SARS-CoV-2 in the knee joint: a cadaver study

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Abstract

Objective

Despite the considerable research efforts being made to learn more about COVID-19, little is known about the presence of SARS-CoV-2 genetic material in biological fluids other than respiratory droplets, blood, and feces. The aim of this post-mortem study was to assess the presence of SARS-CoV-2 RNA in the knee synovial fluid, synovial tissue, and bone tissue of COVID-19 patients in order to discover whether the joint is a possible route of transmission during orthopaedic surgical procedures, and clarify the possible role of SARS-CoV-2 as a directly arthritogenic virus.

Methods

Post-mortem synovial fluid, synovial tissue and bone tissue samples were collected from the knees of five patients who died of COVID-19 in our hospital between September and October 2020, and analysed for the presence of SARS-CoV-2 using a commercial real-time polymerase chain reaction (RT-PCR) panel. Quantitative RT-PCR was used to test post-mortem nasopharyngeal swabs of all of the patients.

Results

No SARS-CoV-2 RNA was detected in any of the knee samples, despite the positivity of the throat swab.

Conclusion

Our findings indicate that SARS-CoV-2 was not detected in knee synovial fluid, synovial membrane or bone. This makes it unlikely that these are potential sources of contagion, and suggests that SARS-CoV-2 is not directly arthritogenic.

Key words

COVID-19, synovial fluid, bone tissue, cadavers, autopsy, arthrocentesis, real-time polymerase chain reaction

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Introduction

SARS-CoV-2 is a novel virus belonging to the coronavirus (CoV) family, and gives rise to coronavirus disease 2019 (COVID-19), which was declared a global pandemic by the World Health Organisation on 11 March 2020. The clinical manifestations of COVID-19 include fatigue, fever, cough, and musculoskeletal symptoms such as arthromyalgia (1). The most severe forms are characterised by life-threatening acute respiratory distress syndrome (ARDS) and multiple organ failure.

Despite the large number of research papers already published, the exact route of viral transmission has not been defined. It has been demonstrated the possibility of transmission from medical staff to medical staff, patient to medical staff, as well as medical staff to patient (2, 3). It is clear that the main route of transmission is exposure to airborne infected droplets (4), but viral RNA has also been isolated in the blood samples and feces of infected patients (5). Furthermore, little is known about the presence of SARS-CoV-2 in the joints either *post-mortem* (6) or *in vivo* (7). To the best of our knowledge, only Lopéz-Gonzalez *et al*. (8) have published a description of acute arthritides occurring during hospitalisation due to COVID-19, and they did not find any SARS-CoV-2 genetic material in the patients' synovial fluid samples.

The aim of this microbiological study was to assess the presence of SARS-CoV-2 RNA in the knee synovial fluid, synovial tissue, and bone tissue of autopsied COVID-19 patients because excluding its presence could reassure healthcare professionals that there is no additional risk of contagion due to invasive joint procedures, if the appropriate protective strategies were adopted, in order to provide the highest level of safety to healthcare workers (9), avoiding occupational transmission of COVID-19 to medical staff. A COVID-19 infection of just one health-care worker could have a dramatic effect for the healthcare system itself, as mentioned by Anelli *et al*. (10); besides, a single COVID-19 infection among essential health-care workers at a hospital might severely reduce the capacity of an entire hospital as testified by the Italian National Federation of Medical Associations (11).

Furthermore, although joint and muscle pain are frequent during the acute phase of the infection, it has not yet been established whether SARS-CoV-2 may play a role in the pathogenesis of various forms of arthritis, directly (12) or indirectly in the form of reactive arthritis (13) as a result of virus-mediated immune system hyperactivation (14).

Materials and methods

Synovial fluid, synovial tissue and bone tissue samples were taken from the knees of five patients who died at ASST-Fatebenefratelli Luigi Sacco University Hospital, Milan (Italy) between September and October 2020. All of the patients were diagnosed as having COVID-19 by means of nasopharyngeal swab samples collected by the hospital's Laboratory of Clinical Microbiology, Virology and Bioemergency during the period of hospitalisation. All of the patients were adults whose certificated cause of death was COVID-19 with pulmonary failure, confirmed by means of a real time polymerase chain reaction (RT-PCR) on a nasopharyngeal swab. Patients with a previous history of knee surgery or a documented diagnosis of arthritis involving the knee were excluded.

The study procedures were approved by our local Ethics Committee (Comitato Etico Milano Area 1, no. 713, 23 June 2020), and informed consent was obtained from the patients' relatives before performing the autopsy.

Synovial fluid and tissue sampling was carried out by the same operator within 72 hours of death, using arthrocentesis followed by an open biopsy through a mini-medial parapatellar access; bone was sampled using a dedicated trocar for bone biopsies at the same location on the medial femoral condyle; and cartilage and subchondral bone were sampled to a depth of at least 2 cm. Furthermore, *post-mortem* nasopharyngeal swabs of all of the patients were tested by means of RT-PCR.

All the samples were stored at -80°C before being analysed using the genetic material detection protocol also used in

Table I. The patients' clinical and demographic characteristics, autopsy timelines and test results.

Patient	Age	Co-morbidities	Date of hospitalisation	Date and time of autopsy	Time between death and autopsy	Post-mortem nasopharyngeal swab (RT-PCR)	Post-mortem RT-PCR		
							Synovial fluid	Synovial tissue	Bone tissue
F1	73	Hypothyroidism, asthma and COPD	10/08/2020	11/09/2020 08,30	24 hours, 10 minutes	Positive	Negative	Negative	Negative
M1	75	Ischaemic heart disease, diabetes type 2, dyslipidaemia, epilepsy, consequences of stroke	14/09/2020	16/09/2020 15,00	54 hours, 20 minutes	Positive	Negative	Negative	Negative
M2	79	Hypertension, dyslipidaemia	18/09/2020	28/09/2020 9,00	24 hours, 45 minutes	Positive	Negative	Negative	Negative
M3	87	Ischaemic heart disease	22/10/2020	29/10/2020 10,30	66 hours, 25 minutes	Positive	Negative	Negative	Negative
M4	89	Ischaemic heart disease, chronic renal failure, atrial fibrillation, dementia	22/10/2020	28/10/2020 11,00	62 hours, 20 minutes	Positive	Negative	Negative	Negative

COPD: chronic obstructive pulmonary disease; RT-PCR: real-time reverse-transcriptase polymerase chain reaction.

our hospital's diagnostic protocols. The QIAMP Viral RNA mini-kit (Qiagen, Hilden, Germany) was used to extract viral RNA from 200 μL of synovial fluid in accordance with the manufacturer's protocol. The presence of SARS-CoV-2 was assessed using a RT-PCR panel containing primers and probes targeting the nucleocapsid (N) gene, the ORF1ab gene, and the envelope E gene (15).

Results

The study group comprised one female and four males, whose average age at the time of death was 80.6 years. Their clinical manifestations upon hospital admission were fever, cough, arthromyalgia, pharyngodynia and respiratory distress, together with chest x-ray and computed tomography findings of COVID-19-associated pneumonia. All five patients had co-morbidities, including cardiovascular disease, hypertension, dyslipidaemia, diabetes and dementia. The mean time between hospital admission and death was 8.2 days: two patients died in our intensive care unit (ICU), and three in our infectious disease ward. All of the deaths were caused by pulmonary failure associated with microembolism confirmed by the final autopsy. The mean time between death and the autoptic biopsies was 46 hours and 24 minutes.

RT-PCR did not detect any evidence of the viral RNA in any of the synovial fluid, synovial tissue and bone samples, whereas all of the *post-mortem* nasopharyngeal swabs tested positive for SARS-CoV-2 (RT- PCR).

In Table I we summarised the main patient clinical characteristics, autopsy timelines and test results.

Discussion

To the best of our knowledge, this is the first paper assessing the presence of SARS-CoV-2 RNA in the knee joints of patients who died because of COVID-19. No SARS-CoV-2 RNA was detected in any of the synovial fluid, synovial tissue or bone tissue samples, although the *post-mortem* nasopharyngeal swabs of all five patients tested positive for SARS-CoV-2 using the same RT-PCR procedure as that used to diagnose COVID-19 diagnosis in living patients. This is an important finding because the virus has been documented in plasma, feces, and urine (5, 7), thus showing that is possible (although unlikely) to be infected by fluids other than respiratory secretions.

The risk of contagion during surgical procedures has not yet been determined. One study conducted at 24 hospitals in Wuhan at the very beginning of the pandemic hypothesised that up to 20% of orthopaedic surgeons were infected by SARS-CoV-2 mainly as a result of airway exposure to infected respiratory droplets (2). Our findings suggest that all healthcare professionals performing surgical procedures on the joints of COVID-19 patients are exposed to a risk of contagion due to exposure to respiratory droplets, blood and body fluids, but not to direct exposure to joint- or bone-related tissues. It is therefore plausible that there is no additional risk associated with invasive knee procedures involving patients with SARS-CoV-2 infection, although it remains recommended to avoid using instruments such as electrocautery or pulsatile lavage to minimise aerosolisation and therefore contamination of the surrounding environment (16). Hence, the milestone that SARS-CoV-2 can be transmitted via a blood-borne pathway remains, but we can conclude that additional precautions secondary to universal precautions against blood-borne pathogens that have been standard of care in the healthcare setting are not necessary (17, 18).

The absence of SARS-CoV-2 RNA in the knee is also clinically relevant. COVID-19 patients may be asymptomatic or have symptoms ranging from mild to severe and other clinical manifestations. Musculoskeletal manifestations such as arthralgias are present in about 15% of COVID-19 patients (19, 20), but the effects of the disease on the musculoskeletal apparatus are still unclear. It is known that viral infections can cause acute poly- or monoarticular arthralgia and arthritis: the list of involved viruses is long and includes parvovirus B19, hepatitis B virus, hep-

atitis C virus, HIV (21). The diagnosis involves epidemiological, clinical and serological analysis, such as type of articular involvement (poly/mono, symmetric/asymmetric), good response to NSAIDs, onset within the first weeks of infection and a self-limiting presence (12). Although the presence of the viral genetic material in the joint may be the most important factor for the pathogenesis (as it occurs with parvovirus B19) (21), the etiopathogenesis of this condition may be immune-related: for example, the immune complex deposition syndrome is important for arthralgias and arthritis in hepatitis B infection. Considered this, it is possible that COVID-19 patients display viral-mediated arthralgias and arthritis, and some sporadic case reports of arthritis in COVID-19 patients (8, 12, 22, 23) may suggest that this possibility is conceivable. The absence of the virus in the knee highlighted by our study suggests that it is unlikely that SARS-CoV-2 has a direct inflammatory action on the joint, but it could induce an inflammation-related reaction, manifesting as a reactive arthritis (24).

The high transmissibility of the virus, along with case fatality estimates ranging from 1% to above 5%, has generated worldwide concern. Patients with comorbid conditions including hypertension, diabetes and pulmonary disease are highly represented among hospitalised patients with COVID-19 disease, suggesting the presence of risk factors that may predispose heightened susceptibility to SARS-CoV-2 infection (25). In the literature there are several studies highlighting that mortality and the risk of developing post-surgical complications increase if there are concomitant comorbidities or an older age (>70 years) in COVID-19 patients (26, 27) undergoing surgical procedures. Nonetheless, this topic is still a matter of debate, as some authors like Vranis and colleagues showed a mortality rate of 10% and a rate of Intensive Care Unit admissions of 18% after surgery, suggesting that patients COVID-19 undergoing surgery do not appear to have a significantly increased risk of mortality with respect to non-surgical COVID-19 patients. This discrepancy in literature could be the result of the type of surgery and of geographic differences in healthcare approaches to pandemic management that may have led to opposite results in different areas (27, 28). In addition, tissue and cellular tropism is key to understand the pathogenesis of SARS-CoV-2. A connection between SARS-CoV-2 and hypertension, in particular, is suggested by the discovery that angiotensin converting enzyme 2 (ACE 2) is the essential receptor for SARS-CoV-2. The expression and subcellular localisation of the SARS-CoV-2 receptor, ACE 2, within the upper (nasal) and lower (pulmonary) respiratory tracts was already investigated in the literature (25) and in the future the presence of ACE 2 receptor could be sought in the knee.

This study has some limitations. The number of patients is small, but nonetheless representative given the paucity of data in the literature. Furthermore, as no samples were taken from other joints, our findings are restricted to the knee. Finally, we did not analyse biological fluids other than synovial and nasopharyngeal fluids, but we feel that the data concerning synovial fluid, synovial tissue and bone tissue are relevant by themselves. Further studies are being conducted in order to investigate the relationship between cytokine titres, inflammation indices, and arthritic manifestations in COVID-19 patients, and provide a rationale for the possible onset of reactive arthritis.

Conclusion

Our findings show that SARS-CoV-2 was not detected in knee synovial fluid, synovial membrane or bone, indicating that it is unlikely that these may be potential sources of contagion, and suggesting that SARS-CoV-2 is not directly arthritogenic.

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