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ORIGINAL ARTICLE

High diagnostic accuracy of subcutaneous Triptorelin test compared with GnRH test for diagnosing central precocious puberty in girls

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Summary

Context The GnRH test is the gold standard to confirm the diagnosis of central precocious puberty (CPP); however, this compound is not always readily available. Diagnostic accuracy of subcutaneous GnRH analogues tests compared to classical GnRH test has not been reported.

Objective To evaluate the diagnostic accuracy of Triptorelin test (index test) compared to the GnRH test (reference test) in girls with suspicion of CPP.

Design A prospective, case–control, randomized clinical trial was performed. CPP or precocious thelarche (PT) was diagnosed according to maximal LH response to GnRH test and clinical characteristics during follow-up.

Patients and Interventions Forty-six girls with premature breast development randomly underwent two tests: (i) intravenous GnRH 100 μg, (ii) subcutaneous Triptorelin acetate (0·1 mg/m^2, to a maximum of 0·1 mg) with blood sampling at 0, 3 and 24 h for LH, FSH and estradiol ascertainment.

Measurements Gonadotrophins and estradiol responses to Triptorelin test were measured by ultrasensitive assays.

Results Clinical features were similar between CPP (n = 33) and PT (n = 13) groups. Using receiver operating characteristic curves, maximal LH response (LH-3 h) under Triptorelin test $\geq$7 IU/l by immunofluorometric assay (IFMA) or $\geq$8 IU/l by electrochemiluminescence immunoassay (ECLIA) confirmed the diagnosis of CPP with specificity of 1·00 (95% CI: 0·75–1·00) and sensitivity 0·76 (95% CI: 0·58–0·89). Considering either LH-3 h or maximal estradiol response at 24 h (cut-off value, 295 pM), maintaining the specificity at 1·00, the test sensitivity increased to 0·94 (95% CI: 0·80–0·99) and the diagnostic efficiency to 96%.

Conclusion The Triptorelin test had high accuracy for the differential diagnosis of CPP *vs* PT in girls providing a valid alternative to the classical GnRH test. This test also allowed a comprehensive evaluation of the pituitary–ovarian axis.

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Introduction

Central precocious puberty (CPP) in girls is characterized by an activation of the hypothalamic–pituitary–ovarian (HPO) axis before 8 years of age.[1] Given the gradual awakening of the GnRH pulse generator, a spectrum of presentations has been found among girls with premature sexual development.[2] Earliest clinical features of CPP are not easily distinguished from idiopathic precocious thelarche (PT) or other intermediate positions along this spectrum where the gonadotrophic axis is not completely activated.[1] These conditions do not always warrant therapy with GnRH agonists.[2,3] Diagnostic confirmation of CPP at early stages of pubertal development, through the demonstration of the activation of the HPO axis, is desirable to consider treatment to optimize outcome.[4,5] Lost to follow-up or inadequate interventions may lead to shorter final height as well as to psychosocial disturbances caused by earlier and inappropriate physical maturation to the age of the girl. These could be prevented with timely treatment.

For the last decades, the GnRH stimulation test has been the gold standard to assess the status of the hypothalamic–pituitary–gonadal axis in children with pubertal disorders.[1] However, GnRH is not readily available worldwide, and GnRH agonists (GnRHa) have been used, albeit not systematically, as an alternative.[1,6] While depot Triptorelin acetate formulation is used as a therapeutic regimen to suppress the 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 h

after administration.[7] Owing to its longer half-life, this effect lasts at least 24 h, making possible to additionally evaluate the sex-hormone secretion by the gonads.[7,8]

To date, no reports are available on the diagnostic accuracy of stimulation tests with subcutaneous aqueous GnRH analogues compared to the gold standard GnRH test, assessed with ultrasensitive gonadotrophin assays, for the diagnosis of CPP in girls. The aim of this study was to evaluate the diagnostic accuracy of a Triptorelin test, based on the administration of a single dose of a rapid-acting, aqueous formulation of Triptorelin acetate, followed by serum gonadotrophins and estradiol (E_2) determination, to ascertain the diagnosis of CPP in girls compared to the classical GnRH test.

Study design, patients and methods

Study design

A diagnostic validation study of Triptorelin acetate test following the guiding principles of Standards for Reporting of Diagnostic Accuracy (STARD) Initiative was conducted.[9] A prospective case–control study in girls with clinical signs of precocious pubertal development, considering peak gonadotrophin and E_2 levels during Triptorelin test as the index test in comparison with maximal LH responses to natural GnRH and clinical follow-up as a reference standard test was performed. For Triptorelin test, we studied gonadotrophin responses using two ultrasensitive assays and evaluated the E_2 response to assess the maturation of the whole HPO axis.

This clinical trial was approved by the Institutional Review Board and the Ethical Committee of the Hospital General de Niños Dr. Ricardo Gutiérrez from Buenos Aires, and by the National Drugs Regulatory Agency from Argentina, and registered in clinicaltrials.gov NCT01278290. Written informed parental consent for all girls and assent from girls >7 years of age were obtained.

Patients

All girls with premature thelarche referred to the Endocrine Division of the Hospital General de Niños Dr. Ricardo Gutiérrez in Buenos Aires, Argentina, a tertiary public hospital, were considered for enrolment in the study. The inclusion criteria were girls between 4 and 8·5 years old, presenting premature thelarche (thelarche onset between 4 and 8 years of age), associated with accelerated growth velocity (above the 90th percentile in the last 6 months) and/or advanced bone age of at least 1·5 years in relation to the chronological age. Girls with previous central nervous system conditions or a pattern of peripheral precocious puberty were excluded. Weight and height were measured in the orthostatic position using a Harpenden stadiometer. *Z*-scores for height and body mass index (BMI) were calculated using the software AUXOLOGY version 1.0 b17 Copyright® 2003 Pfizer (Pfizer Inc., NY, USA). Pubertal development was evaluated according to Tanner's criteria.[10] Bone age was determined according to the method of Greulich & Payle.[11] All patients underwent a pelvic ultrasonography.

Study protocol

All patients were randomized for the sequence to undergo the two tests, with an interval of at least 15 days between them. The randomization was performed using the RANDOM, version 3.01 (Llanidloes, UK) program Copyright JH Abranson 1995–2000 Programs for Epidemiologist (PEPI).

Reference standard test. GnRH 100 μg/m^2 (LHRH Ferring® Gonadorelin Acetate; Ferring GmbH, Kiel, Germany) was given as an intravenous bolus. LH and FSH were determined at baseline, 30 and 60 min. E_2 was measured only at baseline.

Index test. Aqueous Triptorelin Acetate, 0·1 mg/m^2, to a maximum of 0·1 mg (Gonapeptyl® Daily; Ferring GmbH), in a prefilled syringe was administrated subcutaneously into the left tricipital fold region. Blood samples were obtained at baseline and after 3 and 24 h of the injection. This sampling time was chosen according to the kinetics of Triptorelin acetate.[7,8] LH, FSH and E_2 serum concentrations were measured in all samples. Serum was obtained by centrifugation and stored at −20 °C until assayed. All samples from an individual girl were measured in the same run assay.

Reference standard and index tests assessment

The reference standard was the maximal LH response to GnRH test by an immunofluorometric assay (IFMA), in addition to the patient's clinical assessment during follow-up. We defined as case group (CPP) those girls having peak LH $\geq$ 6 IU/l.[12] Control group (PT) was composed of girls showing peak LH below 6 IU/l. To confirm the PT condition, follow-up of these patients was performed including clinical visits every 3 months, and pelvic ultrasonography and bone age evaluations every 6 months until the age of 9 year. Patients, initially considered as PT by a peak LH <6 IU/l (IFMA) on GnRH test that having <8 years old and showing pubertal progression throughout the follow-up, underwent a second GnRH test 6 months after the first evaluation. They were considered as having CPP if the second test confirmed a pubertal pattern. Patients with CPP were recommended to start treatment on long-acting GnRHa to arrest pubertal progression. Upon diagnosis of CPP, a CNS MRI was performed to rule out any pathological condition.

The main outcome measures for index test were maximal LH and FSH concentrations measured by two ultrasensitive methods: IFMA and ECLIA and maximal E_2 responses under Triptorelin test.

Hormonal assays

Serum LH and FSH concentrations under the Triptorelin test were determined by two ultrasensitive assays: (i) Immunofluorometric assay (IFMA, DELFIA; PerkinElmer, Inc. by Wallac Oy, Turku, Finland). The functional sensitivities were 0·05 and 0·10 IU/l, according to the second WHO IS 80/552 for LH and IRP 94/632 for FSH, respectively. Intra- and interassay

coefficients of variation for IFMA method were $\leq 3{\cdot}2$ and $\leq 7{\cdot}3\%$ for LH and $\leq 2{\cdot}3$ and $\leq 5{\cdot}2\%$ for FSH and (ii) Electrochemiluminescence assay (ECLIA, LH ref. 11732234 and FSH ref. 11775863; Roche Diagnostics GmbH, Mannheim, Germany) using a Cobas e411 analyzer. The functional sensitivities of both LH and FSH assays were 0·10 IU/l, according to the second WHO IS 80/552 for LH and the second IRP 78/549 for FSH. Intra-and interassay coefficients of variation were $\leq 1{\cdot}8\%$ for LH and $\leq 4{\cdot}2\%$ for FSH.

Linear regression of Passing Bablok and Bland & Altman concordance analyses were performed using Method Validator (MultiQC; www.multiqc.com). LH measurement by ECLIA was positively biased compared to IFMA method (ECLIA LH = 1·17 × IFMA LH + 0·07). FSH by ECLIA showed a positive bias compared to IFMA results (ECLIA FSH = 1·20 × IFMA FSH + 0·016). Owing to these between-assay differences, cut-off values for serum LH and FSH were analysed separately.

Estradiol concentrations were determined by ECLIA (Roche Diagnostics GmbH) using a Cobas e411, with functional sensitivity of 36·7 pM, intra-assay CVs $\leq$ 3·8% and interassays CVs $\leq$ 6·5%. When serum LH, FSH or E_2 levels were undetectable, the value of the limit of quantification of the assay was attributed.

Statistical analyses

Mann–Whitney's test was used to compare clinical features differences between patients with CPP and PT. Log-transformed data of LH, FSH and E_2 responses to Triptorelin test were analysed by two-way ANOVA and *post hoc* Tukey's test. Receiver operating characteristic (ROC) curves for the main outcome measures were determined to obtain their optimal cut-off point for the diagnosis of CPP (GRAPHPAD Prism version 4.00 for Windows; GraphPad Software, San Diego, CA, USA). Sensitivity was defined as the proportion of patients with CPP having a pubertal response to the index test (peak LH or E_2 above cut-off value); specificity was the proportion of PT patients with peak LH or E_2 below cut-off value; positive predictive value (PPV) was the proportion of patients with an index test above cut-off value having CPP; negative predictive value was the proportion of patients with an index test below the cut-off point having PT. Diagnostic efficiency was the percentage of true findings (CPP with pubertal responses plus PT with prepubertal responses divided by the number of total girls).

Spearman correlation was carried out between maximal LH response obtained under both Triptorelin and GnRH tests and between maximal LH and E_2 responses under the Triptorelin test. $P < 0{\cdot}05$ was considered significant.

Results

Forty-six girls were included in the study between December 2009 and June 2011. All patients underwent both the GnRH and the Triptorelin tests within an interval of 18 ± 3 days. Upon randomization, 21 girls started with the GnRH test (8 PT and 13 CPP), and 25 girls with the Triptorelin test (5 PT and 20 CPP, Fisher test for sequence $P = 0{\cdot}2$). According to the reference test, 33 patients were classified as CPP and 13 as PT (Table 1). At the beginning of the study, 31/33 CPP girls had peak LH $\geq$ 6 IU/l and two showed peak LH < 6 IU/l in the first GnRH test; however, these two girls showed pubertal progression during follow-up, and a second GnRH test showed a pubertal LH response. We performed ROC curve with the data of the maximum LH response on the initial GnRH test and we found that peak LH of 5·7 IU/l by IFMA has 100% of specificity and 94% of sensitivity for the diagnosis of CPP. All CPP patients had normal CNS MRI. The 13 PT patients were followed-up during 18 ± 6 months (mean ± SD). They showed no pubertal progression or regression of breast development, decrease in growth velocity and no increase in the relative advancement in bone age upon re-evaluation 6–12 months later.

At the beginning of the study, both groups had similar clinical features: chronological age, age at onset of thelarche, Tanner stage, bone age, bone age advancement, height, BMI, growth velocity and uterine length (Table 2). LH, FSH and E_2 levels – measured by IFMA and ECLIA at baseline, 3 and 24 h after Triptorelin 0·1 mg subcutaneous administration in CPP and PT girls – are shown in Table 3. Basal LH was significantly higher in CPP than in PT, yet with a significant overlap between groups (50% of CPP patients had baseline LH within the PT range). The overlap of FSH and E_2 baseline values between the two groups was even higher (55% and 60%, respectively). The maximal gonadotrophin levels were observed at 3 h in both groups, and serum levels persisted above baseline at 24 h. Mean LH at 3 h was almost four times higher in CPP than in PT ($P < 0{\cdot}001$). FSH levels were not significantly different between groups. E_2 was not significantly different from baseline levels at 3 h but increased 11-fold in CPP and fivefold in PT at 24 h (E_2-24 h). The E_2-24 h response was significantly higher in CPP than PT ($P < 0{\cdot}001$).

The most appropriate cut-off values, determined by ROC curves for the diagnosis of CPP using Triptorelin test, with 100% specificity were LH at 3 h (LH-3 h) 7 IU/l (IFMA) and 8 IU/l (ECLIA) and E_2-24 h 295 pM (80 pg/ml) (Table 4, Fig. S1). LH-3 h post-Triptorelin (index test) showed a significant correlation with peak LH post-GnRH (reference test), both measured by IFMA (Fig. 1). Figure 1 also shows that a cut-off

Table 1. LH, FSH and estradiol (E_2) levels during GnRH test measured by IFMA for CPP and PT girls

	CPP $n = 33$		PT $n = 13$	
	Mean ± SEM	Range	Mean ± SEM	Range
Basal LH (IU/l)	0·82 ± 0·24	0·05–5·95	0·09 ± 0·02	0·05–0·27
Peak LH (IU/l)	13·06 ± 1·93	5·11–55·4	3·43 ± 0·35	1·1–5·61
Basal FSH (IU/l)	3·36 ± 0·38	0·57–8·3	1·46 ± 0·26	0·48–3·33
Peak FSH (IU/l)	13·42 ± 1·25	5·71–36·5	10·23 ± 1·14	5·72–21·4
Basal E_2 (pM)	77 ± 11	37–290	40 ± 1·5	37–55

CPP, central precocious puberty; PT, precocious thelarche.

Table 2. Clinical features of CPP and PT groups

Parameters	CPP (n = 33)	PT (n = 13)	P
Cronological age, years	7·4 (4–8·5)	8 (5·5–8·4)	NS
Age at onset of thelarche, years	6·4 (3·5–7·7)	6·6 (4–7·9)	NS
Breast Tanner stages, n*			
II	0 (0)	2 (15)	NS
III	33 (100)	11 (85)	NS
Bone age, years	9·5 (6–11)	9·8 (5–11)	NS
Bone age advancement, years	1·9 (0·1–3·5)	1·8 (−0·5 to 3)	NS
Height, z score	1·4 (−0·4 to 3)	1·5 (−0·2 to 2·7)	NS
Body mass index, z score	1 (−0·5 to 2·7)	1·7 (0·1 to 2)	NS
Growth velocity, z score	2·6 (0·3–7)	2·5 (−0·5 to 3·6)	NS
Uterine length, mm	38 (20–53)	32 (25–56)	NS

Data are presented as median (range).
CPP, Central precocious pubety; PT, Precocious thelarche.
*Figures in parentheses represent percentage.

of 7 IU/l for Triptorelin test correctly classified all PT patients, and 25 of 33 CPP girls (sensitivity 76%), being the overall diagnostic efficiency of 83%. These results were identical when LH-3 h $\geq$ 8 IU/l by ECLIA was used.

There was a significant correlation between the pituitary (LH-3 h) and ovarian (E_2-24 h) responses under Triptorelin test (Spearman R: 0·67, $P < 0·001$, Fig. 2). E_2-24 h presented high specificity and sensitivity, resulting in a PPV of 100% for CPP. Interestingly, six of the eight CPP patients with LH-3 h below the cut-off figures showed E_2 levels >295 pM (80 pg/ml) (Fig. 1). Therefore, in those patients with LH-3 h below the cut-off value, considering E_2-24 h raised the sensitivity of the Triptorelin test to 94%, maintaining the PPV at 100% (Table 4). Taking into account, the cut-off values of these two biochemical markers together, LH-3 h or E_2-24 h, the overall diagnostic efficiency raised to 96%. It is important to note that the first GnRH test did not detected the activation of the HPO axis in two patients of 33 CPP; however, the Triptorelin test showed pubertal response that was confirmed by the clinical progression and the second GnRH test.

The Triptorelin test was well tolerated. Most patients reported only little sharp local pain just at the precise time of application, and no systemic side effects were observed.

Discussion

The main objective of the current study was to determine the diagnostic efficiency of the Triptorelin test compared to the GnRH test for CPP diagnosis in girls. To get robustness and applicability, we followed STARD criteria.[9] We also used two ultrasensitive assays employed worldwide, especially in paediatric assistance.

This is the first prospective clinical trial that showing diagnostic efficiency of a subcutaneous Triptorelin acetate test for the diagnosis of CPP. In addition, the Triptorelin test provides a comprehensive evaluation of the pituitary–ovarian axis.

We have shown that the Triptorelin test is a simple and highly efficient test to differentiate between CPP and PT girls. We have used a prefilled syringe formulation that allows administrating the exact dose, avoiding dilution errors. A cut-off value of 7 IU/l (IFMA) or 8 IU/l (ECLIA) for LH-3 h allowed us to exclude the PT diagnosis, because all PT patients showed LH levels below the cut-off value, rending almost null the possibility of false positive results. However, the sensitivity of LH-3 h for CPP diagnosis was not optimal because eight of 33 CPP girls had

Table 3. LH, FSH and estradiol levels measured by IFMA and ECLIA assays at baseline, 3 and 24 h after Triptorelin 0·1 mg subcutaneous administration for CPP and PT girls

	IFMA					ECLIA				
	CPP, n = 33		PT, n = 13			CPP, n = 33		PT, n = 13		
	Mean ± SEM	Range	Mean ± SEM	Range	P	Mean ± SEM	Range	Mean ± SEM	Range	P
LH (IU/l)										
Baseline	0·53 ± 0·15	0·05–3·8	0·08 ± 0·01	0·05–0·13	<0·001	0·74 ± 0·21	0·1–5·4	0·12 ± 0·01	0·1–0·24	<0·001
3 h	12·7 ± 1·7*	3·7–42·6	3·3 ± 0·47*	1–6·7	<0·001	14·5 ± 1·8*	4·4–46·9	3·86 ± 0·53*	1·16–7·6	<0·001
24 h	7·9 ± 1·3†	1·2–29·9	1·2 ± 0·15†	0·66–2·5	<0·001	8 ± 1·3†	1·6–31·3	1·7 ± 0·21†	0·89–3·5	<0·001
FSH (IU/l)										
Baseline	3·2 ± 0·29	0·88–6·89	1·52 ± 0·18	0·44–2·56	0·001	3·8 ± 0·34	1·2–8·1	1·8 ± 0·21	0·53–3·1	0·001
3 h	19·7 ± 1·2*	11·1–41·4	17·1 ± 1·8*	3·55–24·3	ns	23·9 ± 1·4*	13·2–49·9	18·3 ± 2*	4·01–28·9	NS
24 h	13·2 ± 0·89†	5·44–26·7	7·2 ± 0·77†	3·7–11·5	0·001	15·8 ± 1†	6·3–30·4	8·5 ± 0·9†	4·28–14.5	0·001
Estradiol (pM)										
Baseline						54 ± 6	37–198	39 ± 1	37–53	NS
3 h						70 ± 5	37–158	46 ± 3	37–60	<0·01
24 h						550 ± 60†	125–1340	130 ± 23†	37–290	<0·001

CPP, central precocious puberty; PT, precocious thelarche.
*$P < 0·001$ *vs* baseline.
†$P < 0·001$ *vs* baseline and 3 h.

Table 4. Diagnostic accuracy of LH-3 h and estradiol (E_2)-24 h levels under Triptorelin test for central precocious puberty diagnosis in girls with signs of precocious sexual development

Biochemical marker	Assay	Cut-off	Sensitivity	Specificity	PPV	NPV	DE
LH-3 h	IFMA	≥7 IU/l	0·76 (0·58–0·89)	1·00 (0·75–1·00)	1·00 (0·86–1·00)	0·61 (0·38–0·82)	0·83
	ECLIA	≥8 IU/l	0·76 (0·58–0·89)	1·00 (0·75–1·00)	1·00 (0·86–1·00)	0·61 (0·38–0·82)	0·83
E_2-24 h	ECLIA	≥295 pM	0·79 (0·61–0·91)	1·00 (0·75–1·00)	1·00 (0·87–1·00)	0·65 (0·41–0·85)	0·85
LH-3 h or E_2-24 h	IFMA	LH ≥ 7 IU/l or E_2 ≥ 295 pM	0·94 (0·80–0·99)	1·00 (0·75–1·00)	1·00 (0·89–1·00)	0·87 (0·60–0·98)	0·96
	ECLIA	LH ≥ 8 IU/l or E_2 ≥ 295 pM	0·94 (0·80–0·99)	1·00 (0·75–1·00)	1·00 (0·89–1·00)	0·87 (0·60–0·98)	0·96

The 95% confidence intervals are shown in parentheses.
PPV, positive predictive value; NPV, negative predictive value; DE, diagnostic efficiency.

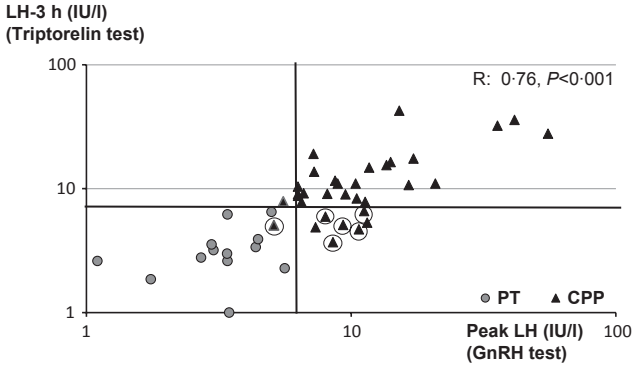

Fig. 1 Correlation between LH-3 h (Triptorelin test) and peak LH (GnRH test) measured by IFMA presented on a log scale. Solid lines represent the cut-off values: LH-3 h: 7 IU/l and peak LH: 6 IU/l. CPP girls marked in circles showed pubertal E_2-24 h response after Triptorelin administration. PT, precocious thelarche; CPP, central precocious puberty.

levels below the cut-off. The ovarian response determined by E_2-24 h sample with a cut-off 295 pM (80 pg/ml) raises the test sensitivity and confirms that marked ovarian steroidogenic activation is present in CPP girls. The use of both biochemical markers has the best diagnostic accuracy (96%) comparing with the reference standard test. Taking into account the LH-3 h 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 resulting in significant time and costs reduction. If LH-3 h is below cut-off, it is advisable to assess the ovarian response determined by E_2-24 h sample. In our study, estradiol 24-h measurement complemented the LH response to the analogue for the pubertal characterization in 6/33 CPP girls, which confirmed the lack of activation of HPO axis in 13/13 PT.

It is important to note that we intentionally selected the cut-off values with maximal specificity and PPV s to avoid potential treatment of a benign condition as precocious thelarche. Even with this approach, we were able to correctly diagnose 44 of 46 girls with suspected CPP or TP.

Confirming the HPO axis activation in CPP patients is essential to determine an appropriate therapeutic option, emphasizing the need to find out a reliable test for this purpose. Despite the high sensitivity of the third-generation LH assays, early signs of precocity in girls may occur in the face of undetectable or prepubertal levels of basal LH concentration.[13–15] Our results also show that the basal serum LH diagnostic efficiency is low.

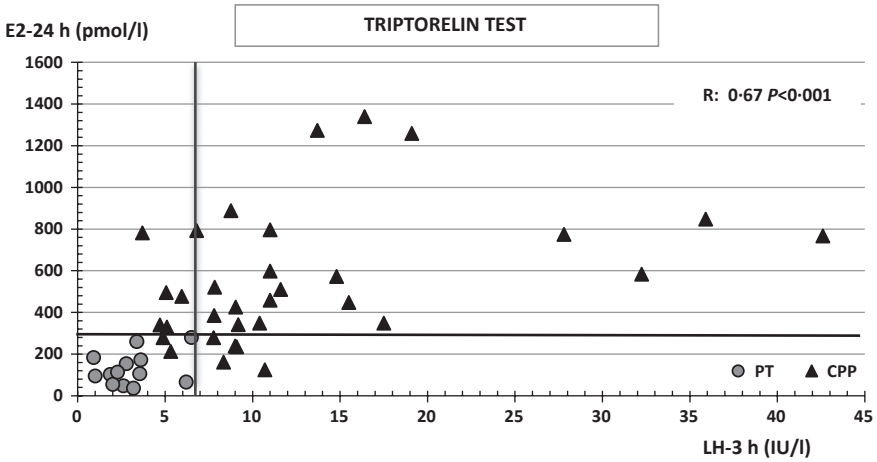

Fig. 2 Correlation between E_2-24 h and LH-3 h responses after Triptorelin administration. Solid lines represent the cut-off values: estradiol: 295 pM and LH: 7 IU/l (IFMA). PT, precocious thelarche; CPP, central precocious puberty.

The unavailability of commercial natural GnRH in some countries restricts its use and encourages the evaluation of new diagnostic strategies to confirm the activation of the HPO axis. Some studies have been published on the use of GnRHa for assessing the onset of precocious pubertal development, most of them employing aqueous Leuprolide acetate. In these reports, however, the use of non ultrasensitive assays, the absence of a gold standard test as a reference test and the lack of a defined end-point when making a retrospective evaluation of patients[16–19] introduce potential biases that might affect the clinical usefulness of the reported results.

To our knowledge, up to now, two studies have reported data on the HPO response to subcutaneous Triptorelin acetate in girls with precocious sexual development.[18,20] Only one of them[18] was focused on Triptorelin test as a method to diagnose CPP. However, this study was retrospective, no GnRH test as reference standard was used and no E_2 response was assessed.

The advantage of using a GnRH analogue test over the classical GnRH test is to assess the ovarian response providing a comprehensive evaluation of the HPO. Previous studies in girls with premature sexual development have shown that the robust stimulating effect on the HPO achieved by a single injection of GnRHa induces an E_2 rise which is proportional to the degree of endogenous activation of the axis.[21,22]

In our study, eight CPP patients had LH-3 h levels below cut-off values in response to Triptorelin test; however, six of these girls showed E_2-24 h above 295 pM (80 pg/ml). In these six girls, the findings could be due to a delayed LH peak or more probably – as it was reported by Garibaldi *et al.*[14] due to the fact that patients who are in early stages of CPP have an E_2 secretion dissociated from LH secretion. Moreover, Sathasivam *et al.*[19] using aqueous Leuprolide acetate have shown that it is not infrequent that LH and E_2 responses be discordant in girls with precocious puberty, so they also recommended the 24 h sample for measuring serum E_2, despite the inherent practical difficulties. They found that a Leuprolide E_2 stimulated levels >184 pM are observed in patients with pubertal progression. Our higher E_2 stimulated levels might be due to the superior relative potency of the Triptorelin compared to that of the Leuprolide. All these findings highlight the usefulness of evaluating together the pituitary and the ovarian responses. The E_2 level shows that the ovarian steroidogenic synthesis is activated and it complements the gonadotrophin parameters for pubertal characterization.

We are aware of that one weakness of our study might be that we did not include normal prepubertal girls. However, according to the recommendation of the STARD initiative for studies of diagnostic accuracy, to compare the test under evaluation with a reference standard test both should be performed in subjects who are suspected of having the condition of interest. Therefore, PT patients were considered as control group because including normal prepubertal girls could lead to biased results.[9]

Pubertal peak LH cut-off value after stimulation by classical GnRH test could be variable depending on the immunoassay used and the design of the study to obtain it.[1] In our study, analysing the peak LH on the initial GnRH test by a ROC curve, we found that a cut-off value of 5·7 IU/l by IFMA had a specificity of 100% and a sensitivity of 94% for the diagnosis of CPP. It is very important to consider that our cut-off was obtained in girls with suspected premature sexual precocity, in contrast to other investigators, that have taken as threshold the 97 percentile of the peak LH from a very small group of girls with Tanner I.[12,23,24]

Our PT patients have similar clinical features to the entity referred by other authors as "unsustained" puberty,[2] or "exaggerated thelarche",[21] "thelarche variant".[25] Irrespective of the term used to name this kind of condition, the main point is that these patients do not have a pubertal activation of the HPO axis, so they do not warrant therapy with GnRH agonist.[2]

In conclusion, the Triptorelin test has high diagnostic accuracy for the differential diagnosis of central precocious puberty *vs* precocious thelarche in girls with signs of precocious pubertal development. LH-3 h levels $\geq$7 IU/l by immunofluorometric assay or $\geq$8 IU/l by electrochemiluminescence immunoassay confirm the activation of the hypothalamic–pituitary–ovarian axis in central precocious puberty girls and allow to exclude the precocious thelarche diagnosis. When LH-3 h levels are below cut-off, taking into consideration the estradiol 24 h levels if they rise above 295 pM (80 pg/ml) after the Triptorelin administration is a very useful tool to confirm the diagnosis of CPP.

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Conflict of interest

No authors has any potential conflict of interest to declare.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Receiver operator characteristics (ROC) curves constructed to determine the optimal cutoff points for: (A) maximal LH response to Triptorelin test (LH-3 h) by IFMA (ECLIA data was coincident) and (B) maximal estradiol response to Triptorelin test (E_2-24 h).