Case report - a cornea donor with a positive SARS-CoV-2 finding

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Case Report

Keywords: SARS-CoV-2, Cornea, tissue donation, detection methods, safety

Posted Date: October 7th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-88018/v1>

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Abstract

The safety of the patient is the highest priority when providing a recipient with a tissue transplant. When screening a potential donor, the presence of infectious diseases is therefore also considered, which requires special attention in view of the current SARS-CoV-2 pandemic. Reliable test systems for the detection of the novel virus and its determination during all phases of infection are a prerequisite for a reliable statement on the risk of transmission of the virus through a corneal transplant. The case of a 70-year-old cornea donor with a positive SARS-CoV-2 result, which is documented here, demonstrates the difficulty of a meaningful donor test.

Background

Corneal donation is subject to legal regulations that ensure the safety and quality of the tissue and serve to protect the recipient. Donor testing must be adapted in case of newly emerging pathogens.

On 31.12.2019, the *World Health Organization* (WHO) was informed of cases of pneumonia of unknown aetiology (China). The causative virus was designated as *severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2). On 11.03.2020 the WHO classified the disease caused by the virus as a pandemic.

The German federal authority for tissue establishments, the Paul Ehrlich Institute, demands preventive measures when assessing the suitability of potential donors. These refer to confirmed contacts with SARS-CoV-2 infected persons and past confirmed SARS-CoV-2 infections [1]. Extended donor testing is at the discretion of the tissue establishments. All donations to the German Society for Tissue Transplantation gGmbH (DGFG) are subject to a strict risk assessment.

Currently, the risk of SARS-CoV-2 transmission through tissue transplantation is not known.

Case Presentation And Test Methods

A 70-year-old woman was reported as a potential tissue donor by a cooperating hospital. The cause of death was intoxication. The medical and social history was unremarkable, with no evidence of pneumonia or other infections. As a standard procedure, a nasopharyngeal swab was taken upon admission to hospital to test for SARS-CoV-2, but the results were not available at the time of donation. The ophthalmologic tissues and a post-mortem swab were collected 36 h after death. After the donation, the hospital informed the tissue establishment of the positive SARS-CoV-2 result of the swab taken at the time of admission of the donor [Tab. 1].

The post-mortem swab taken during the donation was negative after qRT-PCR examination. Based on the positive result of the pre-mortem swab, qRT-PCR analysis of the donor cornea and the culture medium was initiated. Both tests were negative. Consequently, the pre-mortem serum of the donor was tested for

the SARS-CoV-2 antibodies IgA and IgG using "Anti-SARS-CoV-2-Elisa" (Euroimmun AG). SARS-CoV-2-IgA antibodies were detected (ratio: 2.35; according to manufacturer's data: positive from ratio 1.1). IgG-antibodies were not detected. Further analysis of the donor serum using various lateral flow assays did not detect SARS-CoV-2-IgM or SARS-CoV-2-IgG [Tab. 2].

To reconcile the different test results of the hospital and the tissue establishment, the sampling conditions were compared. The swabs were each taken with dry swab in the nasopharyngeal region. The storage times were comparable. The interim storage was at 2-8°C. Both test laboratories used different test kits. The laboratory commissioned by the hospital used a test based on the protocol of the Charité Berlin, which detects the genome regions of the E gene (envelope gene) and RdRp gene (RNA-dependent RNA polymerase gene). The qRT-PCR test of the positive detection from the hospital was weakly positive (CT value between 38-40) and thus in the borderline range. The confirmatory test from the combined detection of the E gene (CT value 37.8) and the RdRp gene (CT value 38.9) was also in this range. The laboratory of the Mitteldeutsche Corneabank Halle (MCH) used the "Anchor SARS-CoV-2 PCR Kit" (Anchor Diagnostics). This test detects the N gene (nucleoprotein) and the S gene (spike protein). The post-mortem swab was tested negative for SARS-CoV-2 with this test system. In order to exclude sensitivity differences between the tests, the sample blank of the post-mortem smear was tested with the "LightMix® Modular SARS-CoV (COVID19)" (Roche Diagnostics), which also detects the genome regions of the E gene and the RdRp gene. With this test, the post-mortem sample was also tested negative for SARS-CoV-2 [Tab.1].

Discussion

In the context of the corona pandemic, the question arises whether and with which diagnostic possibilities the safety of corneal transplants can be increased. For this reason, MCH routinely tests a pooled nasopharyngeal/conjunctival swab for SARS-CoV-2 using qRT-PCR in all tissue donors post-mortem.

In the present case, the weakly positive PCR signal from the pre-mortem smear suggested that the donor's infection with SARS-CoV-2 had already largely subsided. The donor probably had no or only mild symptoms, since both the medical history taken at the hospital and by the tissue establishment were unremarkable. Due to the borderline CT value of the positive pre-mortem test, the negative result of the post-mortem test is not unexpected. The respective CT values of the individual tests [Tab.1] correspond to the data described in the literature [2]. A false positive result is therefore excluded. Re-examination of the pre-mortem swab specimens was not possible due to a lack of reserves.

To date, over 50 donors have been tested by post-mortem nasopharyngeal/conjunctival swab and all results have been negative. This could be due to the fact that all potential donors are subject to a strict risk assessment and that the general infection rate in the donor region is very low. In the DGFG network, this is the only case in which a SARS-CoV-2 infection has been detected.

The risk of viral infection via tissue transplantation cannot be assessed yet. The suitability of existing tests for post-mortem analysis is unclear. However, initial studies on COVID-19-dead patients showed that SARS-CoV-2 can be detected in cornea and conjunctiva, although with comparably lower virus RNA concentrations [3]. Analyses of the infectivity of PCR-positive samples showed that virus growth from samples with a CT value >24 and >8 days after onset of symptoms was not successful [4]. Analyses of the correlation between viral load and the ability to cultivate the viruses in cell culture as a measure of infectivity can be used to derive threshold values above which there is very little or no infectivity. The Robert Koch Institute has recommended that a control examination should be initiated to assess further measures if the CT value exceeds 30 [5]. Currently, it must be assumed that transmission of the virus via transplanted tissue such as the cornea is potentially possible. Based on the anamnesis, symptomatic patients are not available as tissue donors, so that patients in the pre-symptomatic phase and symptom-free patients remain as risk groups. To what extent these patients represent a risk of transmission is not yet clear. Therefore, it would be important for recipient protection to know whether a donor is infected with SARS-CoV-2.

This case clearly shows the diagnostic gap in the test methods used, since with largely identical methods the virus could not be detected 45 h after the first test. First indications suggest that in a SARS-CoV-2 infection specific antibodies are only detected after seroconversion from day 7 to 14. The IgA-positive but IgG-negative serum result indicates an acute or recent infection with SARS-CoV-2. The detection of antibodies alone is therefore not recommended for acute diagnosis. It is not yet clear over what period of time SARS-CoV-2 specific IgM, IgA and IgG antibodies are detected, especially since viral RNA may still be detectable in the nasopharynx despite cured COVID19 disease. In comparative studies of infected patients with mild vs. severe symptoms, IgA antibodies were not detected until 8 days after the onset of symptoms in mildly progressing SARS-CoV-2 infections, with a correlation between the intensity of the symptoms and the onset of detection [6]. In cases with weak symptoms, IgG antibody detection may be absent or delayed.

The case presented in this paper illustrates the following facts:

In the case of a asymptomatic patient, the importance of routine clinical swabbing at patient admission is clearly demonstrated, as the right test must be performed at the right time [7]. With the presented case, no statement can be made whether the post-mortem swab test is suitable for testing for SARS-CoV-2. There is no valid proof that this method can reliably detect post-mortem infection with SARS-CoV-2. Whether a proposed test of the extracted cornea or culture medium by qRT-PCR [8] would be suitable for SARS-CoV-2 detection requires further analysis.

Conclusions

After seroconversion [7], an analysis of the antibodies could help to identify a low-symptom infection and reduce the diagnostic gap. The sensitivity of the lateral flow assays also tested is clearly inferior to other methods, e.g. ELISA.

Whether an infection with SARS-CoV-2 even at low viral load means that corneal tissue is affected and whether a transplantation represents a possible risk of infection should be the subject of further investigations.

Declarations

Ethics and Consent: The reported data are results from an analysis of data that are collected as part of standard procedures in relation to cornea donation, thus not requiring approval by the Ethics Committee. All data were documented after receiving informed consent of the relatives of the cornea donors according to German legislation. The authors confirm that the study and data accumulation conform with all federal or state laws and that the study was in adherence to the tenets of the Declaration of Helsinki.

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Tables

Table 1: Overview of swabs

	hospital	corneabank
sample	nasopharyngeal-swab (dry swab)	nasopharyngeal-/ conjunctival-swab, pooled (dry swab)
time of sampling	pre-mortem	post-mortem
results	E-Gen: CT - 37,8 (tested twice)* ¹ RdRp-Gen: CT - 38,9* ¹	N-Gen: negative* ² S-Gen: negative* ² E-Gen: negative* ³ RdRp-Gen: negative* ³
period: sampling until test	22 h	19 h
time interval: test hospital/ test corneabank	45 h	

*¹ test based on the protocol of the Charité Berlin

*² "Anchor SARS-CoV-2 PCR Kit" (Anchor Diagnostics GmbH, Hamburg)

*³ „LightMix® Modular SARS-CoV (COVID19)" (Roche Diagnostics Deutschland GmbH/ TIB MOLBIOL GmbH Berlin)

Table 2: Period of sample analysis

hospital			comeabank		
sample	time of sampling	result	sample	time of sampling	result
nasopharyngeal-swab	admission	positive (weakly)	nasopharyngeal-/conjunctival-swab	45 h after admission	negative
serum	admission	IgA: positive* ¹ IgG: negative* ¹ (LFA IgG/ IgM: negative)* ²	cornea and culture medium	9 Tage after admission	negative

*¹ "Anti-SARS-CoV-2-Elisa" (Euroimmun AG, Lübeck)

*² Lateral Flow Assays: „mö-screen 2019-NCOV“ (möLab GmbH, Langenfeld), „Rapid 2019-NCOV IgG/IgM Combo Test Card“ (Xiamen Boson Biotech Co. Ltd; Xiamen/ China), „COVID-19 IgG/IgM Rapid Test Kit“ (Wuhan UNscience Biotechnology Co. Ltd; Wuhan/China)