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RAPID COMMUNICATION

Study of SARS-CoV-2 in semen and urine samples of a volunteer with positive naso-pharyngeal swab

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Abstract

Introduction The recent appearance of SARS-CoV-2 in Wuhan in 2019 has started a pandemic which has involved over a million people worldwide. A matter of debate is the possible viral detection in different body fluids than respiratory droplets. Thus, we evaluated the possible presence of SARS-CoV-2 in semen and urine samples of a volunteer with confirmed COVID-19.

Materials and methods A 31-year-old man with fever, myalgia, anosmia, and ageusia was tested and found positive for SARS-CoV-2 through a pharyngeal swab. Eight days after he provided semen and urine samples in which viral RNA presence was measured using a Real time RT PCR system (RealStar SARS-CoV-2 RT-PCR, Altona Diagnostics) targeting E and S viral genes.

Results and discussion Semen and urine samples search for SARS-CoV-2 RNA was negative. Although this should be interpreted cautiously, it may be possible that either the viral clearance kinetics in these matrices matches the progressive clinical recovery of the patient or that the virus was never present in these fluids at the time of the laboratory diagnosis.

Keywords SARS-CoV-2 · Semen fluid · Pharyngeal swab · COVID-19

Introduction

Coronaviruses (CoV) are single-stranded RNA viruses with peculiar glycoprotein spikes around the viral envelope that give a crown-like appearance in electron microscopy. The coronavirus family includes several genera (from alpha to delta) with tropism for a variety of tissues and potentially pathogenic for various animal species, including humans [1]. In recent years, several coronaviruses have caused epidemics in various regions of the world (2002–2003, SARS-CoV epidemic in China; 2012, MERS-CoV epidemic in Saudi Arabia) and the appearance of a new viral strain (SARS-CoV-2) in 2019, in the Chinese region of Wuhan, started a pandemic which has involved over a million people worldwide,

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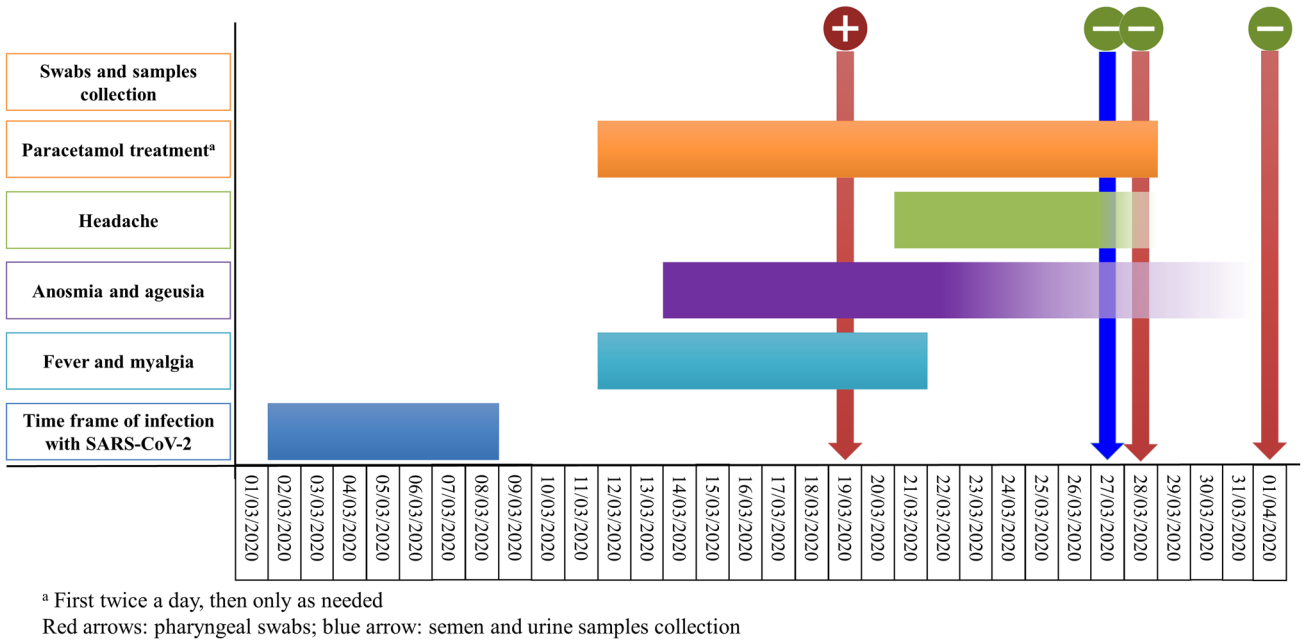
causing over 50,000 deaths [2]. The rapid geographical spread together with high transmissibility and serious clinical manifestations of the Coronavirus disease (COVID-19) [3], have led governments and health authorities to take serious measures to try to contain the pandemic.

At the same time, the researchers took global action on various objectives, including the study of viral transmission pathways and the search/validation of diagnostic methods available to identify affected subjects early [3–5]. SARS-CoV-2 seems to have a strong interaction capacity for the angiotensin two converting enzyme (ACE2): the wide expression in different human tissues (as well as in the lung also in the intestine, testicle, kidney, etc.,) would also justify different theoretical modes of transmission of the virus in addition to respiratory droplets [5]. In fact, viral RNA has also been identified in other biological samples such as feces, urine and blood [6, 7]. However, the viral presence in other biological fluids such as semen has never been tested and this gap of knowledge has raised several concerns in reproductive medicine. Thus, we evaluated the possible presence of SARS-CoV-2 in semen and urine samples collected by a 31-year-old man with COVID-19 confirmed through a naso-pharyngeal swab.

Materials and methods

Patient

A 31-year-old man voluntarily participated in the study and signed informed consent. Two kinds of samples were provided for SARS-CoV-2 testing (semen and urine). The patient's medical history featured a recent diagnosis of dyslipidemia, for which treatment with Simvastatin 20 mg/die was started about one year prior to SARS-CoV-2 diagnosis. Other relevant entries included a diagnosis of androgenetic alopecia currently treated with topical finasteride 1 mg/die and a knee trauma which required surgical reconstruction of the anterior cruciate ligament in 2012. The subject had no relevant andrological pathologies. He referred a state of well-being until March 12th 2020, when he experienced the onset of fever above 38 °C and myalgia (predominantly in the interscapular region) in the absence of chills, pharyngodynia, cough and dyspnea. Since he previously visited a site where COVID-19 cases were reported, he started home isolation, as indicated by current COVID-19 protocols, and began treatment with oral paracetamol 1 g twice a day. The further onset of anosmia and ageusia together with the persistence of fever compelled the execution of a pharyngeal swab on March 19th, which was positive for SARS-CoV-2. The clinical conditions showed a slow but stable improvement with complete resolution reported on March 31st. Figure 1 shows the detailed development of the clinical picture



[a] First twice a day, then only as needed
Red arrows: pharyngeal swabs; blue arrow: semen and urine samples collection

Fig. 1 Temporal description of COVID-19 symptoms and SARS-CoV-2 testing in the volunteer subject

from the onset until resolution. Control pharyngeal swabs were repeated on March 28th and April 1st.

Sample collection and analyses

Semen and urine samples were collected at home inside sterile containers by the patient on March 27th after granting his informed consent and were brought to the Laboratory of Virology, Department of Molecular Medicine, "Sapienza" University of Rome from a researcher wearing appropriate personal protective equipment. Viral RNA from 140 µl of seminal fluid *in toto* and of urine was extracted using QIAamp viral RNA kit (Qiagen) according to manufacturer's protocol. Ten µl of extracted RNA was reverse-transcribed and simultaneously amplified using a Real time RT PCR system (RealStar SARS-CoV2 RT PCR, Altona Diagnostics) targeting E and S viral genes.

Results and discussion

In this study, for the first time, we studied SARS-CoV-2 in semen and urine samples of a 31-year-old man who volunteered for analyses. The subject, after experiencing symptoms compatible with COVID-19 performed a first pharyngeal swab on March 19th where SARS-CoV-2 RNA was detected. Since he experienced a mild disease, he was isolated at home and received only treatment with paracetamol. Eight days after, when symptoms were partially improved, he agreed to provide a semen and urine sample for testing. In both these samples, we did not detect the presence SARS-CoV-2 RNA. Further pharyngeal swabs were performed on March 28th and April 1st, both of them resulting negative.

The different Coronaviridae genera are capable to induce infections in human and vertebrates: while γ-CoV and δ-CoV mainly affect birds, the α-CoV and β-CoV can affect human and various mammals by infecting respiratory, gastrointestinal, and central nervous system. SARS-CoV-2 belong to the β-CoV [1, 8]. The onset of clinical manifestations of SARS-CoV-2 seem to begin in less than a week and are commonly characterized by fever, cough, nasal congestion, asthenia, anosmia, and ageusia. The infection can progress in a severe lung disease with dyspnea, with the development of an atypical pneumonia that corresponds to bilateral ground-glass opacity detected in chest CT scans that might require the subject hospitalization [9].

The viral presence in different body fluids, secretions, and excreta defines the infectious state of the patient. If virus is detected in naso- or oro-pharyngeal swabs, the subject has to be isolated until resolution of the disease and at least two consecutive negative swabs are required to define recovery.

However, current practice has focused mainly on viral clearance from respiratory secretions and little is known about the possible concurrent presence and clearance in different body fluids [6].

Apart from naso-/oro-pharyngeal swabs, the presence of SARS-CoV-2 RNA has also been reported in different biological samples such as feces, urine and blood. Feces, in particular, seem to contain viral RNA in a high percentage of cases and it was reported a longer period of viral clearance than pharyngeal swabs [6]. Instead, the percentage of patients with the presence of viral RNA in urine and blood appears to be fairly low [6, 7, 10]. An improved knowledge of viral diffusion through both respiratory and extra-respiratory routes is of high interest in the management of these patients. To date, SARS-CoV-2 presence in seminal fluid has not been investigated, although it may represent a relevant information in reproductive medicine. Zhou et al. indicated that SARS-CoV-2 enters cells through the same receptor for SARS-CoV, which has been identified in ACE2 [11]. Testicular tissue expresses a certain degree of ACE2, however, if this could cause a testicular involvement during a systemic infection and/or if it could lead to consequences on spermatogenesis still has to be determined. In any case, ACE2 expression patterns in different tissues suggests the possibility of different extra-respiratory viral transmission routes through several body fluids, also including the seminal fluid [5]. The possibility of testicular involvement was previously investigated for SARS-CoV, which may be generalized to SARS-CoV-2 due to strict relation between the two viruses, but we wish to stress that evidence is limited and conflicting [12, 13].

To clarify this, we aimed to evaluate the possible presence of SARS-CoV-2 in urogenital fluids (seminal fluid and urine) of an infected subject. Both these samples were found negative for the viral mRNA. It should be stressed that our volunteer has collected these biological samples only on the eighth day after the first positive swab, but this was necessary due to the impaired health condition of the subject during the peak of the symptoms.

Nonetheless, this result allows us to formulate two hypotheses: (1) if the virus had been present in the seminal fluid, at the peak of the infection, our results could indicate that SARS-CoV-2 clearance kinetics coincides with the progressive clinical recovery experienced by the patient or (2) the virus was never present in the seminal fluid at the time of the laboratory diagnosis.

In our opinion, this information can provide a useful tool for clinicians which can be reassured that recovering patients have limited chance to spread the virus through semen and urine. Furthermore, this may be a relevant topic in reproductive medicine, with implications on artificial reproduction and, especially, semen cryopreservation to evaluate the safety of male gametes cryopreserved in this period.

However, these findings must be interpreted cautiously as, to date, this is the only attempt to determine the

SARS-CoV-2 presence in seminal fluid with currently available molecular methods in medical literature. Moreover, the analysis was conducted only eight days after the first positive swab in a subject with a relatively mild viral disease which did not require hospitalization. We cannot exclude whether in a more severe disease and/or in case of samples collected in the acute phase (if possible) the virus could have been detected in semen and urine of the subject. Another issue of interest is the possibility of andrological consequences of infected subjects. One of the aims of the future research could be the study of the possible testicular involvement with local inflammation and disruption of testicular environment that might lead to consequences on spermatogenesis and autoimmunization with development of anti-sperm antibodies. This hypothesis needs to be confirmed through a follow-up, in particular in young-adult in reproductive age with history of COVID-19 disease to determine possible short- and long-term consequences for their andrological health.

Author contributions FL and DP conceived the work; FP and DP drafted the article; SC and FB provided patient care; LM and OT performed molecular analyses; AL, FL and GA critically reviewed the paper.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethics approval This work was approved by the Ethical Committee Policlinico Umberto I-"Sapienza" University of Rome.

Research involving human participants All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Appropriate informed consent was acquired from the participating subject.

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