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

Authors: Katarzyna Ożegowska, Urszula Mantaj, Patrycja Rojewska, Maciej Osiński, Leszek Pawelczyk, Małgorzata Kędzia

Article type: Research letter

Received: July 24, 2020.

Accepted: October 2, 2020.

Published online: October 5, 2020.

ISSN: 1897-9483
Can Total Thrombus-formation Analysis System (T-TAS®) better predict coagulation disturbances than conventional laboratory measurements in patients with polycystic ovary syndrome?

Katarzyna Ożegowska¹, Urszula Mantaj², Patrycja Rojewska³, Maciej Osiński², Leszek Pawelczyk¹, Małgorzata Kędzia²

1 Division of Infertility and Reproductive Endocrinology, Department of Gynecology, Obstetrics and Gynecological Oncology, Poznan University of Medical Sciences; Poznań, Poland.

2 Division of Reproduction, Poznan University of Medical Sciences, Poznań, Poland.

3 Department of Gynecological Endocrinology, Poznan University of Medical Sciences, Poznań, Poland.

T-TAS® in detection of coagulation disturbances in PCOS patients.

Contact information:

Katarzyna Ożegowska, MD, PhD
Division of Infertility and Reproductive Endocrinology, Department of Gynecology, Obstetrics and Gynecological Oncology, Poznan University of Medical Sciences
Polna 33, 60-535 Poznań, Poland
Tel. 61-8-419-412
k.ozegowska@gmail.com
katarzyna.ozegowska@ump.edu.pl

Conflict of interest: none declared
“What’s new?”

In this study, we analyzed and compared conventional coagulation measurements with the results of Total Thrombus-formation Analysis System (T-TAS®),

The main feature of the present study was assessment of whole blood thrombogenicity using T-TAS® in patients with the diagnosis of polycystic ovary syndrome (PCOS). There are many methods used for the assessment of the hemostasis, however standard coagulation assays are insensitive to detect hypercoagulation adequately and thus remain insufficient to evaluate the thrombotic risk. T-TAS®, that enables to evaluate in vitro thrombus formation process under flow condition makes this diagnosis more accurate.

This paper indicated that T-TAS® is a novel method, that better diagnoses coagulatory disturbances in patients with PCOS, which are not detected by the standard coagulatory panel. What is also worth underlying is the fact, that among PCOS patients increased triglycerides (TG) concentration seems to be the most substantial factor causing their procoagulatory status, detected with T-TAS®. As far as we know, this is the first study presenting the use of T-TAS® in PCOS patients.
INTRODUCTION

Polycystic ovary syndrome (PCOS) is an endocrine disorder characterized by anovulation, hyperandrogenism, and polycystic ovaries on the ultrasound and associated with metabolic disorders[1,2]. It also presents with a tendency of thrombosis formation: hypercoagulability and low fibrinolysis[3]. PCOS presents with decreased prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time, and elevated levels of D-Dimers and fibrinogen[4].

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

The aim of our study was the assessment of coagulatory disturbances in women with PCOS using both conventional methods and T-TAS®.

PATIENTS AND METHODS

Patients diagnosed with PCOS according to Rotterdam criteria[1,2], were divided into two groups: “PCOS-obese”- 26 overweight and obese patients [body mass index (BMI)>25kg/m²] and 23 normal weight (PCOS-normal) and compared with 11 healthy controls, which included healthy volunteers with no menstrual cycle irregularities, no clinical hyperandrogenism and no polycystic ovarian morphology on the ultrasound. Controls did not have hormonal evaluation. Medical history and clinical examination were taken. Blood samples for analysis were drawn and analyzed according to standard protocols[2].

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

Statistical analyses were performed using the Statistica version 10 PL software (StatSoft, Inc., Tulsa, OK, USA). Testing for normality of data distribution was performed using the Shapiro-Wilk test. The t-test was used to measure the significance of the difference between 2 continuous variables when data fitted a normal distribution with results presented as mean (SD). In nonnormally distributed data, comparisons were made using the Mann-Whitney test, with results presented as the median and interquartile range (IQR)- first quartile and third quartile. Spearman’s rank correlation coefficient was used to test the relationship between two variables when the data did not follow a normal distribution. A P value of less than 0.05 was considered significant.

Anthropometric, metabolic, and hormonal characteristics were provided to the editor as supplementary material (Table S1, S2, S3).

The Ethics Committee of Poznan University of Medical Sciences (Poznan, Poland) approved the study. We obtained consent form from all the patients.

RESULTS

The median age in the study groups and the control group was 24.0 (21.0-25.8), 25.5 (21.0-29.0), and 23 (22.7-26.5) years, respectively (p=0.06). The PCOS-obese group had significantly higher waist circumference (WC), hip circumference (HC), waist-to-hip ratio (WHR) (p<0.001). No differences were observed in WC, BMI, WHR between PCOS-normal and controls (p<0.001) (Table S1).

Both PCOS groups had significantly higher fasting glucose levels than controls, but no significant differences were observed between study groups. Interestingly the levels of HbA1C were similar in PCOS-normal and control groups, but they both were significantly lower than in the PCOS-obese group (p<0.001). Total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), C-reactive protein (CRP) were significantly different in the PCOS-obese group, comparing to PCOS-normal and controls (TC<0.04; all p<0.001) (Table S2).
We observed significantly higher levels of luteinizing hormone (LH) and estradiol in the PCOS-obese compared to PCOS-normal (p<0.001 and p<0.012, respectively). There were no other differences in hormonal parameters between those groups (Table S3).

Table 1 shows a comparison of blood count, standard, and novel coagulatory parameters. Leukocyte concentration differed significantly between the control group and PCOS-obese (p<0.001). PCOS-obese had significantly higher erythrocytes than the control group and PCOS-normal group (p<0.001 and p=0.04). There was a difference in hematocrit level between the control group and PCOS-obese, PCOS-normal (p<0.001; p=0.02, respectively). Fibrinogen levels differed between PCOS-obese and PCOS-normal groups (p<0.001).

All the groups did not differ significantly according to commonly used coagulation panel [APTT, DD, PT, international normalized ratio (INR), prothrombin index (PI)], except fibrinogen levels. However, when we take into account the new parameters, we observed significant differences: T10 and OT were shorter in PCOS-obese vs. controls (p<0.012, p=0.02, respectively) as well as in PCOS-normal vs. controls (both p<0.001). AUC30 for flow pressure computed to assess platelet thrombogenicity was significantly higher in both PCOS-obese and PCOS-normal than in controls (both p<0.001). Such differences were not observed between both study groups (both p=1.00). Thus we assume, that not body weight, but PCOS itself is the factor predisposing to changes in coagulation parameters detected with this novel technique.

The Spearman correlation in all the studied subjects correlated coagulation panel variables and T-TAS® results with BMI and WHR in. BMI correlated with hematocryte (Rs=0.28; p=0.03); erythrocytes (Rs=0.34; p<0.02) and fibrinogen concentration (Rs=0.39; p<0.001). There was no significant correlation of the BMI with T-TAS®, parameters. WHR correlated only with HT (Rs=0.28; p=0.03), APTT [Rs=(-0.28); p=0.03] and fibrinogen (Rs=0.27; p=0.04) levels. Spearman correlation showed no significant correlation of T10, AUC30 and OT with glucose [(Rs=(-0.17); p=0.18); (Rs=0.2; p=0.09); (Rs=(-0.17); p=0.19), respectively], insulin [(Rs=(-0.02); p=0.87); (Rs=0.02; p=0.89); (Rs=(-0.01); p=0.99), respectively] or HbA1c [(Rs=(-0.17); p=0.18); (Rs=0.18; p=0.16); (Rs=(-0.03); p=0.17), respectively] and hormonal profile: FSH [(Rs=(-0.1); p=0.25); (Rs=0.09; p=0.55); (Rs=(-0.05); p=0.74), respectively]; LH [(Rs=(-0.09); p=0.53); (Rs=0.11; p=0.49); (Rs=(-0.16); p=0.30), respectively]; E2 [(Rs=0.008); p=0.95); (Rs=(-0.05); p=0.75); (Rs=0.06; p=0.71), respectively]; T [(Rs=0.2; p=0.12); (Rs=(-0.19); p=0.20); (Rs=0.16; p=0.29), respectively].
T10, AUC30, or OT did not correlate with lipid profile parameters: TC [(Rs=(-0.2); p=0.09); (Rs=0.2; p=0.10); (Rs=0.1; p=0.12), respectively], HDL [(Rs=(-0.009); p=0.94); , Rs=(-0.001); p=0.99); (Rs=(-0.02); p=0.83), respectively], LDL [(Rs=(-0.04); p=0.73); (Rs=0.06; p=0.63); (Rs=(-0.05); p=-0.69), respectively].

Interestingly the only parameter in the whole group that presented the significant correlation with T-TAS® parameters was the TG concentration: AUC30 (Rs=0.3; p=0.02), T10 [Rs=(0.3); p=0.03] and OT [Rs=(-0.29); p=0.21]. We did not observe it, when divided into separate groups.

**DISCUSSION**

This is the first study assessing the whole blood thrombogenicity using T-TAS® in patients with PCOS.

Yildiz et al., like in our study, showed that commonly used coagulation parameters were comparable to the control population, and glucose intolerance and insulin levels were not confounding factors to hypofibrinolysis in PCOS[7]. Manneras-Holm et al. showed similarly to us that higher levels of fibrinogen in women with PCOS correlated positively with BMI[8].

Elevation of TG in both PCOS groups resulted in the elevation of AUC30 and lower T10 and OT parameters. Traditional analytic methods did not detect his finding. Lipid abnormalities may activate platelet adhesion, coagulation pathway, and inhibit fibrinolysis. PCOS patients have an atherogenic lipid profile, with the tendency to elevated TG[8]. Plasma TG can increase the expression of PAI-1, which predisposes to antifibrinolytic state and impaired lipid profile predicts better type 2 diabetes later in life than obesity[9]. TG was also found to be a determinant of hyperandrogenism conditions in PCOS patients[10].

T-TAS® offers an advantage for rapidly assessing thrombus formation in more physiological conditions using whole blood underflow[11]. Importantly, T-TAS® has all the five requirements of flow assays methods, as described by Roest et al.[12]. There are no previous studies evaluating T-TAS® parameters in PCOS patients, so it is impossible to compare those results with any other studies.

The main findings of our study were as follows:
(1) Non-Obese and Obese Patients with PCOS did not differ according to standard coagulation parameters, except fibrinogen levels;

2) There were significant differences in hemostatic parameters diagnosed with T-TAS® between the PCOS and control group;

3) No significant influence of the BMI and WHR on T-TAS parameters.

4) Increased TG concentration seems to be the substantial factor causing procoagulatory status, detected with T-TAS®

Our study has limitations: small sample size, as a result of the high costs of the T-TAS® procedure and the preliminary form of the study and lack of hormonal analysis in the control group.

Studies using larger sample sizes to control such parameters would help explain the effect of coagulation parameters in the PCOS group. More studies are needed to explore whether T-TAS® coagulation assays can be used to predict and prophylactic prevention of thromboembolism in that population and could be applied to reducing, for example, the risk of subsequent adverse pregnancy outcomes.


Table 1. Standard and novel coagulatory characteristics of subjects in the polycystic ovary syndrome-obese, polycystic ovary syndrome-normal and control group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PCOS-obese (n=26) [1]</th>
<th>PCOS-normal (n=23) [2]</th>
<th>Control (n=11) [3]</th>
<th>P-value*</th>
<th>P-value (comparison) #</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard coagulatory parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E, T/L</td>
<td>4.9 (3.9-5.6)</td>
<td>4.7 (4.0-5.4)</td>
<td>4.4 (4.2-4.8)</td>
<td>0.014</td>
<td>1 vs.2 0.04; 1 vs.3 &lt;0.001; 2 vs.3 0.04</td>
</tr>
<tr>
<td>L, G/L</td>
<td>8.2 (4.7-21.3)</td>
<td>6.5 (4/3-9.8)</td>
<td>5.9 (3.6-7.7)</td>
<td>&lt;0.001</td>
<td>1 vs.2 0.16; 1 vs.3 &lt;0.001; 2 vs.3 0.3</td>
</tr>
<tr>
<td>HT, L/L</td>
<td>0.4 (0.4-0.5)</td>
<td>0.4 (0.3-0.5)</td>
<td>0.4 (0.4-0.4)</td>
<td>&lt;0.001</td>
<td>1 vs.2 0.24; 1 vs.3 &lt;0.001; 2 vs.3 0.02</td>
</tr>
<tr>
<td>PLT, G/L</td>
<td>291.0 (204.0-453.0)</td>
<td>267.0 (175.0-410.0)</td>
<td>272.0 (194.0-351.0)</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>AT, %</td>
<td>99.0 (79.0-114.0)</td>
<td>97.5 (72.0-128.0)</td>
<td>101.0 (84.0-128.0)</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>APTT, s</td>
<td>29.8 (26.0-33.9)</td>
<td>30.7 (25.9-50.3)</td>
<td>29.4 (25.7-37.6)</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>DD, ng/mL</td>
<td>232.2 (25.0-624.0)</td>
<td>159.0 (112.3-505.0)</td>
<td>253.0 (108.0-371.0)</td>
<td>0.4</td>
<td>1 vs.2 &lt;0.001; 1 vs.3 0.13; 2 vs.3 1.0</td>
</tr>
<tr>
<td>fibrinogen, g/L</td>
<td>3.2 (2.1-5.4)</td>
<td>2.6 (1.8-4.7)</td>
<td>2.8 (2.3-3.4)</td>
<td>0.04</td>
<td>1 vs.2 &lt;0.001; 1 vs.3 0.13; 2 vs.3 1.0</td>
</tr>
<tr>
<td>PT, s</td>
<td>11.8 (10.8-13.8)</td>
<td>12.1 (11.0-14.9)</td>
<td>11.9 (10.3-12.6)</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>INR</td>
<td>1.1 (0.9-1.3)</td>
<td>1.1 (1.0-1.4)</td>
<td>1.1 (0.9-1.1)</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>PI, %</td>
<td>93.2 (79.7-101.9)</td>
<td>91.3 (73.8-100.0)</td>
<td>92.4 (87.3-106.8)</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td><strong>Novel coagulatory parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T10, s</td>
<td>301.0 (108.0-585.0)</td>
<td>279.0 (105.0-1302.0)</td>
<td>668.0 (203.0-1190.0)</td>
<td>&lt;0.001</td>
<td>1 vs.2 1.00; 2 vs.3 &lt;0.001; 1 vs.3 &lt;0.012</td>
</tr>
<tr>
<td>AUC30</td>
<td>1920.1 (1470.8-2214.9)</td>
<td>1941.7 (414.0-2173.0)</td>
<td>1358.9 (42.0-2074.0)</td>
<td>&lt;0.001</td>
<td>1 vs. 2 1.00; 2 vs.3 &lt;0.001; 1 vs.3 &lt;0.001</td>
</tr>
<tr>
<td>OT, s</td>
<td>426.0 (170.0-782.0)</td>
<td>406.0 (261.0-1690.0)</td>
<td>800.5 (293.0-1412.0)</td>
<td>&lt;0.001</td>
<td>1 vs.2 1.00; 1 vs.3 0.02; 2 vs.3 &lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as median (interquartile range).

*Kruskal-Wallis test

#Kruskal-Wallis test with z Dunn-Bonferronic multiple comparison test;

Abbreviations: AUC30, area under the curve for the first 30 minutes; APTT, activated partial thromboplastin time; AT, antithrombin; DD, d-dimers; E, erythrocytes; HT, hematocrit; INR, international normalised ratio; OT, occlusion time; PI, prothrombin index; PLT, platelets; PT,
prothrombin time; T10, time of blood clot formation initiation
Contributon statement:

KO designed the model and the framework of the study, helped to recruit the patients, took consent forms, patients history, anthropometric measurements performed the analytic calculations, wrote the manuscript.

UM helped to recruit the patients, took consent forms, patients history, anthropometric measurements, blood samples,

PR helped to recruit the patients

MO helped with the idea and design of the study, helped to recruit the patients, took consent forms, patients history, anthropometric measurements

LP encouraged to investigate and supervised the findings of this work, evaluated the final version of the manuscript

MK verified the analytical methods, interpretation of the results, encouraged to investigate and supervised the findings of this work, evaluated the final version of the manuscript

All authors provided critical feedback and helped shape the research, analysis and manuscript.