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SARS-CoV-2 RNA may rarely be present in a uterine cervix LBC sample at the asymptomatic early stage of COVID 19 disease

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Abstract

Objective: Currently, it is thought that uterine cervix mucosal samples present a low risk of SARS-CoV-2 exposure. So far, there is no evidence of SARS-CoV-2 detection in Papanicolaou (Pap) smears. Nevertheless, clinicians could be exposed unaware to the coronavirus while performing and handling a Pap smear. We aimed to retrospectively evaluate the presence of SARS-CoV-2 RNA in cervical liquid-based cytology (LBC) samples in women who tested positive for a nasopharyngeal COVID-19 PCR test.

Methods: From our laboratory database, we identified patients with data on a cervical cancer screening LBC sample paired with a positive nasopharyngeal COVID-19 PCR test. Relevant LBC samples taken within an incubation period of 14 days and post-onset RNA shedding interval of 25 days were subsequently tested for SARS-CoV-2 RNA using RT-PCR tests.

Results: The study group consisted of 102 women. Of those, 23 LBC samples were tested. SARS-CoV-2 RNA was detected in one LBC sample from a 26-year-old asymptomatic woman taken six days before reporting headaches and knee arthralgia with a positive nasopharyngeal SARS-CoV-2 RT-PCR test.

Conclusions: It is possible to detect SARS-CoV-2 RNA in cervical LBC samples at an early asymptomatic stage of COVID-19. In general, this finding is infrequent in asymptomatic women who tested SARS-CoV-2 positive within an incubation of 14 days and a post-onset RNA shedding period of 25 days. We fully support the current thinking that cervical LBC samples from asymptomatic women pose a low risk of SARS-CoV-2 exposure and can be handled in the frame of good microbiological practice and procedures.

KEYWORDS

coronavirus, COVID-19, LBC, PAP smear, PCR, SARS-CoV-2

1 | INTRODUCTION

Unprecedented circumstances caused by the COVID-19 pandemic have led to changes in cytology laboratory workflow. These consist of a well-documented, significant drop in the overall number of processed specimens, including Pap smears.[1-3] At the same time, re-evaluations of laboratory biosafety procedures were promptly made and implemented worldwide.[4-10] Some cytological specimens may contain SARS-CoV-2 mRNA. Based on the frequency of SARS-CoV-2 mRNA detection and reports on successful virus cultivation, cytology specimens were categorised by Chen et al[8] into a high-risk group (nasopharyngeal and oropharyngeal swabs, all types of

bronchoscopic samples, blood, teardrops) and an intermediate-risk group (pleural and pericardial effusion, urine), with all the other specimens falling into a low-risk category. Currently, uterine cervix mucosal samples are considered to present a low risk of SARS-CoV-2 exposure.[8] So far, there is no evidence of SARS-CoV-2 detection in Pap smears. Nevertheless, clinicians, nurses, and cytotechnologists could be unaware that they were exposed to coronavirus while performing and handling a Pap smear. To the best of our knowledge, the infectious potential of Pap smears has not been studied yet. We aimed to retrospectively evaluate the presence of SARS-CoV-2 RNA in cervical LBC samples in women whose nasopharyngeal swabs tested positive for SARS-CoV-2.

2 | METHODS

At Biopticka laboratory we performed a cross-search of the Pap smear database with a regular yearly workload of some 800 000 tests and the COVID-19 PCR test database with more than 155 000 tests performed, peaking at 3522 tests per day on 23 October 2020 (overall positivity rate 21.8%). Patients with a cervical cancer screening liquid-based cytology (LBC) sample taken from 01 October 2020 to 19 November 2020 and paired with a recorded positive COVID-19 PCR test were identified. LBC was evaluated using the ThinPrep Imaging system with Image processor (Hologic). The study was performed with the approval of the local ethical committee.

2.1 | SARS-CoV-2 testing

Nasopharyngeal swabs (COVID-19 PCR test) were taken by trained staff at a dedicated sampling room using a collection kit containing a flexible brush, and virus transport and preservation medium (Biologix). The viral RNA from the sample was isolated from 300 μL of the media using a Maxwell® RSC Viral Total Nucleic Acid Purification kit on a Maxwell automated system (Promega). Real-time PCR detection of the SARS-CoV-2 virus was performed on a CFX96™ detection system using Allplex™ SARS-CoV-2 Assay (Seegene, South Korea), targeting three viral genes (N gene, E gene, RdRp gene) and an exogenous internal control. The results were automatically evaluated by the SARS-CoV-2 Viewer program for Real time Instruments V3 (Seegene, South Korea).

Gynaecologists took cervical LBC samples as part of routine cervical cancer screening, and 2 mL of the LBC medium was centrifuged. Viral nucleic acid isolation was performed from 300 μL of the concentrated sample using a Maxwell RSC Viral Total Nucleic Acid Purification kit on a Maxwell automated system (Promega). Detection of SARS-CoV-2 was performed using Allplex SARS-CoV-2 Assay (Seegene) on the CFX96. The results were automatically evaluated by the SARS-CoV-2 Viewer program for Real time Instruments V3 (Seegene). For a positive sample, the isolation and detection were repeated in triplicate, and the final result was provided as a set of calculated mean values for each gene. Another confirmatory detection was performed using the Xpert Xpress SARS-CoV-2 test (Cepheid Europe) aiming at the N2 and E genes, with 2 mL of an LBC medium centrifuged, and 1.8 mL of supernatant discarded. The Xpert Xpress SARS-CoV-2 test was automatically performed and evaluated by GeneXpert instrument systems using 200 μL of the concentrated LBC sample.

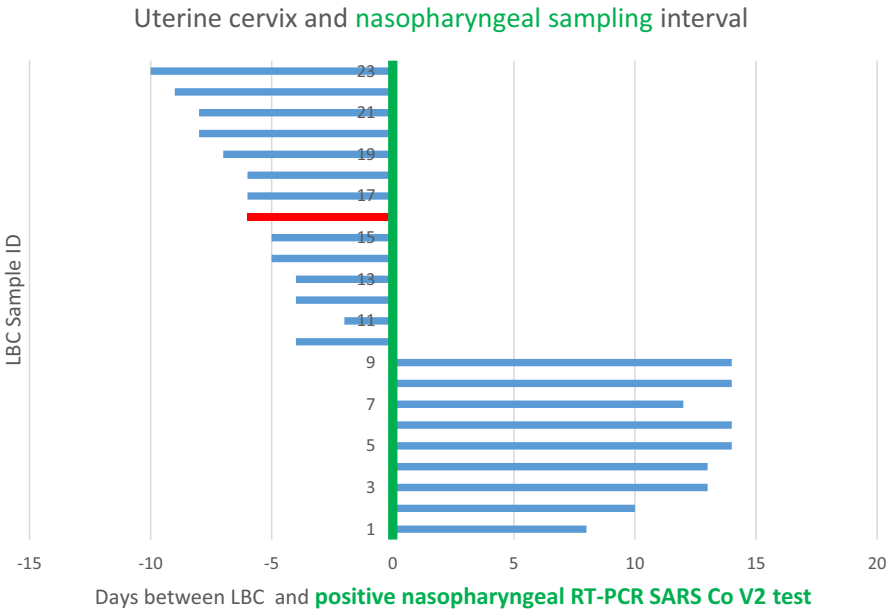
2.2 | Study group adjustment

Of all cases, only those presenting with a biologically relevant time interval between cervical and nasopharyngeal sampling determined by incubation and post-onset RNA shedding period were analysed. An incubation period's exact time interval was set to 14 days following the recommended safety limit suggested by Backer et al based upon the actual calculated incubation period of 2.11-11.1 days.[11] The time interval of 25 days for possible post-onset RNA shedding was based on Liu et al, who reported that mild cases were found to have an early viral clearance, with 90% of these patients repeatedly testing negative on RT-PCR by day ten post-onset. In severe cases, they observed the RNA shedding as late as day 25 post-onset.[12]

3 | RESULTS

We identified 102 women aged 20 to 64 years with data on paired nasopharyngeal swab and cervical LBC samples. Of those, 23 cases fell into a predetermined sampling time interval forming two groups. There were 14 LBC samples which preceded the positive nasopharyngeal COVID-19 PCR test by up to 14 days (2 to10 days). The other nine LBC samples were taken within 25 days (8 to 14 days) after the positive nasopharyngeal COVID-19 PCR test (Table 1). All remaining samples were excluded as their separation interval (1 to 7 months) was not within the established time frame. No woman reported any significant virosis-associated symptoms, including loss of the sense of taste and smell at the time of the visit with her gynaecologist. We detected SARS-CoV-2 RNA in one of 23 (4%) LBC samples. That sample was taken six days before the positive COVID-19 PCR test (Table 1). Respective cycle threshold (ct) values for individual genes were as follows: for RdRp gene, 39.82; for E gene, 37.23; for N gene, 36.9. Nucleic acid isolation and detection was then repeated in triplicate (isolation 2, 3, and 4), and all three replicates yielded SARS-CoV-2 positive results, with the following mean ct values: for RdRp gene, 37.09; for E gene, 37.75; for N gene, 37.57 (Table 2). The viral RNA load for the RdRp gene was low, representing approximately ten copies of targeted viral RNA in 1 μL of isolated nucleic acid (quantified using a calibration curve of viral nucleic acid of known concentrations donated by the National Reference Laboratory for Respiratory Pathogens, Czech Republic). Also, confirmatory SARS-CoV-2 detection using the Xpert Xpress test yielded a positive result, with

TABLE 1 Regular cervical cancer screening liquid-based cytology (LBC) samples from 23 women taken 2 to 10 d before or 8 to 14 d after SARS-CoV-2 positive nasopharyngeal swab test using RT-PCR method



Positive cervical LBC sample is highlighted in red.

TABLE 2 Results of repeated isolation and detection of the SARS-CoV-2 positive cervical liquid-based cytology (LBC) sample

Isolation number	ct (E gene)	ct (N gene)	ct (RdRp gene)	Final result
2	37.92	36.31	35.82	POSITIVE
3	37.72	38.1	37.79	POSITIVE
4	37.61	38.31	37.65	POSITIVE
Mean ct	37.75	37.57	37.09	

Note: Viral nucleic acid isolation was performed from 300 μL of concentrated LBC sample using a Maxwell® RSC Viral Total Nucleic Acid Purification kit on a Maxwell automated system (Promega). Detection of SARS–CoV–2 was performed using Allplex™ SARS–CoV–2 Assay (SeeGene, South Korea) on a CFX96™. The results were automatically evaluated by the SARS-CoV-2 Viewer program for Real time Instruments V3 (Seegene, South Korea). Cycle threshold (ct) values of 3 SARS-CoV-2 genes (E gene, N gene, and RdRp gene) are reported for each isolation/detection, as well as a mean value for the triplicate.

ct values for the E gene and N2 gene being 39.8 and 40.2, respectively. The 26-year-old woman felt well and reported no symptoms at the time of her visit to the gynaecologist's office. Six days later, she presented to the general practitioner with viral symptoms complaining of headache and knee arthralgia. A COVID-19 PCR test was indicated.

The viral RNA load of resulting nasopharyngeal swab was low—the ct value was 31.76 for the RdRp gene, 30.40 for the E gene, and 31.02 for the N gene—representing about 500 copies of targeted viral RNA in 1 μL of isolated nucleic acid (Supplementary Material 1,2).

4 | DISCUSSION

To the best of our knowledge, this is the first dedicated study reporting the presence of SARS-CoV-2 RNA in a Pap test sample.

Herein we report one patient (1/23; 4%) with SARS-CoV-2 RNA shedding into a cervical sample. The sample RNA load was low, both in the cervical LBC sample and subsequent nasopharyngeal swab. Based on the clinical correlation, we assume that the patient's Pap test was taken at an asymptomatic early stage of COVID-19. Our findings add to the current view that LBC samples pose a low risk of SARS CoV-2 exposure even when taken during the incubation period of 14 days or within 25 days following a positive nasopharyngeal test. Interestingly, in 9 of 102 initially identified patients, we acknowledge a phenomenon of unexpectedly early gynaecologist appointment and Pap test sampling occurring 8 to 14 days following a positive SARS-CoV-2 PCR test. Patients reported no respiratory or virosis-associated symptoms. The reason for their gynaecological appointment is unknown as it was deemed unrelated to the aim of the study. Nevertheless, this interesting observation could be further elucidated by future epidemiological studies.

The RNA of SARS-CoV-2 has frequently been detected in some human tissues and body fluids, including the upper and lower respiratory tract, teardrops, blood, faeces, and perianal skin. Likewise, but less frequently, it has been found in pleural and pericardial effusions and urine.[13-17] It has rarely been reported in ascites,[18] cerebrospinal fluid,[19] semen,[20] and maternal milk.[21] Of interest, maternofoetal transmission of some other human coronaviruses has been reported.[22]

If not paired with virological in vitro study, isolated interpretation of all the above-mentioned findings is equivocal. Likewise, the

detection of SARS-CoV-2 RNA in the LBC Pap test sample presented by this study does not equate to detecting a viable virus with infectious potential.[23]

To limit possible SARS-CoV-2 exposure, high-grade ethanol should be added to any non-ethanol-based fixation medium when handling pulmonary and oral LBC samples that fall into a high-risk category.[8-10] This approach is not warranted in the case of low-risk samples, including PreservCyt-containing vials for cervical LBC.[8] Despite size limitation, this study supports that view. More extensive population-based and in vitro studies could further improve our understanding of possible SARS-CoV-2 infection biology in the uterine cervix.

To conclude, we have demonstrated it is possible to detect SARS-CoV-2 RNA in a cervical LBC sample at an early asymptomatic stage of COVID-19. In general, this finding is infrequent. It occurred in an asymptomatic woman who then tested SARS-CoV-2 positive six days following the Pap test. Larger studies should address the true incidence and biological significance of this phenomenon. Although limited by size, this study lends further support to current thinking that cervical LBC samples pose a low risk of SARS-CoV-2 exposure and, as currently recommended, can be safely handled in the frame of good microbiological practice and procedures.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHORS' CONTRIBUTIONS

Ondrej Ondič: conceptualisation, project administration, data curation, draft writing and review. Kateřina Černá: conceptualisation, methodology data curation. Iva Kinkorová-Luňáčková: data curation, validation, methodology. Jana Němcová: conceptualisation, methodology, draft writing, data curation. Bořivoj Mejchar: methodology, data validation. Jan Chytra: methodology, validation. Jiří Bouda: funding acquisition, methodology, validation.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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