Development and Validation of a Prediction Rule for Growth Hormone Deficiency Without Need for Pharmacological Stimulation Tests in Children With Risk Factors

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Introduction: Practice guidelines cannot recommend establishing a diagnosis of growth hormone deficiency (GHD) without performing growth hormone stimulation tests (GHST) in children with risk factors, due to the lack of sufficient evidence.

Objective: Our goal was to generate an evidence-based prediction rule to diagnose GHD in children with growth failure and clinically identifiable risk factors.

Methods: We studied a cohort of children with growth failure to build the prediction model, and a second, independent cohort to validate the prediction rule. To this end, we assessed the existence of: pituitary dysgenesis, midline abnormalities, (supra)sellar tumor/surgery, CNS infection, traumatic brain injury, cranial radiotherapy, chemotherapy, genetic GHD, pituitary hormone deficiencies, and neonatal hypoglycemia, cholestasis, or hypogenitalism. Selection of variables for model building was performed using artificial intelligence protocols. Specificity of the prediction rule was the main outcome measure in the validation set.

Results: In the first cohort (n=770), the resulting prediction rule stated that a patient would have GHD if (s)he had: pituitary dysgenesis, or two or more anterior pituitary deficiencies, or one anterior pituitary deficiency plus: neonatal hypoglycemia or hypogenitalism, or diabetes insipidus, or midline abnormalities, or (supra)sellar tumor/surgery, or cranial radiotherapy ≥18 Gy. In the validation cohort (n=161), the specificity of the prediction rule was 99.2% (95% CI: 95.6–100%).

Conclusions: This clinical rule predicts the existence of GHD with high specificity in children with growth disorders and clinically identifiable risk factors, thus providing...
compelling evidence to recommend that GHD can be safely diagnosed without recurring to GHST in neonates and children with growth failure and specific comorbidities.

Keywords: multiple pituitary hormone deficiencies, pituitary dysgenesis, short stature, growth failure, midline abnormalities

INTRODUCTION

Growth is a good indicator of a child’s health, and growth failure prompts the pediatrician to search for nutrition disorders, subclinical chronic diseases, or hormone deficiencies. Growth hormone deficiency (GHD) is characterized by the insufficient production of growth hormone (GH), which leads to deficient insulin-like growth factor 1 (IGF1) synthesis and secretion leading to growth failure in children. An accurate diagnosis is crucial for timely initiation of treatment in order to optimize child growth and adult height and to avoid co-morbidities resulting in impaired quality of life (1).

Auxologic evaluation lies at the basis of the diagnosis of GHD in children (2–4), and the Growth Hormone Research Society clearly defined in its consensus guidelines released in year 2000 the clinical criteria that should prompt immediate investigation of GHD in childhood and adolescence (5). Many national endocrine societies have set up procedures to diagnose GHD, and the health authorities of several countries have established national or regional boards that review and monitor GH prescriptions (2, 6). Multiple GH stimulation tests (GHSTs) have been designed to evaluate GH sufficiency in children in an attempt to reach the most accurate diagnosis of GHD (7, 8). Although they have limitations, GHSTs are still used as the gold standard for the diagnosis of pediatric GHD in most countries (1, 8).

The guidelines of the US Pediatric Endocrine Society advocate for restricting GH testing (1). For instance, in newborns with hypoglycemia and/or neonatal cholestasis the diagnosis of GHD is an emergency, and GHSTs may be dangerous (9). In a child with growth failure, the presence of micropenis and cryptorchidism or of craniofacial midline abnormalities are other putative predictors of GH deficiency (1, 5). Acquired GHD may be suspected in patients with intracranial tumors, severe traumatic brain injury or cranial radiotherapy in whom a common co-morbidity is hypothalamic obesity, associated with blunted response during GHSTs (10). However, none of these studies provide predictive values that can guide medical decisions. Therefore, due to the lack of sufficient evidence, the guidelines cannot recommend establishing the diagnosis of GHD without GHSTs in patients with these conditions (1, 11).

Pharmacological GHSTs remain a standard practice in pediatric patients—usually due to requirements from health systems (6)—even in children with clearly identifiable potential risk factors for GHD (12–14) in whom the implementation of GHSTs might be considered redundant (1, 6, 15). The aim of the present study was to assess predictors of GHD in children with growth failure by analyzing a large cohort of pediatric patients in whom GHSTs had been performed in a tertiary referral center. Our primary objective was to develop and validate an accurate clinical prediction rule with high enough specificity to allow confirmation of the diagnosis of GHD in children without recurring to GHSTs. We, therefore, designed and validated a multivariable prediction model in accordance with the Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD) statement (16).

PATIENTS AND METHODS

Study Design and Data Sources
We performed a study designed to develop and validate a prediction rule to diagnose GHD with the highest specificity rate in children with growth failure and clinically identifiable risk factors, who underwent GHSTs. We analyzed clinical and biochemical characteristics and brain imaging findings in all patients younger than 18 years of age who underwent GHSTs at the Division of Endocrinology of the Hospital de Niños Ricardo Gutierrez, a tertiary pediatric public hospital in the city of Buenos Aires, Argentina, between August 1, 2004 and July 31, 2014. Additionally, we validated the predictive rule in a second, independent cohort including GHSTs performed between February 1, 2017 and January 31, 2019.

We included GHSTs performed in patients who:

1. Met the criteria required for performing a GHST according to the Summary Statement of the Growth Hormone Research Society (5), as follows:
   a. - Severe short stature, defined as a height >3 SD below the mean, or
   b. - Height >1.5 SD below the mid-parental height, or
   c. - Height >2 SD below the mean and a height velocity over 1 year >1 SD below the mean for chronological age, or a decrease in height SD of >0.5 over 1 year in children over 2 years of age, or
   d. - In the absence of short stature, a height velocity >2 SD below the mean over 1 year or >1.5 SD sustained over 2 years, or
   e. - Signs indicative of an intracranial lesion, or
   f. - Signs of multiple pituitary hormone deficiency, or
   g. - Neonatal symptoms and signs of GHD.

2. Underwent two sequential GHSTs (arginine-clonidine), or only one test in patients weighing <10 kg.

We excluded GHSTs performed in patients:

1. With incomplete medical records.
2. With poorly controlled chronic disorders (e.g., hypothyroidism, or gastrointestinal, immunological, nephrogenic or hematological diseases).
3. In whom the GHST was performed for re-testing reasons.
Neonatal hypogenitalism: At least two of the following: micropenis, jaundice, neonatal cholestatic.

Penile size was compared to standardized data of the Argentine population (28).

Clinical Evaluation:
Auxologic data and past medical and family history were collected from medical records. Height and weight were expressed as standard deviation scores (SDS) using Argentine standards (25). Growth velocity was assessed considering a period of at least 6 months. Pubertal stage was assessed at the time of GHSTs according to Marshall and Tanner (26, 27).

Penile period of at least 6 months. Pubertal stage was assessed at the 

Hormone Measurements:
Plasma GH was measured using a chemiluminescent immunometric assay (ICMA; Immulite 2000; Siemens Healthcare Diagnostics, Gwynedd, UK) at baseline, and 30, 45, 60 and 90 min after iv administration of arginine-HCl (0.5 g/kg), intravenous clonidine (0.1 mg/m²) (29). Intra- and inter-assay coefficients of variation were <4%. GH standards were IS-80/505 from 2004 to 2011 (17) and rhGH IS 98/574 from 2012 to 2019 (18). Total IG1 was measured by radioimmunoassay (30) and, since October 2009, by ICMA (Immulate 2000, Siemens) (31). Serum levels of T4, free T4, T3, TSH, cortisol, ACTH, and prolactin were determined by electrochemiluminescence (Elecsys Cobas e411; Roche, Indianapolis, IN, USA) (22, 32).

Imaging:
Pre- and post-gadolinium enhanced T1- and T2-weighted images of magnetic resonance imaging (MRI) studies of the brain and hypothalamic-pituitary region were evaluated. When MRI was not performed, it was considered as “missing value.”

Prediction Model-Building Procedures and Statistical Analysis:
We followed the TRIPOD guideline (16) for development, validation, and reporting of the proposed score. Most of the work was done using the KNIME software, version 3.7.0 (which is open source under GNU General Public License), and we also performed calculations using Microsoft Excel® for Microsoft 365 MSO version 16.0.13001.20266 and GraphPad Prism® version 8.4.3.

It is key to the analysis that we did not look for an unbiased model. The predictive criteria were intended to detect cases with GHD with the highest specificity. Type II errors (false negatives) were tolerated since, in real world practice, those cases would be subsequently diagnosed by the GHSTs. Conversely, type I errors (false positives) would have a very high impact since a patient

TABLE 1 | Definitions of predictors used in the model building.

| Predictor                     | Operational definition                                                                 | Reference |
|-------------------------------|----------------------------------------------------------------------------------------|-----------|
| Pituitary dysgenesis          | MRI of the hypothalamic-pituitary region, pre- and post-gadolinium enhanced T1- and T2 weighted images, with at least two of the following: anterior pituitary hypoplasia or aplasia, interrupted or hypoplastic stalk, ectopic or absent posterior lobe (including empty sella) | (15)      |
| Clinical or radiological craniofacial midline abnormalities | Cleft lip and palate, single central incisor, agenesis of the nasal septum, septo-optic dysplasia, Rieger syndrome, holoprosencephaly, transphenoidal myelomeningocele, hydrocephalus, Chiari type 1 malformation |           |
| Sellar or suprasellar tumor/surgery | Imaging study of the CNS indicating the existence of a mass in the sellar or suprasellar region, or surgical report indicating compromise of sellar or suprasellar region, except for pituitary microadenoma |           |
| Central nervous system infection | Meningitis, meningoencephalitis, encephalitis, pyogenic ventriculitis |           |
| Severe traumatic brain injury | Glasgow Coma Scale score ≤8 | (20)      |
| Cranial radiotherapy          | ≥18 Gy | (21)      |
| Chemotherapy                  | Use of mono- or poly-chemotherapy for at least 6 months |           |
| Familial or sporadic GHD of genetic etiology | Index case and one or more first-degree relatives with GHD in the family, or index case with a pathogenic mutation |           |
| TSH deficiency                | Serum basal free T4 < 0.8 ng/dl with TSH ≤ 10 mIU/l in patients under 2 months of age and ≤ 6.5 mIU/l in older infants | (22)      |
| ACTH deficiency               | Serum basal cortisol < 6.5 µg/dl with low or normal plasma ACTH | (22)      |
| Prolactin deficiency          | Serum basal prolactin < 2.5<sup>th</sup> centile for age and sex | (22)      |
| Central diabetes insipidus    | Polyuria associated with a urinary/plasma osmolality ratio <1.5 and plasma osmolality >300 mosmol/l | (22)      |
| Neonatal persistent hypoglycemia | Plasma glucose <50 mg/dl (≤ 2.8 mmol/l) days 3–28 of age (i.e., the period of transitional glucose regulation of postnatal days | (23)      |
| Neonatal cholestatic jaundice | Conjugated bilirubin/total bilirubin >0.15 | (24)      |
| Neonatal hypogenitalism       | At least two of the following: micropenis defined as penile length <-2.5 standard deviation scores for age, cryptorchidism and micro-orchidism defined as testis volume <1 ml | (22)      |
would be diagnosed as having GHD without undergoing GHST and receive GH treatment. Therefore, the model should have the highest specificity (low rate of false positives) while keeping an acceptable sensitivity (rate of false negatives) to be clinically relevant. Since the predictors we considered were mostly binary, we could not construct ROC curves. Therefore, to develop and validate an accurate clinical prediction rule intended to diagnose GHD with the highest specificity in children without recurring to GHSTs, we used the following methodology (33, 34):

**Step 1: Data Exploration**

Data gathered from clinical experience and from existing criteria (1, 5) included the 15 dichotomous variables as potential predictors for diagnosing GHD without GHSTs defined in Table 1. We also included auxologic data (height and weight) and IGF1 and IGFBP3 serum levels, as continuous variables. Data exploration (cohort 2004–2014) consisted of: a) establishing linear dependencies between variables, using Pearson’s chi-squared test and product-moment coefficients for discrete and continuous variables respectively (a summary of all pairwise correlations is presented in Supplementary Figure 1); b) analyzing distributions of each continuous variable (distribution summary in Supplementary Figure 2), and c) establishing a-priori probabilities for GHD using a frequency table (Supplementary Table 1) for every variable (for instance, see a-priori for insipid diabetes in Supplementary Table 2, this analysis was done for each nominal variable). Data exploration and metrics used followed those previously described by Gelman (35). Finally, a model was automatically built using an information gain algorithm. We chose to construct a decision tree using the algorithm described by Quinlan (36). Decisions trees have the advantage that they have a graphical representation, and they give relevant information when inspected. The decision tree was subsequently used in the step 2 to select the predictors to be used in the final model.

**Step 2: Feature Selection**

Finally, we calculated conditional probabilities (from frequency tables) for the cases where two variables could give similar information. All variables selected by statistical means were checked from the point of view of clinical criteria.

Feature selection was done by combining statistical analyses with clinical criteria. First, from a quantitative point of view, we analyzed the decision tree built during step 1 and selected the variables that maximized entropy reduction. We also built a random forest to derive an analysis of feature relevance in order to validate the robustness of the set of conditions selected, as described (37). The algorithm used in KNIME to establish variable importance using random forests was the Tree Ensemble Learner (Supplementary Table 3), as previously established (38). Finally, all variables selected by statistical means were checked from the point of view of clinical criteria.

**Step 3: Model Building**

Model building was done in an iterative way: a model built using a machine learning algorithm was discussed from the point of view of clinical criteria. The model was then refined to build a new version. The process was iterated until the prediction rule was satisfactory from both points of view: the quantitative analysis and the clinical criteria.

We started by building a decision tree using the machine learning algorithm mentioned in step 2, fed only with the selected predictors. The result was discussed from the point of view of clinical criteria. The model was then refined to build a new version, by adjusting the parameters of the algorithm. The algorithm used in KNIME to build the decision trees was the Decision Tree Learner node. Some characteristics of this algorithm are as follows: numeric splits are always binary, dividing the domain in two partitions at a given split point. Nominal splits can be either binary or they can have as many outcomes as nominal values. The quality measure used for split calculation was the gain ration, no pos pruning method was used during the execution and the minimum number of records per node was set to 2. No root column was forced. We “pruned” the branch that had more false positives from the refined model, which led to lower sensitivity and higher specificity, which was the original goal. The final tree is shown in Supplementary Figure 3.

**Step 4: Validation**

In order to validate the prediction model, a dataset from a different cohort of patients (2017–2019) was used. This second cohort was independent from the first one, and the data set had not been used in the derivation of the model nor the analysis.

Validation included three axes: a) Safety, interpreted as no false positives. We aimed to keep type I error near 0 in the validation cohort; b) Usefulness, translated to sensitivity of the model, set at >0.2, meaning that the prediction rule would provide a diagnosis in at least 20% of all patients with suspected GHD, and c) significance: to establish significance, the null hypothesis H0 was that the proposed criterion did not imply GHD (as tested by GHSTs), and hence it was independent. We did not assume any further conditions nor particular distribution of the data. Statistical significance was not analyzed in the original dataset (2004–2014) since these data were used to derive the diagnostic procedure and to perform exploratory analysis of the features. For the validation data (2017–2019), we set an α-value of 0.00001 in order to build a very conservative model.

**RESULTS**

A total of 1,006 GHSTs were eligible for evaluation: 770 out of the 834 in the 2004–2014 cohort used to build the prediction model, and 161 of the 172 in the 2017–2019 cohort used to validate the model, could be analyzed (Figure 1).

Clinical characteristics of the 2004–2014 cohort are summarized in Table 2. It includes GHSTs performed in 491 boys and 279 girls (1.8:1), 79% were prepubertal, with a median age of 7.74 years and median height SDS of – 2.51. Of the 770 GHSTs analyzed, 150 (19.5%) yielded results defined as GHD. The auxologic features were similar in patients without GHD (controls) and patients with GHD (cases), and within the latter,
clinical features were similar in those predicted by the model and those not predicted. Both groups included congenital and acquired conditions (Supplementary Table 4). An MRI, used to define “pituitary dysgenesis” (Table 1) was available in 218 of the 770 patients (120 of 150 with GHD and 98 of 620 without GHD).

In this cohort of 770 patients, after tuning the classification model trained using the 15 potential dichotomic predictors, we selected 9 variables of interest, with an odds ratio (OR) >5 and a p-value <0.0001 (Table 3); “cranial radiotherapy,” with an OR=4.4, was also selected given its clinical relevance. The continuous variables height, weight, and serum levels of IGF1 and IGFBP3 were not informative enough to be considered in the model (Table 2). We used a gain ratio, entropy reducing algorithm to automatically build a decision tree from the 10 selected variables, without reduce error pruning or limits on minimum number of records. Anterior pituitary hormone (TSH, ACTH, prolactin) deficiencies were categorized as 0 (no deficiency), 1 (any one deficiency), or ≥1 (multiple pituitary hormone deficiency) (Supplementary Figure 3). This model reached a 99.2% specificity (Table 4, “decision tree” column). Its sensitivity (49.3%) was above the required threshold, and overall accuracy was 89.5%, with a resulting F-measure = 0.646. Cross validation, with a test set of 20% of the cases randomly selected using stratified sampling, showed similar results, which remained consistent after repeating the random selection and changing the parameters (for instance, Gini index instead of gain ratio, or by using pruning). Alternative models, such as Naïve Bayes, also gave similar results.

The conditions “pituitary dysgenesis” and “≥1 anterior pituitary hormone (TSH, ACTH or prolactin) deficiency” were selected by the entropy reduction algorithms as the first variables to analyze. Interestingly, this selection was consistent with relevant clinical criteria. We also built a random forest (37) to derive an analysis of feature relevance, that reinforced the robustness of the set of conditions selected (Supplementary Table 3). Finally, we also calculated conditional probabilities for the cases where two variables could give similar information.
The resulting prediction rule stated that a patient, who met the criteria required for performing GHSTs according to the Summary Statement of the Growth Hormone Research Society (5), was a case (GHD associated to risk factors) if (s)he met the following conditions:

1. Pituitary dysgenesis on MRI, or
2. Two or more anterior pituitary hormone (TSH, ACTH or prolactin) deficiencies, or
3. At least one anterior pituitary hormone (TSH, ACTH or prolactin) deficiency plus one of the following:

TABLE 2 | Clinical characteristics of the 2004–2014 cohort, used for model building, classified according to growth hormone stimulation test (GHST) results.

| Clinical characteristic | Total cohort | No GHD | GHD (all) | GHD (predicted by model) | GHD (not predicted by model) |
|-------------------------|--------------|--------|----------|--------------------------|----------------------------|
| No. (%)                 | 770(100)     | 620(80.5) | 150(19.5) | 61(7.9 of total cohort;40.7 of all GHD) | 89(11.6 of total cohort;59.3 of all GHD) |
| Gender M/F, No. (% M)   | 491(279)(63.8) | 403(217)(65.0) | 88(62)(58.7) | 41(20)(67.2) | 46(43)(51.7) |
| Age at GHST median, years(IQ range) | 7.74(5.22;11.08) | 7.82(5.52;11.11) | 7.15(3.52;10.89) | 6.42(3.21;11.85) | 7.37(4.08;10.16) |
| Pubertal stage at GHST, No.(% I) | | | | | |
| I                       | 609(79.1) | 486(78.4) | 123(82.0) | 47(77.0) | 76(85.4) |
| II                      | 99(12.9) | 83(13.4) | 16(10.7) | 8(13.2) | 8(9.0) |
| III                     | 51(6.6) | 43(6.9) | 8(5.3) | 3(4.9) | 5(5.6) |
| IV                      | 10(1.3) | 7(1.1) | 3(2.0) | 3(4.9) | 0(0.0) |
| V                       | 1(0.1) | 1(0.2) | 0(0.0) | 0(0.0) | 0(0.0) |
| Height SDS, median(IQ range) | | | | | |
| -2.51(-3.02; -2.20) | -2.58(-3.18; -2.08) | -2.53(-3.39; -2.00) | -2.61(-3.06; -2.08) |
| Weight SDS, median(IQ range) | | | | | |
| -2.30(-2.77; -1.71) | -2.03(-2.78; -0.74) | -1.87(-2.86; -0.14) | -2.07(-2.73; -1.15) |
| BMI SDS, median(IQ range) | | | | | |
| -0.50(-0.94; -0.24) | -0.62(-0.96; -0.34) | 0.00(-0.76; 0.51) | 0.21(-0.53; 0.72) | 0.13(-0.87; 0.3) |
| IGHD/MPHD, No. (% IGHD) | 86(64)(73.0) | 18(45)(29.5) | 69(81)(25.5) | 89(11.6) |
| IGFI SDS, median(IQ range) | | | | | |
| -1.46(-3.46; -0.59) | -2.92(-4.68; -1.77) | -3.48(-4.68; -2.59) | -2.55(-4.61; -1.23) |
| IGFBP3 SDS, median(IQ range) | | | | | |
| -1.38(-2.28; -0.66) | -2.88(-3.32; -2.19) | -3.22(-3.72; -2.50) | -2.76(-3.09; -1.51) |

F, female; GHD, growth hormone deficiency; IGHD, isolated GHD; IQ, interquartile; M, male; MPHD, multiple pituitary hormone deficiency; SDS, standard deviation score.

TABLE 3 | Categorical distribution and odds ratios for growth hormone deficiency of predictors used for model building (cohort 2004–2014).

| Predictor | Stratum | Full cohort n=770 | GHD n=150 | No GHD n=620 | OR (95% CI) | P value |
|-----------|---------|-------------------|----------|--------------|-------------|---------|
| Pituitary dysgenesis | Yes | 38 | 38 | 0 | = | <0.0001 |
| Clinical or radiological craniofacial midline abnormalities | Yes | 36 | 19 | 17 | 13.2 | <0.0001 |
| Suprasellar or sellar tumor/surgery | Yes | 734 | 131 | 603 | 2.6 | 10.4 |
| Central nervous system infection | Yes | 6 | 0 | 6 | 0.0 | 6.03 |
| Severe traumatic brain injury | Yes | 3 | 1 | 2 | 2.1 | 0.479 |
| Cranial radiotherapy | Yes | 20 | 10 | 10 | 4.4 | 0.002 |
| Chemotherapy | Yes | 21 | 10 | 11 | 4.0 | 0.003 |
| Familial or sporadic GHD of genetic etiology | Yes | 749 | 140 | 609 | 1.7 | 9.0 |
| TSH deficiency | Yes | 766 | 147 | 619 | 1.9 | 164.3 |
| ACTH deficiency | Yes | 43 | 42 | 1 | 240 | 0.7 |
| Prolactin deficiency | Yes | 32 | 32 | 0 | = | <0.0001 |
| Central diabetes insipidus | Yes | 738 | 118 | 620 | 43.5 | = |
| Neonatal hypoglycemia | Yes | 749 | 133 | 616 | 6.6 | 54.5 |
| Neonatal cholestatic jaundice | Yes | 30 | 18 | 12 | 9 | 6.9 |
| Neonatal hypogentalism | Yes | 9 | 5 | 4 | 5.3 | 0.017 |

CI, confidence interval; GHD, growth hormone deficiency; OR, odds ratio. P value of Fisher’s exact test.
TABLE 4 | Performance of the prediction rule for diagnosing growth hormone deficiency (GHD).

| Decision tree (2004–2014 cohort) | Prediction rule (2004–2014 cohort) | Validation (2017–2019 cohort) |
|---------------------------------|-------------------------------------|-----------------------------|
| Specificity, % (95% CI)         | 99.2 (98.1–99.7)                    | 100 (99.4–100)              | 99.2 (95.6–100) |
| Sensitivity, % (95% CI)         | 49.3 (41.5–57.3)                    | 40.7 (33.1–48.7)            | 55.6 (39.6–70.5) |
| Positive PV, % (95% CI)         | 93.7 (86.6–97.3)                    | 100 (94.1–100)              | 95.2 (77.3–99.8) |
| Positive LR                    | 61.2                                | >1.000                      | 69.4 |
| NNT (95% CI)                   | 1.21 (1.15–1.36)                    | 1.14 (1.11–1.26)            | 1.19 (1.11–1.62) |
| Accuracy, %                    | 89.5                                | 88.4                        | 89.4 |

LR, likelihood ratio; NNT, number needed to test with prediction rule to diagnose GHD; PV, predictive value.

a. Neonatal symptoms of pituitary deficiency (hypoglycemia or hypogotalism)
b. Central diabetes insipidus
c. Clinical or radiological craniofacial midline abnormalities
d. Suprasellar or sellar tumor/surgery
e. Cranial radiotherapy ≥18 Gy

The proposed criteria were very conservative for specificity (Table 4, “prediction rule” column), and missed 89 cases of GHD of the 2004–2014 cohort, which could then be diagnosed using GHSTs. Therefore, the proposed predictive rule diagnosed 61 GHD cases, which represents 40.7% of all GHD patients.

We validated the predictive rule in an independent cohort of patients (2017–2019). Clinical characteristics of this second cohort are summarized in Table 5. It includes GHSTs of 111 boys and 50 girls (2.2:1), 87% prepubertal, with a median age of 7.67 years and median height SDS of −2.57. This validation group was similar to the 2004–2014 cohort in terms of age, gender, or proportion of pathological tests compared, as well as in IGF1 serum levels. GHD was diagnosed in 36 patients (22.4%). An MRI was available in 46 of the 162 patients (27 of 36 with GHD). In this validation cohort (2017–2019), the prediction rule showed a specificity of 99.2%, and its positive predictive value was 95.2% (Table 4, “validation” column). The false-positive case according to our rule was a 10-year-old boy with height at −3.98 DS, IGF1 level 78 ng/ml (reference for age 60–370), who showed one peak GH level of 6.71 ng/ml at GHST, very close to the cutoff value, and no other remarkable feature. Given the severe growth deficiency, a therapeutic trial with GH treatment resulted in 1.06 DS gain in height after 1 year. GHD could not be ruled out in this patient despite the GHST result. The positive likelihood ratio of the prediction rule was 69.4, and the number needed to test with the rule was 1.19.

Finally, we tested significance following the methodology presented. The a priori probability of a GHD case in the second cohort was 36/161 (22.4%). We can safely assume that individual patients are independent cases, so we have a set of Bernoulli Trials under a binomial distribution. Thus, the p-value given by the cumulative function was <0.000001.

TABLE 5 | Clinical characteristics of the 2017–2019 cohort, used to validate the predictive rule, classified according to Growth Hormone Stimulation Test (GHST) results.

| Clinical characteristic          | Total cohort | No GHD | GHD (all) | GHD (predicted by model) | GHD (Not predicted by model) |
|---------------------------------|-------------|--------|----------|--------------------------|-----------------------------|
| No. (%)                         | 161 (100)   | 125 (77.6) | 36 (22.4) | 21 (13.0 of total cohort; 58.3 of all GHD) | 15 (9.3 of total cohort; 41.7 of all GHD) |
| Gender M/F, No. (% M)           | 111/50 (68.9) | 87/39 (69.6) | 24/12 (66.7) | 14/7 (66.7) | 10/5 (66.7) |
| Age at GHST median, years (IQ range) | 7.67 (5.1; 11.2) | 7.64 (5.2; 11.02) | 8.83 (7.3; 11.29) | 6.32 (3.57; 11.16) | 9.35 (5.49; 11.31) |
| Pubertal stage at GHST, n       | I           | 140 (87.0) | 109 (87.2) | 31 (86.1) | 20 (95.2) |
|                                | II          | 7 (5.6) | 5 (6.5) | 2 (5.6) | 0 (0.0) |
|                                | III         | 2 (1.3) | 6 (7.2) | 2 (5.6) | 1 (4.8) |
|                                | IV          | 1 (0.6) | 0 (0.0) | 1 (2.7) | 0 (0.0) |
|                                | V           | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Height SDS, median (IQ range)   | −2.57 (~3.07; −2.02) | −2.62 (~3.01; −2.18) | −2.35 (~3.69; −0.92) | −2.01 (~3.18; −0.37) | −2.54 (~3.70; −1.59) |
| Weight SDS, median (IQ range)   | −2.20 (~2.80; −1.43) | −2.40 (~2.9; −1.7) | −1.25 (~2.13; 0.3) | −1.00 (~1.8; 0.8) | −2.00 (~2.8; 0.1) |
| BMI SDS, median (IQ range)      | −0.30 (~0.88; −0.10) | −0.56 (~0.94; −0.28) | 0.80 (~0.44; 1.10) | 0.90 (~0.25; 1.20) | 0.1 (~0.58; 0.45) |
| IGHDI/MPHD, No. (%GHD)         | 20/16 (55.6) | 0/0 | 20/16 (55.6) | 5/16 (23.8) | 15/0 (100) |
| IGF1 SDS, median (IQ range)     | −0.39 (~1.45; −0.43) | −0.09 (~1.02; 0.48) | −1.78 (~2.80; −0.50) | −2.07 (~2.78; −1.13) | −1.06 (~3.04; −0.32) |

F, female; GHD, growth hormone deficiency; IGHD, Isolated GHD; IQ, interquartile; M, male; MPHD, multiple pituitary hormone deficiency; SDS, standard deviation score.

DISCUSSION

In this study, we identified clinically relevant risk factors for GHD in children, which were applied to build a robust clinical prediction rule to diagnose GHD, which could avoid resorting to GHSTs, in pediatric patients with growth failure and comorbidities. Our conclusion is based on the scientific evidence provided by the use of strict diagnostic criteria and
clearly defined and accurately measured exposure variables in the
analysis of a large cohort of GHSTs performed in a tertiary
pediatric hospital.

The Endocrine Society clinical practice guideline on
“Hypothalamic–Pituitary and Growth Disorders in Survivors
of Childhood Cancer” advises using the same provocative
testing to diagnose growth hormone deficiency in childhood
cancer survivors as are used for diagnosing growth hormone
deficiency in the non-cancer population as an ungraded good
practice statement (11). On the other hand, the “Guidelines for
the treatment of children with GHD, idiopathic short stature,
and primary insulin-like growth factor I deficiency” of the
Pediatric Endocrine Society (PES) suggest that in patients with
auxological criteria, hypothalamic-pituitary defects and
deficiency of at least one additional pituitary hormone, GHD
diagnosis could potentially be established without performing
GHSTs (1). However, due to the insufficient level of evidence
according to the Grading of Recommendations, Assessment,
Development, and Evaluation (GRADE) consensus (39), these
recommendations could only be considered as conditional. Our
study provides evidence to increase the strength of these
recommendations.

We built a model on the knowledge generated by experts over
the last 20 years (1, 5, 39) (and references therein), and applied a
rigorous mathematical and machine-learning approach. Feature
selection was based on the combination of statistical analyses
with clinical criteria, used to refine the model in an iterative way
until the prediction rule was satisfactory from both the statistical
analysis and the clinical criteria, in a cohort of 700 GHSTs
performed in our center between 2004 and 2014. Since the
prediction rule was intended to diagnose GHD without the
need for a GHST, and therefore type I errors would result in a
false diagnosis of GHD leading to GH treatment in real world
practice, we set goals of high specificity and positive predictive
value for our rule. Of all the potential risk factors considered
during model building, we identified the presence of pituitary
dysgenesis on MRI or the existence of two or more anterior
pituitary hormone deficiencies (TSH, ACTH, or prolactin) as
specific enough to diagnose GHD without resorting to GHSTs in
children meeting the criteria required for GHST by the
Summary Statement of the Growth Hormone Research Society (5).
Alternatively, if only one (TSH, ACTH, or prolactin) deficiency
was present, the coexistence of central diabetes insipidus,
neonatal symptoms of pituitary deficiency (hypoglycemia or
hypoglycaemia), sellar or suprasellar surgery or tumor
(excluding microadenomas), clinical or radiological craniofacial
midline abnormalities, or cranial radiotherapy ≥18 Gy, also led
to a safe diagnosis. Gonadotropin deficiency was not considered
in the analysis because its ascertainment may prove challenging
in prepubertal patients.

To test the clinical applicability of the prediction rule, we
validated our results using an independent cohort of 161 GHSTs
performed between 2017 and 2019. Auspiciously, specificity was
99.2% in this second cohort, supporting the safety of our
prediction rule. As expected, sensitivity was relatively low,
reaching 55.6% in the validation cohort, indicating that almost
half of the patients with GHD would only be identified after
referring to GHSTs. Nonetheless, the sensitivity of the prediction
rule applied to children meeting the criteria required for GHST
by the Summary Statement of the Growth Hormone Research
Society (5) would reduce in approximately half of the cases with
GHD the need to perform a relatively invasive endocrine test,
which underscores the clinical relevance of our results.

An unexpected, clinically relevant result is that only 20% of
the children undergoing provocative tests, due to a suspicion of
GHD, proved to be GH deficient (150 of 770 in the first cohort
and 36 of 161 in the validation cohort). This is particularly
significant given that only patients meeting the rigorous criteria
defined by the Growth Hormone Research Society for
prescribing GHSTs to children (5) were included in our study.
This may be explained by the stringent criteria used to ascertain
GHD in our center. Indeed, the diagnosis of GHD was based on
peak GH levels <6.1 ng/ml between 2004 and 2011 (17), or <4.7
ng/ml between 2012 and 2019 (18), according to previously
validated cutoff values (19).

Key strengths of this study are the high number of patients
included in the construction of the predictive model as well as in
the independent validation sample. It should also be stressed that
strict criteria were used to define cases and controls: as
mentioned above, the diagnosis of GHD was based on
stringent cut-off levels for peak GH in GHSTs. A meticulous
analysis of inclusion and exclusion criteria was performed to
avoid inclusion bias. The population sample is representative of
patients seeking advice from pediatric endocrinologists at
referral centers for the assessment of short stature, which
renders our results widely applicable.

Our study also has some limitations related to its design.
Frequently, observational studies are prone to missing
information in their datasets. To minimize memory bias, we
limited the assessment of potential predictors to risk factors that
were routinely reported in the clinical charts or were available
from electronic records of endocrine laboratory or imaging study
in our hospital. However, we cannot exclude that risk factors
have been missed and, therefore, not included in the prediction
rule we generated. Particularly, very few patients had a confirmed
genetic diagnosis. This may explain why the condition “familial
or sporadic GHD of genetic etiology” was not prioritized by our
model. Also, MRI studies were not available in all patients;
nonetheless, false positive prediction of GHD due to pituitary
dysgenesis did not occur in any of the 98 controls (no GHD,
Table 3) who underwent MRI.

In summary, this study developed an algorithm that led to the
construction of a predictive rule for decision-making in the
diagnosis of GHD in children typically seeking advice from
pediatricians for growth failure, on the basis of a reduced
number of clinically relevant and easily identifiable risk factors.
The application of this rule avoids the need for GHSTs in a
significant proportion of the patients in whom testing to assess
GHD is presently indicated, and it is especially important for a
subgroup of labile or vulnerable patients, such as infants, very
low weight patients and children with oncological conditions or
other comorbidities.
DATA AVAILABILITY STATEMENT
The original contributions presented in the article are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT
The studies involving human participants were reviewed and approved by Comité de Ética en Investigación, Hospital de Niños Ricardo Gustiérrez, Buenos Aires, Argentina. Written informed consent from the participants’ legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS
FC, RG, DY, IB, GF, and RR conceived the study and designed the analysis plan. FC, DY, and RR did the statistical analyses. FC, RG, DY, GF, and RR wrote the manuscript. SMB, MS, MR, MB, AK, DB, PP, IB, and GF contributed to obtain and interpret the data. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL
The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2020.624684/full#supplementary-material

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