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# The procoagulant phospholipid dependent clotting time and the initiation phase of thrombin generation test are mandatory for the evaluation of the risk for severe early onset preeclampsia

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# Thrombosis Research

The procoagulant phospholipid dependent clotting time and the initiation phase of thrombin generation test are mandatory for the evaluation of the risk for severe early onset preeclampsia.

--Manuscript Draft--

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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Dear Editors,

following your recommendations, we modified our paper and transformed it to a Letter to the Editor by including only the part of the predictors of preeclampsia severity in order to keep the main message and to respect the limit of 1500 words. As recommended, we included one Table and one Figure.

Kind regards

Grigoris Gerotziafas

**The procoagulant phospholipid dependent clotting time and the initiation phase of thrombin generation test are mandatory for the evaluation of the risk for severe early onset preeclampsia.**

Patrick Van Dreden<sup>1,2</sup>, Eleftheria Lefkou<sup>1,3</sup>, Aboubakar Ka<sup>1</sup>, Konstantinos Sfakianoudis<sup>4</sup>, Aurélie Rousseau<sup>1,2</sup>, Grigoris T Geroziakas<sup>1,5</sup>

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**Keywords:** preeclampsia, procoagulant phospholipid, thrombin generation, pregnancy

## Dear Editor

Preeclampsia occurs in up to 7% of pregnancies in Western Countries and is a leading cause of maternal and fetal morbidity and mortality.<sup>1</sup> Classification of preeclampsia is based on the timing of symptoms' onset and the severity of the clinical status. Accordingly, early and late preeclampsia are characterized by symptom onset before or after 32-34 weeks of gestation respectively.<sup>2</sup> Pathogenesis of preeclampsia is characterized by abnormal placental vascular development with impaired invasion of the uterine spiral arteries by trophoblasts, resulting in fetal complications and maternal endothelial dysfunction.<sup>3</sup> Intact antithrombotic properties of the endothelial cells and efficient regulation of thrombin generation and platelet activation at the microenvironment of the uterus are necessary conditions for successful trophoblast invasion and development of fetomaternal circulation during normal pregnancy.<sup>4,5</sup> Prompt identification of pregnant women at risk of preeclampsia worsening could help to anticipated therapeutic intervention and improvement of the clinical outcome. The ROADMAP-Early Onset Preeclampsia study (ROADMAP-EOP) aiming to respond to this unmet need assessed the clinical relevance of biomarkers of hypercoagulability and endothelial activation for prompt identification of pregnant women at high risk of preeclampsia.

An observational single center retrospective case-control study was conducted in pregnant women diagnosed with preeclampsia from July 2020 to July 2021. Pregnant women were retrospectively enrolled upon EOP diagnosis and stratified in mild EOP group (n=34) and severe EOP group (n=15 according to the criteria of the International Society for the Study of Hypertension in Pregnancy<sup>6</sup>). Eligible women were included in the study before initiation of any treatment for preeclampsia. Exclusion criteria were: age  $\leq 18$  years, patients receiving prophylactic or therapeutic anticoagulant therapy, or aspirin, or with personal history of venous or arterial thrombosis, inflammatory or autoimmune disorders, cancer, diabetes, obesity, chronic hypertension, renal, or hepatic diseases, acute infections, known inherited thrombophilia.

The control group (n=35) consisted of women with uncomplicated pregnancy. Blood samples were collected at the time of clinical diagnosis of preeclampsia, before starting any treatment, in the frame of routine hematological exploration. Citrated platelet-poor plasma (PPP) was prepared within 1 hour of blood collection by double centrifugation (2 x 15 minutes at 2500 g) at room temperature to perform routine blood coagulation tests. The residual volume of PPP was stored in aliquots at -80°C until analysis. All

women were assessed with thromboelastometry, *Calibrated Automated Thrombogram*<sup>®</sup>, tissue factor activity (TFa), Procoagulant phospholipid dependent clotting time (Procoag-PPL), Proteins S, TFPI, D-dimer, antithrombin, thrombomodulin, fibrinogen prothrombin time and activated partial thromboplastin time. Primary and secondary end points were severe EOP and EOP respectively. Principal component analysis (PCA) was performed to check for associations among the biomarkers and the clinical outcome. The number of patients included in the study was calculated so that the model for the prediction of significant CAD had to be constructed according to the rule of thumb, the so-called events per variable (EPV) 10-1.<sup>7,8</sup>

The study was conducted in Greece, in accordance with the Helsinki declaration. All haematological tests were performed in the frame of routine monitoring of patients. This is a non-interventional data-based research. There is no processing of indirectly identifiable data, or chaining with data from other sources, or long-term patient follow-up for this research. This study was approved by the institutional review board (Reference 1.0 of 03/07/2019) according to the Greek regulations. All participants gave consent to participate.

There were no significant differences regarding age, gravidity, gestational age and the body mass index (BMI) between EOP groups and normal pregnancy as well as within the EOP groups. Patients had significantly higher blood pressure than the control group. Platelet counts were similar in healthy pregnant women, and mild EOP group, and significantly lower in severe EOP ( $p < 0.05$ ). No other statistically significant differences were found between-groups.

We performed exploratory data analysis of 26 biological predictors by applying PCA for making predictive models for the primary and secondary end points (mild EOP and EOP). The PCA showed that the repartition of data allowed to discriminate the patients with EOP from healthy pregnant women as well as patients with mild EPO from those with severe EOP. When biomarkers of hypercoagulability were considered according to the severity of EOP in PCA analysis the lag-time and PPL clotting time were independently associated with the risk of severe EOP (Table 1).

The increase of the lag-time by 1 min was associated with a 20.2-fold increase of the risk of severe EOP. The marginal effects curve shows that for a lag-time longer than 2.6 min, the probability of severe EOP was higher than 50% (Figurer 1, Frame A).

Longer Procoag-PPL clotting time was associated with lower risk of severe EOP. The marginal effects curve, showed that for Procoag-PPL clotting time longer than 58.75 sec the probability of severe EOP decreased by 50%. (Figure 1; Frame B).

These data suggest that a biological score composed of the Procoag-PPL<sup>®</sup> clotting time and thrombin generation (Thrombogram-Thrombinoscope<sup>®</sup> PPP-reagent 1 pM-TF assay) could be a useful tool to identify pregnant women with high probability of EOP. An external validation of the proposed methodology needs to be performed in a large prospective observational study.

The implication of plasma hypercoagulability, enhanced thrombin generation and impaired fibrinolysis and endothelial cell activation in the manifestation of severe preeclampsia has been shown in recent studies.<sup>9,10</sup> The findings of the ROADMAP-EOP study support this concept.

The present study has some limitations. The monocentric design did not allow the evaluation of the potential bias induced by local practices in the management of pregnant women at high risk of vascular complications. This issue must be explored in a prospective study and this is the aim of the ongoing ROADMAP-EOP project. The findings of the present study are restricted to the assessment of procoagulant phospholipids and thrombin generation in plasma using only the assays and the reagents employed. The performance of other methods which measure thrombin generation (i.e. in-house assays, other combinations of reagents or other techniques available in the market) should be assessed in suitably designed prospective studies.

In conclusion, the Procoag-PPL<sup>®</sup> clotting time and thrombin generation assay reflect cellular derived hypercoagulability and allow accurate screening of pregnant women for the prompt identification of those at risk of EOP and the evaluation of its severity. This concept has to be validated in a large prospective cohort trial.



## Tables and Figures

Predictors	Odds ratio	95% Confidence Interval	p-value
Lag-time (min)	20.2	1.58 - 47.2	0.033
Procoag-PPL (sec)	0.89	0.80 - 0.98	0.020

**Table 1.** Principal component analysis for the risk of severe EOP.

**Figure 1.** Marginal effects curve of predictors for severe EOP following PCA analysis. The plot shows the predicted values for the response at each value of the lag-time (frame A) and Procoag-PPL clotting time (frame B).

### Declarations

### Founding Source

There is no founding source for this study

### Acknowledgments

The authors wish to thank Stago for offering assays and reagents for the measurement of the studied biomarkers of hypercoagulability in the frame of institutional and unconditional collaboration with the Research Group “Cancer, Haemostasis-Angiogenesis” INSERM UMR\_S 938, Sorbonne Université.

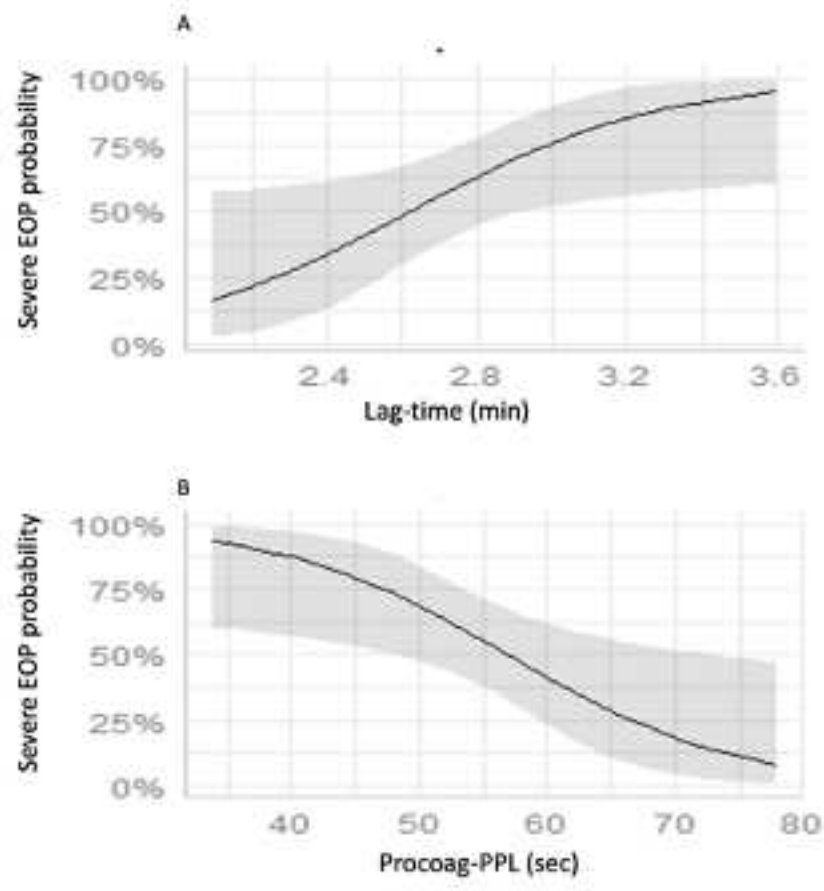
This paper is part of the Project ROADMAP-EOP study for the identification of biomarkers of hypercoagulability relevant for the risk of vascular complications in pregnancy.

### Conflicts of Interest

The authors, except Dr P. Van Dreden have no conflict of interest to declare for this study. Dr P. Van Dreden is employee of Stago.

### References

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women were assessed with thromboelastometry (on fresh citrated whole blood on ROTEM delta, Tem Innovations GmbH, Werfen, using the INTEM® reagent), Calibrated Automated Thrombogram® from Diagnostica Stago, (Gennevilliers, France), tissue factor activity (TFa), Procoagulant phospholipid dependent clotting time (Procoag-PPL), Proteins S, TFPI, D-dimer, antithrombin, thrombomodulin, fibrinogen prothrombin time and activated partial thromboplastin time. All assays were from Diagnostica Stago, (Asnières, France). Primary and secondary end points were severe EOP and EOP respectively. Principal component analysis (PCA) was performed to check for associations among the biomarkers and the clinical outcome. The number of patients included in the study was calculated so that the model for the prediction of significant CAD had to be constructed according to the rule of thumb, the so-called events per variable (EPV) 10-1.<sup>7,8</sup>

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**Figure 1.** Marginal effects curve of predictors for severe EOP following PCA analysis. The plot shows the predicted values for the response at each value of the thrombogram lag-time which is informative on the initiation phase of thrombin generation (frame A) and Procoag-PPL clotting time which is inversely correlated with the concentration of pro-coagulant phospholipids in plasma (frame B).

### Declarations

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There is no founding source for this study

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75012 Paris, France  
Paris 23/6/2022

Dear Editors,

We wish to thank the reviewers for their comments on the Letter to the Editor entitled “The procoagulant phospholipid dependent clotting time and the initiation phase of thrombin generation test are mandatory for the evaluation of the risk for severe early onset preeclampsia”.

Kind regards

Grigorios Gerotziafas

#### **Answers to review**

We added, in the main text, in the Table and the Figure the definition on the lag time of thrombin generation.

