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## SARS-CoV-2 infection and impact on female genital tract: An untested hypothesis



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### ABSTRACT

COVID pandemic consists one of the most challenging medical realities. Apart from affecting respiratory system, current evidence has demonstrated multiorgan manifestations that SARS-Cov-2 infection may actually have. However, one of the medical hypotheses not yet thoroughly tested is the impact on female reproductive system and more specifically cervix. No large observational studies have been performed to test presence of SARS-Cov-2 in cervical samples, while potential correlation and impact on HPV infection has not yet been examined. In this context, our research team has already planned to begin a prospective observational study regarding detection rates of SARS-CoV-2 genetic material in cervical cytology. The collected specimen will be analyzed for the presence of COVID-19 genetic material and in case of positive results, HPV typing will be performed as well in order to detect potential correlations between SARS-CoV-2 infection and HPV-infection. We would therefore like to launch our idea to control for SARS-CoV-2 infection in cervical specimen as well as examine potential correlation with HPV infection. Potential scientific proof of such hypothesis would change much regarding follow-up of HPV-positive patients while also triggering further research regarding aetiopathogenetic pathways of COVID. Communication of such a medical hypothesis could potentially motivate colleagues worldwide to expand their interest also on the research of SARS-CoV-2 cervical infection, in an effort to optimize our level of knowledge towards this new threatening and unknown reality of SARS-CoV-2.

### Background

Viral diseases continue to emerge and represent a serious public health issue. In the last two decades, several viral epidemics such as the severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002, H1N1 influenza in 2009 and respiratory syndrome coronavirus (MERS-CoV) in 2012 have been reported. Just a few months ago, the SARS-CoV-2 (Coronavirus Disease-2019) appeared in China and was officially reported by WHO Country Office in China at the last day of 2019, although trails of the viral infection can be traced back in the literature from November 2019 where the first COVID-19 cases were described as “pneumonia of unknown etiology” [1]. Later, SARS-CoV-2 was identified in samples of bronchoalveolar lavage fluid from a patient in Wuhan and was confirmed as the cause of that pneumonia called now as novel coronavirus–infected pneumonia (NCIP) [2].

The current pandemic outbreak of the SARS-CoV-2 has spread from China to many other countries. On 31st January 2020, the WHO Emergency Committee declared a global health emergency based on growing number of NCIP cases especially in China (9692) which in midst of February 2020 had increased rates of morbidity and mortality, whereas the incidence in other Asian countries, in Europe and North America was still low. On the last day of February, WHO raised the threat to the COVID-19 epidemic to the “very high” level. On 11th March 2020, as the number of COVID-19 cases outside China has

increased 13 times and the number of countries involved has tripled with more than 118,000 cases in 114 countries and over 4,000 deaths, WHO declared the COVID-19 a pandemic [3] with the total confirmed cases worldwide as of 18th July reaching almost 14 million.

The case detection rate is increasing daily, even though world governments have implemented countermeasures. Health organizations coordinate information flows and issues directives and guidelines to best mitigate the impact of the threat. At the same time, scientists around the world work tirelessly. However, there is still much research to be done regarding the exact aetiopathogenetic mechanisms, clinical manifestations as well as further complications caused by COVID infection.

### The hypothesis/theory

The present article aims to set the medical hypothesis of potential impact of SARS-CoV-2 infection on female genital tract status as well as its impact and correlation with HPV infection.

The pathogenic mechanism of SARS-CoV-2 infection is still unclear. The initial clinical sign of the COVID-19 which allowed case detection was pneumonia. In a study that analyzed 138 COVID-19 patients, the most common clinical features were fever (99%), fatigue (70%), dry cough (59%), anorexia (40%), myalgias (40%), dyspnea (31%) and sputum production (27%) [4]. Headache, sore throat and rhinorrhea,

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anosmia and dysgeusia have also been reported as less common symptoms [5].

In symptomatic patients, the clinical manifestations usually start after less than a week, consisting of fever, cough, nasal congestion, fatigue and other signs of upper respiratory tract infections. The infection can progress to severe disease with dyspnea and severe chest symptoms corresponding to pneumonia. Pneumonia mostly occurs in the second or third week of a symptomatic infection. Typical signs include decreased oxygen saturation, blood gas deviations, changes visible through chest X-rays and other imaging techniques, with ground glass abnormalities, patchy consolidation, alveolar exudates and interlobular involvement, eventually indicating deterioration. Lymphopenia appears to be common, and inflammatory markers are elevated [6,7].

As mentioned above, most patients with severe COVID-19 present with NCIP-related symptoms. However, there is already evidence supporting the multi-organ impact of the virus. In fact, there are studies indicating that, apart from the respiratory system, SARS-CoV-2 genetic material can be detected in blood and plasma samples implying viral invasion to the host's hematological system [8,9]. It is widely agreed that COVID-19 can also have adverse effects on cardiovascular health, causing or aggravating damage to the heart [10].

Furthermore, Puelles et al, when conducting autopsies on 27 people with COVID-19, found that the virus was also detected at lower levels in the kidneys, liver, heart, brain and blood. By scrutinizing databases of genetic activity, the team found that 3 genes known to encourage SARS-CoV-2 infection are highly active in kidney cells. Recent studies revealed high expression levels of the cell-surface protein angiotensin-converting enzyme 2 (ACE2), the main receptor for the SARS-CoV-2 spike protein, in lung, intestines, and kidney, as well as in 2.4% of urothelial cells, potentially increasing their susceptibility to infection with SARS-CoV-2 and possible subsequent viral cystitis [11]. Furthermore, the plausibility of direct renal cellular damage from SARS-CoV-2 is implied by studies that detected SARS-CoV-2 in urine samples from infected patients [12,13].

Also, a significant number of patients reported gastrointestinal (GI) symptoms such as diarrhea, nausea, vomiting and abdominal pain, with some reporting these symptoms as their sole presenting complaint. The incidence of GI symptoms, alongside the detection of SARS-CoV-2 RNA in stool samples of infected patients [14] suggest that ACE2 receptors highly expressed in the GI tract are another target for SARS-CoV-2 infection. Furthermore, Chen et al found that a total of 28 patients tested positive for SARS-CoV-2 RNA in stool specimens, and this was not associated with the presence of GI and the severity of illness while viral RNA has been detected in feces from children as well [11,15].

Mild and transient liver injury, as well as severe liver damage may also occur in COVID-19. Wong et al. indicated that 14.8–53.1% of COVID-19 patients had abnormal levels of alanine aminotransferase, aspartate aminotransferase and bilirubin during the disease [16]. Besides, it has been suggested that viral invasion of the central nervous system by SARS-CoV-2 is possible by the synapse-connected route leading to several neurological complications including ataxia, seizures, neuralgia, unconsciousness, acute cerebrovascular disease and encephalopathy [17].

From all the above, it is clear that there is strong evidence indicating the multi-organ involvement of COVID-19. Therefore, as impact of SARS-CoV-2 infection has been already demonstrated for multiple organs, it would be reasonable to hypothesize the potential impact it may have on female genital tract as well.

The correlation between SARS-CoV-2 infection and female genital tract has not yet been established. However, there are some initial signs. First of all, Li et al. investigated whether SARS-CoV-2 could be detected in semen. They discovered that, among 38 men with confirmed COVID-19 infection, 15.8% were positive for SARS-CoV-2 in semen specimens [18,19]. Although the percentage is not relatively high and the physiology of male reproductive system is different from that of female, it is still impressing that a virus predominantly found in

respiratory system affects semen. Therefore, the question “then why not cervix?” may be reasonable raised.

Secondly, we should always take into consideration the severe impact of cervix by viruses, specifically HPV virus. Cervix is the main anatomical part of female genital tract system that is affected by virus. Women represent a huge spectrum of gynecological pathology, however, it is only cervix so much seriously affected by a virus (HPV). Just to remind, this virus infection has been proven as the absolute aetiopathogenic cause of the leading cause of gynaecological cancer mortality, which is the cervical cancer [21].

Despite the variety of studies already performed to investigate multi-organ manifestations of SARS-CoV-2 infection, cervix has not yet been attributed significant attention. Currently, there is only one study from Cui et al. having been performed, including only 35 patients. Authors reported that they did not find SARS-CoV-2 in the genital tract of patients of reproductive and postmenopausal age, diagnosed with COVID-19 with all vaginal and cervical swabs reported as negative for SARS-CoV-2 [20]. However, this study dates from March 2020, while we are already in August 2020, pandemic is still developing and no larger observational studies have been performed.

Apart from the diagnosis of COVID-19 itself on vaginal swabs, that would be a significant finding, it would be also interest to study whether there is any correlation between SARS-CoV-2 infection and Human Papilloma Virus (HPV) infection. Papillomaviruses have little in common with coronaviruses in terms of genetics, however, they may share common characteristics in terms of transmission process or life cycle. Furthermore, it would be interesting to study the potential of higher susceptibility of HPV-infected epithelium cells to SARS-CoV-2 in comparison with HPV-negative cells.

To summarize, given the fact that the complete pathogenic mechanism of SARS-CoV-2 infection is still unclear while it has been found that the viral genetic material is detected in various anatomical sites of the host, it seems that there is much place available for studies examining whether COVID-19 genetic material can be detected in cervical cytology samples. Therefore, we could verify if cervix may belong to the group of organs that are invaded and infected by COVID-19 or not and if there is any potential interaction with HPV, adding this way valuable information onto the ever-emerging landscape of medical knowledge on COVID-19, encapsulating its multi-organ impact.

## Evaluation of the hypothesis/idea

In this context, our research team has already planned to begin a prospective observational study regarding detection rates of SARS-CoV-2 genetic material in cervical cytology. There will be included clinical cervical samples collected from patients of the Cervical Pathology Clinic at the 2nd Obstetrics and Gynecology Department of Aristotle University of Thessaloniki (Hippokration General Hospital). Our center consists a large tertiary department with specialized Unit for Cervical Pathology and specialized Laboratory of Cervical Cytology. Our study protocol includes ectocervical and endocervical sampling that will be performed using a plastic Ayre's Spatula and a Cytobrush (CooperSurgical) respectively. After sampling, the device will be directly immersed in a vial containing PreservCyt collection fluid (Hologic), according to manufacturer's instructions. Cervical exfoliated cell samples will be collected by brushing from the cervical surface before colposcopic evaluation and punch biopsy. Cytological material will be collected in proper methanol-based fixative PreservCyt/ThinPrep solution (Hologic) for the long-term storage of cells (Cytolyt solution). The collected specimen will be analyzed for the presence of COVID-19 genetic material and in case of positive results, HPV typing will be performed as well in order to detect potential correlations between SARS-CoV-2 infection and HPV-infection. Viral nucleic acid extraction and purification will be carried out with suitable molecular biology kit on an automated nucleic acid extractor system for elimination of human error and potential sample contaminations. Viral

amplification and detection protocols both for HPV and SARS-CoV-2 will be done through a low-density microarray-based platform that is validated for clinical use and in vitro diagnostics with high sensitivity and specificity for its purpose including positive and negative reaction controls to avoid false positive or negative results. Sample processing is straightforward, and the analysis and interpretation of results will be performed automatically by a state-of-the-art microarray reader running tailor-made software. In case of positive results, correlations will be examined in terms of colposcopic impression and histopathologic diagnosis of the corresponding cases. All patients will fill in a questionnaire with personal and clinical information related to the study in order to correlate laboratory results with demographics and lifestyle parameters such as smoking, number of sexual partners, nutrition habits, and alcohol consumption.

### Empirical data

There is no empirical data to report on this issue. However, it would be interesting to report a case treated by the Colposcopy Unit of our Department. This was a patient followed up because of known HPV infection. She was affected by SARS-CoV-2 infection in April 2020, fully recovered and was readmitted to our Colposcopy Unit in June for regular follow-up. It was discovered then that cervical epithelium had developed a lesion tested by biopsy and finally diagnosed to be CIN1. Patient is 32 years old and is only followed-up. Nobody may support definitely the hypothesis of correlation between COVID infection and development of lesion. However, it is true that, during the whole patient follow-up in last two years, this was the first time she was diagnosed with lesion. Potentially, it is not the SARS-CoV-2 infection itself, but the dysregulation of immune system, which is already considered as known factor for cervical pathology occurrence. However, it is anyway a motivation to further test what exactly is happening in female patients affected by SARS-CoV-2 in terms of cervical pathology.

### Consequences of the hypothesis and discussion

Potential observation of SARS-CoV-2 presence in cervix may have two main clinical implications.

The first implication concerns the presence of the virus itself. It is still important, in the general context of trying to encounter with this pandemic, to know as much aspects of its manifestations as possible. Clinical apparition in cervix may trigger further basic research, potentially leading to discovery of new pathogenetic pathways this virus may act via.

The second implication concerns its impact on HPV infection. Women infected with HPV are regularly followed-up in a very stratified algorithm to exclude the development of a new cervical lesion. It is really possible that this follow-up should actually be altered in case of SARS-CoV-2 infection. Potential alterations should concern intervals of follow-up, prognosis of low and high-grade lesions as well as surgical treatment. It would not be exaggerating to make the hypothesis, for example, that SARS-CoV-2 positive patients should be anyway followed-up more regularly in order to optimize early detection of lesions. This may not only be due to the general dysregulation of female autoimmune system, but also on certain interactions between the two viruses. Furthermore, we should always keep in mind that an increasing number of patients are vaccinated towards HPV, which sets another interesting aspect on the overall issue.

We would therefore like to launch our medical hypothesis and scientific protocol to control for SARS-CoV-2 infection in cervical specimen as well as examine potential correlation with HPV infection. We strongly believe that COVID-19 could actually not make an exception from invading genitourinary system. However, this is only a theoretical assumption based on common sense but without any strong evidence until now. Communicating such ideas could potentially trigger

colleagues worldwide to expand their interest also on the research of SARS-CoV-2 cervical infection, in an effort to optimize our level of knowledge towards this new threatening and unknown reality of SARS-CoV-2.

We hereby confirm that there is no conflict of interest regarding the submitted medical hypothesis «SARS-CoV-2 infection and impact on female genital tract: an untested hypothesis» submitted by Eleftherios Vavoulidis, PhD, Chrysoula Margioulou-Siarkou, PhD, Stamatios Petousis, PhD, Prof. Konstantinos Dinas, PhD.

### 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.

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