Endokrynologia Polska/Polish Journal of Endocrinology
Tom/Volume 62; Numer/Number 2/2011
ISSN 0423–104X

The utility of the gonadotrophin releasing hormone (GnRH) test in the diagnosis of polycystic ovary syndrome (PCOS)

Ocena przydatności testu z gonadoliberyną (GnRH) w diagnostyce zespołu policystycznych jajników (PCOS)

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Abstract

Introduction: Polycystic ovary syndrome (PCOS) is characterised by increased frequency of hypothalamic GnRH pulses leading to a relative increase in LH synthesis by the pituitary. As GnRH stimulation can reveal a relative LH excess, we have endeavoured to assess whether GnRH test might be useful in the diagnosis of PCOS.

Material and methods: The study involved 185 subjects: a PCOS group, n = 151, all with *oligo-* or *amenorrhoea*, aged (mean ± SD) 24.8 ± ± 5.4 years, BMI 24.5 ± 6.0 kg/m^2; and regularly menstruating controls, n = 34, aged 26.6 ± 5.0 years, BMI 24.6 ± 5.5 kg/m^2. In 121 subjects with PCOS and in 32 controls, serum LH and FSH were measured before (0 minutes) and 30 and 60 minutes after GnRH stimulation (100 μg i.v.). Insulin resistance was assessed by HOMA and Insulin Resistance Index derived from glucose and insulin concentrations during 75 gram oral glucose tolerance test.

Results: Women with PCOS had higher testosterone (p = 0.0002), androstendione (p = 0.0021), 17OH-progesterone (p < 0.0001) and were more insulin resistant. Raised concentrations of at least one androgen were, however, found only in 58.1% of women with PCOS. Baseline and stimulated LH concentrations were higher in PCOS (9.09 ± 5.56 *vs* 4.83 ± 1.71 IU/L, 35.48 ± 31.4 *vs* 16.30 ± 6.68 IU/L, 33.86 ± 31.8 *vs* 13.45 ± 5.2 IU/L, at 0, 30 and 60 mins post GnRH, respectively, p < 0.0001). There was no difference in baseline or stimulated FSH concentrations between groups. Relative increases of LH or FSH in comparison to respective baseline values were similar in both groups. There was, however, a marked increase in LH/FSH ratio in PCOS in comparison to controls ($LH_{0\ min}/FSH_{0\ min}$ 1.59 ± 0.95 *vs* 0.76 ± 0.2, $LH_{30\ min}$/ /$FSH_{30\ min}$ 4.07 ± 3.0 *vs* 1.89 ± 0.79, $LH_{60\ min}/FSH_{60\ min}$ 3.56 ± 2.58 *vs* 1.55 ± 0.63, p < 0.0001 at all time points). Further analysis revealed that $LH_{30\ min}/FSH_{30\ min}$ > 2.11 or $LH_{60\ min}/FSH_{60\ min}$ > 1.72 had 78.3% and 87.5% sensitivity and 81.7% and 81.3% specificity for the diagnosis of PCOS, respectively.

Conclusions: Women with PCOS have higher baseline and GnRH-stimulated LH concentrations. GnRH stimulation results in an increase in LH/FSH ratio in women with PCOS. Therefore we postulate that this phenomenon might be potentially useful as an additional tool in the diagnosis of PCOS. **(Pol J Endocrinol 2011; 62 (2): 120–128)**

Key words: *polycystic ovary syndrome, GnRH test, insulin resistance, oligomenorrhoea*

Streszczenie

Wstęp: Test z gonadoliberyną (GnRH) może ujawnić względną przewagę syntezy LH nad FSH, w wyniku zwiększonej częstości podwzgórzowych pulsów GnRH w zespole policystycznych jajników (PCOS, *polycystic ovary syndrome*). W pracy podjęto zatem próbę oceny, czy stymulacja przysadki przez GnRH może być przydatna w diagnostyce PCOS.

Materiał i metody: Badaniem objęto 185 osób: kobiety z PCOS, n = 151, z zaburzeniami miesiączkowania o typie *oligo-* lub *amenorrhoea*, w wieku (średnia ± SD) 24,8 ± 5,4 lat, wskaźnikiem masy ciała (BMI, *body mass index*) 24,5 ± 6,0 kg/m^2 oraz kobiety z grupy kontrolnej, n = 34, wiek 26,6 ± 5,0 lat, BMI 24,6 ± 5,5 kg/m^2. U 121 kobiet z PCOS oraz u 32 kobiet z grupy kontrolnej oznaczono LH i FSH przed (0 minut) oraz w 30 i 60 minut po podaniu GnRH (100 μg i.v.). Insulinooporność oceniono za pomocą modelu HOMA oraz za pomocą indeksu insulinooporności (IRI), obliczanego na podstawie stężeń glukozy i insuliny w doustnym teście tolerancji glukozy (75 g).

Wyniki: Kobiety z PCOS miały wyższe stężenia testosteronu całkowitego (p = 0,0002), androstendionu (p = 0,0021), 17OH-progesteronu (p < 0,0001) oraz charakteryzowały się większą insulinoopornością. Podwyższone stężenia któregoś z androgenów obserwowano u 58,1% kobiet z PCOS. Wyjściowe oraz stymulowane stężenia LH były wyższe u kobiet z PCOS (9,09 ± 5,56 *v.* 4,83 ± 1,71 IU/L, 35,48 ± 31,4 *v.* 16,30 ± 6,68 IU/L, 33,86 ± 31,8 *v.* 13,45 ± 5,2 IU/L, odpowiednio w 0, 30 i 60 minucie testu, p < 0,0001). Nie było różnic pomiędzy wyjściowymi oraz stymulowanymi stężeniami FSH między grupami. Stosunki stężeń LH oraz FSH po stymulacji przez GnRH w porównaniu z wartościami wyjściowymi były zbliżone w obu badanych grupach. Zaobserwowano znaczący wzrost stosunku LH/FSH u kobiet z PCOS, w porównaniu z grupami kontrolną ($LH_{0\ min}/FSH_{0\ min}$ 1,59 ± 0,95 *v.* 0,76 ± 0,2, $LH_{30\ min}/FSH_{30\ min}$ 4,07 ± 3,0 *v.* 1,89 ± 0,79, $LH_{60\ min}/FSH_{60\ min}$

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3,56 ± 2,58 *v.* 1,55 ± 0,63, p < 0,0001 we wszystkich punktach czasowych). Stosunek $LH_{30\ min}/FSH_{30\ min}$ > 2,11 lub $LH_{60\ min}/FSH_{60\ min}$ > 1,72 charakteryzował się odpowiednio czułością 78,3% i swoistością 87,5% oraz czułością 81,7% i swoistością 81,3% dla kobiet z PCOS.
Wnioski: U kobiet z PCOS stwierdza się wyższe stężenia LH, zarówno przed, jak i po stymulacji przez GnRH. Stosunek stężeń LH do FSH ulega istotnemu zwiększeniu po stymulacji przez GnRH u kobiet z PCOS. Autorzy wnioskują, że ocena stosunku LH/FSH po stymulacji przez GnRH może być przydatna, jako badanie dodatkowe w diagnostyce PCOS.
(Endokrynol Pol 2011; 62 (2): 120–128)

Słowa kluczowe: *zespół policystycznych jajników, test GnRH, insulinooporność, oligomenorrhea*

Introduction

Polycystic ovary syndrome (PCOS) is the commonest endocrinopathy of women of reproductive age, affecting 4–7% of this population [1, 2]. Clinically it can present as oligo-/amenorrhoea, hyperandrogenism/hirsutism and/or fertility problems [1–3].

Current ESHRE/ASMR consensus defines PCOS by the so-called Rotterdam criteria [4]. According to these, PCOS can be diagnosed if a woman fulfills at least two of three criteria: i.e. oligo/anovulation, clinical or biochemical hyperandrogenism and/or polycystic ovaries on ultrasound imaging, when other causes of menstrual irregularities and hyperandrogenaemia have been ruled out [4]. In some cases, diagnosis of PCOS may be, however, still controversial, particularly in women with irregular periods and polycystic ovaries on ultrasound, but with androgens within the reference range. Increased androgen synthesis in PCOS, both of ovarian and adrenal origins [5], stems from abnormalities on several levels of the hypothalamo-pituitary-ovarian axis. The most prominent abnormality involves an increased frequency of pituitary LH pulses resulting from an increased frequency of pulsatile hypothalamic GnRH secretion. The above mentioned dysfunction of hypothalamic GnRH pulse generator leads to a relative increase of LH rather than FSH secretion by the pituitary [6], that in turn results in an increased LH-stimulated androgen secretion [7, 8]. More than 20 years ago, it was also noted that administration of a GnRH analogue allows the demonstration of abnormalities of ovarian steroidogenesis in PCOS, namely an increase of 17-hydroxy-progesterone to androstendione ratio after GnRH stimulation [9, 10]. On the strength of that, some authors [10, 11] formulated the concept of so-called *functional ovarian hyperandrogenism* that was supposed to be characteristic for PCOS and that could be demonstrated after stimulation by GnRH analogue (originally by nafarelin) [9–11]. The same authors [9–11] also described increased LH concentrations after GnRH analogue stimulation, but these changes were described in a qualitative, rather than a quantitative, manner. We have endeavoured to quantitatively investigate the issue of an increased LH release after GnRH stimulation.

Aim of the study

The aim of the study was to assess LH and FSH secretion after GnRH stimulation in women with PCOS, and in regularly menstruating healthy controls. We decided to compare both the changes in LH and FSH concentrations to their respective baseline values as well as the changes of LH/FSH ratio after GnRH stimulation. Thus, we have endeavoured to assess whether assessment of gonatrophin secretion after GnRH stimulation might provide some useful information for the diagnosis of PCOS.

Material and methods

The study involved 185 women admitted to the Department of Endocrinology & Metabolic Diseases of the 'Polish Mother' Memorial Research Institute and the Medical University of Lodz between 2006 and 2009. Of these, 151 women aged 24.8 ± 5.4 years (mean ± SD) were diagnosed with PCOS according to the Rotterdam criteria [4]. All these women had had oligo- or amenorrhoea and polycystic ovaries on pelvic (intravaginal) ultrasound, while the majority also had clinical hyperandrogenism (acne and/or hirsutism). The control group consisted of 34 healthy, regularly menstruating, women aged 26.8 ± 5.0 years (Table I).

All hormonal investigations were performed between the third and sixth day of either a spontaneous or a progestagen-induced menstruation (in the latter case menstrual bleeding was typically obtained after ten-day administration of dydrogesterone (Duphaston®) or micronised progesterone (Luteina®). Endocrine investigations involved measurements of LH, FSH, oestradiol, total testosterone, androstendione, di-hydroepinadrosterone sulphate (DHEAS), 17-hydroxy-progesterone, TSH, free T3 and free T4. The presence of hyperprolactinaemia was excluded after assessment of a nine time point prolactin day curve, as previously described [12]. Hypercortisolaemia was excluded either on the basis of a midnight serum cortisol below 50 nmol/L (1.8 μg/dL) or on cortisol suppression below 50 nmol/L after an overnight 1.0 mg dexamethasone suppression test [13]. Furthermore, in all subjects we performed a 75 gram oral glucose tolerance test (OGTT) with glu-

Table I. *Demographic characteristics of women with polycystic ovary syndrome (PCOS), n = 151, and regularly menstruating controls (n = 34)*

Tabela I. *Statystyki opisowe dla danych demograficznych pacjentek z grupy kontrolnej (n = 34) i z PCOS (n = 151)*

	Group	n	Mean	Median	SD	Min	Max	p value
Age (years)	PCOS CONTR	151 34	24.8 26.8	24.0 27.0	5.4 5.0	16.0 17.0	49.0 38.0	*0.83*
BMI [kg/m^2]	PCOS CONTR	151 34	24.5 24.5	22.7 22.1	6.0 5.5	14.5 18.1	43.6 39.0	*0.99*

Table II. *Concentrations of selected hormones in women with PCOS and controls (CONTR)*

Tabela II. *Stężenia badanych hormonów u pacjentek z PCOS i w grupie kontrolnej (CONTR)*

	Group	n	Mean	Median	SD	Min	Max	p value
Oestradiol [pg/mL]	CONTR PCOS	32 151	45.49 65.31	35.09 47.60	31.69 62.06	12.05 15.0	190.4 406.7	***0.018***
Testosterone [ng/mL]	CONTR PCOS	32 151	0.53 0.77	0.59 0.72	0.29 0.40	0.16 0.20	1.50 2.07	***0.0002***
DHEAS [μg/dL]	CONTR PCOS	32 133	264.5 288.4	249.7 261.6	115.3 129.2	70.4 70.0	517.7 662.5	***0.38***
Androstendione [ng/mL]	CONTR PCOS	31 124	2.23 3.25	2.15 2.78	0.93 1.80	0.79 0.10	5.04 10.00	***0.0021***
17OH-progesterone [ng/mL]	CONTR PCOS	30 120	0.54 1.25	0.46 1.00	0.23 0.93	0.10 0.30	1.30 6.29	***< 0.00001***

cose and insulin measurements at 0, 60 and 120 minutes. Subsequently, insulin resistance parameters were calculated, i.e. HOMA, (where HOMA = fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5) [14] and an Insulin Resistance Index (IRI) based on the assessment of glycaemia and insulinaemia during OGTT. The product of the glucose area under the plasma glucose curve and insulin area under plasma glucose curve is used as an index of insulin resistance, calculated through the formula: 2/[1/(INSp × GLYp)]+1, where INSp and GLYp are the measured insulin and glycaemic areas [15]. This method has a good correlation with the gold standard of assessment of insulin resistance, i.e. the euglycaemic hyperinsulinaemic clamp technique [16].

Hirsutism was assessed according to the Ferriman-Galwey scale [17], where a score above eight was considered significant. All pelvic ultrasound examinations were performed at the Department of Ultrasound Diagnositics of the 'Polish Mother' Memorial Research Institute, where diagnosis of polycystic ovaries was based on the presence of either 12 or more follicles measuring 2–9 mm in diameter, or increased ovarian volume (> 10 cm^3) [18].

In 121 patients diagnosed with PCOS, and in 32 controls, we performed a GnRH test that involved intravenous administration of 100 μg of synthetic GnRH (Relisorm®) by Serono lub LHRH® by Ferring. Blood samples for the measurements of LH and FSH (electrochemiluminescence method (ECLIA) by Elecsys 2010 analyser) were taken before (0 minutes) and 30 and 60 minutes after GnRH administration.

Statistical analysis

Depending on distribution characteristics of the analysed parameters, we employed either a *t*-test in cases of normal distribution or a Mann-Whitney test if distribution characteristics were not normal. Correlation analysis was performed by the means of Spearman rank correlation method. Statistical significance was assumed for $p < 0.05$. All analyses were performed using Statistica 8.0 software.

Results

Women with PCOS and regularly menstruating controls were matched for their age and BMI (Table I). Mean prolactin concentrations were higher in women with PCOS, but failed to reach statistical significance (data not shown). As expected, women with PCOS had higher concentrations of total testosterone, androstendione, 17-hydroxy-progesterone and oestradiol (Table II). It should be noted, however, that androgen concentrations remained within the reference range in a significant number of women with PCOS despite, on aver-

age, higher values than in controls. In particular, raised concentrations of total testosterone were noted only in 38.6% of women with PCOS, androstendione in 34.7% and DHEAS in only 28.8%. Raised concentration of at least a single androgen was observed in 58.1% of women with PCOS. Interestingly, women with PCOS also had significantly (i.e. around two-fold) higher 17-hydroxysprogesterone/androstendione ratio. This was statistically highly significant ($p < 0.001$, see Fig. 1).

There were no differences in glucose levels during OGTT between women with PCOS and controls; women with PCOS had, however, higher concentrations of fasting insulin and were more insulin-resistant (IRI: 0.93 ± 0.38 *v.* 0.78 ± 0.34, for PCOS and controls, respectively, $p = 0.045$). HOMA index was higher in women with PCOS, although this was of borderline statistical significance ($p = 0.06$) (Table III). Both baseline and GnRH-stimulated LH concentrations were significantly higher in women with PCOS, but there were no differences in FSH concentrations between women with PCOS and controls (Table IV, Fig. 2 and 3).

Relative proportions of GnRH-stimulated versus baseline gonadotrophin concentrations, i.e. $LH_{30\ min}/LH_{0\ min}$, $LH_{60\ min}/LH_{0\ min}$, $FSH_{30\ min}/FSH_{0\ min}$, $FSH_{60\ min}/FSH_{0\ min}$, were similar in PCOS and controls, though there was a trend towards higher $LH_{60\ min}/LH_{0\ min}$ ratio in women with PCOS (3.76 ± 2.0 IU/L *v.* 3.28 ± 2.59 IU/L, $p = 0.053$), Table V. In women with PCOS, we observed significantly raised LH/FSH ratio at all three time-points (Table V, Fig. 4). Furthermore, there was a further increase in LH/FSH ratio after GnRH stimulation in women with PCOS (Table 5, Fig. 4), i.e. baseline LH/FSH ratio was 1.59 ± 1.0 in PCOS and 0.79 ± 0.2 in controls, but 4.09 ± 2.99 and 3.56 ± 2.58 for PCOS, and 1.89 ± 0.79, and 1.55 ± 0.63 for controls at 30 and 60 minutes after GnRH stimulation, respectively ($p < 0.001$, Table V, Fig. 4).

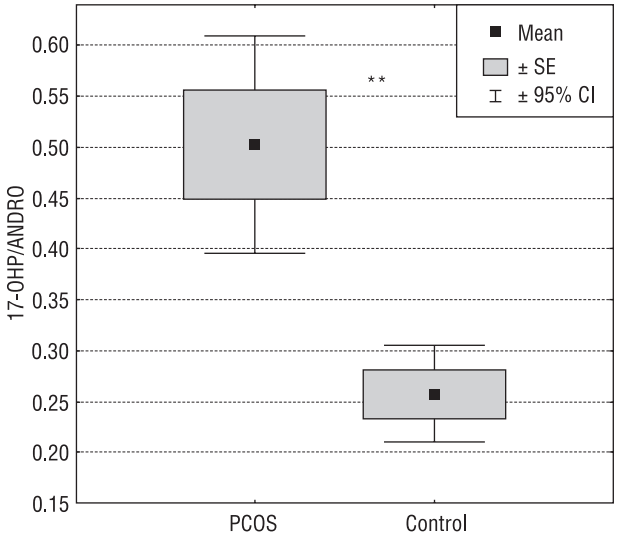

Figure 1. *17-hydroxyprogesterone [ng/mL] to androstendione [ng/mL] ratio in women with PCOS and controls*

Rycina 1. *Wskaźnik stosunku stężeń 17-OH-progesteronu [ng/ml] do androstendionu [ng/ml] w u kobiet z PCOS i w grupie kontrolnej*

Table III. *Glucose, insulin and insulin resistance indices during oral glucose tolerance test (OGTT) in women with PCOS and controls (CONTR)*

Tabela III. *Glikemia, insulinemia w teście OGTT oraz wskaźniki insulinooporności u kobiet z PCOS i w grupie kontrolnej (CONTR)*

	Group	n	Mean	Median	SD	Min	Max	*p value*
Glucose_0′ [mg/dL]	PCOS CONTR	151 34	80.0 80.8	81.0 81.5	9.4 6.9	80.0 68.0	99.00 96.00	*0.77*
Glucose_60′ [mg/dL]	PCOS CONTR	145 34	112.6 112.6	109.0 113.0	36.1 31.0	140.0 93.0	242.0 213.0	*0.93*
Glucose_120′ [mg/dL]	PCOS CONTR	145 34	97.1 93.8	95.0 94.0	29.7 23.7	110.0 48.0	261.0 159.0	*0.65*
Insulin_0′ [mIU/mL]	PCOS CONTR	144 34	8.95 7.34	6.97 5.51	6.35 6.54	0.38 2.00	41.84 31.92	***0.043***
Insulin_60′ [mIU/mL]	PCOS CONTR	143 34	67.01 51.74	44.34 35.80	56.74 40.50	4.74 16.4	307.50 192.00	*0.18*
Insulin_120′ [mIU/mL]	PCOS CONTR	143 34	49.81 44.29	35.43 31.09	47.86 51.01	6.10 13.10	360.00 300.0	*0.61*
IRI	PCOS CONTR	132 34	0.93 0.78	0.84 0.69	0.38 0.34	0.15 0.33	1.88 1.68	***0.045***
HOMA [mmol/L × μIU/mL]	PCOS CONTR	144 34	1.81 1.48	1.41 1.16	1.36 1.32	0.37 0.36	8.37 5.99	*0.060*

Table IV. *Descriptive statistics for LH and FSH concentrations before and after GnRH stimulation in women with PCOS and controls*

Tabela IV. *Statystyki opisowe parametrów LH i FSH w teście z GnRH u pacjentek z PCOS i w grupie kontrolnej*

	Group	n	Mean	Median	SD	Min	Max	*p value*
LH_0′ [IU/L]	PCOS CONTR	151 34	9.04 4.83	7.80 4.57	5.81 1.68	0.46 1.08	34.00 922	***< 0.00001***
LH_30′ [IU/L]	PCOS CONTR	121 32	35.38 16.30	24.00 16.23	31.32 6.68	5.92 5.34	200.00 32.59	***< 0.00001***
LH_60′ [IU/L]	PCOS CONTR	120 32	33.86 13.45	21.67 12.79	31.78 5.22	4.87 5.01	200.00 28.51	***< 0.00001***
FSH_0′ [IU/L]	PCOS CONTR	148 32	5.80 6.44	5.45 6.01	2.48 2.02	1.14 2.15	24.49 12.11	***0.047***
FSH_30′ [IU/L]	PCOS CONTR	121 32	8.90 8.84	7.83 8.11	4.84 2.73	3.24 3.61	42.58 15.28	*0.312*
FSH_60′ [IU/L]	PCOS CONTR	120 32	9.95 9.04	8.43 8.61	8.21 3.00	3.58 4.05	73.24 17.36	*0.723*

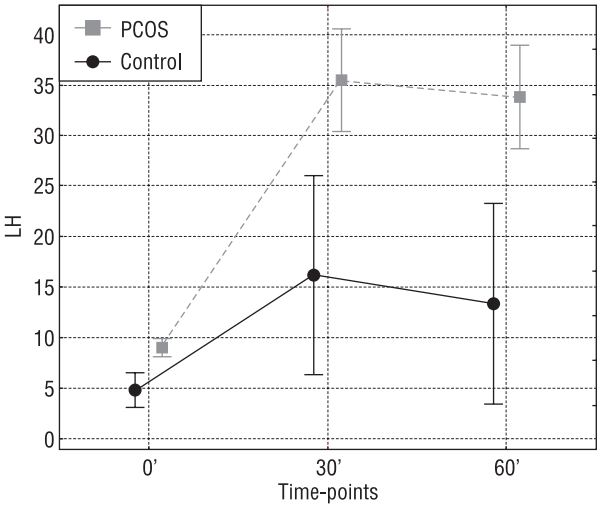

Figure 2. *Mean LH concentrations [IU/L] during GnRH test in women with PCOS and in regularly menstruating controls. Vertical bars represent 95% confidence intervals in the respective time-points*

Rycina 2. *Schematyczne przedstawienie rozkładów średnich wartości LH [jm./L] w teście z GnRH — w grupie z PCOS i w grupie kontrolnej. Pionowe słupki przedstawiają 95-procentowe przedziały ufności dla średnich wartości w odpowiednich punktach czasowych*

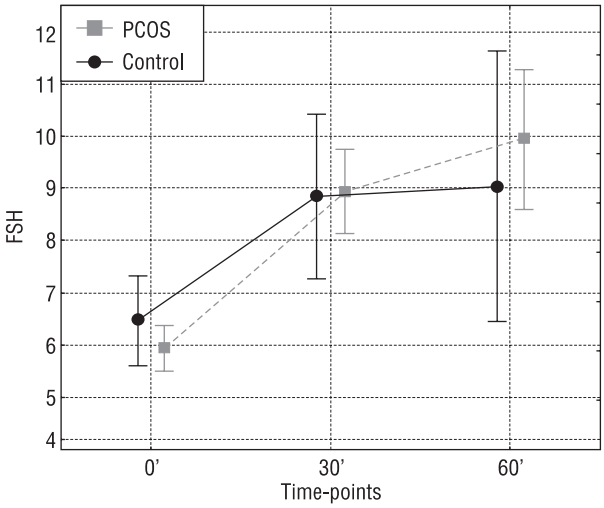

Figure 3. *Mean FSH concentrations [IU/L] during GnRH test in women with PCOS and in regularly menstruating controls. Vertical bars represent 95% confidence intervals in the respective time-points*

Rycina 3. *Schematyczne przedstawienie rozkładów średnich wartości poziomów FSH [jm./l] w teście z GnRH — w grupie z PCOS i w grupie kontrolnej. Pionowe słupki przedstawiają 95-procentowe przedziały ufności dla średnich wartości w odpowiednich punktach czasowych*

Results of sensitivity and specificity analysis for LH/FSH ratio are presented in Table VI. Receiver operated characteristics analysis (ROC curves) was employed in order to define the best discriminatory cut-off point for GnRH-stimulated LH/FSH ratio. This revealed that $LH_{30\,min}/FSH_{30\,min} > 2.11$, or $LH_{60\,min}/FSH_{60\,min} > 1.72$, had 78.3% sensitivity and 87.5% specificity (for $LH_{30\,min}/FSH_{30\,min}$) as well as 81.7% sensitivity and 87.5% specificity ($LH_{60\,min}/FSH_{60\,min}$) for diagnosis of PCOS (Fig. 5A and B). In contrast, baseline LH/FSH ratio above 2.0, although highly specific, had a very low sensitivity for the diagnosis of PCOS, i.e. only 23% (Table VI). Interestingly, ROC analysis for the baseline 17-hydroxy-progesterone to androstendione ratio demonstrated 68.4% sensitivity and 64.3% specificity for the diagnosis of PCOS for 17-hydroxy-progesterone [ng/mL]/androstendione [ng/mL] ratio > 0.244.

Table V. *Descriptive statistics for concentrations of LH and FSH in relation to their respective baseline values after GnRH stimulation and for the baseline LH/FSH ratio as well as LH/FSH ratio after GnRH stimulation in women with PCOS and controls (CONTR)*

Tabela V. *Statystyki opisowe stężeń LH i FSH w stosunku do wartości wyjściowych po stymulacji przez GnRH, oraz stosunku LH do FSH w teście z GnRH u pacjentek z PCOS i z grupy kontrolnej (CONTR)*

	Group	n	Mean	Median	SD	Min	Max	*p value*
LH_30'/0'	PCOS CONTR	121 32	3.99 3.89	2.13 3.30	7.83 2.88	1.08 1.59	12.99 17.56	*0.264*
LH_60'/0'	PCOS CONTR	120 32	3.76 3.28	3.06 2.78	2.00 2.59	1.10 0.94	11.89 15.91	*0.053*
FSH_30'/0'	PCOS CONTR	121 32	1.52 1.38	1.41 1.33	0.53 0.21	0.81 1.08	5.80 1.98	*0.211*
FSH_60'/0'	PCOS CONTR	120 32	1.68 1.42	1.47 1.39	1.14 0.25	0.86 0.96	12.96 2.13	*0.061*
LH/FSH_0'	PCOS CONTR	121 33	1.59 0.76	0.99 0.76	1.00 0.20	0.23 0.26	5.28 1.06	***< 0.00001***
LH/FSH_30'	PCOS CONTR	121 32	4.09 1.89	3.18 1.75	2.99 0.79	0.51 0.72	16.99 5.25	***< 0.00001***
LH/FSH_60'	PCOS CONTR	120 32	3.56 1.55	2.77 1.44	2.58 0.63	0.43 0.60	13.65 4.24	***< 0.00001***

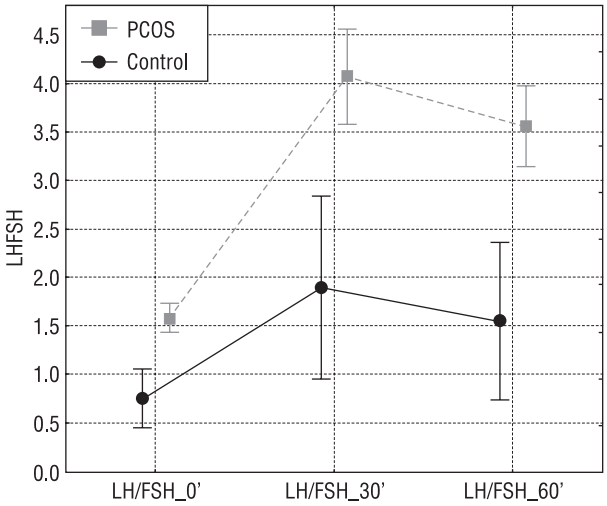

Figure 4. *Mean values of LH/FSH ratio during GnRH test in women with PCOS and in regularly menstruating controls. Vertical bars represent 95% confidence intervals in the respective time-points*

Rycina 4. *Schematyczne przedstawienie rozkładów średnich wartości stosunków LH/FSH w teście z GnRH — w grupie z PCOS i w grupie kontrolnej. Pionowe słupki przedstawiają 95-procentowe przedziały ufności dla średnich wartości w odpowiednich punktach czasowych*

Table VI. *Sensitivity and specificity of diagnosis of PCOS based on selected cut-off points of baseline and GnRH-stimulated LH to FSH ratio*

Tabela VI. *Czułość i swoistość rozpoznania PCOS na podstawie oceny stosunku wyjściowego LH do FSH w teście z GnRH*

LH/FSH ratio during GnRH test	Sensitivity (%)	Specificity (%)
LH/FSH_'0' > 2.0	23	100
LH/FSH_30' > 2.11	78.3	87.5
LH/FSH_60'> 1.72	81.7	81.3
17-OHP/androstendione > 0.244	68.4	64.3

Discussion

The results of our study suggest that assessment of pituitary LH and FSH reserve after GnRH stimulation might be used to confirm the diagnosis of PCOS in some patients. The study was performed on a large number of women with PCOS, while the number of controls (matched for age and BMI) was similar to other leading studies on gonadotrophin secretion and GnRH stimulation in women with PCOS [10, 19, 20].

It is well known that relative proportions of pituitary secretion of LH and FSH are determined by both frequency and amplitude of hypothalamic GnRH pulses [20, 21]. Very frequent GnRH pulses (i.e. every 8–30 minutes) result in preferential transcription of proteins for alpha subunit. Pulse frequency of about 30–60 minutes results in preferential transcription of LH beta subunit, while less frequent pulses (i.e. every 120–240 minutes) lead to predominant transcription of FSH beta subunit [21]. In the normal menstrual cycle, GnRH pulse

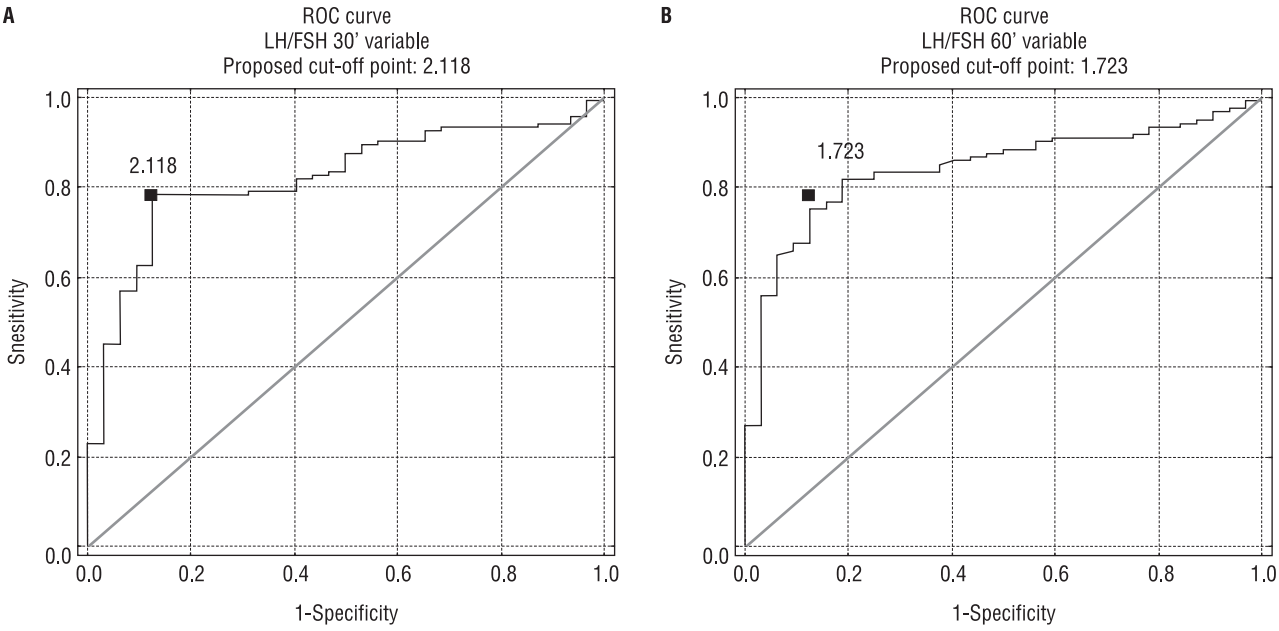

Figure 5. *Receiver operating characteristics (ROC) curve analysis for finding the cut-off for optimal diagnostic accuracy (considered as the highest proportion of correctly classified subjects) of LH/FSH ratio during GnRH test. The highest sensitivity (78.3% and 81.3%) is obtained for LH/FSH > 2.110 at 30 minutes of GnRH test (**A**) and for LH/FSH > 1.723 at 60 minutes of GnRH test (**B**). Respective sensitivity is 87.5% and 81.3%. **A.** ROC curve for assessment of cut-off point for maximal sensitivity for LH/FSH ratio at 30 minutes of GnRH test; **B.** ROC curve for assessment of cut-off point for maximal sensitivity for LH/FSH ratio at 60 minutes of GnRH test*

Rycina 5. *Analiza optymalnej czułości oceny stosunku stężeń LH do FSH w teście z GnRH dla optymalnej swoistości. Najwyższa czułość diagnostyczna (odpowiednio 78,3% i 81,7%) jest osiągana dla stosunku LH/FSH > 2,110 w 30. minucie testu (A) oraz dla stosunku LH/FSH > 1,723 w 60. minucie testu z GnRH (B). Odpowiednio swoistość wynosi 87,5 i 81,3%. **A.** Ocena punktu odcięcia dla maksymalnej czułości testu z GnRH dla stosunku LH/FSH w 30. minucie testu z GnRH (krzywa ROC); **B.** Ocena punktu odcięcia dla maksymalnej czułości testu z GnRH dla stosunku LH/FSH w 60. minucie testu z GnRH (krzywa ROC)*

frequency increases from about 90-100 minutes to about every 60 minutes through a follicular phase. Gradual increase in GnRH pulse frequency facilitates LH secretion culminating in an ovulatory LH surge [22, 23]. In contrast, increased progesterone secretion in a luteal phase results in an increase in FSH synthesis as a result of less frequent hypothalamic GnRH secretion (approximately one pulse every 3–5 hours), through mechanisms involving opioid receptors [24–27] and possibly other factors such as kisspeptin [28].

PCOS is characterised by an increase in both frequency and amplitude of hypothalamic GnRH pulses, generated at a frequency of about once every 60 minutes, i.e. the frequency observed only in the late follicular phase in healthy women [29, 30]. This is accompanied by resistance to inhibitory effects of progesterone on the frequency of hypothalamic GnRH secretion [31, 32]. Furthermore, a similar situation has been observed in hyperandrogenic adolescent girls even in the setting of regular menstrual cycles [33, 34].

As a result of the above described phenomena, PCOS is characterised by a relative overproduction of LH in relation to FSH. Hence, an increased LH to FSH ratio was once suggested to be used as an additional parameter confirming the diagnosis of polycystic ovary syndrome [6, 35]. There was, however, no agreement as to the optimal cut-off point for the baseline LH to FSH ratio, and various authors suggested various cut-off values (e.g. LH/FSH > 1 [36], LH/FSH > 2 [37] or even LH/FSH > 3 [38]). Meta-analysis of several studies demonstrated, however, very poor positive predictive value for baseline LH/FSH ratio i.e. only 18% [39], which effectively precluded the use of this index as a valid diagnostic tool. In our series, 74% of women with PCOS had higher baseline LH than FSH concentrations, but for the most frequently quoted cut-off point of LH/FSH > 2 [37] we obtained only a 23% sensitivity (and 43% sensitivity for LH/FSH > 1.5), despite high (100%) specificity for the diagnosis of PCOS.

In such circumstances, there is a question as to why the sensitivity of baseline LH/FSH ratio is so low despite evidence for increased LH synthesis in PCOS. As it turns out, women with PCOS, as well as more pronounced insulin resistance and hyperandrogenism, have relatively lower LH concentrations [20, 40–44], despite preserved increased GnRH pulse frequency in those who are more obese and insulin-resistant [42]. The same phenomenon was also observed in our study,

where there was a weak ($r = –0.21$), but still significant ($p < 0.05$) correlation between BMI and baseline LH concentrations as well as between LH and insulin resistance index ($r = –0.31$).

In our study, a relative increase of LH concentrations is proportional to (higher) baseline LH concentrations in women with PCOS, resulting in higher stimulated LH concentrations in this group, albeit without significant differences in either baseline or stimulated FSH levels. This is in accordance with results of other studies [20, 45]. Such a situation results in a more pronounced increase of GnRH-stimulated LH/FSH ratio in women with PCOS than in controls. This, in turn, leads to a dramatic (almost four-fold) increase in the diagnostic sensitivity of GnRH-stimulated LH/FSH ratio. For instance, results of ROC analysis reveal 81.7% sensitivity with 81.3% specificity for LH/FSH ratio above 1.72 post GnRH stimulation. This is much better than sensitivity of total testosterone assays (around 40% [46]) and is even higher than the sensitivity of all androgen analyses (58.1%) observed in our study. One must be also aware that assessment of androgen concentrations within the female range by the means of standard androgen assays is fraught with methodological problems, including very high coefficients of variation reaching as much as around 35% [47–49], while the more accurate mass spectrometry method [50] is still very rarely used in Poland. If such assay-dependent variability were employed e.g for the diagnosis of diabetes, than for fasting glucose concentration of 136 mg/dL, the obtained results would range from 88 mg/dL (entirely normal) to as much as 183 mg/dL (poorly controlled diabetes)!

According to the current consensus [4], diagnosis of PCOS requires exclusion of other causes of oligo-/amenorrhoea and hyperandrogenism (e.g. hyperprolactinaemia, hypercortisolaemia, late-onset congenital adrenal hyperplasia). In such a context, we note that in our study more than 40% of the women with PCOS according to the Rotterdam criteria [4] had androgens within the reference range i.e. on the basis of coexistence of oligo/amenorrhoea and polycystic ovaries, they would not be classified as having PCOS according to the recent Androgen Excess Society criteria [51].

We suggest that assessment of GnRH-stimulated LH/FSH ratio may be potentially useful, particularly in that group of women.

As we have already described, Barnes et al. [9] and Ehrmann et al. [10] have suggested that the GnRH test might be useful in the diagnosis of PCOS, but the issue of the optimal GnRH test protocol remains unresolved. In particular, there is a question whether GnRH test should be performed according to the protocol involving assessment of 17-hydroxy-progesterone/androstendione ratio around 24-hours post GnRH agonist stimulation, as described before [9, 10]. As it turns out, however, increased GnRH stimulated 17-hydroxy-progesterone/androstendione ratio is observed only in slightly less than 50% of women with PCOS [52]. That is also in accordance with earlier studies involving ovarian and adrenal vein catheterisation [5], where frank ovarian hyperandrogenism was observed in only around 50% (10/21) of women with PCOS. Interestingly this is slightly lower than the diagnostic utility of baseline 17-hydroxy-progesterone/androstendione ratio above 0.244 (ROC analysis) observed in our study, where we observed 68.4% sensitivity and 64.3% specificity for the diagnosis of PCOS.

In summary, our study demonstrated a marked increase in GnRH-stimulated LH/FSH ratio in women with PCOS diagnosed according to the Rotterdam criteria [4]. Although the results of our study need to be confirmed in women with hyperandrogenism, polycystic ovaries and regular menses, we suggest that assessment of GnRH stimulated LH/FSH ratio might be potentially useful as a confirmatory test for the diagnosis of PCOS in some patients, such as women with polycystic ovaries and oligo-/amenorrhoea, but with androgens within the reference range. In such cases, LH/FSH ratio above 2.11 at 30 minutes post GnRH stimulation, or above 1.72 at 60 minutes post GnRH stimulation, would support a diagnosis of PCOS.

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