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Circulating IGF-I, IGFBP-3 and the IGF-I/IGFBP-3 Molar Ratio Concentration and Height Outcome in Prepubertal Short Children on rhGH Treatment over Two Years of Therapy

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Recombinant human growth hormone therapy · IGF-I · IGF-I/IGFBP-3 molar ratio · Prepuberty · Children

Abstract
Objective: To investigate the occurrence of abnormally elevated values of biomarkers of growth hormone (GH) action in short children on recombinant human GH (rhGH) therapy. ***Methods:*** Sixty-three prepubertal short children were examined: 31 with GH deficiency (GHD), 25 small for gestational age (SGA), and 9 with Turner syndrome (TS). The main outcomes were the following: standard deviation score (SDS) values of IGF-I, IGFBP-3, and IGF-I/IGFBP-3 molar ratio before, at the 1st and at the 2nd year on rhGH and Δheight (Ht)-SDS to evaluate GH treatment efficacy (adequate 1st-year ΔHt SDS: >0.4 SDS for GHD and >0.3 SDS for non-GHD). ***Results:*** Seventy-eight percent of GHD, 78% of SGA and 55% of TS children had adequate 1st-year ΔHt SDS. In GHD, 88% of IGF-I SDS and IGFBP-3 SDS that were ≤–2.0 SDS at baseline normalized on treatment. Abnormal IGF-I values >+2.0 SDS were observed in 52% of SGA and in 55% of TS patients on rhGH. Within each group, the IGF-I/IGFBP-3 molar ratio increased significantly from pretreatment and throughout therapy, remaining within normal range for most patients. ΔIGF-I/IGFBP-3 molar ratio SDS were significantly higher in children with an adequate response ($p < 0.01$). ***Conclusion:*** Non-GHD groups presented markedly elevated concentrations of GH biomarkers on rhGH and normal IGF-I/IGFBP-3 molar ratio in most patients. Since there is a lack of consensus regarding the molar ratio usefulness, we think that interventions towards a more physiological IGF-I serum profile should be implemented.

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Introduction

Recombinant human growth hormone (rhGH) therapy is licensed worldwide for the treatment of children with growth hormone deficiency (GHD) and Turner syndrome (TS) and of children born small for gestational age (SGA) among other disorders. Treatment modalities and dosing were geared mainly to optimize efficacy and currently, there is general consensus about the recommended doses for each disorder [1–3]. Response to rhGH treatment varies largely among growth disorder etiologies [1–3]. Long-term safety has mostly been evaluated by postmarketing surveillance studies performed by pharmaceutical companies [4–6]. Significant side effects of rhGH treatment in children are rare, thus the overall safety profile of therapy remains favorable [1–3]. Most studies of children on rhGH therapy focus on auxological outcomes, while accurate circulating biomarkers of GH action such as IGF-I are not routinely monitored during rhGH treatment despite consensus guideline recommendations [1, 3]. Abnormally high levels of total IGF-I have been reported in short children on rhGH for both GHD and non-GHD patients [7, 8], leading in some studies to titrate rhGH dose accordingly [9]. The molar ratio between total IGF-I and IGFBP-3 (IGF-I/IGFBP-3) has been suggested to indirectly reflect free IGF-I [10–12]. Although the IGF-I/IGFBP-3 molar ratio was proposed as a potential biomarker to ascertain safety of rhGH [8], this index is not usually included when monitoring children on rhGH therapy.

In the present study, we aimed to investigate the course and the occurrence of elevated values of circulating total IGF-I, IGFBP-3 and the IGF-I/IGFBP-3 molar ratio concentrations in a cohort of prepubertal GHD, SGA and TS children on rhGH treatment and their relationship with variables of GH treatment efficacy during the first 2 years of therapy.

Materials and Methods

We conducted a retrospective study in a group of 63 prepubertal children with growth disorders referred to the Endocrinology Division of the Ricardo Gutiérrez Children's Hospital of Buenos Aires from 2011 to 2015.

This investigation was part of the regular follow-up of rhGH-treated children in our center. All data were obtained retrospectively from clinical charts. The study was approved by the local Institutional Review Board of the Children's Hospital Ricardo Gutiérrez of Buenos Aires and the need for informed consent was waived owing to the observational design of the study in which all the procedures followed the standard care of our patients with GHD and non-GHD disorders.

GHD diagnosis was based on the assessment of auxology along with supporting evidence from biochemical and neuroradiological studies. All patients underwent provocative tests of GH secretion using pharmacological stimuli of sequential arginine (0.5 g/kg body weight) and clonidine (100 μg/m^2 body surface) tests. A maximal GH-stimulated level <6.0 ng/mL (calibrated against WHO 80/505, IMMULITE® 2000 system, Siemens Healthcare Diagnostics Products Ltd., Gwynedd, UK) or <4.7 ng/mL (calibrated against WHO 98/574, IMMULITE® 2000 system, Siemens Healthcare Diagnostics Products Ltd.) after pharmacological test categorized short children with GHD (n = 31) [13, 14]. Alternatively, during the newborn period, a random GH <10 ng/mL under hypoglycemia identified GHD in neonates when associated with other pituitary deficiencies [15]. Thirty-seven children with GHD were included. Twenty-one presented multiple pituitary hormone deficiencies (5 with acquired organic pituitary lesions). Multiple pituitary hormone deficiency patients had been clinically and biochemically monitored to guarantee that the doses and the compliance to treatment had been adequate to reassure physiological conditions. The remaining 16 patients had isolated GHD.

Twenty-five SGA patients were included, defined as having a birth weight and/or length below –2.0 standard deviation score (SDS) of the population reference mean for gestational age and absence of catch-up growth after the age of 2 or 3 years, when born at term or preterm, respectively [16]. Two SGA patients in whom pretreatment serum IGF-I SDS values fell above +2.0 SDS were excluded from the study.

Nine TS patients were included. Diagnosis was performed based on clinical phenotype and confirmed by abnormal karyotype.

Data of clinical and biochemical assessments were obtained at baseline and at the end of the 1st and 2nd year on rhGH treatment for each patient. At each visit, height (Ht), Ht velocity, pubertal stage, bone age, and body mass index (BMI) were assessed. BMI SDS and Ht SDS were calculated according to WHO and local standards [17–19]. Pubertal staging was assessed according to Tanner [20, 21]. Serum fasting morning samples were obtained at each visit to measure IGF-I and IGFBP-3. These values and those of calculated IGF-I/IGFBP-3 molar ratio were expressed as SDS according to our normal control group, taking into account age and pubertal stage [22]. rhGH was administered subcutaneously daily before bedtime at a dose of 0.33 mg/kg/week in the SGA and TS groups, whereas in GHD patients, the dose ranged from 0.14 to 0.26 mg/kg/week. Compliance to rhGH therapy was ascertained by a thorough interrogation at each visit.

We calculated the 1st-year and 2nd-year response of Ht (ΔHt SDS) to evaluate GH treatment efficacy. According to published data [23, 24], we considered poor or inadequate a 1st-year response of Ht <0.4 SDS for GHD and <0.3 SDS in girls with TS or in SGA children.

Hormonal Assays

IGF-I and IGFBP-3 concentrations were measured by a two-site chemiluminescent immunometric assay (IMMULITE® 2000 system, Siemens Healthcare Diagnostics Products Ltd.). Intra- and interassay coefficients of variation were <5.5% for IGF-I and <7.2% for IGFBP-3 serum measurements. Both IGF-I and IGFBP-3 assays were the same throughout the whole study period. The IGF-I/IGFBP-3 molar ratio was calculated based on a molecular mass of 7.6 kDa for IGF-I and 29 kDa for IGFBP-3, respectively.

Table 1. Clinical and auxological features of GHD, SGA and TS children before and throughout rhGH treatment

	GHD	SGA	TS
Baseline (n = 63)			
Mean age (range), years	4.9 (0.6–15.5)	6.4. (4.4–10.4)	7.0 (3.9–12.1)
Gender (boys/girls)	19/12	15/8	0/9
Mid-parental Ht SDS	–0.4	–0.4	–0.52
Ht SDS	–2.9±1.2	–3.0±0.8	–2.4±0.8
BMI SDS	0.7±1.7	–0.9±1.0	0.4±1.2
1st year on rhGH treatment (n = 63)			
Tanner stage (prepubertal/pubertal)	30/1	21/2	9/0
Ht SDS	–1.9±1.3***	–2.3±0.8***	–1.9±0.9*
ΔHt SDS (vs. pretreatment)	0.8±0.7	0.7±0.3	0.4±0.4
BMI SDS	1.0±2.4	–0.7±1.0	0.5±1.0
2nd year on rhGH treatment (n = 63)			
Tanner stage I (prepubertal/pubertal)	28/3	21/2	9/0
Ht SDS	–1.1±1.3***,#	–1.9±0.8***,#	–2.0±0.8**
ΔHt SDS (2nd vs. 1st year on rhGH)	0.6±0.9	0.3±0.2	0.1±0.2
BMI SDS	1.2±0.4	–0.7±1.2	0.4±1.4

GHD, growth hormone deficiency; SGA, small for gestational age; TS, Turner syndrome; rhGH, recombinant human growth hormone; Ht, height. Data are expressed as the mean ± SD unless stated otherwise. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ versus pretreatment; # $p < 0.01$ versus 1st year on rhGH.

Statistical Analysis

Data distribution of hormone serum levels were tested for normality using the Shapiro-Wilk test. Serum IGF-I was log-transformed to reach normal distribution. ANOVA for repeated measures followed by an a posteriori Tukey test was used to evaluate the variation of auxological and hormonal variables within each group over the study period. ANOVA and the Tukey post-test including rhGH doses as covariable were used to evaluate between-group differences. We set a cutoff value of +2.0 SDS as a hypothetical measure of safety for circulating IGF-I, IGFBP-3, and the IGF-I/IGFBP-3 molar ratio. Values above +3.0 SDS were also analyzed. The proportion of elevated IGF-I, IGFBP-3 and IGF-I/IGFBP-3 molar ratio SDS among groups was assessed by the χ^2 exact text. Spearman correlation was used. The level of significance was set at $p < 0.05$. Statistical analyses were performed using GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, CA, USA).

Results

The clinical and auxological features of GHD, SGA, and TS children at the start of therapy and at the end of the 1st and 2nd year of rhGH are shown in Table 1. Ht SDS improved significantly throughout treatment in all groups although with a lesser extent for TS girls ($p < 0.001$ for GHD and SGA; $p < 0.01$ for TS). First-year ΔHt SDS on rhGH was adequate in 78, 78, and 55% of GHD, SGA, and TS children, respectively, and it correlated negatively with chronological age at the start of treatment (r = –0.31, $p < 0.05$). In GHD, 1st- and 2nd-year ΔHt SDS were similar (1st-year gain: 0.84 ± 0.69 vs. 2nd-year gain: 0.62 ± 0.97, $p = 0.30$) while 2nd-year gain in Ht SDS was lower for SGA and TS groups compared to 1st year on rhGH (2nd-year gain: 0.29 ± 0.22 vs. 1st-year gain: 0.61 ± 0.38, $p < 0.001$). In the 2nd year on rhGH, we observed that ΔHt SDS was significantly higher in patients with adequate 1st-year gain in Ht than in those with poor response (adequate responders: 0.45 ± 0.71 vs. poor responders: 0.06 ± 0.29, $p < 0.05$).

Serum IGF-I, IGFBP-3 and the IGF-I/IGFBP-3 molar ratio features for GHD, SGA and TS children before and at the end of the 1st and 2nd year on rhGH treatment are shown in Table 2 and Figure 1. A direct association was observed for IGF-I SDS (r = 0.41, $p < 0.001$), IGFBP-3 SDS (r = 0.50, $p < 0.0001$) and IGF-I/IGFBP-3 molar ratio SDS (r = 0.30, $p < 0.05$) with rhGH doses during treatment. At baseline, IGF-I and IGFBP-3 were abnormally low (≤–2.0 SDS) in 74 and 45% of GHD patients, respectively, while they were within normal range in most SGA children (IGF-I ≤–2.0 SDS: 17% and IGFBP-3 ≤–2.0 SDS: 18%) and TS girls (IGF-I ≤–2.0 SDS: 33% and IGFBP-3 ≤–2.0 SDS: 22%). On treatment, IGF-I SDS changed from low to normal in 88% of GHD children. A considerable in-

Table 2. Serum IGF-I, IGFBP-3 and IGF-I/IGFBP-3 molar ratio profile in GHD, SGA, and TS children at the start of therapy and on rhGH

	GHD	SGA	TS	*p*
Baseline ($n = 63$)				
IGF-I SDS	−3.6±1.6	−0.6±1.5***	−0.7±2.2***	<0.0001
IGFBP-3 SDS	−2.1±1.6	−0.4±1.8**	−1.1±1.4	<0.001
IGF-I/IGFBP-3 molar ratio SDS	−0.7±0.4	−0.2±0.6*	−0.3±0.8	<0.05
1st year on GH treatment				
IGF-I SDS	−0.1±2.0	1.6±1.3**	1.9±1.6*	<0.001
ΔIGF-I SDS (vs. pretreatment)	3.4±1.9	2.2±1.4 *	2.6±2.0	<0.05
IGFBP-3 SDS	−1.3±1.9	1.5±1.9***	1.3±2.2**	<0.0001
ΔIGFBP-3 SDS (vs. pretreatment)	1.3±1.3	1.9±1.6	1.9±1.4	0.33
IGF-I/IGFBP-3 molar ratio SDS	0.3±1.1	0.6 ±1.0	1.6±1.2**#	<0.01
ΔIGF-I/IGFBP-3 molar ratio SDS (vs. pretreatment)	1.0±0.9	0.9±0.8	1.7±1.6	0.15
2nd year on GH treatment				
IGF-I SDS	0.4±2.3	1.9±1.1**	2.5±1.3*	<0.001
ΔIGF-I SDS (2nd vs. 1st year)	0.5±2.1	0.4±1.4	0.2±1.2	0.92
IGFBP-3 SDS	−0.4±1.7	2.0±1.4***	1.5±1.9***	<0.0001
ΔIGFBP-3 SDS (2nd vs. 1st year)	0.8±1.9	0.8±1.9	0.7±1.3	0.99
IGF-I/IGFBP-3 molar ratio SDS	−0.2±1.1	1.1±1.2**	1.3±1.2**	<0.001
ΔIGF-I/IGFBP-3 molar ratio SDS (2nd vs. 1st year)	−0.5±1.5	0.6±0.9*	−0.2±1.0	<0.05

GHD, growth hormone deficiency; SGA, small for gestational age; TS, Turner syndrome; rhGH, recombinant human growth hormone. Data are expressed as the mean ± SD of standard deviation scores (SDS) according to our reference data. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ versus GHD; # $p < 0.05$ versus SGA.

Table 3. Proportion of elevated IGF-I, IGFBP-3 and IGF-I/IGFBP-3 molar ratio (either >+2.0 SDS or >+3.0 SDS) in GHD, SGA and TS children on rhGH

	GHD, %	SGA, %	TS, %	χ^2 test (*p*)
1st year on GH treatment				
Cutoff >+2.0 SDS				
IGF-I SDS	19	52	55	<0.0001
IGFBP-3 SDS	0	40	50	<0.0001
IGF-I/IGFBP-3 molar ratio SDS	4	9	33	<0.0001
Cutoff >+3.0 SDS				
IGF-I SDS	3	13	22	<0.001
IGFBP-3 SDS	3	22	37	<0.0001
IGF-I/IGFBP-3 molar ratio SDS	0	5	11	<0.05
2nd year on GH treatment				
Cutoff >+2.0 SDS				
IGF-I SDS	7	54	67	<0.0001
IGFBP-3 SDS	3	54	67	<0.0001
IGF-I/IGFBP-3 molar ratio SDS	4	22	22	<0.01
Cutoff >+3.0 SDS				
IGF-I SDS	0	13	22	<0.0001
IGFBP-3 SDS	0	27	11	<0.0001
IGF-I/IGFBP-3 molar ratio SDS	0	5	0	<0.05

GHD, growth hormone deficiency; SGA, small for gestational age; TS, Turner syndrome; rhGH, recombinant human growth hormone; SDS, standard deviation score.

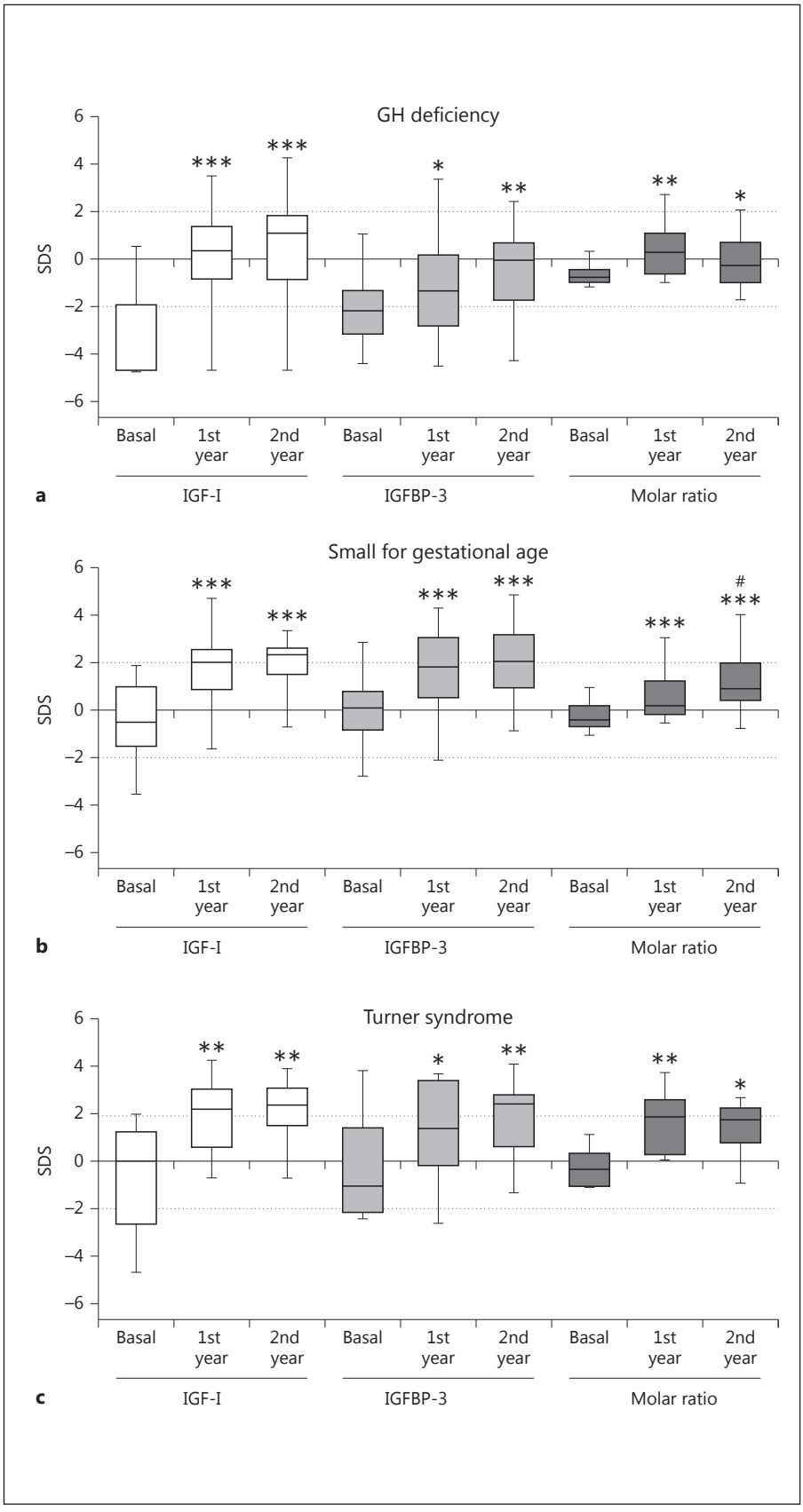

Fig. 1. IGF-I, IGFBP-3 and IGF-I/IGFBP-3 molar ratio in GH deficiency (**a**), small for gestational age (**b**) and Turner syndrome children (**c**) at pretreatment and during the first 2 years on rhGH therapy. Values are expressed as standard deviation score (SDS). The dotted lines denote the ±2.0 SDS. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus pretreatment and # $p < 0.05$ versus 1st year.

crease of 3.4 SDS for IGF-I was observed for GHD children as a group while a moderate increase of 1.0 SDS on average was observed for IGFBP-3 and the IGF-I/IGFBP-3 molar ratio values. Six GHD patients had IGF-I above +2.0 SDS at the 1st year of therapy (Table 3); in only 1 patient did IGF-I and the IGF-I/IGFBP-3 molar ratio remain elevated over the 2 years of treatment. All SGA and TS children had significantly increased IGF-I and IGFBP-3 values over the 1st year of therapy. At the end of the 1st year of therapy, 12/23 (52%) SGA patients and 5/9 (55%) TS girls had increased IGF-I values from normal range to ≥+2.0 SDS (Table 3). A low proportion of non-GHD patients (20% on average) had IGF-I and IGFBP-3 ≥+3.0 SDS. Within each group, the IGF-I/IGFBP-3 molar ratio increased significantly from pretreatment and during the course of rhGH therapy, remaining within normal range for most of the patients.

When biochemical data were analyzed according to adequate or poor 1st-year gain in Ht (ΔHt SDS), we found that ΔIGF-I SDS ($p < 0.05$), IGF-I/IGFBP-3 molar ratio SDS ($p < 0.05$), and ΔIGF-I/IGFBP-3 molar ratio SDS ($p < 0.01$) were significantly higher in children with an adequate response than those with a poor response (Fig. 2). In the GHD group, GH doses were significantly higher in patients with adequate 1st-year gain in Ht (0.20 ± 0.03 mg/kg/week) than in those with a poor response (0.16 ± 0.02 mg/kg/week) ($p < 0.01$).

The proportion of elevated IGF-I and IGFBP-3 concentrations (either >+2.0 SDS or >+3.0 SDS) were significantly higher in SGA and TS children than in the GHD group (Table 3).

Three GHD patients (1/3 with a poor 1st-year Ht response) and 2 SGA children with adequate response entered into puberty during the study. Data were reanalyzed after excluding these 5 children. We found that Ht, IGF-I, IGFBP-3, and IGF-I/IGFBP-3 molar ratio (all parameters expressed as SDS) still improved significantly throughout treatment in GHD and SGA groups ($p < 0.0001$). SGA children had a significantly higher proportion of IGF-I SDS (above +2.0 SDS: 42.9% in the 1st and 2nd year) and IGFBP-3 SDS (9.5% in the 1st year and 38% in the 2nd year on rhGH) than the GHD group (IGF-I SDS: 14.2% and IGFBP-3 SDS: 3.5%; $p < 0.0001$). According to the 1st-year Ht response, we found that children with adequate 1st-year ΔHt SDS had higher ΔIGF-I SDS (3.58 ± 1.49 vs. 2.37 ± 1.66 SDS, $p < 0.05$), IGF-I/IGFBP-3 molar ratio SDS (0.64 ± 1.04 vs. –0.31 ± 0.43 SDS, $p < 0.015$) and ΔIGF-I/IGFBP-3 molar ratio SDS (1.06 ± 0.86 vs. 0.24 ± 0.57 SDS, $p < 0.05$) than poor responders.

Discussion

This study evaluated serum IGF-I, IGFBP-3 and IGF-I/IGFBP-3 molar ratio in GHD, SGA and TS prepubertal children prior to and over the first 2 years of rhGH therapy in order to assess the course of these parameters in relation to auxological changes and the proportion of abnormally elevated levels of serum GH action markers. We found that most children on recommended rhGH doses improved ΔHt over the 1st year on treatment. The increment in Ht continued for GHD and SGA groups at the 2nd year of therapy in association with increments in IGF-I and IGFBP-3 concentrations towards normal range in GHD and with high values in SGA patients. Interestingly, TS girls had a striking proportion of IGF-I and IGFBP-3 levels above +2.0 SDS without a significant improvement in Ht between the 1st and the 2nd year of rhGH. In spite of serum IGF-I and IGFBP-3 discrepancies among groups, most children had normal IGF-I/IGFBP-3 molar ratio values during treatment.

The 1st-year growth on rhGH constitutes the strongest indicator of long-term Ht outcomes [2]. We found that 1st-year ΔHt SDS correlated negatively with the age at the start of therapy, supporting the importance of the early diagnosis and onset of replacement therapy. There is still a lack of consensus regarding definitions of poor response to treatment [2]. According to the definition of Ranke et al. [24], we were able to find unsatisfied responses in our cohort of prepubertal children to a similar extent as other authors. In GHD patients with adequate 1st-year Ht response, GH doses were significantly higher than in those with poor responses. Unfortunately, we did not rule out whether other causes could also explain differences in Ht response such as poor compliance as well as an intrinsic impaired responsiveness to GH in non-GHD groups. Ht gain continued to improve over the 2nd year of treatment in most GHD and SGA patients, while it reached a plateau in the TS group. Increment in Ht at the 2nd year was higher in patients with adequate 1st-year gain in Ht, thus reassuring that the magnitude of 1st-year ΔHt SDS gain is a good indicator of future growth outcome [2].

Circulating IGF-I is useful to monitor compliance and efficacy in response to rhGH therapy, while there is no consensus about IGFBP-3 nor about IGF-I/IGFBP-3 molar ratio [2, 3, 25]. We found that IGF-I and IGFBP-3 significantly increased in GHD, SGA and TS children on rhGH therapy in a dose-dependent manner. The increment was more evident for IGF-I than for IGFBP-3, especially for GHD patients as compared to SGA probably

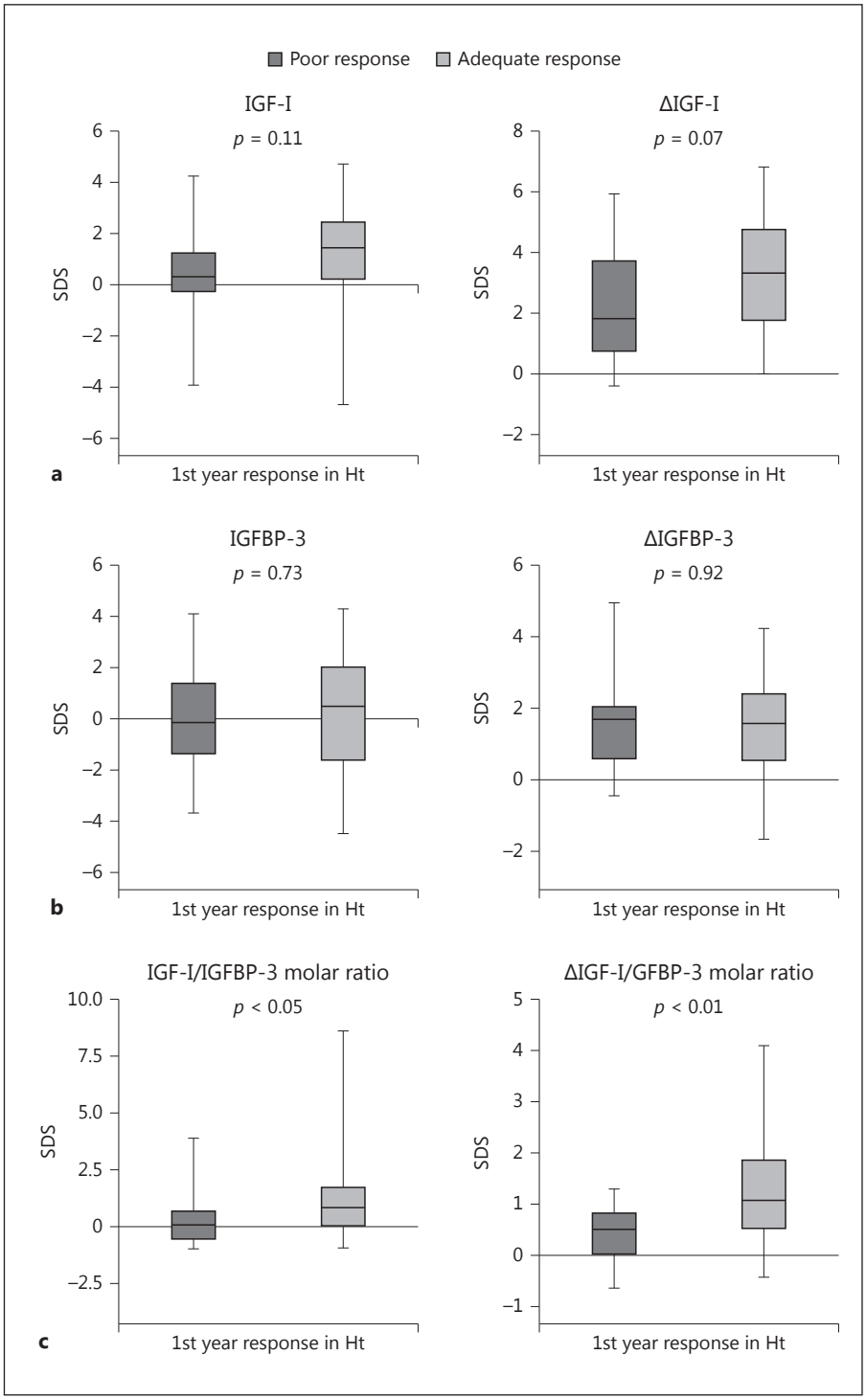

Fig. 2. IGF-I and ΔIGF-I (**a**), IGFBP-3 and ΔIGFBP-3 (**b**), and IGF-I/IGFBP-3 molar ratio and ΔIGF-I/IGFBP-3 molar ratio (**c**) according to the expected growth response (adequate or poor) of prepubertal children to rhGH therapy.

as a consequence of the differences in IGF-I concentration at the start of therapy and/or GH sensitivity [26]. Most IGF-I and IGFBP-3 concentrations remained within normal range in GHD patients, while SGA and TS groups had a statistically higher occurrence of circulating levels above +2.0 SDS (hypothetical measure of safety) or even above +3.0 SDS. This was not totally explained by dosing differences for each primary diagnosis

nor by baseline IGF-I and IGFBP-3 concentrations. Differences between GHD and non-GHD groups were still present after correcting IGF-I and IGFBP-3 SDS by rhGH doses. Of note, 75% of non-GHD patients with elevated IGF-I throughout treatment had IGF-I SDS values before treatment above –0.5 SDS. Taking this into account, non-GHD children on rhGH appear to be a group at higher risk of having markedly elevated serum IGF-I and IGFBP-3 levels. Consequently, titration of rhGH dose might be recommended in these patients in order to reach more physiological IGF-I concentrations on treatment. The number of observations in our TS cohort is small. Since rhGH treatment surveillance data in TS girls are still scarce, we found it useful to analyze the TS group separately from the SGA group. Although controversial, elevated serum IGF-I concentrations may predict risk of long-term safety [27–31]. Therefore, rhGH dosing reduction has been recommended if IGF-I is repeatedly above the upper limit of the laboratory-defined normal range for the age or pubertal stage of the patient [2, 3].

In spite of higher IGF-I and IGFBP-3 levels in SGA and TS children compared to the GHD group, we found that most IGF-I/IGFBP-3 molar ratio levels fell within the normal range. Children with an adequate 1st-year gain in Ht had higher IGF-I/IGFBP-3 molar ratio SDS and ΔIGF-I/IGFBP-3 molar ratio SDS than poor responders, thus probably reflecting a higher proportion of free IGF-I in them. These results were also maintained after excluding children who entered into puberty during the study period. It is still of concern that elevated IGF-I/IGFBP-3 molar ratio values were present in 19% of the whole group of children (12/63), being more frequently found in TS girls. In GHD children, IGF-I/IGFBP-3 molar ratio above cutoff concentrations were due to an unequal increase in IGF-I with respect to IGFBP-3, thus probably reflecting the known higher sensitivity of IGF-I gene to GH action [26]. The molar ratio between total IGF-I and IGFBP-3 has been suggested to indirectly reflect unbound IGF-I availability and it has been shown to change in the same direction as free IGF-I in conditions with primary abnormalities in GH secretion [10–12]. Despite this, IGF-I/IGFBP-3 molar ratio is not commonly included as a tool for monitoring patients on rhGH therapy. There is no consensus statement regarding its usefulness probably due to the lack of solid reference intervals. Moreover, as a consequence of proteases degradation, IGFBP-3 is present in biological fluids in several lower-molecular-size fragments recognized by immunoassays but which are not functional (24 and 19 kDa) [32, 33]. This problem inherent to immunoassays probably limits IGFBP-3 measurement and consequently, the appropriate assessment of IGF-I/IGFBP-3 molar ratio. Of concern, IGF-II in circulation occupies the binding sites on circulating IGFBP-3 and IGFBP-3 complexes 3-fold more than IGF-I, therefore, IGF-II levels are an important bias. On the other hand, insulin plays a critical role in the GH-IGF-IGFBP system by affecting the hepatic IGF-I production and its bioactivity through its impact on IGFBP-1 and -2 concentrations. In addition, insulin upregulates hepatic GH binding probably through an increment in GH receptor availability [12, 33–35]. Both TS girls and SGA children may be at a higher risk of developing glucose metabolism abnormalities [36]. Taking into account the above-mentioned insulin actions, the molar ratio between IGF-I and IGFBP-3 should be less informative in non-GHD disorders and therefore, we strongly recommend to look at IGF-I levels to further assess safety in TS and SGA children on rhGH therapy. For the above mentioned, it is unclear whether calculated molar ratios reflect in vivo status of the IGF-I/IGFBP-3 system. Measurement of free IGF-I may have strengthened the findings of the present work. Unfortunately, this method is not available in our laboratory.

In summary, most of our cohort of prepubertal children on rhGH treatment had satisfied Ht gain responses at the 1st year on rhGH treatment regardless of the etiology. The majority of GHD children normalized IGF-I and IGFBP-3 values within the 1st year of rhGH therapy and these GH biomarkers were maintained within normal range over the 2nd year associated with Ht gain improvement. However, non-GHD groups presented a considerable proportion of markedly elevated IGF-I and IGFBP-3 concentrations since the end of the 1st year of rhGH with most IGF-I/IGFBP-3 molar ratios within normal range. Since many factors may affect the IGF-I/IGFBP-3 molar ratio, its calculation seems to be less helpful. The long-term risk of high IGF-I is not resolved yet. We think proper interventions towards a more physiological serum IGF-I profile should be implemented.

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Disclosure Statement

The authors declare that they have no conflict of interest to disclose.

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