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# ROLE OF GONADOTROPIN-RELEASING HORMONE AND HUMAN CHORIONIC GONADOTROPHIN STIMULATION TESTS IN DIFFERENTIATING PATIENTS WITH HYPOGONADOTROPIC HYPOGONADISM FROM THOSE WITH CONSTITUTIONAL DELAY OF GROWTH AND PUBERTY

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**Precis :** Gonadotropin-releasing hormone (GnRH) tests in combination with both a 3-day and 19-day HCG test may aid in differentiating between a diagnosis of Constitutional Delay of Growth and Puberty (CDGP) and Hypogonadotropic Hypogonadism (HH) in adolescent males.

**Abstract**

*Background :*

Delayed puberty can be due to either constitutional delay of growth and puberty (CDGP) or hypogonadotropic hypogonadism (HH). Differentiating between the two using current testing can be difficult. We assessed the utility of a GnRH test in combination with a 3-day and 19-day HCG test to discriminate between the two conditions.

*Methods:*

A retrospective analysis of 43 boys with pubertal delay who required pubertal induction with testosterone. All were followed through puberty, and 29 were subsequently diagnosed with CDGP and 14 with HH. A standard GnRH test (2.5 μg/kg) was undertaken followed by either a short [3 day; n=38 (13 HH; 25 CDGP)], extended [19 day; n=31 (12 HH; 19 CDGP)], or both [n=27 (11 HH; 16 CDGP)] HCG stimulation test. Receiver Operating Characteristic (ROC) analysis was performed to assess the performance of the tests.

*Results:*

Peak testosterone concentrations to both 3-day and 19-day HCG tests were significantly lower in patients with HH compared with CDGP. The 19 day test performed better than the 3-day test, and a combination of the LHRH, 3-day and 19 day HCG test [peak LH cut-off 2.8 U/l, peak 3-day testosterone cut-off 1.04 μg/l (3.6 nmol/L), peak 19 day testosterone cut-off 2.75 μg/l (9.5nmol/L)] gave a sensitivity and a specificity of 100%

*Conclusions:*

Our data suggest that a GnRH test in combination with both a 3-day and 19-day HCG test may aid in differentiating between CDGP and HH.

## INTRODUCTION

Delayed puberty in boys is one of the commonest causes for referral to a paediatric endocrinologist. The prevalence is approximately 5% at 14 years of age with 0.1% remaining prepubertal 3 years later[1]. The differential diagnosis lies between constitutional delay of growth and puberty (CDGP), which is common, and hypogonadotropic hypogonadism (HH), which is rare (prevalence 0.025%) [2]. The commonest cause of HH is Kallmann syndrome (1 in 10,000 males). Other causes include isolated HH, tumours of the hypothalamus and pituitary, syndromes such as Bardet-Biedl and Prader-Willi, and mutations in genes that are implicated in pituitary development (eg. *HESX1*, *SOX2*, *SOX3*, *PROP1*, *LHX3*), as well as mutations in leptin and the leptin receptor. Monogenic causes have been extended further with the identification of mutations in a number of genes in the hypothalamo-pituitary-gonadal axis such as *LHβ*, *FSHβ*, *GnRHR*, *KAL-1*, *Kisspeptin*, *GPR54*, *FGFR1*, *NELF*, *prokineticin 2* and *prokineticin receptor 2*[3].

At the time of referral, it is often difficult to distinguish boys with CDGP from HH, as they may share similar clinical and hormonal features. Differentiation is not possible on unstimulated serum testosterone and gonadotropin concentrations as there is considerable overlap. As a result, a variety of physiological and stimulation tests have been proposed such as nocturnal luteinizing hormone (LH) sampling[4], prolactin response to TRH (thyrotropin-releasing hormone), daily urine excretion of follicle stimulating hormone (FSH)[5] and gonadotropin releasing hormone (GnRH) and human chorionic gonadotropin (HCG) stimulation tests[6]. Despite the variety of tests reported, no single test has been shown to differentiate between the two conditions with 100% sensitivity and specificity. Only the demonstration of a complete and spontaneous recovery can distinguish CDGP from HH and few studies have verified outcomes in adulthood or at the end of pubertal induction. Recent studies have however shown reversibility of gonadotropin secretion in 10% of a cohort of young men with hypogonadotrophic hypogonadism, 20% of whom had evidence of genetic mutations in *FGFR1* and *GnRHR*[7].

Analysis of test performance requires a gold standard for comparison. To date, no single test fulfils the criteria required to make a diagnosis of HH, although advances in the understanding of the genetic basis of pubertal development offers the possibility of a more refined diagnostic process and a gold standard with which to compare endocrine tests[8]. At present, genetic disorders of pubertal development only account for approximately 10% of cases[9], so assessment will continue to rely on clinical evaluation, often post pubertal induction. In this study, we have re-evaluated the role of GnRH and HCG testing in the diagnosis of HH by comparing responses to testing with long-term clinical outcomes. In addition, we have considered the performance of the HCG test when extended from its more conventional 3 day duration to that of 19 days. We have also recorded pre-test testicular volumes as well as those at diagnostic follow-up.

## PATIENTS AND METHODS

*Patients*

We audited the clinical outcome data in 43 males who presented with delayed puberty and had been treated with testosterone with assessments made of the HPG axis some 3 – 5 years previously. All patients had presented to the London Centre for Paediatric Endocrinology at Great Ormond Street Hospital for Children and University College London Hospitals. Ethical Committee approval for the retrospective review was obtained at both hospitals. A diagnosis of HH was made in those that had undergone no spontaneous pubertal development by 15 years of age, had required testosterone therapy for initiation and completion of pubertal development and who required subsequent therapy after re-evaluation as adults to maintain secondary sexual characteristics. CDGP was diagnosed in those who were treated with testosterone for pubertal induction, but progressed through puberty and attained adult secondary sexual characteristics, not requiring testosterone as adults, or in those who progressed spontaneously through puberty. Additionally, 35 patients also underwent an LHRH stimulation test allowing a comparison between the serum gonadotropin responses between the 2 groups.

Testicular volumes were recorded in both groups at the time of testing as well as at the final follow-up visit.

Endocrine assessment of the HPG axis was undertaken using an intravenous bolus of 2.5 μg/kg GnRH (HRS, Intrapharm, UK) in 35 of 43 patients (10 HH; 25 CDGP) with blood samples drawn at 0, 20 and 60 minutes following GnRH administration for the measurement of serum LH and FSH

concentrations. This was followed by either a short [3 day; n=38 (13 HH; 25 CDGP)], extended [19 days; n=31 (12 HH; 19 CDGP)], or both [n=27 (11 HH; 16 CDGP)] HCG stimulation tests. HCG [Pregnyl, Organon Laboratories Ltd, Cambridge Science Park, Milton Road, Cambs, CB4 OFL] was administered intramuscularly at a dose of 1500 units after the completion of the GnRH test and again on days 2 and 3 for the short (3 day) test and, in addition, on days 8, 11, 15 and 18 for the extended (19 day) test. A blood sample for the measurement of serum testosterone concentration was drawn before the GnRH test (D 0) and then 24 hours after the day 3 (D 4) and day 18 (D 19) HCG injections.

*Hormone Assays*

LH, FSH and testosterone were measured using the Abbot Architect assay (Abbot Diagnostics).

*Statistical Analysis*

All data are expressed as means and standard deviation (SD). Between group comparisons were performed using Student's t-test. The Chi squared test was used to compare frequencies of occurrences. Correlation analysis was performed using Pearson's correlation coefficient. Test performance was assessed using principles outlined by Sox[10]. The groups were also compared to assess the positive predictive value at various cut-offs of rise in testosterone. The receiver operating characteristic (ROC) curve was used to depict and determine the trade-off between the true and false positive rates for the tests studied[11]. The area under the ROC curve for each test was used to compare tests using the principle that the test with the greatest area under its ROC curve is the better test[12].

We analysed the peak LH response at 20 minutes as it was significantly greater than that at 60 minutes (p= 0.003). We also used the peak FSH response at 20 minutes as there was no statistical difference between the 20 and 60 minute concentrations (p=0.88), and the 20 minute sample reflects secretion of LH and FSH in response to GnRH whereas the 60 minute sample might reflect a combination of synthesis and secretion.[13]

**RESULTS**

*General*

All patients with HH, having failed to develop spontaneous puberty, received pubertal induction with testosterone therapy and are currently post-pubertal and requiring long-term testosterone supplementation to maintain normal adult serum testosterone concentrations. The CDGP group attained spontaneous puberty or required testosterone treatment for pubertal induction; all subsequently progressed through puberty with increasing testicular volumes and pubertal staging. They maintained adult pubertal staging with serum testosterone concentrations within the normal adult range, and none require exogenous testosterone.

*Unstimulated hormone concentrations*

Table 1 details the unstimulated hormone concentrations in the two groups. The unstimulated serum testosterone concentrations were not significantly different between patients with HH {mean 0.3 (0.17) µg/l [1.0 (0.6) nmol/l]} and CDGP {mean 0.4 (0.3) µg/l [1.5 (1.1) nmol/l]} (p = 0.08) (Table 2). The area under the ROC for unstimulated testosterone was 0.63 (0.16) (Figure 1). The estimated concentration with the best discrimination was 0.14 µg/l (0.5 nmol/l) (sensitivity 93%, specificity 100%), which was below the sensitivity of the assay. The unstimulated serum FSH concentration was significantly lower in HH [mean 0.9 (0.8) U/l] compared with CDGP [mean 2.2 (1.3) U/l] (p = 0.007). The unstimulated serum LH concentration was not significantly different between the groups [HH mean 0.7 (0.7) U/l; CDGP mean 0.9 (0.9) U/l; p = 0.47] (Table 2). The ROC for LH was 0.53 (0.11) and for FSH 0.76 (0.09). However, no valid LH cut-off could be established and the best derived for FSH was 0.9 U/l (sensitivity 88.5%, specificity 55%).

*3 day (Short) HCG stimulation*

Short HCG stimulation was performed in 38 of 43 patients. Patients with HH had significantly lower D4 serum testosterone concentrations as compared with patients with CDGP (Table 2). An absolute serum testosterone concentration on Day 4 of 1.04 µg/l (3.6 nmol/l) offered the best sensitivity [92%] and specificity [92%] for the diagnosis of HH (Figure 2A). The positive predictive value of this cut-off was 86%.

*19 day (Extended) HCG stimulation*

31 patients underwent extended HCG stimulation. The D19 serum testosterone concentrations were significantly lower in HH patients compared to those with CDGP (Table 2). An absolute serum testosterone concentration on D19 of 2.75 µg/l (9.5 nmol/l) provided optimal sensitivity [92%] and specificity [95%] for the diagnosis of HH (Figure 2B). The positive predictive value for this cut off was 92%.

*Combination of short and extended HCG stimulation*

11 patients with HH and 16 with CDGP underwent both 3 day and 19 day HCG stimulation. The area under the ROC was 0.92 (0.05) for the 3-day HCG stimulation and 0.98 (0.02) for the 19 day study (Figure 1). Given the moderately greater area for the 3-week study it would seem preferable to use this to define gonadal responsivity. No patient with HH had both 4 and 19 day testosterone values that were above the respective cut-offs. Additionally, no patient with CDGP had both 4 and 19 day testosterone values that were below the respective cut-offs.

***GnRH* stimulation test**

The peak serum LH and FSH response to GnRH stimulation was significantly lower in the patients with HH p<0.001 (Table 2). The area under the ROC was greater for LH [0.88 (0.06)] than FSH [0.70 (0.10)] and yielded an optimal peak response cut-off point of 2.8 U/l for LH (sensitivity 90%, specificity 84%, positive predictive value 69%) (Figure 2C) and 3.7 U/l for FSH (sensitivity 90%, specificity 52%, positive predictive value 41%). There was no difference between the groups in terms of the time of the FSH (Chi squared 0.48, P=0.41) and LH (Chi squared 0.41, P=0.66) peak responses.

***Approach to diagnosis***

Using the "rule-in" approach to diagnose HH with an absolute D4 serum testosterone cut-off, an absolute D19 serum testosterone cut-off and a peak serum LH cut-off of 1.04 µg/l (3.6 nmol/l), 2.75 µg/l (9.5nmol/l) and 2.8U/L respectively resulted in a sensitivity of 100% and a specificity of 100% as 9/9 patients with HH who underwent all 3 tests did not achieve these cut-offs and none with CDGP (n=16) failed all 3 tests. If only the 3-day HCG and the LH response to GnRH test were used, the sensitivity decreased to 90% although the specificity remained unchanged at 100%. On the other hand, if only the 3 day and the 19 day tests are used with cut-offs as above, the sensitivity decreases to 83%, although the specificity remains at 100%, with a positive predictive value of 100%.

*Testicular volumes*

The testicular volumes were documented at time of testing in both groups as well as at the final follow-up visit. The mean age of testing in the HH group was 12.6 years (SD 2.4) whilst that in the CDGP group was 13.4 years (1.6). The mean age at the final follow-up visit was 17.6 years (SD 3.1) in the HH group compared with 18.1 years (1.4) in the CDGP group. The initial mean testicular volume in the HH group was 1.3 ml (SD 0.5) compared with a mean initial testicular volume in the CDGP group of 2.4 ml (0.8) (Unpaired t-test P < 0.001). At the time of the final follow-up visit, the mean testicular volume in the HH group was 3.7 ml (2.2), whilst that in the CDGP group was 13.1 ml (3.3) (p< 0.001). There was a significant increase in testicular volumes in both the HH and CDGP groups (paired t-test P<0.001).

Using a cut-off testicular volume of 3 ml for the diagnosis of HH in isolation, we achieved a sensitivity of 93% with a specificity of 45%. Using a combination of testicular volumes less than 3 ml and a 3-day HCG peak testosterone < 1.04 µg/l for a diagnosis of HH, the sensitivity was 92% with a specificity of 92%. Using the "rule-out" approach to exclude HH (testicular volumes > 3 ml and testosterone > 1.04 µg/l) the sensitivity is 92% with 87% specificity.

## DISCUSSION

Presentation of adolescents in the peri-pubertal period with pubertal delay can be diagnostically challenging. In practice, a decision is often made to treat these adolescents with testosterone in order to optimise their growth and pubertal progress in a timely fashion, and reassess later in terms of diagnosis. However, a definitive diagnosis would be desirable from the viewpoint of long-term prognosis for fertility, and to alleviate anxiety in adolescents with CDGP. A number of tests have been evaluated for their potential to differentiate between HH and CDGP. Of these, use of HCG and GnRH appear to be most widely used but, when used in isolation, demonstrate poor discrimination between the two conditions [14-16]. Other options include overnight sampling for LH secretion, and the use of the pulsatile administration of GnRH, both of which are

time-consuming, expensive and difficult to perform on an ambulatory basis[17], as well as the prolactin response to TRH stimulation[18], estimation of daily excretion of urinary FSH[19], free α-subunit measurement[20], and the use of GnRHa[21-24]. All of these tests have relatively poor specificity due to overlap between the two groups.

It has been suggested that the GnRH test be used in conjunction with the HCG test to differentiate between CDGP and HH [15]. Our data suggest that this may be a useful approach to the diagnostic question as the peak serum testosterone response to either 3-day or 19-day HCG stimulation was significantly lower in those with HH. ROC analysis revealed that unstimulated serum testosterone was unhelpful in diagnosis, but generated cut-off points for Day 4 and Day 19 serum testosterone concentrations of 1.04 and 2.75 μg/l (3.6 and 9.5 nmol/l) respectively. Individually, this translates into positive predictive values for HH of 86% for the Day 4 test and 92% for the Day 19 study.

Historically, the extended HCG test has been used in children with undescended testes to assess the testicular response to long-term HCG, in addition to enabling testicular descent[24]. Although the extended test has been evaluated in children with either a micropenis or cryptorchidism[25], Adiyaman et al. did not formally compare the two tests. Additionally, to the best of our knowledge, the 19-day HCG test has not been evaluated in children with significant pubertal delay. Although the 19-day HCG test does prolong the evaluation of the patient, given the better test performance, we believe that the test is justified, although a good response on day 4 after the 3-day test could lead to termination of the extended test if results were available rapidly.

Our data also suggest that information from GnRH testing can be of value, particularly when combined with HCG testing. Unstimulated serum FSH concentrations were significantly lower in patients with HH, as were peak serum FSH concentrations in response to GnRH stimulation. In contrast, unstimulated LH concentrations were not different between the groups whilst the peak serum LH concentration was again significantly lower in the patients with HH. ROC analysis suggested that the peak serum LH performed better than the peak FSH response, with an optimal cut-off value of 2.8IU/l (positive predictive value of 69%) for serum LH concentration at 20 minutes. Combining the GnRH and the two HCG tests led to a sensitivity and specificity of 100%. If only the 3-day HCG test and the LH response to GnRH are used, the sensitivity decreases to 90%, although the specificity remains at 100%. These observations on the limitation of the 3 day HCG test support the observations of Degros et al[16] who derived similar cut-off points to ourselves, and noted that some 29% of children lay in the borderline area of the HCG test between a clear diagnosis of HH or CDGP, a finding echoed by Kauschansky et al[15].

Although we found that prepubertal testicular volumes in those with HH were slightly lower than those with CDGP [1.3 (SD 0.5) vs 2.4 (SD 0.8) ml] the wide range would lead to considerable overlap in testicular sizes between the two groups, and hence testicular volumes could not on their own differentiate between the two groups. Testicular volumes of less than 3 ml on presentation identified patients with HH with a sensitivity of 93% but 16 out of 29 CDGP children also had testes less than 3 ml at presentation. The use of initial testicular volumes in combination with the peak testosterone response to the 3 day HCG did not lead to improved diagnosis of HH.

The testicular volumes at the last follow-up visit were clearly larger in the CDGP group as would be expected. Nevertheless it is important to note that the HH group increased their testicular volumes to a mean of 3.7 (SD 2.2) mls.

Although observation over time may resolve the diagnosis itself, other issues such as growth, psychological consequences and societal pressures with respect to delay/ lack of pubertal development may necessitate earlier investigation. Confirmation of the diagnosis may also be required to alert the physician to the possibility of other hormonal deficiencies as well as a progressive underlying lesion such as a tumour, and the patient may also benefit from understanding the diagnosis and implications for future fertility. The data that we present suggest that combining a 19 day HCG test with a conventional GnRH test may be of benefit in the differentiation of HH from CDGP.

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**Legends for Figures**

**Figure 1**

Receiver operator characteristics for day 0 and days 4 and 19 post-HCG stimulation serum testosterone concentrations in 43 patients presenting with delayed puberty

**Figure 2**

Scatter plots showing A) 3 day plasma testosterone (μg/L) in HH and CDGP, B) 19 day plasma testosterone (μg/L) in HH and CDGP and C) Peak LH (IU/L) in HH and CDGP.

**Table 1**

Serum testosterone, FSH and LH concentrations in patients with hypogonadotropic hypogonadism (patients 1-14) and constitutional delay of growth and puberty (patients 15-43)

| Patient | Investigation | | Discharge/ diagnosis | | Serum testosterone (µg/l) | | | Serum FSH (U/l) | | | Serum LH (U/l) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Post HCG stimulation | | Minutes | | | Minutes | | |
| | Age Years | testic vol mls r/l | Age Years | testic vol mls r/l | Day 0 | Day 4 | Day 19 | 0 | 20 | 60 | 0 | 20 | 60 |
| 1 | 10.6 | 1/2 | 15.4 | 2/4 | 0.2 | 0.29 | 1.04 | 0.5 | 2.7 | 3.6 | 0.7 | 1.7 | 1.1 |
| 2 | 11.2 | 1/1 | 18.0 | 4/4 | 0.2 | 0.2 | 1.15 | - | - | - | - | - | - |
| 3 | 10.3 | 1/1 | 16.3 | 3/5 | 0.2 | 0.2 | 1.6 | 2.0 | 3.0 | 3.6 | 0.7 | 2.4 | 2.1 |
| 4 | 12.0 | 0/1 | 17.4 | 0/1 | 0.2 | 0.2 | 0.6 | 0.2 | 0.2 | 0.2 | 0.7 | 0.7 | 0.7 |
| 5 | 11.6 | 1/1 | 17.8 | 1/1 | 0.2 | 0.45 | 0.29 | 0.3 | 0.7 | 1.4 | 0.7 | 1.2 | 1.0 |
| 6 | 15.8 | 1/1 | 18.6 | 1/1 | 0.61 | 0.84 | 1.13 | 0.2 | 1.6 | 3.0 | 0.1 | 0.8 | 1.0 |
| 7 | 12.7 | 1/1 | 16.2 | 4/4 | 0.23 | 1.01 | 2.7 | 1.3 | 3.7 | 6.3 | 0.1 | 1.4 | 1.8 |
| 8 | 14.3 | 2/2 | 17.5 | 5/6 | 0.35 | 0.78 | 2.14 | 0.6 | 1.4 | 1.5 | 0.1 | 0.1 | 0.1 |
| 9 | 16.9 | 3/0 | 20.4 | 4/5 | 0.78 | 3.01 | 2.42 | 2.6 | - | - | 2.5 | - | - |
| 10 | 11.6 | 2/2 | 18.9 | 10/8 | 0.2 | 0.35 | 3.03 | - | - | - | - | - | - |
| 11 | 10.3 | 1/2 | 14.3 | 5/5 | 0.2 | 0.2 | 2.02 | 1.5 | 4.2 | 6.8 | 0.7 | 1.8 | 1.9 |
| 12 | 14.3 | 2/2 | 18.5 | 4/4 | 0.2 | 0.5 | 1.33 | 0.1 | 0.6 | 0.6 | 0.1 | 0.8 | 0.6 |
| 13 | 14.0 | 1/1 | 19.6 | 2/2 | 0.4 | 0.64 | - | 0.6 | 2.8 | 4.2 | 0.7 | 6.1 | 5.3 |
| 14 | 11.8 | 2/2 | 17.5 | 4/4 | 0.2 | - | 2.02 | - | - | - | - | - | - |
| 15 | 10.7 | 1/2 | 16.2 | 12/12 | 0.2 | 0.69 | 7.43 | 1.4 | 3.4 | 6.0 | 0.7 | 3.8 | 3.5 |
| 16 | 13.8 | 3/1 | 17.9 | 15/15 | 0.69 | 3.32 | 4.33 | 4.6 | 8.4 | 9.9 | 1.0 | 24.4 | 19.7 |
| 17 | 15.8 | 2/2 | 19.4 | 12/12 | 0.84 | 5.6 | 7.43 | 3.0 | 5.3 | 4.2 | 3.7 | 19.7 | 15.8 |
| 18 | 12.3 | 2/2 | 18.1 | 10/12 | 0.2 | 2.08 | 2.77 | 1.0 | 2.7 | 3.2 | 0.7 | 19.7 | 12.8 |
| 19 | 13.7 | 1/1 | 16.5 | 8/10 | 0.43 | 2.02 | 4.24 | 2.5 | 5.6 | 6.1 | 0.3 | 6.2 | 5.6 |
| 20 | 13.4 | 3/3 | 16.8 | 15/15 | 0.26 | 1.07 | 5.52 | 4.5 | 8.0 | 10.3 | 0.1 | 2.3 | 2.0 |
| 21 | 10.1 | 2/2 | 15.3 | 10/12 | 0.52 | 3.41 | 6.32 | 2.2 | 6.8 | 9.8 | 0.7 | 5.7 | 3.8 |
| 22 | 13.5 | 3/2 | 17.7 | 10/12 | 0.69 | 3.58 | 8.18 | 3.6 | 6.0 | 7.1 | 0.5 | 10.5 | 9.3 |
| 23 | 14.0 | 3/4 | 19.4 | 20/25 | 0.69 | 6.06 | 6.82 | 4.8 | 6.4 | 8.2 | 1.4 | 15.1 | 15.7 |
| 24 | 16.7 | 2/2 | 20.3 | 10/12 | 0.06 | 1.73 | 3.32 | 2.2 | 2.9 | 3.4 | 0.1 | 1.0 | 1.3 |
| 25 | 16.0 | 2/2 | 19.8 | 15/15 | 0.26 | 2.63 | 5.38 | 3.5 | 6.4 | 8.3 | 0.2 | 2.0 | 2.0 |
| 26 | 12.4 | 2/2 | 17.1 | 18/18 | 0.2 | 0.49 | 4.39 | 0.9 | 2.8 | 3.8 | 0.2 | 8.3 | 6.4 |
| 27 | 16.8 | 4/4 | 19.5 | 14/16 | 0.52 | 15.1 | 13.8 | 0.1 | 16.9 | 18.4 | 1.3 | 3.2 | 3.9 |
| 28 | 11.2 | 2/2 | 18.0 | 20/20 | 0.2 | 1.79 | 4.31 | 3.1 | 8.1 | 13.6 | 0.7 | 3.1 | 3.0 |
| 29 | 14.7 | 2/2 | 19.4 | 12/15 | 1.18 | 4.91 | 4.19 | 1.9 | 3.0 | 3.0 | 2.7 | 18.2 | 14.6 |
| 30 | 11.1 | 2/2 | 15.2 | 12/10 | 0.2 | 1.73 | 4.82 | 1.1 | 6.2 | 6.7 | 0.7 | 8.9 | 3.6 |
| 31 | 11.9 | 4/5 | 17.6 | 15/15 | 0.31 | 1.39 | - | 3.5 | 6.5 | 8.8 | 0.1 | 3.9 | 3.2 |
| 32 | 13.1 | 2/2 | 18.9 | 15/15 | 0.2 | 6.27 | - | 1.1 | 1.4 | 1.8 | 0.7 | 10.4 | 8.9 |
| 33 | 15.7 | 3/3 | 18.2 | 15/15 | 0.92 | 6.0 | - | 1.1 | 1.4 | 1.5 | 2.2 | 9.6 | 8.2 |
| 34 | 16.9 | 3/2 | 19.1 | 12/12 | 0.23 | 7.4 | - | 2.9 | 3.6 | 5.0 | 0.6 | 5.6 | 8.2 |
| 35 | 15.4 | 2/3 | 18.8 | 15/15 | 0.1 | 1.62 | - | 2.1 | 3.3 | 4.7 | 0.1 | 6.0 | 6.0 |
| 36 | 11.4 | 1/2 | 18.5 | 8/10 | 0.2 | 1.27 | - | - | - | - | - | - | - |
| 37 | 12.7 | 2/3 | 17.3 | 12/12 | 0.2 | 1.13 | - | 1.0 | - | - | - | - | - |
| 38 | 11.3 | 2/2 | 17.9 | 10/10 | 0.46 | 4.28 | - | 1.5 | - | - | - | - | - |
| 39 | 14.6 | 2/3 | 19.2 | 12/12 | 0.2 | 2.89 | - | 0.8 | 1.4 | 1.9 | 0.7 | 9.2 | 7.6 |
| 40 | 11.4 | 2/2 | 16.5 | 10/12 | 0.29 | - | 2.45 | 1.5 | 2.8 | 4.9 | 0.1 | 0.3 | 0.6 |
| 41 | 15.2 | 1/2 | 19.6 | 10/12 | 0.46 | - | 3.64 | 3.1 | 4.8 | 5.6 | 1.1 | 9.4 | 7.8 |
| 42 | 16.5 | 4/4 | 19.1 | 8/10 | 1.13 | - | 4.77 | 1.5 | 2.3 | 2.5 | 2.7 | 16.0 | 15.2 |
| 43 | 10.6 | 2/4 | 17.4 | 8/8 | 0.61 | - | - | 1.1 | - | - | 0.1 | - | - |

To convert testosterone from µg/l to nmol/l, multiply by 3.46.

**Table 2**
Serum testosterone, LH and FSH concentrations in patients with hypogonadotropic hypogonadism (HH) and constitutional delay of growth and puberty (CDGP)

| | HH | CDGP | P |
|---|---|---|---|
| **Serum testosterone (µg/l)** | | | |
| Day 0 | 0.29 (0.17) (n=14) | 0.43 (032) (n=29) | 0.08 |
| Day 4 | 0.75 (0.75) (n=13) | 3.53 (3.12) (n=25) | <0.00001 |
| Day 19 | 1.9 (0.8) (n=12) | 5.49 (2.6) (n=19) | 0.00001 |
| **Serum FSH (U/l)** | | | |
| Unstimulated | 0.9 (0.8) (n=11) | 2.2 (1.3) (n=28) | 0.007 |
| Peak (20 min) | 2.2(1.5) | 5.1(3.3) | <0.001 |
| **Serum LH (U/l)** | | | |
| Unstimulated | 0.7 (0.7) (n=11) | 0.9 (0.9) (n=26) | 0.47 |
| Peak (20 mins) | 1.7(1.7) | 8.9(6.6) | <0.001 |

To convert testosterone from µg/l to nmol/l, multiply by 3.46.

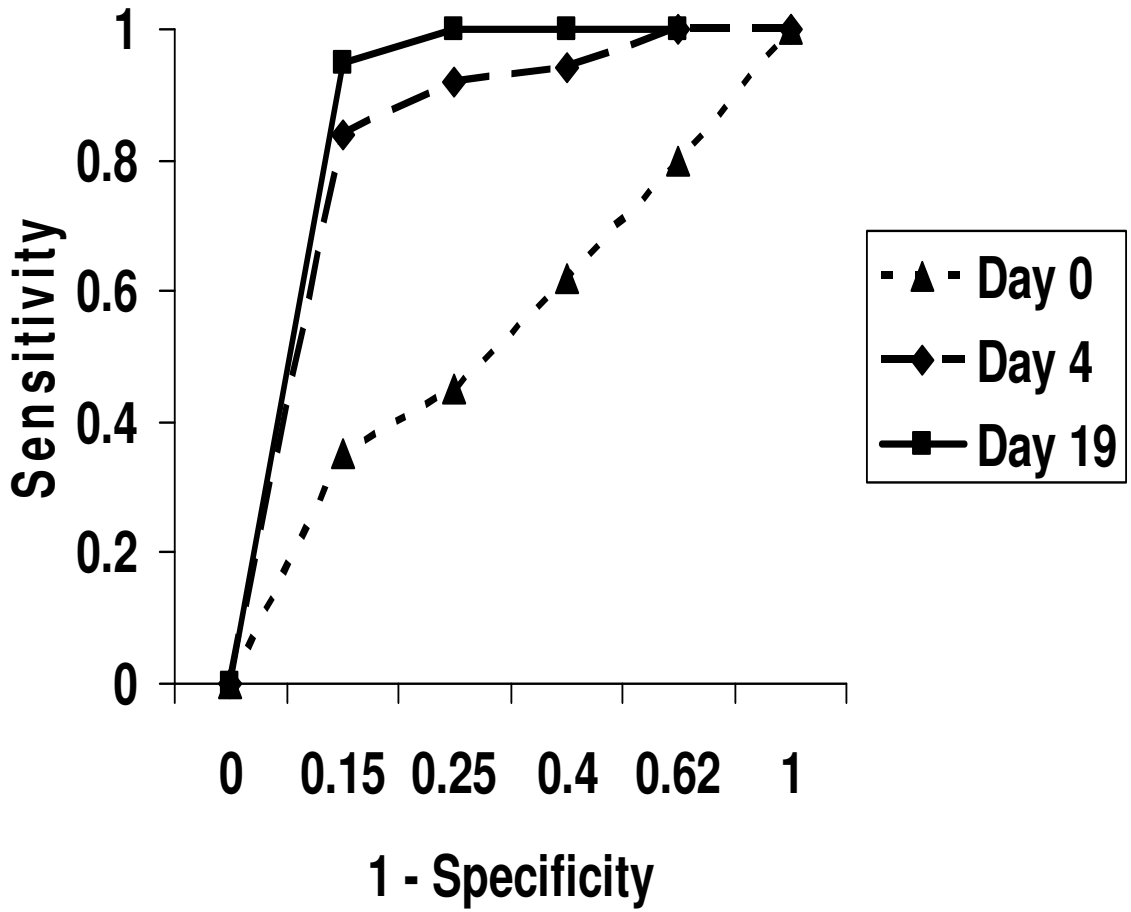
Sensitivity
1
0.8
0.6
0.4
0.2
0
0
0.15
0.25
0.4
0.62
1
1 - Specificity
Day 0
Day 4
Day 19

Figure 1


16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Testosterone mcg/L
Cut off 1.04mcg/L
HH
CDGP

Figure 2a


16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Testosterone mcg/L
Cut off 2.75mcg/L
HH
CDGP

Figure 2b

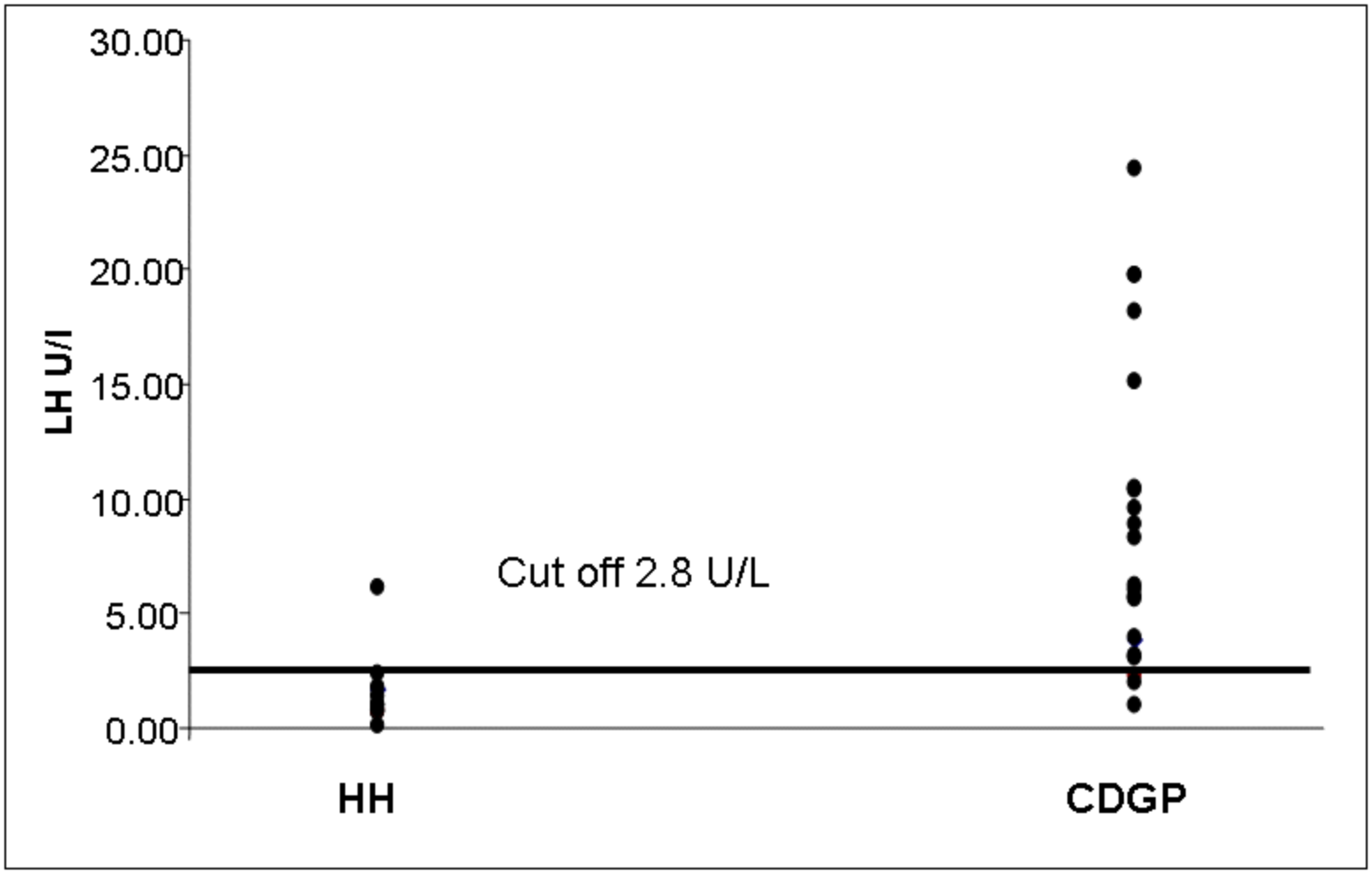


Figure 2c