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.

Background

Precocious puberty (PP) is defined in girls as the onset of breast development (Tanner stage B2) before the age of 8 years, and in boys as testicular volumes of more than 3ml (Tanner stage G2) before the age of 9 years [1]. The aim of stimulation testing is to measure the peak gonadotropin (luteinising hormone [LH] and follicle stimulating hormone [FSH]) concentrations after stimulation of the hypothalamic-pituitary-gonadal (HPG) axis, in order to diagnose central precocious puberty (CPP). However, when there is a lack of commercial availability of gold standard recombinant gonadotropin-releasing hormone (rGnRH), this guideline outlines the use of GnRH analogue (GnRHa) stimulation testing as a suitable alternative to investigate CPP.

Investigation of Central Precocious Puberty

CPP should be diagnosed based on clinical features of pubertal development with biochemical evidence of HPG axis activation. In conjunction with clinical features, basal LH and FSH concentrations may be sufficient to confirm CPP if sufficiently elevated [2-5], although this diagnostic information is more sensitive in males than females [4]. In the early phase of HPG axis activation, girls with CPP are capable of clinically relevant oestradiol (E2) production, which may occur in the face of low basal LH secretion and low LH:FSH ratios. Thus, if basal gonadotropin concentrations, in conjunction with clinical and radiological data, are not sufficient to make a diagnosis of CPP, measurement of peak gonadotropin concentrations after stimulation is required [5]. Various GnRHa preparations have been investigated in this context, including Triptorelin acetate and Leuprorelin (or Leuprolide) acetate [4, 6-10].

Literature Review

i. Triptorelin acetate for stimulation of the HPG axis

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

A randomised controlled trial by Freire et al. evaluated the diagnostic accuracy of 0.1 mg/m^2 of rapid-acting Triptorelin (to a maximum dose of 0.1 mg) given subcutaneously, versus standard rGnRH 100 mcg/m^2 administered intravenously, in girls with suspected CPP (n=46) [7]. This study found that Triptorelin 0.1 mg/m^2 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 a specificity of 100% (95% CI: 75–100%) and sensitivity of 76% (95% CI: 58–89%). LH-3h post-Triptorelin (index test) showed a significant correlation with peak LH post-rGnRH (reference test), both measured by IFMA ($r= 0.76$, $p<0.01$). In girls with LH-3h concentrations below the cut-off value (n=8/33), E2-24h > 295 pmol/L (80 pg/mL) raised the sensitivity of the Triptorelin test to 94% (95% CI: 80-99%), maintaining the positive predictive value (PPV) at 100%.

A further retrospective study of girls with CPP who had undergone GnRHa stimulation testing with 100 mcg of subcutaneous Triptorelin (n=101) 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.

More recent studies have proposed that a lower peak LH cut-off may maintain diagnostic accuracy. A 2023 retrospective study of a Korean cohort comparing 74 girls with CPP to 72 girls with idiopathic premature thelarche (IPT) determined a peak LH of > 4.52 IU/L at 120 minutes measured by ICMA maintained 100% sensitivity with 95.8% specificity [12]. However, the study acknowledged the value of peak LH concentrations at 180 minutes in patients for whom there is a high clinical suspicion of CPP who have not met diagnostic cut-off LH values at 120 minutes.

A further 2021 study by Vukovic et al. prospectively compared GnRHa to rGnRH stimulation testing in 32 girls with CPP and 28 with IPT. They determined a peak LH value of ≥ 3.4 IU/L by ICMA at 180 minutes after GnRHa administration was 96.9% sensitive and 89.3% specific in diagnosing CPP, with a PPV of 91.2% and NPV of 96.2% [13].

The Triptorelin test was well-tolerated among cohorts with no systemic side effects observed.

ii. Leuprorelin acetate for stimulation of the HPG axis

Leuprorelin acetate has been evaluated as a suitable substitute for rGnRH in the diagnosis of CPP. Ibanez et al. analysed the effects of a single injection (500 mcg subcutaneous) of Leuprorelin acetate on gonadotropin secretion compared with rGnRH in 32 children (11 males and 21 females), in conjunction with clinical evidence of CPP and compared these with pre-pubertal and pubertal controls [10]. A peak serum LH response > 8 IU/L occurred in patients with clinical features of progressive puberty and in patients with Tanner stage II puberty 3 hours after Leuprorelin acetate challenge.

A further retrospective analysis by Yazdani et al. evaluated stimulation testing with 20 mcg/kg of subcutaneous Leuprorelin acetate in 58 girls and 13 boys with clinical features of CPP [4]. Compared to stimulated LH concentrations at 1 hour, the LH concentration > 5 mIU/mL at 3 hours had improved sensitivity (83% vs 73%) without compromising specificity (97% vs 100%). This cut-off also had optimal sensitivity and specificity when compared to a lower cut-off of 3 mIU/mL or a higher cut-off of 7 mIU/mL.

Other Considerations

Investigation of premature adrenarche, without breast development in girls or testicular enlargement in boys, should aim to exclude excess androgen production (e.g. due to late virilising congenital adrenal hyperplasia) rather than HPG axis activation [14].

Summary and Recommendations

- The gold standard for diagnosis of CPP is rGnRH stimulation testing in combination with clinical assessment of Tanner staging, testicular volumes, x-ray bone age, and ultrasound pelvis.

- In the instance of a lack of availability of rGnRH, alternative stimulation testing with GnRHa is recommended.

In conjunction with clinical judgment and regular follow up, the following protocols are suggested:

GnRHa Stimulation Test Protocol Using Triptorelin or Leuprorelin

Principle:

GnRH secreted by the hypothalamus is responsible for the release of LH and FSH from the anterior pituitary gland. This test is performed to assess the gonadotropin secretion in response to GnRHa stimulation.

Indication

For the diagnosis and follow-up of gonadotrophin-dependent premature sexual maturation.

Precautions

- Avoid human chorionic gonadotropin injections prior to testing.
- Do not perform following priming for growth hormone stimulation test.

Preparation

Fasting is not required unless combined with growth hormone stimulation testing (without priming).

Timing

Perform at any time of day convenient to the patient and clinical team.

Protocol

i. At time 0 minutes, draw basal serum samples for LH, FSH, E2 in females, testosterone in males.

ii. Administer subcutaneously:
   - Triptorelin acetate 0.1 mg/m^2 (maximum dose 0.1 mg)
     or
   - Leuprorelin acetate 20 mcg/kg

iii. At time 180 minutes, draw peak serum samples for LH and FSH.

iv. **Females only:* If peak LH concentrations at 180 minutes do not reach diagnostic cut-offs outlined below, draw serum E2 at time 24 hours.

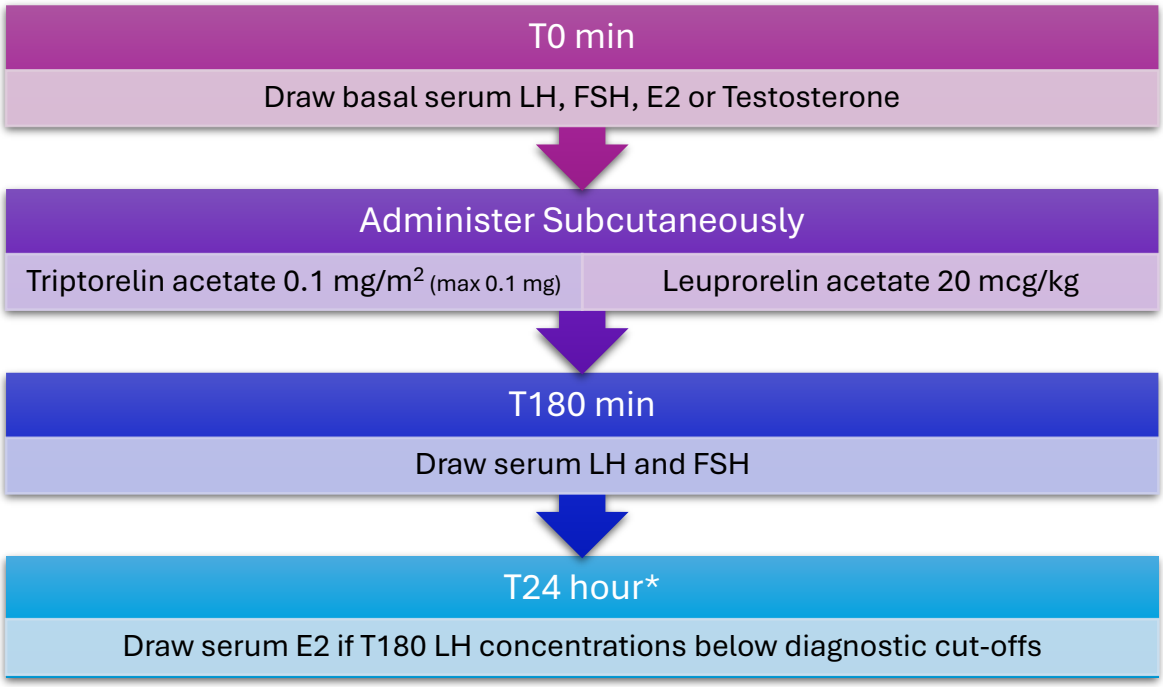

Figure 1: GnRHa stimulation testing protocol **Females only*

Interpretation

Results should be interpreted in conjunction with specialist clinical biochemistry based on the assay used within each centre (See *Appendix 1* for assay sensitivities and specificities), while also considering clinical features of the individual patient (Tanner staging, testicular volumes, uterine length and ovarian volumes, growth velocity, bone age), as well as additional biochemical markers of puberty (E2 or T).

i. *Suspected Precocious Puberty*

Biochemistry	Assay	Triptorelin	Leuprorelin
T180 Peak LH	ICMA	> 4.5 IU/L	> 5 IU/L
	IFMA	> 7 IU/L	
	ECLIA	> 8 IU/L	
T180 Peak FSH		Elevation < LH response	Elevation < LH response
24h Oestradiol		> 295 pmol/L > 80 pg/mL	> 295 pmol/L > 80 pg/mL

ii. *Gonadotropin-Independent Precocious Puberty*

- Suppressed basal LH and FSH with no response to GnRHa at T180
- Elevated oestradiol or testosterone

iii. *Adequately Treated Precocious Puberty*

- Suppressed basal LH and FSH with no response to GnRHa at T180

iv. *Premature Thelarche and Thelarche Variant*

- T180 FSH-predominant response
- T180 LH in pre-pubertal ranges

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Appendix 1: Sensitivities and specificities of GnRHa versus GnRH stimulation testing

	Triptorelin				**Leuprorelin**			**rGnRH**		
	LH Peak	Sensitivity (%)		Specificity (%)	LH Peak	Sensitivity (%)	Specificity (%)	LH Peak	Sensitivity (%)	Specificity (%)
		Pre-E2	24h E2**							
ICMA	4.5*	100		95.8	5	83	97	5	90-100	90-100
IFMA	7	76	94**	100	5	ND	ND	5		
ECLIA	8			100	5	ND	ND	5		

* At 120 minutes
** Females only
Pre-E2 = sensitivity and specificity before measuring oestradiol 24 hours after GnRHa stimulation
24h E2 = sensitivity and specificity after measuring oestradiol 24 hours after GnRHa stimulation
Abbreviations: E2 = oestradiol; ECLIA = electrochemiluminescence immunoassay; ICMA = immunochemiluminometric assay; IFMA = immunofluorometric assay; LH = luteinising hormone; ND = No data; rGnRH = recombinant gonadotropin-releasing hormone