RESEARCH LETTER

Can the Total Thrombus-formation Analysis System (T-TAS) better predict coagulation disorders than conventional laboratory measurements in patients with polycystic ovary syndrome?

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Introduction Polycystic ovary syndrome (PCOS) is an endocrine disorder characterized by anovulation, hyperandrogenism, and polycystic ovaries on ultrasound and associated with metabolic disorders.[1,2] It also exhibits a tendency to thrombus formation: hypercoagulability and decreased fibrinolysis.[3] Patients with PCOS present with shorter prothrombin time (PT), activated partial thromboplastin time (APTT), and thrombin time as well as elevated levels of D-dimer and fibrinogen.[4]

Commonly used methods to assess hemostasis do not predict the thrombotic risk, because they are insensitive to hypercoagulation. The Total Thrombus-formation Analysis System (T-TAS) is a microchip flow-chamber system that mimics blood flow in the vessels, enabling the quantitative assessment of the platelet thrombus formation process and whole-blood thrombogenicity.[5]

In this study, we analyzed and compared classic coagulation measurements with the results obtained with the use of the T-TAS. The main objective of the present study was to assess whole blood thrombogenicity using the T-TAS in patients diagnosed with PCOS.

Patients and methods Patients diagnosed with PCOS according to the Rotterdam criteria,[1,2] were divided into 2 groups—"PCOS-obese" (26 overweight and obese patients [body mass index (BMI) >25 kg/m^2]) and "PCOS-normal" (23 individuals with normal weight)—and compared with 11 controls including healthy volunteers without menstrual cycle irregularities, clinical hyperandrogenism, or polycystic ovarian morphology on ultrasound. Controls did not have hormonal evaluation performed. Medical history was taken and clinical examination was carried out. Blood samples for analysis were drawn and analyzed according to standard protocols.[2]

The T-TAS (Fujimori Kogyo, Zacros, Japan) is an automated microchip-based flow-chamber system developed for an easy and quick assessment of platelet thrombus formation under flow conditions at a shear rate of 240 s^{-1}, equipped with a special atheroma (AR) microchip.[5] The system analyzes thrombus formation with a simple procedure using 2 microchips with different thrombogenic surfaces: a platelet chip coated with type I collagen and an AR chip coated with type I collagen plus tissue thromboplastin. The process of thrombus formation inside the 2 chips was analyzed by monitoring the flow pressure change. The area under the curve (AUC30) for flow pressure was calculated to assess platelet thrombogenicity inside the microchips. Considering the first 30 minutes for the AR tested at a flow rate of 10 µl/min, AUC30 was described as AR10-AUC30.[6] In each patient, blood samples were analyzed for thrombus formation AUC30, time of thrombus formation initiation (T10), and occlusion time (OT)—a time of complete thrombus formation inside the AR chip.

Statistical analyses were performed using the Statistica software, version 10 PL (StatSoft,

Inc., Tulsa, Oklahoma, United States). Normality of data distribution was tested using the Shapiro–Wilk test. The *t* test was used to measure the significance of the difference between 2 continuous variables when data fitted normal distribution, and the results were presented as mean (SD). Non-normally distributed data were compared using the Mann–Whitney test, and the results were expressed as median interquartile range (IQR). The Spearman rank correlation coefficient was used to test the relationship between 2 variables if data did not follow a normal distribution. A *P* value less than 0.05 was considered significant. The anthropometric, metabolic, and hormonal characteristics of the study participants were provided in Supplementary material, *Tables S1–S3*.

The ethics committee of Poznan University of Medical Sciences (Poznań, Poland) approved the study. All patients provided written informed consent to participate in the study.

Results The median age in the PCOS-obese and PCOS-normal groups and the control group was 24 (21–25.8), 25.5 (21–29), and 23 (22.7–26.5) years, respectively ($P = 0.06$). The PCOS-obese group had a significantly greater waist circumference, hip circumference, and waist-to-hip ratio (WHR) ($P < 0.001$). No differences were observed in waist circumference, BMI, and WHR between the PCOS-normal group and controls ($P < 0.001$) (Supplementary material, *Table S1*).

Both PCOS groups had significantly higher fasting glucose levels than controls, but no significant differences were observed between the study groups. Interestingly, the levels of hemoglobin A_{1C} were similar in PCOS-normal and control groups, but they both were significantly lower than in the PCOS-obese group ($P < 0.001$). The levels of total cholesterol (TC), triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and C-reactive protein significantly differed in the PCOS-obese group compared with the PCOS-normal and control groups (TC < 0.04; all $P < 0.001$) (Supplementary material, *Table S2*).

We observed significantly higher levels of luteinizing hormone and estradiol in the PCOS-obese group compared with the PCOS-normal group ($P < 0.001$ and $P < 0.012$, respectively). There were no other differences in hormonal parameters between those groups (Supplementary material, *Table S3*).

Blood count as well as standard and novel coagulation parameters in the study groups are presented in **TABLE 1**. Leukocyte count significantly differed between controls and the PCOS-obese group ($P < 0.001$). The PCOS-obese group had a significantly higher erythrocyte count than the control and PCOS-normal groups ($P < 0.001$ and $P = 0.04$, respectively). There was a difference in the hematocrit level between controls and the PCOS-obese and PCOS-normal groups ($P < 0.001$ and $P = 0.02$, respectively). Tthe PCOS-obese and PCOS-normal groups differed in terms of fibrinogen levels ($P < 0.001$).

All study groups did not significantly differ with regard to the commonly used coagulation panel (APTT, D-dimer level, PT, international normalized ratio, prothrombin index), except for fibrinogen levels. However, considering the new parameters, we observed significant differences: T10 and OT were shorter in the PCOS-obese group versus controls ($P < 0.012$ and $P = 0.02$, respectively) as well as in the PCOS-normal group versus controls (both $P < 0.001$). AUC30 for flow pressure calculated to assess platelet thrombogenicity was significantly higher in both PCOS-obese and PCOS-normal groups than in controls (both $P < 0.001$). Such differences were not observed when we compared the PCOS-obese group with the PCOS-normal group (both $P > 0.99$). Thus, we assumed that PCOS, rather than body weight, is a factor predisposing to changes in coagulation parameters detected with the novel technique.

In all study subjects, there were correlations among coagulation panel variables, T-TAS results, BMI, and WHR. The body mass index correlated with hematocrit ($R = 0.28$; $P = 0.03$), red blood cell count ($R = 0.34$; $P < 0.02$), and fibrinogen levels ($R = 0.39$; $P < 0.001$). There was no significant correlation of BMI with T-TAS parameters. The waist-to-hip ratio was associated only with hematocrit ($R = 0.28$; $P = 0.03$), APTT ($R = –0.28$; $P = 0.03$), and fibrinogen ($R = 0.27$; $P = 0.04$) levels. The Spearman correlation test showed no significant correlation of T10, AUC30, or OT with the levels of glucose ($R = –0.17$, $P = 0.18$; $R = 0.2$, $P = 0.09$; $R = –0.17$, $P = 0.19$; respectively), insulin ($R = –0.02$, $P = 0.87$; $R = 0.02$, $P = 0.89$; $R = –0.01$, $P = 0.99$; respectively), and hemoglobin A_{1C} ($R = –0.17$, $P = 0.18$; $R = 0.18$, $P = 0.16$; $R = –0.03$, $P = 0.17$; respectively), and the hormonal profile: follicle-stimulating hormone ($R = –0.1$, $P = 0.25$; $R = 0.09$, $P = 0.55$; $R = –0.05$, $P = 0.74$; respectively), luteinizing hormone ($R = –0.09$, $P = 0.53$; $R = 0.11$, $P = 0.49$; $R = –0.16$, $P = 0.3$; respectively), estradiol ($R = –0.008$, $P = 0.95$; $R = –0.05$, $P = 0.75$; $R = 0.06$, $P = 0.71$; respectively), testosterone ($R = 0.2$, $P = 0.12$; $R = –0.19$, $P = 0.2$; $R = 0.16$, $P = 0.29$; respectively).

T10, AUC30, and OT did not correlate with lipid profile parameters: TC ($R = –0.2$, $P = 0.09$; $R = 0.2$, $P = 0.1$; $R = 0.1$, $P = 0.12$; respectively), high-density lipoprotein cholesterol ($R = –0.009$, $P = 0.94$; $R = –0.001$, $P = 0.99$; $R = –0.02$, $P = 0.83$; respectively), low-density lipoprotein cholesterol ($R = –0.04$, $P = 0.73$; $R = 0.06$, $P = 0.63$; $R = –0.05$, $P = –0.69$; respectively).

Interestingly, the triglyceride level was the only parameter in the whole group that showed a significant correlation with T-TAS parameters: AUC30 ($R = 0.3$, $P = 0.02$), T10 ($R = –0.3$, $P = 0.03$), and OT ($R = –0.29$, $P = 0.21$). We did not observe this correlation after dividing the study cohort into subgroups.

TABLE 1 Standard and novel coagulation characteristics of the study patients in the polycystic ovary syndrome–obese, polycystic ovary syndrome–normal, and control groups

Variable	PCOS-obese (n = 26; group 1)	PCOS-normal (n = 23; group 2)	Control (n = 11; group 3)	*P* value[a]	*P* value (comparison)[b]
Standard coagulation parameters					
RBC, T/l	4.9 (3.9–5.6)	4.7 (4–5.4)	4.4 (4.2–4.8)	0.014	1 vs 2: 0.04; 1 vs 3: <0.001; 2 vs 3: 0.04
WBC, g/l	8.2 (4.7–21.3)	6.5 (4.3–9.8)	5.9 (3.6–7.7)	<0.001	1 vs 2: 0.16; 1 vs 3: <0.001; 2 vs 3: 0.3
Hematocrit, l/l	0.4 (0.4–0.5)	0.4 (0.3–0.5)	0.4 (0.4–0.4)	<0.001	1 vs 2: 0.24; 1 vs 3: <0.001; 2 vs 3: 0.02
Platelets, g/l	291 (204–453)	267 (175–410)	272 (194–351)	0.1	–
Antithrombin, %	99 (79–114)	97.5 (72–128)	101 (84–128)	0.4	–
APTT, s	29.8 (26–33.9)	30.7 (25.9–50.3)	29.4 (25.7–37.6)	0.8	–
D-dimer, ng/ml	232.2 (25–624)	159 (112.3–505)	253 (108–371)	0.4	–
Fibrinogen, g/l	3.2 (2.1–5.4)	2.6 (1.8–4.7)	2.8 (2.3–3.4)	0.04	1 vs 2: <0.001; 1 vs 3: 0.13; 2 vs 3: 1.0
PT, s	11.8 (10.8–13.8)	12.1 (11–14.9)	11.9 (10.3–12.6)	0.6	–
INR	1.1 (0.9–1.3)	1.1 (1–1.4)	1.1 (0.9–1.1)	0.6	–
PI, %	93.2 (79.7–101.9)	91.3 (73.8–100)	92.4 (87.3–106.8)	0.4	–
Novel coagulation parameters in the Total Thrombus-formation Analysis System					
T10, s	301 (108–585)	279 (105–1302)	668 (203–1190)	<0.001	1 vs 2: 1.00; 2 vs 3: <0.001; 1 vs 3: <0.012
AUC30	1920.1 (1470.8–2214.9)	1941.7 (414–2173)	1358.9 (42–2074)	<0.001	1 vs 2: 1; 2 vs 3: <0.001; 1 vs 3: <0.001
OT, s	426 (170–782)	406 (261–1690)	800.5 (293–1412)	<0.001	1 vs 2: 1; 1 vs 3: 0.02; 2 vs 3: <0.001

Data are presented as median (interquartile range).

a Kruskal–Wallis test

b Kruskal–Wallis test with the *z* Dunn–Bonferroni multiple comparison test

Abbreviations: AUC30, area under the curve for the first 30 minutes; APTT, activated partial thromboplastin time; INR, international normalized ratio; OT, occlusion time; PI, prothrombin index; PT, prothrombin time; RBC, red blood cells; T10, time of blood clot formation initiation

Discussion To our knowledge, this is the first study assessing whole blood thrombogenicity using the T-TAS in patients with PCOS.

Yildiz et al,[7] as in our study, showed that the levels of commonly used coagulation parameters in patients with PCOS were similar to those observed in the control population, and glucose intolerance and insulin levels did not constitute confounding factors to hypofibrinolysis in PCOS. Similar to our study, Manneras-Holm et al[8] showed that higher levels of fibrinogen in women with PCOS positively correlated with BMI.

Elevated triglyceride levels in both PCOS groups resulted in an increased AUC30 and lower values of T10 and OT. Classic laboratory investigations did not detect it. Lipid abnormalities may activate platelet adhesion and the coagulation pathway and inhibit fibrinolysis. Patients with PCOS have an atherogenic lipid profile, with a tendency towards elevated triglyceride levels.[8] The plasma triglyceride level can increase the expression of plasminogen activator inhibitor 1, which predisposes to the antifibrinolytic state, and an abnormal lipid profile predicts type 2 diabetes later in life better than obesity does.[9] The triglyceride level has also been found to determine hyperandrogenic conditions in patients with PCOS.[10]

The T-TAS offers the advantage of rapidly assessing thrombus formation in more physiological conditions, using whole blood under flow conditions.[11] Importantly, the T-TAS meets all 5 requirements of flow assay–based methods, as described by Roest et al.[12] To date, no studies have evaluated T-TAS parameters in patients with PCOS, so it is impossible to compare our results with any other reports.

To conclude, the main findings of our study were as follows: 1) nonobese and obese patients with PCOS did not differ in terms of standard coagulation parameters, except for fibrinogen levels; 2) there were significant differences in T10, AUC30, and OT between both PCOS groups compared with controls; 3) no significant association of BMI and WHR with T-TAS parameters was noted; and 4) an increased triglyceride level seems to be a relevant factor contributing to the procoagulant state detected with the T-TAS.

Our study had some limitations. We included a small sample as a result of the high costs of the T-TAS procedure, the study had a preliminary design, and no hormonal analysis was conducted in the control group. Studies using larger sample sizes to control such parameters would help to explain the effect of coagulation disorders, reflecting them in T-TAS parameters, on still unexplained disturbances occurring in patients with PCOS, such as implantation failure, elevated miscarriage rates, as well as a tendency to develop cardiovascular complications. Further research is needed to explore whether T-TAS parameters can be used for the prediction and prevention of thromboembolism in that population and could be used to reduce, for example, the risk of subsequent adverse pregnancy outcomes.

SUPPLEMENTARY MATERIAL

Supplementary material is available at www.mp.pl/paim.

ARTICLE INFORMATION

CONTRIBUTION STATEMENT KO designed the model and framework of the study, helped to recruit patients, obtained consent forms, took patients' history, collected anthropometric data, performed analytic calculations, and wrote the manuscript. UM helped to recruit patients, obtained consent forms, took patients' history, and collected anthropometric data and blood samples. PR helped to recruit patients. MO contributed to the concept and design of the study, helped to recruit patients, obtained consent forms, took patients' history, and collected anthropometric data. LP conceived the study concept and evaluated the final version of the manuscript. MK verified the analytical methods and the interpretation of the results, conceived the study concept, and evaluated the final version of the manuscript. All authors provided critical feedback and contributed to shaping the study, analysis, and the manuscript.

CONFLICT OF INTEREST None declared.

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