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Expert Opinion

Pharyngeal sampling for PCR-testing in the investigation of SARS-COV-2 vertical transmission in pregnancy

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A B S T R A C T

The novel COVID-19 global pandemic has raised, among many others, major concerns regarding the impact of infection during pregnancy. Current evidence suggests that vertical transmission from mother to baby, antenatally or intrapartum, does occur, but is uncommon. According to the published reports of infants born to COVID-19-affected mothers, as well as the anecdotal experience of current practices worldwide, it appears that investigations regarding the potential of SARS-COV-2 vertical transmission in pregnancy have so far been based, to a large extent, on PCR testing of neonatal pharyngeal swab samples.

Given that the transplacental route of intrauterine transmission for SARS-COV-2 is less likely to immediately involve the upper respiratory tract of the newborn, contrary to what happens after birth, it would be advisable to include appropriate biological samples, such as cord blood, placenta, amniotic fluid and neonatal blood, along with the pharyngeal samples, in order to contribute significantly to such investigations. It is important to point out that negative PCR tests of neonatal pharyngeal samples do not exclude the possibility of intrauterine viral transmission, while positive pharyngeal swabs are more likely to reflect intrapartum or postpartum contaminants, rather than antenatal intrauterine transmission, in the absence of other criteria.

Revision and enhancement of the so far prevailing practices appear important, in order to facilitate the development of good clinical practice for managing neonates and ensuring safety of families and healthcare providers.

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Introduction

The COVID-19 global pandemic has raised, among many others, major concerns regarding the impact of infection during pregnancy, which in turn result in regular revision of guidelines and recommendations, based on emerging evidence and acquired knowledge.

One major concern in COVID-19-affected pregnancies is the potential of vertical viral transmission (transmission from mother to baby antenatally or intrapartum). This article aims to underline the limitations of the diagnostic procedures used to evaluate the potential risk of SARS-COV-2 vertical transmission, with focus on the use of neonatal pharyngeal swabs.

Routes of vertical viral transmission

The main routes of vertical transmission of a viral infection are from mother-to-fetus across the placenta (transplacental) and from mother-to-newborn by direct contact of the baby with the genital tract during delivery (intrapartum).

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The transplacental route of infection implies that viruses can gain access to the decidua and placenta by ascending from the lower genital tract or, more commonly, via hematogenous transmission. Viral tropism for the decidua and placenta is then dependent on viral entry receptor expression in these tissues, as well as on the maternal immune response to the virus [1]. Angiotensin-Converting enzyme-2 (ACE2) identified as the surface receptor of SARS-COV-2 has been shown to be expressed in human placentas [2], thus allowing to assume that viral tropism and spreading of the virus through the placenta can be possible.

In the transplacental mode of transmission, viruses passing through the maternal-fetal interphase manage to cross the placental barrier and enter the fetal circulation, while they may also gain access to the amniotic cavity. Hence viral nucleic acids may be detected in the cord blood and amniotic fluid [3] as well as in fetal tissues, indicating fetal infection.

Evidence of SARS-COV-2 vertical transmission – Review

Current evidence suggests that vertical transmission from mother to baby, antenatally or intrapartum, does occur, but is uncommon [4]. Initial evidence was based on the presence of IgM for SARS-COV-2 in neonatal serum at birth [5,6], arguing that since IgM macromolecules do not cross the placenta, their presence is likely to represent a neonatal immune response to intrauterine infection. This evidence, however, has been challenged and remains under debate [7].

We have reviewed all the previous reports that came to our knowledge, of infants born to COVID-19-affected mothers in the early phase of the pandemic, appearing in the published literature from February 9 to April 20, 2020 [8–18], and comment on the samples used for PCR-testing in relation to the investigation of SARS-COV-2 transmission. According to those reports, and based on our anecdotal experience of the practices followed in Greece as well as other European countries, it appears that PCR testing had been based, to a large extent, on neonatal pharyngeal swabs. Out of a total of 114 neonates, tested during the first wave of the COVID-19 pandemic, 95 from China [8–16] and 19 from the U.S.A. [17,18], in only a small subset of 14 cases (12.2 %) described in four reports [8,9,16,18] were cord blood and/or amniotic fluid included in the samples. Placental tissues were examined in six cases [10,16], along with the pharyngeal swabs, paired with cord blood and amniotic fluid in three cases [9] or isolated in the remaining three (10), whereas vaginal secretions were examined in a total of six cases [5,9,18] (Details on sampling, as it appears in published case series and reports, are shown in Table 1). It is of note that several authors mentioned the lack of cord blood testing among the limitations of their studies. In all the above cases, PCR-testing of all sorts of samples was negative, apart from four neonates, one reported by Yu et al. [16] and three by Zeng et al. [13], who tested positive in the pharyngeal samples taken on day 2 and 4 of life; in these four infants it was assumed that viral acquisition was more likely to have occurred after birth.

In the late phase of the pandemic, isolated published reports [19,20] appear to confirm the potential of intrauterine transplacental hematogenous transmission, by detecting SARS-CoV-2 genome in the umbilical cord blood among other biological samples. In addition, the report by Vivanti et al. documents a rare case of intrauterine transmission with severe clinical impact on the newborn [19].

### Biological samples in the investigation of SARS-COV-2 vertical transmission

To date, SARS-COV-2 genome has been detected by molecular PCR testing in the placenta, both fetal and maternal side, umbilical cord blood, amniotic fluid, vaginal secretions, breast milk, as well as in neonatal serum and (naso)pharyngeal and rectal swabs [19–21].

Regardless of the incidence of SARS-COV-2 vertical transmission, which is likely to be low, or the clinical significance of such transmission, it is important to establish reliable criteria for its documentation. In this respect, the criteria proposed by Shah, et al. in April 2020 [22] contribute to the classification of cases of congenital infection as “confirmed, probable, possible, unlikely or not infected.” It is of note that, according to these criteria, the absolute confirmation of SARS-COV-2 intrauterine transmission to the newborn is based on the detection of viral genome in the

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**Table 1**

| Source (Ref. Nr) | Place of origin/Date of publication in 2020 | Infants in the study | Infants tested | Sample neonatal/(maternal) | Infants PCR(+) |
|-----------------|---------------------------------------------|----------------------|----------------|-----------------------------|---------------|
| Zhu H, et al. [11] | Wuhan/Febr 9 | 10 | 9 | throat swab | 0 |
| | Wuhan/Febr 12 | 9 | 6 | throat swab | 0 |
| Chen S, et al. [9] | Wuhan/March 1 | 3 | 3 | throat swab placenta | 0 |
| Lei D et al. [8] | Wuhan/March 2 | 9 | 4 | throat swab cord blood, amniotic fluid/(vaginal secretions) | 0 |
| Chen Y, et al. [10] | Wuhan/March 16 | 4 | 3 | throat swab | 0 |
| Yu N, et al. [15] | Wuhan/March, 24 | 7 | 3 | throat swab cord blood, placenta | 1 (throat s. on day 2 |
| Dong L et al. [4] | Wuhan/March, 24 | 1 | 1 | nasopharyngeal swab neonatal blood – also tested for antibodies/(vaginal secretions) | 0 (serum antibodies +) |
| Zeng H, et al. [5] | Wuhan/March, 26 | 6 | 6 | throat swab neonatal blood tested for antibodies nasopharyngeal swab anal swab | 0 (serum antibodies +) |
| Zeng L, et al. [12] | Wuhan/March, 26 | 33 | 33 | throat swab amniotic fluid/(vaginal secretions) | 3 on day 2&4 |
| Iqbal SN, et al. [17] | Washington DC/April, 1 | 1 | 1 | throat swab amniotic fluid/(vaginal secretions) | 0 |
| Breslin N, et al. [16] | New York/ April 9 | 18 | 18 | nasopharyngeal swab | 0 |
| Liu W, et al. [13] | Wuhan/April, 13 | 19 | 19 | throat swab, urine, faeces | 0 |
| Chen L, et al. [14] | Wuhan/April, 17 | 68 | 8 | throat swab | 0 |
| **Total** | | **188** | **114** | | |
umbilical cord blood, neonatal blood or amniotic fluid, regardless of the neonatal pharyngeal swab positivity or negativity.

Moreover, the need has emerged to distinguish between mother-to-fetus intrauterine transmission or mother-to-neonate intrapartum transmission or early postnatal contamination of the newborn. In this regard, in line with other authors [22,23], we believe that it appears more useful to avoid the terms “vertical” or “horizontal” transmission and rather develop diagnostic criteria that classify transmission as intrauterine transplacental infection of the fetus or newborn, neonatal infection acquired intrapartum, or neonatal infection acquired postnatally, which provides meaningful information as to the route of transmission. This classification, distinguishing between intrauterine / antenatal, intrapartum and early postnatal transmission of SARS-COV-2, requires a careful and meaningful interpretation of laboratory testing on various biological samples, in association with the clinical data. Therefore, the test method for investigating whether there has been intrauterine transmission of SARS-COV-2, would be to detect the virus in a combination of aseptic samples from cord blood, placenta, amniotic fluid, and, in cases of abortion or stillbirth, fetal tissues. It is obvious that testing restricted to neonatal pharyngeal swab samples, which has been the prevailing practice as of the time of this writing, cannot exclude or confirm the potential risk of intrauterine antenatal viral transmission.

Another way used to find the virus “aseptically” is the first pass meconium, given that the GI tract, similarly to the respiratory tract, is lined with ACE2 receptors. The interpretation of neonatal rectal swabs as a possible marker of vertical transmission is still unclear.

Interpretation of neonatal pharyngeal swabs

Testing the neonatal pharyngeal swab for SARS-COV-2 genome, in order to investigate the potential risk of vertical transmission in pregnancy, is a practice which has so far prevailed. This practice appears inadequate for the exclusion or confirmation of the intrauterine transplacental route of transmission, and has more value for identifying intrapartum and early postnatal acquisition of the virus.

In fact, detection of SARS-COV-2 RNA in neonatal pharyngeal samples may reflect the following: 1) Viral transmission occurring after birth through droplets from the immediate environment. It is important to point out that a positive pharyngeal sample most likely reflects contamination from the room that is cohorted by a SARS-COV2 infected mother. 2) Intrapartum contamination may occur through direct contact with the genital tract at vaginal delivery, as is the case with other viruses, Herpes Simplex Virus being the best example [1]. Intrapartum transmission of SARS-COV-2 is likely to occur due to exposure to maternal blood, vaginal secretions, urine or feces, this also depending on the infectivity of the virus in the mother’s genital fluids. 3) Viral acquisition may occur following intrauterine aspiration or swallowing of infected amniotic fluid, in the context of a viral amniotic infection. Regarding the latter, infection of the amniotic cavity is commonly encountered in ascending bacterial infections, but has also been causally related to a variety of viruses, more or less common and devastating for the fetus as CMV and Zika virus [1,3]. With regard to SARS-COV-2, viral genome in the amniotic fluid has been recently reported in only 2 cases [19,21], notably with a low viral load, as compared to the placenta [19]. Our review of the published literature in the early phase of the pandemic shows no evidence of the virus in 11 cases where the amniotic fluid was tested (Table 1); similarly, there was no such evidence in a few later cases where the amniotic fluid was available for testing [20]. These data suggest that aspiration or swallowing of infected amniotic fluid by the fetus in utero is less likely to account for a positive pharyngeal swab after birth.

In conclusion, given that the hypothetical route of infection for SARS-COV-2 in utero is less likely to immediately involve the upper respiratory tract, contrary to what happens after birth, it would be desirable to openly clarify to the public and healthcare providers involved, that negative PCR tests of neonatal pharyngeal samples do not exclude the possibility of intrauterine viral transmission. On the other hand, positive pharyngeal swabs are more likely to reflect intrapartum or postpartum contaminants, rather than antenatal intrauterine transmission, in the absence of other criteria. Appropriate biological samples, namely cord blood, placenta, amniotic fluid and neonatal blood, in association with pharyngeal and rectal swabs, may provide more meaningful information and contribute significantly to the investigation of the potential risk of intrauterine viral transmission.

In view of the ongoing increase in COVID-19-related deliveries, an enhancement of the current procedures and practices appears even more important, in order to facilitate the development of good clinical practice for managing neonates and ensuring safety of families and healthcare providers.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Racicot K, Mor G. Risks associated with viral infections during pregnancy. J Clin Invest 2017;127:1591–9.
[2] Valdes G, Neves A, Anton L, et al. Distribution of angiotensin-(1-7) and ace2 in human placenta of normal and pathological pregnancies. Placenta 2006;27:200–7.
[3] Gervasi M-T, Romero R, Bracalente G, J Matern Fetal Neonatal Med 2012;25:2002–13.
[4] Royal College of Obstetricians and Gynaecologists. Coronavirus (COVID-19) infection in pregnancy. Version 12. 2021. Updated October 20, 2020 https://www.rcog.org.uk/globalassets/documents/guidelines/2020-10-14-coronavirus-covid-19-infection-in-pregnancy-v12.pdf.
[5] Dong L, Tian J, He S, et al. Possible vertical transmission of SARS-CoV-2 from an infected mother to her newborn. JAMA 2020, doi:http://dx.doi.org/10.1001/ jama.2020.4621.
[6] Zeng H, Xu C, Fan J, et al. Antibodies in infants born to mothers with COVID-19 pneumonia. JAMA 2020, doi:http://dx.doi.org/10.1001/ jama.2020.4851.
[7] Wang C, Zhou Y-H, Yang H-X, Poon LC. Intrauterine vertical transmission of SARS-CoV-2: what we know so far. Obstet Gynecol 2020, doi:http://dx.doi.org/10.1016/j.ogc.2020.
[8] Chen H, Guo J, Wang C, et al. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. Lancet 2020, doi:http://dx.doi.org/10.1016/S0140-6736(20)30360-3.
[9] Lei D, Wang C, Li C, et al. Clinical characteristics of COVID-19 in pregnancy: analysis of nine cases. Chin J Perinat Med 2020;23:225–31.
[10] Chen S, Huang B, Luo D, et al. Pregnant women with new coronavirus infection: clinical characteristics and placental pathological analysis of three cases. Chin J Perinat Med 2020;49:E909.
[11] Chen Y, Peng H, Wang L, et al. Infants born to mothers with a new coronavirus (COVID-19). Front Pediatr 2020;8:., doi:http://dx.doi.org/10.3389/fped.2020.00010.
[12] Zhu H, Wang L, Fang C, et al. Clinical analysis of 30 neonates born to mothers with 2019-nCoV pneumonia. Transl Pediatr 2020;9:51–60 https://doi.org/10.21037/tp.2020.02.06.
[13] Zeng L, Xia S, Yuan W, et al. Neonatal early-onset infection with SARS-CoV-2 in 33 neonates born to mothers with COVID-19 in Wuhan, China. JAMA 2020, doi:http://dx.doi.org/10.1001/jamapediatrics.2020.0878 Pediatr.
[14] Liu W, Wang J, Li W, Zhou Z, Liu S, Rong Z. Clinical characteristics of 19 neonates born to mothers with COVID-19. Front Med 2020, doi:http://dx.doi.org/10.1007/s11684-020-00777-x.
[15] Chen L, Li Q, Zheng D, Jiang H, Wei Y. Clinical characteristics of pregnant women with Covid-19 in Wuhan, China. N Engl J Med 2020, doi:http://dx.doi.org/10.1056/NEJMc2009526.
[16] Yu N, Li W, Kang Q, et al. Clinical features and obstetric and neonatal outcomes of pregnant patients with COVID-19 in Wuhan, China: a retrospective, single-centre, descriptive study. Lancet Infect Dis 2020, doi:http://dx.doi.org/10.1016/S1473-3099(20)30376-6.

[17] Breslin N, Baptiste C, Gyamfi-Bannerman S, et al. COVID-19 infection among asymptomatic and symptomatic pregnant women: two weeks of confirmed presentations to an affiliated pair of New York City hospitals. AJOG MFM 2020, doi:http://dx.doi.org/10.1016/j.ajogmf.2020.100118.

[18] Iqbal Sn, Overcash R, Mokhtari N, et al. An uncomplicated delivery in a patient with Covid-19 in the United States. N Engl J Med 2020, doi:http://dx.doi.org/10.1056/NEJMc2007605.

[19] Vivanti AJ, Vauloup-Fellous C, Prevot S, Zupan V, Suffee C, Do Cao J, et al. Transplacental transmission of SARS-CoV-2 infection. Nat Commun 2020;11:1–7, doi:http://dx.doi.org/10.1038/s41467-020-17436-6.

[20] Fenizia C, Biasin M, Cetin I, Vergani P, Mileto D, Spinillo A, et al. Analysis of SARS-CoV-2 vertical transmission during pregnancy. Nat Commun 2020;11, doi:http://dx.doi.org/10.1038/s41467-020-18933-4.

[21] Zamaniyan M, Ebadi A, Aghajanpoor S, Rahmani Z, Haghsenas M, Azizi S. Preterm delivery, maternal death, and vertical transmission in a pregnant woman with COVID-19 infection. Prenat Diagn 2020;40:1759–61, doi:http://dx.doi.org/10.1002/pd.5713.

[22] Shah PS, Diambomba Y, Acharya G, Morris SK, Bitnun A. Classification system and case definition for SARS-CoV-2 infection in pregnant women, fetuses, and neonates. Acta Obstet Gynecol Scand 2020;99:365–8, doi:http://dx.doi.org/10.1111/aogs.13870.

[23] Blumberg DA, Underwood MA, Hedriana HL, Lakshminrusimha S. Vertical transmission of SARS-CoV-2: what is the optimal definition? Am J Perinatol 2020;37:769–72, doi:http://dx.doi.org/10.1055/s-0040-1712457.