

**Effect of SARS-CoV-2 estimated nasopharyngeal viral load on
perinatal outcomes in pregnant women affected by COVID-19
during the third trimester (VALOROUS study)**

(part of the multi-center epidemiological study

***“Prevalence and consequences of SARS-CoV-2 infection in pregnant
women, fetuses and neonates” [In French : Prévalence et conséquence
de l’infection à SARS-CoV-2 chez la femme enceinte, le fœtus et le
nouveau-né] – n. 2020-A00924-35).***

Principal Investigator

Prof. Daniele De Luca (MD, PhD) - ORCID ID: 0000-0002-3846-4834

Service de Pédiatrie et Réanimation Néonatale,

Hôpital “A. Béclère”, GHU Paris Saclay - APHP

157 rue de la Porte de Trivaux, 92140 Clamart, France

Tel: +33 (0)145374837 - Fax: +33 (0)145374546 - Email: dm.deluca@icloud.com

Last protocol English version n.2/august 2021

BACKGROUND

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has interested a relevant number of pregnant women worldwide. SARS-CoV-2 mother-to-child transmission is possible, although rare,¹ and clinical features of neonatal COVID-19 have been described.² Some studies suggest an increased incidence of stillbirths during pandemics³ and viral load is known to be associated with COVID-19 clinical severity and negative outcomes in non-pregnant patients.^{4–6} Nonetheless, the relationship between SARS-CoV-2 viral load and perinatal outcomes in pregnant women affected by COVID-19 in late pregnancy is unknown. We sought to investigate whether or not nasopharyngeal SARS-CoV-2 viral load has any effect on perinatal outcomes, when COVID-19 is diagnosed in the third trimester of pregnancy.

STUDY PURPOSES

The objectives of this study are to verify the hypotheses that maternal viral load in the airways is associated with perinatal outcomes in women affected by COVID-19 in the third trimester of pregnancy.

STUDY DESIGN

This is a multicenter, international, observational, retrospective, cohort study conducted in the following European academic tertiary referral perinatal center:

- Department of Women and Newborn Health, Paris Saclay University Hospitals, APHP, Paris (France)
- Fetal Medicine Unit, Saint George's Hospital, London (United Kingdom)
- Department of Translational Medical Sciences, University of Naples Federico II, Naples (Italy)

- Department of Obstetrics and Gynecology, University Hospital Brugmann, Université Libre de Bruxelles, Brussels (Belgium)

These centers have been chosen by contacts between peers, for their interest and expertise on perinatal COVID-19 research.

POPULATION

All women affected by COVID-19 during the third trimester of pregnancy will be eligible.

To be included patients had fulfill the following criteria: 1) diagnosis of COVID performed according to World Health Organization criteria; 2) viral load estimated by real-time polymerase chain reaction cycle threshold (Ct) for any viral gene. Exclusion criteria were the following: 1) fetal congenital malformations; 2) fetal with major genetic or chromosomal abnormalities

DATA COLLECTION

Data will be prospectively collected into customized, secured, electronic spreadsheets by local investigators in each recruiting center. Data will be completely anonymous in accordance with local and European privacy regulations, with only local investigators maintaining an identification log, separate from the spreadsheet used for data collection.

The following data will be collected: basic maternal and neonatal demographics, birth weight Z-score, time between COVID-19 diagnosis and delivery, 5' Apgar score, cord pH, estimated viral load for any vital gene, COVID-19 severity. These variables were chosen in order to keep a pragmatic design and make from the different centers easy to merge.

LABORATORY METHODS

Nasopharyngeal swabs will be obtained following US Center for Disease Control and Prevention guidelines.¹ Extraction and amplification will be performed with commercial

assays validated for SARS-CoV-2 diagnosis by WHO or local healthcare authorities.

Manufacturer's recommendations will always be followed. The SARS-CoV-2 load will be estimated for any viral gene, according to each laboratory protocol. RT-PCR technique will be performed according to European Center for Disease Prevention and Control.⁷

OUTCOMES

The following will be the primary outcomes and their association with the estimated viral load will be tested:

- Gestational age at the birth
- Birth weight
- 5'Apgar score
- Cord pH (cord pH will only be available in center routinely measuring it and with enough expertise)

STATISTICS AND CALCULATIONS

Being the first study on the topic a formal sample size calculation is unfeasible. We decided to enroll a convenience sample size of at least 100 patients as it has been done in the earlier study on SARS-CoV-2 load in non-pregnant patients.⁴

Data distribution will be tested, and data will be described and compared accordingly.

Basic population details will be summarized with descriptive statistics. Perinatal outcomes will be analyzed with general linear models with gamma distribution and/or finite mixture general linear distribution, considering maternal age and time between COVID-19 diagnosis and delivery, as covariates as these variables are known to influence perinatal outcomes. The recruiting center will also be included as covariate. Additionally, machine learning process analysis will be performed, if appropriate. Results will be expressed as crude and adjusted B coefficient (with 95% confidence interval). If adequate, results will be

graphically shown in scatter plots with trendline generated by local regression smoothing procedure (Epanechnikov's kernel with at least 85% of span). Analyses were done with R software rel.4.1. p -values <0.05 was considered significant.

WHY THE PROJECT IS IMPORTANT AND JUSTIFIED

Viral load is an important determinant of COVID-19 severity. However, the perinatal consequences of COVID-19 have been discovered late. This was due to the higher proportion of older age patients and the effect of lockdowns during 2020 masking the effect of COVID-19 on pregnancies. Now that we know that the disease may have severe consequences on pregnant women and that prematurity is amongst these,³ it becomes important to measure this risk. The same way the estimated viral load has contributed to the risk estimation for non-pregnant patients, we feel important to have this information for pregnant women and perinatal outcomes. This has a dual importance since these perinatal outcomes are strongly linked with the need for neonatal critical care and the number of available neonatal intensive care unit (NICU) beds may be not sufficient at least in some settings. Organization of care becomes important in order to avoid NICU beds shortage, particularly during pandemics, since babies needing critical care will not be reduced by the outbreak itself.⁸

ETHICAL CONSIDERATIONS

This is a multicenter, international, observational, retrospective, cohort study approved by the ethical committee (CPP Sud Méditerranée n.2020-A00924-35, final approval on nov 3, 2020 with approval for retrospectively collected data) of the coordinating center (Department of Women and Newborn Health, Paris Saclay University Hospitals, APHP, Paris (France)) within its research activities about COVID-19 pandemics and perinatology. Other participating centers will seek local IRB approval if needed according to local

Regulations. Women consent will also be obtained following local regulations. The study will be registered in the ISRCTN Registry and protocol details will be available there.

Although this is not considered necessary for an observational study, authors feel that this is increasing the quality of data as it reduces the chance of publication bias and selective data reporting. The study is pragmatic as the participation did not change the clinical management, which will be provided according to local protocols, essentially based on optimal prenatal care and international guidelines.

The study does not modify the routine care in anyway and does not carry any risk for the patients. Collected data are those already registered in the clinical care and no others are recorded. All data will be totally anonymous with only local investigators maintaining a separated log to identify patients.

Data will be collected in secured computers dedicated only to research purpose and transmitted using secured files. Local and European privacy regulations will be respected.

The study has no sponsor of any type and no honorarium is previewed for the participation to the study. Authors are performing the study for free during their worktime and they do not have any conflict of interest to disclose in relation to the project.

COMMUNICATION AND PUBLICATION PLAN

Video- or phone-conferences and in-person meetings are planned between the study investigators to update on the project, as needed. E-mail communication will be regularly used in-between. Collaborating investigators are already in contact since longtime and have participated together in other research and training project, so communication will not

face any particular problem. Results will be shared by email and videoconference with all authors and a group authorship will be created. Order of authors will be decided by consensus guided by the International Committee of Medical Journals Editors guidelines.⁹

Results will be partially presented in main obstetrical and/or neonatal congresses worldwide. Final results will be published in one or more articles in top international journals in the field of pediatrics or critical care. STROBE guidelines will be followed.¹⁰

DATA DEFINITION

- COVID-19: diagnosis provided according to WHO criteria
- Gestational age is based on the postmenstrual date and early gestation ultrasound findings.
- Birth weight will be considered as the weight measured in the delivery room after newborn stabilization
- Cord pH is measured from the umbilical artery at the delivery

REFERENCES

1. Vivanti A, Vauloup-Fellous C, Prevot S, et al. *Transplacental Transmission of SARS-CoV-2 Infection*. In Review; 2020. doi:10.21203/rs.3.rs-28884/v1
2. Raschetti R, Vivanti AJ, Vauloup-Fellous C, Loi B, Benachi A, De Luca D. Synthesis and systematic review of reported neonatal SARS-CoV-2 infections. *Nature Communications*. 2020;11(1). doi:10.1038/s41467-020-18982-9
3. Khalil A, von Dadelszen P, Draycott T, Ugwumadu A, O'Brien P, Magee L. Change in the Incidence of Stillbirth and Preterm Delivery During the COVID-19 Pandemic. *JAMA*. 2020;324(7):705. doi:10.1001/jama.2020.12746

4. The Massachusetts Consortium for Pathogen Readiness, Fajnzyblber J, Regan J, et al. SARS-CoV-2 viral load is associated with increased disease severity and mortality. *Nat Commun.* 2020;11(1):5493. doi:10.1038/s41467-020-19057-5
5. Zacharioudakis IM, Prasad PJ, Zervou FN, et al. Association of SARS-CoV-2 Genomic Load with Outcomes in Patients with COVID-19. *Annals ATS.* 2021;18(5):900-903. doi:10.1513/AnnalsATS.202008-931RL
6. Westblade LF, Brar G, Pinheiro LC, et al. SARS-CoV-2 Viral Load Predicts Mortality in Patients with and without Cancer Who Are Hospitalized with COVID-19. *Cancer Cell.* 2020;38(5):661-671.e2. doi:10.1016/j.ccell.2020.09.007
7. <https://www.ecdc.europa.eu/en/all-topics-z/coronavirus/threats-and-outbreaks/covid-19/laboratory-support/questions>.
8. De Luca D. Managing neonates with respiratory failure due to SARS-CoV-2. *The Lancet Child & Adolescent Health.* 2020;4(4):e8. doi:10.1016/S2352-4642(20)30073-0
9. <http://www.lcmje.org>.
10. von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol.* 2008;61(4):344-349. doi:10.1016/j.jclinepi.2007.11.008