

COVID-19 in Pregnant Patients at Term: Evolution of Coagulation Factors in Mild Forms throughout the Pandemic: Conventional Analysis and Logistic Regression

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ABSTRACT

Aim: The evolution of coagulation factors in pregnant patients infected with SARS-CoV-2 during the pandemic is still debated. However, few studies have been carried out to evaluate the silent alterations of blood values in mild forms of the disease.

Methods: A total of 153 pregnant patients with an asymptomatic form of COVID-19 and 306 healthy pregnant patients, who were admitted for delivery in our hospital between April 1, 2020 and March 1, 2022, were studied. The blood values harvested closest to the time of delivery were considered.

Results: There was a significant variability in values of fibrinogen, prothrombin time, though these were still within normal limits.

Conclusions: Pregnant patients with mild forms of COVID-19 displayed some blood alterations, even if they were asymptomatic for COVID-19.

Keywords: mild COVID-19, pregnancy, fibrinogen, prothrombin time.

ABBREVIATIONS

COVID-19=coronavirus disease 2019

SARS-CoV-2=severe acute respiratory syndrome coronavirus 2

INTRODUCTION

The clinical behavior of COVID-19 disease was mostly mild (1). Pregnant patients often show less symptoms and present a milder form of COVID-19 compared with non-pregnant patients, but they have a higher risk of admittance to the intensive care unit. SARS-CoV-2 infected pregnant patients may have a higher risk of adverse perinatal outcomes (2, 3).

The evolution of coagulation factors in pregnant patients infected with SARS-CoV-2 during the pandemic is still debated. During the pandemics, pregnant women needed special attention, and laboratory changes relevant to evaluate the coagulation state included D-dimer, prothrombin time (PT), activated partial thromboplastin time (APTT), platelet count and fibrinogen (4).

Debates regarding the different aggressivity of different variants of concern of the SARS-CoV-2 virus have gone on for some time. These have increased or decreased both the severity of the disease and its transmissibility. In comparison with the pre-pandemic period, stillbirths and maternal and neonatal deaths remained stable, but the need for ventilatory support and/or ICU admission among women with pneumonia increased during the Alpha-variant period com-

pared with the wild-type period (5). In France, the risk of developing a severe-to-critical infection was three times greater during the Alpha period than the wild-type one (6) and in Brazil, the risk was twelve times greater during the Gamma period than the wild-type one (6). Nobody compared them in Romania so far.

Infection with a mild form of COVID-19 may suggest that, since the disease is mild, with almost no symptoms, there will be no ongoing danger after recovery. Serwin (7) showed that, even in mild forms of COVID-19, there were blood biochemical alterations similar to those observed in patients with symptomatic COVID-19 disease at one-month follow-up.

The aim of this work was to study the coagulation factors in pregnant patients at term, in asymptomatic forms of disease, in different periods of time during the pandemic, when different virus variants of concern were present. □

MATERIALS AND METHODS

In a case-control study, all pregnant patients who were admitted for delivery at term (37-41 weeks of gestation) in "Elena Doamna" Obstetrics and Gynecology University Hospital in Iasi, Romania, from April 1st 2020 to March 1st 2022, were included. Only patients who tested positive for SARS-CoV-2 RT-PCR and presented a mild form of infection were included in the present study, while those with blood diseases (leukemia, thrombocytopenia), systemic diseases (kidney failure) or autoimmune diseases (thyroiditis, lupus) were excluded from the study.

Patients were considered to have a mild form of disease if they had no symptoms (e.g., cough, fever, sore throat, headache or diarrhea).

There were four groups of patients, according to the period of time they were admitted and the type of virus that was circulating: group 1 (all 2020, original SARS-CoV-2 virus), group 2 (spring 2021, alpha variant), group 3 (autumn 2021, delta variant) and group 4 (spring 2022, delta and omicron variants) (8). There were time intervals (months) between these groups when there were no SARS-CoV-2 positive patients requiring hospital admission. Though we labeled group 2 as spring 2021 and group 3 as autumn 2021, this was done to simplify understanding and description. The real time intervals were as follows: group 1 spanned from April 2020 to December 2020, group 2 from January 2021 to June 2021, group 3 from August 2021 to November 2021 and group 4 from December 2021 to March 2022 (the latter is ongoing, but we stopped gathering data on March 1st 2022). There were 43 patients in group 1, 24 in group 2, 48 in group 3 and 38 in group 4, or 153 patients altogether. For each group of patients, we chose a control group, having double the number of the study group: starting with the day of admittance of the first patient in each study group, we calculated the consecutive patients until number was fulfilled. Patients who had no complete blood analysis values were not considered in the control groups.

The two groups were similar as regarded age, gestation number, parity number and gestational age ($P>0.05$). The patients in the four study and four control groups showed no differences in age, gestation number, gestational age or parity number ($P>0.05$).

Patients' blood was harvested for analysis and RT-PCR testing upon admittance. When required, blood analysis was repeated or other blood components were determined. In this study, the blood values harvested closest to the time of delivery were considered. We performed a comparison of coagulation factors between the COVID-19 pregnant patients and healthy pregnant patients, then between the four groups of COVID-19 patients. Then we applied logistic regression to study the impact of the five coagulation factors on the probability of SARS-CoV-2 infection in patients.

Written informed consent was obtained from all patients involved. The study was approved by the Ethical Committee of "Elena Doamna" Obstetrics and Gynecology University Hospital in Iasi (Approval number 4/ April 2nd, 2020).

The SPSS version 18 was used, from PASW Statistics for Windows, SPSS Inc., Chicago, IL, USA. To describe the data, we calculated the mean values and standard deviations, as well as the median and quartile values. The non-parametric Kruskal–Wallis test was used for comparisons and Shapiro–Wilk test was used for normality examination. For statistical decision, a 0.05 p value was considered the cutoff for significance. $\square$

RESULTS

Coagulation factors

Coagulation factors in the two groups show significant differences (Table 1).

We further studied these values in the four groups of COVID-19 pregnant patients. There was a significant difference between the values of fibrinogen and prothrombin time in the four groups of COVID-19 pregnant patients (Table 2). The differences in fibrinogen and prothrombin time between the four groups were further stu-

Values	COVID n=153	Control n=306	P
APTT	33.1	31.6	0.012
Fibrinogen	348.1	344.4	0.857
Prothrombin time	12.53	13.22	0.003
INR	1.00	1.03	0.003
Prothrombin activity	103.45	98.12	0.004

TABLE 1. Mean values of coagulation factors in the study and control groups

APTT=activated partial thromboplastin time;

INR=international normalized ratio.

Values /Groups	1	2	3	4	P
APTT	31.99	34.67	34.42	31.64	0.056
Fibrinogen	308.20	363.50	342.40	380.70	<0.01
Prothrombin time	12.95	12.46	12.43	12.21	<0.01
INR	1.00	0.99	0.99	1.01	0.35
Prothrombin activity	100.22	104.70	106.86	102.02	0.49

APTT=activated partial thromboplastin time;

INR=international normalized ratio.

TABLE 2. Mean values of coagulation factors values in the four groups of COVID-19 pregnant patients

TABLE 3. P values of significant differences between the four groups of patients, taken two by two, for fibrinogen and prothrombin time values

Groups compared	Fibrinogen	Prothrombin time
1-2	.00	.23
1-3	.00	.08
1-4	.00	.005
2-3	1.00	1.00
2-4	.02	1.00
3-4	.00	1.00

Group 1=year 2020, group 2=spring 2021, group 3=autumn 2021, group 4=spring 2022. Significance values adjusted by the Bonferroni correction for multiple tests

died (Table 3). Only values of fibrinogen in spring 2021 were close to those obtained in autumn 2021. All other values showed a tendency toward significant growth between 2020 patients and 2022 patients. There was a significant difference between the prothrombin time values of 2020 and spring 2022. However, all the other groups showed close values, and all values slowly decreased from 2020 to spring 2022.

Both fibrinogen and prothrombin time revealed increases in the coagulability of pregnant patients at term, from 2020 to 2022, as a result of changes in SARS-CoV-2 virus variants, even if the values were still within normal limits and patients were asymptomatic.

Logistic regression

We defined the two possible outcomes for the studied pregnant patients: SARS-CoV-2 positive (coded with 1) and SARS-CoV-2 negative (coded with 0).

In order to find which of the five studied coagulation factors were changing the probability of outcome (SARS-CoV-2 infection), we have used both forward selection and backward elimination methods. From the five coagulation factors studied by us, we found out that only three covariate (APTT, prothrombin time and prothrombin activity), grouped by two, were significant.

According to forward selection method, the significance and effect measured by the coefficients are described in Table 4. Variables con-

TABLE 4. Forward selection. Coagulation factors

Coefficients	B	Sig.	OR: Exp(B)	95% C.I. for EXP(B)	
				Lower	Upper
APTT	.084	.000	1.088	1.038	1.140
Prothrombin activity	.013	.009	1.013	1.003	1.023
Constant	-4.740	.000	.009		

OR=odd ratio; Sig.=significance p; C.I.=confidence interval
Variables considered for analysis: APTT, fibrinogen, prothrombin time, INR, prothrombin activity

sidered for analysis included APTT, fibrinogen, prothrombin time, INR, and prothrombin activity; APPT varies between 20-40 units. Increasing APPT with 1 unit will have a risk of getting the infection in the Odd Ratio (OR) space of 1.088. Increasing APPT by 5, the result will be $OR = \exp(5 \cdot 0.084) = \exp(0.42) = 1.52$. Prothrombin activity varies between 50-139; for prothrombin activity, $OR = 1.013$. Increasing prothrombin activity by 10 will generate $OR = \exp(0.013 \cdot 10) = \exp(0.13) = 1.14$.

By backward elimination method we computed the coefficients (Table 5). APPT is again significant and the effect is similar: OR for 1 unit increase is 1.092, OR for 5 units increased is $OR(+5) = 1.55$. Prothrombin time ranges between 10 and 135. The effect is negative and the probability of getting the virus is lowered if prothrombin time is increasing with 1, $OR = 0.807$. Increasing prothrombin by 10 means $OR = \exp(-0.214 \cdot 10) = \exp(-2.14) = 0.117$ or the chance of not getting the virus is reflected by the $OR = 1/0.117 = 8.49$.

We split the data in the four groups defined by the time period: 1 (year 2020), 2 (spring 2021), 3 (autumn 2021) and 4 (spring 2022). In order to find which of the covariates were changing the probability of outcome (SARS-CoV-2 infection) between the four time periods/groups studied by us, we again used both forward selection (Table 6) and backward elimination me-

TABLE 5. Backward elimination. Coagulation factors

Coefficients	B	Sig.	Exp(B)	95% C.I. for EXP(B)	
				Lower	Upper
APTT	.088	.000	1.092	1.041	1.144
Prothrombin time	-.214	.010	.807	.686	.949
Constant	-.800	.522	.449		

TABLE 6. Forward selection. Coagulation factors and time periods

Group	Coefficients	B	Sig.	OR Exp(B)	95% C.I. for EXP(B)	
					Lower	Upper
1	Fibrinogen	-0.13	.047	.987	.974	1.000
	Prothrombin time	-0.682	.001	.506	.337	.758
	Constant	12.438	.001	252280.4		
2	Constant	-4.498	.010	.011		
	Fibrinogen	0.011	.026	1.011	1.001	1.021
3	Constant	-6.65	.000	.001		
	APTT	0.183	.000	1.201	1.097	1.314

thods. The same variables, including APTT, fibrinogen, prothrombin time, INR, and prothrombin activity, were introduced in the analysis.

For the last period denoted by 4 (spring 2022) there were no significant coefficients. From backward elimination method we found similar results, the algorithm converged in the same points. □

DISCUSSION

In the pandemic generated by SARS-CoV-2, due to the physiologic hypercoagulability generated by pregnancy, pregnant women were at higher risk (9-11) for both themselves and their babies than non-pregnant patients. Pregnant patients with COVID-19 were exposed to an increased risk of thromboembolism with much higher D-dimer levels than uninfected pregnant ones (12).

Most pregnant women had mild forms of disease (11, 13-18). Still, disseminated intravascular coagulation did complicate mild or asymptomatic COVID-19 in pregnant patients (19). Our work, however, showed that the mild form may be less mild than has been assumed.

From the coagulation factors studied by us, we showed that fibrinogen and prothrombin time had a significant evolution throughout the pandemic. Fibrinogen values significantly increased from 2020 to spring 2022, and prothrombin time slowly decreased, even if they were still within normal limits. There was a gradual increase in the capacity of virus variants to stimulate coagulation. Pregnant patients already have a hypercoagulability state, and each variant

increased their risk of thrombosis. This was in accordance with Garcia-Espinoza (1), who found that fibrinogen and D-dimers remained unchanged in Covid-19 positive Mexican pregnant patients. It was also in accordance with Januszewski (20), who found a longer activated partial thromboplastin time (APTT), a reduced prothrombin time (PT), and lower platelet count at initial presentation in SARS-CoV-2 infected pregnant patients. On the other hand, reverse evolution, meaning increasing prothrombin time, associated with decrease in fibrinogen, indicated a negative evolution during COVID-19 (21), and would have been much worse.

Iba (22) reported increased fibrinogen and normal prothrombin time at the beginning of SARS-CoV-2 infection. We reported them as normal in the mild form of infection, but with significant variability between different viral variants.

By logistic regression we found out that, out of the five coagulation factors studied by us, only three covariates (APTT, prothrombin time and prothrombin activity), grouped by two, were significant in predicting the output. Taking into account all data, we found that increasing APPT with 1 unit would generate OR=1.088, and increasing APPT by 5, OR=1.52. For prothrombin activity, OR=1.013, and increasing prothrombin activity by 10 would generate OR=1.14. By backward elimination method, APPT is again significant and the effect is similar: for 1 unit increase OR=1.092, and for increase by 5, OR=1.55. Prothrombin time has a negative effect and the probability of getting the virus is lowered if prothrombin time is increasing with 1, OR=0.807. Increasing prothrombin by 10 means OR=0.117, or the chance of not getting the virus is reflected by the OR=1/0.117=8.49.

For group 4 (spring 2022) there are no significant coefficients. The effect of fibrinogen and prothrombin time is positive in group 1 (2020), that is the increase of covariates generated a decrease of the probability of getting the virus. Fibrinogen has a negative effect in group 2, it is a risk factor and also APTT represents a risk factor in group 3. □

CONCLUSION

From all the coagulation factors studied by us, there was a significant difference only between the values of fibrinogen and prothrombin

time in the four groups of COVID-19 pregnant patients. Regarding fibrinogen, only its values obtained in spring 2021 were close to those measured in autumn 2021; all other fibrinogen values show a tendency toward significant growth between 2020 patients and 2022 ones. Regarding prothrombin time, there was a significant difference between its values measured in 2020 and those obtained in spring 2022. However, all the other groups showed close prothrombin time values, and all values slowly decreased from 2020 to spring 2022.

According to forward selection method, increasing APPT with 1 unit will have a risk of getting the infection in the Odds Ratio (OR) space of 1.088. For prothrombin activity: OR=1.013. By backward elimination method, APPT is again significant and the effect is similar: OR for 1 unit increase is 1.092. The effect is negative and the probability of getting the virus is lowered if prothrombin time is increasing with 1, OR=0.807.

In mild forms of COVID-19, in pregnant patients at term, significant variability in values of fibrinogen and prothrombin time occurred – though still within normal limits. $\square$

Conflicts of interest: none declared.

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Institutional Review Board statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of “Elena Doamna” Obstetrics and Gynecology University Hospital in Iasi, Romania (approval number 4/ April 2nd, 2020).

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