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Mild or Moderate COVID-19 during Pregnancy Does Not Affect the Content of CD34+ Hematopoietic Stem Cells in Umbilical Cord Blood of Newborns

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The study included umbilical cord blood samples (n=64) intended for cryogenic storage of hematopoietic stem cells and obtained from patients with a history of mild and moderate forms of COVID-19 during pregnancy. The control group was composed of samples (n=746) obtained from healthy women in labor. A comparative analysis of the volume of cord blood collected, the total leukocyte count, the relative and absolute content of cells with the $CD34^+$/$CD45^+$ phenotype revealed no significant differences between the groups.

Key Words: *COVID-19; pregnancy; umbilical cord blood; hematopoietic stem cells; CD34*

Since December 2019, a new coronavirus disease (COVID-19) caused by SARS-CoV-2 virus has spread rapidly around the world and already in March 2020, WHO has given it the status of a pandemic. In mild forms of the disease, its main, and sometimes the only, symptoms are the loss of smell and taste, fever, cough, and shortness of breath, followed in severe cases by massive damage to the lung tissue and the development of respiratory failure.

Although the lungs are the main target of coronavirus, the disease can affect other organs and systems: the liver, gastrointestinal tract, kidneys, cardiovascular, hematopoietic, and CNS [1,9,16,28,32,33,3]. Another target for SARS-CoV-2, apparently, is the placenta, because both trophoblast cells and placental vascular endothelial cells express (although not always and/or weakly) the angiotensin-converting enzyme receptor (ACE-2) and serine protease TMPRSS2, necessary for virus penetration into the cell [22,32]. At the same time, even in the absence of the virus directly in the placental tissue, signs of its lesion (placentitis, changes in the morphology of chorionic villi, impaired perfusion, infiltration by inflammatory cells) are detected in about 50% of COVID-19 cases [22,29]. The consequences of placental damage include pregnancy complications such as miscarriage, intrauterine fetal development delay or premature birth [2,10,18,25]. In severe forms of COVID-19, an additional risk factor for the pregnant woman and fetus becomes a cytokine storm, an acute increase in the concentration of proinflammatory cytokines: IL-6, soluble IL-2 receptor (IL-2R), IL-1β, monocytic chemoattractant protein (MCP-1), and TNFα [8,21].

Pregnant women are at approximately the same risk of SARS-CoV-2 infection as the general population [27]. However, even though pregnant women more often (in 60-88% cases) carry COVID-19 asymptomatically or in a mild form [10,31], almost a third of them (31.5%) need medical help with subsequent hospitalization [7]. This is partly due to the physiological features of pregnant women and many comorbid conditions that require closer attention in clinical settings.

According to some estimations, the number of babies born during 9 months of the pandemic reaches 116 million, which puts forward the problem of

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"COVID pregnancy" [27]. However, many aspects of SARS-CoV-2 effect on pregnant woman and the effects of COVID-19 on the fetus remain insufficiently studied. This first of all refers to the effect of coronavirus disease of a pregnant woman on the developing fetus and, later, on the newborn [6,15]. The effect of COVID-19 during pregnancy on the parameters of umbilical cord blood (UCB) remains unexplored.

The aim of this work was a comparative study of $CD34^+$ hematopoietic progenitor cells content in UCB of children born by healthy women and those with COVID-19 during pregnancy, as a marker of newborn maturity and hematopoietic system state.

MATERIALS AND METHODS

Patients. A comparative retrospective study included 64 patients of the V. I. Kulakov National Medical Research Center for Obstetrics, Gynecology, and Perinatology, who have concluded an agreement and signed an informed consent for the collection and cryogenic storage of hematopoietic stem cells for scientific research and who suffered a mild-to-moderate coronavirus infection during pregnancy in the period from June 2020 to December 2021. The control group was composed by 746 healthy women in labor who preserved cord blood cells during the same time interval. Comparative characteristics of both groups are presented in Table 1.

Collection of umbilical cord blood and isolation of nucleated cells. Cord blood was collected *in utero* into blood collection bags (RaviMed) with the anticoagulant CPDA-1. The blood was transported to the laboratory and processed within 24 h after delivery.

The fraction of nucleated cells was isolated by double centrifugation in accordance with the registered medical technology (FS No. 2009/387 from November 23, 2009) and the current standard operating procedures of the Cord Blood Bank. After sedimentation of red blood cells and volume reduction, nucleated cells were resuspended in autologous plasma with the addition of 10% DMSO and 1% dextran 40, aliquoted in cryotubes, and subjected to programmable freezing followed by cryogenic storage in liquid nitrogen [22].

Aliquots of cord blood before separation and the obtained nucleated cells were used for hematological analysis (Abacus 5, Diatron MI PLC) and flow cytometry (isolated nucleated cells only). The volume of the collected cord blood was determined after weighing the bag with anticoagulated blood and subtracting the weight of the system from the obtained value. Similarly, the correction coefficient was calculated to obtain absolute values of the leukocyte content.

Flow cytometry. The relative content of hematopoietic progenitor cells (HPC) was determined using flow cytometry (FACSCalibur, BD) using CellQuest Pro software according to the ISHAGE protocol [11]. FITC- and PE-conjugated monoclonal antibodies to CD45 and CD34 (Beckman Coulter) were used in the concentration recommended by the manufacturer. In each duplicative sample, 100,000 $CD45^+$ cells were analyzed (Fig. 1). The absolute number of HPC was calculated

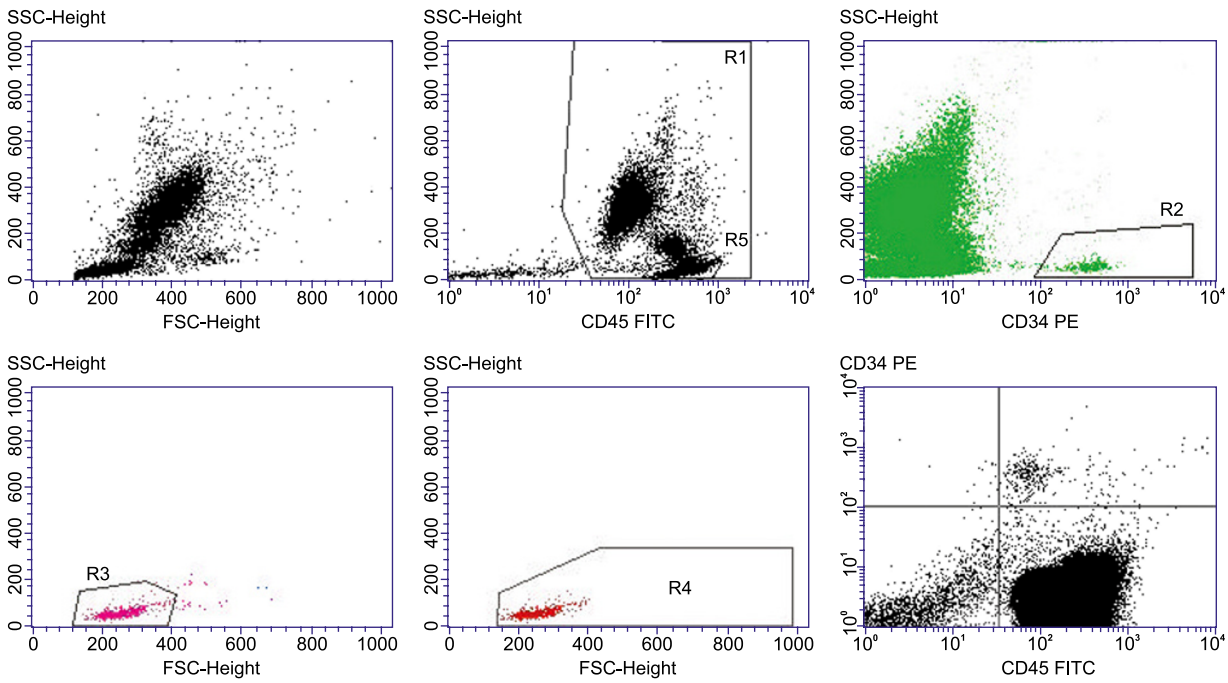

Fig. 1. Representative protocol of HPC analysis by flow cytometry.

TABLE 1. Characteristics of Pregnant Women of the Studied Groups

Parameter	Control group (*n*=746)		COVID-19 group (*n*=64)		*p*
	M±*SD*	min-max	*M*±*SD*	min-max	
Maternal age, years	34.2±5.3	19-55	33.3±4.9	21-44	0.2033
Gestational age, weeks	38.8±1.4	28-42	38.9±1.3	35-41	0.7786
Birth weight, g	3383±472.8	1330-4880	3415±446.6	2220-4354	0.5970

by multiplying the data of flow cytometry (the proportion of HPC among leukocytes) by the absolute number of leukocytes according to hematological analysis (the so-called two-platform method).

Statistical analysis. The means and their deviations were calculated using Microsoft Excel and GraphPad Prism 6.0 software. The significance of the differences between the groups was evaluated using the Mann–Whitney test for two groups and Kruskal–Wallis test for three groups. The Spearman's test was applied to evaluate correlations between the studied parameters. The differences were considered significant at $p<0.05$.

RESULTS

The study included 64 UCB samples intended for cryogenic storage of HPC and obtained from patients with a history of COVID-19 during pregnancy. The age of mothers ranged from 21 to 44 years (mean 33.3±4.9 years), the gestational age was 38.9±1.3 weeks (min-max 35-41 weeks) (Table 1). Most of the patients had mild or moderate COVID-19 in the second and third trimester of pregnancy: 30 (46.9%) and 24 (37.5%), respectively.

The control group included 746 UCB samples obtained from healthy women in labor (age 19-55 years, gestational age 28-42 weeks) (Table 1) hospitalized for delivery in the same period. When forming the control group, samples with a volume <27 ml (see data on the COVID-19 group) were excluded from the analysis. Comparative analysis of patients' age, gestational age, and birth weight in the main and control groups revealed no significant differences (p=0.20, p=0.78 and p=0.60, respectively).

The mean volume of UCB harvested (without anticoagulant) was 69.3±24.8 ml in the COVID-19 group and 67.9±27.2 ml in the control (Table 2). The absolute number of leukocytes before separation was $1018.0\pm482.9\times10^6$ and $991.7\pm498.6\times10^6$, respectively. There were no significant differences either in the volume of UCB collected (p=0.7) or in the total leukocyte count (p=0.69).

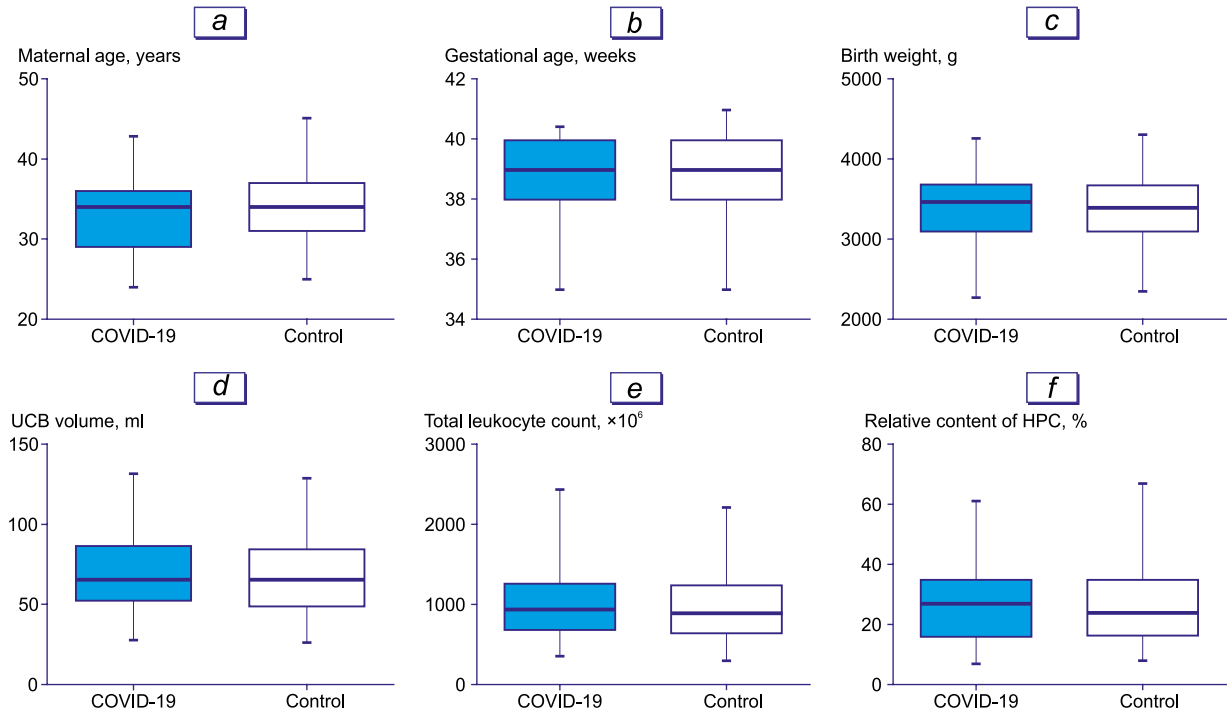

Fig. 2. Comparative analysis of patients' characteristics and UCB parameters in the COVID-19 and control groups.

TABLE 2. UCB Parameters in COVID-19 and Control Group

Parameter	Control group (*n*=746)		COVID-19 group (*n*=64)		*p*
	M±*SD*	min-max	*M*±*SD*	min-max	
UCB volume, ml	67.9±27.2	18-239	69.3±24.8	27-152	0.6961
Leukocytes, $\times 10^6$	991.7±498.6	124.8-3892	1018±482.9	343.2-3303	0.6911
$CD34^+$, %	0.2783±0.1893	0.03-3.27	0.2851±0.1468	0.07-0.62	0.7811
$CD34^+$, $\times 10^6$	2.971±3.135	0.2-46.9	3.005±2.381	0.5-13.5	0.9341

The content of $CD34^+/CD45^+$ HPC varied from 0.07 to 0.62% in the COVID-19 group and from 0.03 to 3.27% in the control group (0.29±0.15 and 0.28±0.19%, respectively), which corresponds to the data obtained by other authors [4]. The statistical analysis revealed no significant differences in relative and absolute ($3.0\pm2.4\times10^6$ *vs* $3.0\pm3.1\times10^6$) HPC content between the studied groups (p=0.78 and p=0.93, respectively) (Fig. 2). Higher HPC content in some UCB samples of the control group (0.63% or more, n=22) was detected mainly at gestation ages <38 weeks (n=13, 59.1%) and/or birth weight >3800 g (n=10, 45.5%), and practically did not affect the results of statistics.

The search for possible correlations between UCB parameters and the trimester of pregnancy in which coronavirus infection occurred did not yield positive results in any of the studied values (total leukocyte count, r=0.096, p=0.46; relative HPC content, r=-0.027, p=0.84; absolute HPC content, r=0.034, p=0.79) (Fig. 3).

Over the past few decades, UCB has been regarded as a viable graft source and an effective alternative to bone marrow transplantation [13,17]. It is not surprising that the main attention of researchers was paid to predictors of obtaining the largest amount of both the UCB itself and the leukocytes including various stem/progenitor cell populations [24,36]. The factors that significantly affect these parameters can include the duration of pregnancy and body weight of the newborn. The highest concentration of $CD34^+$ cells was detected in UCB samples collected at gestation term <32 weeks [20,30], which is consistent with the data obtained in the course of this work during the analysis of the control group. The factor negatively affecting the content of HPC is the number of previous births – the content of progenitor cells was maximum in UCB from primiparous and decreased with each subsequent birth [24]. As for other factors that can influence UCB characteristics (mother's age, child's sex, blood type, method of delivery, *etc.*), the data available in the literature are contradictory.

It is noteworthy, preeclampsia also can have a pronounced negative impact not only on the content of HPC and other progenitor cell populations (including endothelial progenitor cells), but also on their "stemness" and ability to differentiate [12,19,34]. As endothelial dysfunction plays a key role in the pathogenesis of both preeclampsia and COVID-19, there may be a lot in common between these two diseases [1,3,5,26,38].

As for the effect on UCB parameters of the presence of infectious (including viral) diseases in a pregnant woman, such data are practically absent in the literature – acute or chronic infections are a contraindication for UCB cell banking for safety reasons of the use of blood products. The only pathogenic virus for which it was possible to find information about the effect on UCB composition was cytomegalovirus (CMV), whose presence in the transplant or

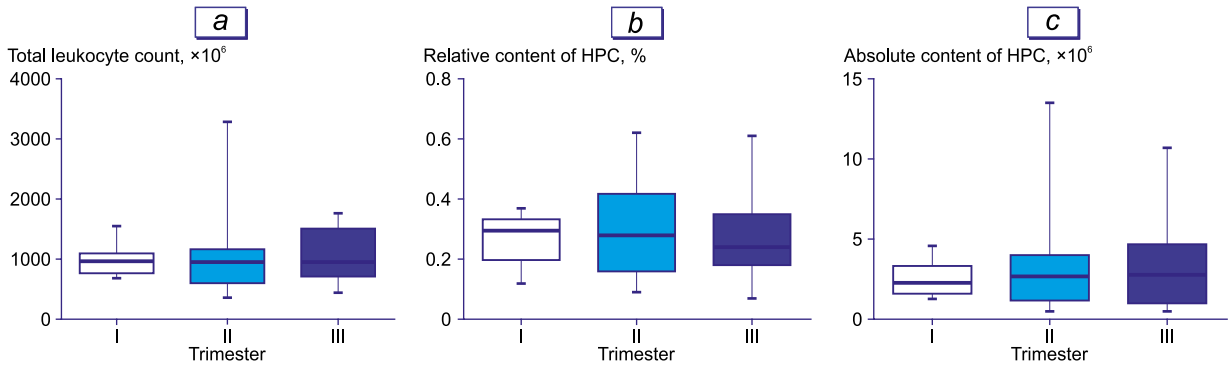

Fig. 3. Total leukocyte count (*a*), relative (*b*) and absolute (*c*) HPC content in UCB samples depending on the pregnancy trimester in which COVID-19 was diagnosed.

reactivation during chemotherapy can destroy the newly formed hematopoietic system of the recipient and/or lead to no less dramatic consequences [37]. According to available data, acute CMV infection in a pregnant woman is accompanied by a significant (almost 2-fold) decrease in the number of $CD34^+$ cells in the newborn's cord blood [14].

Nevertheless, during this preliminary study, we were unable to identify any significant differences in HPC content in the UCB samples of newborns from healthy women in labor and those who underwent mild and moderate forms of COVID-19 during pregnancy. The insufficient number of cases and the absence of patients with severe forms of the disease in the studied group did not allow us to detect possible correlations with the gestational period at which the pregnant woman was infected and other parameters of newborn health, which requires further research.

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