members of professional associations and expert groups should insist that only assays be used that meet their quality requirements. Laboratories should use only assay-specific and sufficiently validated limits and reference intervals as well as independent targets of quality control samples, and participate in external interlaboratory tests. Clinicians should make treatment decisions only in full knowledge of a laboratory's methodology and quality. An additional suggestion would be that journals admit for publication only studies that involve quality-assured assays.

Acknowledgments: The article is based on the findings of the expert workshop "Quality assurance in endocrinological diagnosis and therapy", held in Hohenkammer, Bavaria, on June 1 and 2, 2012. Workshop participants: Matthias Auer, Martin Bidlingmaier, Michael Droste, Diana-Alexandra Ertl, Harald Etzrodt, Gwendolin Etzrodt-Walter, Berthold P. Hauffa, Renate Hiefinger-Schindlbeck, Jürgen Kratzsch, Sylvia Omran, Stephan Petersenn, Michael B. Ranke, Robert Rivest, Christian J. Strasburger, Peter J. Trainer, Stefan van Buuren, Henri Wallaschofski, Detlef Waller.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.
Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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