Does plasma IGF-BP3 measurement contribute to the diagnosis of growth hormone deficiency in children?

La mesure du plasma IGF-BP3 contribue-t-elle au diagnostic du déficit en hormone de croissance chez l’enfant ?

S. Kayemba-Kay’s, S. Epstein, P. Hindmarsh, A. Burgueta, P. Ingrand, R. Hankard

Paediatric Endocrinology Unit, Department of Pediatrics, Poitiers University Teaching Hospital, 86021 Poitiers, France
Paediatric Endocrinology Department, Institute of Child Health, University College, London WC1N 1EH, United Kingdom
Medical Informatics & Statistics Department, Poitiers University Teaching Hospital, 86021 Poitiers, France

Résumé

Objectif. – Évaluer l’apport du dosage plasmatique de l’IGF-BP3 au diagnostic du déficit en GH chez l’enfant. Population et méthodes. – Étude rétrospective d’enfants vus en consultation d’endocrinologie pédiatrique pour bilan de petite taille et/ou suivi post-irradiation, et qui avaient eu au moins un test de stimulation de la GH. Les enfants porteurs d’hypothyroïdie, de syndrome de Laron ou de Kowarski, de malnutrition sévère, d’insuffisance rénale chronique et d’insuffisance hépatique ont été exclus. Résultats. – Cinquante-huit enfants ont été recrutés et classés en deux groupes : GHD [+], (19 cas) et GBD [–], (39 cas). Les dosages d’IGF-I et IGF-BP3 ont été réalisés chez 88 % et 63 % cas respectivement, les deux groupes étaient comparables en ce qui concernent l’âge, le sexe, l’IMC, la taille cible, le stade pubertaire et l’âge osseux. Le pic de GH était significativement différent entre les deux groupes (GHD [+], 41.8 ± 21.7 mUI/L versus 11.5 ± 5.9 mUI/L pour le groupe GHD [–], p < 0.00001). Aucune différence n’a été mise en évidence entre les deux groupes pour les scores d’IGF-I et d’IGF-BP3. Il existait en revanche, une corrélation positive entre le score d’IGF-I et celui d’IGF-BP3 (r = 0.50 ; p < 0.0016).

Conclusion. – Dans cette étude, le dosage d’IGF-BP3 n’a pas permis de différencier les enfants ayant un déficit en GH de ceux qui étaient indemnes. Nous ne recommandons pas son usage en routine.

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Mots clés : IGF-BP3 ; GHD ; Enfants ; Diagnostic ; Contribution

Abstract

Objective. – To audit the contribution of plasma IGF-PB3 measurement to the diagnosis of growth hormone deficiency (GHD) in children. Population and methods. – Retrospective case study including boys and girls aged 0 to 18 years who attended our paediatric endocrinology clinic for short stature and/or post-irradiation follow-up, and had at least one GH provocative testing. Children with hypothyroidism, Laron or Kowarski syndromes, severe malnutrition, chronic renal failure and liver failure were excluded. Results. – Fifty-eight children were enrolled and grouped as GHD [+], (19 cases) and GDH [–], (39 cases). IGF-I and IGF-BP3 assay was carried out in 88% and 62% cases respectively, both groups were comparable for age, sex, BMI, target height, pubertal stage and bone age. There was a significant difference in peak GH between GDH [–] and GHD [+], groups (41.8 mUI/L ± 21.7 versus 11.5 ± 5.9 mUI/L, P < 0.00001, respectively). No difference was found between groups with regards to IGF-I Z-scores and IGF-BP3 Z-scores. There was, however, a positive correlation between IGF-I Z-scores and IGF-BP3 Z-scores (r = 0.50; P < 0.0016). IGF-BP3 measurement could not differentiate between GHD [+], and GHD [–], groups. Conclusions. – Measurement of plasma IGF-BP3 level contributes poorly to the diagnosis of GHD. We do not recommend it in routine use.

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Keywords: IGF-BP3; GHD; Children; Diagnosis; Contribution

* Corresponding author.

E-mail addresses: k.kayemba-kays@chu-poitiers.fr, kayembakays@yahoo.com.au (S. Kayemba-Kay’s).

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1. Introduction

Growth hormone deficiency (GHD) is conventionally diagnosed, in children, by the association of several parameters that are short stature below –2.0 SD, low growth velocity appreciated preferably over a period of a year, delayed bone age and is confirmed by finding of diminished peak of plasma concentrations of growth hormone following GH provocation test.

Although short stature is common complaint in paediatric endocrinology clinics, GHD remains, however, relatively rare with estimated prevalence of about 1/3000 [1].

In routine practice, ascertainment of GH secretion status relies upon different and numerous pharmacological tests which utilize various types of stimuli (insulin hypoglycaemia, glucagon, arginine, beta-xolol-clonidine, L-Dopa, etc.), interpretation and comparison of which are rather difficult. Although consensus exists on the definition of GHD in children [2], the diagnostic process remains, in itself, a matter of debate: pharmacological tests utilized show both inter-assay variability and lack of reproducibility, besides one child may pass one test and, yet fail another [3,4].

To remedy to this drawback, assessment of spontaneous GH secretion over 24 hours has been suggested as alternative. In spite of it being more physiological, spontaneous GH secretion is costly, time consuming and its results have shown poor correlation with those obtained with provocative pharmacological tests in some studies [5–8]. Additionally, interpretation of a single GH profile is to be carried out with caution [9].

To add to the diagnostic power of provocative pharmacological tests in identifying children with GHD, measurement of both IGF-I and IGF-BP3 plasma concentrations have been suggested as complements [10–15].

In recent years, however, some studies have questioned the contribution of IGF-BP3 assay to the diagnosis of GHD in children [16–19].

The diagnosis of GHD in children carries a number of important clinical consequences as it may subsequently lead to testing of other pituitary functions, performing MRI scanning of hypothalamic pituitary region, genetic screening, and ultimately lead to long-term treatment, etc. GH stimulation can also be risky and induce serious side effects [20]. All these consequences obviously add to economic burden of our health systems.

The above discrepancies along with lack of a single reliable and indisputable diagnostic test capable of identifying children with GHD have led some authors to advocate for GH provocative tests to be simply abandoned [21].

This study was therefore undertaken to audit the contribution of plasma IGF-BP3 measurement to the diagnosis of GHD in a population of children who attended our paediatric endocrinology clinics for short stature and/or post-irradiation therapy follow-up, and underwent growth hormone stimulation tests.

2. Population and methods

This is a retrospective study conducted in the Paediatric Endocrinology Unit, Department of Paediatrics, Poitiers University Teaching Hospital over a period of a year.

2.1. Population

The study population consisted of boys and girls aged 0 to 18 years who underwent at least one pharmacological GH provocative test. Children were seen in our clinics for short stature, or for follow-up following cranial radiotherapy for brain tumor or leukemia. We excluded those children with diagnosis of Laron syndrome, Kowarski syndrome and those with pathologies known to interfere with plasma concentrations of IGF-1, IGF-BP3 (anorexia nervosa chronic malnutrition, unstable type 1 diabetes mellitus, chronic inflammatory disease, chronic renal insufficiency or severe hepatic insufficiency).

2.2. Methods

IGF-I and IGF-BP3 plasma concentrations were measured in those children who were tested on the same day of initial GH provocative testing, prior to GH therapy initiation.

2.2.1. Test performed

Diagnosis of GHD was based on finding of peak plasma concentrations of GH less than 20 mUI/L, and complete GHD (peak GH < 10 mUI/L) following two different pharmacological provocative tests (Ornithine and Clonidine-Betaxolol tests respectively). GH assay was performed using immune radiologic measurement assay (IRMA), which utilizes two monoclonal antibodies that recognizes two epitopes that are different from GH. The first antibody adheres to the tube while the second serves as tracer; at the end of incubation GH contained in the sample fixes the antibodies, and the free material is discarded. The resultant radioactive signal is proportional to GH concentration present.

Our laboratory used kits from AGH CTK from Sorin (Laboratoires Sorin™, France S.A), with intra-assay coefficient of variation (CV) between 1.9 to 3.9%, and inter-assay CV 2.3 to 5.5%, sensitivity of 0.3 mUI/L. From 2006, calibration was changed to IS 98-574, with intra-assay CV between 1.9 and 3.9%, inter-assay CV between 2.5 and 4.1% and a sensitivity of 0.45 mUI/L.

2.2.2. IGF-I assay

Since 2001, our laboratory uses RIA-CT assay without extraction (Mediagnost GmbH, Tubingen, Germany). CV intra-assay less than or equal to 6.4%; CV inter-assay between 7.4 and 9.3%, lowest limit of sensitivity at 0.1 ng/mL.

2.2.3. IGF-BP3 assay

IGF-BP3 assay was performed after sample was centrifuged and stored at –20 °C. by Pasteur Cerba laboratories. The technique employed was IRMA (Immunotech™ SAS, Beckland Coulter™). Intra-assay CV less than or equal to 6.4%; CV inter-assay between 7.4 and 9.3%, lowest limit of sensitivity at 0.1 ng/mL. Both results of IGF-1 and IGF-BP3 were compared to reference normal ranges for age and gender provided by the
manufacturers and reference laboratories (Mediagnost, GmbH, Tubingen, Germany, and our hospital laboratory for IGF-I and Pasteur Cerba Laboratory for IGF-BP3). We also calculated the sensitivity and specificity of IGF-BP3 to evaluate its value as predictor of GHD in the study population.

2.3. Variables collected

From each patient’s record we collected following data: age, sex, height in cm and standard deviation (SD) according to French curves by Sempé et al. [22], weight in kilogram, BMI, pubertal stage according to Tanner staging [23], target height [father’s height + mother’s height ± 13]/2, bone age evaluated according to Greulich and Pyle method [24], past history of small for gestational age (SGA), for those children who had received radiotherapy, we also collected data on their pathology, treatment received and maximum irradiation received in grays. Height was recorded in supine position with infants stadiometer for those children aged less than 2 years, and with wall stadiometer to the nearest 0.5 cm in older children, weight in kilogram was recorded using an electronic scale (SECA®, France).

3. Statistics

Statistics were performed using SAS™ version 8 program. IGF-I and IGF-BP3 are expressed as SD for age and sex and also as Z-scores. To calculate IGF-I Z-scores, we used normal values established by Mediagnost GmbHTM, Tubingen, Germany; for IGF-BP3 Z-scores, we utilized reference curves from Diagnostic systems Laboratories™ (Diagnostic systems Laboratories Inc., Webster, TX, USA). Continuous variables are expressed as mean ± SD, qualitative variables are expressed as number (n) and percentage. Non-parametric test performed as studied values have non-Gaussian distribution and study population small. Values for age, height Z-score, BMI, target height, IGF-I Z-score and IGF-BP3 Z-score and peak GH were compared by Wilcoxon tests. Spearman rank test was used to study correlation, P < 0.05 was considered as level of significance.

4. Results

Sixty children met our inclusion criteria, two were excluded due to missing data, remaining 58 cases were grouped as GHD (–): 39 cases (group 1), and GHD (+): 19 cases (group 2). Characteristics of our study population are summarized in Table 1: age, sex, height Z-scores, target height, pubertal stage and bone age were comparable in both groups.

There were 36 boys and 22 girls (Table 1). Forty-five children were prepubertal (15 from group 1 and 30 from group 2), eight were early pubertal (Tanner 2), two were Tanner 3, and three were Tanner 4. Thirteen children were intrauterine growth retarded, five children had received cranial irradiation (three from group 1, and two from group 2), with total irradiation varying from 12 to 56 grays.

All patients were euthyroid, two were receiving thyroid hormone supplementation (one for panhypopituitarism secondary to semi lobar holoprosencephaly, the other for secondary hypothyroidism following cranial radiotherapy for medulloblastoma). Two patients were receiving hydrocortisone (the one with hypopituitarism, and one with past history of chronic myeloid leukemia). Three patients had organic cause to their GHD (one holoprosencephaly, one craniopharyngioma and one pituitary stalk interruption). Two patients from group 2 were not substituted with recombinant GH (rhGH) because of recurrence of medulloblastoma, seven patients from group 1 were receiving rhGH (four intrauterine growth retardation without catch-up growth, one Noonan syndrome and two for severe idiopathic short stature).

Peak GH plasma concentrations were statistically different between group 1 (41.8 ± 21.7 mUI/l) and group 2 (11.5 ± 5.9 mUI/l), P < 0.0001.

IGF-I assay could be performed in 51 (88%) cases of the study population, whereas IGF-BP3 assay results were available only for 36 (62%) patients. No difference was found between both groups with regard to IGF-I Z-scores (P = 0.72) and IGF-BP3 Z-scores (P = 0.64) as summarized in Tables 2 and 3. In addition, we found no correlation between peak GH plasma concentrations following the first pharmacological test and IGF-I Z-scores (P = 0.34) or with IGF-BP3 Z-scores (P = 0.44). A correlation was however found between IGF-I Z-scores and IGF-BP3 Z-scores (r = 0.50; P < 0.0016).

Table 1

Study population characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>GHD (+) n = 19</th>
<th>GHD (–) n = 39</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12 (63%)</td>
<td>24 (62%)</td>
<td>NS</td>
</tr>
<tr>
<td>Female</td>
<td>7 (37%)</td>
<td>15 (38%)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>9.0 ± 3.8 [0.4-14.5]</td>
<td>9.4 ± 4.1 [2.1-18.3]</td>
<td>NS</td>
</tr>
<tr>
<td>Height Z-score</td>
<td>-1.9 ± 1.3</td>
<td>-2.2 ± 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>17.9 ± 4.2</td>
<td>17.0 ± 5.8</td>
<td>NS</td>
</tr>
<tr>
<td>Target height</td>
<td>165.5 ± 10.5</td>
<td>164.3 ± 9.4</td>
<td>NS</td>
</tr>
<tr>
<td>Pubertal stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>79%</td>
<td>77%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>16%</td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5%</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>IUGR</td>
<td>11%</td>
<td>28%</td>
<td></td>
</tr>
<tr>
<td>Cranial irradiation</td>
<td>16%</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>GH therapy</td>
<td>89%</td>
<td>18%</td>
<td></td>
</tr>
</tbody>
</table>

Table 2

IGF-BP3 values in the two groups.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>GHD (+)</th>
<th>GHD (–)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of tests</td>
<td>12 (63%)</td>
<td>26 (666%)</td>
<td>0.72</td>
</tr>
<tr>
<td>IGF-BP3 (mg/L)</td>
<td>2.3 ± 0.9</td>
<td>2.4 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>IGF-BP3 (Z-score)</td>
<td>-2.1 ± 2.0</td>
<td>-1.5 ± 1.0</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Results presented as mean ± SD.
Table 3
Correlation coefficients between IGF-I Z-score, IGF-BP3 Z-score and peak GH.

<table>
<thead>
<tr>
<th></th>
<th>IGF-I (Z-score)</th>
<th>IGF-BP3 (Z-score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak GH</td>
<td>0.14 (P = 0.34)</td>
<td>0.13 (P = 0.44)</td>
</tr>
<tr>
<td>IGF-BP (Z-score)</td>
<td>0.5 (P = 0.0016)</td>
<td>1</td>
</tr>
</tbody>
</table>

Both IGF-BP3 absolute values (in milligram per litre) as well as IGF-BP3 Z-scores did not discriminate between group 1 (2.3 ± 0.9 mg/L; –2.1 ± 2.0 respectively) and group 2 (2.4 ± 0.6 mg/L; –1.5 ± 1.0, respectively) (P = 0.72 and P = 0.64 respectively).

5. Discussion

Measurement of plasma IGF-BP3 concentration was proposed as a complementary tool for the diagnosis of growth hormone deficiency (GHD) in children, with regard to its regulation by the GH and stability of its plasma levels in comparison to that of IGF-I, whose plasma level vary in certain conditions (malnutrition, chronic diseases, liver disease, hypothyroidism, diabetes, renal insufficiency) [25].

Measuring both IGF-I and IGF-BP3 is more reproducible, easier and cheaper than pharmacological tests. In addition, both markers show minimal diurnal variation so that a single sample taken at any time of the day gives reliable assessment. Drawbacks with IGF-I and IGF-BP3 assessments include their dependency on gender, age, pubertal status, BMI and lack of specificity in children less than 6 years of age [9].

In 1990, Blum et al. reported a sensitivity of 97% and specificity of 95% for IGF-BP3 assay’s performance in the diagnosis of growth hormone deficiency [14]. Subsequent studies conducted by several other researchers were, unfortunately, unable to reach similar levels of specificity and sensitivity [11,15,26–28]. Instead, discordant results were reported: with absence of discriminating power of IGF-BP3 assay in the diagnosis of growth hormone deficiency for some [16,18,19,29–31], and lack of correlation between plasma IGF-BP3 concentrations and peak plasma GH concentrations for others [18,29]. Our findings contradict those reported earlier by Blum et al. [14], but are comparable to those of Cianfarani et al. who found a correlation between IGF-I Z-scores and IGF-BP3 Z-scores (r = 0.51; P < 0.001) [18].

The main aim of our study was to verify whether IGF-BP3 assay as used in common practice contributes to the diagnosis of growth hormone deficiency in children, our results suggest that this biological marker, like IGF-I, does not, in our study population at least, differentiate children with growth hormone deficiency from those without. In attempt to add to the strength of our results, we evaluated both IGF-BP3 absolute values expressed in milligrams, as well as IGF-BP3 Z-score with reference to normal values provided by our reference laboratories; IGF-BP3 has a specificity of 92% and sensitivity of only 25% in this study. These low specificity and sensitivity are insufficient for a diagnostic tool, our results, hence, corroborate those reported by other studies [16,19,30].

This study being retrospective, presents a number of limits such as those linked to loss of data, changes in laboratory techniques over time for both IGF-I and IGF-BP3 assays with consequent variability of their results, etc. Besides, reference curves we utilized to calculate IGF-BP3 Z-scores were established from a population of American children different from French children with regard to body mass index (BMI) in particular. Juul et al. have shown that a correlation exists between plasma IGF-BP3 concentrations and BMI [32]. Another weakness is that IGF-BP3 reference curves provided by the laboratory were not adjusted to pubertal stage, which is known to influence plasma IGF-BP3 concentrations independently from patient’s age [32].

In spite of the above-mentioned weaknesses, our study reflects daily clinical reality and carries the advantage of questioning the practical contribution of IGF-BP3 assay to the diagnosis of GHD in paediatric population. More research is needed to standardize both IGF-I and IGF-BP3 assay techniques, taking into consideration several parameters such as age, sex, BMI, pubertal stage, etc.

Consensus exists in France that diagnosis of GHD in children should be based on finding of peak plasma GH concentration less than 20.0 mUI/L (10 ng/L) following two pharmacological tests (one with a single pharmacological stimulus, the second with combined two pharmacological stimuli) in a child with short stature (height below –2.0 SD), low growth velocity and delayed bone age. Clinicians are asked to accept this definition, but no consensus exists with regard to pharmacological tests, assay techniques, antibodies, reagents to be utilized, so that different tests are performed (insulin test, Clonidine-Betaxolol, etc) in different centres with results that cannot be compared adequately.

Diagnosing GHD in children can be straightforward in those children with clear-cut clinical conditions such as familial GHD, hypoglycaemia, micro phallus, cranial irradiation, septo-optic dysplasia, interrupted pituitary stalk syndrome, hypothalamic and pituitary tumours. Even in these conditions, confirmation of clinical and radiological diagnosis requires pharmacological tests whose specificity and sensitivity are rather hypothetical. Moreover, GH secreted by the pituitary gland is heterogeneous, so that the amount of it measured in an immunoassay can vary [33–36]. GHD can be a result of total or relative absence of GH secretion from the pituitary gland or a consequence of secretion of abnormal GHS.

Although not perfect for the diagnosis of GHD, assays of both IGF-I and IGF-BP3 plasma concentrations can, however, be of help for the titration of GH dose in treated children; this aspect being beyond the scope of our study.

This study gave us also the opportunity to find that although diagnostic kits manufacturers recommend that user laboratories establish local reference values from tests over a large local population of healthy children, this is not always the case.
6. Conclusion

IGF-BP3 measurement is still recommended in routine endocrine practice and, therefore, expected to improve the diagnosis of GHD in children, our results suggest, however, that this marker is unable to differentiate children with GHD from those without. We believe additional research is necessary to establish reference values that take into account different remarks we have formulated above. Finally, following the results of this audit we have discarded IGF-BP3 measurement from initial routine screening of short children. We hope improvement will come in near future.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

References

