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The influence of SARS-CoV-2 on the immune system elements and on the placental structure. Clinical, histological and immunohistochemical study

CRISTINA JANA BUSUIOC[1)], GABRIELA-CAMELIA ROŞU[1)], GEORGE-LUCIAN ZORILĂ[2)], LAURENŢIU MOGOANTĂ[1,3)], ANCA-MARIA ISTRATE-OFIŢERU[1)], DANIEL PIRICI[1)], ILONA MIHAELA LILIAC[1)], LARISA IOVAN[1)], ELENA IULIANA ANAMARIA BERBECARU[4)], MARIA-CRISTINA COMĂNESCU[5)], SERGIU MARIAN CAZACU[6)], DOMINIC-GABRIEL ILIESCU[2)]

[1)]*Department of Histology, University of Medicine and Pharmacy of Craiova, Romania*

[2)]*Department of Obstetrics and Gynecology, University of Medicine and Pharmacy of Craiova, Romania*

[3)]*Romanian Academy of Medical Sciences, Craiova Subsidiary, Romania*

[4)]*PhD Student, Doctoral School, University of Medicine and Pharmacy of Craiova, Romania*

[5)]*Department of Anatomy, University of Medicine and Pharmacy of Craiova, Romania*

[6)]*Department of Gastroenterology, University of Medicine and Pharmacy of Craiova, Romania*

Abstract

Background: The effects of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection during pregnancy remain relatively unknown. *Aim*: We present this original paper where we analyzed 60 parturients, at term, 30 without associated infection (C-) and 30 with associated infection (C+), present at birth. *Methods*: We analyzed the blood count and placental microscopic structure through classical and immunohistochemical staining and observed the placental areas affected by the presence of SARS-CoV-2. *Results*: SARS-CoV-2 infection was accompanied by a decrease in the number of lymphocytes, the number of platelets and the presence of placental structural changes, identifying extensive areas of amyloid deposits, placental infarcts, vascular thrombosis, syncytial knots, with a decrease in placental vascular density and the presence of infection in the cells located at decidual level, at syncytiotrophoblast level and at the level of the cells of the chorionic plate, still without overcoming this barrier and without causing any fetal infection in the analyzed cases. *Conclusions*: This study shows that the invasion of SARS-CoV-2 in the placenta can produce significant structural changes, with a decrease in placental vascular density that can have significant implications on proper fetal perfusion. Also, the presence of immunoreactivity at the level of decidua, the placental villi, as well as the chorionic plate proves that the virus can overcome the maternal–fetal barrier. However, in the analyzed cases there were no fetal infections at birth, which may show that local placental factors can be a protective filter for the fetus.

Keywords: placenta, SARS-CoV-2 infected cells, immune system.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a betacoronavirus causing the pandemic that started in 2019. The virus causes an imbalance of the inflammatory response, with a reduction in the number of lymphocytes and innate defense, with the triggering of an inflammatory response that leads to the presence of endothelial damage, coagulopathies, and thrombosis in several tissues of infected individuals [1, 2]. The specific risks and effects of SARS-COV-2 in pregnant women remain unknown. No adverse results were noted among a small group of pregnant women who presented with infection at the end of the third trimester in Wuhan, China [1, 2]. The placenta can be one of the affected organs and the produced changes can influence the good maternal–fetal perfusion. However, in the vast majority of pregnancies, newborns are not infected when tested with the nasopharyngeal test. However, pregnant women with symptomatic SARS-CoV-2 infection are more likely to be admitted to intensive care units, and maternal death rates are statistically higher compared to infected but non-pregnant women [3]. Preterm births were more frequent in women with confirmed SARS-CoV-2 infection, but there was no increase in early neonatal deaths [4]. Prospective and retrospective studies showed that pregnant women infected with SARS-CoV-2 presented a higher risk of adverse events, including a higher rate of Caesarean (C)-section delivery, but also increased postpartum complications [5–7]. Vertical transmission from mother to fetus was reported in a few cases [8–13], but most studies did not report any viral transmission to the fetus [14–22]. Several studies detected placental infection in women who tested positive for the virus at delivery or before delivery. In some cases, it was found that the placenta showed signs of inflammation, with increased vascular malperfusion by the presence of thrombi within the intravillous vessels [13, 20–22]. In this study, we analyzed the blood count values of patients infected with SARS-CoV-2,

as well as their placental structures to determine the influence of the infection on the number of immune cells and the impact on the placental tissue.

Aim

The purpose of this study was to analyze the influence of SARS-COV-2 infection on the blood count values, to analyze whether the maternal environment could have been involved in this infection, but also to analyze the effects of the placental infection in terms of placental structure, villous vascularization, and the presence of the virus in cells in different placental areas, with the probability of maternal–fetal transmission of the virus.

Patients, Materials and Methods

Patients and statistical analysis

This retrospective study included 60 pregnant patients hospitalized within the Obstetrics–Gynecology Clinic II of the Emergency County Clinical Hospital of Craiova, Romania, between 2020–2023.

The inclusion criteria of the patients in this study were: primiparous women, without previous abortions, without associated medical pathologies, aged between 20–35 years old.

The exclusion criteria of patients in the study were younger than 20 years old or older than 35 years old, multiple births, patients who have an obstetric history of spontaneous miscarriage or on request, associated pathologies, such as: pre-existing high blood pressure to pregnancy or gestational high blood pressure, pre-existing diabetes mellitus to pregnancy or gestational diabetes, documented thrombophilia or other documented pathologies, except of SARS-CoV-2 infection, which could influence proper placental and fetal development.

The 60 patients were divided into two groups: the first group included patients without SARS-CoV-2 (C-) at the time of birth and 30 patients who were confirmed with SARS-CoV-2 before the time of birth within a maximum of 24 hours period (C+), confirmed by real-time reverse-transcriptase–polymerase chain reaction (rRT-PCR) test-qualitative evaluation (kit utilized: MP2606-0100 Euro RealTime SARS-CoV-2 EC-EUROIMMUN).

Informed consent was obtained from all subjects. The patients signed a consent form for personal data use. The study was conducted according to the guidelines of the Declaration of Helsinki.

All 60 patients had a eutocic delivery. Postpartum, placental fragments were collected and sent to the Research Center for Microscopic Morphology and Immunology within the University of Medicine and Pharmacy of Craiova (UMPhCv) to be processed and their microscopic morphology assessed (https://eeris.eu/ERIF-2000-000G-1574).

Histopathological analysis

We performed the histological preparation and examination within the Department of Histology of UMPhCv, in the Research Center for Microscopic Morphology and Immunology. After fixation of the biological material in 10% neutral buffered formalin, tissue fragments were routinely processed for paraffin embedding and sectioning into 5 µm thick sections.

The classical Hematoxylin–Eosin (HE) staining highlighted the placental decidual structures, the placental villi, and the chorionic plate, while the Periodic Acid Schiff–Hematoxylin (PAS–H) staining highlighted the amyloid deposits in the placental structure. First of all, for both the classical HE staining and immunolabeling, simple or poly-L-lysine-treated slides were utilized to collect 5 µm-thick tissue sections, obtained with the help of the Microm HMB350 microtome on a water-based sections-transfer system. They were placed in xylene baths (three baths × 15 minutes) for deparaffinization, in alcohol baths with decreasing concentrations (100%, 90%, 70%, five minutes each) for dehydration, then the sections were hydrated with the help of distilled water (dH_2O) (three baths × five minutes); for the classical HE staining, the nuclei were labeled with Hematoxylin, and the cytoplasm of the cells with Eosin, and for the special staining, initially underwent antigen retrieval by microwaving in a specific pH 6 0.1 M citrate buffer or pH 9 ethylenediaminetetraacetic acid (EDTA) solution, as specified by the manufacturer (Table 1). The tissue sections were incubated in a 3% hydrogen peroxide (H_2O_2) solution for 30 minutes to block endogenous peroxidase that could interfere with signal detection, and then in a 3% skimmed milk saline solution to block the antibody nonspecific binding sites. Next, the primary antibodies were applied on the slides after obtaining the specific solution (Table 1) for 18 hours in the refrigerator at 4°C. After this time, the slides with the tissue sections were left at room temperature (RT), then washed with phosphate-buffered saline (PBS), followed by incubation with Horseradish peroxidase (HRP)-labeled secondary antibodies specific for the species of the primary antibodies (Nichirei Biosciences, Inc., Tokyo, Japan). The last step of the reaction was the actual detection of the signal utilizing 3,3'-Diaminobenzidine (DAB), after which the sections were counterstained with Mayer's Hematoxylin, dehydrated, clarified in xylene, and covered with a special slide-covering environment (Canada balm), for imaging and analysis.

Table 1 – ***Immunohistochemical panel of antibodies used***

Antibody	Manufacturer	Clone	Antigenic exposure	Secondary antibody	Dilution	Labeling
Anti-CD34	Dako	QBEnd/10	Citrate, pH 6	Monoclonal mouse anti-human CD34 Class II	1:50	Neoformed blood vessels
Anti-SARS-CoV-2	Abcam	Polyclonal	EDTA, pH 9	Polyclonal rabbit	1:1000	Spike glycoprotein

CD34: Cluster of differentiation 34; EDTA: Ethylenediaminetetraacetic acid; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

Method of analysis

For every patient, the following data was recorded: age, rural (R) or urban (U) environment, hemoglobin (Hb), number of leukocytes, number of lymphocytes, number of platelets before birth, depending on the group they belonged, the average values and standard deviations (SDs) were calculated, and the results were represented graphically with the help of the Microsoft Excel program. Comparative statistical analysis evaluated the differences between the

patients without SARS-CoV-2 and those diagnosed with SARS-CoV-2 infection, through the use of a two-tailed Student's *t*-test.

Also, the histopathology of placental sections was analyzed. The presence of placental changes was observed (calcifications, syncytial knots, villous infarcts, amyloid deposits, thrombosis). Regarding the immunolabeled sections, the number of cluster of differentiation 34 (CD34)-positive intravillous vessels/400× magnification was analyzed, the average value of the number of vessels per four images obtained for each case and the presence of anti-SARS-CoV-2 antibody immunoreactivity in the maternal side (decidua), in the chorionic villus and the fetal side (chorion) was calculated. Depending on the level of immunolabeling, the positive/negative cases were recorded in Microsoft Excel, the values being recorded as a percentage.

Results

The age of patients in C+ group was between 20 and 34 years old, with a mean value of 25.30 years old (±4.2 years old), and the age of patients in C- group varied between 20 and 34 years old, with a mean value of 29.10 years old (±3.68 years old). Using the *t*-test, we observed that there were statistically significant differences between the two groups, $t(30)=-3.347$, $p<0.005$ (Figure 1).

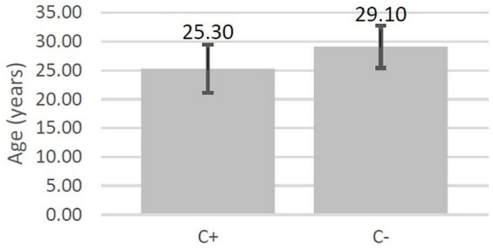

Figure 1 – ***The average age of the patients included in the study, depending on the group they belong to. Statistically significant differences were present between the two groups, t(30)=-3.347, p<0.005. C+: Patients with SARS-CoV-2 infection at birth; C-: Patients without SARS-CoV-2 infection at the time of birth; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.***

Regarding the patient origin environment, we noticed that in the case of the C+ group, 46.67% of the patients came from the R environment and 53.33% from the U environment, and in the case of the patients from the C- group, 60% of they came from R environment and 40% of them from U environment (Figure 2).

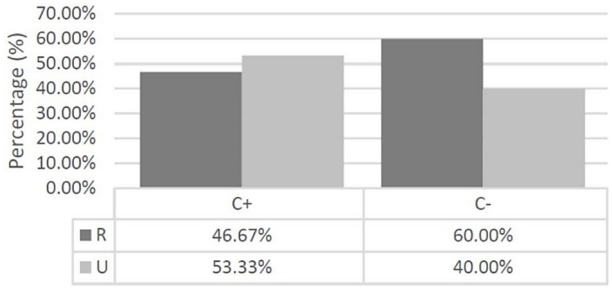

Figure 2 – ***The origin environment for the patients included in the study. C+: Patients with SARS-CoV-2 infection at the time of birth; C-: Patients without SARS-CoV-2 infection at the time of birth; R: Rural; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; U: Urban.***

Regarding the mean value of Hb of the patients from the two groups included in the study, we observed that in the case of the C+ group, it varied between 8.9 g/dL and 12.3 g/dL, with a mean value of 10.87 g/dL (±0.9 g/dL), and in the case of C- group, it varied between 10.2 g/dL and 12.3 g/dL, with a mean value of 11.33 g/dL (±0.79 g/dL). We noticed that there were statistically significant differences between the two groups, $t(30)=-2.776$, $p<0.005$ (Figure 3).

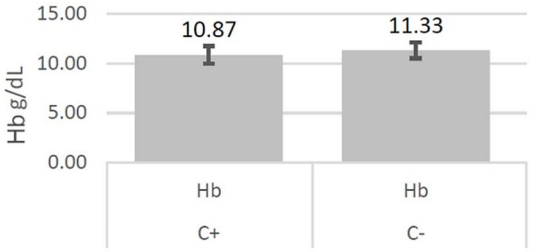

Figure 3 – ***Mean values of Hb depending on the patient group. We noticed that there were no statistically significant differences between the two groups, t(30)= -2.776, p<0.005. C+: Patients with SARS-CoV-2 infection at the time of birth; C-: Patients without SARS-CoV-2 infection at the time of birth; Hb: Hemoglobin; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.***

The values of leukocytes were analyzed according to the SARS-CoV-2 infection status at the time of birth. Thus, in the case of C+ group, we observed that the values were between 7800/μL and 13 400/μL, with a mean value of 11 046.67/μL (±1626.66/μL). In the case of the C- group, leukocyte values varied between 8600/μL and 13 000/μL, with a mean value of 11 203.33/μL (±1634.22/μL). We observed that there were no statistically significant differences between the C+ and C- groups regarding the number of leukocytes, $t(30)=-0.314$, $p>0.005$ (Figure 4).

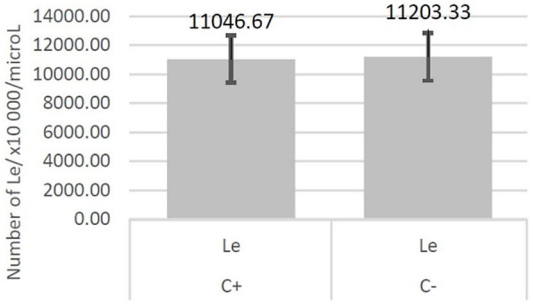

Figure 4 – ***Mean values of leukocytes depending on the group of patients. We observed that there were no statistically significant differences between the two groups, t(30)=-0.314, p>0.005. C+: Patients with SARS-CoV-2 infection at the time of birth; C-: Patients without SARS-CoV-2 infection at the time of birth; Le: Leukocytes; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.***

The lymphocyte values varied according to the SARS-CoV-2 infection status at the time of birth. Thus, in the case of C+ group, we observed that the values were between 450/μL and 2000/μL, with a mean value of 987.33/μL (±313.7/μL), and in the case of the C- group, lymphocyte values varied between 2900/μL and 4800/μL, with a mean value of 3906.67/μL (±601.68/μL). We detected statistically significant differences between the C+ and C- groups regarding the number of lymphocytes, $t(30)=-24.901$, $p<0.001$ (Figure 5).

Platelet values also varied depending on the infection status. In the case of C+ group, we observed that the values were between 56 000/μL and 320 000/μL, with a mean value of 144 333.33/μL (±74 157.03/μL), and in the C- group, platelet values varied between 213 000/μL and 450 000/μL, with a mean value of 300 723.33/μL (±62 254.15/μL). We found statistically significant differences between the C+ and C- groups regarding the number of platelets, $t(30)=-8.099$, $p<0.001$ (Figure 6).

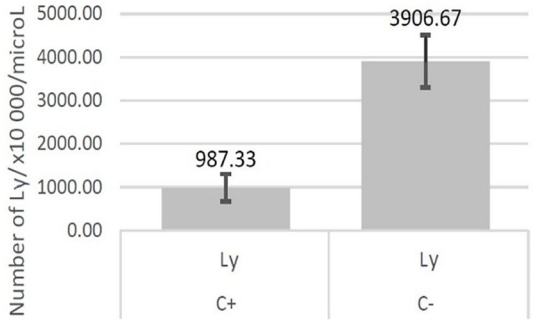

Figure 5 – ***Mean values of Ly depending on the group of patients. We observed that there were statistically significant differences between the two groups, t(30)= -24.901, p<0.001. C+: Patients with SARS-CoV-2 infection at the time of birth; C-: Patients without SARS-CoV-2 infection at the time of birth; Ly: Lymphocytes; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.***

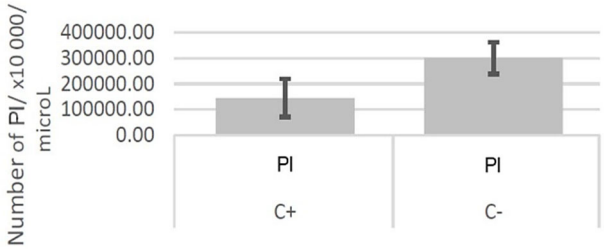

Figure 6 – ***Mean values of the number of Pl depending on the patient group. We observed that there were statistically significant differences between the two groups, t(30)=-8.099, p<0.001. C+: Patients with SARS-CoV-2 infection at birth; C-: Patients without SARS-CoV-2 infection at the time of birth; Pl: Platelets; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.***

Regarding the histopathological analysis, we noticed that in the case of the C+ group, for the classical HE staining there were extensive areas of calcifications (Figure 7A), of thrombosis (Figure 7B), of placental infarcts (avascular areas, pale pink) (Figure 7C), of extravillous and intravillous fibrinoid deposition (bright fine granular pink areas), syncytial knots (blue areas on the periphery of the placental villi) (Figure 7D), compared to C- group, where these changes were minimal (Figure 7E). The extensive areas of fibrinoid deposition in the C+ group cases were also highlighted with PAS–H staining, where they were intensely labeled in bright pink (Figure 7F).

Regarding the intravillous vascularization, we observed that in the case of patients in the C+ group, the number of CD34+ vessels/400× magnification varied between 12 and 32 CD34+ vessels/400× magnification, with a mean value of 22.39 CD34+ vessels/400× magnification (±5.35 vessels), and in the case of C- group patients, the number of CD34+ vessels/400× magnification varied between 24 and 48 CD34+ vessels/400× magnification, with a mean value of 34.97 CD34+ vessels/400× magnification (±7 vessels). We observed that there was a statistically significant difference between the two groups of patients (C+ and C-), regarding the number of CD34+ intravillous vessels/400× magnification, $t(31)=-9.182$, $p<0.001$ (Figure 8; Figure 9, A and B).

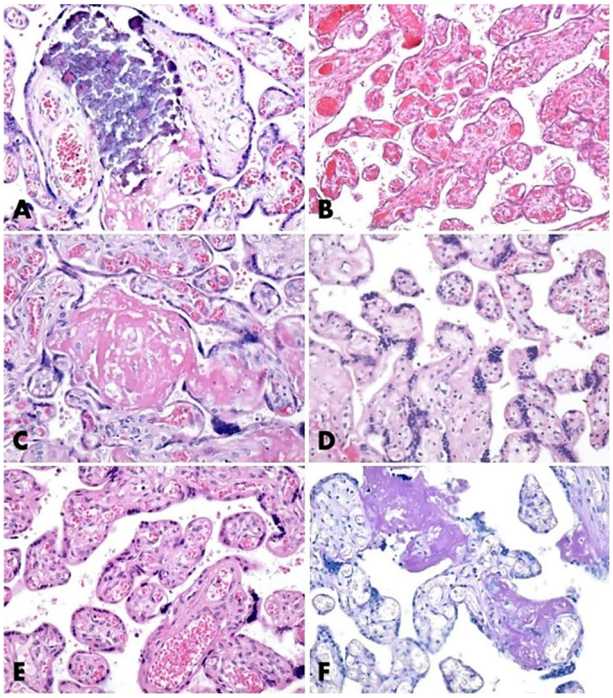

Figure 7 – ***Microscopic changes present in placental structures infected with SARS-CoV-2, from C+ group, compared to placental structures from C- group: (A) Extensive areas of intra- and extravillous calcifications; (B) Numerous intravillous vessels with intravascular thrombosis; (C) Placental infarction area (avascular areas, pale pink); (D) Numerous syncytial knots (the blue areas on the periphery of the placental villi); (E) Normal villi structures in C- group; (F) Areas of fibrinoid deposit (bright pink). HE staining: (A–E) ×200. PAS–H staining: (F) ×200. C+: Patients with SARS-CoV-2 infection at birth; C-: Patients without SARS-CoV-2 infection at birth; HE: Hematoxylin–Eosin; PAS–H: Periodic Acid Schiff–Hematoxylin; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.***

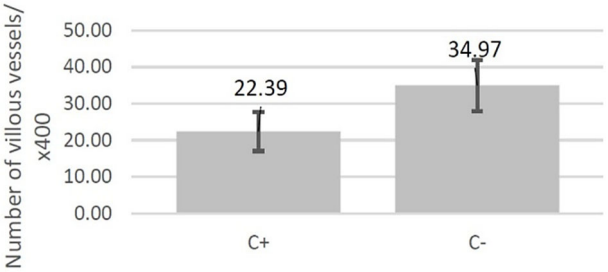

Figure 8 – ***The mean value of the vascular density (CD34+ intravillous vessels/400× magnification) of the patients included in the study, depending on the group they belong to. We observed that there was a statistically significant difference between the two groups, t(31)=-9.182, p<0.001. C+: Patients with SARS-CoV-2 infection at the time of birth; C-: Patients without SARS-CoV-2 infection at the time of birth; CD34: Cluster of differentiation 34; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.***

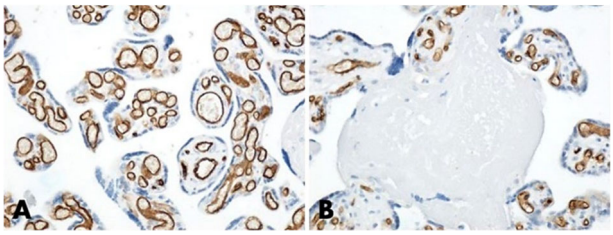

Figure 9 – *Microscopic patternss of intravillous CD34+ vascular density: (A) Placental villi with normal CD34+ vascular patterns from C- group; (B) Placental villi with vascular patterns CD34+ changes, low vascular densities due to frequent infarcts, present in C+ group. Anti-CD34 antibody immunolabeling: (A and B) ×400. C+: Patients with SARS-CoV-2 infection at the time of birth; C-: Patients without SARS-CoV-2 infection at the time of birth; CD34: Cluster of differentiation 34; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.*

Referring to the SARS-CoV-2 infection at the placental level, we made a schematic image of the placental, maternal, and fetal areas so that we could understand the level at which the virus reached, and a positive immunoreaction occurred. The maternal placenta includes the decidual area, the middle area includes the villous ramifications, and the fetal placenta includes the chorionic plate (chorion) (Figure 10).

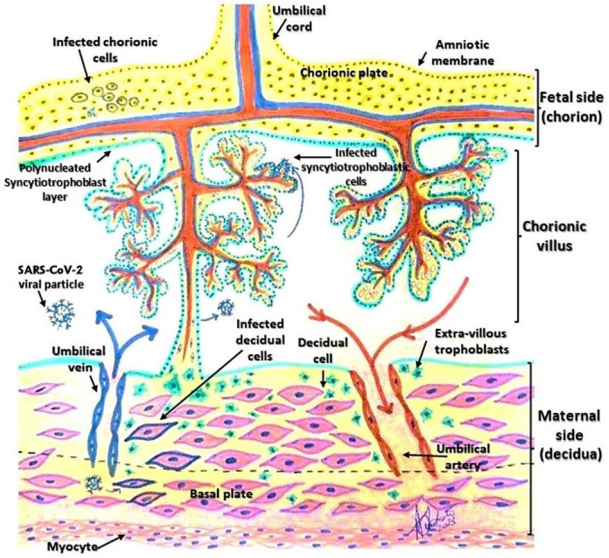

Figure 10 – *Schematic representation of the placental structure, the types of layers, the cells present, the direction of the vascular flow towards the fetus via the umbilical vein (marked in blue), blood loaded with oxygen, nutrients, and possible viral particles and from the fetus towards the maternal basal plate through the umbilical arteries (marked in red), blood loaded with metabolic products and carbon dioxide. Also, we presented the probability of infection of cells from different placental layers with SARS-CoV-2. SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.*

We, thus, observed that all cases from the C- group presented a negative immunoreaction for the anti-SARS-CoV-2 antibody, 60% of the C+ cases presented a positive immunoreaction at the level of the maternal side (decidua), 40% of the C+ cases presented positive immunoreaction both at the maternal side (decidua) and at chorionic villus level and only 13.33% of the C+ cases presented positive immunoreactivity at all three levels: maternal side (decidua), chorionic villus, and at fetal side (chorion) level (Figure 11; Figure 12, A–F).

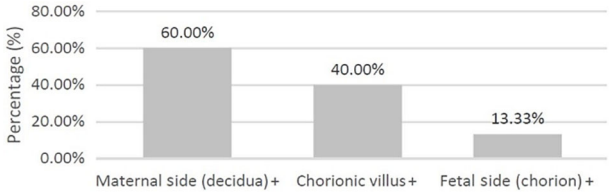

Figure 11 – *Percentage representation for placental immunoreactivity for SARS-CoV-2 infection. SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.*

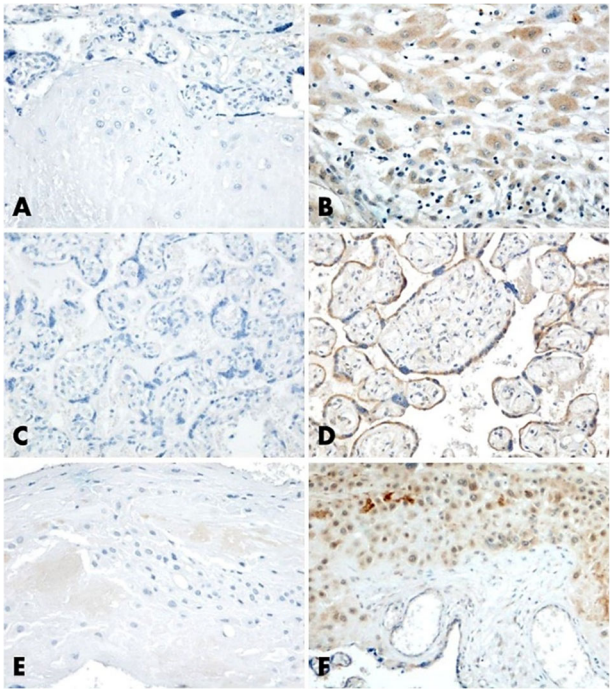

Figure 12 – *Microscopic patterns of maternal and fetal placental areas concerning immunoreactivity to the anti-SARS-CoV-2 Ab: (A) Maternal side area (decidua) from C- group, with decidual cells negative for the anti-SARS-CoV-2 Ab; (B) Maternal side area (decidua) from C+ group, with decidual cells positive for the anti-SARS-CoV-2 Ab; (C) Chorionic villus area from C- group, with syncytiotrophoblastic cells negative for the anti-SARS-CoV-2 Ab; (D) Chorionic villus area from C+ group, with syncytiotrophoblastic cells positive for the anti-SARS-CoV-2 Ab; (E) Fetal side area (chorion) from C- group, with cellularity showing negative immunoreactivity to the anti-SARS-CoV-2 Ab; (F) Fetal side area (chorion) from C+ group, with cellularity showing positive immunoreactivity to the anti-SARS-CoV-2 Ab. Anti-SARS-CoV-2 Ab immunomarking: (A, B, D–F) ×200; (C) ×100. Ab: Antibody; C+: Patients with SARS-CoV-2 infection at the time of birth; C-: Patients without SARS-CoV-2 infection at the time of birth; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.*

Discussions

The placenta is a vital organ that ensures the connection between mother and fetus. Viral infections can cause placental dysfunction, which can create pregnancy-related complications, increasing morbidity and mortality for both mother and fetus [23–25]. Also, placental changes can

influence the proper future development of the child, with the presence of subsequent chronic diseases [26].

Although the vast majority of newborns from mothers infected with SARS-CoV-2 did not become infected [14–22], our study shows that SARS-CoV-2 can be frequently detected in the placenta. In the placental tissues that were strongly infected, the virus was located in the decidual cells, in the chorionic villi, especially in the syncytiotrophoblast (ST), and also in the chorionic plate. The placenta can be infiltrated by maternal immune cells with the transcriptional signs of inflammation and procoagulant factors that contribute to the formation of vascular thrombi, the presence of areas of placental infarction and the decrease in vascular density, as we also observed in our study in the patients from C+ group.

Studies showed that the detection of the presence of the virus in the placenta depends on the time when the infection was present. The closer the infectious moment is, the more the number of positive placentas for the viral infection increases [9, 27]. Thus, in our study, half of the patients were positive at birth for SARS-CoV-2 infection, 60% of them presented infected cells at the decidual level, 40% both in decidua and placental villi, especially in structure of the ST and only in 13.33% of the cases of C+ group we detected infected cells in decidua and placental villi, both in the ST and chorionic plate cells.

The infection of the placenta is followed by an extensive infiltration with maternal immune cells, leading to a conglomeration of macrophages and T-lymphocytes both at the decidual level, as well as in the maternal space and in the fetal chorionic villi, which contributes to the creation of a proinflammatory and procoagulant environment and the presence of thrombosis intravillous vascular and implicitly placental infarcts [23–25], a fact also observed in the histopathological analysis of placentas from C+ group.

However, massive infiltration of macrophages alongside fibrin deposition was recently observed in lung tissue from patients with severe SARS-CoV-2. This raises the possibility of a common immunopathology leading to the recruitment and activation of macrophages that cause tissue injury, including placental ones [28]. Further studies of placentas from women with SARS-CoV-2 may help establish a histological feature associated with placental infection [29].

The placenta has many critical functions, including protecting the fetus from infection, pathogens, and maternal disease [30, 31]. Its role is essential in the proper development of pregnancy, especially considering that the blood flow to this organ constitutes a substantial part of the cardiac flow [18]. The fetal placenta plays a key role in protection by forming a selective protective barrier system that prevents the movement of pathogens from mother to fetus, through the maternal–fetal circulation, which exceeds the ST, which has an essential role in autophagy and resistance to viral infections [32–34].

Maternal–fetal interaction during coronavirus disease 2019 (COVID-19) includes morphopathological changes in the infected placenta, although some research has revealed the presence of SARS-CoV-2 in the placenta without detectable microscopic abnormalities. Currently, several studies suggested placental infection with SARS-CoV-2 and viral presence was confirmed by PCR (placental tissue/amniotic membrane), immunohistochemistry, and *in situ* hybridization assays (tissue sections fixed in formalin and embedded in paraffin). The placental morphopathological patterns from the COVID-19 patients came from the third trimester. The most frequent changes were represented by vascular malperfusion, as we also observed in this study, where there was a significant difference of approximately 30% between the mean values of CD34+ vascular densities between the two groups (C+ and C-), fetal vascular thrombosis and maternal vascular malperfusion, massive infection with generalized inflammation (presence of M2 macrophages, cytotoxic and helper T-cells and activated B-lymphocytes), massive fibrin deposition and intervillous and intravillous thrombosis. These abnormalities resulted from direct cell infection, systemic inflammation with massive cytokine secretion, hypercoagulable state, and maternal hypoxia [35–39]. To prove that these changes were caused by the presence of the viral infection, we used immunomarking which highlighted the presence of a synthetic peptide within human coronavirus SARS-CoV-2 spike glycoprotein (C-terminal tail) in the structure of the decidual cells, in the ST, as well as in the basal plate, thus observing that the placenta can be affected in different proportions by the virus and that it can be completely crossed and sometimes it can even lead to vertical fetal infection [8–13], although in this study, this situation was not present.

In terms of age, we selected patients between 20 and 35 years old to avoid being associated with placental changes caused by chronic pathologies, common at advanced ages [40–45]. However, between the two groups (C+ and C-), we noticed that the average age of the patients in the C+ group was approximately four years lower compared to the C- group. Thus, it seems that younger patients can be more frequently infected.

Regarding the origin environment, we observed that most patients from the C+ group were from the U environment, and those from the R environment were associated with the viral infection in a smaller proportion. This fact can be due to U congestion, by means of public transport, by the abundance of commercial spaces, which can be factors associated with viral transmission [46].

For C+ patients, the blood count is predictive for assessing the severity and prognosis of the disease [47]. Hematological parameters and inflammatory indices are associated with severe disease. C+ patients may have significant cytopenia, mainly severe lymphopenia and excessive depletion of CD8+ T-cells, leading to an immunocompromised state and cytokine storm [48]. Different studies showed that the severity of the disease is associated with numerous hematological abnormalities, many of which are warning signs for unfavorable clinical outcomes [49, 50]. Common hematological abnormalities in patients infected with SARS-CoV-2 include changes in the number of platelets, white blood cells, Hb, coagulation/fibrinolytic changes and lymphopenia [51]. Low lymphocytes, low platelets, and clotting problems are the most common hematological disorders [52–54]. Understanding the hematological characteristics of patients with COVID-19 may help with early triage and prediction of disease severity [47, 52, 53]. In this study, there were no significant differences between the two groups regarding the mean values of Hb or leukocytes. Still, we highlighted statistically significant differences in the case of lymphocytes, where

in the case of the C+ group they were approximately six times lower lymphopenia, compared to the C- group, where their values were within normal limits; similarly, we reported in the case of platelets, where mean values were recorded in the C+ group half compared to the C- group, being frequently associated with thrombocytopenia. Other studies showed that hematological features of patients with COVID-19 in Ethiopia, reported total cytopenia in 41% of cases, while lymphopenia was present in 72.2% of cases. Severe cytopenia was more frequent in patients with severe symptoms than in those with moderate conditions [55]. Another study conducted in Debre Markos Isolation and Treatment Center, Ethiopia, reported that leukocytosis and lymphopenia were the predominant hematological abnormalities in COVID-19 patients [56].

All these hematological changes can have an impact on all the organs, as well as on the placental structure and the proper utero–placental vascular perfusion, with possible consequences on the fetal condition and increasing the risk of early delivery before 37 weeks or by C-section [57].

Conclusions

The presence of the SARS-CoV-2 in pregnant patients was associated with younger maternal age and coming from a U environment, as being important risk factors. The viral infection changes the hematological parameters, leading to lymphopenia, thrombocytopenia, with unfavorable maternal and fetal consequences and predisposes the maternal body to severe forms of the disease. Viral particles show the ability to cross the placental structure, being able to infect decidual cells, ST, and chorionic plate cells. Still, vertical transmission from mother to fetus was rarely associated. Thus, the placenta can be considered a fetal protection filter against SARS-CoV-2 infection. The pro-inflammatory microenvironment created by the immune reaction produced by the infection of the placental structures leads to the presence of placental calcifications, amyloid deposition, the presence of intravascular thromboses and implicitly placental infarcts, the decrease in vascular density and utero–placental malperfusion that can have consequences on the fetal condition and growth the risk of premature birth or C-section delivery.

Conflict of interests

The authors declare that they have no conflict of interests.

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Authors' contribution

Cristina Jana Busuioc and Gabriela-Camelia Roşu equally contributed to this article.

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Corresponding authors

Anca-Maria Istrate-Ofiţeru, MD, PhD, Department of Histology, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Romania; Phone +40764–836 619, e-mail: ancaofiteru92@yahoo.com

Maria-Cristina Comănescu, MD, PhD, Department of Anatomy, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Romania; Phone +40721–285 546, e-mail: cristinacomanescu85@gmail.com

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