Lipid accumulation product as a marker of cardiometabolic susceptibility in women with different phenotypes of polycystic ovary syndrome

Ivana Božić-Antić¹, Dušan Ilić¹, Jelica Bjekić-Macut², Tamara Bogavac¹, Danijela Vojnović-Milutinović³, Biljana Kastratovic-Kotlica⁴, Nataša Milić⁵, Olivera Stanojlović⁶, Zoran Andrić² and Djuro Macut¹

¹Clinic of Endocrinology, Diabetes and Metabolic Diseases, Clinical Centre of Serbia, ²UMC Bežanijska kosa, Faculty of Medicine, ³Institute for Biological Research “Siniša Stanković”, ⁴Clinic of Obstetrics and Gynecology, Clinical Centre of Serbia, ⁵Institute of Medical Statistics, and ⁶Institute of Medical Physiology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia

Correspondence should be addressed to D Macut
Email
djmacut@gmail.com

Abstract

Objective: There are limited data on cardiometabolic risk factors and the prevalence of metabolic syndrome (MetS) across the different PCOS phenotypes in Caucasian population. Lipid accumulation product (LAP) is a clinical surrogate marker that could be used for evaluation of MetS in clinical practice. The aim of the study was to analyze metabolic characteristics and the ability of LAP to predict MetS in different PCOS phenotypes.

Design: Cross-sectional clinical study analyzing 365 women with PCOS divided into four phenotypes according to the ESHRE/ASRM criteria, and 125 healthy BMI-matched controls.

Methods: In all subjects, LAP was determined and MetS was diagnosed according to the National Cholesterol Education Program/Adult Treatment Panel III (NCEP-ATP III), the International Diabetes Federation (IDF) and the Joint Interim Statement (JIS) criteria. Logistic regression and ROC curve analyses were used to determine predictors of MetS in each PCOS phenotype. All analyses were performed with age and BMI adjustment.

Results: All PCOS phenotypes in comparison to controls had higher prevalence of MetS assessed by NCEP-ATP III criteria, and only classic phenotypes when IDF and JIS criteria were used. All phenotypes had the same prevalence of MetS irrespective of used definition. LAP and exhibited the highest diagnostic accuracy and was an independent predictor of MetS in all phenotypes.

Conclusion: LAP is an independent and accurate clinical determinant of MetS in all PCOS phenotypes in our Caucasian population. All PCOS phenotypes, including non-classic ones, are metabolically challenged and with cardiovascular risk, particularly phenotype B.

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Introduction

Polycystic ovary syndrome (PCOS) is a multisystem endocrinopathy that affects 15–25% reproductive-aged women according to ESHRE/ASRM criteria, and is characterized by various reproductive, obstetric and cardiometabolic features. ESHRE/ASRM definition

considers polycystic ovary morphology (PCOM), oligo/anovulation (ANOV) and hyperandrogenism (HA) as equal criteria (1). When combined, these criteria form four different phenotypes: A (ANOV, HA, PCOM), B (ANOV, HA), C (HA, PCOM) and D (ANOV,

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PCOM). Several studies that have so far analyzed PCOS phenotypes have found differences in anthropometric, hormonal and metabolic characteristics between PCOS phenotypes (2–5). This implies different cardiometabolic risk factors in different PCOS phenotypes as well. A common conclusion is that phenotypes A and B have a higher metabolic risk than other phenotypes (6–8), but the data are inconsistent.

Frequent metabolic complications of PCOS are obesity, insulin resistance, dyslipidemia, dysglycemia, hypertension and the consequent development of low-grade inflammation (9). These cardiovascular risk factors are considered as independent predictors of cardiovascular morbidity and mortality (9) and usually cluster together into metabolic syndrome (MetS). Because of the high cost of treatment of associated morbidities (10), PCOS not only has significant health implications *per se*, but it also represents a significant economic burden on the general healthcare. However, patients with PCOS are still rarely evaluated for presence of metabolic and cardiovascular risk factors in routine clinical practice (11), and a long-term follow-up of cardiovascular outcomes is missing. One possible explanation could be that the procedures for its evaluation are both time- and money-consuming. Specifically, the evaluation of the presence of MetS, regardless of the MetS definition used should include measuring waist circumference (WC), blood pressure, high-density cholesterol (HDL), triglycerides, fasting glucose, as well as a 2-h oral glucose tolerance test (OGTT) (12). The additional problem is related to the lack of recommendation on which MetS definition should be used with specific populations, like with PCOS. Therefore, there is a need for a simple and accurate method for the assessment of cardiometabolic risk in PCOS women.

Lipid accumulation product (LAP) represents a clinical index based on the assessment of waist circumference (WC) and serum triglycerides (TG), which estimates visceral lipid accumulation. LAP is considered to be an independent predictor of metabolic syndrome (MetS), cardiovascular diseases and type 2 diabetes mellitus (T2DM) in healthy population (13, 14). In women with PCOS, LAP was shown to be a marker of increased cardiovascular risk (15), and was associated with impaired glucose tolerance (IGT) (16), MetS (17), and non-alcoholic fatty liver disease (NAFLD) (18). Recently, we have shown a strong association between LAP and MetS in Caucasian PCOS population. Moreover, LAP had the highest diagnostic accuracy in comparison to other

MetS determinants regardless of which MetS definition was used (NCEP-ATP III, IDF or JIS) (19). Since there is emerging evidence of variability of cardiometabolic risks in different PCOS phenotypes (8, 20), the aim of this study was to evaluate the metabolic characteristics and prevalence of MetS, as well as to analyze the ability of LAP to predict MetS in different phenotypes of women with PCOS.

Subjects and methods

Subjects

We analyzed 365 women with PCOS (age: 25.48 ± 5.21 years, BMI: 25.05 ± 6.24 kg/m²) and 125 healthy, BMI matched women (age: 30.35 ± 5.62 years, BMI: 25.41 ± 5.16 kg/m²). Subjects were referred to the outpatient endocrine clinic for evaluation of irregular menstrual cycles, infertility, obesity, hirsutism or acne. PCOS was defined according to the ESHRE/ASRM definition that requires the presence at least two of the following three criteria: (1) oligo/amenorrhea or anovulation; (2) clinical and/or biochemical hyperandrogenism; and (3) morphologically polycystic ovaries on ultrasound (21). Clinical hyperandrogenism (hirsutism) was evaluated by Ferriman–Galway (FG) score system (22), and was considered to be present when FG score was ≥ 8 , irrespective of the concomitant presence of acne or alopecia (23). Free androgen index (FAI) was calculated by the formula $((\text{testosterone} \times 100)/\text{SHBG})$ with both testosterone and SHBG expressed in nmol/L (24). Biochemical hyperandrogenism (hyperandrogenemia) was defined by presence of FAI >6 and/or serum total testosterone (TT) >2 nmol/L. These cut-off values were based on examination of 56 non-selected, non-hirsute women presenting for routine controls who had regular menstrual cycles, and morphologically normal ovaries (25). Concomitantly, these cut-offs also corresponded to 95% percentile of FAI and serum TT of analyzed healthy control women in this study. In all subjects, we excluded the presence of non-classical 21-hydroxylase deficiency, hyperprolactinemia, Cushing's disease, hypothyroidism, diabetes mellitus and history of drug or alcohol abuse. No patients had received any hormone treatment for at least 3 months before the study.

Control group of healthy women were volunteers referred after regular yearly gynecological examination. They were normoandrogenic, without hirsutism, had regular, ovulating menstrual cycles and morphologically normal ovaries on ultrasound.

Methods

In all subjects, we measured systolic and diastolic blood pressure (SBP and DBP, respectively), and waist circumference (WC) at the level of umbilicus. Body mass index (BMI, kg/m²) was calculated by formula: weight (kg)/height (m)². After a 12-h overnight fast, in the follicular phase of the menstrual cycle (between days 3 and 7) for women with regular menstrual cycles and irrespective on the menstrual cycle for those with oligo/amenorrhea, blood samples were taken for determination of total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG), fasting plasma glucose (FPG), insulin, serum total testosterone (TT), sex-hormone-binding protein (SHBG). Samples for hormonal analyses were frozen at -80°C until measurement. In all subjects, 2-h oral glucose tolerance test (OGTT) with 75g glucose was performed on the second day of examination, and serum glucose was determined at 0 min and 120 min of the test. Serum glucose (mmol/L) was measured by the glucose-oxidase method (Randox, UK). TC (mmol/L) and TG (mmol/L) were determined using standard enzymatic methods (TC: cholesterol oxidase, Randox, TG: glycerol-3-phosphat oxidase, Randox). HDL (mmol/L) was measured by direct method (Randox), and LDL (mmol/L) determined by the Friedewald formula (26). Serum insulin (mU/L) concentrations were determined by radioimmunoassay (RIA INSULIN (PEG), INEP, Belgrade, Serbia, intra- and interassay CV were 2.5 and 7.7%, respectively). Serum TT (nmol/L) was measured by radioimmunoassay (TESTOCT2, CIS Bio international, Gif-sur-Yvette Cedex, France; intra- and interassay CVs 4.5 and 5.1%, respectively). SHBG (nmol/L) was measured by radioimmunoassay (SHBG-RIACT, CIS Bio international, Gif-sur-Yvette Cedex; intra- and interassay CVs were 3.9 and 4.7%, respectively). Insulin resistance (IR) was estimated by the homeostasis model assessment of IR (HOMA-IR=insulin (mIU/L)×glucose (mmol/L)/22.5) (27). LAP was calculated using formula: ((WC-58)×triglycerides) (28), where 58 is the minimum WC value for women according to the Third National Health and Nutrition Examination Survey (NHANES III). All subjects were assessed as having MetS using three different international criteria: NCEP-ATP III (29), IDF (30) and Joint Interim Statement (JIS) criteria (31). According to NCEP-ATP III, the diagnosis of MetS was established if any three or more of the following criteria were satisfied: 1) WC: ≥88 cm, 2) triglycerides (TG) ≥1.7 mmol/L, 3) SBP ≥130 and/or DBP ≥85 mmHg, 4) fasting HDL <1.3 mmol/L, and 5) fasting plasma glucose (FPG) ≥6.1 mmol/L. The IDF definition of

the MetS for the Euroid population considered central adiposity (defined as WC 80 cm) as an obligatory factor for the diagnosis of the MetS, plus two of the following criteria: TG ≥1.7 mmol/L, or specific treatment, low HDL (<1.3 mmol/L) or specific treatment, high blood pressure (SBP >130 and/or DBP ≥85 mmHg) or treatment of diagnosed hypertension, and FPG ≥5.6 mmol/L or T2DM. According to the JIS criteria, the diagnosis of MetS was established as the presence of any three of the following criteria: 1) central adiposity (WC ≥80 cm), 2) TG ≥1.7 mmol/L, or specific treatment, 3) HDL <1.3 mmol/L or specific treatment, 4) SBP ≥130 and/or DBP ≥85 mmHg or treatment of diagnosed hypertension, and 5) FPG ≥5.6 mmol/L or previously diagnosed T2DM that is under treatment.

The study was approved by the Institutional Ethical Committees and written consent was obtained from all subjects.

Statistical analyses

Statistical analysis was performed using the Statistical Package for the Social Sciences software (SPSS, version 17.0; SPSS Inc, Chicago, IL, USA). As there were significant differences in age and BMI between PCOS phenotypes and control group, all statistical analyses were adjusted for age and BMI, except BMI and WC, which were adjusted only for age. Accordingly, results are presented as ANCOVA-mean (95% confidence interval (95% CI)). Normality of data distribution of continuous variables was tested by the Kolmogorov-Smirnov test. Data with skewness or kurtosis were logarithmically transformed in order to achieve normal distribution.

Binary logistic regression analyses were performed in order to analyze the determinants of MetS defined by NCEP-ATP III criteria and LAP in each PCOS phenotype. Only significant determinants from univariate analyses entered multivariate analyses, performed by *Forward LR* method.

Receiver operating characteristic (ROC) curves were generated for each continuous variable to identify the performance of classic determinants of MetS, as well as LAP. The areas under the curves (AUCs) are provided with standard error of mean (S.E.M.) and 95% confidence intervals (95% CI). ROC curves, a plot of the sensitivity (SEN) (true positive) versus 1-specificity (SP) (false positive) for each potential indicator tested, determine the ability of a screening measure to correctly identify individuals based on their classification by a reference test.

Table 1 Clinical characteristics of PCOS phenotypes and control group.

	Phenotype A (n=207)	Phenotype B (n=40)	Phenotype C (n=80)	Phenotype D (n=38)	Controls (n=125)	Controls vs Phenotype ^s	Phenotype vs Phenotype ^s
Age (years)*	25 (21–29)	24 (22–29)	25 (21–30)	25 (22–28)	30 (27–34.5)	A, B, C, D	NS
BMI (kg/m ²)*	25.46 (24.66–26.26)	27.21 (25.42–29.00)	25.76 (24.49–27.03)	21.77 (19.92–23.61)	24.60 (23.53–25.68)	B, D	A-D B-D C-D
WC (cm)*	83.79 (81.67–85.91)	86.87 (82.10–91.64)	84.32 (80.99–87.65)	75.38 (70.60–80.18)	80.02 (77.31–82.73)	A, B, C	A-D B-D C-D
SBP (mmHg)	120.67 (119.03–122.30)	119.63 (115.72–123.55)	121.90 (119.33–124.47)	116.53 (112.69–120.37)	113.25 (111.12–115.37)	A, B, C	C-D
DBP (mmHg)	79.34 (78.06–80.62)	77.89 (74.82–80.96)	79.48 (77.46–81.49)	75.76 (72.46–79.06)	71.41 (69.75–73.07)	A, B, C, D	A-D C-D
FG (mmol/L)	4.71 (4.64–4.77)	4.62 (4.47–4.77)	4.73 (4.63–4.83)	4.69 (4.54–4.84)	4.82 (4.73–4.91)	NS	NS
FI (mU/L)	16.50 (15.25–17.75)	21.43 (18.44–24.41)	16.09 (14.12–18.05)	16.11 (13.15–19.06)	12.74 (11.15–14.35)	A, B, C, D	A-B B-C B-D
HOMA-IR	3.49 (3.20–3.78)	4.67 (3.99–5.35)	3.44 (2.99–3.89)	3.46 (2.79–4.13)	2.78 (2.42–3.15)	A, B, C, D	A-B B-C B-D
TC (mmol/L)	5.11 (4.97–5.26)	5.09 (4.78–5.41)	5.20 (4.97–5.43)	5.03 (4.70–5.36)	4.73 (4.54–4.92)	A, B, C	NS
HDL (mmol/L)	1.35 (1.31–1.39)	1.36 (1.27–1.46)	1.38 (1.32–1.45)	1.34 (1.25–1.44)	1.37 (1.32–1.43)	NS	NS
LDL (mmol/L)	3.18 (3.05–3.31)	3.16 (2.87–3.44)	3.30 (3.10–3.51)	3.17 (2.88–3.47)	2.96 (2.79–3.14)	NS	NS
TG (mmol/L)	1.23 (1.13–1.32)	1.28 (1.05–1.50)	1.25 (1.09–1.40)	1.17 (0.95–1.40)	0.90 (0.77–1.03)	A, B, C, D	NS
TT (nmol/L)	3.00 (2.87–3.14)	2.70 (2.41–2.99)	2.50 (1.83–2.95)	1.50 (2.29–2.71)	1.47 (1.16–1.78)	A, B, C	A-C A-D B-D C-D
SHBG (nmol/L)	38.61 (35.25–41.98)	35.02 (27.48–42.56)	41.95 (36.69–47.22)	43.20 (35.40–51.01)	55.86 (51.46–60.25)	A, B, C, D	NS
FAI (%)	11.81 (10.68–12.94)	10.80 (8.26–13.34)	8.69 (6.92–10.46)	5.15 (2.52–7.77)	3.56 (2.08–5.03)	A, B, C, D	A-C A-D B-D C-D
LAP	36.81 (33.13–40.48)	40.62 (32.58–48.65)	30.62 (24.96–36.27)	36.93 (28.68–45.18)	21.77 (17.22–26.32)	A, B, C, D	NS

* $P < 0.05$; Except for *, all analyses are age and BMI adjusted; BMI and WC analyses were only age adjusted; NS, non significant.

BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; FG, fasting glucose; FI, fasting insulin; HOMA-IR, homeostasis model assessment of insulin resistance; TC, total cholesterol; HDL, high-density cholesterol; LDL, low-density cholesterol; TG, triglycerides; TT, total testosterone; SHBG, sex hormone binding globulin; FAI, free androgen index; LAP, lipid accumulation product.

Values for each AUC can be between 0 and 1, with a value of 0.5 indicating that the diagnostic test is no better than chance. We considered that the parameters possessed an accurate diagnostic sensitivity when the AUC value was >0.75 (32). We defined the best cut-off value as the value with the highest proportion of positives and negatives classified correctly by the test. A P value less than 0.05 was considered statistically significant.

Results

In our PCOS group, 207 (57%) had phenotype A, 40 (11%) phenotype B, 80 (22%) phenotype C and 38 (10%) phenotype D. Clinical parameters are presented in Table 1.

PCOS phenotypes in comparison to controls showed BMI to be significantly higher for phenotype B and significantly lower for phenotype D, while it was similar for phenotypes A and C. At the same time phenotypes A and C, as well as phenotype B, had greater WC. Although women with phenotype D had lower BMI, their WC was similar as in controls. All PCOS phenotypes had higher DBP, TG, fasting insulin, HOMA-IR, FAI and LAP, and lower SHBG in comparison to controls, while there were no differences in fasting glucose, HDL and LDL. Hyperandrogenic phenotypes (A, B, C) in comparison to controls had higher SBP, TC and TT.

Comparison between PCOS phenotypes did not show differences in fasting glucose, lipids, SHBG and LAP. While hyperandrogenic phenotypes had the same BMI and WC, phenotype B showed the highest fasting insulin and HOMA-IR. At the same time, phenotype D had lower BMI, WC, TT and FAI in comparison to hyperandrogenic phenotypes.

Impaired fasting glucose (IFG) was more prevalent in PCOS than controls (1.4% vs 0%, $P=0.045$). Of four PCOS women with IFG, two (50%) had phenotype A, one (25%) had phenotype C and one (25%) had phenotype D. Impaired glucose tolerance (IGT) was confirmed in ten (2.7%) women with PCOS. IGT was equally prevalent among hyperandrogenic PCOS phenotypes (seven (1.9%) with phenotype A, two (0.5%) with phenotype B and one (0.3%) with phenotype C, $P=0.440$) as well between all PCOS phenotypes and controls ($P=0.523$). Healthy controls did not show dysregulation of glucose metabolism, and none of the PCOS women had T2DM.

Age and BMI adjusted prevalence of MetS according to different international definitions are presented in Table 2. After age adjustment, all hyperandrogenic phenotypes in comparison to controls had higher

prevalence of MetS defined by NCEP-ATP III and JIS criteria, while A and B phenotypes had higher prevalence when IDF criteria were used. After combined age and BMI adjustment, only phenotypes A and B had higher prevalence of MetS when IDF and JIS criteria were used, while all PCOS phenotypes had higher prevalence of MetS when NCEP-ATP III criteria were used.

In comparison to hyperandrogenic phenotypes, phenotype D had the lowest age adjusted prevalence of MetS when IDF and JIS criteria were used, but it differed only from phenotypes A and B when NCEP-ATP III criteria were used. After combined age and BMI adjustment was applied, all phenotypes had the same prevalence of MetS, irrespective of criteria used.

When the prevalence of the standard individual components for the definition of MetS (WC ≥ 80 cm, HDL < 1.29 mmol/L, FG ≥ 5.6 mmol/L and FG ≥ 6.1 mmol/L) was analyzed with combined age and BMI adjustment, there were no differences between PCOS phenotypes and controls, as well among PCOS phenotypes (data not shown). As PCOS women and controls had similar body weight and BMI, and no different prevalence of WC ≥ 80 cm as a criterion for IDF and JIS definitions, we decided to use only NCEP-ATP III criteria for further analyses.

Univariate logistic regression analyses showed that LAP was a significant determinant of MetS in all PCOS phenotypes (Table 3). Additionally, standard MetS criteria were significant determinants only in phenotype A (Table 3). In order to avoid problem with collinearity, we omitted WC and TG from multivariate logistic regression analyses, and showed that LAP was an independent determinant of MetS defined by NCEP-ATP III criteria in all phenotypes. Additional independent determinants of MetS were DBP in phenotype A, and SBP in phenotype C (Table 3).

ROC curve analysis showed that LAP had the highest diagnostic accuracy for determination of MetS by NCEP-ATP III criteria in all PCOS phenotypes (Table 4). For phenotype A, LAP cut-off value of 30.63 had SN: 100%, SP: 81%, PPV: 59%, NPV: 100%. For phenotype B, LAP cut-off value of 39.65 had SN: 100%, SP: 83%, PPV: 73.3%, NPV: 100%. For phenotype C, LAP cut-off value of 24.40 had SN: 100%, SP: 75%, PPV: 50%, NPV: 100%. For phenotype D, LAP cut-off value of 25.49 had SN: 100%, SP: 87.5%, PPV: 33%, NPV: 100%.

Discussion

The results of our study have confirmed that compared with standard determinants of MetS, lipid accumulation

Table 2 Age and age plus BMI adjusted prevalence of metabolic syndrome in PCOS phenotypes by using three definitions: NCEP-ATP III, IDF and JIS.

	Phenotype A (n=207)		Phenotype B (n=40)		Phenotype C (n=80)		Phenotype D (n=38)		Controls (n=125)		Controls vs Phenotype [§]		Phenotype vs Phenotype [§]	
	%*	% [‡]	%*	% [‡]	%*	% [‡]	%*	% [‡]	%*	% [‡]	P*	P [‡]	P*	P [‡]
ATP III	22.3	21.3	28.3	21.3	18	16	6.9	18.4	2.7	4.6	A, B, C	A, B, C, D	A-D B-D	NS
IDF	25.3	24.1	34.1	26.8	21.3	18.6	7	20.2	9.7	11.2	A, B	A, B	A-D B-D C-D	NS
Joint	25.9	24.7	33.4	25.5	23.1	20.8	7.1	20.0	9.5	11.6	A, B, C	A, B	A-D B-D C-D	NS

[§]P < 0.05; *age adjusted; [‡]age plus BMI adjusted; NS, non significant.

NCEP-ATP III, National Cholesterol Education Program/Adult Treatment Panel III; IDF, International Diabetes Federation; JIS, Joint Interim Statement.

product has the highest diagnostic accuracy in predicting MetS in Caucasian PCOS population and in each phenotype. Moreover, we have confirmed that both hyperandrogenic and non-hyperandrogenic PCOS phenotypes carry a similar metabolic risk.

MetS represents a collection of cardiometabolic risk factors that expose the subject to a five times higher risk of developing type 2 diabetes mellitus (T2DM) (33), and a two times higher risk of cardiovascular morbidity and mortality (34). Moreover, the risk of atherosclerosis and cardiovascular diseases grows with an increasing number of MetS determinants (35). The prevalence of MetS in PCOS women does show ethnic variation, but it is 2–4 times higher than that in healthy women (36). As the prevalence of MetS rises with BMI and age (37), it could be presumed that women with PCOS have increased cardiometabolic risks during their reproductive period and menopause, although longitudinal studies are lacking.

There is emerging evidence that PCOS phenotypes differ in anthropometric, hormonal and metabolic characteristics (2–5), which points to diverse cardiometabolic risks and consequences the phenotypes may experience. A few studies that evaluated metabolic characteristics of PCOS phenotypes suggested that classic phenotypes A and B have a worse metabolic profile than phenotypes C and D (6–8, 20), while some studies showed either no differences between phenotypes (38) or a higher MetS prevalence in phenotype D in comparison to the hyperandrogenic phenotypes (39). Obesity is among the most important modifiers of phenotypic expression of PCOS. However, even normal-weighted women with PCOS have increased abdominal/visceral adipose tissue, which correlates with insulin resistance, dyslipidemia, hypertension and MetS (40). In accordance with the fact that WC is a marker of abdominal/visceral adiposity (41), we have shown that all PCOS phenotypes have increased abdominal adiposity in comparison to healthy women, regardless of age.

Dyslipidemia is a common metabolic consequence of both obesity and PCOS (18, 42). In our population, only hyperandrogenic phenotypes had increased TC, while all phenotypes had increased TG in comparison to controls, regardless of age and BMI. As insulin is the main regulator of SHBG and TG production (43), their respective low and high levels in all analyzed PCOS phenotypes are in accordance with the increased IR in all phenotypes, regardless of age and BMI. Our results are in line with other studies showing the same lipid levels in all phenotypes after age and BMI adjustment (4, 6, 38, 44, 45). Interestingly, all our PCOS phenotypes and controls

Table 3 Logistic regression analysis of standard and new predictors of metabolic syndrome in PCOS phenotypes according to NCEP-ATP III criteria.

	Phenotype A			Phenotype B			Phenotype C			Phenotype D		
	P	RR	95% CI	P	RR	95% CI	P	RR	95% CI	P	RR	95% CI
Univariate logistic regression analysis												
LAP	<0.001	1.077	1.051–1.103	0.007	1.089	1.024–1.157	<0.001	1.065	1.030–1.101	<0.001	5.235	0.000–6.757
WC	<0.001	1.128	31.089–1.169	0.002	1.133	1.047–1.227	0.001	1.090	1.037–1.146	0.740	1.330	0.973–1.819
TG	<0.001	8.684	4.175–18.062	0.003	9.363	4.547–16.987	0.096	1.456	0.936–2.265	0.001	6.439	0.001–7.381
HDL	<0.001	0.001	0.000–0.005	0.015	0.004	0.000–0.351	0.002	0.006	0.000–0.139	0.088	0.001	0.000–3.390
SBP	<0.001	1.107	1.067–1.149	0.154	1.042	0.985–1.104	<0.001	1.210	1.098–1.333	0.269	0.931	0.820–1.057
DBP	<0.001	1.152	1.093–1.213	0.109	1.079	0.983–1.185	<0.001	1.281	1.115–1.471	0.810	0.981	0.839–1.147
FG	0.002	3.013	1.490–6.095	0.105	4.532	0.729–28.187	0.127	2.441	0.775–7.687	0.717	1.537	0.150–15.715
Multivariate logistic regression analysis												
LAP	<0.001	1.075	1.046–1.104	0.007	1.089	1.024–1.157	0.003	1.063	1.021–1.106	<0.001	5.235	0.000–6.757
DBP	0.001	1.143	1.054–1.240	-	-	-	-	-	-	-	-	-
SBP	-	-	-	-	-	-	0.003	1.193	1.060–1.343	-	-	-

LAP, lipid accumulation product; WC, waist circumference; TG, triglycerides; HDL, high density cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; FG, fasting glucose; NCEP-ATP III, National Cholesterol Education Program/Adult Treatment Panel III.

Table 4 Area under ROC curve for identification of determinants for metabolic syndrome using NCEP-ATP III criteria in PCOS phenotypes and controls.

Determinant	Phenotype A		Phenotype B		Phenotype C		Phenotype D		Controls	
	AUC (95% CI)	P	AUC (95% CI)	P	AUC (95% CI)	P	AUC (95% CI)	P	AUC (95% CI)	P
LAP	0.950 (0.920–0.981)	<0.001	0.972 (0.922–1.023)	<0.001	0.931 (0.868–0.993)	<0.001	1.000 (0.998–1.015)	0.020	0.929 (0.865–0.992)	<0.001
WC	0.917 (0.871–0.962)	<0.001	0.894 (0.770–1.019)	0.001	0.836 (0.713–0.960)	<0.001	0.963 (0.884–1.042)	0.031	0.843 (0.737–0.948)	0.001
TG	0.874 (0.813–0.936)	<0.001	0.967 (0.909–1.025)	<0.001	0.917 (0.839–0.994)	<0.001	1.000 (0.896–1.003)	0.020	0.851 (0.718–0.984)	0.001
HDL	0.879 (0.830–0.928)	<0.001	0.831 (0.696–0.965)	0.001	0.866 (0.789–0.943)	<0.001	0.943 (0.866–1.020)	0.037	0.859 (0.774–0.944)	0.001
DBP	0.774 (0.690–0.858)	<0.001	0.631 (0.401–0.860)	0.260	0.851 (0.740–0.963)	<0.001	0.491 (0.072–1.054)	0.966	0.802 (0.662–0.942)	0.004
SBP	0.780 (0.693–0.868)	<0.001	0.614 (0.371–0.856)	0.326	0.872 (0.760–0.983)	<0.001	0.370 (0.131–0.872)	0.256	0.708 (0.524–0.891)	0.050
FG	0.636 (0.522–0.749)	0.015	0.658 (0.437–0.879)	0.172	0.632 (0.470–0.794)	0.156	0.648 (0.405–0.891)	0.491	0.641 (0.422–0.860)	0.183

LAP, lipid accumulation product; WC, waist circumference; TG, triglycerides; HDL, high-density cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; FG, fasting glucose; NCEP-ATP III, National Cholesterol Education Program/Adult Treatment Panel III.

had high prevalence of low HDL after age adjustment (range 33–58%), as well as after combined age and BMI adjustment (range 36–51%), exposing our population of PCOS women to a more pronounced cardiovascular risk.

Our PCOS women with phenotype B were significantly more insulin-resistant and hyperinsulinemic in comparison to other phenotypes, regardless of age and BMI. Some studies showed higher IR in hyperandrogenic PCOS phenotypes (8, 20).

The results regarding the prevalence of dysglycemia (impaired fasting glucose, impaired glucose tolerance and T2DM) in analyzed PCOS women are comparable to the results of other authors (6, 46). Our study was designed as a cross-sectional on four clinical PCOS phenotypes that combine hyperandrogenic and non-hyperandrogenic women. Hence, we do not exclude the possibility that our cohort of PCOS women are at risk of developing T2DM in their future life. Moreover, the deterioration of glucose tolerance toward T2DM could be expected among our cohort of subjects with substantial proportion of MetS, which is considered as an independent predictor of T2DM (47). In effect, it has been shown that 30% to 40% of subjects with normal fasting glucose and normal glucose tolerance could have beta-cell dysfunction and insulin resistance for a long period of time before dysglycemia would manifest (48, 49).

As far as we know, this is the first study that has analyzed age and combined age and BMI adjusted prevalence of MetS in four classical PCOS phenotypes using three different international criteria. In hyperandrogenic phenotypes, lower MetS prevalence defined by NCEP-ATP III criteria compared with other two definitions is the result of a different cut-off level used for WC criterion.

The results regarding metabolic outcomes in PCOS phenotypes A and B are similar among studies (44, 50). However, our women with phenotype C demonstrate the same metabolic risks prevalence (hyperlipidemia, hypertension, insulin resistance) after age and BMI adjustment as women with phenotypes A and B. Moreover, PCOS women with phenotype D had the same age adjusted MetS prevalence irrespective of international criteria used, as well as a higher age and BMI adjusted MetS prevalence when NCEP-ATP III criteria were applied. Our results suggest that BMI adjustment abolishes the difference between hyperandrogenic and non-hyperandrogenic phenotypes, and that even the non-hyperandrogenic phenotype D is characterized by an unfavorable metabolic profile.

It is proposed that LAP, a simple clinical marker of lipid over-accumulation in adults, could be used as a

marker of IR, MetS, cardiovascular risk and mortality in non-PCOS populations (13, 28, 51) as well as in PCOS population (15, 16, 52). Previously we showed that LAP was the strongest predictor of MetS defined by three different international criteria in Caucasian women with PCOS (19). In the present study, we have confirmed that LAP is an independent predictor of MetS in each PCOS phenotype. ROC curve analysis showed that of all standard determinants of MetS, LAP had the best discriminative ability for MetS in all PCOS phenotypes as well in controls. Although LAP includes TG and WC, those MetS determinants had lower discriminative power than LAP. All PCOS phenotypes showed higher LAP in comparison to healthy controls after age and BMI adjustment, but without any difference among the phenotypes. These findings are in accordance with MetS prevalence in our PCOS cohort defined by NCEP-ATP III criteria.

Different LAP cut-off values obtained for each PCOS phenotype with similar SN and SP are in accordance with different metabolic characteristics of the phenotypes. The main value of LAP as a predictor of MetS yields the NPV of 100% for all proposed cut-offs. Accordingly, our results imply that LAP is a simple time-saving clinical marker for the accurate evaluation of MetS defined by NCEP-ATP III criteria. Therefore, LAP could be used in the routine clinical practice in metabolically vulnerable patients such as women with PCOS, as well as for the evaluation of MetS in PCOS phenotypes.

We are aware of the fact that the use of radioimmunoassay for the analysis of total testosterone concentration is a limitation of our study. While the characteristics of liquid chromatography–tandem mass spectrometry (LC–MS/MS) make steroid hormone analysis a more favorable method for the differentiation of women with PCOS (53, 54), it is expensive for extensive clinical application and is not readily available in the clinical setting in our country. Moreover, a recent study has concluded that the total testosterone measurement using radioimmunoassay and LC–MS/MS differentiated PCOS women from controls in a similar fashion (55).

In conclusion, we have found that LAP is an independent, simple and accurate clinical determinant of the existence of MetS in all phenotypes of our analyzed PCOS population. Moreover, we have confirmed that all PCOS phenotypes, including the non-hyperandrogenic ones, are metabolically susceptible, and carry the potential for the development of abdominal obesity, insulin resistance and cardiovascular morbidity.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

D M and I B-A contributed to the conception and design of the study, to the acquisition, analysis and interpretation of data and to drafting the article. D I, J B-M, T B, D V-M, B K-K, O S and Z A contributed to the acquisition of data and revised the article critically for important intellectual content. N M contributed to the statistical analysis and interpretation of data. All authors gave their final approval of the version to be published.

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Clinical Study	I Božić-Antić and others	Lipid accumulation product in PCOS phenotypes	175:6	560
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