Guideline: GnRH Analogue Stimulation Testing to Investigate Precocious Puberty

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Scope

This guideline is intended for general paediatricians and paediatric endocrinologists who are regularly investigating children with symptoms and signs of precocious puberty. Precocious puberty is defined in girls as the onset of breast development (Tanner stage B2) before the age of 8 years, and in boys as testes volume enlargement more than 3mls (Tanner stage G2) before the age of 9 years [1]. The aim of stimulation testing is to measure the peak gonadotropin (lutenizing hormone, LH and follicle stimulating hormone, FSH) concentrations after stimulation of the hypothalamic-pituitary-gonadal (HPG) axis, in order to diagnose central precocious puberty. However, in view of lack of availability of the gold standard recombinant gonadotropin-releasing hormone (GnRH), this guideline outlines the use of GnRH analogues for stimulation testing to investigate precocious puberty.

Investigation of Central precocious puberty

Central precocious puberty (CPP) can be diagnosed based on clinical features of pubertal development with evidence of hypothalamic-pituitary-gonadal activation. Basal gonadotropin (lutenizing hormone, LH and follicle stimulating hormone, FSH) concentrations may be sufficient to confirm CPP if sufficiently elevated [2-5], and this diagnostic information is more sensitive in males than females [4]. However, girls with central precocious puberty in the early phase of activation of the hypothalamic-pituitary-gonadal axis are capable of clinically relevant oestradiol (E2) production, which may occur in the face of low LH secretion and low LH/FSH ratios. Thus, if basal concentrations alongside clinical and radiological data are not sufficient to make a diagnosis of CPP, then measurement of peak gonadotropin concentrations after stimulation is required [5].

Gonadotropin-releasing hormone (GnRH) has been used as the gold standard stimulation test for diagnosing CPP [1]. However, as over recent years the availability of recombinant GnRH has been limited, GnRH analogues (GnRHa) have been considered for the investigation of peak LH and FSH concentrations following stimulation testing. Several different GnRHa preparations have been investigated for their use in HPG axis stimulation for investigation of CPP, including Triptorelin acetate and Leuprorelin (or Leuprolide) acetate [4, 6-10].

Triptorelin acetate for stimulation of the HPG axis

While depot Triptorelin acetate formulation is used as a therapeutic regimen to suppress the hypothalamic-gonadotrophic axis, the rapid-acting, aqueous formulation of Triptorelin acetate has an acute stimulatory effect on the gonadotrophs when given as single dose, with gonadotrophins reaching maximum levels 3 hours after administration [11].

A randomised controlled trial by Freire et al. evaluated the diagnostic accuracy of 0.1mg/m^2 of rapid-acting Triptorelin to a maximum of 0.1 mg given subcutaneously, versus standard GnRH 100 microgram/m2 administered intravenously, in girls with suspected CPP (n=46) [7]. This study found that 0.1mg/m^2 of rapid-acting Triptorelin with LH response measured at 3 hours post administration and a cut off of >7 IU/l by immunofluorometric assay (IFMA) or >8 IU/l by electrochemiluminescence immunoassay (ECLIA) confirmed the diagnosis of CPP with specificity of 100% (95% CI: 75–100%) and sensitivity 76% (95% CI: 58–89%). LH-3h post-Triptorelin (index test) showed a significant correlation with peak LH post-GnRH (reference test), both measured by IFMA ($r= 0.76$, $p<0.01$).

In patients with LH-3 h below the cut-off value (n=8/33), considering E2-24h >295 pM (80 pg/ml) raised the sensitivity of the Triptorelin test to 94% (95% CI: 80-99%), maintaining the positive predictive value (PPV) at 100%. Taking into account the cut-off values of these two biochemical markers together, LH-3h or E2-24h, the overall diagnostic efficiency raised to 96%. It was recommended that, taking into account the LH-3h specificity of 100% in the CPP diagnosis, if it is possible to obtain the results of LH during the day of sampling, it would be unnecessary to take the sample at 24 h. If LH-3h is below cut-off, it is advisable to assess the ovarian response determined by a E2-24h sample.

Of note, the first GnRH test did not detect the activation of the HPG axis in 2/33 patients with CPP; however, the Triptorelin test showed pubertal response that was confirmed by the clinical progression and the second GnRH test, performed 6 months after the first evaluation.

A separate retrospective study reviewed girls that had undergone GnRHa stimulation testing with 100 micrograms of subcutaneous Triptorelin (n=101) and identified that a peak LH of >6 IU/l at 1 hour post administration provided the most appropriate cut off level in diagnosing CPP, with a sensitivity of 89.1% and a specificity of 91.3% [8]. Of note this study did not evaluate LH concentrations more than 2 hours post administration.

The Triptorelin test was well tolerated, and no systemic side effects were observed [7]. Patients diagnosed with CPP following a subcutaneous Triptorelin test can then be started on GnRHa treatment [12].

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Leuprorelin acetate for stimulation of the HPG axis

Leuprorelin acetate has also been evaluated as a suitable substitute for GnRH in the diagnosis of central precocious puberty.

Ibanez et al. analysed the effects of a single injection (500 microgram subcutaneous) of the GnRH agonist Leuprorelin acetate on gonadotropin secretion compared with those induced by a GnRH test in 32 children (11 males and 21 females) with pubertal disorders (precocious breast development, advanced puberty with predicted adult height <-2SDS and delayed puberty) [9]. Patients were divided into 4 groups: A: progressive puberty, B: Non-progressive puberty, C: pre-pubertal controls and D: pubertal controls. A peak serum LH response >8 IU/L occurred in patients with progressive puberty and in patients with Tanner stage II puberty, 3 hours post Leuprorelin acetate challenge. This response was consistently seen in patients with progressive puberty and pubertal controls. In patients with non-progressive puberty and in pre-pubertal controls, the LH peak occurred between 3 and 6 hours after injection.

A more recent retrospective analysis by Yazdani et al. evaluated stimulation testing with 20 microgram/kg of subcutaneous Leuprorelin acetate in children with premature sexual development (n=107) [4]. Group A with premature thelarche and non-progressive puberty included 21 girls, Group B with premature adrenarche and non-progressive puberty included 15 subjects (12 girls and 3 boys) and Group C with central progressive puberty included 71 children (58 girls and 13 boys). In the group with central precocious puberty the main findings were: a) In boys basal LH (>0.1 mIU/mL), testosterone concentrations (≥10 ng/dL), basal and stimulated LH/FSH ratios (at 1 and 3h) have excellent sensitivity and specificity (all 100%); b) In girls basal LH > 0.1 mIU/ml, basal and stimulated LH/FSH ratios and basal estradiol (≥1.5 ng/dL) have low sensitivity though excellent specificity; c) Compared to stimulated LH concentration at 1h, the LH concentration > 5mIU/ml at 3h had better sensitivity (83% vs 73%) without compromising specificity (97% vs 100%). This cut off also has optimal sensitivity (83%) and specificity (97%) when compared to a lower cut off of 3mIU/ ml or a higher cut off of 7mIU/ml.

Summary

The gold standard for diagnosis of CPP is stimulation testing with GnRH, in combination with clinical assessment, x-ray bone age and ultrasound pelvis. However, recently recombinant GnRH has been unavailable in the UK and therefore alternative tests with GnRH analogues have been assessed. There is generally a paucity of good quality data, and timing and absolute concentration of the LH cutoff value for diagnosis of CPP varies between studies and depending on the GnRH analogue assessed. Whilst clinical judgment and follow up continues to be of great importance in the evaluation of precocious puberty, the following protocols are suggested:

- For subcutaneous Triptorelin acetate, 0.1mg/m^2 to a maximum of 0.1 mg, a cut off of LH of >**7 IU/l** by IFMA or >**8 IU/l** by ECLIA *at 3 hours post administration* should be used to confirm the diagnosis of CPP. If LH-3h is below cut-off, it is advisable to assess the ovarian response determined by a E2-24h sample (E2-24h >295 pM (80 pg/ml))
- For subcutaneous Leuprorelin acetate (Prostap SR; Takeda UK Ltd); 20 microgram/kg a cut off of LH of >**5 IU/** *at 3 hours post administration* should be used to confirm the diagnosis of CPP.

GnRH analogue tests summary protocols:

Background: This test is performed to assess the ability of the pituitary gland to secrete gonadotrophins in response to GnRH analogue stimulation.

Indication: Diagnosis and follow-up of gonadotrophin-dependent premature sexual maturation.

Prior arrangements: Obtain GnRH analogue from pharmacy.

Special Precautions: None.

Patient preparation: No need to fast. The test can be performed at any time of day.

A - Triptorelin test

Dose: Triptorelin acetate (Gonapeptyl Depot; Ferring GmbH); **0.1mg/m^2** (maximum 0.1 mg)

Test procedure:
1-Collect basal (time 0) samples (see below).
2-Then administer Triptorelin acetate subcutaneously

Sampling:
Basal (time 0) plasma LH, FSH, oestradiol or testosterone.
Repeat plasma LH and FSH at 180 min.

Interpretation:
A cut off of LH of >**7 IU/l** by IFMA or >**8 IU/l** by ECLIA *at 3 hours post administration* should be used to confirm the diagnosis of CPP
If LH-3h is below cut-off, it is advisable to assess the ovarian response determined by a E2-24h sample (E2-24h >295 pM (80 pg/ml))

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B - Leuprorelin test

Dose: Leuprorelin acetate (Prostap SR; Takeda UK Ltd); **20 microgram/kg**

Test procedure:
1-Collect basal (time 0) samples (see below).
2-Then administer Leuprorelin acetate subcutaneously

Sampling:
Basal (time 0) plasma LH, FSH, oestradiol or testosterone.
Repeat plasma LH and FSH at 180 min.

Interpretation:
A cut off LH >5IU/ml at 3 hours post administration should be used to confirm diagnosis of CPP.

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