Research Article

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Diagnostic Value of Peak-to-Basal Difference or Ratio of Growth Hormone in Children with Growth Hormone Deficiency

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Abstract
Introduction: Growth hormone deficiency (GHD) is a rare but important cause of short stature in children. Although GH stimulation tests remain the gold standard for diagnosis, establishing a definitive diagnosis continues to be challenging. In this study, we aimed to evaluate the diagnostic performance of the peak-to-basal ratio and difference for identifying GHD in children.
Materials-Methods: We retrospectively analyzed 265 patients with short stature who were evaluated for GHD with growth hormone stimulation tests. ΔGH was defined as the difference between peak and basal GH levels. The GH ratio was calculated as the ratio of peak to basal GH levels.
Results: Data were collected from 265 patients (182 prepubertal) with a median age at presentation of 10.6 years (IQR: 6.13-12.42), of whom 46.7% were female. In total, 146 patients met the diagnostic criteria for GHD. ΔGH and GH ratio during the L-Dopa and Clonidine stimulation tests were significantly lower in the GHD group ($p<0.001$). A ΔGH cutoff of ≤7.08 in the clonidine test demonstrated excellent discriminative ability, with both sensitivity and specificity above 80%, and an AUC close to 0.9, suggesting that this parameter may may provide supportive diagnostic information for GHD.
Conclusion: To the best of our knowledge, ΔGH has been explored only in a limited number of studies. This study investigated diagnostic accuracy of difference (ΔGH) or ratio of peak-to-basal GH on a large cohort of children with short stature. The supportive diagnostic performance observed in our cohort suggests that ΔGH is clinically useful in routine practice.
Keywords: Growth hormone deficiency, growth hormone stimulation tests, short stature, GH ratio, ΔGH

What is already known on this topic?
Growth hormone deficiency (GHD) is a relatively rare but important cause of short stature in children, and its diagnosis remains challenging due to the limitations of GH stimulation tests.

What this study adds?
In this study, we evaluated the diagnostic performance of basal-to-peak ratio and basal-to-peak difference derived from L-Dopa and clonidine stimulation tests. Our findings indicate that ΔGH (peak-to-basal difference), particularly in the clonidine test, demonstrated excellent diagnostic performance and may serve as a reliable adjunct to conventional peak GH cut-offs in clinical practice.

Introduction

Short stature is one of the most common reasons for referral to pediatric endocrinology clinics (1). Short stature is defined as a height below -2 standard deviation scores (SDS) for age and sex (2).
Growth hormone deficiency (GHD) is one of the most important causes of short stature in children, and accounts for approximately 10% of cases presenting with short stature. Its prevalence ranges from 1/4,000 and 1/10,000 according to the reports from worldwide (3,4). Although relatively rare, an accurate and early diagnosis of growth hormone deficiency is crucial, as recombinant growth hormone (rhGH) replacement therapy is highly effective. Conversely, a misdiagnosis may lead to unnecessary economic costs and expose patients to avoidable adverse effects (5).
The diagnosis of GHD typically relies on evidence from clinical, auxological, radiological, biochemical assessments and endocrine dynamic tests (1). Although the assessment of spontaneous growth hormone (GH) release is considered the best approach, difficulties associated with technicalities and standardization of results make it challenging (6). A diagnosis of GHD requires a failure to respond to two separate stimulation tests (1).

Growth hormone stimulation tests are still the gold standard for the diagnosis of growth hormone deficiency, but controversies remain regarding diagnostic criteria (7). One of the major challenges in the diagnostic process is the uncertainty regarding the cut-off values used to define GHD. The limited availability of reference data on growth hormone secretion in normally growing children, the variability in assay methodologies over time, all contribute to this vagueness (6).

With the advent of monoclonal antibody testing and the implementation of newer standards, GH assay results are approximately 40% lower than those obtained with older immunoassay-based methods. Consequently, the diagnostic cutoffs for GH deficiency should be reduced accordingly. However, no universally accepted threshold has yet been established (1).

In this study, we aimed to evaluate the diagnostic performance of the peak-to-basal ratio and peak-to-basal difference for identifying GHD in children.

Materials-Methods

Study design and patients:

We retrospectively analyzed 265 patients with short stature who were evaluated for GHD with growth hormone stimulation tests at the Department of Pediatric Endocrinology, Izmir Dr. Behçet Uz Children's Hospital, between December 2022 and August 2025. All patients were followed up in our pediatric endocrinology clinic at a tertiary referral hospital in western Turkey. A structured questionnaire was used to systematically evaluate all clinical, hormonal and radiological data. The Standard Deviation Scores (SDS) of weight, height, body mass index (BMI), and midparental height were measured according to Turkish children's reference values (8).

Patients with chronic systemic illnesses, chronic conditions affecting growth, untreated or inadequately treated hypothyroidism or other endocrine disorders were excluded from the study. Mild, well-controlled hypothyroidism was allowed if thyroid function had been normalized before testing. In addition, patients with incomplete data regarding GH stimulation tests, biochemical parameters were also excluded from the study.

Hormonal and biochemical measurements:

Serum GH concentrations were measured by chemiluminescent immunoassay, using Siemens Healthineers IMMULITE 2000 xpi Immunoassay System. Results were expressed in ng/mL. After an overnight fast, L-Dopa was administered orally at a dose of 10 mg/kg (maximum 500 mg), and blood samples were obtained at 0, 30, 60, 90, and 120 minutes for GH measurement. Following overnight fasting, clonidine was administered orally at a dose of 0.15 mg/m² (maximum 300 mg) body surface area between 08:00 and 09:00, blood samples were collected at baseline and at 30, 60, 90, and 120 minutes. The highest GH value obtained during the test was defined as the peak GH concentration. Children with peak GH value <10 ng/mL in the first stimulation test underwent a second stimulation test on a seperate day. GHD was diagnosed when the peak GH concentration was <10 ng/mL in at least two different stimulation tests. ΔGH was calculated as the difference between peak and basal GH levels in the L-Dopa and Clonidine stimulation tests. The GH ratio was calculated as the ratio of peak to basal GH levels during the L-Dopa and Clonidine stimulation tests.

Statistical analyses:

Statistical analyses of the data were performed using SPSS for Windows, version 25.0 (IBM Corp., Armonk, NY, USA). Distribution of data was evaluated using the Kolmogorov-Smirnov (K-S) test. For numerical comparisons, the Student's t-test or Mann-Whitney U- tests were used to assess differences between the two groups according to the normal distribution of the measured parameters. Categorical variables were analyzed with the Chi-Square (χ^2) test. Receiver operating characteristic (ROC) curves were used to define the cutoff values for the ratios and delta of growth hormone levels in clonidine and L-Dopa tests that yielded the highest sensitivity and specificity. Data were presented as mean ± standard deviation (SD) or median and interquartile range (IQR, 25th - 75th percentile). In all statistical tests, *p*-values < 0.05 were considered as statistically significant.

Results

A total of 317 patients with short stature who underwent GH stimulation testing were included in the analysis. Fifty-two patients with a peak GH >10 ng/mL in the first stimulation test were excluded from the study. Data were collected from 265 patients (182 prepubertal) with a median age at presentation of 10.6 years (IQR: 6.13-12.42), of whom 46.7% were female. In total, 146 patients met the diagnostic criteria for GHD. Cohort with GHD consisted of 146 children, including 79 male and 67 female, median aged 10.3 years (IQR: 6.3-12.6). These children had a median height SDS of -2.64 (IQR: -3.02 – -2.37) and a mean BMI SDS of -0.55±1.07. The GH peak responses to L-Dopa and Clonidine stimulation were 3.09 and 5.14 ng/mL, respectively.

Table 1 summarizes the demographic, clinical, and laboratory findings of the patients, comparing diagnosed with GHD to those without GHD. Chronological age, age by height, bone age, weight SDS, height SDS, mid-parental height (MPH) SDS, IGF-1 SDS, and IGFBP-3 SDS were similar between the groups.

As expected, peak GH responses during both the L-dopa and clonidine stimulation tests were significantly lower in patients with GHD compared with those without GHD. ΔGH (the difference between peak and basal GH levels) in the stimulation tests were significantly lower in the GHD group ($p<0.001$). GH ratio (the ratio of peak-to-basal GH levels) during the L-Dopa and Clonidine stimulation tests were significantly lower in the GHD group ($p<0.001$).

ROC analysis revealed that the cutoff value of GH ratio in the L-Dopa stimulation test ≤9.98 supports good diagnostic prediction with 57.2% sensitivity and 63.3% specificity (AUC ± SE, 0.627 ± 0.038; p = 0.001) (Figure 1). According to ROC curve analysis, a cutoff value of ≤4.04 for the ΔGH in the L-Dopa stimulation test yielded a diagnostic accuracy of 82.2% sensitivity and 60.9% specificity (AUC ± SE, 0.735 ± 0.036; p = 0.001) (Figure 2).

ROC curve analysis showed that a GH ratio cutoff value of ≤27.4 in the Clonidine stimulation test provided good diagnostic performance, with 66% sensitivity and 49.4% specificity (AUC ± SE, 0.610 ± 0.039; p = 0.001) (Figure 3). ROC curve analysis demonstrated that a cutoff value of ΔGH ≤7.08 in the clonidine stimulation test provided good diagnostic accuracy, with 81.3% sensitivity and 86.2% specificity (AUC ± SE, 0.892 ± 0.029; p < 0.001) (Figure 4).

Discussion

In this study, we investigated the diagnostic performance of the GH ratio and ΔGH in L-Dopa and clonidine stimulation tests for identifying growth hormone deficiency in children. Our findings demonstrated that ΔGH and GH ratio may provide additional supportive value, particularly ΔGH in the clonidine test.

A diagnosis of GHD is established when peak GH responses are subnormal in at least two independent stimulation tests (4,9). As expected, in our cohort, the peak GH levels were lower in children with GHD than in non-GHD.

GHD accounts for only a small proportion of children with short stature, but misdiagnosis is common and may expose children to unnecessary treatment (6). Despite the dramatic changes in GHD treatment since the 1960s, diagnosing growth hormone deficiency remains challenging (6,10). Previous studies have also reported the challenges of GH stimulation tests. Furthermore, GH secretion may be influenced by factors such obesity, undernutrition, sex, age and puberty (1,6,11). In fact, due to the inherent limitations of GH stimulation tests, the Pediatric Endocrine

Society guidelines recommend against using GH stimulation test results as the sole diagnostic criterion for GHD in children and emphasize the importance of integrating auxological, biochemical, and imaging findings in the diagnostic process (12).
GH stimulation tests remain controversial, due to their low sensitivity and specificity, which further reduce their diagnostic reliability (13). Traditionally, a cut-off of <10 μg/L has been used in children. However, some experts have proposed lowering this threshold to <7 μg/L. Although diagnostic guidelines have been revised over the past decades and peak GH cut-off values have been modified accordingly, these thresholds remain largely arbitrary, particularly in pediatric populations (14).
The difficulties in performing GH stimulation tests, the potential adverse effects, reference data for growth hormone secretion in normally growing children, the high false-positive rates and the uncertainty of consensus on cut-off values have led researchers to explore new diagnostic strategies (4,7,11,15,16).
The rationale for using ΔGH or GH ratio lies in their ability to capture dynamic responsiveness rather than relying on a single stimulated peak, which may be influenced by pre-test conditions, body composition, or assay variability. In our cohort, ΔGH improved the discriminatory of the tests, supporting the concept that such dynamic indices may offer supplementary information rather than serving as independent diagnostic tools. One possible explanation for false-negative results in GH stimulation testing is the occurrence of a spontaneous physiological GH peak shortly before the test (9,17), which may blunt the stimulated response. In such cases, considering the increase relative to the basal value may provide a better reflection of the pituitary reserve and secretory capacity than absolute peak concentrations alone.
In children with peak GH responses <5 ng/mL, the diagnosis of GHD is clearer (2,7,9,12). However, when peak GH values is between the range of 5-10 ng/mL, the diagnosis becomes more challenging and requires additional values and supportive criteria. Our study extends previous findings by identifying a ΔGH cutoff of ≤7.08, although its clinical utility remains dependent on the reference peak GH threshold used to define GHD. In our cohort, a ΔGH value of ≤7.08 in the clonidine stimulation test demonstrated reasonable discriminative performance, with sensitivity (81.3%) and specificity (86.2%) both exceeding 80% and an AUC approaching 0.9. These results suggest that ΔGH may provide supportive diagnostic information, particularly when evaluating children within the borderline peak GH range of 5–10 μg/L. However, although ΔGH showed discriminative ability in this subgroup, it did not confer a clear diagnostic advantage over conventional peak GH criteria. Therefore, ΔGH should be interpreted as an adjunctive rather than a primary diagnostic parameter.
To the best of our knowledge, ΔGH has rarely been investigated in the diagnostic work-up of pediatric GHD. Borges et al. (18) addressed this parameter and reported that both GH peak concentrations and ΔGH were significantly lower in children with GHD compared to non-GHD groups. Our study extends these findings by identifying a ΔGH cutoff (≤7.08) with supportive performance, thereby providing novel evidence that this parameter may serve as reliable criteria.
This study has certain limitations. First, the retrospective, single-center design inherently restricts the generalizability of our findings, as patient characteristics, clinical approaches, and stimulation protocols may differ across institutions. Second, only L-Dopa and clonidine stimulation tests were used; the proposed ΔGH and GH ratio thresholds were not validated against more reliable reference tests such as the insulin tolerance test (ITT). Third, subgroup analyses by pubertal stage, sex, BMI and MRI characteristics were limited. Another limitation of our study is that GH deficiency was defined based on stimulation test results rather than structural or genetic confirmation. However, this definition is consistent with most clinical studies in the field, as the insulin tolerance test or MRI-based criteria are not routinely available for all patients. Finally, longitudinal outcomes, particularly growth response to rhGH treatment, were not available. Prospective, multicenter studies are needed to examine whether baseline ΔGH and GH ratio predict rhGH treatment response.

Conclusion

The strong supportive diagnostic performance observed in our cohort suggests that ΔGH is clinically useful in routine practice. However, validation in larger, multicenter studies is needed before it can be widely adopted.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Dr. Behcet Uz Children's Hospital (protocol code: 2025/15-07 and date of approval: 2.10.2025)

Informed Consent Statement: Informed consent was obtained from all subjects and parents involved in the study. Written informed consent has been obtained from the patients and parents to publish this paper.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Conflicts of Interest: The authors declare no conflicts of interest.

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Table 1. The demographic, clinical, and laboratory findings of the patients

	GH deficiency (n=146)	Normal (n=119)	p value
Gender (male / female)	79 / 67	63 / 56	0.846
Prepubertal / Pubertal	102 / 45	80 / 38	0.781
Chronological age (years)	10.3 (6.3-12.6)	9.0 (5.9-12.3)	0.398
Age by height (years)	7.59 (4.45-9.98)	6.84 (3.83-9.45)	0.321
Bone age (years)	8.0 (4.0-11.0)	7.0 (3.5-11.0)	0.468
Weight, SDS*	-1.89±1.04	-2.19±0.88	0.008
Height, SDS	-2.64 (-3.02 – -2.37)	-2.69 (-3.16 – -2.29)	0.863
BMI, SDS*	-0.55±1.07	-0.83±0.88	**0.020**
MPH, SDS*	-1.26±0.98	-1.39±0.91	0.342
IGF-1, SDS	-1.61 (-2.27 – -1.15)	-1.46 (-2.21 – -0.79)	0.121
IGFBP-3, SDS	-0.41 (-1.05 – 0.27)	-0.22 (-0.63 – 0.34)	0.051
L-Dopa			
Peak GH, L-Dopa	3.09 (1.79 - 4.60)	6.49 (4.11 – 11.25)	**<0.001**
ΔGH, L-Dopa	2.37 (0.53 – 3.77)	5.15 (2.21 – 10.70)	**<0.001**
GH ratio, L-Dopa	7.78 (1.69 – 28.19	16.6 (5.2 – 91.6)	**0.001**
Clonidine			
Peak GH, Clonidine	5.14 (3.20 – 7.11)	13.05 (11.4 – 15.70)	**<0.001**
ΔGH, Clonidine	4.59 (2.34 – 6.44)	11.79 (9.59 – 14.40)	**<0.001**
GH ratio, Clonidine	12.77 (4.10 – 38.8)	26.15 (8.80 – 68.5)	**0.006**

*normal distribution (Student's t-test)
Data are given as mean ± SD or median (IQR 25-75 percentile).
SDS: standard deviation score; GH: growth hormone; BMI: body mass index; MPH: Midparental height; IGF-1: insulin-like growth factor 1; IGFBP-3: insulin-like growth factor binding protein 3; GH: growth hormone
ΔGH: Peak GH – basal GH in the L-Dopa and Clonidine provocation tests.
GH ratio: ratio of peak-to-basal GH in the L-Dopa and Clonidine provocation tests.

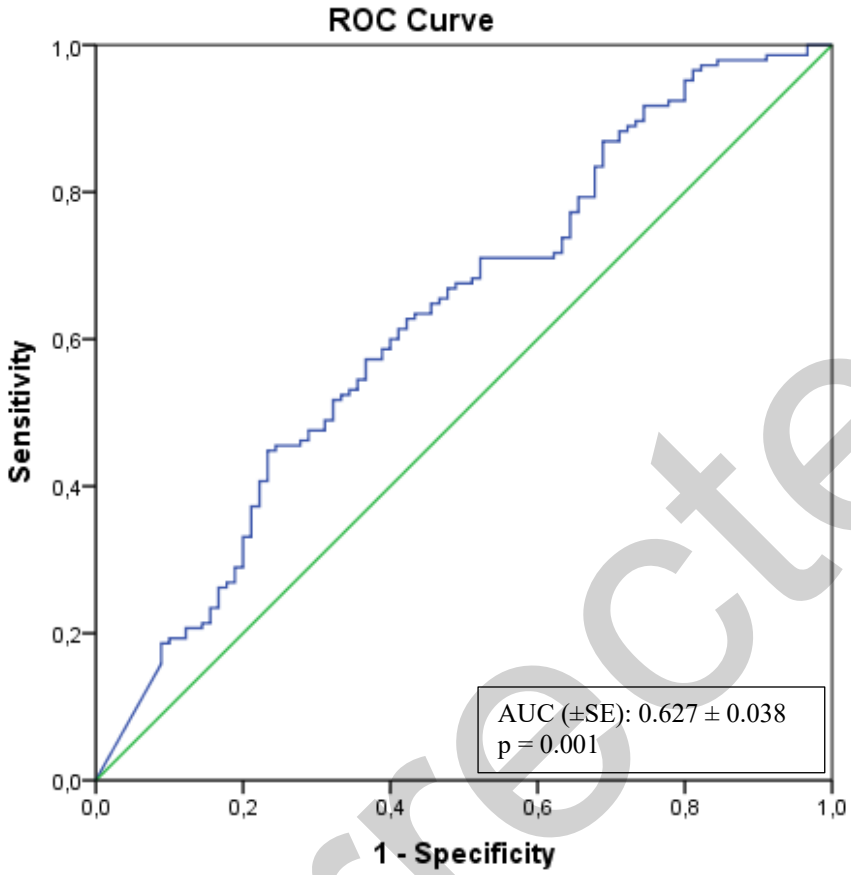

Figure 1. ROC curve analysis of the GH ratio in the L-Dopa stimulation test

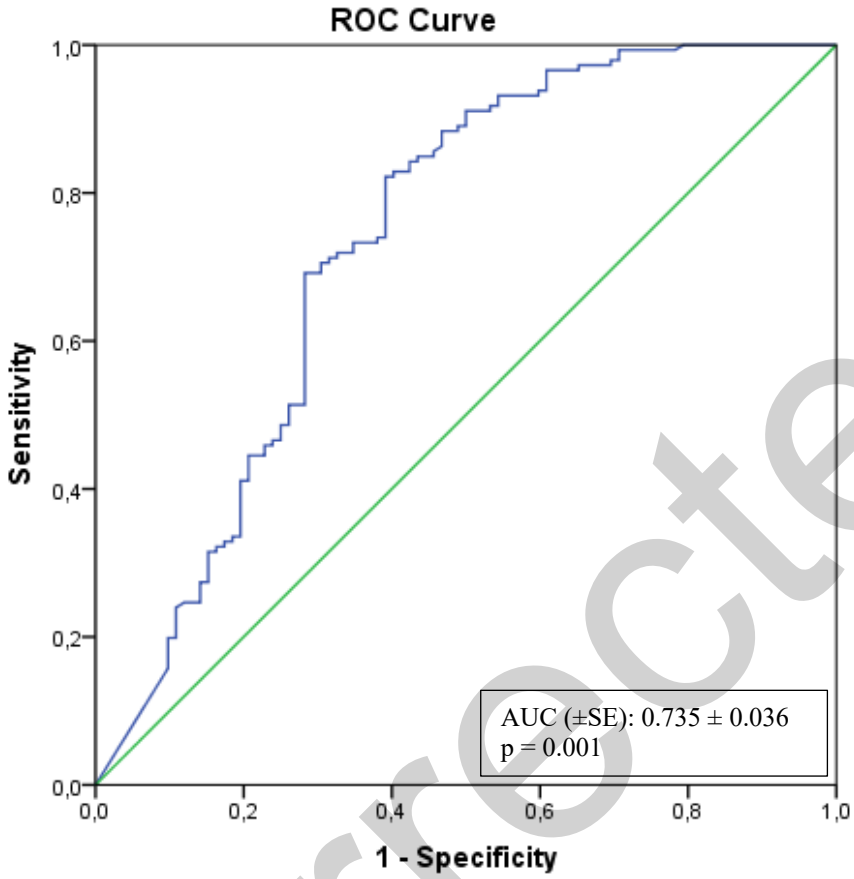

Figure 2. ROC curve analysis of the ΔGH in the L-Dopa stimulation test

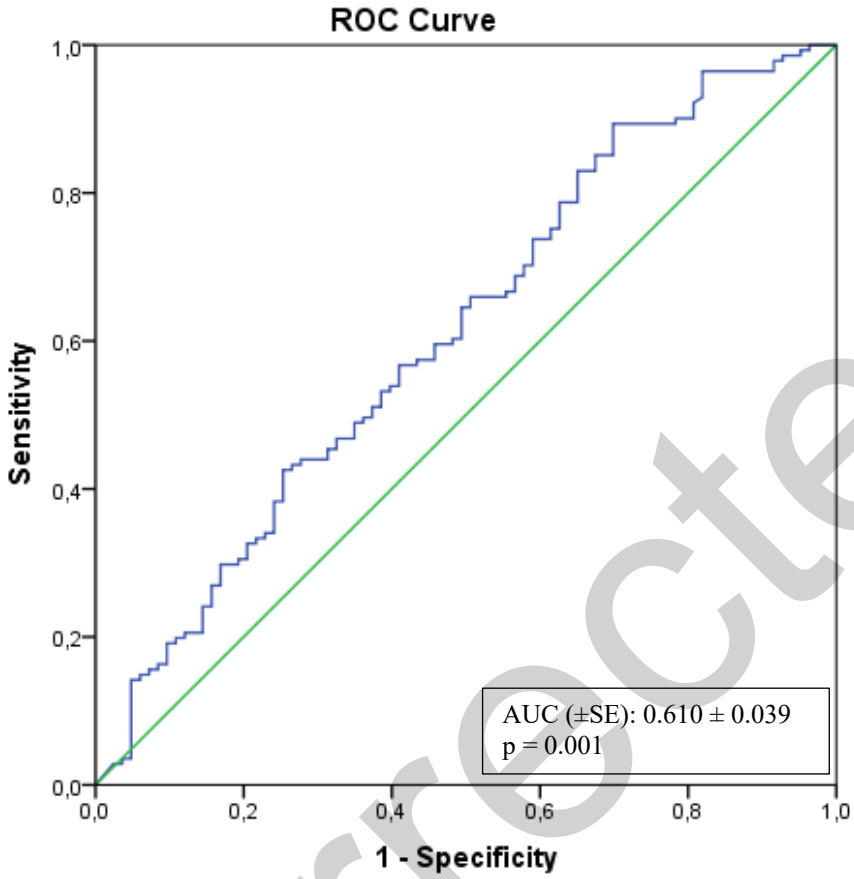

Figure 3. ROC curve analysis of the GH ratio in the Clonidine test

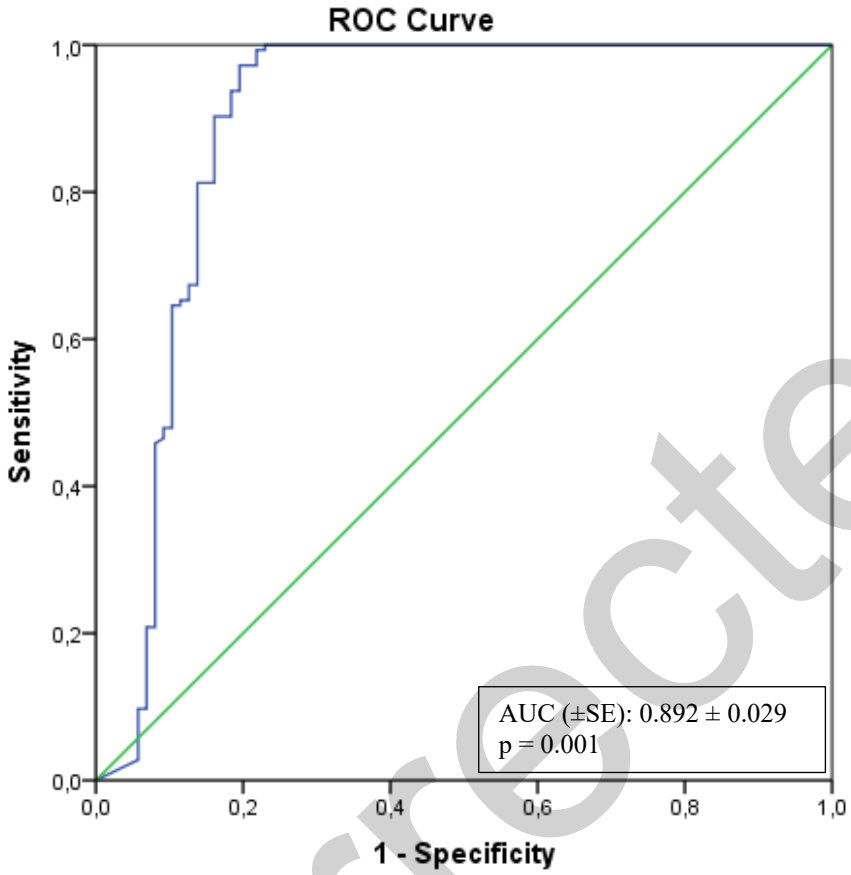

Figure 4. ROC curve analysis of the ΔGH in the Clonidine stimulation test