SARS-CoV-2 is not detectable in the vaginal fluid of women with severe COVID-19 infection

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Abstract

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is mainly spread through respiratory droplets or direct contact. But the infection condition of genital system is still unknown. This study aimed to evaluate whether or not SARS-CoV-2 is found in the vaginal fluid of women with COVID-19 illness.

Methods: 10 women with confirmed severe COVID-19 pneumonia admitted to in Tongji Zhongfa Hospital Intensive care union (ICU) ward from Feb 4, 2020 to Feb 24, 2020 were included. Clinical records, laboratory results, and computer tomography (CT)-scan examination were retrospectively reviewed. The evidence of genital infection potential was accessed by testing for the presence of SARS-CoV-2 in vaginal fluids obtained from vaginal swab samples. Reverse transcriptase polymerase chain reaction (RT-PCR) was used to confirm the SARS-CoV-2 infection in vaginal fluids.

Results: The clinical characteristics of these ten women were similar to those reported severe COVID-19 patients. All ten patients were tested for SARS-CoV-2 in vaginal fluid, and all samples tested negative for the virus.

Conclusion: Findings from this small group of cases suggest that no SARS-CoV-2 virus existing in the vaginal fluids of severe COVID-19 patients.

Keywords: COVID-19 pneumonia; SARS-CoV-2; vaginal fluid; clinical features;
Introduction
Since December 2019, the outbreak of a viral pneumonia caused by the 2019 novel coronavirus disease has been declared by WHO as a global public health emergency and now a pandemic. The disease was first reported in Wuhan, Hubei Province, China, and now it has spread to more than 100 other countries. SARS-CoV-2 is mainly spread through respiratory droplets or direct contact. Previous reports have found that, SARS-CoV-2 has been found in the testis of male patients, but not in ovary or uterus. In contrast, Ebola has been found in vaginal fluid of female patients. The infection condition of SARS-CoV-2 in genital system is now unknown. The aim of this study was to investigate whether women with severe COVID-19 illness had signs of the SARS-CoV-2 virus in their vaginal fluid.

Method:
Study oversight
Written consent was obtained from all eligible participants. All eligible patients were invited to participate in the study, and those who agreed were provided with a written informed consent form to sign. Ethical approval for the research was sought and obtained from the Research Ethical Committee at the Peking Union Medical College Hospital in 2020, No. JS-2331.

Data sources and collection
All medical records and compiled data for hospitalized patients was obtained from Feb 4, 2020 to Feb 24, 2020 in Tongji Zhongfa Hospital ICU ward which is being managed by Peking Union Medical College Hospital. All ten patients were postmenopausal women and were diagnosed with severe COVID-19 pneumonia. Clinical laboratory examinations including blood analyses, serum biochemical tests, coagulation function tests Chest CT examinations were performed. Vaginal swabs were obtained between 17 to 40 days after the onset of SARS-CoV-2 infection. Vaginal swabs were inserted 2–3 cm into the vagina and rotated for 3–5 s. Swabs were transferred to the laboratory immediately and RT-PCR procedures were completed within 2 hours. We obtained, collected clinical findings, laboratory and radiological findings, therapeutic interventions and outcomes for all the patients. All data were checked by three researchers.

Laboratory confirmation
COVID-19 was diagnosed on the basis of WHO interim guidance World Health Organization. A confirmed case of COVID-19 was defined as a positive result on RT-PCR assay of nasal and/or pharyngeal swab specimens. RT-PCR assays were performed in accordance with the protocol established by the WHO.

Results
Between Feb 4, 2020 to Feb 24, 2020, ten postmenopausal women were admitted with confirmed severe COVID-2 pneumonia in Tongji Zhongfa Hospital ICU ward. All of them were residents of Wuhan. The patients, aged 52 to 80, were initially admitted due
to symptoms of fever or cough. They were transferred to the ICU because of severe illness and all met criteria for, severe pneumonia according to the definition of illness severity by WHO interim guideline for COVID-19\textsuperscript{11} (Table 1). All patients had lymphopenia and eosinopenia, with evidence of liver and/or kidney dysfunction. All laboratory tests from the time of disease onset and the time of vaginal sampling are presented in Table 2). The presence of SARS-CoV-2 in vaginal fluid was assessed in ten patients. None were found to be positive for SARS-CoV-2 by RT-PCR assay.

**Discussion**

Most symptoms of patients with COVID-19 are related to the respiratory system. SARS-CoV-2 is mainly spread through respiratory droplets or close contact. There are few studies about the presence of viruses in body fluids\textsuperscript{3,12}, and no reports about the presence of virus in vaginal fluids has been reported. In our study, 10 postmenopausal women in the ICU with severe COVID-19 were tested for SARS-CoV-2 in vaginal fluid with RT-PCR assay and all samples were negative for the virus. These protective barriers against pathogens in postmenopausal women become weaker, leading to increased susceptibility to bacteria and viruses in postmenopausal women\textsuperscript{13}.

Previous studies have explored the effects of other epidemic viral infections on the female reproductive tract. A study by Prisant et al in France confirmed the presence of Zika virus in the female reproductive tract\textsuperscript{14}. Rodriguez et al\textsuperscript{15} found Ebola virus in the vaginal fluids of a patient recovering from Ebola virus infection 33 days of onset of illness.

Severe acute respiratory syndrome (SARS) caused by SARS-CoV was first discovered in November 2003 in Guangdong, China. The lungs are the organs that sustain the most severe damage. As the disease progresses, patients show multiple organ invasion. Autopsy results of patients who died of SARS showed that SARS-CoV was found in the lungs, trachea / bronchus, stomach, small intestine, and distal renal tubules \textsuperscript{8}. Autopsy results showed virus impact on the reproductive system of both men and women. Xu et al\textsuperscript{4} observed that orchitis was present in some men with SARS. The pathology of male SARS patients showed widespread germ cell destruction, almost no spermatozoon in seminiferous tubules, and leukocyte infiltration. But the presence of the virus was not clearly detected in the testis \textsuperscript{5,16}. However, there is no clear study showing that SARS-CoV invades the female reproductive tract, and there is no report on detection of SARS-CoV in vaginal fluids. The autopsy pathological results of SARS patients have not found the virus in the ovaries and uterus, and one report did not assay vaginal fluids\textsuperscript{5}.

SARS-CoV-2 may have similar transmission mechanisms to SARS-CoV. As the disease progresses, limited research results indicate the presence of the virus in the stools of
patients with COVID-19, which also suggests another possible route of transmission for the virus. At present, there is no report on the presence of SARS-CoV-2 in the female reproductive tract. The issue is particularly important, because it could help inform the risks of sexual transmission and mother-to-child transmission. In our study, we included 10 female patients of COVID-19. Unlike Ebola virus or Zika virus, SARS-CoV-2 was negative in the vaginal fluids of women with severe pneumonia.

This study was the first to attempt to detect SARS-CoV-2 in vaginal fluid. All the tested women were postmenopausal women. Even when patient's respiratory symptoms are severe, the results of vaginal swabs were negative. Therefore, it is possible that SARS-CoV-2 does not enter the vaginal fluid. This finding suggests that the likelihood of transmitting SARS-CoV-2 to sexual partners through virus in vaginal fluids may be low.

There is a single publication in which no SARS-CoV-2 was found in amniotic fluid or umbilical cord blood of women with COVID-19. In the same study, neonatal throat swabs were also negative. Taken together, these findings suggest that the risk of vertical transmission from pregnant women to newborn delivered by cesarean section is low. Our research did not find SARS-CoV-2 by PCR in vaginal fluids as late as 40 days after disease onset in women who were still requiring ICU care for ongoing COVID-19 illness, therefore it is speculated that the risk of vertical transmission during vaginal delivery might also be very low.

The study has several limitations. First, the number of patient is small. In addition, only RT-PCR is used for detection of SARS-CoV-2 in this study. In theory, even if there is only one copy of the virus in the template, it is likely to be detected. However, in practice, if the amount of virus is small, the amplification result may not be ideal. In this research, we only recruited postmenopausal women, and the vaginal swab were taken on the 17 days or more after the onset of the disease. More research about possible differences in the pathophysiology of SARS-CoV-2 in the female genital tract of premenopausal women is needed.
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Author contributions
L Zhu, TS Li: Study concept and design, Obtaining funding, Critical revision of the manuscript for important intellectual content
A Morse: Critical revision of the manuscript for important intellectual content
X Liu, M Xiao, J Xie, W Cao, ZY Liu, TS Li: Acquisition of data
L Qiu, YH Xie: Writing of the manuscript, Statistical analysis

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Table 1 Clinical features and CT findings of the postmenopausal women with severe COVID-19

|                          | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 | Patient 7 | Patient 8 | Patient 9 | Patient 10 |
|--------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|------------|
| Age (year)               | 57        | 65        | 52        | 80        | 71        | 63        | 73        | 66        | 55        | 75         |
| Epidemiologic exposure   | Lived in Wuhan | Lived in Wuhan | Lived in Wuhan | Lived in Wuhan | Lived in Wuhan | Lived in Wuhan | Lived in Wuhan | Lived in Wuhan | Lived in Wuhan | Lived in Wuhan |
| Menopause                | Yes       | Yes       | Yes       | Yes       | Yes       | Yes       | Yes       | Yes       | Yes       | Yes        |
| Chronic illness          | No        | Hypertension; Diabetes Type II | No | Hypothyroidism | No | Diabetes Type II, Hypothyroidism | No | Obesity | Coronary heart disease, Hypertension, Diabetes Type II |
| Severity of pneumonia    | Severe    | Severe    | Severe    | Severe    | Severe    | Severe    | Severe    | Severe    | Severe    | Severe     |
| Initial Symptoms         | Fever, Cough, | Fever, Cough, | Fever, Cough | Fever, Cough | Fever, Cough | Fever, Cough | Fever, Cough | Fever, Cough | Fever, Cough | Fever, Cough |
| Lowest SpO2 (%)          | 65        | 70        | 94        | 84        | 94        | 92        | 91        | 93        | 80        | 99         |
| Diagnosis method         | Real-time PCR | Real-time PCR | Real-time PCR | Real-time PCR | Real-time PCR | Real-time PCR | Real-time PCR | Real-time PCR | Real-time PCR | Real-time PCR |
| Days from diagnosis to sampling | 25        | 26        | 31        | 40        | 27        | 24        | 17        | 28        | 33        | 30         |
|                     | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---------------------|---|---|----|----|----|----|----|----|----|
| Antiviral therapy   | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Chest CT scans      | Multiple bilateral ground-glass opacities and consolidation | Bilateral ground-glass opacities | Bilateral ground-glass opacities prominent on the left | Bilateral ground-glass opacities and consolidation | Bilateral ground-glass opacities prominent on the right | Regional bilateral ground-glass opacities | Bilateral ground-glass opacities prominent on the left | Bilateral ground-glass opacities | Bilateral ground-glass opacities |
|                     |                             |                         |                                           |                                              |                                             |                                             |                                             |                                             |
| Laboratory findings | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 | Patient 7 | Patient 8 | Patient 9 | Patient 10 |
|---------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Leucocyte count (x10^9/L) [3.5-9.5] | 10.5 | 15.63 | 6.7 | 3 | 9.2 | 1 | 14.35 | 9.5 | 4 | 5.6 | 7 | 20.3 | 11.05 | 5.6 | 4 | 6.2 | 9 | 6.3 | 4 | 7.4 | 2 | 5.0 | 8 | 12.73 | 15.06 | 13.13 | 6.1 | 5 | 12.61 |
| Hemoglobin (g/L) [115-150] | 109 | 80 | 99 | 88 | 125 | 86 | 160 | 77 | 123 | 85 | 98 | 73 | 128 | 110 | 126 | 74 | 124 | 96 | 90 | 96 |
| Neutrophil count (x10^9/L) [1.8-6.3] | 9.3 | 3 | 13 | 12 | 6.2 | 4 | 13 | 67 | 8.0 | 8 | 5.0 | 4 | 18 | 44 | 9.4 | 8 | 5.3 | 4 | 8 | 4.8 | 9 | 11 | 88 | 6.0 | 1 | 4.1 | 2 | 7.0 | 4 | 12 | 55 | 10 | 88 | 5.5 | 1 | 12 | 02 |
| Eosinophil count (x10^9/L) | 0 | 0 | 2 | 0 | 0.0 | 4 | 0.0 | 1 | 0 | 0.0 | 1 | 0.0 | 3 | 0 | 0 | 0.0 | 1 | 0 | 0.1 | 7 | 0 | 0 | 0 | 0.0 | 2 | 0 | 0 | 0.2 | 2 | 0 | 0 | 0 |
|                     | 0.02-0.52 | 0.1-0.9 | 0.2-0.3 | 0.2-0.4 | 0.4-0.5 | 0.6-0.7 | 0.7-0.8 | 0.8-0.9 | 0.9-1.0 | 1.0-1.1 | 1.1-1.2 | 1.2-1.3 | 1.3-1.4 | 1.4-1.5 | 1.5-1.6 | 1.6-1.7 | 1.7-1.8 | 1.8-1.9 | 1.9-2.0 |
|---------------------|-----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Lymphocyte count   | 0.71      | 0.91    | 0.29    | 0.91    | 0.41    | 0.51    | 0.91    | 0.21    | 0.91    | 0.41    | 0.51    | 0.91    | 0.21    | 0.91    | 0.41    | 0.51    | 0.91    | 0.21    |
| (x10^9/L) [1.1-3.2] |           |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Platelet (x10^9/L)| 116.8     | 227.1   | 79.1    | 176.1   | 141.1   | 212.1   | 223.1   | 312.1   | 288.1   | 73.1    | 230.1   | 138.1   | 118.1   | 134.1   | 5.08    | 53.1    | 344.1   | 500.1   | 148.1   | 185.1   |
| [125-350]          |           |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| C-reactive protein | 116.8     | 320.8   | 66.8    | 57.8    | 270.8   | 232.1   | 142.5   | 73.7    | 166.7   | 105.5   | 41.1    | 66.8    | 49.8    | 41.1    | 66.8    | 167.6   | ND      | 17.1    | 42.4    |
| protein(mg/L)[0-5] |           |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| D-dimer(ug/mL)<0.5 | >21       | 5.49    | 2.84    | 1.28    | 1.41    | 4.21    | 12.08   | 4.37    | 2.83    | 5.53    | 18.19   | 4.47    | 2.13    | 4.96    | >21     | 11.12   | ND      | 2.93    | 2.43    | 3.67    |
| Prothrombin time(s)| 18.2      | 14.9    | 17.2    | 18.1    | 14.8    | 16.1    | 13.7    | 14.3    | 15.8    | 15.3    | 16.9    | 14.4    | 18.6    | 15.1    | 18.1    | 16.2    | 15.9    | 12.8    | 19.7    |         |
| [11.0-16.0]        |           |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
|                      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| International        | 1.4 | 1.1 | 1.4 | 1.4 | 1.3 | 1.2 | 1.0 | 1.1 | 1.2 | 1.1 | 1.3 | 1.1 | 1.5 | 1.1 | 1.5 | 1.2 |
| normalized           | 9   | 5   | 7   | 4   | 2   | 7   | 2   | 9   | 3   | 5   | 1   | 9   | 6   | 5   | 8   | 5   |
| ratio[0.80-1.31]     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Activated            | 42  | 39  | 45  | 39  | 44  | 38  | 30  | 39  | 71  | 45  | 77  | 40  | 53  | 43  | 38  | 35  |
| partial             | 1   | 8   | 2   | 2   | 6   | 8   | 4   | 1   | 3   | 7   | 4   | 8   | 3   | 3   | 3   | 1   |
| Thromboplastin       | 6   | 5   | 3   | 4   | 2   | 8   | 8   | 5   | 6   | 4   | 2   | 6   | 2   | 6   | 2   | 1   |
| time(s)[28.0-43.5]   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Fibrinogen           | 1.2 | 6   | 5.9 | 4.4 | 4.7 | 8.3 | 4.2 | 8.5 | 4.7 | 6.1 | 5.4 | 6.2 | 5.2 | 6.0 | 6.2 | 5.4 |
| (g/L)[<5]            | 6   | 3   | 2   | 1   | 3   | 4   | 3   | 5   | 3   | 2   | 2   | 2   | 4   | 3   | 1   | 3   |
| Erythrocyte          | 23  | ND  | ND  | 91  | 54  | 112 | 64  | 74  | 65  | ND  | 55  | 110 | 80  | ND  | 54  | 18  |
| sedimentation        |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| rate(mm/h) [<20]     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Albumin(g/L)[35-50]  | 29  | 32  | 32  | 27  | 28  | 26  | 34  | 33  | 36  | 32  | 29  | 31  | 28  | 31  | 34  | 27  |
|                     | 5   | 6   | 6   | 7   | 8   | 1   | 3   | 3   | 3   | 7   | 6   | 7   | 6   | 3   | 9   | 3   |
|                     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|                  | 0.4 | 1   | 1.3  | 1.8  | 2.2  | 2.3  | 3.3  | 3.5  | 4.7  | 8.3  | 3.2  | 0.2  | 4.5  |
|------------------|-----|-----|------|------|------|------|------|------|------|------|------|------|------|
| Bilirubin (umol/L) [3-22] |    |     |      |      |      |      |      |      |      |      |      |      |      |
| Serum alanine aminotransferase (U/L) [9-52] |    |     |      |      |      |      |      |      |      |      |      |      |      |
| Serum aspartate aminotransferase (U/L) [14-36] |    |     |      |      |      |      |      |      |      |      |      |      |      |
| Serum alkaline phosphatase (U/L) [38-126] |    |     |      |      |      |      |      |      |      |      |      |      |      |
|                                      | 7.1 | 10.6 | 10.5 | 6.7 | 11.4 | 7    | 9    | 11.5 | 6.3 | 9.4 | 10 | 7.1 | 6.8 | 6.5 | 4.7 | 6.1 | 5.5 | 5  | 4.4 | 19.2 |
|-------------------------------------|-----|------|------|-----|------|------|------|------|-----|-----|----|-----|-----|-----|-----|-----|-----|----|-----|-----|
| Blood urea nitrogen(mmol/L) [2.9-8.2] |     |      |      |     |      |      |      |      |     |     |    |     |     |     |     |     |     |    |     |     |
| Creatinine(umol/L)[4-106]           | 46  | 113  | 58   | 46  | 71   | 46   | 93   | 65   | 66  | 66  | 80 | 51  | 66  | 112 | 57  | 38  | 76 | 44  | 70  | 131 |
| Uric acid(umol/L)[155-357]          | 171 | 252  | 100  | 109 | 233  | 80   | 409  | 199  | 209 | 240 | 94 | 259 | 125 | 184 | 88  | 293 | 142| 180 | 453 |
| Sodium(mmol/L)[136-145]             | 142 | 149  | 148  | 146 | 135  | .8   | 130  | .1   | 143 | .1  | 135 | .6  | 146 | .7  | 141 | .4  | 137 | .3  | 146 | .4  | 154 | .1  |
| Potassium(mmol/L)[3.5-5.2]          | 4.5 | 5    | 3.4  | 4.2 | 4.3  | 4.7  | 4.1  | 4.6  | 3.1 | 4.8 | 4.5 | 3.6  | 4.3 | 4.3  | 4.8 | 4.4  | 3.4 | 3.7 | 3 |
| Detection of SARS-CoV in             | (-) | (-)  | (-)  | (-) | (-)  | (-)  | (-)  | (-)  | (-) | (-) | (-) | (-)  | (-)  | (-)  | (-) | (-)  | (-) | (-) | (-) | (-) |

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|                          | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) |
|--------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Detection of SARS-CoV in |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| blood by RT-PCR          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Detection of SARS-CoV in |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| vaginal fluid by         |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| RT-PCR                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

ND, not detected; (-), negative;