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Hirsutism and oligomenorrhea are appropriate screening criteria for polycystic ovary syndrome in adolescents

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Abstract
We evaluated the association of hirsutism and oligomenorrhea (persistent menstrual cycles > 45 days) as screening criteria for the detection of biochemical hyperandrogenism (BH) and polycystic ovaries (PCOM) during adolescence and determined which androgens, granulosa cell hormone, ultrasonographic parameters have the best association with polycystic ovary syndrome (PCOS). Hirsute girls with oligomenorrhea (N=26 Hirs/Oligo group) and non-hirsute girls with regular cycles (N=63, C group) were studied. Prevalence of BH and PCOM, diagnostic performance of androgens and ultrasound parameters for PCOS diagnosis were analyzed. BH and PCOM prevalence were higher in the Hirs/Oligo girls than in the C girls (76.9% versus 25.5%; 92.3% versus 33.3%, respectively; p<0.0001). A complete PCOS phenotype (Hirs/Oligo with and BH and PCOM) was observed in 73.1% of the Hirs/Oligo group. The presence of both BH and PCOM was observed in 7.9% of the C group. The parameters with the best diagnostic performance were free androgen index ≥6.1, testosterone ≥2.4 nmol/L, follicle number ≥12 and ovarian volume ≥10 ml anti-Müllerian hormone (AMH) exhibited a low diagnostic accuracy. Hirsutism and persistent menstrual cycle over 45 days are highly associated with BH and PCOM suggesting that the presences of both criteria are necessary for the diagnosis of PCOS during adolescence.

Introduction
Defining appropriate diagnostic criteria for diagnosis of PCOS for young adolescents is a relevant problem. Menstrual irregularities, polycystic ovaries, lower rates of ovulation and higher androgen and anti-Müllerian hormone (AMH) levels are frequently observed in adolescents compared with adult women [1,2].

The use of adult diagnostic criteria could erroneously overdiagnose polycystic ovary syndrome (PCOS) during adolescence and it is necessary to develop specific criteria for the diagnosis during this period of life [3]. Recently, the American Academy of Pediatrics (AAP) and the American College of Obstetrics and Gynecology (ACOG) published specific criteria for the diagnosis of menstrual disturbances during adolescence that may identify girls with a higher risk of ovarian dysfunction [4].

We performed a cross-sectional study to evaluate an adapted clinical NICHD criterion of PCOS for adolescents, as screening tools to detect biochemical hyperandrogenism (BH) and polycystic ovaries during adolescence and to determine the clinical phenotypes, threshold values of androgen levels and ultrasonographic patterns in girls with hirsutism and oligomenorrhea compared with non-obese adolescent girls.

Materials and methods
Subjects
We studied girls (n=89) who were at least 1 year past menarche and ≤20 years of age. Twenty-six of these girls exhibited oligomenorrhea and hirsutism (Hirs/Oligo). Hirsutism (Ferriman–Gallwey [FG] scores ≥8) [5]. Oligomenorrhea: persistent menstrual cycle lengths equal ≥45 days [4]. The Hirs/Oligo girls were recruited in the gynecological and pediatric endocrinology clinics of San Borja Arriarán Hospital. Twenty-two girls (86.7%) of the Hirs/Oligo girls were naïve for treatment. Four girls (13.3%) ceased medication at least two months prior to recruitment. Exclusion criteria were as previously described [6].

Sixty-three non-hirsute girls (FG score ≤6) with regular menses (cycle length between 21 and 45 days) were recruited as Control Group (C) from nearby schools. All the C girls who were 6 years past menarche (n=6) had menstrual cycles less than 35 days. The exclusion criteria for this group were similar to the Hirs/Oligo group. Girls with severe acne, obesity, premature puberty or intrauterine growth retardation were also excluded. Twenty-four (92.3%) of the Hirs/Oligo girls and 61 (96.8%) of the C girls were 2 years past menarche at the moment of recruitment.
The protocol was approved by the Institutional Review Board of the San Borja Arriaran Hospital. The parents and adolescent girls ≥18 years signed an informed consent form, and the volunteers gave their written assent.

**Study protocol**

Clinical evaluations, blood sampling and ultrasonographic examinations were performed during early follicular phase. The cycle length was calculated as mean length of the last six menstrual cycles. The body mass index (BMI), FG score and waist-to-hip ratio (WHR) were determined. Standard deviation scores were calculated for BMI (BMI-SDS) using current NCHS standard curves. Overweight and obesity were defined as having a BMI between the 85th and 94th percentiles or over the 95th percentile, respectively.

Blood sample was obtained for the measurement of sex steroids, gonadotropins, insulin, sex hormone-binding globulin (SHBG), AMH and inhibin B (INHB). Homeostatic model assessment of insulin resistance (HOMA-IR) values was calculated.

**Ultrasonographic analysis**

Transabdominal ultrasound measurements were performed by a single observer as previously described [1,7].

**Laboratory assays**

Total testosterone (T) was measured by direct RIA (DIAsource ImmunoAssays Louvain-La-Neuve, Belgium) as previously described [1]. The RIA DIAsource kit was compared with a liquid chromatography–tandem mass spectrometry (LC–MS/MS) assay as previously reported [2]. The correlation between the T levels measured by RIA (DIAsource) and by LC–MS/MS was Spearman’s $r = 0.7548$ ($p < 0.0001$). Data and regression analyses identified the following equation for converting the testosterone level from the DIAsource value to the LC–MS/MS value T (ng/ml, LC–MS/MS) = $0.534 \times T$ (ng/ml, DIAsource) ($p < 0.0001$) (online Supplementary Figure).

Androstenedione, 17-hydroxyprogesterone (17OHP), dehydroepiandrosterone sulfate (DHEAS), Estradiol (E2), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were measured by RIA from Siemens Healthcare Diagnostics (USA) [8]. The free androgen index (FAI) was calculated [1]. Serum AMH was assayed using the AMH/MIS ELISA kit (ImmunoTech-Beckman, Marseilles, France) as previously described [9]. INHB was measured using a specific two-site ELISA assay (Beckman Coulter Inc.) as previously described [1,10]

**Definition of BH, PCOM and other PCOS phenotypes**

BH was defined by cut-off values that we previously demonstrated to be diagnostic for PCOS in adult women (T ≥ 2.1 nmol/l, free androgen index (FAI) ≥ 4.5% and/or DHEA-S ≥ 9214 nmol/l) [11]. Polycystic ovarian morphology (PCOM) was identified according to the Rotterdam consensus criteria [12]. Different phenotypes are described depending on the presence of hirsutism, oligomenorrhea, BH and PCOM.

**Statistical analysis**

The normality of the distributions was evaluated using the Kolmogorov–Smirnov test. A2, 17OHP, LH, SHBG and HOMA-IR required logarithmic transformation to normalize the distribution. The remaining hormones passed the normality test. Analyses of all continuous variables were adjusted by BMI-SDS using linear regression. Differences in the proportions were assessed with the $\chi^2$ test.

Receiver operating characteristic (ROC) analysis was used to determine the ability of hormonal markers and cut-off values to diagnose PCOS. Differences between ROCs were analyzed according to the method described by Hanley and McNeil [13]. A significance level of 5% was used. All statistical calculations were performed using SPSS for Windows version 18.0 and GraphPad Prism version 6.0. The data are reported as the arithmetic mean ± standard deviation (SD), with the exception of the FG score, which is reported as the median, minimum and maximum.

**Results**

The clinical, hormonal and ultrasonographic characteristics of the Hirs/Oligo and control groups adjusted by BMI-SDS are presented in Table 1. The Hirs/Oligo group exhibited higher BMI, obesity prevalence, FG scores and longer menstrual cycles ($p < 0.001$). After adjusting for BMI-SDS, the Hirs/Oligo girls exhibited higher T, FAL, DHEAS, LH, 17OHP and INHB levels, LH/FSH ratios, OV and total FN (all $p < 0.0001$) and lower SHBG ($p < 0.0001$) levels compared with the C group. The AMH levels were similar between the groups. However, when only girls with PCOM were considered, higher AMH levels were observed in the C group than in the Hirs/Oligo group ($P = 0.033$). The fact that only two girls in the Hirs/Oligo group did not have PCOM precluded a statistical analysis to compare AMH levels in the girls of both the groups without PCOM.

**Table 1. Clinical, hormonal and ultrasonographic characteristics of the Hirs/Oligo and control groups.**

|                      | Hirs/Oligo group | Control group |
|----------------------|------------------|---------------|
| **n**                | 26               | 63            |
| **Age** (years)      | 17.3 ± 1.9       | 16.6 ± 1.5    |
| **BMI-SDS**          | 1.0 ± 1.0*       | 0.4 ± 0.8     |
| **Obesity** ( % )    | 6 (23.1) [5]     | 0 (0)         |
| **Waist/hip ratio**  | 0.81 ± 0.09 [6]  | 0.78 ± 0.06   |
| **Menstrual cycle**  | 78.1 ± 37.1 [7]  | 30.1 ± 2.9    |
| **Ferriman–Gallwey score** | 11.0 (8.0–23.0) [8] | 2 (0.0–6.0) |
| **Testosterone** (nmol/l) | 2.8 ± 1.4 [9] | 1.7 ± 0.7  |
| **SHBG** (nmol/l)    | 42.7 ± 23.1 [10] | 68.9 ± 41.1 |
| **Free androgen index** | 9.3 ± 9.7 [11] | 3.1 ± 2.2  |
| **AMH** (pmol/l)     | 6075.3 ± 2790 [12] | 4791.8 ± 1943 |
| **Androstenedione** (ng/ml) | 13.3 ± 5.9 | 12.6 ± 8.0  |
| **17OHP** (ng/ml)    | 4.6 ± 3.0 [13]  | 3.6 ± 3.3     |
| **LH** (mIU/ml)      | 9.2 ± 7.7* [14] | 4.0 ± 2.3     |
| **FSH** (mIU/ml)     | 6.0 ± 2.2        | 5.9 ± 1.7     |
| **LH/FSH**           | 1.5 ± 1.1 [15]  | 0.7 ± 0.5     |
| **Estradiol** (pmol/ml) | 128.1 ± 96.9 | 169.2 ± 86.3 |
| **Glucose** (nmol/l) | 4.4 ± 0.4        | 4.4 ± 0.6     |
| **Insulin** (mIU/ml) | 7.5 ± 4.9        | 6.1 ± 2.3     |
| **HOMA-IR**          | 1.5 ± 0.9        | 1.2 ± 0.5     |
| **AMH** (pmol/l)     | 47.3 ± 29.8      | 45.6 ± 29.7   |
| **AMH in those with PCOM** | 48.9 ± 30.6 | 73.3 ± 29.2 |
| **AMH in those without PCOM** | 28.8 ± 6.2 | 31.7 ± 18.2 |
| **Inhibin B** (pg/ml) | 164.7 ± 62.9 [16] | 59.8 ± 29.7 |
| **Ovarian volume** (ml) | 13.5 ± 6.7 | 7.4 ± 3.3  |
| **Follicle number** (N) | 17.7 ± 6.7 | 8.6 ± 4.3  |

Data are presented as the mean ± SD. Ferriman–Gallwey scores are presented as median (minimum to maximum). SD: standard deviation; BMI: body mass index; BMI-SDS: body mass index – standard deviation score.

* $p < 0.01$ adjusted for BMI-SDS.

$|p| = 0.05$ adjusted for BMI-SDS.

$|p| < 0.0001$ adjusted for BMI-SDS.

$|p| < 0.0001$ adjusted for BMI-SDS.

$|p| < 0.05$ adjusted for BMI-SDS.
The presence of the different PCOS diagnostic criteria and phenotypes is presented in Table 2. BH was observed in a higher proportion of the Hirs/Oligo group than the C group (76.9% versus 25.4%; \( p < 0.0001 \)). The most frequently elevated androgen was T and FAI (65.4% versus 22.2% and 61.5% versus 12.7% for the Hirs/Oligo and C girls, respectively; \( p < 0.0001 \)). The prevalence of elevated DHEA-S was similar in both the groups (8.0 versus 3.2%; \( p = 0.59 \)). PCOM was observed in 92.3% and 33.3% of the Hirs/Oligo and C girls, respectively (\( p < 0.0001 \)).

Table 2. Prevalence of biochemical hyperandrogenism, polycystic ovarian morphology and the presence of different PCOS phenotypes in girls with PCOS defined by hirsutism and oligomenorrhea and a control group with regular menses and without hirsutism.

|                      | Hirs/Oligo group | Control group |
|----------------------|------------------|--------------|
| Biochemical hyperandrogenism (BH) | 20 (76.9)* | 16 (25.4) |
| PCOM by follicle number (%) | 24 (92.3)* | 21 (33.3) |
| PCOM by volume (%) | 18 (69.2)* | 11 (17.5) |
| PCOM by follicle number (%) | 23 (88.5)* | 16 (25.4) |
| PCOM by volume + follicle number (%) | 17 (65.3)* | 6 (9.5) |
| PCOS phenotypes | | |
| H-O-BH-PCOM | 19 (73.1)* | 0 (0) |
| H-O-PCOM | 5 (19.2)* | 0 (0) |
| H-O-BH | 1 (3.8) | 0 (0) |
| H-O | 1 (3.8) | 0 (0) |
| BH-PCOM | 0 (0) | 5 (7.9) |

H: hirsutism; O: oligomenorrhea; BH: biochemical hyperandrogenism; PCOM: polycystic ovarian morphology.

*% \( p < 0.0001 \) in the e-PCOS group versus the control group.

All the Hirs/Oligo girls fulfilled the NIH diagnostic criteria for PCOS. Almost three-fourths (73.1%) of them exhibited both BH and PCOM. One-fourth of the Hirs/Oligo girls (26.9%) did not have either BH or PCOM or both. The presence of both BH and PCOM was observed in 7.9% of the C group.

The abilities of different hormone levels and ultrasound characteristics to diagnose PCOS are presented in Table 3. FAI and T had the best diagnostic performances (cut-off values FAI \( \geq 6.1 \) and T \( \geq 2.1 \) nmol/l) by LC–MS/MS [online-only Supplementary Figure 1]). Using these new cut-off values, 73.1% and 12.7% of the Hirs/Oligo and C girls, respectively, were hyperandrogenic (\( p < 0.0001 \)), reducing in a 50% the prevalence of BH in the C girls with a minor decrease in the BH prevalence in the Hirs/Oligo girls.

The AMH AUC exhibited a good diagnostic performance for PCOS. The best cut-off value of INHB was 110.6 pg/ml. AMH exhibited a low diagnostic accuracy for PCOS (AUC = 0.5, \( p = 0.7 \)). However, when girls with PCOM were excluded from the C group, the AMH AUC exhibited significant diagnostic accuracy (AUC = 0.74, \( p = 0.007 \)). In this scenario, the best AMH threshold was 61.5 pmol/l, which corresponded to S = 66.7%, Sp = 75, PPV = 55.1%, NPV = 57.2% and LR = 1.2.

**Discussion**

This study evaluated the phenotypic, hormonal and ultrasonographic characteristics of hirsute and oligomenorrheic adolescents compared with a group of non-obese control girls. We found that the simultaneous presence of persistent menstrual cycles longer than 45 days and hirsutism is necessary as screening criteria for PCOS during this stage of life. These diagnostic criteria are associated with a high prevalence of BH and PCOM, similar to those described in adult PCOS women [14,15].

Table 3. Comparison of receiver operating characteristic (ROC) plot analysis of different hormonal and ultrasonographic criteria for PCOS in adolescents.

| Hormone                        | Area under the curve/p value | Cut-Off value | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Likelihood ratio |
|--------------------------------|-------------------------------|---------------|----------------|----------------|---------|---------|-----------------|
| **Androgen and SHBG**          |                               |               |                |                |         |         |                 |
| Free androgen index            | 0.832/<0.0001                 | 4.5           | 61.5           | 87.3           | 82.9    | 69.4    | 4.9             |
| T                              |                               | 5.5           | 57.7           | 92.1           | 88.0    | 68.5    | 7.3             |
| 61.4                           |                               | 57.7          | 93.7           | 90.2           | 68.9    | 9.1     |                 |
| 7.4                            |                               | 65.4          | 84.6           | 74.6           | 82.9    | 3.3     |                 |
| 2.4                            |                               | 65.4          | 85.7           | 82.1           | 71.2    | 4.6     |                 |
| 2.9                            |                               | 65.4          | 88.9           | 84.7           | 69.8    | 5.5     |                 |
| **Granulosa cell hormone**     |                               |               |                |                |         |         |                 |
| Inhibin B                      | 0.942/<0.0001                 | 104.4         | 92.3           | 93.7           | 93.6    | 92.4    | 14.5            |
| 111.7                          |                               | 88.5          | 85.3           | 72.7           | 94.9    | 89.2    | 18.6            |
| **Ultrasound Criteria**        |                               |               |                |                |         |         |                 |
| Follicle number (N)            | 0.877/<0.0001                 | 11            | 88.5           | 66.7           | 72.7    | 85.3    | 2.7             |
| 12.4                           |                               | 84.6          | 74.6           | 76.9           | 82.9    | 3.3     |                 |
| 13                             |                               | 84.6          | 74.6           | 76.9           | 82.9    | 3.3     |                 |
| 14                             |                               | 84.6          | 74.6           | 76.9           | 82.9    | 3.3     |                 |
| 25                             |                               | 84.6          | 74.6           | 76.9           | 82.9    | 3.3     |                 |
| **Ovarian volume (ml)**        | 0.849/<0.0001                 | 7             | 88.5           | 49.2           | 63.5    | 81.1    | 1.7             |
| 10.4                           |                               | 84.1          | 84.1           | 82.3           | 78.5    | 4.9     |                 |
| 11                             |                               | 84.1          | 84.1           | 82.3           | 78.5    | 4.9     |                 |
| 12                             |                               | 84.1          | 84.1           | 82.3           | 78.5    | 4.9     |                 |
| 14.7                           |                               | 84.1          | 84.1           | 82.3           | 78.5    | 4.9     |                 |

Note: PPV: positive predictive value; NPV: negative predictive value; AUC: Area under the curve.

*Cut-off values with the best compromise between sensitivity, specificity and likelihood ratio.
We determined that 80% of the Hirs/Oligo girls exhibited BH. This is similar to the 75% incidence of BH observed in adult PCOS women diagnosed with NIH criteria [16]. Furthermore, T and FAI, but not DHEAS or androstenedione, were elevated in these groups.

The hormones that should be measured for PCOS diagnosis in adolescents have not yet been established. Our data suggest that DHEAS and androstenedione levels have a low diagnostic utility for PCOS diagnosis during adolescence.

Other areas of uncertainty regarding PCOS diagnosis during adolescence include the cut-off values for androgens that should be used to label a girl as hyperandrogenic [17,18]. We determined that testosterone \( \geq 2.4 \text{ nmol/l} \) and FAI \( \geq 6.1 \% \) exhibited the best diagnostic performance. Although these values are higher than those previously reported by other groups [19–21], they are similar to reports of PCOS in obese adolescents [22]. A cautious analysis of the assay of androgens should be performed in girls without clinical hyperandrogenism. Using the criteria for adults, we observed that BH was present in 25.4% of the C girls. However, when BH was analyzed using the higher cut-off values established in our study, only 12.7% of the C girls were classified as hyperandrogenic.

PCOM was observed in 92.3% of the Hirs/Oligo girls, similar to reports regarding adult PCOS women and significantly higher than the 33% prevalence observed in the C group. We determined that the Hirs/Oligo girls exhibit a threefold increase in the prevalence of PCOM relative to girls in the C group. These data differ from those of previously reported one, who observed that only 35% of girls with PCOS exhibited PCOM [23].

OV and FN exhibited similar diagnostic accuracy. The cut-off levels associated with PCOS in adolescents for OV and FN were 10 ml and 12 follicles, respectively, similar to those reported for adult women. This was an unexpected result, as higher OV and FN values have been reported for adolescents compared with adult women [24,25].

AMH levels are elevated in adult women with PCOS and may thus be used as a diagnostic feature [26]. We found that AMH levels are positively associated with the follicular pool and PCOM in the Hirs/Oligo and healthy girls and are not associated with a hyperandrogenic state (Supplementary Table 1) [1,27]. The diagnostic utility of this single exam for diagnosing PCOS is significantly lower than previously reported in adults [28]. Hart et al. described a similarly low diagnostic performance of AMH for diagnosing PCOS during adolescence. Therefore, the AMH levels during the second decade of life are more closely associated with PCOM and increased follicular mass than with hyperandrogenism [27,29].

INHB exhibited the best performance for the diagnosis of PCOS. This differs from data reported in adult PCOS women, who exhibit a closer association with elevated AMH levels [30]. However, a positive correlation between INHB, FN and OV has been described in adolescents [1,28].

In conclusion, we demonstrated that the presence of hirsutism and a persistent menstrual cycle length \( \geq 45 \) days are appropriate screening elements for PCOS in adolescents, which are strongly associated with BH and PCOM. We show that borderline androgen levels can be observed in a subset of healthy nonobese girls with regular menstrual cycles. The fact that some C girls simultaneously exhibited PCOM and BH should lead to research on the long-term consequences of this phenotype. However, at this moment, it is premature to classify these girls as having PCOS. Good diagnostic accuracy for predicting PCOS was observed for T, FAI, OV, FN and INHB but not for DHEAS, A2 or AMH levels. Ultrasonographic threshold levels similar to those described for adult women were observed in this group of adolescents.

Declarations of interest

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