Endocrine characteristics, body mass index, and metabolic syndrome in women with polycystic ovary syndrome

Jian Li[1], Qi Wu[2], Chi Chiu Wang[2,3,4], Rui Wang[5], Ernest H. Y. Ng[6], Jian-Ping Liu[7], Ben Willem. J. Mol[8], Xiao-Ke Wu[1]*, Wentao Li[9], PCOSAct Study Group[10]

[1]Department of Obstetrics and Gynecology, First Affiliated Hospital, Heilongjiang University of Chinese Medicine, Harbin, China, e-mail: liamjiam@gmail.com

[2]Department of Obstetrics and Gynaecology, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong, e-mail: wuqidonice@link.cuhk.edu.hk

[3]Reproduction and Development Laboratory, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong, e-mail: ccwang@cuhk.edu.hk.

[4]School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, e-mail: ccwang@cuhk.edu.hk.

[5]Robinson Research Institute and Adelaide Medical School, University of Adelaide, North Adelaide, Australia, e-mail: r.wang@adelaide.edu.au.

[6]Department of Obstetrics and Gynecology, The University of Hong Kong, Hong Kong, e-mail: nghye@hku.hk.

[7]Centre for Evidence-Based Chinese Medicine, Beijing University of Chinese Medicine, Beijing, China, e-mail: liujp@bucm.edu.cn

[8]Department of Obstetrics and Gynaecology, Monash University, Monash Medical Centre, 246 Clayton Road, Clayton, Victoria 3168, Australia, e-mail: ben.mol@monash.edu.

[9]Department of Obstetrics and Gynaecology, Monash University, Monash Medical Centre, 246 Clayton Road, Clayton, Victoria 3168, Australia, e-mail: wentao.li@monash.edu.

[10]PCOSact Study group: Hong-Ying Kuang, Hong-Li Ma, Jing-Shu Gao, Liang-Zhen Xie, Li-Hui Hou, Zhen-Xing Hu, Xiao-Guang Shao, Jun Ge, Jin-Feng Zhang, Hui-Ying Xue, Xiao-Feng Xu, Rui-Ning Liang, Hong-Xia Ma, Hong-Wei Yang, Dong-Mei Huang, Yun Sun, Cui-Fang Hao, Shao-Min Du, Cai-Fei Ding, Ya-Qin Gao, Tai-Xiang Wu, Heping Zhang, Elisabet Stener-Victorin, Richard S. Legro.

Short title: Overweight, but not lean women with PCOS bear a significantly higher risk of MS

Keywords: polycystic ovary syndrome (PCOS), metabolic syndrome (MS), body mass index (BMI), free androgen index (FAI), sex hormone-binding globulin (SHBG), anti-mullerian hormone (AMH)

Word count: 2519

Number of tables: 2

Number of figures: 2

Conflict of interest

BWM is supported by a NHMRC Practitioner Fellowship (GNT1082548). BWM reports consultancy for ObsEva, Merck KGaA and Guerbet. All authors declare no financial relationships with any organisations that might have an interest in the submitted work in the previous three years, and no other relationships or activities that could appear to have influenced the submitted work.

Funding support

National Public Welfare Projects for Chinese Medicine (201507001, 201107005), the National Key Discipline of Chinese Medicine in Gynecology during the year of 2009–2016 to the First Affiliated Hospital, Heilongjiang University of Chinese Medicine. Health and Medical Research Fund (06171026) from Food and Health Bureau, The Government of the Hong Kong Special Administrative Region to the Chinese University of Hong Kong. The Australian National Health and Medical Research Council (NHMRC) funded Centre for Research Excellence in Polycystic Ovary Syndrome (APP1078444).

Corresponding authors and reprint request to:

Xiao-Ke Wu, PhD

Department of Obstetrics and Gynecology,

First Affiliated Hospital, Heilongjiang University of Chinese Medicine,

26 Heping Road, XiangFang

Harbin, 150040, Heilongjiang, China.

Phone: +86 137 9603 6734

Abstract

Context: Polycystic ovary syndrome (PCOS) is reported to be associated with an increased risk of metabolic syndrome (MS). However, it is unknown if that association is linked with the individual clinical characteristics of PCOS, or that it confounded by body mass index (BMI).

Objective: We aimed to evaluate the associations of endocrine and ultrasound characteristics with MS in women with PCOS. We hypothesized that these associations are modified by BMI.

Design and setting: Secondary analysis of baseline data from a randomized controlled trial of PCOS for ovulation induction.

Participants: Oligo-anovulatory Chinese women with PCOS according to Rotterdam 2003 criteria complicated with or without MS according to Chinese Diabetes Society criteria.

Main Outcome Measure: Association of MS with baseline clinical and biochemical measurements.

Results: Among 947 women with PCOS, 153 (16.2%) were diagnosed with MS. The prevalence of MS in women with normal (<24) and increased (≥24) BMI was 3.6% and 30.5%, respectively. In all women, high free androgen index (FAI≥5%) was positively associated with MS (OR 2.06, 95%CI 1.11-3.82). High FAI was positively associated with MS among women with increased BMI (OR 3.37, 95%CI 1.78-6.37), but the association was not significant in women with normal BMI (OR 1.27, 95%CI 0.34-4.70). The presence of polycystic ovary (PCO) morphology was negatively associated with MS (OR 0.52, 95%CI 0.26-1.03) in all women (normal BMI OR 0.42, 95%CI 0.11-1.67; increased BMI OR 0.54, 95%CI 0.23-1.28, respectively). Luteinizing hormone (LH), Sex hormone binding globulin (SHBG), and anti-mullerian hormone (AMH) were negatively associated with MS. The associations of FAI, SHBG, and AMH in relation to MS were significantly modified by BMI.

Conclusions: The prevalence of MS is low in lean women with PCOS. In women with PCOS, FAI is positively associated with MS, while PCO morphology is negatively associated. The

77 presumed cardiovasculair risk in women with PCOS is mediated by BMI, and does not hold for
78 lean women.

Introduction

Polycystic ovary syndrome (PCOS), characterized by anovulation, infertility, and androgen excess, is the most common endocrine disorder in women of reproductive age[1]. These manifestations typically provide the impetus to seek medical evaluation. However, metabolic comorbidities are commonly found in women with PCOS and could be more important determinants of overall and long-term health[2-4].

Metabolic syndrome (MS) is a clustering of disorders including abdominal obesity, high blood pressure, high blood glucose, high serum triglycerides, and low high-density lipoprotein (HDL) levels, which is associated with the risk of developing cardiovascular disease and type 2 diabetes. In general, women with PCOS have a higher chance to develop MS (odds ratio 2.2) compared to women without PCOS[5-7]. However, PCOS is a heterogeneous disorder in terms of its link with metabolic disorder. The association is much stronger in the classic PCOS phenotype that presents hyperandrogenism and oligo-anovulation than in the ovulatory or non-hyperandrogenic phenotype[8-10]. On the basis of traditional phenotyping, personalized diagnosis according to clinical characteristics would capacitate more accurate foreseeing of the long-term health consequences of PCOS, thereby facilitating the peace of mind of women with PCOS and individualized treatment strategy. There is limited evidence indicating that characteristics including age, acanthosis, and free androgen index (FAI) are positively associated with MS, while sex hormone-binding globulin (SHBG) is inversely associated with the risk of MS in women with PCOS[11]. However, the application of these findings is hampered by the small sample size of the study, likely confounding, and various diagnostic criteria of PCOS used.

Overweight and obesity are the most important factors responsible for the metabolic heterogeneity of PCOS[1]. While an excess of androgen secretion is a prerequisite for developing PCOS, obesity is suggested to be an effect magnifier for hyperandrogenism, allowing obese

104 women with mild hyperandrogenism to develop PCOS[1,12]. In the general population, obese
105 adolescent and adult women are 8 to 17 times more likely to be identified as MS than their lean
106 counterparts[12,13]. Obesity was found to be associated with MS in adolescents with PCOS but not
107 in adolescents without PCOS [14]. Moreover, increased prevalence of MS was found in overweight
108 or obese women with PCOS (OR 1.88, 95% 1.16, 3.04) but not in lean women (OR 1.45, 95% CI
109 0.35, 6.12)[15]. Thus, it is reasonable to hypothesize that BMI is an effect modifier for the
110 associations between clinical characteristics and MS in women with PCOS.

111 In this study, we utilized the baseline data of a large-scale, multicenter, randomized control trial
112 of PCOS to determine the associations of endocrine and ultrasound characteristics of PCOS with
113 MS and examine interactions between BMI and clinical characteristics.

Materials and Methods

Participants

This is a secondary analysis of the baseline characteristics of Chinese women with PCOS participated in the PolyCystic Ovary Syndrome Acupuncture plus Clomiphene Trial (PCOSAct)[16]. PCOSAct was a large-sample, multi-center, randomized controlled trial of ovulation induction in women with PCOS conducted between 2012 and 2015 in mainland China. The trial was registered in ClinicalTrial.gov (NCT01573858) and chictr.org.cn (ChiCTR-TRC-12002081). The study protocol had been described elsewhere[17], and the main results were published in details[16]. In this trial, participants were women diagnosed with PCOS according to the modified Rotterdam criteria[18], who all had oligo-or anovulation (OA) with either clinical/biochemical hyperandrogenism (HA) or polycystic ovary (PCO) morphology, but no HA and PCO without OA (ovulatory PCOS). Exclusion criteria were other endocrine disorders, use of hormonal or other medication including Chinese herbal prescriptions in the past 2 months, miscarriage or given birth within 6 weeks, and breastfeeding within the last 6 months.

Demographic and clinical data

Participating women underwent an interview at baseline to obtain information on socio-demographics, health history, reproductive history, and menstruation. Oligomenorrhea was defined as an intermenstrual interval >35 days and <8 menstrual bleedings in a year, and amenorrhea was defined as an intermenstrual interval >90 days[19]. Participants also underwent a complete physical examination at baseline, including weight and height measurement, waist circumference, systolic blood pressure (SBP), diastolic blood pressure (DBP), skin and hair condition, and transvaginal ultrasound examination of the ovaries. We computed body mass index (BMI). Hirsutism was scored in accordance with the modified Ferryman-Gallwey (mF-G

score[20]. Clinical HA was defined as mF-G score ≥5[21]. Acne was measured using a standard acne lesion assessment diagram and definition[22]. Acanthosis nigricans (AN) severity was evaluated according to the neck severity scale[23]. PCO morphology was diagnosed by transvaginal ultrasound when at least one ovary had a volume of >10cm^3 or there were 12 or more follicles measuring 2-9 mm in diameter[24].

Biochemical data

Baseline laboratory measurements were performed after an overnight fast. Sex steroids and gonadotropins, total testosterone (TT), free testosterone (FT), sex hormone binding globulin (SHBG), estradiol, progesterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), glucose, triglyceride, and high-density lipoprotein (HDL) were measured at the core laboratory in Heilongjiang University of Chinese Medicine. All sex hormones were analyzed by electro-chemiluminescent immunoassays, except for FT, which was measured by radioimmunoassay (RIA). Glucose and lipid profiles were measured by enzymatic methods. Anti-mullerian hormone (AMH) was measured by Ultra-Sensitive AMH ELISA assay (Wester, Tx, USA) at the laboratory of reproduction and development in the Chinese University of Hong Kong, Prince of Wales Hospital. Baseline FAI was calculated as a percentage ratio of total testosterone to SHBG values. Biochemical HA was defined as TT ≥1.67 nmol/L or AFI >5%[25].

Outcome

MS was diagnosed according to the most updated China Diabetes Society (CDS) criteria[26] with at least three of the following: 1) abdominal obesity defined as waist circumference ≥85 cm for women; 2) hyperglycemia defined as fasting peripheral glucose ≥6.1 mmol/L or plasma glucose at 2h after glucose load ≥7.8 mmol/L and/or diagnosed with diabetes and receiving treatment; 3) hypertension defined as blood pressure ≥130/85 mmHg and/or having been confirmed with

hypertension and receiving treatment; 4) fasting triglycerides ≥1.7 mmol/L; 5) fasting HDL-C <1.04 mmol/L.

Statistical analysis

Data were described as frequencies and percentages for categorical data; or median and interquartile range (IQR) for numerical data after examining normality. We first analyzed the associations between individual characteristics of PCOS (age, acne, acanthosis, menstrual cycles, duration between menstruation, oligo-amenorrhea, hirsutism, TT, FT, FAI, PCO morphology, LH, FSH, progesterone, estradiol, SHBG, and AMH) and MS with univariable logistic regression in all women. As there were missing values for TT (4.06%), FAI (5.07%), PCO morphology (4.77%), LH (4.26%), FSH (4.26%), progesterone (4.46%), estradiol (4.16%), SHBG (4.56%), and AMH (1.72%), we performed multiple imputation using chained equations (50 iterations) prior to multivariable analysis. We then performed multivariable logistic regression to calculate the odds ratio (OR) and 95% confidence interval (95% CIs) for the association between clinical and endocrine variables related to MS risk. Further, we performed subgroup analysis (low BMI<24 and high BMI≥24) by including significant variables in the previous multivariable regression. We used the medians of hormones as cut-offs in the subgroup analysis to highlight the difference in their associations with MS between the two subgroups. We then computed multiplicative interaction between variables included in the subgroup analysis and BMI. We also performed a sensitivity analysis by equally dividing participants according to quartiles of BMI (BMI<21.0, 21.0≤BMI≤23.7, 23.7≤BMI≤26.7, BMI≥26.7) in all women. Finally, receiver operating characteristic curve with both discrimination and calibration analyses was performed in both subgroups (low BMI<24 and high BMI≥24). Statistical significance for all analyses was defined as a two-tailed P value of less than 0.05. Data analysis was performed using Stata Version 13.0 (Stata Corp., College Station, TX).

Results

Out of the 1000 women recruited in PCOSAct, metabolic status was available in 947 women with PCOS, of whom 153 (16.2%) were diagnosed with MS according to the criteria of CDS. Compared with oligomenorrhea women with PCO morphology alone, those who had clinical or biochemical HA with (OR 2.39, 95%CI 1.61-3.55) or without (OR 2.58, 95%CI 1.28-5.20) PCO morphology had elevated risk of MS.

In all women, amongst all endocrine characteristics, high FAI (≥5%) was positively associated with MS (OR 2.06, 95% CI 1.11-3.82), while LH, SHBG, and AMH were negatively associated with MS (OR 0.93, 95% CI 0.89-0.98; OR 0.98, 95% CI 0.96-0.99; OR 0.95, 95% CI 0.92-0.99, respectively). (Table 1) The association between PCO morphology and MS was borderline significant (OR 0.52, 95%CI 0.26-1.03). Clinical HA, TT, FT, FSH, estradiol, and progesterone were not significantly associated with MS.

In subgroup analysis, the prevalence rates of MS in women with low (<24) and high (≧24) BMI were 3.56% and 30.54%, respectively. In women with high BMI, high FAI (≥5%) was positively associated with MS (OR 3.37, 95% CI 1.78-6.37), but PCO morphology was not significantly associated with MS (OR 0.54, 95% CI 0.23-1.28). In women with low BMI, neither FAI (OR 1.27, 95% CI 0.34-4.70) nor PCO morphology (OR 0.42, 95% CI 0.11-1.67) was significantly associated with MS. In women with low BMI, low median SHBG (≤33.90pmol/L) was positively associated with MS (OR 3.98, 95%CI 1.06-14.91), but the OR inverted to 0.61 (95% CI 0.32-1.14) in women with high BMI. Low AMH (≤11.42 ng/ml) was associated with MS (OR 1.60, 95% CI 1.03-2.48) in women with high BMI and the association was likely stronger in women with low BMI (OR 2.15, 95% CI 0.74-6.29). (Table 2) The p-values for the interaction of BMI in relation to FAI, PCO morphology, LH, SHBG, and AMH were 0.014, 0.537, 0.081, 0.005, and 0.005,

208 respectively. We also observed the pattern of effect modification of BMI in the sensitivity analysis.
209 (Figure 1)

210 ROC analysis incorporating age, FAI, PCO morphology, LH, SHBG, and AMH presented good
211 discrimination (AUC 0.84, 0.75-0.93) and calibration in women with low BMI. In women with high
212 BMI, the discrimination (AUC 0.63, 0.58-0.69) and calibration of ROC were poor. (Figure 2)

Discussion

In this study, we demonstrated that in women with PCOS, the prevalence of MS is strongly dependent on BMI, with MS being present in 3.6% in normal weight women and 30.5% in overweight women. PCO morphology was negatively associated with MS, but the association was not statistically significant in either normal weight or overweight women. High FAI was positively associated with MS only in women with high BMI, but not in women with low BMI. SHBG was positively associated with MS in women with BMI <24 but negatively associated with MS in women with increased BMI. The associations of FAI, AMH, and SHBG in relation to MS were significantly modified by BMI.

It had been documented that there was an inverse association of SHBG with MS incidence in women with and without PCOS[27,28], but not the FAI[29]. In Ko's study, the MS was defined according to International Diabetes Federation criteria and the sample size was small (n=25), which had a high chance to incur inaccurate estimations due to bias. Here, we comprehensively evaluated the association of clinical and endocrine characteristics of PCOS with MS using high-quality data of a large randomized controlled trial. To further minimize bias and increase statistical power, we dealt with the missing data by employing multiple imputation prior to multivariable analysis. We not only found that SHBG and FAI were associated with MS in women with PCOS, but also discovered that BMI was an effect modifier for the associations between FAI, AMH and SHBG and MS in women with PCOS. Nevertheless, the present study still has several limitations. First, the risk of MS for ovulatory women with HA and PCO was not available to evaluate. Second, this was a cross-sectional study, and thus the findings warrant to be validated in future cohort studies. Finally, we could not evaluate the effect modification of BMI in women without PCOS.

Clinical HA, namely hirsutism, ranges from 6.1% to 10% in women with PCOS in China, yet the prevalence of biochemical HA could be as high as 21.1%[21,30]. Biochemical HA was found to be independently associated with the risk for MS (OR 2.1) and obesity (OR 1.7) among women with PCOS[31,32]. For clinical HA, there have been inconsistent findings for the association of MS and hirsutism which was defined as mFG score of $\geqslant$8[33,34]. In this study, instead of clinical HA, we found that biochemical HA defined according to FAI$\geqslant$5%, was positively associated with MS. Apart from HA, the MS development in women with PCOS depends on several factors such as high BMI. Obesity is an independent risk factor for many diseases including MS, diabetes mellitus, and cardiovascular diseases. Our study demonstrated that overweight PCOS women with high FAI bear a significantly higher risk of MS, but the risk in lean women with PCOS is low. Apart from medication, the management of both PCOS and MS includes lifestyle changes, especially for weight optimization which has multiple clinical and personal benefits. Weight loss in overweight subjects could reduce the risk of suffering diabetes and death from cardiovascular causes by 28% [36]and 21%[35], respectively. At present, clinicians often only focus on infertility in women with PCOS, resulting in an underestimation of the risk of MS and other long-term diseases. In general, women with PCOS have an 11-fold increase in the prevalence of MS compared with their age-matched controls[37]. Although MS screening and precautionary measures are required for women with PCOS, given the low prevalence of MS in lean patients and the effect modification of BMI found in this study, when it comes to MS screening and prevention, it is reasonable to focus on overweight women with PCOS, especially those with high FAI. Such an attempt may save medical input and avoid unnecessary psychological stress imposed on lean PCOS patients concerning long-term MS hazards.

Our study shows that in PCOS women with low BMI, the diagnosis of PCOS according to the Rotterdam criteria, apart from infertility, has limited value for the prognosis and treatment in these population. The Rotterdam criteria, widely used to diagnose the PCOS, expanded the

criteria from the National Institutes of Health (NIH) to include polycystic ovaries, resulting in approximate 2- to 3-fold increase of prevalence[38,39]. This rise is due to the inclusion of more phenotypes, along with the increases in obese population, disease awareness, and sensitivity of tests. The over-diagnosis tends to result in a rise in milder cases[40]. Indeed, polycystic ovaries are present in many women in general population without PCOS[41]. Acne and oligomenorrhoea are also common features of pubertal development in adolescence[42]. Menstrual irregularity and sporadic high free testosterone may occur in a substantial proportion of younger women[7]. In addition to the questionable using of the criteria in adolescents and young women, more importantly, non-hyperandrogenic phenotypes of PCOS do not have the similar associated adverse implications as the hyperandrogenic phenotypes, and labelling low BMI women with PCOS might negatively impact their physical and psychological health, inducing fear and anxiety about future fertility, MS and long term health[43]. Our study shows that applying a one-size-fits-all diagnostic criteria to heterogeneous presentations of symptoms in PCOS is not justified.

In summary, we found that the prevalence of MS is low in lean women with PCOS. FAI, PCO morphology, LH, SHBG, and AMH are associated with MS in women with PCOS. BMI is an effect modifier for the associations of FAI, SHBG, and AMH in relation to MS. Labeling PCOS appears to be unnecessarily for low BMI women. These findings echo future cohort studies.

Acknowledgments

The content is solely the responsibility of the authors and does not necessarily represent the official views of the State Administration of Traditional Chinese Medicine of People's Republic of China. The Steering Committee (SC) members included Xiao-Ke Wu, Jiang-Ping Liu, Tai-Xiang Wu, Ernest HY Ng, Elisabet Stener-Victorin and Heping Zhang, and Richard S Legro (Chair). The Data and Safety Monitoring Board (DSMB) members included Esther Eisenberg, Wei-Liang Weng, Su-Lun Sun, Wei Zou and Zi-Dan Chen, and Robert Rebar (Chair). Meizhuo Zhang in Yale, contributed to the randomization scheme and training of our study personnel. Jin-Ying Fu, Chang-Ling Zhu and Xiao-Hong Wang participated in the patient recruitment at local sites of Henan, Wenzhou and Xuzhou. Other personnel with administrative resource supports included Song-Jiang Liu, Gui-Yuan Wang, Yan-Qiu Du, Yang Xia, Shu-Lai Li, Ke-Qiu Zhang, and Jian-Hua Shen. Yan Li, Wen-Juan Shen, Wei Li and Jing Cong were involved in protocol preparation and blood sample management in Harbin office and core laboratory. We thank the Reproductive Medicine Network Steering Committee of the National Institutes of Health for sharing the protocol and case-report forms from the Pregnancy in Polycystic Ovary Syndrome II study.

References

1. Escobar-Morreale HF. Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment. *Nat Rev Endocrinol*. 2018;14(5):270-284.

2. Wang ET, Calderon-Margalit R, Cedars MI, Daviglus ML, Merkin SS, Schreiner PJ, Sternfeld B, Wellons M, Schwartz SM, Lewis CE, Williams OD, Siscovick DS, Bibbins-Domingo K. Polycystic Ovary Syndrome and Risk for Long-Term Diabetes and Dyslipidemia. *Obstet and Gynecol*. 2011;117(1):6-13.

3. Faloia E, Canibus P, Gatti C, Frezza F, Santangelo M, Garrapa GG, Boscaro M. Body composition, fat distribution and metabolic characteristics in lean and obese women with polycystic ovary syndrome. *J Endocrinol Invest*. 2014;27(5):424-429.

4. Barry JA, Kuczmierczyk AR, Hardiman PJ. Anxiety and depression in polycystic ovary syndrome: a systematic review and meta-analysis. *Hum Reprod*. 2011;26(9):2442-2451.

5. Li R, Zhang Q, Yang D, Li S, Lu S, Wu X, Wei Z, Song X, Wang X, Fu S, Lin J, Zhu Y, Jiang Y, Feng HL, Qiao J. Prevalence of polycystic ovary syndrome in women in China: a large community-based study. *Hum Reprod*. 2013;28(9):2562-2569.

6. Wu X, Chang H, Zhang Y, Yang X, Hou L. Epidemiology of Polycystic Ovary Syndrome. *Science Technology Review*. 2010;28(21):101-105.

7. Zhuang J, Liu Y, Xu L, Liu X, Zhou L, Tang L, Kang D, Guo W, He M, Yang F, Qiu D. Prevalence of the Polycystic Ovary Syndrome in Female Residents of Chengdu, China. *Gynecol Obstet Invest*. 2014;77(4):217-223.

8. Moghetti P, Tosi F, Bonin C, Di Sarra D, Fiers T, Kaufman JM, Giagulli VA, Signori C, Zambotti F, Dall'Alda M, Spiazzi G, Zanolin ME, Bonora E. Divergences in insulin resistance between the different phenotypes of the polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2013;98(4):E628-637.

9. Moran L, Teede H. Metabolic features of the reproductive phenotypes of polycystic ovary syndrome. *Hum Reprod Update*. 2009;15(4):477-88.

10. Barber TM, Wass JAH, McCarthy MI, Franks S. Metabolic characteristics of women with polycystic ovaries and oligo-amenorrhoea but normal androgen levels: implications for the management of polycystic ovary syndrome. *Clin Endocrinol (Oxf)*. 2007;66(4):513-517.

11. Li R, Yu G, Yang D, Li S, Lu S, Wu X, Wei Z, Song X, Wang X, Fu S, Qiao J. Prevalence and predictors of metabolic abnormalities in Chinese women with PCOS: a cross- sectional study. *BMC Endocr Disord.* 2014; 14:76. doi: 10.1186/1472-6823-14-76.

12. Lim SS, Kakoly NS, Tan JWJ, Fitzgerald G, Bahri Khomami M, Joham AE, Cooray SD, Misso ML, Norman RJ, Harrison CL, Ranasinha S, Teede HJ, Moran LJ. Metabolic syndrome in polycystic ovary syndrome: a systematic review, meta-analysis and meta-regression. *Obes Rev*. 2018;83:3078. doi:10.1111/obr.12762.

13. Esmaillzadeh A, Mirmiran P, Azadbakht L, Etemadi A, Azizi F. High Prevalence of the Metabolic Syndrome in Iranian Adolescents. *Obesity (Silver Spring)*. 2006;14(3):377-382.

14. Ervin RB. Prevalence of metabolic syndrome among adults 20 years of age and over, by sex, age, race and ethnicity, and body mass index: United States. *Natl Health Stat Report*. 2009;5(13): 1-7.

15. Rossi B, Sukalich S, Droz J, Griffin A, Cook S, Blumkin A, Guzick DS, Hoeger KM. Prevalence of metabolic syndrome and related characteristics in obese adolescents with and without polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2008;93(12):4780-4786.

16. Wu XK, Stener-Victorin E, Kuang HY, Ma HL, Gao JS, Xie LZ, Hou LH, Hu ZX, Shao XG, Ge J, Zhang JF, Xue HY, Xu XF, Liang RN, Ma HX, Yang HW, Li WL, Huang DM, Sun Y, Hao CF, Du SM, Yang ZW, Wang X, Yan Y, Chen XH, Fu P, Ding CF, Gao YQ, Zhou ZM, Wang CC, Wu TX, Liu JP, Ng EHY, Legro RS, Zhang H; PCOSAct Study Group. Effect of Acupuncture and Clomiphene in Chinese Women With Polycystic Ovary Syndrome: A Randomized Clinical Trial. *JAMA*. 2017;317(24):2502-2514.

17. Kuang H, Li Y, Wu X, Hou L, Wu T, Liu J, Ng EH, Stener-Victorin E, Legro RS, Zhang H. Acupuncture and clomiphene citrate for live birth in polycystic ovary syndrome: study design of a randomized controlled trial. *Evid Based Complement Alternat Med*. 2013;2013:527303.

18. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod*. 2004;19(1):41-47.

19. Norman RJ, Dewailly D, Legro RS, Hickey TE. Polycystic ovary syndrome. *Lancet*. 2007;370(9588):685-697.

20. Zhao X, He Z, Mo Y, Chen X, Chen Y, Yang D. Determining the normal cut-off levels for hyperandrogenemia in Chinese women of reproductive age. *Eur J Obstet Gynecol Reprod Biol*. 2011;154(2):187-191.

21. Zhao X, Ni R, Li L, Mo Y, Huang J, Huang M, Azziz R, Yang D. Defining hirsutism in Chinese women: a cross-sectional study. *Fertil Steril*. 2011;96(3):792-796.

22. Tan JK, Tang J, Fung K, Gupta AK, Thomas DR, Sapra S, Lynde C, Poulin Y, Gulliver W, Sebaldt RJ. Development and Validation of a Comprehensive Acne Severity Scale. *J Cutan Med Surg*. 2007;11(6):211-216.

23. Burke JP, Hale DE, Hazuda HP, Stern MP. A quantitative scale of acanthosis nigricans. *Diabetes Care*. 1999;22(10):1655-1659.

24. Balen AH, Laven JSE, Tan S-L, Dewailly D. Ultrasound assessment of the polycystic ovary: international consensus definitions. *Hum Reprod Update*. 2003;9(6):505-514.

25. Al Kindi MK1, Al Essry FS, Al Essry FS, Mula-Abed WA. Validity of serum testosterone, free androgen index, and calculated free testosterone in women with suspected hyperandrogenism. *Oman Med J*. 2012;27(6):471-474.

26. Chinese Diabetes Society. Guidelines for the Prevention and Treatment of Type 2 Diabetes in China (2017 Edition). *Chin J Diabetes Mellitus*. 2018;10(1):4-67.

27. Chen MJ, Yang WS, Yang JH, Hsiao CK, Yang YS, Ho HN. Low sex hormone-binding globulin is associated with low high-density lipoprotein cholesterol and metabolic syndrome in women with PCOS. *Hum Reprod*. 2006;21(9):2266-2271.

28. Fenske B, Kische H, Gross S, Wallaschofski H, Völzke H, Dörr M, Nauck M, Keevil BG, Brabant G, Haring R. Endogenous Androgens and Sex Hormone–Binding Globulin in Women and Risk of Metabolic Syndrome and Type 2 Diabetes. *J Clin Endocrinol Metab*. 2015;100(12):4595-4603.

29. Ko JK, Li HW, Lam KS, Tam S, Lee VC, Yeung TW, Ho PC, Ng EH. Serum adiponectin is independently associated with the metabolic syndrome in Hong Kong, Chinese women with polycystic ovary syndrome. *Gynecol Endocrinol*. 2015;32(5):390-394.

30. Zhang HY, Guo CX, Zhu FF, Qu PP, Lin WJ, Xiong J. Clinical characteristics, metabolic features, and phenotype of Chinese women with polycystic ovary syndrome: a large-scale case–control study. *Arch Gynecol Obstet*. 2013;287(3):525-531.

31. Guo M, Chen ZJ, Macklon NS, Shi YH, Westerveld HE, Eijkemans MJ, Fauser BC, Goverde AJ. Cardiovascular and metabolic characteristics of infertile Chinese women with PCOS diagnosed according to the Rotterdam consensus criteria. *Reprod Biomed Online*. 2010;21(4):572-580.

32. Zhao X, Zhong J, Mo Y, Chen X, Chen Y, Yang D. Association of biochemical hyperandrogenism with type 2 diabetes and obesity in Chinese women with polycystic ovary syndrome. *Int J Gynaecol Obstet*. 2010;108(2):148-151.

33. Aswini R, Jayapalan S. Modified Ferriman–Gallwey Score in Hirsutism and its Association with Metabolic Syndrome. *Int J Trichology*. 2017;9(1):7-13.

34. Marcondes JA, Hayashida SA, Barcellos CR, Rocha MP, Maciel GA, Baracat EC. Metabolic syndrome in women with polycystic ovary syndrome: prevalence, characteristics and predictors. *Arq Bras Endocrinol Metabol*. 2007;51(6):972-979.

35. Look AHEAD Research Group, Gregg EW, Jakicic JM, Blackburn G, Bloomquist P, Bray GA, Clark JM, Coday M, Curtis JM, Egan C, Evans M, Foreyt J, Foster G, Hazuda HP, Hill JO, Horton ES, Hubbard VS, Jeffery RW, Johnson KC, Kitabchi AE, Knowler WC, Kriska A, Lang W, Lewis CE, Montez MG, Nathan DM, Neiberg RH, Patricio J, Peters A, Pi-Sunyer X, Pownall H, Redmon B, Regensteiner J, Rejeski J, Ribisl PM, Safford M, Stewart K, Trence D, Wadden TA, Wing RR, Yanovski SZ. Association of the magnitude of weight loss and changes in physical fitness with long-term cardiovascular disease outcomes in overweight or obese people with type 2 diabetes: a post-hoc analysis of the Look AHEAD randomised clinical trial. *Lancet Diabetes Endocrinol*. 2016;4(11):913-921.

411 36. Feldman AL, Griffin SJ, Ahern AL, Long GH, Weinehall L, Fhärm E, Norberg M,
412 Wennberg P. Impact of weight maintenance and loss on diabetes risk and burden: a
413 population-based study in 33,184 participants. *BMC Public Health*. 2017;17(1):170.

414 37. Dokras A, Bochner M, Hollinrake E, Markham S, VanVoorhis B, Jagasia DH.
415 Screening Women With Polycystic Ovary Syndrome for Metabolic Syndrome. *Obstet*
416 *Gynecol*. 2005;106(1):131-137.

417 38. March WA, Moore VM, Willson KJ, Phillips DIW, Norman RJ, Davies MJ. The
418 prevalence of polycystic ovary syndrome in a community sample assessed under
419 contrasting diagnostic criteria. Hum Reprod. 2010;25(2):544-551.

420 39. Yildiz BO, Bozdag G, Yapici Z, Esinler I, Yarali H. Prevalence, phenotype and
421 cardiometabolic risk of polycystic ovary syndrome under different diagnostic criteria.
422 Hum Reprod. 2012;27(10):3067-3073.

423 40. Teede HJ, Joham AE, Paul E, Moran LJ, Loxton D, Jolley D, Lombard C. Longitudinal
424 weight gain in women identified with polycystic ovary syndrome: results of an
425 observational study in young women. *Obesity (Silver Spring)*. 2013;21(8):1526-1532.

426 41. Johnstone EB, Rosen MP, Neril R, Trevithick D, Sternfeld B, Murphy R,
427 Addauan-Andersen C, McConnell D, Pera RR, Cedars MI. The polycystic ovary
428 post-rotterdam: a common, age-dependent finding in ovulatory women without
429 metabolic significance. *J Clin Endocrinol Metab*. 2010;95(11):4965-4972.

430 42. Morris S, Grover S, Sabin MA. What does a diagnostic label of “polycystic ovary
431 syndrome” really mean in adolescence? A review of current practice recommendations.
432 *Clin Obes*. 2016;6(1):1-18.

433 43. Copp T, Jansen J, Doust J, Mol BW, Dokras A, McCaffery K. Are expanding disease
434 definitions unnecessarily labelling women with polycystic ovary syndrome? *BMJ*.
435 2017;358:j3694. doi:10.1136/bmj.j3694.

Legends

Figure 1. Sensitivity ananlysis: adjusted OR of endocrine characteristics with metabolic syndrome in women with PCOS in subgroups. Patients were divided into four subgroups according to the quartiles of BMI of all patients. Prevalence of MS: BMI<21.02, 0% (0/237); 21.02≤BMI<23.71, 6.20% (15/242); 23.71≤BMI<26.71, 21.10% (50/237); BMI≥26.71, 38.10% (88/231).

Figure 2. ROC discrimination and calibration of metabolic syndrome in women with PCOS by BMI. Indicators: Age, FAI, PCO morphology, LH, SHBG, AMH. A, BMI<24 kg/m2, AUC: 0.84 (0.75-0.93); B, BMI⩾24 kg/m2, AUC: 0.63 (0.58-0.69).

Table 1. Association of metabolic syndrome and phenotypes in women with PCOS

PCOS phenotype	CDS MS		OR (95%CI)
	negative	positive	
Oligomenorrhea + PCO	341 (46.46)	40 (26.49)	Reference
Oligomenorrhea + hyperandrogenism	43 (5.86)	13 (8.61)	2.58 (1.28-5.20)
Oligomenorrhea + hyperandrogenism + PCO	350 (47.68)	98 (64.90)	2.39 (1.61-3.55)

n (%) are presented.
Abbreviations: CDS MS, China Diabetes Society Criteria for Metabolic Syndrome; PCO, polycystic ovary morphology.

Table 2. Association of metabolic syndrome and clinical characteristics in women with PCOS

Variable	CDS MS		Unadjusted OR (95%CI)	Adjusted OR (95%CI)[a]
	Negative (n=794)	Positive (n=153)		
Age (years)	28 (26, 30)	29 (26, 31)	1.08 (1.03-1.14)	1.06 (1.01-1.13)
Clinical characteristics				
Acne n(%)				
No	535 (67.38)	111 (72.55)	Reference	Reference
Yes	259 (32.62)	42 (27.45)	0.78 (0.53-1.15)	0.68 (0.45-1.05)
Acanthosis (score)				
1	666 (83.88)	111 (72.55)	Reference	Reference
□2	128 (16.12)	42 (27.45)	2.00 (1.34-2.99)	1.53 (0.96-2.43)
Menstrual cycles (time/yrs)	6 (5, 8)	6 (4, 8)	0.91 (0.83-0.99)	0.94 (0.80-1.10)
Duration between menstruation (days)	60 (45, 72)	60 (45, 90)	1.00 (1.00-1.01)	1.00 (0.99-1.01)
Oligo-amenorrhea				
Oligomenorrhea n(%)	710 (89.42)	127 (83.01)	Reference	Reference
Amenorrhea n(%)	84 (10.58)	26 (16.99)	1.73 (1.07-2.79)	1.18 (0.51-2.72)
Clinical hyperandrogenism				
Hirsutism (F-G score)				
F-G score <2	288 (36.27)	46 (30.07)	Reference	Reference
F-G score 2≤ and <5	305 (38.41)	60 (39.22)	1.23 (0.81-1.87)	0.97 (0.61-1.52)
F-G score ≥5	201 (25.31)	47 (30.72)	1.46 (0.94-2.28)	1.14 (0.70-1.87)
Biochemical hyperandrogenism				
Total testosterone (nmol/L)				
<1.67nmol/L	432 (54.89)	74 (48.68)	Reference	Reference
1.67≤ and <2.39nmol/L	260 (33.04)	57 (37.50)	1.28 (0.88-1.87)	1.24 (0.78-1.97)
≥2.39 nmol/L	95 (12.07)	21 (13.82)	1.29 (0.76-2.20)	1.43 (0.70-2.88)
Free testosterone (pg/mL)				
<3.00 pg/mL	632 (79.60)	113 (73.86)	Reference	Reference
≥3.00 pg/mL	162 (20.40)	40 (26.14)	1.38 (0.93-2.06)	0.99 (0.62-1.59)
Free androgen index				
<5%	448 (57.58)	37 (24.34)	Reference	Reference
≧5%	330 (42.42)	115 (75.66)	4.22 (2.84-6.27)	2.06 (1.11-3.82)
Polycystic ovary morphology [b]				
No	57 (7.62)	14 (9.21)	Reference	Reference
Yes	691 (92.38)	138 (90.79)	0.81 (0.44-1.50)	0.52 (0.26-1.03)
Hormones				
LH (mU/mlL	9.65 (6.39, 14.76)	8.2 (4.71, 11.36)	0.92 (0.89-0.96)	0.93 (0.89-0.98)
FSH (mU/mL)	6.06 (5.11, 7.08)	5.57 (4.73, 6.85)	0.87 (0.78-0.97)	0.96 (0.84-1.11)
Progesteone (nmol/L)	1.74 (1.22, 2.44)	1.69 (1.16, 2.27)	0.99 (0.96-1.03)	0.99 (0.94-1.04)
Estradiol (pmol/L)	200.2 (159, 267.9)	195.3 (164, 250.5)	1.00 (1.00-1.00)	1.00 (1.00-1.00)
SHBG (pmol/L)	36.2 (23.5, 59.4)	22.5 (5.89, 13.84)	0.96 (0.95-0.97)	0.98 (0.96-0.99)
AMH (ng/mL)	11.87 (7.54, 16.2)	8.99 (5.89, 13.84)	0.94 (0.91-0.97)	0.95 (0.92-0.99)

Median (IQR) or n (%) are presented.

Abbreviations: CDS MS, China Diabetes Society Criteria for Metabolic Syndrome; CI, confidence interval; IQR, interquartile range; OR, odds ratio; FAI, free androgen index; LH, luteinizing hormone; FSH, follicle-stimulating hormone; SHBG, sex hormone binding globulin; AMH, Anti-Müllerian hormone

[a] Results based on multiple imputation; multivariable analysis including age, acne, acanthosis, menstrual cycles, durations between menstrual cycles, oligo-amenorrhea, hirsutism, total testosterone, free testosterone, FAI, polycystic ovary morphology, LH, FSH, progesterone, estradiol, SHBG, and AMH.

[b] Polycystic ovaries morphology was defined by an antral follicle count of 12 or more or by a volume of more than 10 cm^3 in at least 1 ovary.

Table 3. Association of metabolic syndrome and clinical characteristics in women with PCOS by BMI.

Variable	BMI<24			BMI≧24		
	CDS MS negative (n=487)	CDS MS positive (n=18)	OR (95% CI) [a,b]	CDS MS negative (n=307)	CDS MS positive (n=135)	OR (95% CI) [a]
Age (years)	28 (26, 30)	29 (27, 32)	1.17 (1.01-1.36)	28 (26, 30)	29 (26, 31)	1.05 (0.98-1.11)
FAI						
<5%	328 (68.91)	8 (44.44)	Reference	120 (39.74)	29 (24.64)	Reference
≧5%	148 (31.09)	10 (55.56)	1.27 (0.34-4.70)	182 (60.26)	105 (78.36)	3.37 (1.78-6.37)
PCOM [b]						
No	44 (9.89)	3 (16.67)	Reference	13 (4.29)	11 (8.21)	Reference
Yes	401 (90.11)	15 (83.33)	0.42 (0.11-1.67)	290 (95.71)	123 (91.79)	0.54 (0.23-1.28)
LH [c]						
>9.33 mU/mL	285 (58.52)	10 (55.56)	Reference	134 (43.65)	51 (37.78)	Reference
≦9.33 mU/mL	202 (41.48)	8 (44.44)	0.94 (0.32-2.77)	173 (56.35)	84 (62.22)	1.36 (0.87-2.12)
SHBG [c]						
> 33.90 pmol/L	335 (70.23)	6 (33.33)	Reference	94 (30.92)	33 (24.44)	Reference
≦33.90 pmol/L	142 (29.77)	12 (66.67)	3.98 (1.06-14.91)	210 (69.08)	1020 (75.56)	0.61 (0.32-1.14)
AMH [c]						
> 11.42 ng/ml	270 (55.44)	6 (33.33)	Reference	154 (50.16)	52 (38.52)	Reference
≦11.42 ng/ml	217 (44.56)	12 (66.67)	2.15 (0.74-6.29)	153 (49.84)	83 (61.48)	1.60 (1.03-2.48)

Median (IQR) or n (%) are presented.
Abbreviations: CDS, china diabetes society; CI, confidence interval; OR, odds ratio; FAI, free androgen index; LH, luteinizing hormone; SHBG, sex hormone binding globulin; AMH, Anti-Müllerian hormone; PCOM, polycystic ovary morphology
[a] Results based on multiple imputation; multivariable analysis including age, PCOM, FAI, LH, SHBG, and AMH.
[b] Polycystic ovaries were defined by an antral follicle count of 12 or more or by a volume of more than 10 cm^3 in at least 1 ovary.
[c] Median of all patients as cut-off.

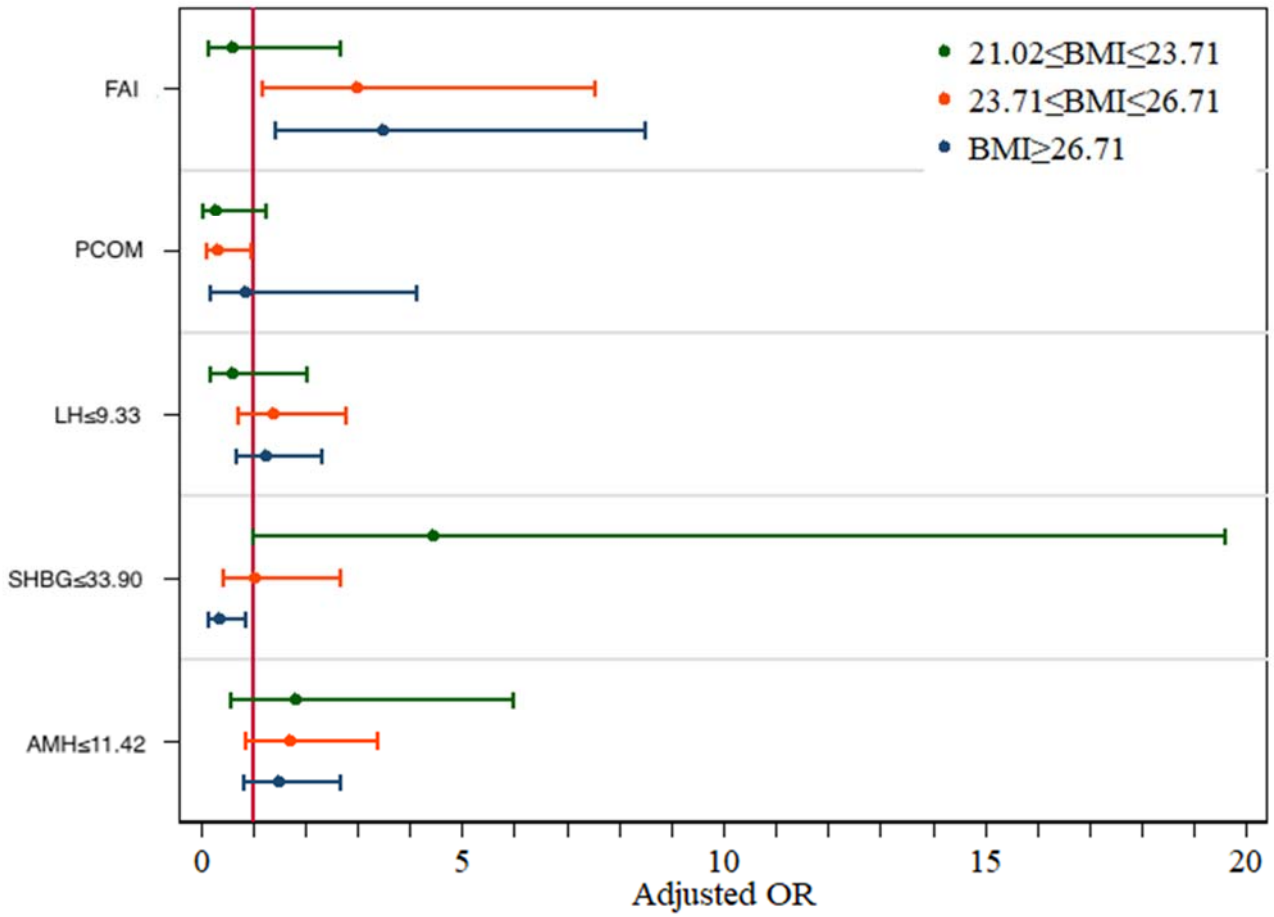
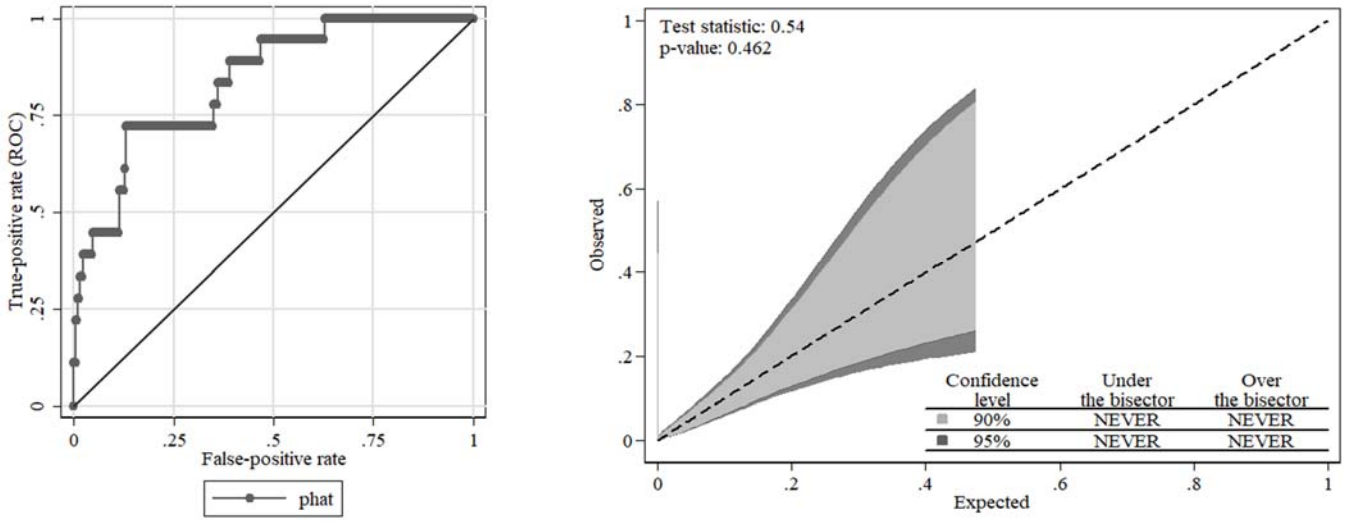


Figure 1. Sensitivity ananlysis of metabolic syndrome in women with PCOS according to quatiles of BMI.
Note: Patients were divided into four subgroups according to the quartiles of BMI of all patients
Prevalence of CDS: BMI<21.02, 0% (0/237); 21.02≤BMI<23.71, 6.20% (15/242); 23.71≤BMI<26.71, 21.10% (50/237); BMI≥26.71, 38.10% (88/231).

A. BMI<24



B. BMI ≥24

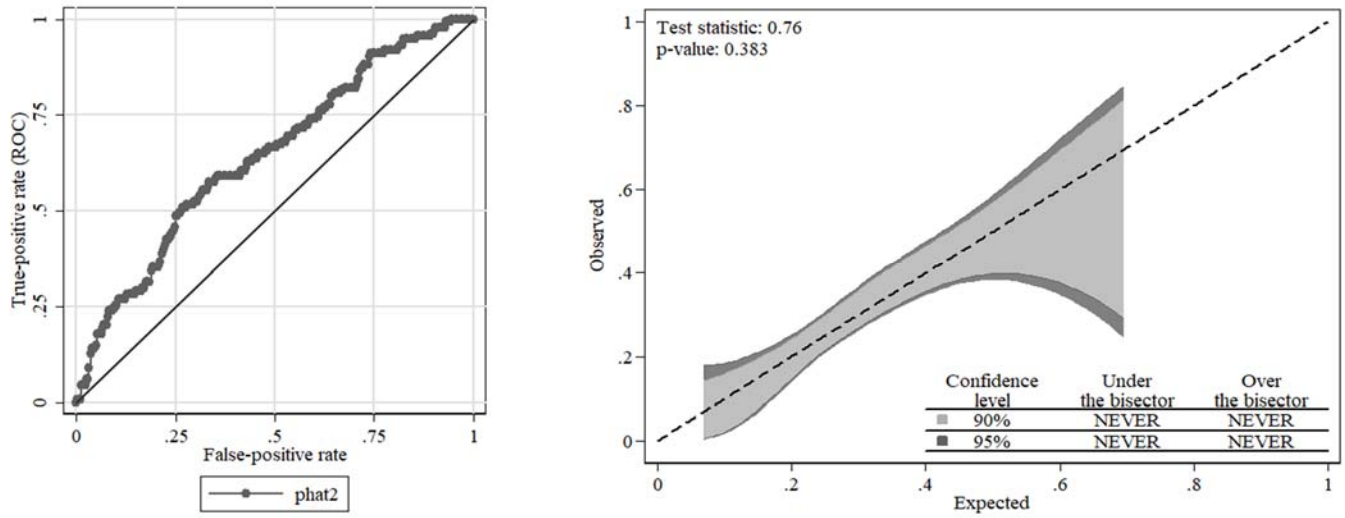


Figure 2. Prediction value of metabolic syndrome in women with PCOS by BMI.
Indicators: Age, FAI, PCO, LH, SHBG, AMH. A, BMI<24 kg/m2, AUC: 0.84 (0.75-0.93); B, BMI≥24 kg/m^2, AUC: 0.63 (0.58-0.69).