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PhD THESIS

**REGIONAL STUDY REGARDING PREGNANCY
OUTCOME IN SARS-COV-2 POSITIVE PREGNANT
PATIENTS AND IPD META-ANALYSIS ON
VERTICAL TRANSMISSION ON THE
CORONAVIRUS**

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RESUMEE

The appearance of COVID-19 disease was unexpected for the scientific and medical communities. Although much more under control thanks to revolutionary breakthroughs, particularly in the field of prevention, the pandemic causes fewer fatalities than in previous years. In spite of the significant progress made in a very short amount of time in terms of diagnosing, treating, and preventing the SARS-CoV-2 infection in adults, pregnant women, and children, the infection of the fetus is still not completely understood.

Pregnant women, who are known to be at a greater risk for severe illness when they are symptomatic, may be adversely affected by SARS-CoV-2 infection, and this can have catastrophic consequences. According to the research that is currently available, pregnant women are more susceptible to severe COVID-19 complications [6], [7], [8]. These complications include acute respiratory distress syndrome, acute renal failure, thrombo-embolic events, and other unfavorable cardiac events. As a direct consequence of this, these women have a significantly increased risk of requiring invasive respiratory support, admission in an ICU, or ECMO. Infected pregnant patients who are in poor condition may need to have labor induced, which may result in preterm delivery with all of the problems that come along with it.

Vertical transmission is defined as the transfer of the pathogen from the mother to the fetus. The infection can occur during pregnancy (via the transplacental route), during birth (when the fetus is in contact with the mother's reproductive tract), or during breastfeeding. Congenital infections are vertically transmitted from mother to fetus during pregnancy, birth, or breastfeeding.

As far as the SARS-CoV-2 is concerned, there is still a lot of controversy regarding its potential of in-utero transfer mostly. Molecular research, based on prior studies of other SARS viruses, dismissed the likelihood of vertical transmission at the beginning of the pandemic, but recent data emerging from infected fetuses begs to difference. Generally speaking in order for transplacental transmission to occur, 2 conditions are mandatory: maternal viremia (even for a short period of time) and

placental tropism. When it comes to viruses, the concept of placental tropism can be interpreted in a number of different ways, recent studies suggesting the high affinity of the virus towards the ACE-2 and NRP1 receptor. Placental infection could occur through clathrin mediated endocytosis, through direct invasion secondary to severe injury of the syncytiotrophoblast or through ascending route.

There is still a lot of conflicting data in the literature related to vertical transmissions, and large studies report a rate of 1.8% to 5.3% of vertical transmission, however none of the studies mention by which standard was vertical transmission diagnosed. Three diagnostic algorithms for maternal-fetal infection have been published thus far; these call for staged collection, usually under sterile conditions (amniotic fluid before the rupture of the membranes, blood from the umbilical cord, and newborn venous blood), at specific times after birth. It may also be advised to evaluate the placenta using PCR, electron microscopy, immunohistochemistry or in situ hybridization. In the absence of regional guidelines, the arduous nature of implementing the recommendations may result in the underreporting of this infection.

However, it is important to identify the coronavirus' vertical transmission since recent research on infants born to women who had the virus while pregnant indicates a higher risk of neurodevelopment delay, and ophthalmological sequela.

This thesis provides a comprehensive study of SARS-CoV-2 prenatal infection in the mother and in the fetus, focusing mostly on the fetal outcomes in case of congenital infection. The topic is significant and timely, particularly in light of the fact that more and more researchers have reported a higher incidence of neurodevelopmental delay in one-year-old children born to infected mothers during pregnancy, as well as a higher incidence of ophthalmological sequelae in these cases.

The thesis analyzes data pertaining to birth outcomes in pregnant women with SARS-CoV-2 infection, as well as the occurrence of vertical transmission of the virus and potential risk factors for unfavorable fetal/neonatal prognosis in cases of congenital infection. The significance of the latter aspect of the research lies in its practical implications for medical professionals, specifically obstetricians and neonatologists, who can use this information to guide their management of such cases. The scientific

objectives of this thesis are to present the most recent findings on the molecular basis of vertical transmission of the SARS-CoV-2 infection, to investigate the mode of termination of pregnancies with infection at delivery, and to examine the effects of maternal infection on newborns, with a focus on infected newborns.

There are two primary components to the current thesis.

The general part contains a study of what is currently known regarding vertical transmission of the virus. The main subjects of the study include algorithms for diagnosing congenital coronavirus infection as well as the molecular mechanism of transplacental transmission, both of which are contentious issues in the specialist literature.

The specific part included a 20-month regional inquiry that was designed to look at how births turned out when the pregnant woman was infected and what were the neonatal consequences. The research was broadened internationally since there were only two instances of neonates that tested positive for SARS-CoV-2 at the time of birth throughout the study period. This chapter's second part has looked at all published cases of vertical transmission and has since undergone IPD meta-analysis. The research first looked at whether actual vertical transmission occurred (using accepted standards) and what were the consequences of the infection on the fetus/newborn in these situations. Further, potential risk factors for adverse fetal/neonatal outcome such as: stillbirths, presence of symptoms at the time of birth, neonatal death and the existence of 2019-nCoV sequelae at the time of newborn discharge from the hospital. The results of both studies have been published in papers that are listed on Clarivate Web of Science.

Study number 1, entitled "Regional study on the outcome of pregnancy in cases of maternal infection with SARS-CoV-2", presented in chapter 2 aimed at evaluating the birth of infected patients diagnosed with SARS-CoV-2 infection, focusing in particular on the consequences for newborns.

This retrospective study employed cross-sectional methods. It covered the pandemic's first three waves, from April 1, 2020 to November 20, 2021. It took place in the Obstetrics and Gynecology Department at Timisoara's Clinical County Hospital, a tertiary medical center in western Romania. This hospital was one among the facilities

ordered by the Romanian Ministry of Health to accept and treat western SARS-CoV-2-infected women with obstetrical or gynecological difficulties. This department accepted exclusively SARS-CoV-2-positive patients from April 1 to May 31, 2020. After this time, uninfected patients were admitted up to a quota. The institution began treating SARS-CoV-2 patients. 397 SARS-CoV-2 positive patients were discharged. Some of these patients had gynecological issues, while others were discharged while pregnant or after delivery.

Out of the 272 pregnant women with SARS-CoV-2 positive RT PCR test, 97 patients were eligible, excluding patients with multiple pregnancy, childbirth under 24 weeks and those who had unknown status in terms of vaccination against SARS-CoV-2 infection. The 97 patients were stratified according to their vaccination status and clinical status at the time of delivery. Overall, 35 patients were vaccinated against 2019-nCoV, the Comirnaty vaccine (BioNTech and Pfizer) being administered in all cases, and 62 unvaccinated patients (all showed symptoms). Twelve of the vaccinated showed symptoms of infection, while the others were asymptomatic.

Although some the vaccinated patients had symptoms of coronavirus infection, they were of much smaller scale (rhinorrhea, anosmia, cough and breathlessness) than the non-immunized patients. In the case of unvaccinated patients rhinorrhea, odynophagia, cough and dyspnea were the most common symptoms, followed by CoV-2 neurological symptoms (agnosia, ageusia and anosmia), fatiguability, emesis and fever. The Simulated Chi-square Monte-Carlo test showed a very modest p value of 0.0001, following the comparison of the two groups.

As far as the duration of hospitalization, is concerned the unvaccinated patients stayed between 5-13 days in the hospital, compared to those vaccinated symptomatic (6-10 days) and asymptomatic (4-7 days). Asymptomatic patients had shorter hospitalizations than symptomatic patients, regardless of vaccination status (Mann-Whitney test, $p = 0.032$). As for risk factors for severe maternal prognosis, except for maternal weight before pregnancy and body mass index, there was no statistical difference between vaccinated and unvaccinated women. Vaccinated pregnant women were statistically heavier. In both groups an increased proportion of patients had

associated comorbidities ($p= 0.517$ value). Anemia was the most frequent pathology. Other comorbidities included: thyroid disease, gestational diabetes, thrombophilia, pregnancy-induced hypertension, chronic hypertension, heart and kidney disease. Upon admission, 14 (22.58%) unvaccinated patients presented, according to the computer tomography, lung damage suggestive of severe illness. The lesions affected between 15% - 80% of the lung parenchyma.

Material and methods

Overall, 272 postpartum women were discharged with positive COVID-19 PCR RT tests throughout the study period (01 April 2020–20 November 2021). Before delivery, all SARS-CoV-2 infections were tested. Multiple pregnancies, births under 24 weeks, and unknown SARS-CoV-2 vaccine status were excluded. Exclusion criteria left 97 eligible patients. 35 were COVID-19-vaccinated, whereas 62 were unvaccinated (all exhibited symptoms). 12 of the vaccinated patients were infected at birth, while the others were asymptomatic.

In the unvaccinated group, 7 cases needed invasive oxygen supplementation, compared to 1 in the vaccinated group. Fifteen unvaccinated women were admitted in the intensive care unit whereas in the vaccinated group only one needed intensive care ($p\text{-value}=0.70$), however only unvaccinated patients needed endotracheal intubation. Of the studied risk factors, only the Protein C Reactive (PCR) had predictor properties for risk of admission in the intensive care unit in case of severe COVID-19 disease. In symptomatic cases, a high PCR increased the probability of "ICU transfer" by 1.01 times.

Five COVID-19-related postpartum maternal deaths occurred in unvaccinated individuals. Two patients had thyroid disease, pregnancy-induced hypertension, and gestational diabetes in addition to anemia. Three of these patients were 35 or older. All severe cases received high-flow oxygen therapy in the intensive care unit. Due to the mother's serious condition, all deliveries were surgical, resulting in healthy babies. All newborns were SARS-CoV-2-free after delivery and throughout their hospital stay.

In the studied lot, delivery occurred between 25 and 42 weeks. 25.7% of all births (25 of 97 patients) were preterm. C-section was the major delivery method for 50 (67.57%) of 74 symptomatic patients and 15 (65.22%) of 23 asymptomatic patients. Scarred uterus, failed labor, intrauterine fetal distress, thrombophilia, breech presentation, hypertension, and severe maternal COVID-19 illness were c-section indications. 16 emergency COVID-19 C-sections occurred. Unvaccinated individuals were more likely to deliver owing to this indication (15 vs 1), p -value=0.07.

Symptomatic patients had similar median and mean gestational ages at delivery. Both vaccinated and unvaccinated patients were 36 weeks and 38 weeks gestational. Prematurity rates were similar for unvaccinated and vaccinated women (16/62 vs 9/35, p -value = 0.886). However, unvaccinated patients were more likely to delivered before 32 weeks (9 vs. 4).

Birthweight varied from 800 to 4930g in newborns from women that were not vaccinated and 2750–3625g in newborns from the vaccinated group. Neonates from the first group were heavier (Mean= 3119 g, Median=3380 g versus 2902g and 3150g in the vaccinated group), but no significant difference was found (p -value= 0.28).

Three, third-trimester IUFD cases were identified in the studied lot. In either of the case the mothers were not vaccinated, and all stillbirths were diagnosed at the moment of hospital admission. Either of the cases with stillbirth had no discernible risk factors. In either of the cases vertical transmission could not be demonstrated due to lack of molecular investigations on the placenta and fetus.

Two live newborns had positive RT-PCR at birth, both of which were born to women who had not been vaccinated. The newborns were delivered at 37 and 38 weeks, respectively. One was delivered through c- section because of the risk of uterine rupture on a preexisting uterine scar, whereas the other was delivered vaginally. The newborns had no symptoms throughout their stay in the hospital.

This analysis described the pregnancy outcomes of 97 women who had a positive RT-PCR test for SARS-CoV-2 infection at the time of delivery. An exploratory analysis of maternity care was the primary purpose of this research, which was conducted over the course of a period of twenty months during the COVID-19 pandemic.

In addition to this, it planned to investigate the occurrence of vertical transmission as well as the efficacy of primary prevention.

During the pandemic period that was being studied, the clinic offered high-quality obstetric care to pregnant patients who were affected by COVID-19.

According to the findings of the current study, immunization of pregnant women may play a beneficial role in SARS-CoV-2 positive patients by reducing the likelihood of COVID-19 symptoms, which may have favorable effects on the health of babies and mother bonding. Unvaccinated patients were more likely to deliver through c-section, due to complicated COVID-19 disease than vaccinated patients (15 vs 1), $p\text{-value}=0.07$. Due to the small number of positive newborns and lack of necessary investigations, we were unable to reach a conclusion regarding the topic of vertical transmission, however despite the shortcomings, the contribution that this debriefing analysis makes to the collection of medical evidence points to the benefits of immunization during pregnancy.

Since there were only two neonates with positive RT-PCR at delivery throughout the 20-month research, a meta-analysis of all the reported cases with vertical transmission was attempted to better identify the outcome of pregnancy in case of vertical transmission of the coronavirus[52].

The following research attempts to offer some insight on the topic of coronavirus vertical transmission. The purpose of this research is to identify all cases in which vertical transmission was at least possible according to standard guidelines, and to explore the outcomes of these pregnancies as well as, potential risk factors for poor outcomes. Finally, the research will suggest a simplified approach for screening coronavirus vertical transmission.

Chapter 2 presented Study 2, which tracked every case where congenital infection was considered possible based on established guidelines. The study aimed to investigate the impact of infection on the fetus and identify any potential risk factors that may lead to an unfavorable fetal or neonatal prognosis. The research findings proposed a potential simplified method for screening for vertical transmission of the coronavirus

A data base was created, that included worldwide cases of reported vertical transmission of the coronavirus. Collection of all data was performed in accordance

with the IPD statement. The research looked for all the published cases, where vertical transmission of the coronavirus was reported.

Two search engines were used: PubMed/MEDLINE and Google Scholar. The following keywords were used: ('covid*' OR 'Sars-CoV-2*') AND ('vertical transmission' OR 'in-utero transmission' OR 'congenital transmission' OR 'placental infection'). The search period was from 1 January 2020 to 30 January 2022. Reference lists of all identified sources were searched for additional sources. There were no limitations or restrictions on the sort of study: all forms of evidence were to be considered.

The following inclusion criteria were used to determine the eligible articles: application of the standard criteria (the WHO or NFSOG criteria) in the attempt to diagnose vertical transmission, delivery starting with 24 weeks of gestation, delivery using strict infection control and prevention practices and mother–neonate separation at least for 24 h after birth.

As far as definition of vertical transmission is concerned, only cases with intrauterine fetal exposure or intrapartum fetal exposure were included. Murad Scoring Criteria as well as The Newcastle–Ottawa Scale Coding Manual served as the basis for the evaluation of the clinical trials' levels of quality [60].

Overall, there were 55 case series/reports and three clinical studies included. The study followed the outcome of 82 pregnancies. These pregnancies resulted in 85 The statistical analysis was conducted at a 95% level of confidence and a 5% level of statistical significance.

Median maternal age was 30 in the symptomatic group and 30.5 in the asymptomatic group. Six women had advanced maternal age. All of them were symptomatic at the moment of diagnosis. Symptomatic women's pregnancy outcomes unfolded faster (median -7 days) than asymptomatic mothers' (median -11 days), regardless of pregnancy outcome (livebirth or IUFD diagnosis) ($p = 0.013$). Fever (54.8%) was the most common symptom among sick women. 8 (9.76%) women had severe pneumonia, and 2 needed ventilatory and hemodynamic support. Forty women addressed to the hospital due to pregnancy related issues. In 23% of cases, they reported

reduced fetal movement, 17.07% had painful uterine contractions, 7.32% presented vaginal bleeding and in 2.44% of cases, premature rupture of membranes was diagnosed. Out of the studied lot, 29 women had at least one comorbidity

The research comprised 85 newborns with probable vertical transmission of SARS-CoV-2: 24 symptomatic, 24 asymptomatic, and 37 intrauterine fetal deaths. In addition to respiratory symptoms, these neonates had neurologic, digestive, cutaneous, and fever. **Details about each case was presented in an article published by our group**

In the studied lot gestational age varied from 24 to 41 weeks. There was no difference between median of gestational age at birth in livebirths (34 weeks of gestation), however stillbirths occurred in median at 32 weeks of gestation ($p=0.014$).

The proportion of stillbirths remained high, irrespective of the grade of prematurity. Thirty-four neonates were born less than 28 weeks of gestation. Of these 52.63% were stillborn. In the moderate to late preterm group almost one third of the cases were stillbirths (10 stillborn vs 10 asymptomatic neonates and 9 symptomatic neonates). This was the same issue, as far as term pregnancies were concerned. 7 pregnancies were diagnosed with intrauterine fetal death the rest, 14 were livebirths. Five-minute Apgar Score varied from 2 to 10, however the median did not differ by far, between symptomatic and asymptomatic infected newborns 8.5 vs 9. Livebirth babies had a median birthweight of 2182g (95% Confidence interval: 1847.96; 2375.32), as opposed to stillbirths which had a median birthweight of 1925g (95% Confidence interval: 1611.91; 2239.09); p -value:0.611.

Maternal clinical state at delivery or COVID-19 severity did not affect neonatal outcome, however maternal fever was statistically strongly associated with newborn symptoms (p -value = 0.037). This logistic regression model is significant. Fever during COVID-19 increases the odds of a symptomatic birth (OR: 4.55). Nineteen of 82 women reported diminished fetal movements during hospital admission. Nearly 2/3 of intrauterine fetal death was discovered (p -value=0.001). Logistic regression indicated that diminished awareness of fetal activity strongly predicts stillbirth. Reduced fetal movement increases stillbirth odds by 7.5 times (95% CI: 2.21 - 25.43). The Odds Ratio was 7.84 (95% CI: 2.23 - 27.5) after eliminating very preterm and premature stillbirth.

Statistically speaking, neonatal outcome was dependent on the gestational age at the moment of maternal diagnosis as well as delivery.

Statistically, neonatal outcome depended on gestational age at the time of maternal diagnosis and time of delivery. The three groups (asymptomatic/ symptomatic newborns and stillborn) differed statistically, when these parameters were taken into consideration. Smaller gestational ages increased newborn risk. Infected mothers under 29 weeks had higher stillbirths. Asymptomatic newborns had a mean GA of 35 weeks, whereas COVID-19-positive babies had 33 (p value <0.05). One unit of increase of gestational age at the moments of maternal infection increases the chance of livebirth by 1.14 (95% CI: 1.03 - 1.26).

Secondary neonatal unfavorable outcomes included COVID-19-related neonatal mortality and sequelae. 3 SARS-CoV-2-related fatalities (6.25%) occurred in 48 livebirths, and 2 neonates (4.16%) had neurological signs at discharge.

Extreme prematurity was the only significant predictor of congenital COVID-19 disease death in newborns (p-value=0.022). The median gestational age of the three preterm fetal births was 26, compared to 34 in the group whose infection resolved/was asymptomatic from the start. Neonatal mortality and SARS-CoV-2 infection were associated with lower median Apgar Scores.

Due to the small proportion of secondary adverse neonatal outcomes in the lot, none of the investigated risk factors had statistical significance. However, linear regression models concludes that a higher gestational age at the moment of maternal infection is associated with a higher probability of infection remission at the moment of neonatal hospital discharge.

Although congenital SARS-CoV-2 infection might be asymptomatic in certain instances, adverse neonatal outcomes are highly common. Maternal fever was a powerful predictor of symptoms occurrence in the newborns (OR:4.5). Even after excluding severe preterm neonates, decreased fetal movement in pregnancies with in-utero transmission of the coronavirus was related with a high risk IUFD (OR:7.8). The gestational age at the time of the mother's infection and the time of birth both showed relevance in influencing the primary outcome in the analyzed lot.