Contents

Introduction

Disorders of the Growth Hormone (GH)/Insulin-like Growth Factor-I (IGF-I) axis such as acromegaly and GH deficiency (GHD) in childhood, adolescence and adulthood, are commonly determined by measuring levels of GH, IGF-I and Insulin-like Growth Factor Binding Protein-3 (IGFBP-3)[1-3]. During the last 20 years, several consensus statements have been established describing how to utilise and interpret these parameters in clinical practice[4-13]. However, despite a number of publications discussing the implications of the variability in GH[14-17] and IGF-I[18-22], the ways in which differences between immunoassays can influence the interpretation of these consensus criteria it is not always taken into account[23]. A failure to recognise the impact of those discrepancies can have serious implications for the treatment of a patient.

GH, IGF-I and IGFBP-3 determinations are used by clinicians in the fields of pediatrics, endocrinology, and by general practitioners, diagnosing patients for the first time and monitoring progression of disease or efficacy of therapy. Assays should therefore be robust enough to allow clinicians a meaningful long-term follow-up of patients, for example in those receiving recombinant GH therapy. From their perspective, clinicians should have an understanding of the absolute numbers each assay reports when applying clinical cutoffs, such as during dynamic testing. They should also be aware of the performance characteristics and limitations of various methodologies, for instance when they are presented with low concentrations of analytes especially during suppression tests. Similarly, laboratory scientists working closely with clinicians should be aware of issues affecting assay performance such as pre-analytics, the impact of assay standardisation, and the ways inherent assay features influence results. Assay manufacturers are, in turn, obliged to provide the laboratories with methods which combine efficient workflow, accuracy and minimal variability in results, proof coming from appropriate external quality assessment schemes, and sufficiently detailed reference ranges to allow appropriate target ranges.

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Chapter 1
Physiology

Chapter 1: Physiology

GH secretion by the anterior pituitary gland is pulsatile and diurnal in nature. Secretion is regulated by hypothalamic Growth Hormone Releasing Hormone (GHRH) and somatostatin. GH circulates in various isoforms, which, in addition to their monomeric forms, also form homo- and heterodimers and multimers. The major circulating isoform of GH is the 22kDa (191 amino acids) followed by the 20kD isoform (176 amino acids). GH acts via plasma membrane GH receptors. It has direct effects in some tissues, but mainly stimulates production of the insulin-like growth factors IGF-I in liver and other tissues. IGF-I in turn mediates many of the anabolic and metabolic actions of GH[24-26].

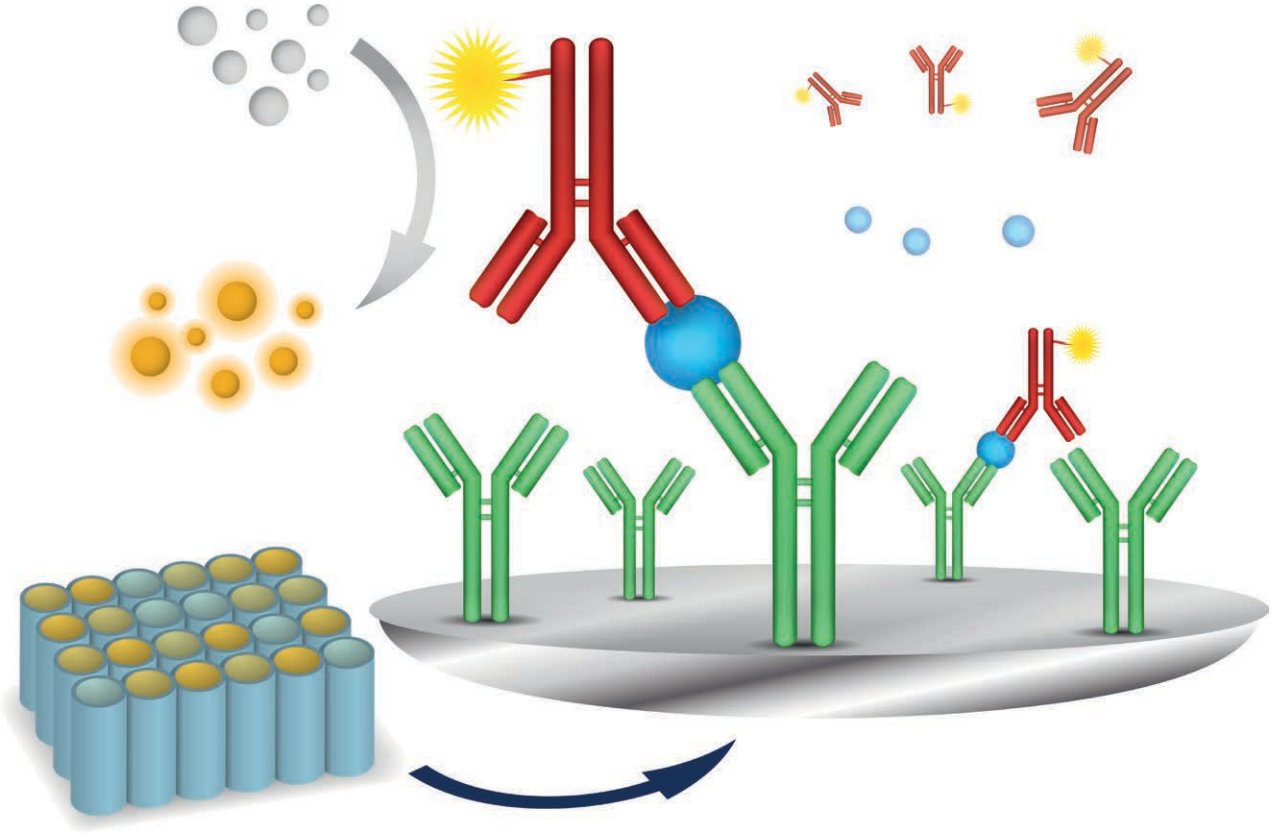
Insulin-like Growth Factor -I (IGF-I) secretion is mostly dependent on Growth Hormone (GH) secretion, with an impact of nutrition and age. IGF-I is a polypeptide (7649Da) with 50% sequence homology to proinsulin and several biological functions similar to those of insulin. As well as mediating the actions of GH, IGF-I has a negative feedback effect on GH release and also inhibits apoptosis. It acts through two transmembrane receptors which are similar in structure to the insulin receptor. The IGFs circulate in blood complexed to six binding proteins (IGFBPs). Insulin-like Growth Factor Binding Protein-3 (IGFBP-3) is the major circulating species and it forms a 150kDa ternary complex which is comprised of an acid labile subunit (ALS), IGFBP-3 and IGF-I or IGF-II. The secretion of IGFBP-3 is GH dependent, being decreased in GH-deficiency and increased in acromegaly[27-29].

Measurement of the GH – IGF axis

Due to the variable levels of GH secretion and its short half-life, single measurements of circulating GH are less meaningful in the assessment of growth-related disorders. Diagnosis is usually confirmed by dynamic testing, i.e. a stimulation test with measurement of peak serum GH concentration or by a suppression test where the ability to suppress serum GH to less than a given value, following oral glucose load, is assessed. The levels of the substances which are under the control of GH (e.g. IGF-I and II, IGFBP-2 and -3, ALS) can also be used as an indirect assessment of GH secretion in the diagnosis of patients with GH disorders. IGF-I and IGBP-3, however, represent the key markers for the evaluation of growth hormone status[30,31].

The fully automated IDS-iSYS growth panel

- The growth panel offers fully automated chemiluminescence assays which measure hGH, IGF-I and IGFBP-3 from a single sample tube
- A fully automated walk-away system
- High throughput
- Continuous loading of samples, reagents and consumables
- Low sample volume
- On-board refrigeration of ready-to-use reagents
- Full reagent, consumable and sample traceability
- Available additional software converts the IGF-I and IGFBP-3 concentrations into Standard Deviation (SD) Scores



IDS-iSYS hGH and IGF-I assays comply with consensus guidelines*

*International consensus workshop on GH and IGF-I at Keswick Hall, Virginia, 2009[6]

IDS-iSYS human Growth Hormone (hGH)

- Calibrated to the WHO International Standard 98/574
- Specific for the 22kDa isoform
- Limit of quantification of 0.05 ng/mL
- No interference from binding proteins, 20kDa isoform and therapeutic analogs
- Assay specific reference data for stimulation and suppression tests

IDS-iSYS Insulin-like Growth Factor-I (IGF-I)

- Calibrated to the WHO International Standard 02/254
- No interference with the binding proteins
- Validation of different sample types
- Largest set of normative data (percentiles, narrow and gender-specific intervals for children, Tanner Stages)

Additional superior assay features

IDS-iSYS human Growth Hormone (hGH) assay

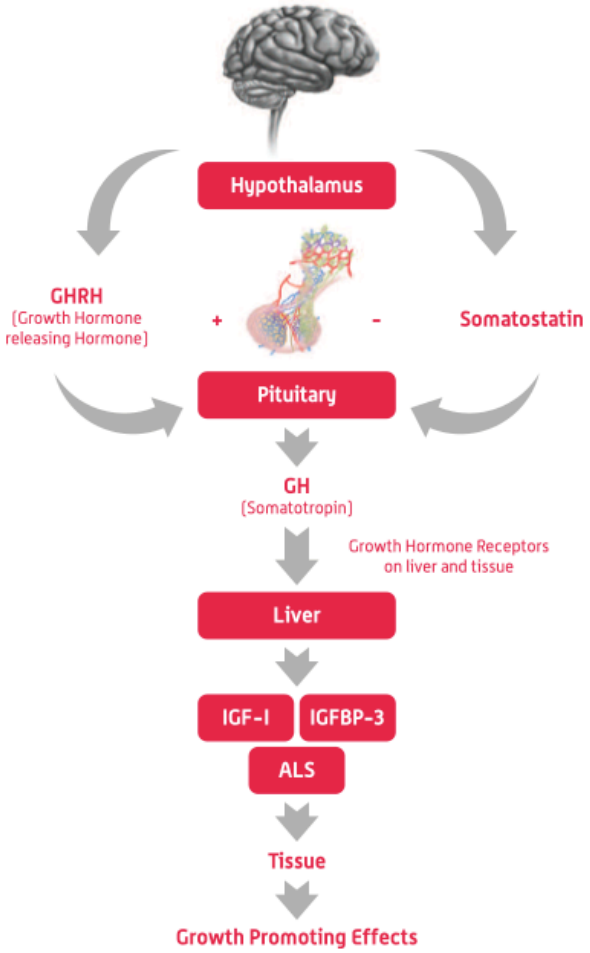
- No interference with Pegvisomant (Somavert®)
- Possibility to monitor pituitary GH secretion in pregnancy
- Monoclonal antibodies providing consistency from lot-to-lot

IDS-iSYS Insulin-like Growth Factor-I (IGF-I) assay

- IGF-II displacement method eliminates the interference of binding proteins
- Two monoclonal antibodies provide high consistency from lot-to-lot
- Ensured lot-to-lot consistency through 3 external laboratories at every lot change
- Validation of sample stability
- Published correlation to existing methods

IDS-iSYS Insulin-like Growth Factor Binding Protein-3 (IGFBP-3) assay

- Two monoclonal antibodies provide high consistency from lot-to-lot
- No cross reactivity with IGF-I, IGF-II, Insulin and all IGFBPs
- Comprehensive normative data (percentiles, narrow and gender-specific intervals for children, Tanner Stages)
- Validation of different sample types and of sample stability
- Published correlation to other methods



Chapter 2
Quality

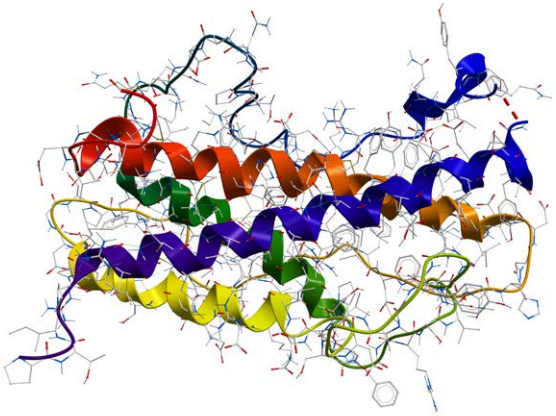
Chapter 2: Quality

The considerable variability that exists between the numerous Growth Hormone (GH) and Insulin-like Growth Factor-I (IGF-I) assays currently available on the market limits the application of consensus guidelines defining treatment algorithms in clinical practice[23]. At a recent international workshop including the Growth Hormone Research Society, the IFCC, the International Society for IGF Research, and the Pituitary Society, it was deemed necessary to have major improvements in the areas of GH and IGF-I assay performance and comparability[6]. In order to achieve this, a consensus statement outlined several recommendations to overcome the obstacles which are currently faced by clinicians and laboratories due to the inherent differences in assay characteristics. These included the use of commutable reference standards, clarity from assay providers on antibody specificities, matrix requirements, and information on interference from binding proteins.

Growth Hormone

Requirements for GH assays:

- Use of the second WHO International Standard (IS) preparation (98/574) with a purity of 96% 22kDa GH[7]. It is recommended that concentrations be reported in mass units[6]. The use of a common, pure, standard reduces the differences between various GH assays[32].
- The assays should use antibodies specific for the 22kDa hGH isoform. To ensure the correct interpretation of results, cross-reactivity to other isoforms should be stated for each GH assay[6,33,17].
- For the measurement of hGH during Pegvisomant (Somavert®) treatment it is essential that the antibodies used do not cross-react with this drug due to the structural homology and the 100-1000-fold drug concentrations used as compared to endogenous GH levels.
- In order to accurately measure low levels of hGH, a limit of quantification (LoQ) of 0.05ng/mL is recommended. A feature which is important in the diagnosis and monitoring of acromegaly by means of the glucose tolerance test[6].
- Each assay´s susceptibility to growth hormone-binding protein (GHBP) interference has to be specified[6]. In some assays GHBP can lead to steric blocking of the epitopes of the antibodies used. This can induce a negative bias of up to 50% even when GHBP concentrations are still in the physiological range[17].



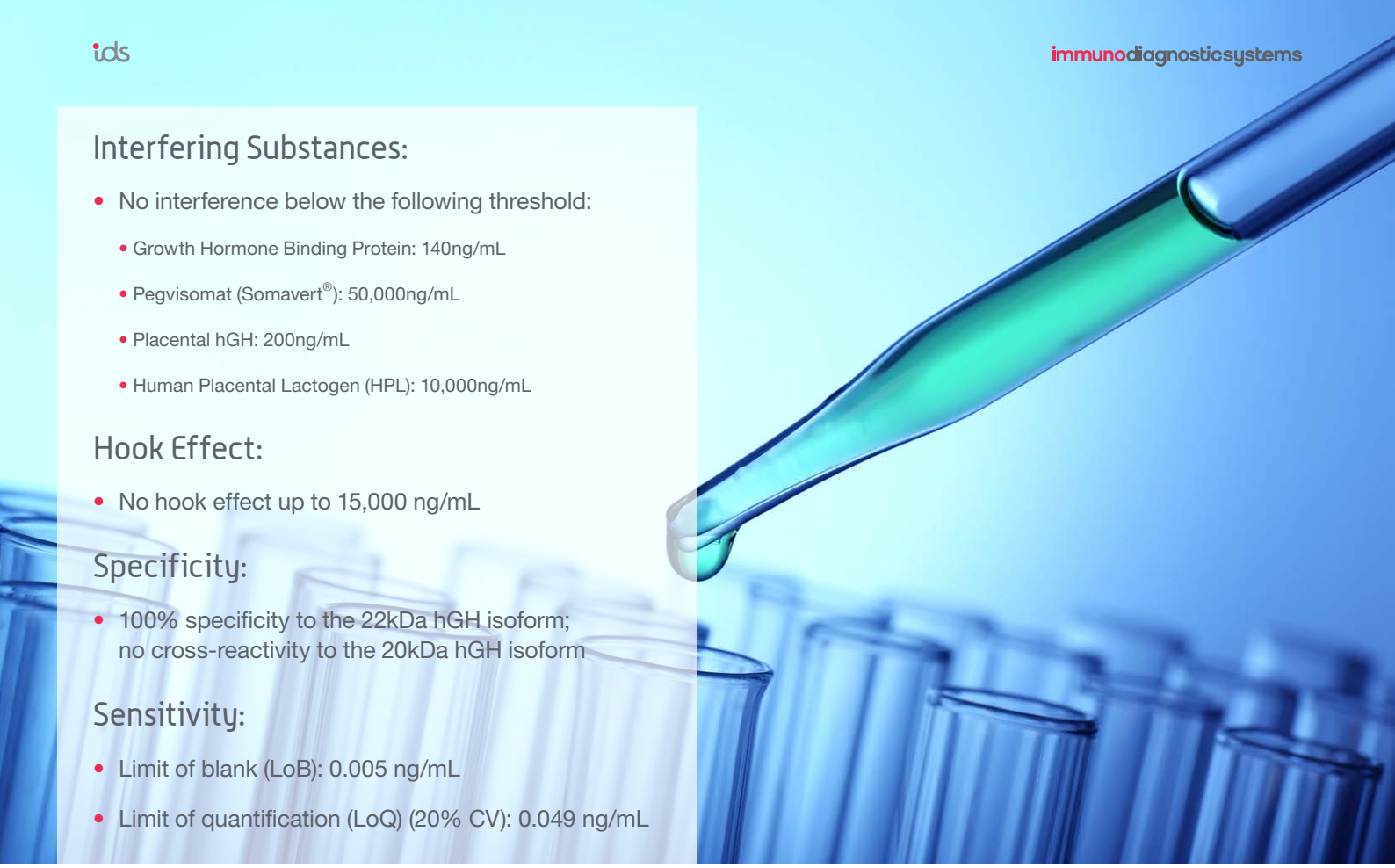
IDS-iSYS human Growth Hormone[34]:

Calibration:

- The assay is calibrated to the WHO International Standard (IS) (98/574) recombinant DNA-derived and consisting only of the 22kDa isoform
- Two monoclonal antibodies are used with well characterized epitopes, high affinity and specificity for the 22kDa isoform[35]

Measurement Range:

- The reportable range is 0.05 to 100ng/mL



Interfering Substances:

- No interference below the following threshold:
  - Growth Hormone Binding Protein: 140ng/mL
  - Pegvisomat (Somavert®): 50,000ng/mL
  - Placental hGH: 200ng/mL
  - Human Placental Lactogen (HPL): 10,000ng/mL

Hook Effect:

- No hook effect up to 15,000 ng/mL

Specificity:

- 100% specificity to the 22kDa hGH isoform; no cross-reactivity to the 20kDa hGH isoform

Sensitivity:

- Limit of blank (LoB): 0.005 ng/mL
- Limit of quantification (LoQ) (20% CV): 0.049 ng/mL

Sample Type:

- Serum (standard sampling tubes or serum separation gel) or plasma (sodium citrate, lithium heparin, sodium heparin, ammonium heparin)

Can be used during pregnancy:

- No cross-reactivity with placental GH or placental lactogen[35]

Can be used during Pegvisomant treatment:

- Measurement of endogenous hGH only[35,36]

Highly quantitative agreement to mass spectrometric measurements:

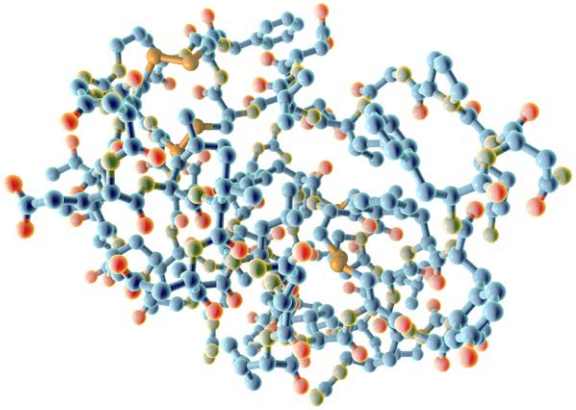
- Correlation was found with $R > 0.94$[37]

Insulin-like Growth Factor-I

Requirements for IGF-I assays:

- The use of the WHO IS 02/254 as a single universally accepted standard. The application of different standards is the main reason for divergent results of different assays[6]. Furthermore the older WHO standard 87/518 has a low purity and therefore assays that are calibrated against it will give concentrations in excess of actual values[38].

- The respective assays' susceptibility to binding protein interference has to be specified. To prevent IGFBP interference, a dissociation method and prevention of re-association is recommended. This method should ideally be validated against a size-exclusion gel chromatography method performed at low pH (the current reference method for eliminating interference of binding protein)[6] and samples should be spiked with the binding proteins in appropriately high concentrations to prove non-interference. Remaining binding proteins can be problematic especially in samples from patients with disproportionately elevated IGFBPs such as type 1 diabetes, liver cirrhosis or chronic renal failure[39]. Results from different assays may therefore also depend on the patient's underlying health status. For instance, studies indicate that sufficient correlation exists between different assays in samples from healthy subjects but not in those with diabetes[40].
- Protocols for sample handling should be provided – especially the sample stability with various storage conditions and the effect of repetitive freezing and thawing. The use of anticoagulants (heparin, citrate, and EDTA) requires validation[6,39].
- Implementation of a quality program to maintaining a batch-to-batch consistency. Lot-to-lot validation studies have to identify a possible change from every new lot to the preceding. Monitoring of the distribution of patient results may be the best way to detect lot-to-lot inconsistencies[41].



IDS-iSYS Insulin-like Growth Factor-I (IGF-I)[42]:

Calibration:

- The calibrator value assignment is traceable to the recombinant WHO International Standard (IS) 02/254
- Two highly specific monoclonal antibodies are utilised[43]

Measurement Range:

- The reportable assay range is 10 to 1,200ng/mL

Hook Effect:

- No hook effect up to levels of 20,000ng/mL

Sensitivity:

- Limit of blank (LoB): 1.9ng/mL
- Limit of quantification (LoQ; 20% CV): 8.8ng/mL

Specificity:

- 100% specificity to IGF-I; < 0.01% cross-reactivity to IGF-II, Proinsulin and Insulin

Sample Pretreatment and Interfering Substances:

- Method of IGF-I displacement with an excess of IGF-II[21,43] is used
- No interference from any of the binding proteins (IGFBP-1 to IGFBP-6) at supra-physiological concentrations[42]
- A close correlation to gel chromatography at low pH (R =0.846)[44], the current reference method for elimination of IGFBP interference[6]

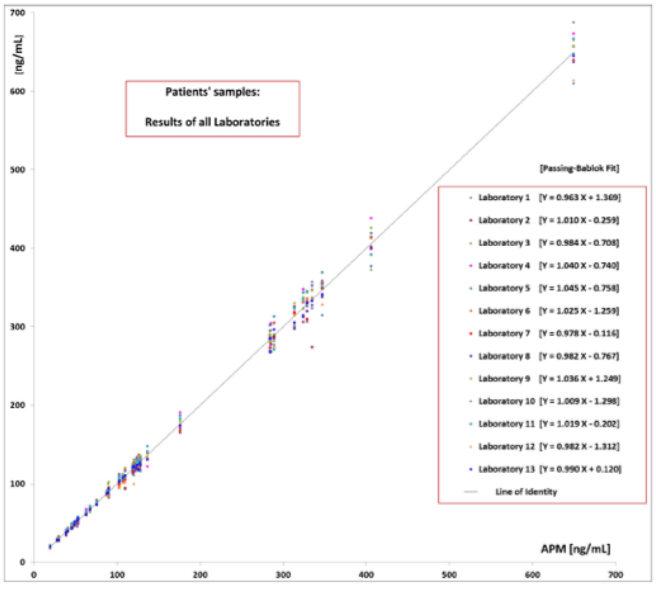


Fig 1. Results of all laboratories: IGF-I of Patients' samples compared to all participants mean (APM).

Sample Type:

- Serum (standard sampling tubes or serum separation gel) or plasma (lithium heparin, sodium heparin, potassium EDTA)[42]. Sodium citrate cannot be used[43]

Sample Stability:

- Up to 5 days at room temperature[45]; in whole blood at room temperature for 24 hours

Concordance to LC-MS/MS:

- Correlation was found with a correlation coefficient R = 0.83[46]

Lot-to-Lot Stability[47]:

- Every new lot is compared to the previous by 3 laboratories with analysis of clinical samples
- A study with 2 sets of clinical samples first measured in 2010 was reanalysed in 2012/2013
- Assessment of all patient median from > 60,000 samples analysed between 2013 and 2014

Evaluation of the between laboratory agreement:

- 52 samples were measured in 13 laboratories across Europe[48].

Insulin-like Growth Factor Binding Protein-3

Requirements for IGFBP-3 assays:

In normal physiological conditions, most IGF-I is bound by IGFBP-3 associated with the acid-labile subunit (ALS) in a 150-kDa ternary complex with a long half-life. While IGF-I inhibits apoptosis, the suppressive effects on cell growth of IGFBP-3 are accomplished via two major mechanisms: limiting the availability of IGF-I and inducing direct pro-apoptotic effects in a variety of tumor cells.

Various proteases have been shown to cleave IGFBP-3. PAPP-A, matrix metalloproteinases, cathepsin, plasmin and thrombin all have the ability to degrade IGFBP-3[49].

- As IGFBP-3 is susceptible to significant proteolysis, immunoassays with the equivalent determination of intact and fragmented IGFBP-3 would be of great value. Enhanced IGFBP-3 proteolysis occurs in pregnancy and in response to a variety of catabolic conditions[50].

It is well known that IGFBP-3 circulates in different forms resulting from glycosylation: two different glycoforms which reduce to a single form after enzymatic deglycosylation. Non-glycosylated rec-human-IGFBP-3 was shown to be identical to fully glycosylated rec-human-IGFBP-3 in its IGF-I binding activity[51].

IDS-iSYS Insulin-like Growth Factor Binding Protein-3[52]:

Calibration:

- The assay is traceable to the National Institute for Biological Standards and Control (NIBSC) material 93/560
- The assay is calibrated with working standards using a recombinant glycosylated human IGFBP-3[53]
- The assay uses two highly specific monoclonal antibodies: a biotinylated anti-IGFBP-3 monoclonal antibody and an acridinium labelled anti-IGFBP-3 antibody

Measurement Range:

- The reportable range is 80 to 10,000ng/mL

Sample Type:

- Serum (standard sampling tubes or serum separation gel) or plasma samples (lithium heparin, sodium heparin, potassium EDTA). Citrate and oxalate plasma cannot be used[53]

Sample Stability:

- Up to 4 days (4 °C and room temperature) and up to four freeze-thaw cycles[53]

Hook Effect:

- No hook effect up to 100,000ng/mL

Sensitivity:

- Limit of blank (LoB): 30 ng/ml
- Limit of quantification (LoQ; 20% CV): 80 ng/ml[52]; (verified externally at <50ng/ml[53])

Specificity:

- < 0.1% cross-reactivity to the IGFBP-1,-2,-4,-5 and -6

Not affected by proteolysis:

- Measures total IGFBP-3 i.e. includes fragments which are present in samples[53]

Detection of the glycosylated form:

- Full recovery of the glycosylated (rec-human)-form spiked in human samples containing high endogenous IGFBP-3 concentrations[53]

Chapter 3
Clinical Indications

Chapter 3: Clinical Indications

GH deficiency and GH excess

The measurement of serum IGF-I and GH is essential in the diagnosis and monitoring of GH deficiency (GHD) and GH excess.

GH secretion is pulsatile and diurnal and may be influenced by exercise and sleep. GH has a short half-life of approximately 20 minutes. Single measurements of serum GH are not usually helpful in assessment of GHD, since levels are usually low in healthy, awake persons.

The diagnosis of GHD, which is the requirement for GH replacement therapy, is confirmed by GH stimulation tests defined as a peak serum GH concentration below a specific cutoff value. Since no single stimulation test has a sensitivity and specificity of 100% at least two tests are recommended. Current GH cutoff levels for children and adolescents are more or less arbitrarily determined[37].

Multiple tests are available:

- Arginine Stimulation Test/ Arginine + GHRH (growth hormone releasing hormone) Test
- Insulin Hypoglycemia Test
- Glucagon Stimulation Test
- Clonidine Stimulation Test

The value of serum IGF-I and IGFBP-3 in the diagnosis of GHD is still under discussion: nevertheless IGF-I, and/or IGFBP-3, concentrations below -1SD score are sufficient for the strong suspicion of GHD according to the German diagnostic guidelines[54]. Because IGF-I is protein bound, its half-life in serum is much longer than GH, and it is not subject to pulsatile variability. It has been shown, that age and the time of onset of GHD have a significant influence on IGF-I levels[55].

Accurate measurement of IGF-I and IGFBP-3 is widely established in the monitoring of GH replacement therapy. Titration of GH dose to maintain these concentrations in their age-related normal ranges is recommended by the consensus guidelines[56]. IGFBP-3 is particularly useful in young children, in whom serum IGF-I levels are in a similar range in GHD and non-GHD[57]. In a recently published systematic review and meta-analysis of 12 studies about the diagnostic values of serum IGF-I and IGFBP-3 for GHD it was stated that they are useful for the diagnosis of GHD and that they can be utilised as auxiliary diagnostic indexes for provocative testing. The pooled sensitivity and specificity of IGF-I in GHD diagnosis was 0.66 and 0.69 respectively, and of IGFBP-3 0.50 and 0.79, respectively[58].

Hypersecretion of GH, commonly due to a pituitary adenoma, leads to gigantism if it occurs before epiphyseal closure and results in acromegaly thereafter.

The majority of patients with acromegaly have GH concentrations greater than 5ng/mL in fasting basal samples drawn at bed rest. But no single elevated GH value can verify the diagnosis of acromegaly. On the other hand, a serum IGF-I level is a tool to assess integrated GH secretion and is useful for screening and diagnosis. If the level is elevated, diagnosis is usually confirmed by functional testing. The inability to suppress serum GH to less than a distinct value after an oral glucose load is considered the diagnostic criterion for acromegaly (oral glucose tolerance test, OGTT). In healthy individuals, GH falls to less than 0.2–0.3 ng/mL one or two hours after glucose[59].

Patients should be followed after treatment of acromegaly to assess the effectiveness of the therapy. Monitoring of serum IGF-I and GH levels in acromegaly patients is essential. Elevation of IGF-I is considered a sensitive and specific indicator for the persistence of disease after therapy[56].

Although in the diagnosis and follow-up of acromegaly and gigantism, IGFBP-3 measurement adds little to IGF-I testing, some studies suggest that IGFBP-3 measurements may be helpful in confirming the diagnosis of acromegaly with coexisting uncontrolled diabetes and distinguishing it from high GH levels attributable to poor control of diabetes[60].

Some disorders with symptoms similar to those of GHD

- Turner syndrome: multiple factors of impaired growth, including abnormalities in the GH/IGF-I and IGFBP axis
- Prader-Willi syndrome: associated with reduced GH secretion and low IGF-I levels
- Silver-Russell syndrome: characterised by growth retardation and occasionally associated with GH deficiency
- Laron syndrome: characterised by insensitivity to GH and very low levels of IGF-I and IGFBP-3
- Small for Gestational Age (SGA) without catch-up growth: GH Therapy with IGF-I and IGFBP-3 monitoring
- SHOX-D (Short stature homeobox gene deficiency): GH Therapy with IGF-I and IGFBP-3 monitoring
- Primary IGF-I deficiency (IGFD): normal GH secretion in the presence of low IGF-I and low IGFBP-3

Epidemiologic investigations

IGF-I and IGFBPs have been extensively studied in epidemiologic investigations. Several studies have associated circulating levels of these proteins with incident diabetes, heart failure, stroke, coronary heart disease, several types of cancer and overall mortality[40].

For example some studies referring to cancer risk:

- Insulin-like growth factors (IGFs) and their binding proteins (IGFBPs) are associated with prostate cancer[61]
- IGF-I concentration is positively associated with risk for prostate cancer[62]
- IGF-I concentrations may be positively associated with risk of differentiated thyroid carcinoma[63]
- Increasing risk of breast cancer with increasing levels of IGF-I and IGF-I:IGFBP-3 ratio[44]
- Subjects with higher serum levels of IGFBP-3 were at reduced risk of lung cancer[64]

Chapter 4
Normative Data

Chapter 4: Normative Data

Growth Hormone

Human Growth Hormone (GH) levels fluctuate during the day as a consequence of the hormone's regulation by several factors, such as nutrition, stress, time of day, sleep, and physical exercise. Additionally, there is an increased pulsatility observed during puberty[65]. Due to this pulsatile secretion, single hormone levels are not very meaningful. In healthy subjects, inter-pulse GH levels often fall below assay sensitivity, making it unclear whether secretion stops or not. On the other hand, the concentration of GH during the peaks may range from 5 to 30ng/mL or more. Peaks typically last from 10 to 30 minutes before returning to basal levels.

Diagnosis and treatment of GH disorders are based on measurements of GH during dynamic (stimulation or suppression) tests such as the clonidine test, the insulin tolerance test, the glucagon and GHRH tests[66]. Stimulation tests have become standard practice in the assessment of children with short stature when deciding the GH secretory status and in the evaluation of the therapeutic benefit of the GH therapy[67]. Due to several factors affecting agreement between immunoassays, the expected level of response and cutoffs to be applied to the various dynamic tests depend on the respective assay used[15]. Depending on the method used, measured GH values can vary remarkably. Discrepancies in results and requirements for harmonisation, such as calibrator preparation, antibody specificities and GHBP interference[54] were discussed in Chapter 2.

Nevertheless, the general definition of growth hormone deficiency has been considered to be a peak stimulation of GH less than 10ng/mL, however peak cutoffs are very much reliant upon age, gender and body mass index (BMI). This cutoff level was increased in the last years from 5ng/mL to 7ng/mL, and to 10ng/mL most recently. A maximum of 8ng/mL has been suggested in Germany[37].

In fact, a recent publication has shown that the diagnostic accuracy of the GH releasing hormone plus arginine test (GARG), is significantly improved by use of BMI- and gender-adjusted cut-offs[72].

The measurement of GH after the oral glucose tolerance test (OGTT) is used for the diagnosis of acromegaly. In general, GH levels below a specific level during OGTT would be viewed as a manifestation of normal GH secretion, while patients with acromegaly will still have elevated GH levels. At present, values <1ng/mL are considered to indicate normal suppression and that such levels exclude acromegaly or establish its remission[68]. There is no general exact value available because this cut-off depends on the assay used[17] and is influenced by gender, age and BMI[68].

As long as the current discrepancies in assay measurements continue, instead of using these arbitrary cutoff levels it is mandatory to use correctly validated, assay-specific reference data for these stimulation tests[6].

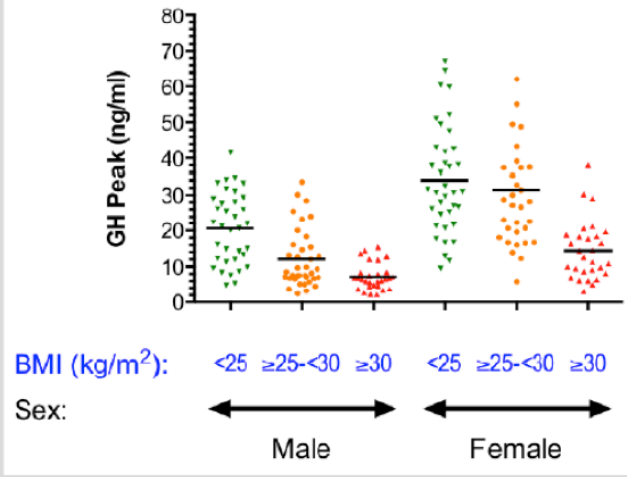


Fig. 1. Figure 1. Peak GH levels during the GARG test in 200 healthy subjects, stratified by sex and BMI. Dark lines represent the means for each group. Abbreviations are: GARG, growth hormone releasing hormone plus arginine; GH, growth hormone.

IDS-iSYS hGH stimulation in children and adolescents[37]

- Discriminant analysis gave a cutoff limit of 7.09 ng/mL for GH stimulation tests in children and adolescents
- For this cutoff the area under the curve relative to the total area in the ROC analysis is 99.1%
- This cutoff is unique for stimulation with arginine, glucagon and insulin

IDS-iSYS hGH stimulation with GHRH plus arginine in adults[69]

- Overall cutoff limit for both sexes of 3.9 ng/mL
- BMI-adjusted for males are 6.5, 3.5 and 2.2 ng/mL (for lean, overweight and obese subjects)
- BMI-adjusted for females are 9.7, 8.5 and 4.4 ng/mL (for lean, overweight and obese subjects)

IDS-iSYS hGH stimulation in adults with the Insulin Tolerance Test (ITT)[73]

- A cut-off of 2.6 ng/mL should be used in adults to diagnose GHD
- The study stressed the importance of appropriate control groups and stringent GH-testing including confirmatory testing in cohorts with low likelihood of GHD such as in Traumatic Brain Injury.

IDS-iSYS hGH suppression with Oral Glucose Tolerance Test[35]

- In healthy subjects, the mean nadir was 0.05 (maximum 0.09) ng/mL in males and 0.11 (maximum 0.34) ng/mL in females
- No nadir value was observed in the treatment-naive patients with acromegaly

IGF-I and IGFBP-3

For the interpretation of IGF-I and IGFBP3 levels it is mandatory to have method-specific normal ranges, and know their variations by age, sex, and pubertal stage. As for GH, method discrepancies also exist for IGF-I assays, owing particularly to interference from IGF binding proteins. IGFBP interference is particularly relevant as concentrations vary in different clinical conditions (see Chapter 2). IGF-I and, to a somewhat lesser extent, IGFBP-3 levels are age-dependent, showing low levels after birth, a pubertal peak and a decline after puberty. In adults the levels decrease continuously with age.

Because IGF-I and IGFBP-3 concentrations show the greatest variability during childhood and puberty, reference interval studies must include sufficient numbers of samples in the age intervals of this period (n>120 for each age group[70]) to provide sufficiently narrow age ranges (every 3 years[6] or ideally at a yearly increment[70]) and include Tanner stages. For ages between 6 and 18 years, gender-specific IGF-I and IGFBP-3 intervals are required[39]. Ethnic origin and Body Mass Index (BMI) only have a minor influence on the measurement of IGF-1 and IGFBP-3[70]. The results of the reference studies must include the 2.5 to 97.5 percentiles and the possibility to calculate the SD scores.

The first normative data for the IDS-iSYS IGF-I assay, based on the recommendation of the consensus conference held in 2009[6] and in a very large population was published by Bidlingmaier et al in 2014[43].

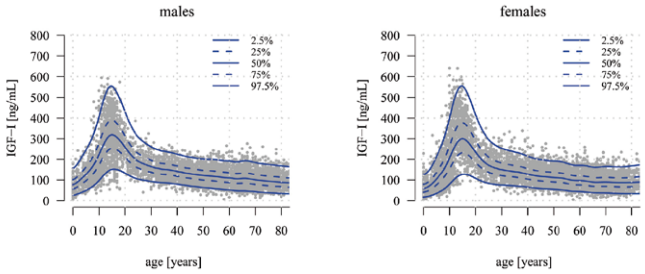


Figure 2. Individual points and fitted percentiles for males (right side) and females (left side). Displayed are the 2.5%, 25%, 50%, 75% and 97.5% percentiles calculated by quantile regression via vector generalized additive models (LMS VGAM method).

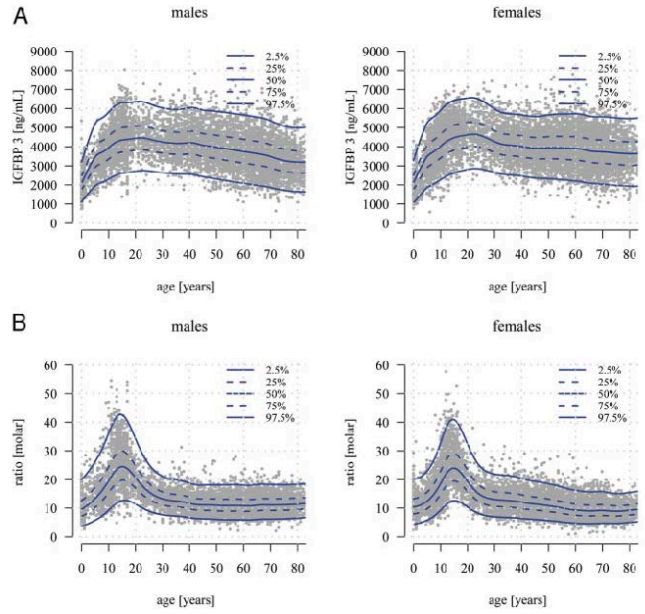


Figure 3. Serum IGFBP-3 (a) and IGF-I/IGFBP-3-ratio (b) values in approximately 15,000 subjects for males (left side)and females (right side). The lines represent the 2.5%, 25%, 50%, 75% and 97.5% percentiles calculated by quantile regression via vector generalized additive models. Ratio = IGF-I[nmol/l] / IGFBP-3[nmol/l]* 100.

IDS-iSYS Insulin-like Growth Factor-I (IGF-I)

- **A multicenter reference study using a large cohort (n = 15 014) of well-characterized subjects[43]**

  Table 1: Details of the Cohorts

- Age- and sex-adjusted reference intervals with 2.5%, 50% and 97.5% percentiles and LMS values
  (necessary to calculate the SD scores)

  Table 2: Percentiles and LMS Charts

  More detailed lists in Supplement Tables 15 and 16, published at http://jcem.endojournals.org

- Percentiles according to Tanner Stages

  Table 3: Percentiles according to Tanner Stages

- Reference intervals in a cohort of 899 adults[71]

  Table 2: Normative reference intervals of IGF-I measured by 6 assay methods according to age range and sex in a cohort of 899 healthy subjects

IDS-iSYS Insulin-like Growth Factor Binding Protein-3 (IGFBP-3)

- **Age- and Sex-Specific Reference Intervals Across Life Span (n = 14 970)[53]**

  Table 1: Details of the Cohorts

- Age- and sex-adjusted reference intervals with 2.5%, 50% and 97.5% percentiles, the LMS values (necessary to calculate the SD scores) and the IGF-1 to IGFBP-3 Ratio

  Tables 2 and 3: Percentiles and LMS Charts for IGFBP-3 and for IGF-1 to IGFBP-3 Ratio

  More detailed lists in Supplement Tables 12 and 13, published at http://jcem.endojournals.org.

- Percentiles according to Tanner Stages[53]

  Table 4: Percentiles according to Tanner Stages

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