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Seminars Article

Sewage surveillance system using urological wastewater: Key to COVID-19 monitoring?

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Abstract

Since December 2019, the emergence of a new Severe Acute Respiratory Syndrome- coronavirus (SARS-CoV-2) has led to a global coronavirus pandemic disease (COVID-19), with devastating consequences for all healthcare worldwide, including urological care. COVID-19 has led to concern among urological healthcare workers about viral presence, detection and routes of transmission during routine clinical practice. The potential presence of (active) virus in bodily fluids of COVID-19 patients remains a continuing topic of debate. Therefore, we highlight viral detection methods and review the presence of SARS-CoV-2 in urine, feces, and semen. Finally, we discuss how excretion of virus particles through urological bodily fluids might be pivotal to epidemiologic monitoring and control of the disease.

Keywords: SARS-CoV-2; Coronavirus; COVID-19; Semen; Feces; Urine; Urology; Oncology

1. Introduction

December 2019 a new coronavirus was first discovered in Chinese patients that presented with cough, fever and severe dyspnoea [1]. Patients showed similar symptoms as the 2003 Severe Acute Respiratory Syndrome caused by a coronavirus (SARS-CoV) [1,2]. On a molecular level, the novel coronavirus showed high resemblance to SARS-CoV and therefore the new strain was named SARS-CoV-2 [3]. SARS-CoV-2 spread rapidly around the globe and by March 2020 the World Health organization (WHO) declared the disease, COVID-19, a pandemic [4]. As of July 2020, there are at least 16 million confirmed cases and more than 640 thousand deaths globally due to COVID-19 and the number of new cases is increasing daily [5]. The viral crisis has caused an enormous flow of COVID-19 patients into hospitals, resulting in drastic effects on regular healthcare, including urological care. As a result, many countries were forced to take firm preventative measures to reduce spread of the virus, [6,7].

Uro-oncological healthcare workers often come into contact with bodily fluids such as urine, feces, and semen. Uncertainty about modes of transmission, SARS-CoV-2-related urological symptoms and viral detection of SARS-CoV-2 in urological bodily fluids has led to questions and concerns among urological healthcare workers worldwide [8]. The WHO has strongly advised hygiene measures and social distancing to reduce viral spread, yet this can be a clear challenge for the urologist during procedures that require close contact with the patient, e.g., urinary cystoscopy, digital rectal examination, semen analyses, or transrectal ultrasound. Thus, it is of utmost importance for urologists to know if SARS-CoV-2 disseminates through

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urine, feces, or semen, as this may affect patient and personal safety. Since it is unclear whether the virus spreads via other bodily fluids than aerosols, the policy of using personal protective equipment in urological clinics is recommended. Over the past few months, contradictory evidence on the presence of (active) virus in bodily fluids was published [9,10]. In addition, it is largely unknown how viral load in bodily fluids is changing over time from the acute to recovery phase of the infection and to what extent patients are actually contagious through feces, urine and semen. The objective was to briefly inform on viral detection and possible pitfalls in humans. We highlight urologically important SARS-CoV-2 related symptoms, whether (active) virus is detected in urine, feces, and semen specimens and discuss if the viral excretion poses a risk for urological healthcare workers. Finally, we discuss how the presence of viral particles in wastewater may play a pivotal role in the epidemiologic monitoring of COVID-19.

2. SARS-CoV-2 detection methods

Sensitivity for detection of SARS-CoV-2 is dependent on test characteristics, the frequency and timing of testing itself, and on the biological nature of this specific coronavirus. The main transmission route of SARS-CoV-2 is via the respiratory tract. Detection of viral RNA in respiratory tract specimens by reverse transcription polymerase chain reaction (RT-PCR) has been the hallmark of COVID-19 diagnosis. Viral RNA in the nasopharyngeal swab becomes detectable as early as day one of symptoms and mostly peaks within the first week of symptom onset. RNA loads often start to decline by week 3 and subsequently can become undetectable [11]. In severely ill hospitalized patients, RT-PCR positivity may persist beyond 3 weeks after onset of illness, whereas most mild cases will then yield a negative RT-PCR result [12]. While there is ample experience with RT-PCR of SARS-CoV-2, many questions remain. Prolonged RNA detection via RT-PCR without clinical signs of disease has been described, and this might prevent the timely discharge of clinically recovered patients from hospitals and complicates discussions on risk of transmission [13−15]. Moreover, while a positive RT-PCR test indicates the presence of viral RNA genome, it does not necessarily mean that the virus is still infectious in nature [13,16].

As SARS-CoV-2 is a novel virus, initial RT-PCR protocols were developed in expert laboratories and implemented in specialized public health care laboratories and hospitals [17,18]. Shortly thereafter, an increasing number of commercial RT-PCR kits became available of which only few have been systematically evaluated [19,20]. Currently, there is a plethora of different commercial RT-PCR tests available including easy-to-use or “point-of-care” assays [21]. However, some FDA-approved tests also appear to have drawbacks and test sensitivity may differ [22]. Since SARS-CoV-2 is an RNA virus with potentially a high mutation rate, the genetic targets of RT-PCR assays are chosen at relatively conserved regions of the viral genome. Most assays consist of a sensitive screening assay that also detects other SARS-related coronaviruses such as the 2003 SARS-CoV and a SARS-CoV-2-specific confirmation assay [19]. Most RT-PCR assays demonstrate a high analytical sensitivity, detecting just a few RNA genomes [19,20]. Clinical sensitivity, however, can be lower, which is predominantly caused by inadequate sampling or timing of sampling [22]. As massive testing is expected to continue in the near future, it is essential that healthcare workers are adequately trained to prevent sampling errors [23].

Up to now, neutralizing antibodies are considered a hallmark of protection against SARS CoV-2, although the durability and robustness of the immune responses against SARS CoV-2 are still under investigation [24,25]. Identification of neutralizing antibodies by a virus neutralization assay is regarded as the gold-standard in coronavirus serology, but this assay is labor-intensive and time-consuming, as it requires qualified personnel and increased biosafety laboratories. For this reason, commercial serological assays with a sensitivity >98% are nowadays readily available to determine antibody responses in a diagnostic setting [26,27]. Laboratory serological assays generally detect IgA, IgM and/or IgG against specific proteins of the virus. Although these antibodies have been found to be positive even as early as the fourth day after symptom onset, higher levels occur in the second and third week of illness. In patients with mild infection, antibody responses generally develop slower than in patients with severe infection [11,13,24]. A recent study shows that a decline in antibody titers is observed within 94 days post onset of infection, but future studies are required to determine the threshold for protection from re-infection [28].

Serological testing against SARS CoV-2 is mostly used to determine if an individual has been exposed to SARS CoV-2, in follow-up of hospitalized patients, or in diagnosing patients with mild to moderate illness who may present late, beyond the first 2 weeks of illness onset [16]. In several assays a cut-off has been determined above which antibodies will be functional (neutralizing), yet there is ongoing debate about the amount and duration of protective immunity against SARS CoV-2 once antibodies can be detected [26,29].

3. The urological practice: SARS-CoV2 in bodily fluids

3.1. Urine

Both the kidney, urinary bladder and prostate (over) express ACE2 receptors and TMPRSS2 proteases which SARS-CoV-2 uses to enter into cells [30−33]. Possibly, due to the high presence of ACE2 receptors in podocytes, which are folded around the capillaries in the renal corpuscle of nephrons in the kidney, COVID-19 may lead to renal inflammation [34]. In many COVID-19 patients admitted into

hospitals, urine analysis shows proteinuria (44%) and hematuria (27%) [35]. In critically ill patients, 15% to 58% patients have severe renal dysfunction, leading to death in 81% to 100% of patients [9,35]. As opposed to severe renal complications and abnormal urine analyses, the remainder of the urinary tract seems largely unaffected by COVID-19. A study of 1,099 patients infected with SARS-CoV-2 reported that patients had no urological symptoms [2]. These findings were confirmed in a recent systematic review containing 20 additional smaller studies and 3,714 positive patients, none of whom reported micturition symptoms [9].

Studies on the detection of SARS-CoV-2 via RT-PCR in urine sediments found ambiguous results. In multiple small cohort studies, a single urine specimen was collected from COVID-19 patients, all of which were negative for detection of SARS-CoV-2 by RT-PCR [36–40]. However, detailed study information is limited, few of the tested patients were considered severely ill and it is often unknown which patients had urine samples tested. More importantly, data on specimen collection and testing after hospital admission (i.e., acute vs. recovery phase) is lacking. Two studies showed that SARS-CoV-2 was absent in multiple urine samples per patient [13,41]. Specimens were longitudinally collected, with up to 6 urines per patient, and viral detection was performed in the first and second week after onset of symptoms, with detailed description of RNA extraction and RT-PCR methods available. Importantly, serial sampling reduces the risk of sampling errors in different phases of disease. In contrary to previous reports, 3 Chinese studies showed that viral genome was detected in a low number of collected urine sediments, 3 of 116 (3%), 4 of 56 (7%), and 1 of 9 (11%) patients respectively [14,42,43]. In the largest study to date investigating viral detection in bodily fluids, 180 urine samples were tested in 96 buccal-swab proven COVID-19 patients [12]. Investigators noted that just one critically ill patient had a positive urine sample 2 weeks after initial symptoms began. However, reviewed studies merely investigated the presence of viral genome and did not investigate viral activity and transmission potential in preclinical research. Notably, scientists from South-Korea recently published that in 2 of 247 (0.8%) urine specimens, samples were actually contagious and virus was able to replicate in vitro – although with a very low viral load [44]. Authors conclude that viral transmission is not impossible through urine specimens, but that the risk is very low. These results are sharply contrasted by the high number of positive urine sediments in the 2003 SARS-CoV outbreak in Hong-Kong, in which SARS-CoV was detected in 42% of septic patients [45,46]. Moreover, SARS-CoV was detected in urine up to 21 days after onset of symptoms with peak-positivity at day 10. In conclusion, only few studies report viral genome detectable in urinary sediment in a low number of cases. Only one study showed potential viral replication *in vitro* from SARS-CoV-2 isolated from urine [44]. Thus, it seems that SARS-CoV-2 transmission via urine might be possible, but probably does not play a major role in COVID-19 spread.

3.2. Feces

To determine the infectivity of feces by SARS-CoV-2, the same RT-PCR based tests are used as in other human secretions. Basically, the presence of viral genome in stool is analyzed without specialized preparation or selection of the material [13,40]. The presence of specific viral genome serves as a surrogate for infectivity, but is no prove for the ability of viral transmission from the feces [47]. To investigate possible enterocyte involvement, human enterocyte organoids were infected with SARS-CoV-2 and the virus showed replication potential in vitro, leading to mRNA expression changes and ACE2-receptor up-regulation [48]. Moreover, the ACE2-receptor expressed in the stratified epithelium of the upper esophagus and in the enterocytes from ileum and colon [49–51]. However, the exact mechanism of COVID-19-induced gastrointestinal (GI) symptoms is still unknown and more research is needed to provide convincing evidence of viral replication in the human gut lining. A review article on GI-symptoms and infectivity published as early as March 2020, described the digestive symptoms of patients defined by positive throat swabs, urinary, or blood test [52]. In an estimate of 500 retrospective cases, the overall incidence varied between 3% and 79%, with diarrhea as the dominant feature in children and adults, followed by anorexia, vomiting, nausea and even GI-bleeding (4%–13%). These features were observed starting several days before, as well as after the RT-PCR diagnosis and lasted on average 4 to 5 days [53]. There appeared to be no relation between viral shedding, intensity of the overall disease and GI-symptoms [40]. Early reports on viral positivity in stool samples are from the Hubei-3 hospital [38]. In 44 of 154 patients, SARS-CoV-2 was detected in feces, while no urinary excretion was observed. In 2 patients with positive stool tests, there were no GI-symptoms. In 36% (5/14) to 53% (39/73) fecal tests became positive, 2 to 5 days later than sputum tests was positive. Fecal excretion persisted after sputum excretion in 23% (17/73) to 82% (54/66) patients for 1 to 11 days [52]. On April 2020, serial measurements were obtained from patients with high SARS-CoV-2 blood and throat titers, but all stool tests were negative [13]. This leaves us with the conclusion that viral particles are found in feces and that stool samples might be infectious, but that the evidence is low and indirect. The GI tract can be involved during a clinical COVID-19 infection, yet possibly gives symptoms only in a low percentage of patients.

3.3. Semen

Vastly increasing numbers of infected patients, uncertainty of the effects of SARS-CoV-2 on the male reproductive system and the lack of data on the possibility of disease

transmission via semen led to recommendations from the major reproductive societies (ASRM, ESHRE, and IFFS) to discontinue all treatments in assisted reproductive care except for urgent cases [54]. RNA and protein expression of the ACE2 receptor has been shown in Sertoli and Leydig cells of the testis [55,56]. Moreover, other viral infections like Zika, Ebola, and Influenza that are known to cross the blood-testis barrier, are found in semen, can cause orchitis with male infertility and the testes could serve as a reservoir of the virus [57]. These findings further reinforce concerns about potential risks of viral transmission via semen, potential damaging effects on the testes and sperm quality, and possible contamination of laboratory personnel during assisted reproduction. Up to now, in 5 independent studies 108 patients have been investigated for the presence of SARS-CoV-2 in semen [58–62]. Four studies investigating both patients in the acute and recovery phase of viral infection showed no evidence of the virus in semen [58–60,62]. Importantly, even SARS-CoV-2 positive patients confirmed by RT-PCR showed no presence of virus in tested semen. In contrary, one study in 38 patients showed the presence of SARS-CoV-2 in 6 of 38 patients; virus was detectable in 4 of 15 in the acute phase and 2 of 23 in the recovering phase [61]. Unfortunately, no detailed information was available on the timing of SARS-CoV-2 diagnosis relative to semen analysis or how the semen was collected and handled. As it has become apparent that the COVID-19 crisis will likely last until the development of a proper vaccine, the reproductive task forces emphasized the continuation of reproductive care. Based on the available data and successful mitigation strategies in some areas, starting from April 2020, the major reproductive societies have sanctioned gradual and judicious resumption and delivery of full reproductive care as this type of care is considered an essential part of human physical and mental well-being [63]. Furthermore, the guideline does not mandate pre-emptive testing in new patients. In summary, while we are aware of difficulties to obtain reproductive research specimens specifically during acute SARS-CoV-2 infections, current data on viral presence in semen is limited and points toward a low viral presence in the male reproductive system. More knowledge on viral interaction with the male reproductive system should be pursued.

3.4. Clinical implications

The above review on COVID-19-related viral elements in urine, feces, and semen suggests that viral presence in bodily secretions specifically handled in the uro-oncological practice is low. However, the hypothesis that SARS-CoV-2 cannot be transmitted from urine, feces, and semen to humans should be further tested in preclinical and epidemiological studies. Current evidence on infectiousness between bodily fluids and humans is limited, thus no definitive recommendations for urological care can be formulated [63]. Therefore, caution is advised in the daily urological practice until more evident data is available. The first phase 1/2 trial with a COVID-19 vaccine shows promising results, but it remains to be seen whether these results endure in a phase 3 trial [64]. Until a working vaccine has been developed and distributed among healthcare workers, personal protective equipment remains strongly recommended in suspected COVID-19 patients [63,65].

4. Preventive measures: Sewage surveillance system

Although the incidence of reported test positivity is low, viral RNA may act as surrogate marker for the presence of virus in the environment [66,67]. SARS-CoV-2 has been found in the sewage, days after wastewater disposal [68–70]. A sewage surveillance system might therefore prove pivotally important in epidemiologic monitoring of the disease, complementary to the information obtained by regional reporting of disease symptoms and serum testing for COVID-specific antibodies [71]. Current RT-PCR-based tests are sufficiently sensitive to illustrate the presence of infected patients in a geographical area by analysis of its wastewater [68]. Rapid and regular testing and isolation of COVID-19 patients is regarded highly important to contain viral spread, and while serum tests are available to identify individual patients, the daily application and determination is logistically challenging, especially in densely populated urban areas [72]. Moreover, the COVID-19 pandemic is significantly complicated and aggravated by viral transmission between non-symptomatic human carriers [73,74]. Excretion of the viral components in feces has been reported 1 to 2 days previous to clinical respiratory symptoms, so sewage control might be an important trigger to targeted individual testing in areas at risk [75]. Though individual patients cannot be identified, this sewage control also allows for the fast instalment of regional/local quarantines and group testing [75]. In a network of 29 sewage treatment plants in The Netherlands, including around the national airport and in provincial capitals, viral detection started soon after the identification of the first symptomatic patients in February 2020 [70]. Analysis of viral spread in wastewater was executed in parallel to the development of the clinical disease by the Dutch National Institute for Public Health and the Environment (RIVM) [76]. Early reports from a trial period in showed peak presence of SARS-CoV-2 in all sewage plants when hospital admissions were highest. A steady decrease in hospital admissions was accompanied by a decreased presence of virus in the sewage as well [76]. Final measurements are expected to be published by the RIVM in July. The RIVM anticipates that a finely meshed network of sewage surveillance centers is needed for early identification of new COVID-19 infections within the general population. Finally, outcomes do not proof that COVID-19 infections can be infectious by wastewater, but do enhance the importance of the advises for basic hygienic discipline, as it enables the use of wastewater for disease monitoring.

5. Conclusions

The presence of SARS-CoV-2 in urological bodily fluids like, urine, feces, and semen is low. Nonetheless, COVID-19 transmission by respiratory shedding still poses a significant healthcare risk. Therefore, it is highly recommended clinicians use personal protective equipment during diagnostic and surgical procedures, especially when patients are symptomatic. Besides, urological healthcare workers are advised to act in accordance with COVID-19 recommendations of the WHO, the national Public Health Institutions and urological guidelines. As countries aim to contain viral transmission between humans, rapid testing and quarantine may not be enough to reduce viral spread. Analyzing wastewater for SARS-CoV-2 using a sewage surveillance system might complement other preventive measures and could act as warning system for return of the virus by early identification of new viral clusters.

Conflict of interest

None of the contributing authors have any conflict of interest, including specific financial interests and relationships and affiliations relevant to the subject matter in the manuscript. No funding was required for this work.

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