

Pregnancy Outcomes in COVID-19: A Prospective Cohort Study in Singapore

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Abstract

Introduction: Pregnant women are reported to be at increased risk of severe coronavirus disease 2019 (COVID-19) due to underlying immunosuppression during pregnancy. However, the clinical course of COVID-19 in pregnancy and risk of vertical and horizontal transmission remain relatively unknown. We aim to describe and evaluate outcomes in pregnant women with COVID-19 in Singapore.

Methods: Prospective observational study of 16 pregnant patients admitted for COVID-19 to 4 tertiary hospitals in Singapore. Outcomes included severe disease, pregnancy loss, and vertical and horizontal transmission.

Results: Of the 16 patients, 37.5%, 43.8% and 18.7% were infected in the first, second and third trimesters, respectively. Two gravidas aged ≥ 35 years (12.5%) developed severe pneumonia; one patient (body mass index 32.9kg/m²) required transfer to intensive care. The median duration of acute infection was 19 days; one patient remained reverse transcription polymerase chain reaction (RT-PCR) positive >11 weeks from diagnosis. There were no maternal mortalities. Five pregnancies produced term live-births while 2 spontaneous miscarriages occurred at 11 and 23 weeks. RT-PCR of breast milk and maternal and neonatal samples taken at birth were negative; placenta and cord histology showed non-specific inflammation; and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific immunoglobulins were elevated in paired maternal and umbilical cord blood (n=5).

Conclusion: The majority of COVID-19 infected pregnant women had mild disease and only 2 women with risk factors (obesity, older age) had severe infection; this represents a slightly higher incidence than observed in age-matched non-pregnant women. Among the women who delivered, there was no definitive evidence of mother-to-child transmission via breast milk or placenta.

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Introduction

Since the first cases of coronavirus disease 2019 (COVID-19) in pregnancy were described,¹ significant concerns have been raised about the potentially increased susceptibility of pregnant women to severe disease,² and the unquantified risk of mother-child transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to the fetus and neonate.³ Despite our experience with other β coronavirus infections in pregnancy, including severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS),⁴ many questions remain regarding the clinical course of COVID-19 in pregnancy. A case series of 116 cases from Wuhan, China, reported a 6.9% incidence of severe pneumonia but no mortality among infected pregnant women, in contrast to the case fatality rate of 1–3% in the general population.⁵ Earlier case series from Italy, the US and Sweden also reported no maternal mortalities and critical care admission in <10% of infected gravidas,⁶ findings similar to systematic reviews involving >160 pregnancies that described favourable maternal and fetal outcomes, possibly related to pregnancy-specific physiological changes that mitigate COVID-19 severity.² More recently, however, there has been growing recognition of unexpected maternal mortality and severe morbidity associated with respiratory and thromboembolic complications affecting both low- and high-resource countries, including 15 reported fatalities in Iran, Brazil and Mexico, and at least 3 in the UK and US.^{7,8} Additionally, the unquantified risks of fetal SARS-CoV-2 infection increase the complexities of prenatal and perinatal management. The evidence for vertical transmission is still inconclusive. Published case series have reported mixed evidence of viral transmission via transplacental, vaginal or breast-milk routes, although the majority of these infections occurred in the late third trimester when the duration of viral exposure was limited.⁹ There is currently insufficient evidence to quantify the risks of vertical transmission when infection occurs at earlier gestations.¹⁰ Here, we present our experience managing 16 pregnant women diagnosed with COVID-19 in all trimesters in Singapore. We discuss the range of clinical manifestations, including trends towards a higher incidence of severe disease compared to age-matched non-pregnant women, and comprehensive analyses of perinatal samples in a subset of postpartum patients that have excluded vertical and horizontal transmission in our cohort so far.

Methods

Study participants

In this nationwide, prospective, multicentre study, we included all pregnant women with COVID-19 diagnosed by reverse transcription polymerase chain reaction (RT-PCR), who were admitted between 15 March 2020 and 22 August 2020 to the National University Hospital, KK Women's and Children's Hospital (KKH), Singapore General Hospital, and the National Centre for Infectious Diseases, all of which were the national receiving centres for COVID-19 maternal infections in Singapore.

Ethics approval

Ethics approval was obtained from National Healthcare Group Domain Specific Review Board. Verbal and written consent was obtained for collection of biological samples for clinical investigations. Informed consent was waived for collection of clinical data under the Infectious Diseases Act (Ministry of Health, Singapore) as part of COVID-19 pandemic investigations. Procedures were followed in accordance with the Declaration of Helsinki (1964, amended 2008) of the World Medical Association.

Screening and diagnosis

Pregnant women with acute respiratory symptoms, who had contact with known COVID-19 cases, had significant travel history, or were exposed to known community clusters, were screened with nasopharyngeal swabs. Only medically indicated, and not universal, screening was performed in Singapore during that time. Diagnosis of SARS-CoV-2 infection was made by RT-PCR of viral nucleic acids utilising the fully automated cobas® SARS-CoV-2 test on the cobas® 6800 Systems (Roche Molecular Systems, Branchburg, US), with selective amplification of ORF1, a non-structural region of the coronavirus genome unique to SARS-CoV-2, with target-specific forward and reverse primers, in line with World Health Organization recommendations.¹¹ Patients were isolated in negative pressure rooms until the diagnosis was confirmed and thereafter transferred to hospitals with maternity services where they were nursed in dedicated isolation wards. Patients were discharged after complete symptom resolution and 2 consecutive negative RT-PCR respiratory samples obtained 24 hours apart, or after at least 21 days had passed from symptom onset, according to updated Ministry of Health advisories (Fig. 1).

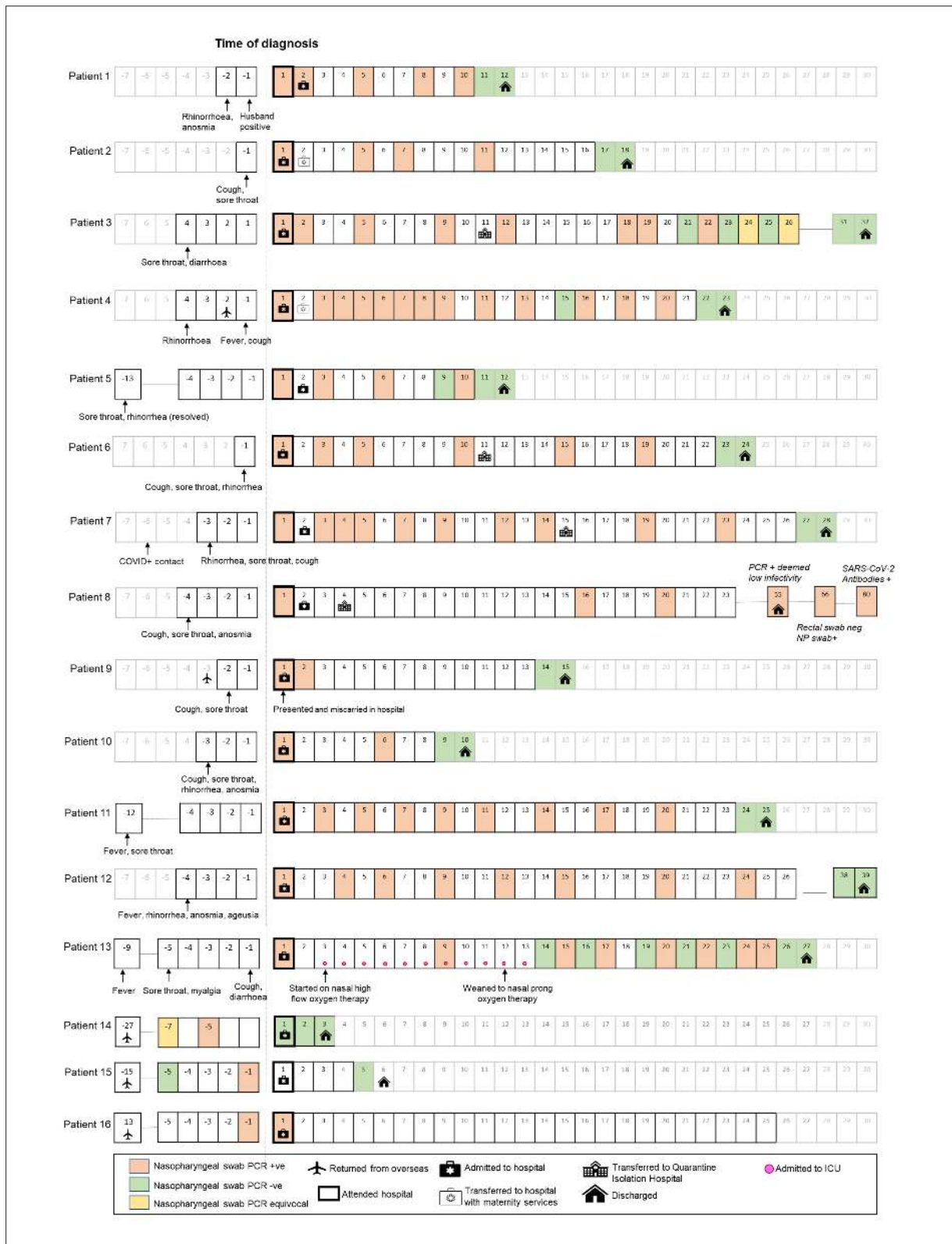


Fig. 1. Timeline for each patient from symptom onset to convalescence. Boxes represent days numbered in reference to the day of admission and are coloured according to nasopharyngeal swab reverse transcription polymerase chain reaction (RT-PCR) results for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Symbols are used to indicate significant events: hospital admission, intensive care unit transfer, transfer to the quarantine isolation hospital and discharge following 2 consecutive negative nasopharyngeal swabs at least 24 hours apart. Patients 8 and 16 remain positive at the time of writing.

Data and sample collection

We obtained demographic, clinical, laboratory and epidemiological data from hospital electronic medical records. Longitudinal data collected included range and duration of presenting complaints, contact history, comorbidities, antenatal history, and pregnancy outcomes including fetal loss, fetal growth and perinatal outcomes. Routine baseline screening included complete blood counts, C-reactive protein (CRP) levels, and liver and renal biochemistry; these were repeated when clinically indicated. Fetal and maternal surveillance during acute infection and convalescence were enhanced to detect adverse effects of COVID-19, if any, and in selected cases included maternal rectal swabs for RT-PCR, and prenatal and postnatal administration of the Edinburgh Postnatal Depression Scale to screen for depression and anxiety. Fetal structural and growth scans were performed at 18, 20–22, 28–32 and 34–36 weeks' gestation. At delivery, perinatal samples were collected to assess for vertical transmission, including maternal blood and vaginal swabs, amniotic fluid and umbilical cord blood (UCB), and swabs of the placental and umbilical cord surfaces, all for SARS-CoV-2 RT-PCR. Placenta and umbilical cord were histologically examined. Maternal and UCB sera were tested for SARS-CoV-2-specific immunoglobulins using the Elecsys Anti-SARS-CoV-2 assay (Roche Diagnostics, Basel, Switzerland), a sandwich immunoassay utilising a recombinant protein representing the nucleocapsid (N) antigen, performed after successful calibration and quality control. A signal cut-off index (COI) ≥ 1.0 indicated seroreactivity, while COI < 1.0 was seronegative.

Statistical analyses

Statistical analysis was conducted using GraphPad Prism version 8.4.2 for Windows (GraphPad Software, San Diego, US) with categorical variables expressed as percentage, and continuous variables expressed as a median (range). Comparison of outcomes was performed with Fisher's Exact test ($p < 0.05$).

Results

Sixteen pregnant women with COVID-19 were included in this study (Table 1). Maternal age ranged from 23–36 years. Diverse ethnicities were represented (Malay, $n=5$; Chinese, $n=3$; Indian, $n=4$; Eurasian, $n=1$; Caucasian, $n=3$). Gestations at diagnosis ranged from 4 weeks (based on the last menstrual period) to 36 weeks, on ultrasound survey at admission (first trimester, $n=6$; second trimester, $n=7$; third trimester, $n=3$). Three patients had significant comorbidities: patient 6 had

gallstone disease and was a hepatitis C carrier with normal levels of transaminases; patients 8 and 14 had well-controlled asthma; and the other patients had no significant comorbidities. Three patients (18.8%) were asymptomatic, while 13 (81.2%) had mild respiratory symptoms on initial assessment at admission, including cough, sore throat, rhinorrhoea, anosmia and ageusia. No patients diagnosed with COVID-19 in the second or third trimesters developed obstetric complications; 87.5% ($n=14$) had mild disease, remained afebrile throughout admission, and did not require supplemental oxygen. The exceptions were 2 women (Patients 9 and 13) who had complicated clinical courses, described separately below.

Two women diagnosed in their first trimester of pregnancy were admitted with their infant children who were breastfeeding and also COVID-19 positive, and 2 women in their third trimester were transferred to the same hospital units as their COVID-19 positive partners, following a national policy of keeping families together. Daily physical examination of the cardiorespiratory system and biweekly fetal heart rate examination (in viable pregnancies > 24 weeks of gestation) were performed. Women in the first trimester did not routinely undergo viability assessment unless they reported bleeding with or without pelvic pain; ultrasound assessments were performed once the woman was RT-PCR negative for SARS-CoV-2, a policy effected to mitigate risk to medical staff. Symptomatic treatment was prescribed for upper respiratory tract symptoms and fever. Multidisciplinary management was provided by infectious disease and maternal-fetal specialists, with additional input from respiratory physicians and psychological support teams (psychiatrists and counsellors) where indicated. Chest radiographs (CXR) were clinically indicated in patients 9, 11, 12 and 13 for persistent fever, despite the initial lung examination being clear in the first 3 patients. The CXR were performed on patients 11 and 12 before their early pregnancies were diagnosed, and these were normal. Some women who were clinically stable with mild or no symptoms, but were still RT-PCR positive, were transferred to a community isolation facility and remained there until they were negative on RT-PCR following national policy. The clinical course from admission to discharge is summarised in Fig. 1.

Nine patients (56.2%) had raised absolute neutrophil count or CRP levels, or both, while only patient 8 had lymphopaenia (0.8×10^9 cells/L) and monocytopenia (0.2×10^9 cells/L) (Fig. 2A). Anaemia was observed in 4 (23%) and ferritin levels were low in 2 (12.5%); renal

Table 1. Characteristics of pregnant women with confirmed COVID-19 infection and pregnancy outcomes

No.	Age (y)	Ethnicity	Parity	Obstetric history	Comorbid status	GA (week, day)	Contact and travel history	Presenting symptoms	Presenting signs	Chest imaging	Supplemental O ₂	Duration RT-PCR+ NP swab (days)	Pregnancy outcome
1	29	Indian	G1P0	-	None	36w 1d	Travelled from UK	Rhinorrhoea Anosmia	Lungs clear bilaterally	Nil	Nil	10	Elective induction at 41w, SVD. Baby well
2	30	Chinese	G3P1	1 LSCS	None	9w	Travelled from UK and France	Sore throat Dry cough	Lungs clear bilaterally	Nil	Nil	17	Ongoing pregnancy
3	36	Caucasian	G3P1	Ectopic pregnancy SVD	None	28w 6d	Exposed through known CO-VID-19 cluster	Sore throat Diarrhoea	Lungs clear bilaterally	Nil	Nil	32	SVD at 39w. Baby well
4	26	Caucasian	G1P0	-	None	29w 5d	Travelled from Spain	Rhinorrhoea Cough	Lungs clear bilaterally	Nil	Nil	22	SVD at 40w Baby well
5	29	Chinese	G2P1	SVD	None	9w 1d	Family member COVID positive	Sore throat Rhinorrhoea	Lungs clear bilaterally	Nil	Nil	11	Ongoing pregnancy
6	34	Malay	G5P1	SVD 1 ectopic pregnancy 2 abortions 1 miscarriage	Hepatitis C carrier Gallstones	24w 5d	Family members COVID positive	Dry cough Sore throat Rhinorrhoea	Lungs clear bilaterally	Nil	Nil	23	Symmetrical SGA at 29w that picked up at 35w SVD at 39w Baby well
7	25	Malay	G1P0	-	None	6w	Exposed through known COVID-19 cluster	Cough Sore throat Rhinorrhoea	Lungs clear bilaterally	Nil	Nil	27	Complete miscarriage 5 days after discharge
8	26	Malay	G1P0	-	Asthma	22w 1d	Exposed through known COVID-19 cluster	Cough Sore throat Anosmia	Lungs clear bilaterally	Nil	Nil	80	SVD at 38w Baby well
9	35	Eurasian	G1P0	-	TCP resolved	23w 3d	Travelled from UK	Sore throat Cough Fever	Lungs clear bilaterally	CXR: clear lung fields CT: opacities	Nil	15	Miscarried on day of admission

Table 1. Characteristics of pregnant women with confirmed COVID-19 infection and pregnancy outcomes (Cont'd)

No.	Age (y)	Ethnicity	Parity	Obstetric history	Comorbid status	GA (week, day)	Contact and travel history	Presenting symptoms	Presenting signs	Chest imaging	Supplemental O ₂	Duration RT-PCR+ NP swab (days)	Pregnancy outcome
10	32	Chinese	G1P0	-	None	10w 2d	Unknown	Rhinorrhoea Anosmia Cough Sore throat	Lungs clear bilaterally	Nil	Nil	10	Ongoing pregnancy
11	35	Caucasian	G2P1	SVD	None	4w	Travelled from UK and Europe	Fever Sore throat	Lungs clear bilaterally	CXR: clear lung fields	Nil	25	Ongoing pregnancy
12	29	Malay	G5P3	SVD Miscarriage	None	4w 2d	Unknown	Fever Rhinorrhoea Anosmia Ageusia	Lungs clear bilaterally	CXR: clear lung fields	Nil	39	Ongoing pregnancy
13	36	Malay	G5P4	LSCS	None	16w	Unknown	Fever Cough Sore throat Diarrhea	Lungs bilateral crepitations	CXR: opacities	Yes (high flow nasal cannula, Venturi mask 40%)	27	Ongoing pregnancy
14	24	Indian	G1P0	-	Asthma	27w 5d	Stayed in India 9/2/20 to 20/6/20	Nil	Lungs clear bilaterally	Nil	Nil	5	Ongoing pregnancy
15	23	Indian	G1P0	-	None	26w 4d	Stayed in India from Feb 2020 to 23/6/2020	Nil	Lungs clear bilaterally	Nil	Nil	5	Ongoing pregnancy
16	27	Indian	G2P1	SVD	None	27w 6d	Stayed in India from Feb 2020 to 9/8/2020	Nil	Lungs clear bilaterally	Nil	Nil	2 (still positive at time of writing)	Ongoing pregnancy

CXR: chest radiographs; LSCS: lower segment caesarean section; TCP: thrombocytopenia; SGA: small-for-gestational age; SVD: spontaneous vaginal delivery; GA: gestational age; RT-PCR: reverse transcription polymerase chain reaction; NP: nasopharyngeal; CT: computed tomography

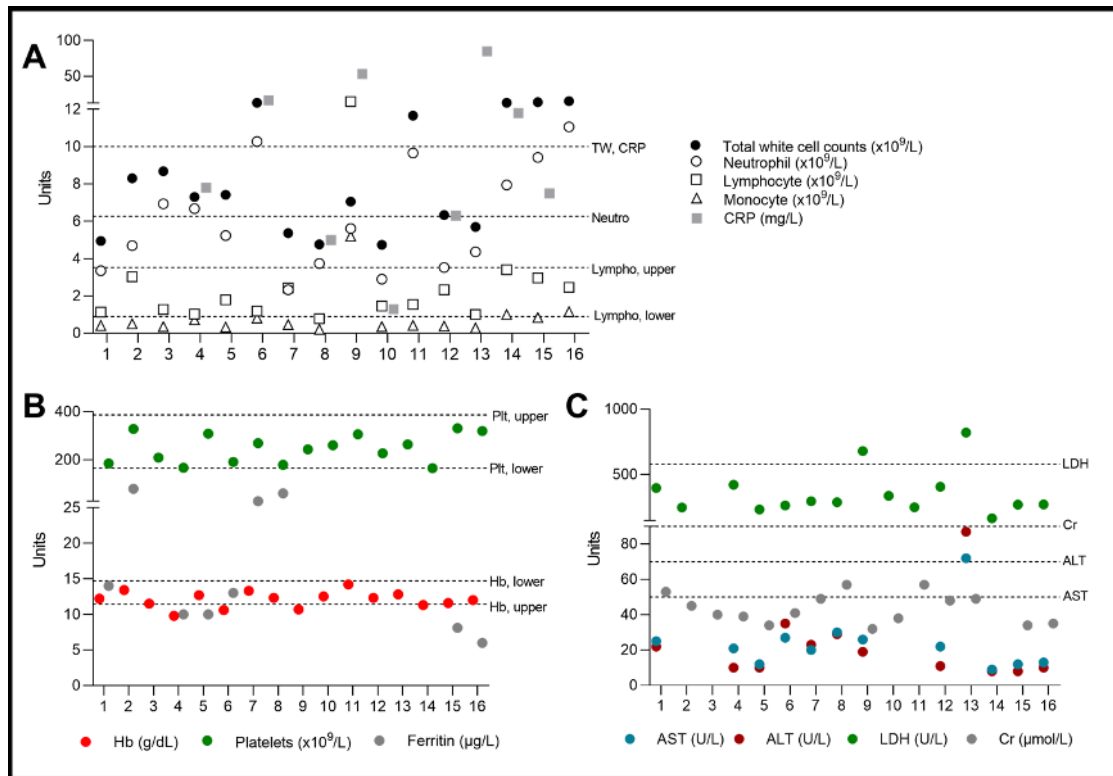


Fig. 2. Biochemistry results for patients 1 to 16 (x-axis) on admission. (A) Patients 9 and 13 who developed severe COVID-19 infection had raised C-reactive protein (CRP) and lactate dehydrogenase (LDH) levels. (B) Haematological indices for all patients were largely normal. (C) Patient 13 had raised aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels which normalised before discharge.

and liver functions were normal in all patients at presentation (Fig. 2B, 2C). The median duration of viral shedding, from the first RT-PCR positive nasopharyngeal swab to the second RT-PCR negative result (or the last documented swab test), was 19 days (range, 2–80 days). Ten patients (62.5%) received stress management education from peer counsellors during hospitalisation and 2 (12.5%) were prescribed anti-anxiety medications by a psychiatrist. Five patients consented to rectal swabs for RT-PCR during convalescence to assess continued SARS-CoV-2 shedding, all of which were negative. Fourteen patients (87.5%) have been discharged at the time of writing. One patient remained admitted while awaiting criteria for discharge. The other patient remained RT-PCR positive 80 days after development of initial symptoms but was deemed no longer at risk of secondary transmission and discharged following a national policy shift from test-based to time-based de-isolation,¹² she also displayed seropositivity (COI, 13.5) despite the positive RT-PCR (Fig. 1). There were no maternal mortalities.

Patient 9 (35 years old) received her initial antenatal care in the UK and reported a low-risk first-trimester

fetal aneuploidy screen and normal fetal anatomy survey at 21 weeks’ gestation. She had multiple uterine fibroids (including a 9.5cm cervical fibroid). She presented to KKH 3 days after arriving from the UK with a recent onset of cough, chest discomfort and lower abdominal pain at 23 weeks 3 days of gestation. Her partner also reported a productive cough. They had no contact with suspected or confirmed COVID-19 cases. On presentation, the patient was febrile (38.9°C), tachypnoeic (20 breaths/min), normotensive and had clear lung fields with no crepitations or rhonchi. There was no uterine tenderness indicative of red degeneration of the fibroid. Her oxygen saturations remained at >94% and she did not require supplemental oxygen. Two consecutive nasopharyngeal swabs were positive for SARS-CoV-2 on RT-PCR. The patient developed painful contractions soon after admission and miscarried approximately 12 hours later. Although the initial CXR was normal, computed tomography of the thorax was performed the following day for persistent postnatal fever, and showed multiple bilateral ground-glass opacities, predominantly subpleural and in the lower pulmonary lobes, some of which demonstrated increased peripheral density (reversed

halo sign; Fig. 3A–3D). Maternal serum and urine, swabs of the fetal ear, nasopharynx and oropharynx, fetal cord blood, and placenta surfaces (fetal and maternal) were negative on RT-PCR for SARS-CoV-2. She was transferred to Singapore General Hospital for further management and received intravenous antibiotics until blood cultures returned negative. She maintained good oxygen saturation levels without the need for supplemental oxygen, remained haemodynamically stable throughout her admission, and was discharged clinically well after 15 days.

Patient 13 (36 years old; body mass index 32.9 kg/m²) presented with generalised myalgia and lethargy (>7 days), and fever, dry cough and sore throat (1 day) at 18 weeks 4 days of gestation. She was febrile (38.3°C), tachycardic (120 beats/min) and had bilateral pulmonary crepitations on chest auscultation with an initial oxygen saturation of 95% on room air, as measured by pulse oximetry. The CXR demonstrated bilateral airspace opacities (Fig. 3E, 3F). The patient's clinical condition worsened over the next 48 hours and she was subsequently transferred to the intensive care unit (ICU) because of an increasing need for support with high flow oxygen. She did not require intubation but remained in ICU for 10 days until being weaned off supplemental oxygen. Transient transaminitis was observed on day 3 of admission (aspartate aminotransferase, 72 U/L; alanine aminotransferase, 87 U/L; lactate dehydrogenase, 822 U/L) and normalised after 19 days (Fig. 2C). Following discharge on day 25 (22 weeks 2 days of gestation) when she was clinically well, the fetal structural ultrasound survey showed normal anatomy and growth.

Of the 9 patients with previsible pregnancies (<24 weeks' gestation), 2 (22.2%) had spontaneous miscarriages. Patient 7 presented with painless vaginal bleeding at 11 weeks amenorrhoea, 5 days after discharge. Serial transvaginal ultrasonography and serum beta human chorionic gonadotropin assays confirmed an early miscarriage at home which was managed expectantly. Clinical surveillance retrospectively confirmed spontaneous complete pregnancy resolution and no products of conception were collected for RT-PCR. Patient 9 had a mid-trimester miscarriage as described above. Patient 6 who was diagnosed with COVID-19 at about 25 weeks' gestation was diagnosed with a symmetrically small-for-gestational-age fetus at 29 weeks (estimated fetal weight at the sixth centile; normal amniotic fluid index and umbilical artery Doppler studies). Fetal growth had normalised by 35 weeks and she subsequently had an uncomplicated term vaginal delivery of a normal-birth-weight infant

at 39 weeks. There were 4 other live births in this cohort. Patients 1, 3, 4 and 8 had otherwise uncomplicated pregnancies with reassuring fetal growth on surveillance, delivering vaginally at 39–41 weeks' gestation, 3 to 11 weeks after the last negative RT-PCR for SARS-CoV-2. In the 5 women who delivered, swabs of the vagina, umbilical cord, maternal and fetal placental surfaces, amniotic fluid, and maternal blood and UCB were negative for SARS-CoV-2 on RT-PCR, and histological examination of the placenta and umbilical cord did not reveal ischaemia, necrosis or funisitis (Table 2). While patient 1 had insufficient colostrum to screen for SARS-CoV-2 prior to discharge, colostrum from patients 4 and 6 was negative on RT-PCR. All mother–baby pairs had elevated SARS-CoV-2 total immunoglobulins. In patient 1, perinatal maternal blood (COI 27.9) and UCB (COI 11.2) were strongly positive. Maternal blood and UCB immunoglobulins in patient 4 were mildly elevated in comparison (COI 1.5 and 2.8, respectively; Table 2). All mothers practised immediate skin-to-skin contact and direct breastfeeding, and mother–baby pairs were discharged well on postnatal day 2.

Discussion

Most patients in our study had a mild clinical course, but 2 women developed severe pneumonia. Recent reports have highlighted the unpredictable clinical course of COVID-19 infection in pregnancy.^{13,14} Severe maternal disease can manifest prenatally or postnatally and trigger abrupt postnatal decompensation, and its presentation may be delayed up to 14 days from symptom onset. Patient 9 had no underlying comorbidities predictive of clinical deterioration.¹⁵ Her clinical status worsened precipitously following delivery, reflecting aggressive disease progression. Patient 13 was obese and both patients 9 and 13 were ≥35 years old; obesity and older age have both been identified as risk factors in maternal deaths, along with diabetes mellitus, cardiorespiratory disease or thromboembolic complications.¹⁴ The median duration of viral shedding in our study was 19 days, although one patient remained positive up to 80 days after initial symptoms. Cohort studies have reported prolonged shedding up to 60 days; putative risk factors for this phenomenon were not present in this patient.¹⁶ Faecal excretion of SARS-CoV-2 for several weeks is well documented, raising concerns of neonatal transmission during vaginal delivery.¹⁷ Rectal swabs were obtained from some of our patients to inform labour management, and all were negative. The mid-trimester pregnancy loss (patient 9) may be partly related to the severe systemic inflammatory response of the acute infection, although there is a paucity of immunological data to prove this.¹⁸ Similarly, with a population incidence of small-for-

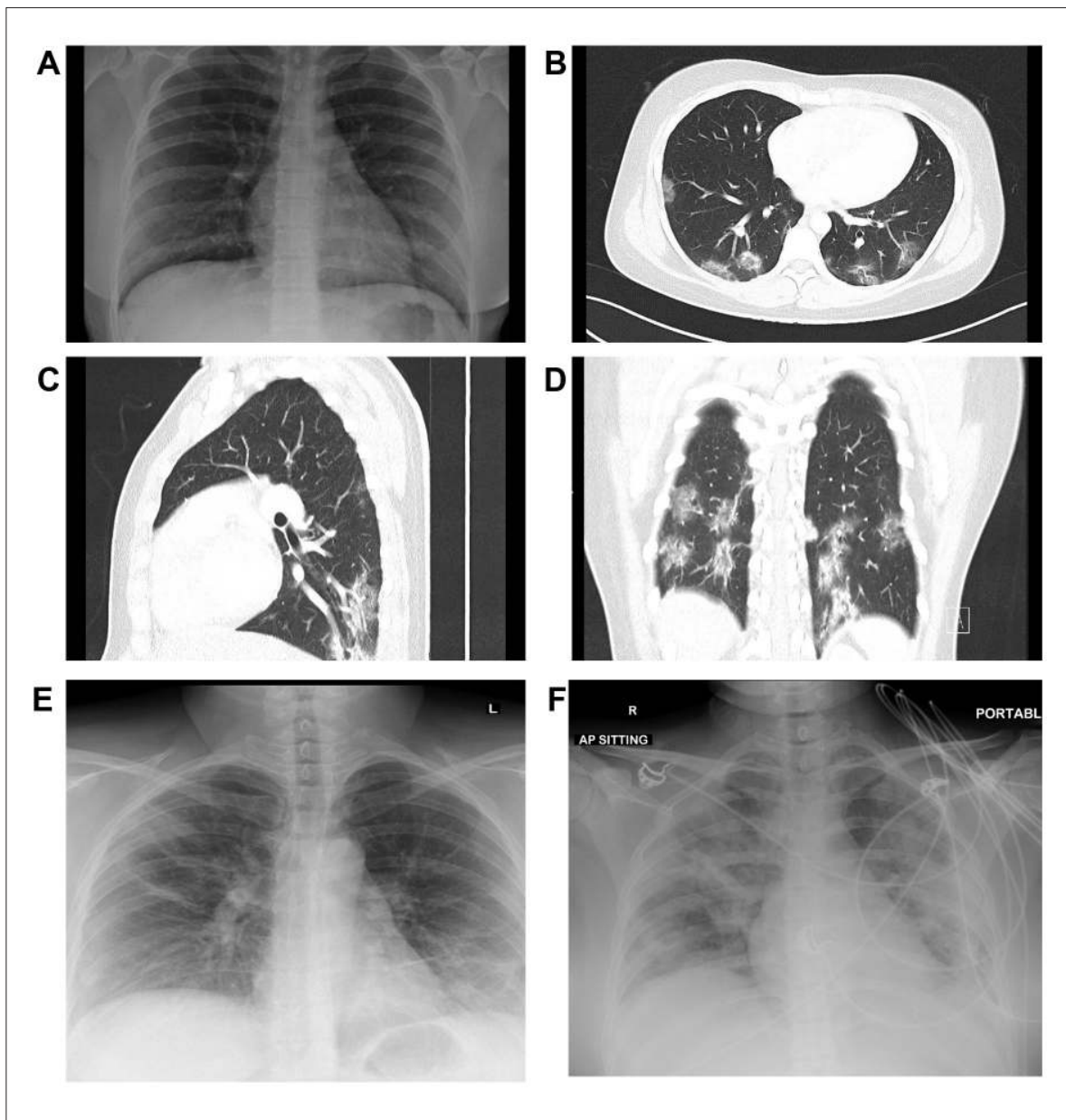


Fig. 3. (A) Chest X-ray for patient 9 performed on the day of admission did not show confluent consolidation or pleural effusion. (B, C) Computed tomography (CT) of the chest performed on the second day showed multiple ground-glass opacities in the peripheries of both lungs, predominantly subpleural and in the lower lobes, with some opacities demonstrating a reversed halo sign. (D) Some denser opacities are peribronchovascular in location, seen as an opacity in the posterior basal segment of the left lower lobe. (E) A chest X-ray for patient 13 performed on the day of admission showed streaky airspace opacities in the right upper to middle zone and left middle to lower zones. (F) A second chest X-ray repeated on day 8 of admission showed interval worsening in bilateral lung consolidation with patchy involvement of both lungs.

gestational-age of 3–10%,¹⁹ the growth restriction in patient 6 cannot be directly attributable to COVID-19. From the systematic screening of perinatal samples, we conclude that there is no evidence thus far of maternal–child transmission in our cohort following second and third trimester infections. Outcomes of first trimester infections are pending at the time of writing.

Peak community transmission of SARS-CoV-2 occurred in Singapore in March 2020 and drastically fell

following strict government measures in April 2020.¹² Our case series and others demonstrate that the incidence and severity of COVID-19 among pregnant women parallel the general population trend.^{13,20} During this period, 256 patients aged between 21 and 35 years were admitted with virologically confirmed COVID-19 (up to 31 March 2020) in Singapore. Non-pregnant females comprised 41.4% (106 of 256) of admissions, 14 of whom developed pneumonia with 1 patient requiring

Table 2. Neonatal or fetal outcomes of women who delivered following a diagnosis of COVID-19 in pregnancy and results of perinatal specimens sent for RT-PCR testing for SARS-CoV-2

No.	GA at delivery (week, day)	Symptom onset to delivery	Maternal NP swab RT-PCR before delivery	Mode of delivery	Fetal / neonatal outcome	SARS-CoV-2 RT-PCR						Histology		Total Ig (COI)	
						Maternal blood	UCB	Vaginal swab	Amniotic fluid	Cord surface swab	Placenta (fetal and maternal) surface swab	Fetal nose, ear / mouth swab	Placenta / umbilical cord tissue	Maternal blood	UCB
1	41w 2d	37d	24d	SVD	Neonate discharged on day 2	Neg	Neg	Neg	-	Neg	Neg	NA	No vasculopathy/inflammation	27.9	11.2
3	39w 4d	75d	43d	SVD	Neonate discharged on day 3 due to jaundice	Neg	Neg	Neg	-	Neg	Neg	NA	Mild non-specific inflammation	2.1	1.5
4	40w 0d	72d	50d	SVD	Neonate discharged on day 2	Neg	Neg	Neg	Neg	Neg	Neg	NA	Mild non-specific inflammation	1.5	2.8
6	39w 4d	104d	81d	SVD	Neonate discharged on day 2	Neg	Neg	Neg	Neg	Neg	Neg	NA	No vasculopathy/inflammation	1.7	1.6
8	38w 0d	115d	56d (from meeting criteria for time-based discharge)	SVD	Neonate discharged on day 2	Neg	Neg	Neg	Neg	Neg	Neg	NA	Mild decidual vasculopathy, no inflammation	Reactive	Reactive
9	23w 3d	2d	Day of delivery	Miscarriage	Deceased	-	Neg	-	-	Neg	Neg	Neg	-	-	-

COI: signal cut-off index; GA: gestational age; Ig: immunoglobulins; NA: not applicable; Neg: negative; NP: nasopharyngeal; SVD: spontaneous vaginal delivery; UCB: umbilical cord blood; RT-PCR: reverse transcription polymerase chain reaction

supplemental oxygen. While there was no difference between pregnant and non-pregnant females developing severe pneumonia (15.4% vs. 13.2%; $p=0.69$), a trend towards a higher incidence of supplemental oxygen use was observed among pregnant women (7.7% vs. 0.9%; $P=0.21$).

The possibility of placental transmission of SARS-CoV-2 remains controversial. In our study, all perinatal tissue specimens were RT-PCR negative. In other studies, SARS-CoV-2 has been identified within the placenta using RT-PCR, viral genome sequencing, immunohistochemistry and electron microscopy, despite RT-PCR being negative for vaginal secretions, amniotic fluid, UCB and fetal tissues.²¹ Caution is advised with these methods as artefacts and other inclusions mimicking viral particles and non-specific staining decrease specificity.²² In addition, studies of placental histology have concluded that placental changes in women with COVID-19 were more likely related to maternal infection and inflammation, rather than fetal infection.¹⁸ Neonatal SARS-CoV-2-specific immunoglobulin M (IgM) may not be conclusive evidence of fetal seroconversion owing to technical limitations and possible transplacental trafficking of maternal IgM when the maternal–fetal interface is breached by inflammation and hypoxia.²³ Evidence in favour of mother–child transmission includes reports of 2 mother–infant pairs who demonstrated positive RT-PCR for SARS-CoV-2 on swabs of maternal and neonatal nasopharynx and from the fetal surface of the placenta,²⁴ and a neonate delivered by caesarean section following maternal COVID-19 infection who then developed neurological manifestations at postnatal day 3. The latter describes multiple positive RT-PCR from placenta, nasopharynx, sera, vagina and pre-membrane rupture amniotic fluid, with a viral load much higher in placental tissue than in maternal blood or amniotic fluid, suggesting congenital COVID-19 infection.²⁵ The main challenge in confirming vertical transmission is related to establishing mechanism, particularly as SARS-CoV-2 cell entry requires colocalisation of angiotensin-converting enzyme 2 and transmembrane protease serine 2 receptors, which are present in negligible levels in placentas.⁹

Our perinatal outcomes reflect the results of a systematic review reporting that the majority of neonates born to mothers with COVID-19 were healthy at birth. Neonates who were symptomatic developed pneumonia or non-specific features, including fever, tachycardia, thrombocytopenia and deranged liver function. The one mortality involved a preterm neonate who developed refractory shock, disseminated intravascular coagulation

and multi-organ failure. Neonatal COVID-19 infection could not be definitively ruled out. At present, we and other researchers have no evidence of COVID-19 teratogenicity following infection in early gestation. While the breast milk from 2 postnatal patients in our cohort was RT-PCR-negative, serial screening may be required to establish this route of transmission following a minimal period of viraemia.²⁶ All 4 delivered patients demonstrated a range of seropositivity in maternal blood and UCB at delivery. Given the absence of viral RNA in UCB and extra-fetal tissues, this finding likely reflects transplacentally trafficked maternal immunoglobulin G. Patient 1 with higher immunoglobulin levels had a shorter infection-to-delivery interval (37 vs. 72 days in patient 4); the lower COI in the other 3 patients may reflect natural maternal antibody decomposition during the longer convalescence.²⁷ Patient 8 had high seropositivity yet prolonged viral shedding, which may be related to physiological deficiencies in gestational immunity, although we have not yet studied her T cell response.²⁸

The primary strengths of this study are: the systematic investigation of all known prenatal COVID-19 cases in Singapore; comprehensive clinical analyses of maternal, neonatal and extra-fetal tissue samples to address the question of vertical transmission; and the inclusion of women affected in their first trimesters in this cohort, although perinatal data are still pending. The main limitations include the lack of universal screening to identify asymptomatic pregnant carriers, as this is not the current national policy given the low community transmission rate in Singapore,²⁹ and the lack of extensive immunological investigations to understand the less aggressive disease progression in our cohort. Cumulative data are valuable in informing evolving maternal risk factors, viable treatment options for severe infection, and transplacental viral transmission.

Conclusion

The comprehensive reporting on all pregnant COVID-19-infected patients managed in public hospitals in Singapore provides a complete review of maternal disease severity. Systematic assessment of prenatal and perinatal samples supports the low likelihood of mother–child transmission if the infection cleared well before delivery, with paired maternal–neonatal seropositivity suggesting transferred immunity. Our Singapore experience reflects a large series with the following findings: a generally low transmission rate; obese and older mothers being more prone to severe disease; no maternal mortality occurring in the hospital setting with close monitoring; and prompt interventional escalation

made possible by a universal admission policy. Prolonged shedding shows variable duration of detectable viral RNA and is an important management consideration, however there is a lack of convincing evidence that this is predictive of actual infectivity.³⁰

This case series is timely and directly addresses the specific concerns shared by pregnant women in Singapore psychologically affected by social distancing and other community measures implemented to curb the spread of COVID-19,³¹⁻³³ even without contracting the infection. The data presented here is generally reassuring from the perspective of pregnancy outcomes, and makes a valuable contribution to inform general pandemic management with a focus on mental, as well as physical, well-being on pregnant and parturient women.

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