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Biological Significance of Anti-GH Antibodies in Children Treated with rhGH

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Keywords
Anti-growth hormone antibodies · Growth hormone deficiency · Growth hormone treatment

Abstract
Background: The occurrence of antidrug antibodies is common in children treated with recombinant human growth hormone (rhGH). However, their clinical significance is unclear. ***Objective:*** This study aimed to examine the clinical significance of anti-GH antibodies by analyzing the phenotype of patients who tested positive in relation to the quantity of anti-GH antibodies. ***Method:*** In this laboratory-based retrospective study encompassing a time span of 6 years, all positive samples were identified, and senders were contacted. Anti-GH antibodies were measured using a radioprecipitation assay; positive samples underwent a confirmatory assay. ***Results:*** Out of a total of 104 samples from 66 patients, positive test results were found in 28 samples from 13 patients. Clinical data were available from all but one. The group with positive test results comprised 6 patients with a normal response to GH provocative tests (group A) and 6 with an insufficient response or with isolated GH deficiency (IGHD) type 1A (group B). Diagnoses in group A were neurosecretory dysfunction, bioinactive GH syndrome and constitutional delay of growth and puberty. Diagnoses in group B were IGHD type 1A, septo-optic dysplasia, and cerebral midline defect with multiple pituitary hormone deficiency. Insufficient growth response to rhGH was absent except in one sibling pair with IGHD type 1A and a patient with cerebral midline defect. These patients had the highest concentrations of anti-GH antibodies. ***Conclusions:*** The biological significance of anti-GH antibodies seems to be limited to patients with high concentrations of anti-GH antibodies. For all other patients, we recommend a careful "wait and see" strategy and monitoring antibody titers.

Introduction

Treatment with biopharmaceuticals can induce an immunogenic response with the development of antidrug antibodies (ADA) [1]. ADA may impact the efficacy of treatment by neutralizing the biological activity of the drug, changing its clearance, or affecting the safety of the treatment by inducing infusion reactions, hypersensitivity reactions, or autoimmune syndromes [1]. The first instances of ADA were against porcine/bovine insulin in diabetic pa-

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tients, which were reported in the 1950s [2], and against extracted human growth hormone (GH) in patients with severe isolated growth hormone deficiency (IGHD) due to homozygous *GH1* deletion (IGHD type 1A), which was reported in the 1970s [3]. The first recombinant growth hormone with an additional methionine residue at its N terminus (met-rhGH) proved to be even more immunogenic than extracted human GH [4]. The production of very pure identical hormones (i.e., rhGH) by recombinant DNA technology enabled the reduction of ADA but not its elimination [5, 6]. Currently, with the advances in biotechnology, regulatory authorities in Europe and the USA require the comprehensive evaluation of ADA responses to biopharmaceuticals for safety reasons [7].

Although the measurement of anti-GH antibodies is a standard procedure in GH drug trials, with a reported prevalence in children varying from 2 to 22% according to recent publications [6, 8, 9], the biological significance of anti-GH antibodies in the absence of very severe GHD (IGHD type 1A) has not been elucidated. Anti-GH antibodies differ in their capacity to neutralize rhGH, although most frequently they are regarded to be nonneutralizing. Therefore, in an individual patient, the significance of a positive test result for anti-GH antibodies is not clear. Nevertheless, therapeutic recommendations such as pausing rhGH treatment [8, 10], changing the rhGH brand [6] and treating with recombinant human insulin-like growth factor (rhIGF)-1 instead of rhGH [4, 11] have been proposed. These recommendations, however, were not evidence based. One important technical aspect of anti-GH antibodies is their interference with GH immunoassays causing false-high GH readings. This problem could be solved by using a mass spectrometry assay instead of an immunoassay [12].

In this study, we retrospectively studied patients with positive tests for anti-GH antibodies in two German laboratories over a span of 6 years (2009–2014). The diagnoses of the patients and the indications of the measurement of anti-GH antibodies were carefully reviewed, and the response to rhGH in the presence of anti-GH antibodies was analyzed.

Materials and Methods

The measurement of anti-GH antibodies in human serum was offered by two laboratories in Germany: the Laboratory of the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics at the University of Leipzig and the Pediatric Hormone Laboratory of the University Children's Hospital in Tübingen. There were no other German laboratories offering the measurement of anti-GH antibodies in pediatric samples. The databases of both laboratories were screened for positive anti-GH antibody tests from patients living in Germany, and the physicians of these patients were contacted.

The study was approved by the Ethical Committee of the Medical Faculty of Tübingen. All patients or their care givers gave written informed consent to their individual physicians before participation.

The following data were extracted from the medical records of the patients: diagnosis; height of the parents; sex of the patient; maturity at birth and mode of delivery; APGAR score; birth weight, length, and head circumference; height and weight at ages 1, 2, and 4 years (from the German booklet for prophylactic pediatric examinations); and growth velocity, bone age, age, height, weight, and pubertal stage at start of rhGH treatment. In addition, we collected data on IGF-1 and IGF binding protein (IGFBP)-3 serum concentrations at the start of rhGH treatment, GH peak values during stimulation tests, MRI morphology of the pituitary gland and the brain, the genetic cause of GHD and the presence of additional pituitary hormone deficiencies.

During follow-up, age, height, weight, pubertal stage, bone age, rhGH brand, GH dose, additional medication, hormone concentrations of IGF-1, IGFBP-3, free thyroxine, thyroid-stimulating hormone, and alkaline phosphatase were documented.

Measurement of Anti-GH Antibodies

The analytical method used for the quantification of anti-hGH antibodies was a radioprecipitation assay modified according to Zeisel et al. [13]. In a one-step procedure, duplicates of the sample (30 μL of serum) were incubated with 170 μL of assay buffer and 100 μL of commercially available 125-iodine-labeled hGH. Anti-hGH antibodies present in the serum are captured by this radioactive ligand. After incubation for 18 h and precipitation of antigen-antibody complexes with 1 mL of 20% PEG 6000/gammaglobulin with 0.5% Tween 20 solution, the samples were centrifuged, and the precipitate was washed with 1 mL of 16% PEG 6000/gammaglobulin/Tween 20 solution. Then, the samples were decanted to remove any nonbound radioactive ligands and measured in a gamma counter. Increases in the radioactive signal were proportional to the concentration of anti-hGH antibodies in the sample. The screening assay enables the identification of putative positive samples using a screening assay cut point with an index of 1.123 [14].

All putative positive samples with an index above the cut point of 1.123 were tested in a confirmatory assay, incubated with and without an excess of 1 μg of rhGH (Eutropin, LG, Frankfurt, Germany). The principle of the confirmatory assay is the inhibition of the binding of 125-iodine-labeled rhGH by an excess of nonlabeled rhGH (Eutropin). This inhibition confirms the specificity of the anti-GH antibodies. The ratio between the radioactive signal without and with inhibition is the quantitative surrogate marker, which is calculated by the following equation: inhibition in percent = [1 – (binding activity with antigen excess/binding activity without antigen excess)] × 100. If the percentage of inhibition reached was equal to or above 5.974%, the sample was declared "confirmed positive" [14].

Statistics

Values are given as the means and standard deviations. SD scores for birth weight and length were calculated according to Niklasson et al. [15] and for height according to Prader et al. [9]. The significance of comparisons was determined using Student's *t* test.

Table 1. Basal characteristics (means ± SD) of the patients grouped according to their initial GH response

	Group A (normal GH response)	Group B (low GH response)	*p*
Patients, *n*	6	6	
Sex (female/male), *n*	0/6	4/2	
Birth weight, SDS	–0.33±0.46	–0.16±0.95	ns
Birth length, SDS	–0.43±0.40	–0.40±2.12	ns
Target height, SDS	–0.57±0.88	–0.73±1.10	ns
Age at GH start, years	9.78±1.85	3.90±3.27	0.033
Height at GH start, SDS	–2.51±0.38	–5.42±1.80	0.003
Bone age delay at rhGH start, years	1.84±1.27	1.06±1.28	ns
GH test peak, ng/mL	11.8±2.0	2.5±3.4 (*n* = 3)	0.001
IGF-1, SDS	–0.89±1.91	<–4.00	0.001
Pituitary gland malformation (yes/no), *n*	0/6	2/4	
MPHD (yes/no), *n*	0/6	2/4	
Monogenic etiology (yes/no), *n*	0/6	5/6	

SDS, standard deviation score.

Results

From 2009 to 2014, a total of 104 serum samples from 66 patients were sent to the two laboratories for the measurement of anti-GH antibodies. Positive test results were found in 28 serum samples from 13 patients (19%). For this study, clinical data were available from 12 positive-tested patients, and 1 positive-tested patient failed to follow-up.

The group with positive antibody tests comprised 6 patients with normal responses to GH provocative tests (GH >8 ng/mL) and 6 patients with insufficient responses or with IGHD type 1A. In group A with a normal GH response, there were 4 patients with neurosecretory dysfunction, 1 patient with bioinactive GH syndrome (without genetic confirmation) and 1 patient with a constitutional delay of growth and puberty (post hoc diagnosis). The 4 patients with neurosecretory dysfunction were tested because of paradoxically elevated GH serum levels during retesting of spontaneous GH secretion. Precisely, the baseline of GH levels was raised to clearly above zero, and the mean GH levels during the night were elevated too. These results were in clear contrast to the initial test results and strongly suggested the presence of anti-GH antibodies. The other 2 patients were tested because the physicians did expect a better growth response than observed.

In group B with low GH response or positive genetics, there were 2 Turkish unrelated sibling pairs with IGHD type 1A with a homozygous 6.7-kB *GH1* gene deletion, 1 patient with septo-optic dysplasia and a *HESX1* mutation, and 1 patient with multiple pituitary hormone deficiency and a severe cerebral midline defect. Testing of the patient with the midline defect was performed because of insufficient catch-up growth after the third month of treatment accompanied by paradoxically elevated basal GH serum levels. The patient with the *HESX1* mutation was tested because the physicians did expect a better growth response than observed. There were no cases of idiopathic isolated GHD in the entire group.

The main clinical data of group A with a normal response to the GH provocation tests ($n = 6$) and of group B with severe GHD ($n = 6$) are shown in Table 1. Compared with the children of group B, the children of group A were older at the start of GH treatment and had higher serum GH and IGF-1 values (which were within the reference), and, in contrast to group B, they had no malformation of the pituitary gland and no additional pituitary hormone deficiencies.

Analysis of the growth charts of each patient (Fig. 1) revealed an insufficient response to rhGH in one sibling pair with IGHD type 1A (patients 10 and 11) and in the child with cerebral midline defect (patient 15). The rhGH treatment of the 2 siblings with IGHD type 1A was stopped after changing brands of rhGH that had no therapeutic effect. The rhGH treatment of the child with midline defect was paused for 3 months and then continued using a different rhGH brand with good efficacy (Fig. 1).

The growth of the other 9 patients, including the second sibling pair with IGHD type 1A, was unaffected by anti-GH

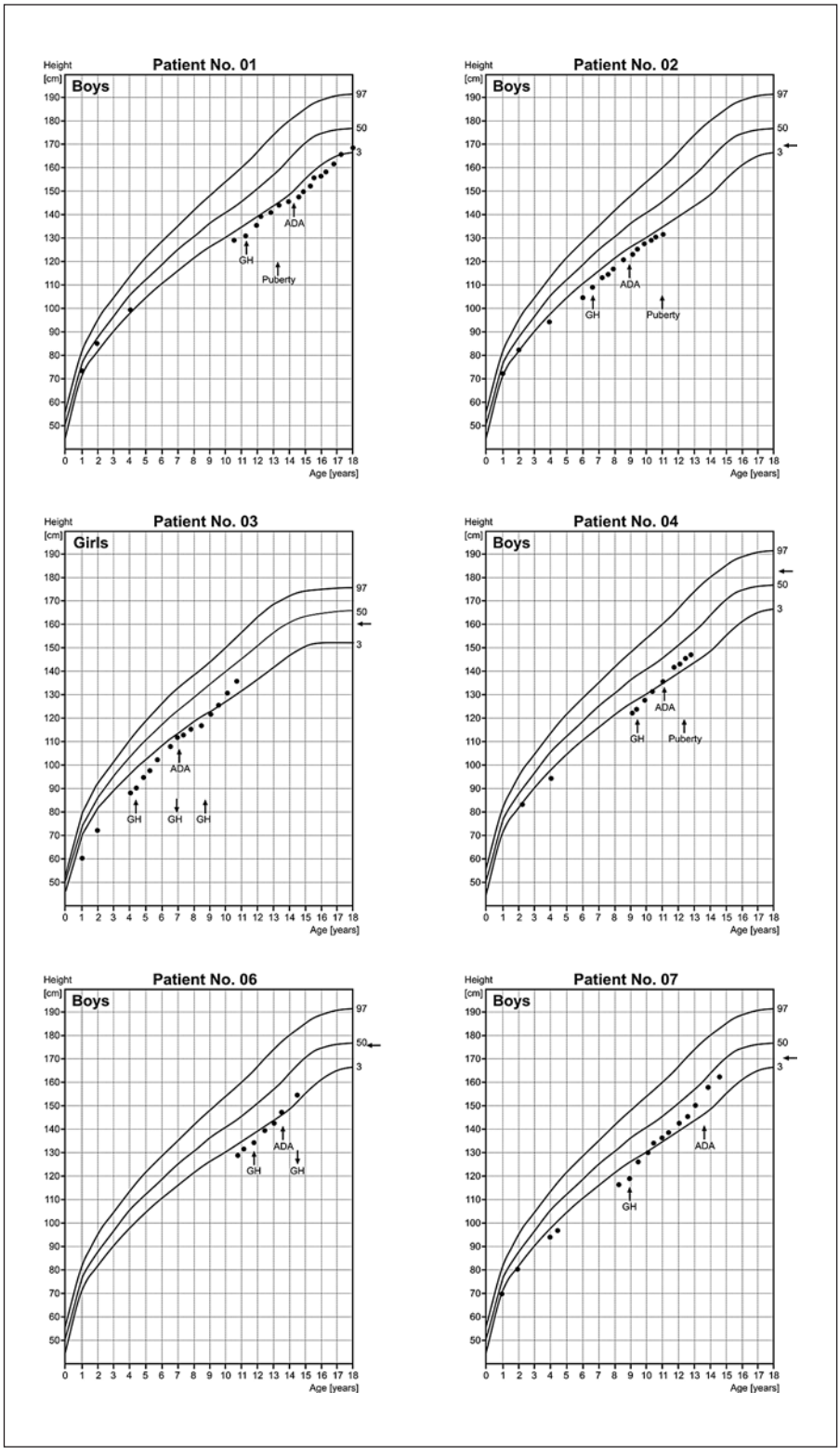
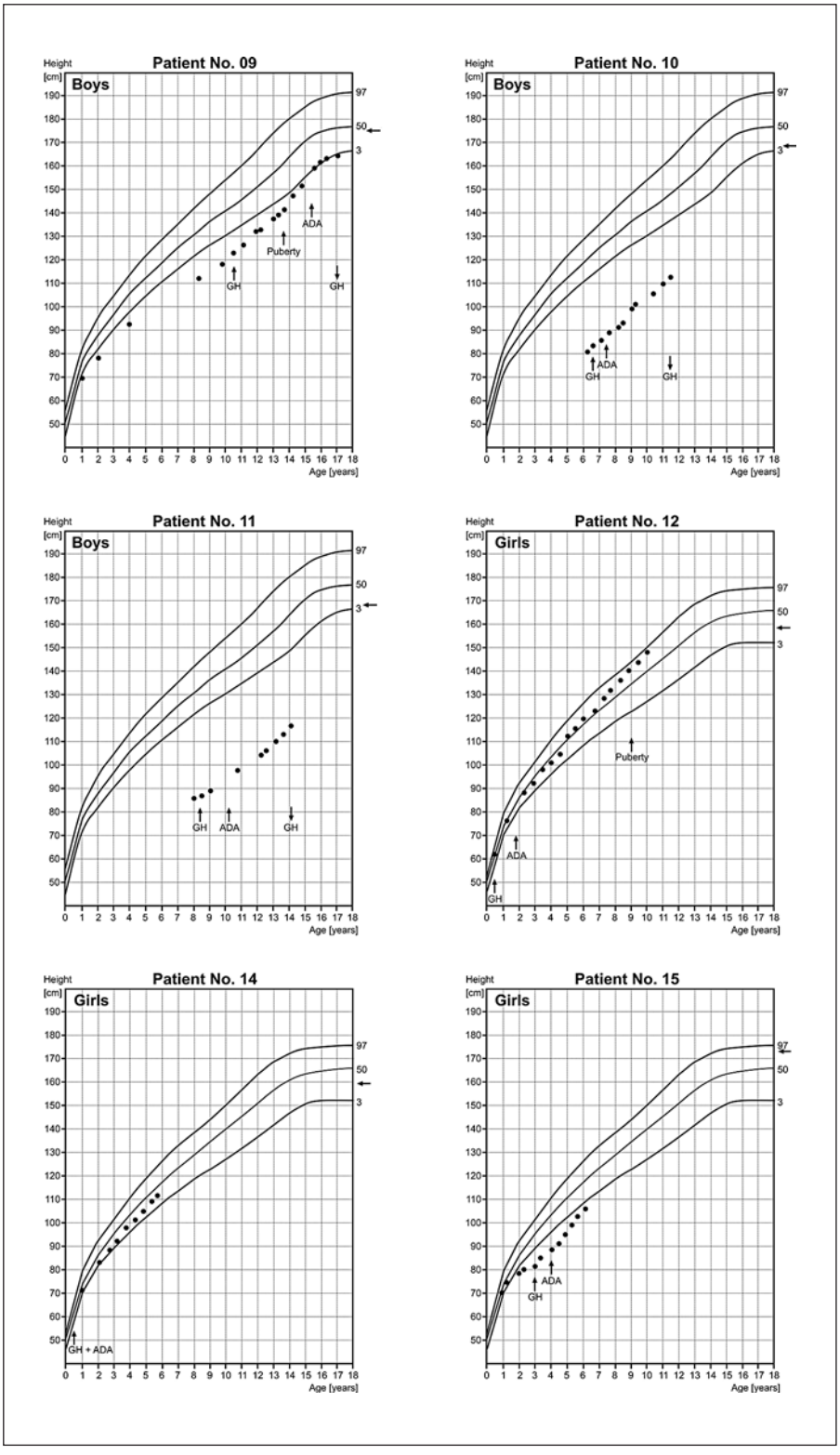


Fig. 1. Individual growth charts of the 12 patients with anti-GH antibodies. Arrows indicate specific time points; "GH" indicates start or end of rhGH therapy; "puberty" indicates the start of puberty; "ADA" indicates the first time that anti-GH antibodies were detected.

(Figure continued on next page.)


Height [cm]
Patient No. 09
Boys
97
50
3
ADA
Puberty
GH
GH
Age [years]
Patient No. 10
Boys
GH
ADA
GH
Patient No. 11
Boys
GH
ADA
GH
Patient No. 12
Girls
Puberty
ADA
GH
Patient No. 14
Girls
GH + ADA
Patient No. 15
Girls
ADA
GH

1

Table 2. Individual anti-hGH antibody test results and the characteristics of the patients and their treatment

Patient	Group	Age, years	rhGH treatment duration, years	RIA value	Inhibition, %	Treatment change
01	A	14.3	3.0	1.547	n.a.	None
02	A	9.0	2.3	1.222	n.a.	None
04	A	11.1	1.7	1.819	41.40	None
06	A	13.6	1.8	1.820	48.05	None
07	A	13.6	4.6	1.540	36.40	None
09	A	15.4	4.9	1.291	22.90	Change of rhGH brand
03	B	7.2	2.8	1.411	25.67	Pause of rhGH for 2 years; then restart
10	B	7.4	0.8	2.880	63.87	Change of rhGH brand; then stop of treatment
11	B	10.4	1.9	2.979	60.65	Change of rhGH brand; then stop of treatment
12	B	1.8	1.3	1.191	13.04	None
14	B	0.6	n.a.	1.205	21.03	None
15	B	4.0	1.0	2.068	48.99	Pause of rhGH for 3 months; then change of rhGH brand

RIA, radioimmunoassay; n.a., not available.

antibodies (Fig. 1). The brand of rhGH was changed in patient 9, although there was no clinical evidence of growth inhibition. Similarly, in patient 3, rhGH treatment was paused for 2 years, although growth velocity had been normal at the time of the detection of anti-GH antibodies.

The quantitative assay results detecting anti-GH antibodies are shown in Table 2. Importantly, the highest radioimmunoassay values and the highest percentages of inhibition were present in those 3 patients (patients 10, 11, and 15) with growth failure when anti-GH antibody tests were positive.

Discussion

This is a laboratory-based study of patients who tested positive for anti-GH antibodies in Germany over a time span of 5 years. The patients were selected for this analysis by their physician because of an insufficient growth response, due to paradoxically elevated GH serum levels during early retesting or due to IGHD type 1A. Interestingly, almost one fifth of the patients in this study carried anti-hGH antibodies (19%). In a previous phase 3 study comparing two different rhGH brands, a clearly low prevalence of anti-hGH antibodies (2–3%) was reported during the first 6–12 months of treatment in the total group of probands screened [16]. In contrast, Rougeot et al. [6] reported up to 22% of GH antibody positives in their comprehensive study, which was a follow-up of rhGH treatment up to 24 months. In their longitudinal study, Rougeot et al. [6] described a rise of anti-GH antibodies during the first 6–9 months of rhGH treatment followed by a decrease to undetectable titers at 30 months. In this study, the median duration of treatment at first detection of anti-GH antibodies was 2.2 years and ranged from <1 to 4.9 years. Different prevalence data at different times of treatment may be explained by the different selection of patients, different assays used, and the different durations of follow-up. However, it is of interest that the percentages found in the unselected group by Rougeot et al. [6] and ours were comparable.

Importantly, positive test results were not associated with therapeutic failure except in 2 siblings with IGHD type 1A and a patient with midline defect and multiple pituitary hormone deficiency. The low clinical significance of anti-GH antibodies may be explained by their low titers, low affinity for rhGH, or a lack of a neutralizing effect. The 3 patients who experienced growth failure in the presence of anti-GH antibodies had the highest titers with the highest inhibitory potency. Similar findings were reported by Pringle et al. [17], who identified 16 patients with anti-GH antibodies but no growth failure when treated with rhGH. In agreement with Pringle et al. [17], Rougeot et al. [6] did not detect an effect on growth velocity in 17 positive-tested patients treated with three different rhGH brands. Massa et al. [4] reported no effect on growth in 18 patients with anti-GH antibodies who were mainly treated with metGH. However, patients with IGHD type 1A make the difference: there are several reports on high titers of anti-GH antibod-

ies and severe growth failure in patients with IGHD type 1A, albeit treated with rhGH [10, 18]. Interestingly, growth failure is not a constant finding in IGHD type 1A; even in the same family, outcomes may vary, indicating that the variable response of the immune system is a major factor [18]. In this study, one sibling pair with IGHD type 1A responded well to rhGH, while the other sibling pair with the same homozygous *GH1* deletion did not respond at all. The first sibling pair had low antibody titers and a low percentage of inhibition in the assay, resembling previous findings.

Limitations of this study include the retrospective design, the relatively small number of positive-tested patients analyzed and the inconsistency of the individual changes of treatment. The strengths of this study include the laboratory-based identification of patients and the complete documentation of longitudinal data of all positively tested patients except one.

In conclusion, the biological significance of anti-GH antibodies seems to be limited to some rare patients with very severe GHD, mostly those with IGHD type 1A. In all other cases, the clinical significance of anti-GH antibodies is still questionable, especially when titers are low. Instead of pausing rhGH treatment, changing the rhGH brand or alternatively treating with rhIGF-1, we recommend monitoring the anti-GH antibody concentrations in these patients and a careful "wait and see" strategy without any change of treatment, which is more rational based on the limited information available. With the upcoming use of long-acting GH, the biological significance of anti-GH antibodies will have to be reassessed [19].

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Statement of Ethics

Subjects or their guardians gave their written informed consent. The study protocol was approved by the research institute's committee on human research. The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Gerhard Binder designed the study, interpreted the data, drafted and revised the manuscript. Laura Heidenreich acquired the data, analyzed the data, revised the manuscript, and approved the final version. Dirk Schnabel analyzed the data, revised the manuscript, and approved the final version. Desire Dunsheimer analyzed the data, revised the manuscript, and approved the final version. Rudolf Oevrink analyzed the data, revised the manuscript, and approved the final version. Wieland Kiess analyzed the data, revised the manuscript, and approved the final version. Antje Körner analyzed the data, revised the manuscript, and approved the final version. Jürgen Kratzsch designed the study, performed the assays, interpreted the data, revised the manuscript, and approved the final version.

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