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LETTER TO THE EDITOR

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Case series of SARS-COV-2 infection in pregnant African women: focus on biological features

To the Editor,

The world is currently facing the coronavirus COVID-19 pandemic that is a global health crisis and the greatest challenge of our time. Therefore, all relevant knowledge about this global threat will contribute to the global effort to mitigate the adverse consequences of this pandemic. To the best of our knowledge, very few studies have reported clinical features of SARS-CoV-2-infected pregnant women,[1–4] and to date, no reports in the African context exist. Data on SARS COV-2 infection impacts on pregnant women living in high tropical disease burden settings are, therefore, of great interest. Here, we report on the coronavirus disease 2019 (COVID-19) features of four pregnant women who were diagnosed with SARS-COV-2 infection.

All women were screened for endemic diseases and common viral infections including Malaria, chikungunya virus, cytomegalovirus, Epstein-Barr virus, dengue virus, hepatitis B virus, hepatitis C virus, human immune-deficiency virus, herpes simplex virus, rubella virus, syphilis, and toxoplasmosis. For all women, we performed an extended blood work analysis, which included analysis of inflammation markers, angiogenic, liver, kidney, pancreatic, and thyroid function markers (Table 1).

The first patient (case no. 1) was a 38-year-old multiparous woman, 31 weeks pregnant with triplets, who consulted for pelvic abdominal pain. She had no history of medical condition or surgery. Her last prenatal check-up was normal and without intercurrent illness. The two ultrasounds, done at Weeks 15 and 23 of her pregnancy, showed perfectly healthy triplet pregnancy. The initial medical examination showed a low blood pressure (100/80 mmHg) and irregular uterine contractions, with a Baumgarten score of 4. The patient was, therefore, hospitalized for observation and underwent oxygen therapy and both a tocolytic treatment with Nicardipine (10 mg/ml) and a fetal pulmonary maturation treatment (dexamethasone 12 mg). After 48 h, the patient developed a fatty cough accompanied by rhinorrhea and myalgia. The pleuropulmonary examination revealed pulmonary condensation syndrome. On the basis of the epidemiological context, the patient nasopharyngeal samples were tested for SARS-COV-2 using real-time polymerase chain reaction (RT-PCR) and yielded a positive result. The blood count did not reveal any abnormalities. C-reactive protein (CRP) concentration was 28 mg/L, which is well above the normal range. C3c was above the normal range at 2 g/L (normal range: 0.8–1.6 g/L). Procalcitonin (PCT) concentration was 1 ng/ml, two-fold above the sepsis threshold (of 0.5 ng/ml). In addition, soluble FMS-like tyrosine kinase 1 (sFlt-a) to placenta growth factor (PlGF) ratio was 27.5 with a negative predictive value of 99.3%, which indicated that the risk of preeclampsia could be ruled out within the week of measurement of the variables. Case 1 was transferred to the COVID-19 management and treatment center, where she was put on the anti-COVID-19 treatment protocol, which consisted of oxygen therapy, hydroxychloroquine (200 mg two times a day for 10 days), azithromycin (500 mg on Day 1 and 250 mg/day for 4 days), vitamin C (500 mg/day for 10 days), and zinc tablets (15 mg/day for 10 days). The mother was declared cured 10 days following the 10 days of treatment. Due to persisting abdominal pain, infants were delivered during an urgent cesarean. The three infants were alive and healthy. The mother died 2 months later from unknown causes.

The second patient (case no. 2) was a 20-year-old, who presented mild flu-like symptoms throughout her last days of pregnancy and tested negative for SARS-COV-2. Childbirth happened without any major difficulties. Two weeks after birth, the patient returned with cough, fever, headache, thoracic pain, stage 2 dyspnea (with 80% oxygen saturation), and signs of viral pneumonia were observed through a chest computed tomography (CT) scan. The blood pressure was normal. Blood work showed mild erythrocytopenia, anemia (7.9 g/dl) with a 24% hematocrit and mild lymphopenia. The PCT level was extremely high at 41.1 ng/ml. CRP concentration was also very high (207 mg/ml). The patient was put on oxygen therapy and the SARS-COV-2 retest by RT-PCR came back positive. The chest CT scan showed a 70% lung invasion. The patient was transferred to the COVID-19 management and treatment center, where she was put on respiratory assistance, but died before the initiation of an anti-COVID-19 treatment protocol. The cause of death was acute respiratory failure associated with a pulmonary.

The third patient (case no. 3) was a 20-year-old, 20 weeks into her first pregnancy, and consulted for persisting cough, high fever, fatigue, and dyspnea. Complementary infections screening showed that the patient was coinfected with SARS-CoV-2 and malaria (parasitemia: 7700 parasites/µl). The patient presented signs of pancytopenia (characterized by a leukopenia associated with neutropenia) and highly elevated biochemical markers such as amylase

TABLE 1 Pregnant cases laboratory results

	Case 1	Case 2	Case 3	Case 4	Reference values (pregnancy)
Gestation (weeks)	32	40	20	22	
Blood cells counts					
Leucocytes (per mm^3)	6160	7640	**3340**	**10,130**	4000–10,000
Red blood cells (per mm^3)	4,090,000	**3,430,000**	**3,890,000**	**3,830,000**	4,000,000–5,000,000
Hemoglobin (g/dl)	12	**7.9**	11.5	**10.0**	11.5–15
Hematocrit (%)	34	**24**	**31**	**28**	37–47
Neutrophils (per mm^3)	3942 (64%)	6418	**1470**	**8813 (87%)**	2000–7500
Lymphocytes (per mm^3)	1294 (21%)	**917**	1140	1013 (10%)	1000–4000
Monocytes (per mm^3)	650 (15%)	650	710	304 (3%)	200–1000
Platelets/Thrombocytes (per mm^3)	296,000	286,000	**80,000**	334,000	150,000–400,000
Pre-eclampsia markers					
PIGF (pg/ml)	503		264	140	
sFIt-1 (pg/ml)	13,827		3658	1276	Exclusion of pre-eclampsia for at least 1 week (regardless of gestational age) NP = 99.3%
sFIt-1/PlGF	27.5		13.9	9.1	
Blood biochemistry					
Amylase (UI/L)			**92.1**	**105.0**	10–45
Aspartate aminotransferase (U/L)	9.8		9.5	33.0	4–42
Alanine aminotransferase (U/L)	27.4		20.4	15.0	2–25
Bilirubin direct (μmol/L)	7.7		**17.6**	**6.0**	0–1.7
Bilirubin total (μmol/L)	10.5		**44.1**	9.5	1.7–18.8
Creatinin (μmol/L)	78.0		70.50	69	35–80
Gamma-Glutamyl Transferase (U/L)	13.0		14.5	**201.5**	3–26
Urea (mmol/L)	1.9		3.1	2.2	2.9–8.2
Uric Acid (μmol/L)	**194**		**192.00**		35–70
Infection-associated markers					
C-reactive protein (mg/L)	**28**	**207**	**208**	**294.3**	0.4–8.1
Procalcitonin (ng/ml)	**1**	**41.1**	**0.41**	**0.20**	≤0.15
Complement C3c	**2**	**-**	**2**	**2.3**	0.8–1.6 g/L
Complement C4	0.34	-	0.36	**0.78**	0.16–0.48 g/L
Infection					
Malaria microscopy	Negative	Negative	**Positive (7700 parasites/μl)**	Negative	
Malaria Rapid Dx test—Serology (rapid chromatographic antigens test)	Negative	Negative	**Positive**	Negative	
HIV test—Serology (immunoluminescence antibody and antigen P24 tests)	Negative	Negative	Negative	Negative	
Hepatitis B virus test—Serology (immunoluminescence Hepatitis C virus [HCV] antibody test)	Negative	Negative	Negative	Negative	

TABLE 1 (Continued)

	Case 1	Case 2	Case 3	Case 4	Reference values (pregnancy)
HCV test—Serology (immunoluminescence HBS antigen test)	Negative	Negative	Negative	Negative	
Syphilis—Serology (Treponema pallidum antigen test by immunoluminescence test and the rapid plasma reagin antigen test)	Negative	Negative	Negative	Negative	
Epstein-Barr virus—Serology (rapid chromatographic antibodies test [IgG/IgM])	Negative	Negative	Negative	Negative	
Cytomegalovirus—Serology (rapid chromatographic antibodies test (IgG/IgM)	Negative	Negative	Negative	Negative	
Herpes simplex virus—Serology (rapid chromatographic antibodies test [IgG/IgM])	Negative	Negative	Negative	Negative	
Chikungunya virus—Serology (rapid chromatographic antibodies test [gG/IgM])	Negative	Negative	Negative	Negative	
Dengue virus—Serology (chromatographic antibodies test [IgG/IgM])	Negative	Negative	Negative	Negative	
Toxoplasmosis—Serology (immunoluminescence antibody test [IgG/IgM])	Negative	Negative	Negative	Negative	
Rubella virus—Serology (immunoluminescence antibody test [IgG/IgM])	Negative	Negative	Negative	Negative	
SARS-COV-2	**Positive**	**Positive**	**Positive**	**Positive**	

Note: Values in bold represent pathological or abnormal values.
Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M.

(92.1 UI/L), direct bilirubin (17.6 µmol/L), total bilirubin (44.1 µmol/L), and uric acid (192 µmol/L). C3c was above range at 2 g/L. PCT was above the normal range (0.41 ng/ml) but below the sepsis range (<0.5 ng/ml). Considerably elevated was the level of CRP (208 mg/ml). Also, the sFlt-1/PlGF ratio was 13.9, excluding the risk of pre-eclampsia. We first initiated an antimalarial treatment consisting of quinine (24 mg/kg/h without exceeding 1500 mg) and paracetamol (1 g every 6 h). The patient was transferred to the COVID-19 management and treatment center, where she received azithromycin, vitamin C, and zinc tablets (as described in case 1). The patient had a total recovery and was declared cured (as indicated by two negatives SARS-CoV-2 PCR tests) after 10 days of the anti-COVID-19 treatment protocol. The posttherapy ultrasounds check-up showed no abnormality in the pregnancy. She gave birth at her 38th week of pregnancy.

The fourth case (case no. 4) was 36 years of age, multiparous, and 22 weeks pregnant, who consulted after 3 days of cough and evolving dyspnea. The medical examination and observation confirmed cough, fever, and dyspnea. Her blood pressure was 160/90 mmHg, and she showed 91% oxygen saturation. The obstetric examination was unequivocal with a progressive monofetal pregnancy. She was diagnosed SARS-COV-2 positive by RT-PCR. Case 4 blood counts were characterized by anemia (10 g/dl and 28% hematocrit) and mild lymphocytosis associated with neutrophilia. Blood biochemistry showed high concentrations of amylase (105 UI/L), gamma-glutamyl transferase (201.5 UI/L), direct bilirubin (6 µmol/L), and uric acid (194 µmol/L). The level of C3c was above range at 2.3 g/L. PCT was a little above the normal range (0.2 ng/ml) but below the sepsis range (<0.5 ng/ml). The concentration of CRP was very high at 294.3 mg/ml. The patient was put on oxygen therapy and transferred to the COVD-19 management and treatment center, where oxygen therapy was associated with azithromycin (500 mg on Day 1 and 250 mg/day for 4 days), vitamin C (500 mg/day for 10 days) and zinc tablets (15 mg/day for 10 days). The patient was declared cured 20 days after the initiation of the anti-COVID-19 treatment protocol. She gave birth at her 40th week of pregnancy.

In SARS-COV-2 infection, the associated antenatal inflammation is clearly the main factor that may create an adverse environment for the mother and the fetus.[5,6] Circulating concentration of Inflammatory factors including complement C3c, PCT, and CRP are elevated in SARS-CoV-2-infected women. Although it was reported that a patient with COVID-19 associated severe acute respiratory distress syndrome was successfully treated with complement C3 inhibitor, our study is one of the first reports showing increased complement C3 in COVID-19. The serum level of complement C3 may have a prognostic value on the course of COVID-19, as both, our report and the one by Gralinski et al.,[7] highlighted complement activation as a part of the inflammatory response cascade in SARS-CoV-2. Also, our data highlights the potential of PCT and CRP as markers of COVID-19 extent of disease and prognosis.[1,8–11] All cases had high CRP concentrations. In three out of four cases, CRP levels were above 200 mg/ml. This was higher than 41.4 mg/ml, the

cut-off identified as independent predictors of COVID-19 severity and adverse outcome by Luo et al.[12] We also observed a stressed hepatobiliary function in two of the mothers. All cases had their level of PCT above the normal range (≤0.15 ng/ml). Moreover, the two cases who had their PCT levels above the sepsis threshold died weeks after giving birth. For pregnant women who had COVID-19, the risk of adverse events persists after birth.

Coronavirus disease 2019 (COVID-19)[13] and Malaria[14,15] are both life-threatening diseases. Our data showed that concomitant COVID-19 and malaria have a great impact on the patient's health condition as illustrated by the clinical observations and the pancytopenia revealed by the blood work. SARS-CoV-2-infected pregnant women should be screened for coinfections based on the local epidemiology. This would help prevent or stop the insidious development of adverse events. In this study, the management of COVID-19 pregnant women is a complex exercise that must combine obstetrics care or intervention and COVID-19 management. In Case 1, the tocolytic treatment and a fetal pulmonary maturation treatment combined with the anti-COVID-19 protocol have helped the live birth of the triplets. In all the cases, infants were born healthy without major complications. The anti-COVID-19 treatment protocol was well tolerated by pregnant women. However, we believe that mothers should be closely observed and followed for weeks or even months after birth.

Although only four cases were described in this study, the clinical and laboratory features of COVID-19 pregnant African women presented here are similar to what has been highlighted and described in the literature.[16,17] The limited number of women in our case series made it difficult to draw a definitive picture. However, the study clearly highlights PCT as a serious candidate marker for adverse outcomes. Further studies are needed to validate PCT, complement C3c, and even CRP as a marker for adverse outcomes in COVID-19 pregnant women. Validating such markers will help develop guidelines to prevent potential SARS-CoV-2 infection' adverse events during or soon after pregnancy.

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AUTHOR CONTRIBUTIONS

Joel F. Djoba Siawaya is the principal investigator who conceived and designed the study. He did the literature search, figures, data collection, data analysis, data interpretation, and writing. Carene A. A. Ndong Sima wrote the manuscript. Ulysse Minkobame was in charge of Patient clinical care and clinical data collection and interpretation. Anicet C. Maloupazoa Siawaya, Amandine Mveang Nzoghe, and Amel K. Alame-Emane were in charge of biological samples collection and analysis. Ofilia Mvoundza Ndjindji, Carinne Zang Eyi, and Armel Ndong Mintsa were involved in data collection, G-Stephane Padzys was involved in data interpretation and writing and Jean-François Meye participated in data interpretation.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

ETHICS STATEMENT

The board of Libreville Mother and Child University Hospital approved the study and written informed consent was obtained from patients.

DATA AVAILABILITY STATEMENT

All materials described in the manuscript will be freely available on-demand to any scientist wishing to use them.

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