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Endocrinological Markers for Assessing Hyperandrogenemia in Women Classified as Having Polycystic Ovary Syndrome (PCOS) according to the Revised 2003 Diagnostic Criteria

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Abstract
The aim of this study was to investigate whether free testosterone estimated by calculation from total testosterone and sex hormone-binding globulin or free androgen indexes were more appropriate markers for assessing hyperandrogenemia in patients with polycystic ovary syndrome (PCOS). 107 Caucasian women were presented at our Division of Gynecological Endocrinology and Reproductive Medicine because of their infertility and hirsutism. Hirsutism was quantitatively assessed using a modified Ferriman–Gallwey score; oligo-ovulation or/and anovulation were assessed; polycystic ovaries were assessed; afterwards women were classified as having PCOS according to the revised 2003 consensus on diagnostic criteria for PCOS or classified as controls; endocrinological parameters were assessed using commercial immunoassays or were calculated. 50 women were classified as having PCOS; 57 women were classified as controls because they did not fulfill the criteria of PCOS. Calculated free and bioavailable testosterone, FAI, total testosterone, free testosterone assessed by immunoassay and DHEAS were significantly increased in women classified as having PCOS. All endocrinological markers for assessing hyperandrogenemia were elevated in the PCOS group regardless if they were assessed using commercial immunoassays or were calculated. Calculated values showed no diagnostic advantage in this study.

Key words: anovulatory infertility, free testosterone, WHO group 2, PCOS, hyperandrogenemia

Introduction

In polycystic ovarian syndrome (PCOS), characterized by chronic anovulation, hyperandrogenemia is the most common cause of normogonadotropic normoestrogenic anovulatory infertility and certainly forms part of WHO group 2 [1, 2]. PCOS is a common and perplexing endocrine disorder in women in their reproductive lifespan, with a general prevalence of up to 10% in all women. However, polycystic ovarian morphology is seen twice as often on ultrasonography, in approximately 22% of all women [2]. PCOS is a syndrome of ovarian dysfunction that is frequently associated with the systemic condition of insulin resistance, with the cardinal features of hyperandrogenism and/or polycystic ovarian morphology [3]. The definition of PCOS has been a matter of controversy, and aspects of the pathophysiology and natural history of the condition remain unclear. Previously established diagnostic criteria were not universally accepted, as there were markedly divergent views of the etiology, pathogenesis, and clinical appearance [2]. The diagnostic criteria for PCOS were recently revised by an expert conference sponsored by the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine [4, 5]. The criteria are characterized by clinical and biochemical signs, as well as by ovarian morphology. PCOS is still a syndrome, and no single diagnostic criterion alone is therefore sufficient for clinical diagnosis [4, 5].

PCOS is diagnosed in the presence of two of three criteria (Table 1) [4, 5]. Polycystic ovaries (PCO) were considered in the consensus view to be one of the possible criteria for PCOS. It is recognized that women with regular cycles and hyperandrogenism may have part of the syndrome. These criteria again recognize that PCOS is also still a diagnosis based on the exclusion of other related disorders [2, 4, 5].

Table 1. Revised 2003 diagnostic criteria for polycystic ovary syndrome [4, 5]. Two out of three symptoms must be present.

1	Oligo-ovulation or/and anovulation
2	Clinical and/or biochemical signs of hyperandrogenism
3	Polycystic ovaries
•	Exclusion of other etiologies (congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome)

The presence of hirsutism was described as the primary clinical indicator of androgen excess, especially when standardized scoring methods are considered [4, 5]. Women with hirsutism should be evaluated endocrinologically, because of possible disorders related to androgen excess [6]. The majority of these women have an androgen excess, but it is unclear which androgen fraction reflects the clinical situation more accurately and correlates best with the symptoms of hyperandrogenemia [6, 7]. The limitations of defining

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androgen excess by measuring circulating androgen levels were considered to be due in part to the inaccuracy and variability of the laboratory measurement methods often used – for example, it was noted that measurement of total testosterone (TT) was not a very sensitive marker of androgen excess and that the direct assays for free testosterone (aFT) available also have limited value. Notwithstanding these limitations, it was considered that measuring free testosterone (FT) by equilibrium dialysis or calculating free testosterone (cFT) from TT and sex hormone–binding globulin (SHBG), or the free androgen index (FAI), were more sensitive methods of assessing hyperandrogenemia in women with possible PCOS [4, 5].

There is good evidence that FT and the hormone fraction nonspecifically bound to albumin in plasma, referred to as the bioavailable testosterone (BT) fraction, reflects the clinical situation more accurately than total hormone levels in plasma [7]. There has been growing interest in estimation of FT [8], and values measured by equilibrium dialysis [9, 10] or by centrifugal ultrafiltration assay [11, 12] are described as being the reference measurement procedures (RMPs) for measuring FT *in vivo*. However, these methods are time-consuming and complex manual procedures that are not routinely practicable in large laboratories, which rely increasingly on automated multiplex assay platforms [8]. Recently, two studies have shown a good correlation between calculated FT values and the values estimated by RMPs [7, 8].

The present study was conducted to investigate whether the biochemical markers of hyperandrogenism recommended by the revised 2003 consensus on the diagnostic criteria for PCOS [4, 5] – free testosterone (FT) calculated from total testosterone (TT) and sex hormone–binding globulin (SHBG) and the free androgen index (FAI) – were more sensitive markers for assessing hyperandrogenemia in women with PCOS.

Materials and Methods

Patient Population

During the study period, from January 2004 to January 2005, 107 Caucasian women were presented at our Division of Gynecological Endocrinology and Reproductive Medicine because of their infertility and hirsutism. The study was approved by the institutional review board at Erlangen University Hospital. All of the patients signed informed consent forms and completed a standard history form with an emphasis on menstrual dating and regularity, hirsutism and acne, gynecologic history, history of infertility and medication, and family history. All of the women underwent a complete physical examination, including weight and height measurement, followed by the usual calculation of the body mass index (BMI) and ultrasound examination of the ovaries.

Assessment of Hirsutism and Oligo-ovulation and/or Anovulation and polycystic Ovaries

The presence of terminal hair in primarily masculine areas was assessed during the first consultation in every patient by a single investigator. The mF-G score was used to describe the hirsutism pattern [13, 14]. The total mF-G score represents the sum of the scores for nine body areas. Only women with an mF-G score of 6 or above were classified as hirsute [13, 14]. The interval between bleeding episodes was assessed. Women with amenorrhea within the previous year were categorized as anovulatory without further testing, and a blood sample was taken immediately. In women with regular cycles of between 26 and 30 days, serum was obtained for hormonal analysis between days 3 and 5 of their menstrual cycle, and they were categorized as ovulatory. Women with cycles longer than 30 days, in addition to having an initial blood sample taken, had their progesterone level measured on days 22 to 24 during the same menstrual cycle. If the progesterone level was 4 ng/mL or less, the women were considered to be anovulatory [6]. A ultrasound examination of the ovaries was performed and polycystic ovaries were diagnosed in accordance with the diagnostic criteria for PCOS [4, 5]. Women were classified as having PCOS only when they fulfilled the diagnostic criteria for PCOS (two out of three symptoms must be present). Women fulfilled not the diagnostic criteria for PCOS were classified as controls.

Exclusion of Related Disorders

Patients who had received hormonal therapy within 3 months of their initial visit were not included in the study. None of the women included in the study had the syndrome of hyperandrogenism, insulin resistance, and acanthosis nigricans (HAIR-AN), Cushing's syndrome, or nonclassical adrenal hyperplasia (NCAH) or an androgen-secreting neoplasm.

Biochemical Measurements

All assays were conducted in our routine diagnostic endocrinology laboratory using established commercial assays routinely monitored by participation in external quality-control programs. Blood samples were immediately assayed for hormonal parameters and albumin in our routine laboratory. Free testosterone was measured using the single-tube Coat-A-Count (^{125}I-labeled) Free Testosterone radioimmunoassay (Diagnostic Products Corp., Los Angeles, California, USA). The calibration range of the assay was 1.9–173 pmol/L, with an analytical sensitivity of 0.52 pmol/L. The CVs were 18.3, 8.5, and 8% at the levels of 4.1, 30.8, and 69.3 pmol/L. The cross-reaction with 5alpha-dihydrotestosterone was 0.041%.

The other hormone parameters were measured with chemiluminescent enzyme immunoassay (Immulite 2000, Diagnostic Products Corp., Los Angeles, California, USA). The calibration range of the total testosterone assay was 0.7–55 nmol/L, with an analytical sensitivity of 0.5 nmol/L. The cross-reaction with 5alpha-dihydrotestosterone was 2%. The calibration range of the DHEAS assay was 0.41–27 μmol/L, with an analytical sensitivity of 0.08 μmol/L. No cross-reactivity with other compounds was known. The calibration range of the SHBG assay was up to 180 nmol/L, with an analytical sensitivity of 0.02 nmol/L.

No cross-reactivity with other compounds was known. The calibration range of the Estradiol assay was 73–7342 pmol/L, with an analytical sensitivity of 55 pmol/L. The cross-reactivity with estradiol-17β valerate was 1.14%. The calibration range of the LH assay was up to 200 mIU/mL, with an analytical sensitivity of 0.05 mIU/mL. The cross-reactivity with human chorionic gonadotrophin (hCG) was 0.20%. The calibration range of the FSH assay was up to 170 mIU/mL, with an analytical sensitivity of 0.1 mIU/mL. The cross-reactivity with thyroid-stimulating hormone (TSH) was 0.01%. Inter- and intrarassay CV`s were always below 11% at mid-range concentrations.

Albumin was regularly measured using routine clinical chemistry methods (values are not shown).

Calculation of Free Testosterone (cFT) and Bioavailable Testosterone (cBT)

Calculations of cFT and cBT were performed using the formula available on the web site of the International Society for the Study of the Aging Male (ISSAM) (http://www.issam.ch/freetesto.htm), from total T and SHBG measured in the same sample from each woman. This method has been described in detail by Vermeulen et al. [7]. FAI was obtained as the quotient 100 TT / SHBG [15].

Statistical Analysis

Statistical analysis was carried out with an unpaired Student's t-test using the Statistical Package for the Social Sciences, version 10.1 for Windows (SPSS, Inc., Chicago, Illinois, USA). A p value of less than 0.05 was considered statistically significant.

Results

The study population consisted of 107 Caucasian women, who showed one or up to three diagnostic criteria for PCOS. Twenty eight women with amenorrhea within the previous year were categorized as anovulatory. Eighteen women with cycles longer than 30 days were categorized as anovulatory because of a progesterone level of less than 4 ng/mL on days 22 or 24 of their present cycle in accordance with Souter et al. [6]. This left forty six women classified as anovulatory. There were sixty two women suffering from hirsutism with an mF-G score of 6 or more as a clinical sign of hyperandrogenism. Furthermore forty nine women fulfilled the ultrasound criteria for polycystic ovaries.

The PCOS group consists of seventeen women because of their polycystic ovaries and their hirsutism; of eighteen women because of their polycystic ovaries and oligo and/or anovulation; and of fifteen women because of their hirsutism and oligo and/or anovulation. The control group consists of thirteen women with oligo and/or anovulation; thirty women with hirsutism; fourteen women fulfilling the ultrasound criteria for polycystic ovaries. But none of the women in the control group fulfilled two or more diagnostic criteria for PCOS.

All clinical parameters and endocrinological values for the 107 women included in the study are shown in Table 2. The general clinical parameters of the BMI, mF-G score, and age did not differ between the two groups of women. In the PCOS – group endocrinological values; in particular, total testosterone and free testosterone measured by direct immunoassays, calculated free and bioavailable testosterone, dehydroepiandrosterone sulfate and free androgen index were

Table 2. Endocrinologic values of all women; control - group and PCOS - group.

	Control – group (n=57)	PCOS – group (n = 50)	P value
Age (y)	32.79 ± 6.18	30.92 ± 6.21	0.12
BMI (kg/m^2)	25.47 ± 5.26	26.65 ± 7.13	0.32
mF-G score	9.25 ± 4.09	9.52 ± 3.74	0.72
TT (nmol/L)	1.94 ± 1.01	2.51 ± 1.05	0.005*
aFT (pmol/L)	6.69 ± 3.56	8.47 ± 4.44	0.02*
cFT (nmol/L)	0.027 ± 0.019	0.039 ± 0.023	0.002*
cBT (nmol/L)	0.67 ± 0.48	0.99 ± 0.58	0.002*
DHEAS (μmol/L)	5.75 ± 3.41	6.93 ± 2.88	0.007*
SHBG (nmol/L)	62.96 ± 38.03	53.63 ± 43.93	0.2
FAI	4.43 ± 3.88	7.19 ± 5.47	0.003*
E (pmol/L)	44.46 ± 33.37	47.17 ± 35.12	0.68
LH mIU/mL	6.82 ± 6.06	8.96 ± 10.34	0.18
FSH (mIU/mL)	11.02 ± 15.04	9.32 ± 17.80	0.59

All values are shown as means, standard deviation, and ranges; * considered significant. BMI, body mass index; mF-G, modified Ferriman-Gallwey (score); TT, total testosterone; aFT, assayed free testosterone; cFT, calculated free testosterone; cBT, calculated bioavailable testosterone; DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone-binding globulin; FAI, free androgen index; E, estradiol; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

significantly increased in comparison with these values of the women classified as controls.

Discussion

This study investigated endocrinological markers for the assessment of hyperandrogenemia in women classified as having PCOS in comparison to women not classified as having PCOS. Women were strictly classified as having PCOS according to the revised 2003 diagnostic criteria [4, 5]. TT, aFT, cFT, cBT, and FAI, as well as DHEAS, were significantly increased in the PCOS – group in comparison with women of the control – group. There seems to be no diagnostic advantage for the calculated values of FT and FAI concerning assessment of hyperandrogenemia in PCOS.

The definition of PCOS has been a matter of controversy. The previously established diagnostic criteria were not universally accepted, as there were markedly disparate views of the etiology, pathogenesis, and clinical appearance of the condition [2]. The role of polycystic ovaries in the diagnosis of PCOS was also debated skeptically. The recently revised definition of PCOS characterizes clinical and biochemical signs as well as ovarian morphology. PCOS is diagnosed in the presence of two of three possible criteria (Table 1) [4, 5]. However the term PCOS describes changes in the ovaries, a diagnosis of PCOS is reasonable without the need for an ultrasound diagnosis of polycystic morphology in the ovaries, provided that the other two criteria are present. Further debate over the diagnostic criteria is therefore inevitable. There may be misinterpretation and underestimation or overestimation of the symptoms, resulting in misleading and simplified use of the term PCOS [16]. Divergent views regarding ovarian ultrasonography are indicative of the controversy over the inclusion of ultrasound findings in the diagnostic criteria for PCOS. Ovarian ultrasonography has significant intraobserver and interobserver variability in the diagnosis of PCOS [17]. In addition, women may receive different diagnoses or treatments depending on the clinical symptoms and on the type of specialist consulted [18].

Despite the debate over the definition of the diagnostic criteria, there is general consensus regarding the limitations of methods of assessing hyperandrogenemia in women with possible PCOS [4, 5, 7, 19, 20]. FT and FAI were considered to be more sensitive methods of assessing hyperandrogenemia in comparison with TT or aFT estimated by direct immunoassays [4, 5]. BT in particular appeared to be intellectually appealing [21]. However, some immunoassays showed a good correlation with the reference measurement procedure (RMP) reported by Van Uytfanghe et al., which measures only a fraction of the FT concentrations [22]. In general, there is discrepancy between TT levels and the clinical situation in women [23]. Vanky et al. found no differences when measuring total testosterone in PCOS patients with regular menstruations, on the one hand, and oligomenorrheic PCOS patients on the other [24]. The RMP for FT is equilibrium dialysis [9,10] or centrifugal ultrafiltration assay [11, 12] for the measurement of FT *in vivo*. However, these are complex and time-consuming procedures that are not widely available. Recently, two studies showed a good correlation between calculable FT values and the RMP, which is much easier to perform [7, 8].

Azziz has proposed a new set of criteria for the diagnosis of PCOS, taking into consideration the high prevalence of polycystic ovaries observed in the disorder, which also includes functional and/or morphological disturbances of the ovaries [25].

The results of the present study show that all endocrinological markers for assessing hyperandrogenemia in women classified as having PCOS are significantly increased. TT, aFT, cFT, cBT, FAI, and also DHEAS were significantly increased in women classified as having PCOS according to the revised 2003 consensus on the diagnostic criteria. This study has limitations and there may be a diagnostic advantage of calculated values in greater study populations. Furthermore due to the definition of PCOS according to the above mentioned criteria, women diagnosed as PCOS reveal a very heterogenic population. Calculable values may be helpful in the clinical diagnosis of PCOS but showing no significant diagnostic advantage in this study [26]. However, calculation of free testosterone (cFT), bioavailable testosterone (cBT), and the free androgen index (FAI) are easy to obtain and should be incorporated into the diagnostic work-up of women with possible hyperandrogenism.

References

1. Laven JS, Imani B, Eijkemans JC, de Jong FH, Fauser BC (2001) Absent biologically relevant associations between serum inhibin B concentrations and characteristics of polycystic ovary syndrome in normogonadotrophic anovulatory infertility. Hum Reprod 16: 1359-64
2. Hart R, Hickey M, Franks S (2004) Definitions, prevalence and symptoms of polycystic ovaries and polycystic ovary syndrome. Best Pract Res Clin Obstet Gynecol 18: 671-83
3. Laven JS, Imani B, Eijkemans MJ, Fauser BC (2002) New approach to polycystic ovary syndrome and other forms of anovulatory infertility. Obstet Gynecol Surv 57: 755-67
4. Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group (2004) Revised 2003 consensus on diagnostic criteria and long term health risk related to polycystic ovary syndrome (PCOS). Hum Reprod 19: 1-7
5. Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group (2004) Revised 2003 consensus on diagnostic criteria and long term health risk related to polycystic ovary syndrome (PCOS). Fertil Steril 81: 19-25
6. Souter I, Sanchez LA, Perez M, Bartolucci AA, Azziz R (2004) The prevalence of androgen excess among patients with minimal unwanted hair growth. Am J Obstet Gynecol 191: 759-67
7. Vermeulen A, Verdonck L, Kaufmann JM (1999) A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 84: 3666-72
8. Ly PL, Handelsman DJ (2005) Empirical estimation of free testosterone from testosterone and sex hormone-binding globulin immunoassays. Eur J Endocrinol 152: 471-8
9. Vermeulen A, Stoica T, Verdonck L (1971) The apparent free testosterone concentration, an index of androgenicity. J Clin Endocrinol Metab 33: 757-67
10. McCann DS, Kirkish LS (1985) Evaluation of free testosterone in serum. J Clin Immunoass 8: 234-6

11. Hammond GL, Nisker JA, Jones LA, Siiteri PK (1980) Estimation of the percentage of free steroid in undiluted serum by centrifugal ultrafiltration-dialysis. J Biol Chem 255: 5023-6
12. Vlahos I, Macmahon W, Sgoutas D, Bowers W, Thompson J, Trawick W (1982) An improved ultrafiltration method for determining free testosterone in serum. Clin Chem 28: 2286-91
13. Ferriman D, Gallwey JD (1961) Clinical assessment of body hair growth in women. J Clin Endocrinol Metab 21: 1440-7
14. Archer JS, Chang RJ (2004) Hirsutism and acne in polycystic ovary syndrome. Best Pract Res Clin Obstet Gynecol 18: 737-54
15. Mathur RS, Moody LO, Landgrebbe S, Williamson HO (1981) Plasma androgens and sex hormone binding globulin in the evaluation of hirsute patients. Fertil Steril 35: 29-37
16. Geisthovel F (2003) A comment on the European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine consensus of the polycystic ovarian syndrome. Reprod Biomed Online 7: 602-5
17. Amer SA, Li TC, Bygrave C, Sprigg A, Saravelos H, Cooke ID (2002) An evaluation of the inter-observer and intra-observer variability of ultrasound diagnosis of polycystic ovaries. Hum Reprod 17: 760-4
18. Cussons A, Stuckey BGA, Walsh JP, Burke V, Norman RJ (2005) Polycystic ovarian syndrome: marked differences between endocrinologists and gynecologists in diagnosis and management. Clin Endocrinol 62: 289-95
19. Rosner W (1997) Errors in the measurement of plasma free testosterone. J Clin Endocrinol Metab 82: 2014-5
20. Boots LR, Potter S, Potter HD, Azziz R (1998) Measurement of total serum testosterone levels using commercially available kits: high degree of between-kit variability. Fertil Steril 69: 286-92
21. Legros RS (2003) Diagnostic criteria in polycystic ovary syndrome. Semin Reprod Med 21: 267-75
22. Van Uytfanghe K, Stöckle D, Kaufman JM, Fiers T, De Leenheer A, Thienpont LM (2005) Validation of 5 routine assays for serum free testosterone with a candidate reference measurement procedure based on ultrafiltration and isotope dilution-gas chromatography-mass spectrometry. Clin Biochem 38: 253-61
23. Azziz R, Sanchez LA, Knochenhauer ES, Moran C, Lazenby J, Stephens KC, Taylor K, Boots LR (2004) Androgen excess in women: experience with over 1000 consecutive patients. J Clin Endocrinol Metab 89: 453-62
24. Vanky E, Kjotrod S, Salvesen KA, Romundstad P, Moen MH, Carlsen SM (2004) Clinical, biochemical and ultrasonographic characteristics of Scandinavian women with PCOS. Acta Obstet Gynecol Scand 83: 482-6
25. Azziz R (2005). Diagnostic criteria for polycystic ovary syndrome: a reappraisal. Fertil Steril 83: 1343-6
26. Mueller A, Dittrich A, Cupisti S, Beckmann MW, Binder H (2006) Is it necessary to measure free testosterone to assess hyperandrogenemia in women? The role of calculated free and bioavailable testosterone. Exp Clin Endocrinol Diabetes 114: 182-7

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