

Detection of SARS-CoV-2 in Different Body Fluids Using RT-PCR – An Autopsy Based Study

HETAL KYADA

PDU Medical College: Pandit Deendayal Upadhyaya Medical College

MAHESH TRANGADIA

PDU Medical College: Pandit Deendayal Upadhyaya Medical College

DIVYESH VADGAMA

PDU Medical College: Pandit Deendayal Upadhyaya Medical College

PRATIK VARU (✉ drpratik5388@gmail.com)

PDU Medical College: Pandit Deendayal Upadhyaya Medical College <https://orcid.org/0000-0003-1151-0922>

SHAILESH BHUVA

PDU Medical College: Pandit Deendayal Upadhyaya Medical College

PRINCE MANVAR

PDU Medical College: Pandit Deendayal Upadhyaya Medical College

BHOOMI RATHOD

PDU Medical College: Pandit Deendayal Upadhyaya Medical College

Research Article

Keywords: SARS-CoV-2, RT-PCR, Tracheal swabs, Vitreous Humor, Cerebrospinal fluid (CSF), Pericardial fluid, Pleural fluid

Posted Date: November 25th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-115745/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

World is in mid of dreaded pandemic – Covid-19 caused by SARS-CoV-2. To curb it tremendous efforts has been put worldwide to enhance scientific knowledge related to SARS-CoV-2. In this study, we collected postmortem samples of tracheal swab, vitreous humor, pleural fluid, CSF and pericardial fluid by conducting complete autopsy on 24 patients with known SARS-CoV-2 infection at department of forensic medicine, P.D.U. Government College, Rajkot (India). RT-PCR was used to detect SARS-CoV-2 from these samples. SARS-CoV-2 was detected from tracheal swab in 54.55% cases and from pleural fluid in 13.34% cases. SARS-CoV-2 wasn't detected from any sample of CSF, vitreous humor and pericardial fluid. Positive and negative cases for postmortem tracheal swabs were analyzed to find out of its relationship with duration of Covid-19 illness. No significant relationship was found between detectability of SARS-CoV-2 in postmortem samples of tracheal swab and duration of Covid-19 illness.

Introduction

On 31st Dec. 2019, Wuhan municipal health commission, China reported cluster of cases of pneumonia in Wuhan, Hubei province. Eventually it was found that cases of pneumonia were caused by severe acute respiratory syndrome corona virus-2 (SARS-CoV-2), a novel corona virus. [1] Since then world has faced dreaded pandemic – Corona Virus Disease-19 (Covid-19), which has taken so many lives. To curb the disease, tremendous efforts have been put worldwide to enhance scientific knowledge regarding SARS-CoV-2 as well as disease caused by it. So far many studies have been conducted for SARS-CoV-2 to understand its virology, epidemiology, pathogenesis, effect on different organs of the body, diagnosis, treatment, vaccine etc. [2-5]

Many researcher highlighted need of autopsies to understand effects of SARS-CoV-2 in the body. [6-8] Many such autopsy based studies have been conducted worldwide so far, which has thrown light on effect of SARS-CoV-2 in body. [4,5,9,10] However, no such autopsy based research data is available for India. To fill this lacuna, we conducted complete autopsies on 24 patients with known SARS-CoV-2 infection in department of forensic medicine, P. D. U. Government medical college, Rajkot (India). We were second only to All India Institute of Medical Science (AIIMS), Bhopal to conduct such research autopsies in India.

So far it is known that SARS-CoV-2 transmits from human to human predominantly via respiratory droplet and through personal contact. Possibility of fecal-oral transmission of virus is also there. [2, 11] Once inside the body, SARS-CoV-2 uses ACE2 receptors of host cells for entry and the serine protease TMPRSS2 for S protein priming. [12] Wang K et al. found novel route of entry of SARS-CoV-2 in host cell in form of CD147-spike protein. [13] Few other receptors like DPP4, also known as CD26 and GRP78 are also found to play role in viral entry into the cell. [14] So, it can be said that tissues expressing ACE2 receptors, TMPRESS2, CD147-spike protein, DPP4, GRP78 etc. are potential targets of SARS-CoV-2. DPP4 receptor has been detected in mononuclear leukocytes, serous cells of submucosal glands, on the surfaces of non-ciliated bronchial epithelial cells, type I and II pneumocyte, alveolar macrophages, vascular endothelial cells, lymphatics and pleural mesothelia. [14] GRP78 is a regulator of endoplasmic reticulum stress due to its role in the unfolded protein response pathway, and it has also been detected in the mitochondria, nucleus, cytosol, and plasma membrane. [14] In study done by Li MY et al., ACE2 expression levels were found to be highest in the small intestine, testis, kidneys, heart, thyroid, and adipose tissue, and were the lowest in the blood, spleen, bone marrow, brain, blood vessels, and muscle. ACE2 showed medium expression levels in the lungs, colon, liver, bladder, and adrenal gland. [15] ACE2 receptors are also expressed in ocular tissues like cornea, conjunctiva, aqueous humor and retina. [16] CD147 is expressed in cornea, conjunctiva, aqueous humor, retina, tear and vitreous humor. [16] All these organs and fluids are potential targets for SARS-Cov-2. So there is need of studies to find out presence of SARS-CoV-2 in these organs and body fluids.

Very few studies have focused on detection of SARS-CoV-2 in vitreous humor, CSF, pleural fluid and pericardial fluid. [17-30] Number of cases were also less in most such studies. In our study, we have collected postmortem samples of vitreous humor, CSF, pleural fluid and pericardial fluid in 24 cases and analyzed them to detect SARS-CoV-2 by RT-PCR. We have also tried to detect presence of SARS-CoV-2 in respiratory tract after death, as one of the objective of our study was to find out relationship between detectability of virus from respiratory tract and duration of Covid-19 illness in fatal cases. As studies have suggested that lower respiratory tract samples provide more positive result than upper respiratory tract samples, we have taken lower tracheal swabs for detection of SARS-CoV-2. [31,32] We have conducted complete autopsies in 24 cases, however, we are discussing only detection of SARS-CoV-2 in body fluids in this research article due to length of study.

Materials And Methods

We have conducted complete autopsy on 24 patients with known SARS-CoV-2 infection from 7th September, 2020 to 3rd November 2020 in department of Forensic Medicine, P. D. U. Government medical college, Rajkot (India). All patients had SARS-CoV-2 infection confirmed either by RT-PCR of nasopharyngeal and oropharyngeal swabs or rapid antigen test of nasopharyngeal swab during hospital admission. Study was started after approval of Institutional Ethics Committee of P. D. U. Government medical college, Rajkot (India).

Written informed consent was obtained from next of kin for all 24 deceased. Demographic, clinical and other relevant data of all patients were evaluated from hospital treatment papers.

To reduce the risk of transmission of infectious pathogens before, during and after postmortem examination, all autopsies were performed according to covid-19 guidelines on dead body management issued by Ministry of Health and Family Welfare, Government of India as well as guidelines by Indian Council of Medical Research (ICMR). [33, 34] Autopsies were performed in a specific covid-19 designated autopsy room with airflow control and airborne infection control procedures including use of appropriated PPE. Neither of researchers involved in autopsy developed any symptom of covid-19. None of the researchers became positive for SARS-CoV-2 on RT-PCR.

Complete autopsy was conducted using routine standard dissection techniques with precaution to avoid aerosol generation. Before starting dissection procedure, vitreous humor was aspirated from posterior chamber of eye using standard 5 ml sterile syringe with 22 gauge needle. Cranial cavity was opened next with chisel and hammer instead of oscillating saw to avoid aerosol generation. After removal of dura, CSF was aspirated from lateral ventricles of brain by standard 5 ml sterile syringe with 22 gauge needle. Neck tissues, thoracic and abdominal cavities were exposed by 'I' shaped incision. Trachea was opened next by putting vertical incision over it from the thyroid cartilage up to the space above the suprasternal notch. Two lower tracheal swabs were obtained through this opening from just above bifurcation of trachea. Pericardial and pleural fluid were collected using standard 5 ml sterile syringe from their respective space after opening of thoracic cavity. All samples were collected with utmost care to avoid contamination. All samples were transferred to viral transport media and were immediately sent to microbiology laboratory of P. D. U. Government medical college, Rajkot (India).

To detect SARS-CoV-2 in postmortem samples of tracheal swab, vitreous humor, CSF, pleural fluid and pericardial fluid, RT-PCR was performed. RT-PCR was done using kit approved by Indian Council of Medical Research (ICMR). Assay detects target genes for SARS-CoV-2 namely RdRp, ORF and N gene. Cycle threshold value of 40 was interpreted as positive for SARS-CoV-2.

Duration of covid-19 illness of patient was calculated from onset of symptom related to covid-19 to death. Duration of illness in positive and negative tracheal swab samples were compared by independent sample t test. Duration of antemortem and postmortem test to detect SARS-CoV-2 was calculated, and such duration for positive and negative swab samples was also compared by independent sample t test.

Data were statistically analyzed using Microsoft excel and SPSS software and presented as means, ranges and percentage.

Result

As shown in table no.1, autopsies were conducted on 24 patients with age ranging from 30 years to 90 years (66.25 ± 14.58). Out of which, 22 were male (91.67%) and 2 were female (8.33%). Duration of covid-19 illness was ranging from 3 days to 21 days (11.13 ± 4.73). Duration between antemortem and postmortem test for detection of SARS-CoV-2 was ranging from 1 day to 18 days (7.54 ± 4.76). Duration between death and preservation of postmortem samples was ranging between 1 hours to 5 hours (Mean duration – 02:55 hours).

Out of 24 cases, SARS-CoV-2 was detected from postmortem samples of tracheal swabs in 14 cases (58.33%). Duration of covid-19 illness in these positive cases was ranging from 3 days to 19 days (9.71 ± 4.43). Duration between antemortem and postmortem test for detection of SARS-CoV-2 was ranging from 1 day to 13 days (6.36 ± 3.92). Duration between death and preservation of postmortem samples was ranging between 1 hours to 4:30 hours (Mean duration – 02:45 hours). Out of 24 cases, postmortem samples of tracheal swabs were negative for SARS-CoV-2 in 10 cases (41.67%). Duration of covid-19 illness in these negative cases was ranging from 5 days to 21 days (13.10 ± 4.63). Duration between antemortem and postmortem test for detection of SARS-CoV-2 was ranging from 2 days to 18 days (9.20 ± 5.51). Duration between death and preservation of postmortem samples was ranging between 1:45 hours to 5 hours (Mean duration – 03:10 hours).

On application of independent sample t-test to compare mean duration of covid-19 illness among positive and negative postmortem tracheal swab, p value of 0.084 was found. On comparison of mean duration between antemortem and postmortem test among positive and negative postmortem tracheal swab, p value of 0.153 was found. As p value is more than 0.05 in both cases, there is no significant statistical difference between duration of covid-19 illness as well as duration between antemortem and postmortem test among positive and negative postmortem tracheal swab.

Out of 24 cases, postmortem samples of pleural fluid was positive for SARS-CoV-2 in 3 cases (12.50%).

Postmortem samples of CSF, vitreous humor and pericardial fluid were negative for SARS-CoV-2 in all cases.

Discussion

SARS-CoV-2 is primarily affecting respiratory system. It can be logically said that viral load should decrease from respiratory tract with time, especially in patients under treatment. However, it needs to be verified scientifically and statistically. For this purpose, we had collected two lower tracheal swabs in each case for detection of SARS-CoV-2 in respiratory tract. SARS-CoV-2 was found from tracheal swabs in 58.33% of cases, while in 41.67% cases RT-PCR couldn't detect presence of virus. Duration of covid-19 illness from onset of symptoms to death was less in cases where SARS-CoV-2 was found from postmortem tracheal swabs (9.71 ± 4.43 days) in comparison with cases where SARS-CoV-2 wasn't found (13.10 ± 4.63). Similarly, duration between antemortem and postmortem test for detection of SARS-CoV-2 was also less in positive cases (6.36 ± 3.92 days) in comparison with negative cases (9.20 ± 5.51 days).

So in comparison of mean of both durations, it seems that viral load has decreased and became undetectable by RT-PCR as duration of covid-19 illness and duration between antemortem and postmortem test for detection of SARS-CoV-2 has increased. However, duration of covid-19 illness was found to be highly variable in both positive as well as negative cases for postmortem tracheal swab. Postmortem tracheal swab was positive where duration of illness was as less as 3 days and as high as 19 days. It was negative where duration was as less as 5 days and as high as 21 days. Similar high variability was observed in duration between antemortem and postmortem swab also. So independent samples t-test was applied to find out statistically significant difference between two. No statistical significant difference was found between duration of covid-19 illness in positive and negative postmortem tracheal swab. Similarly, no statistical significant difference was found between duration from antemortem to postmortem test in positive and negative postmortem tracheal swab. However, as sample size in our study was limited, we suggest that similar studies should be carried out with large sample size.

For duration between death and RT-PCR test for postmortem tracheal swab, there was no major difference between positive cases (Mean duration – 02:45 hours) and negative cases (Mean duration – 03:10 hours). So, it can be said that duration between death and RT-PCR test for postmortem sample hasn't affected result of the test.

Dell'Aquila M et al. [35] had taken 4 postmortem swabs for detection of SARS-CoV-2 – Nasopharyngeal, tracheal and one from each lung parenchyma. SARS-CoV-2 was found in 9 out of 12 cases (75%) in his study. He collected swabs from different parts of respiratory tract. This could be reason for better positivity rate in his study. However, sample size in his study was half of our sample size, so more studies with higher sample size would be necessary to confirm role of multiple swabs and positivity rate in postmortem samples.

On comparison of duration from death to collection of postmortem samples, SARS-CoV-2 was detected as late as 5 hours after death in our study, while in study done by Dell'Aquila M et al, SARS-CoV-2 was detected as late as 120 hours after death. [35] So, it can be suggested that utmost care should be taken while handling dead bodies of persons with SARS-CoV-2 infection, even if they are handled late after the death. However, wider studies would be necessary in order to define the infectiveness of the virus in postmortem tissues.

In our study, SARS-CoV-2 wasn't detected in any postmortem samples of vitreous humor out of 24 cases. None of the patient had any ocular manifestation in our study. SARS-CoV-2 wasn't detected in study done by List W et al. [17] (16 cases), Lauermann P et al. [18] (1 case) and Bayyoud T et al. [19] (1 case). Recent studies have found SARS-CoV-2 in tear and conjunctival swabs in even in patients who hadn't any ocular manifestation. [36, 37] Vitreous humor also expresses CD144 receptor like tear and conjunctiva, which is potential target for SARS-CoV-2. [16] So possibility of detection of SARS-CoV-2 cannot be ruled out from vitreous humor. Recent studies has shown evidence of detection of SARS-CoV-2 in retina. [38] If retinal tissues are inoculated by SARS-CoV-2, possibility of diffusion to vitreous cannot be ruled out. So more studies will be needed with large sample size are needed to find out whether SARS-CoV-2 enters vitreous humor or not.

In our study, 12.50% cases shown presence of SARS-CoV-2 in pleural fluid. Bennet D et al. [20] described case report of 61 year old renal transplant patient, who had shown presence of SARS-CoV-2 in plural fluid by RT-PCR. SARS-CoV-2 was also detected from pleural fluid in study done by Malik MI et al. [21] (1 case), Mei F et al. [22] (1 case), Baek MS et al. [23] (1 case) and Turriziani O et al. [24] (3 out of 8 cases). As pleural mesothelial cell expresses DPP4 (CD26) receptor, which plays a role for entry of a virus inside the cell, pleura is target for SARS-CoV-2. [14] Moreover, virus can diffuse to pleural fluid from lung tissues damaged by SARS-CoV-2 infection.

In our study, SARS-CoV-2 was not found from CSF. None of the patient had shown any neurological symptoms before death in our study. SARS-CoV-2 wasn't found in study of Turriziani O et al. [24] (5 cases) and Neumann B et al. [25] (30 cases). Destras G et al. [26] tested 578 CSF samples of 555 patients received at Lyon university hospital during covid-19 epidemic. Out of these 555 patients, only 23 were confirmed cases of SARS-CoV-2 infection. Two CSF samples were found to be slightly positive for SARS-CoV-2 (cycle threshold [Ct]=32 and Ct=35) in his study. Out of this two cases, in one case blood was positive while brain biopsy was negative for SARS-CoV-2, suggesting possibility of contamination of CSF by blood during lumbar puncture. Espíndola OM et al. [27] tested 8 CSF samples of known cases of SARS-CoV-2 infection, showing neurological symptoms, which were negative in RTPCR. He also reviewed other similar studies involving total 30 patients, apart from 8 patients of his study. Out of total 38 patient, one patient suffering from meningoencephalitis had shown presence of SARS-CoV-2 in CSF. Helms J et al. [28] tested 25 CSF samples of known cases of SARS-CoV-2 infection, showing neurological manifestation. SARS-CoV-2 was detected in only one patient. Blood was negative for SARS-CoV-2 in this patient, which rules out contamination of sample by blood. So, SARS-CoV-2 can be detected in CSF but in very low number of cases. Brain tissues expresses ACE2 receptors, which makes brain a potential target for SARS-Cov-2. [15] Virus can diffuse into CSF from affected nervous tissues. This could be reason for detection of SARS-CoV-2 from CSF.

In our study, SARS-CoV-2 was not found from pericardial fluid. Farina A et al. [29] reported one case of cardiac tamponade in SARS-CoV-2 infected patient, in whom SARS-CoV-2 was detected from pericardial fluid. François Sauer et al. [30] reported 3 cases of pericardial effusion, out of which in one case, SARS-CoV-2 was detected from pericardial fluid. Heart expresses ACE2 receptor. [15] So it is potential target for SARS-CoV-2. Recent studies has shown presence of SARS-CoV-2 in cardiac tissues. [31,32] Virus can diffuse into pericardial fluid form damaged cardiac cell. This could be reason behind detectability of SARS-CoV-2 in pericardial fluid. However, more such studies are needed to get conclusive evidence.

Conclusion

From our study and from comparison with other such studies, it was found that SARS-CoV-2 can be detected from pleural fluid, CSF as well as pericardial fluid, although in less number of cases. SARS-CoV-2 was not detected from vitreous humor in our study as well as in 3 other studies. However, as vitreous humor can be potential target for SARS-CoV-2 due receptors expressed by it and as SARS-CoV-2 has been detected in other ocular tissues like retina, conjunctiva and tear, possibility of detection of SARS-CoV-2 is there. We suggest that more studies should be conducted to detect presence of SARS-CoV-2 in CSF, vitreous humor, pericardial fluid and pleural fluid.

We also found that relationship between duration of covid-19 illness and detectability of virus by RT-PCR is unpredictable, as SARS-CoV-2 was detected from postmortem tracheal swab in cases with long illness and was undetected in cases with short illness. No statistical significant difference was found in our study between duration of covid-19 illness of positive and negative postmortem tracheal swab. We suggest that more such studies with large sample size should be carried out to find out relationship between detectability of SARS-CoV-2 from respiratory tract and duration of illness in fatal cases.

Abbreviations

SARS-CoV-2 – Severe acute respiratory syndrome corona virus-2

Covid-19 – Corona virus disease-19

RT-PCR – Reverse transcription polymerase chain reaction

CSF - Cerebrospinal fluid (CSF)

PPE – Personal protective equipment

RA – Rapid antigen test

Declarations

CONFLICT OF INTEREST: NONE

FUND FOR THE RESEARCH: NONE

ETHICS APPROVAL: The present study was conducted under the ethical approval of institutional ethics committee (Human), P. D. U. Government Medical College, Rajkot, India, vide letter no. 222/20, dated 04/09/2020.

CONSENT TO PARTICIPATE: written informed consent taken from next of kin prior to autopsy.

CONSENT FOR PUBLICATION: written informed consent taken from next of kin prior to autopsy.

AUTHOR CONTRIBUTION:

Dr. Hetal Kyada, Dr. Mahesh Trangadia, Dr. Divyesh Vadgama, Dr. Pratik Varu, Dr. Shailesh Bhuva and Dr. Prince Manvar reviewed the literature, formed the concept of this study and obtained the approval from the Institutional Ethics Committee of the P. D. U. Govt. Medical College, Rajkot.

Dr. Hetal Kyada, Dr. Mahesh Trangadia, Dr. Divyesh Vadgama, Dr. Pratik Varu, Dr. Shailesh Bhuva and Dr. Prince Manvar performed the postmortem examinations and collected the samples.

Postmortem samples were analyzed for detection of SARS-CoV-2 using RT-PCR by Dr. Bhoomi Rathod

Dr. Hetal Kyada, Dr. Mahesh Trangadia, Dr. Divyesh Vadgama, Dr. Pratik Varu, Dr. Shailesh Bhuva and Dr. Prince Manvar analyzed and interpreted the data and drafted the manuscript.

References

1. World Health Organization. Pneumonia of unknown cause – China. Available <https://www.who.int/csr/don/05-january-2020-pneumonia-of-unkown-cause-china/en/>
2. Jin, Y., Yang, H., Ji, W., Wu, W., Chen, S., Zhang, W., & Duan, G. (2020). Virology, Epidemiology, Pathogenesis, and Control of COVID-19. *Viruses*, 12(4), 372. <https://doi.org/10.3390/v12040372>
3. Amawi, H., Abu Deiab, G. I., Aljabali, A. A., Dua, K., & Tambuwala, M. M. (2020). COVID-19 pandemic: an overview of epidemiology, pathogenesis, diagnostics and potential vaccines and therapeutics. *Therapeutic delivery*, 11(4), 245–268. <https://doi.org/10.4155/tde-2020-0035>
4. Wichmann, D., Sperhake, J. P., Lütgehetmann, M., Steurer, S., Edler, C., Heinemann, A., Heinrich, F., Mushumba, H., Kniep, I., Schröder, A. S., Burdelski, C., de Heer, G., Nierhaus, A., Frings, D., Pfefferle, S., Becker, H., Bredereke-Wiedling, H., de Weerth, A., Paschen, H. R., Sheikhzadeh-Eggers, S, Kluge, S. (2020). Autopsy Findings and Venous

- Thromboembolism in Patients with COVID-19: A Prospective Cohort Study. *Annals of internal medicine*, 173(4), 268–277. <https://doi.org/10.7326/M20-2003>
5. Falasca, L., Nardacci, R., Colombo, D., Lalle, E., Di Caro, A., Nicastri, E., Antinori, A., Petrosillo, N., Marchioni, L., Biava, G., D'Offizi, G., Palmieri, F., Goletti, D., Zumla, A., Ippolito, G., Piacentini, M., & Del Nonno, F. (2020). Post-Mortem Findings in Italian Patients with COVID-19 - a Descriptive Full Autopsy Study of cases with and without co-morbidities. *The Journal of infectious diseases*, jiaa578. Advance online publication. <https://doi.org/10.1093/infdis/jiaa578>
 6. Sperhake J. P. (2020). Autopsies of COVID-19 deceased? Absolutely!. *Legal medicine (Tokyo, Japan)*, 47, 101769. <https://doi.org/10.1016/j.legalmed.2020.101769>
 7. Meyerholz, D. K., & McCray, P. B., Jr (2020). Illuminating COVID-19 lung disease through autopsy studies. *EBioMedicine*, 57, 102865. <https://doi.org/10.1016/j.ebiom.2020.102865>
 8. Barth, R. F., Xu, X., & Buja, L. M. (2020). A Call to Action: The Need for Autopsies to Determine the Full Extent of Organ Involvement Associated With COVID-19. *Chest*, 158(1), 43–44. <https://doi.org/10.1016/j.chest.2020.03.060>
 9. Menter, T., Haslbauer, J. D., Nienhold, R., Savic, S., Hopfer, H., Deigendes, N., Frank, S., Turek, D., Willi, N., Pargger, H., Bassetti, S., Leuppi, J. D., Cathomas, G., Tolnay, M., Mertz, K. D., & Tzankov, A. (2020). Postmortem examination of COVID-19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings in lungs and other organs suggesting vascular dysfunction. *Histopathology*, 77(2), 198–209. Advance online publication. <https://doi.org/10.1111/his.14134>
 10. Youd, E., & Moore, L. (2020). COVID-19 autopsy in people who died in community settings: the first series. *Journal of clinical pathology*, jclinpath-2020-206710. Advance online publication. <https://doi.org/10.1136/jclinpath-2020-206710>
 11. Nikolich-Zugich, J., Knox, K. S., Rios, C. T., Natt, B., Bhattacharya, D., & Fain, M. J. (2020). SARS-CoV-2 and COVID-19 in older adults: what we may expect regarding pathogenesis, immune responses, and outcomes. *GeroScience*, 42(2), 505–514. <https://doi.org/10.1007/s11357-020-00186-0>
 12. Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T. S., Herrler, G., Wu, N. H., Nitsche, A., Müller, M. A., Drosten, C., & Pöhlmann, S. (2020). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*, 181(2), 271–280.e8. <https://doi.org/10.1016/j.cell.2020.02.052>
 13. Wang, Ke & Chen, Wei & Zhou, Yu-Sen & Lian, Jian-Qi & Zhang, Zheng & Du, Peng & Gong, Li & Zhang, Yang & Cui, Hongyong & Geng, Jie-Jie & Wang, Bin & Sun, Xiu-Xuan & Wang, Chun-Fu & Yang, Xu & Lin, Peng & Deng, Yong-Qiang & Wei, Ding & Yang, Xiang-Min & Zhu, Yu-Meng. (2020). SARS-CoV-2 invades host cells via a novel route: CD147-spike protein. 10.1101/2020.03.14.988345.
 14. Gelman, R., Bayatra, A., Kessler, A., Schwartz, A., & Ilan, Y. (2020). Targeting SARS-CoV-2 receptors as a means for reducing infectivity and improving antiviral and immune response: an algorithm-based method for overcoming resistance to antiviral agents. *Emerging microbes & infections*, 9(1), 1397–1406. <https://doi.org/10.1080/22221751.2020.1776161>
 15. Li, M. Y., Li, L., Zhang, Y., & Wang, X. S. (2020). Expression of the SARS-CoV-2 cell receptor gene ACE2 in a wide variety of human tissues. *Infectious diseases of poverty*, 9(1), 45. <https://doi.org/10.1186/s40249-020-00662-x>
 16. Chen X, Yu H, et al. (2020) SARS-CoV-2 on the ocular surface: is it truly a novel transmission route? *Br J Ophthalmol*. Aug 11: doi: 10.1136/bjophthalmol-2020-316263.

17. List W, Regitnig P, et al. (2020). Occurrence of SARS-CoV-2 in the intraocular milieu. *Exp Eye Res.* Sep 28; 201:108273. doi: 10.1016/j.exer.2020.108273.
18. Lauermaun, P, Storch, M., Weig, M., Tampe, B., Winkler, M., Hoerauf, H., Feltgen, N., & Hakroush, S. (2020). There is no intraocular affection on a SARS-CoV-2 - Infected ocular surface. *American journal of ophthalmology case reports*, 20, 100884. <https://doi.org/10.1016/j.ajoc.2020.100884>
19. Bayyoud, Tarek MD1,*; Iftner, Angelika2; Iftner, Thomas PhD2; Bartz-Schmidt, Karl Ulrich MD1; Rohrbach, Jens Martin MD1; Ueffing, Marius PhD1,3; Schindler, Michael PhD2; Thaler, Sebastian MD (2020). Absence of Severe Acute Respiratory Syndrome-Coronavirus-2 RNA in Human Corneal Tissues, *Cornea*: June 29, - Volume Publish Ahead of Print - Issue -doi: 10.1097/ICO.0000000000002479
20. Bennett, D., Franchi, F., De Vita, E., Mazzei, M. A., Volterrani, L., Disanto, M. G., Garosi, G., Guarnieri, A., Cusi, M. G., Bargagli, E., Scolletta, S., Valente, S., Gusinu, R., & Frediani, B. (2020). SARS-CoV-2 in pleural fluid in a kidney transplant patient. *Postgraduate medicine*, 1–4. Advance online publication. <https://doi.org/10.1080/00325481.2020.1838817>
21. Malik, M. I., Fox, N., Chopra, A., Hughes, H. Y., Washburn, R., & Huggins, J. T. (2020). Positive Pleural Fluid RT-PCR for Virus Detection in SARS-CoV-2 Pneumonia. *QJM: monthly journal of the Association of Physicians*, hcaa276. Advance online publication. <https://doi.org/10.1093/qjmed/hcaa276>
22. Mei, F., Bonifazi, M., Menzo, S., Di Marco Berardino, A., Sediari, M., Paolini, L., Re, A., Gonnelli, F., Duranti, C., Grilli, M., Vennarucci, G. S., Latini, M. A., Zuccatosta, L., & Gasparini, S. (2020). First Detection of SARS-CoV-2 by Real-Time Reverse Transcriptase-Polymerase Chain Reaction Assay in Pleural Fluid. *Chest*, 158(4), e143–e146. <https://doi.org/10.1016/j.chest.2020.05.583>
23. Baek, M. S., Kim, W. Y., Lee, K. J., & Noh, C. S. (2020). Detection of Severe Acute Respiratory Syndrome Coronavirus 2 in the Pleural Fluid. *Infection & chemotherapy*, Advance online publication.
24. Turriziani, O., Sciandra, I., Mazzuti, L., Di Carlo, D., Bitossi, C., Calabretto, M., Guerrizio, G., Oliveto, G., Riveros Cabral, R. J., Viscido, A., Falasca, F., Gentile, M., Pietropaolo, V., Rodio, D. M., Carattoli, A., & Antonelli, G. (2020). SARS-CoV-2 diagnostics in the virology laboratory of a University Hospital in Rome during the lockdown period. *Journal of medical virology*, 10.1002/jmv.26332. Advance online publication. <https://doi.org/10.1002/jmv.26332>
25. Neumann, B., Schmidbauer, M. L., Dimitriadis, K., Otto, S., Knier, B., Niesen, W. D., Hosp, J. A., Günther, A., Lindemann, S., Nagy, G., Steinberg, T., Linker, R. A., Hemmer, B., Bösel, J., & PANDEMIC and the IGNITE study groups (2020). Cerebrospinal fluid findings in COVID-19 patients with neurological symptoms. *Journal of the neurological sciences*, 418, 117090. <https://doi.org/10.1016/j.jns.2020.117090>
26. Destras, G., Bal, A., Escuret, V., Morfin, F., Lina, B., Josset, L., & COVID-Diagnosis HCL Study Group (2020). Systematic SARS-CoV-2 screening in cerebrospinal fluid during the COVID-19 pandemic. *The Lancet. Microbe*, 1(4), e149. [https://doi.org/10.1016/S2666-5247\(20\)30066-5](https://doi.org/10.1016/S2666-5247(20)30066-5)
27. Espíndola, O. M., Siqueira, M., Soares, C. N., Lima, M., Leite, A., Araujo, A., Brandão, C. O., & Silva, M. (2020). Patients with COVID-19 and neurological manifestations show undetectable SARS-CoV-2 RNA levels in the cerebrospinal fluid. *International journal of infectious diseases: IJID: official publication of the International Society for Infectious Diseases*, 96, 567–569. <https://doi.org/10.1016/j.ijid.2020.05.123>
28. Helms, J., Kremer, S., Merdji, H., Schenck, M., Severac, F., Clere-Jehl, R., Studer, A., Radosavljevic, M., Kummerlen, C., Monnier, A., Boulay, C., Fafi-Kremer, S., Castelain, V., Ohana, M., Anheim, M., Schneider, F., & Meziani, F. (2020). Delirium and encephalopathy in severe COVID-19: a cohort analysis of ICU patients. *Critical care (London, England)*, 24(1), 491. <https://doi.org/10.1186/s13054-020-03200-1>

29. Farina, A., Uccello, G., Spreafico, M., Bassanelli, G., & Savonitto, S. (2020). SARS-CoV-2 detection in the pericardial fluid of a patient with cardiac tamponade. *European journal of internal medicine*, 76, 100–101. <https://doi.org/10.1016/j.ejim.2020.04.045>
30. Sauer, F., Dagnat, C., Couppie, P., Jochum, G., & Leddet, P. (2020). Pericardial effusion in patients with COVID-19: case series. *European Heart Journal: Case Reports*, ytaa287. <https://doi.org/10.1093/ehjcr/ytaa287>
31. Huang Y, Chen S, et al. (2020) SARS-CoV-2 Viral Load in Clinical Samples from Critically Ill Patients. *Am J Respir Crit Care Med*. Jun 1; 201(11):1435-1438. doi: 10.1164/rccm.202003-0572LE.
32. Wang, W., Xu, Y., Gao, R., Lu, R., Han, K., Wu, G., & Tan, W. (2020). Detection of SARS-CoV-2 in Different Types of Clinical Specimens. *JAMA*, 323(18), 1843–1844. <https://doi.org/10.1001/jama.2020.3786>
33. Covid-19: Guidelines on dead body management. Directorate General of Health Services, Ministry of Health & Family Welfare, Government of India. Available: https://www.mohfw.gov.in/pdf/1584423700568_COVID19GuidelinesonDeadbodymanagement.pdf
34. Standard guidelines for medico-legal autopsy in Covid-19 deaths in India. Indian Council of Medical Research. New Delhi. India.
35. Dell'Aquila, M., Cattani, P., Fantoni, M., Marchetti, S., Aquila, I., Stigliano, E., Carbone, A., Oliva, A., & Arena, V. (2020). Postmortem Swabs in the Severe Acute Respiratory Syndrome Coronavirus 2 Pandemic: Report on 12 Complete Clinical Autopsy Cases. *Archives of pathology & laboratory medicine*, 144(11), 1298–1302. <https://doi.org/10.5858/arpa.2020-0362-SA>
36. Xia, J., Tong, J., Liu, M., Shen, Y., & Guo, D. (2020). Evaluation of coronavirus in tears and conjunctival secretions of patients with SARS-CoV-2 infection. *Journal of medical virology*, 92(6), 589–594. <https://doi.org/10.1002/jmv.25725>
37. Kumar, K., Prakash, A. A., Gangasagara, S. B., Rathod, S., Ravi, K., Rangaiah, A., Shankar, S. M., Basawarajappa, S. G., Bhushan, S., Neeraja, T. G., Khandenahalli, S., Swetha, M., Gupta, P., Sampritha, U. C., Prasad, G., & Jayanthi, C. R. (2020). Presence of viral RNA of SARS-CoV-2 in conjunctival swab specimens of COVID-19 patients. *Indian journal of ophthalmology*, 68(6), 1015–1017. https://doi.org/10.4103/ijo.IJO_1287_20 Lauermann P, Storch M, et al. There is no intraocular affection on a SARS-CoV-2 - Infected ocular surface. *Am J Ophthalmol Case Rep*. 2020 Dec;20:100884. doi: 10.1016/j.ajoc.2020.100884. Epub 2020 Aug 20. PMID: 32839739; PMCID: PMC7439011.
38. Casagrande, M., Fitzek, A., Püschel, K., Aleshcheva, G., Schultheiss, H. P., Berneking, L., Spitzer, M. S., & Schultheiss, M. (2020). Detection of SARS-CoV-2 in Human Retinal Biopsies of Deceased COVID-19 Patients. *Ocular immunology and inflammation*, 28(5), 721–725. <https://doi.org/10.1080/09273948.2020.1770301>
39. Escher, F., Pietsch, H., Aleshcheva, G., Bock, T., Baumeier, C., Elsaesser, A., Wenzel, P., Hamm, C., Westenfeld, R., Schultheiss, M., Gross, U., Morawietz, L., and Schultheiss, H.-P. (2020) Detection of viral SARS-CoV-2 genomes and histopathological changes in endomyocardial biopsies. *ESC Heart Failure*, 7: 2440– 2447. <https://doi.org/10.1002/ehf2.12805>
40. Puelles, V. G., Lütgehetmann, M., Lindenmeyer, M. T., Sperhake, J. P., Wong, M. N., Allweiss, L., Chilla, S., Heinemann, A., Wanner, N., Liu, S., Braun, F., Lu, S., Pfefferle, S., Schröder, A. S., Edler, C., Gross, O., Glatzel, M., Wichmann, D., Wiech, T., Kluge, S., Huber, T. B. (2020). Multiorgan and Renal Tropism of SARS-CoV-2. *The New England journal of medicine*, 383(6), 590–592. <https://doi.org/10.1056/NEJMc2011400>

Table

Table-1: Age and sex wise distribution of cases, type of antemortem test used for detection of SARS-CoV-2, duration of covid-19 illness, duration between antemortem and postmortem test, duration between death and preservation of postmortem

samples and result of RT-PCR for SARS-CoV-2 in postmortem samples

No.	Sex	Age	Type of Antemortem test for detection of SARS-CoV-2 RT-PCR / RA	Duration of covid-19 illness (in days)	Duration between antemortem and postmortem test (in days)	Duration between death and preservation of postmortem samples (in hours and minutes)	Result of RT-PCR in postmortem samples				
							Tracheal Swab	CSF	Vitreous Humor	Pleural Fluid	Pericardial Fluid
1	M	85	RA	11	6	03:00	P	N	N	N	N
2	M	67	RT-PCR	5	1	03:00	P	N	N	P	N
3	M	59	RA	11	10	04:30	P	N	N	N	N
4	M	30	RT-PCR	13	9	04:10	N	N	N	N	N
5	F	90	RT-PCR	5	4	02:30	N	N	N	N	N
6	M	61	RA	14	12	01:45	N	N	N	N	N
7	M	59	RT-PCR	21	18	03:40	N	N	N	N	N
8	M	86	RT-PCR	12	2	05:00	N	N	N	N	N
9	M	58	RT-PCR	9	2	01:00	P	N	N	N	N
10	F	80	RT-PCR	19	12	03:00	P	N	N	N	N
11	M	65	RA	10	9	02:25	P	N	N	N	N
12	M	66	RT-PCR	6	3	01:45	P	N	N	N	N
13	M	65	RA	6	5	03:45	P	N	N	N	N
14	M	70	RA	12	11	02:35	N	N	N	N	N
15	M	80	RA	16	13	02:30	P	N	N	P	N
16	M	65	RA	3	1	03:45	P	N	N	N	N
17	M	70	RA	13	5	03:35	N	N	N	N	N
18	M	77	RA	12	9	04:05	P	N	N	P	N
19	M	76	RA	14	10	03:15	N	N	N	N	N
20	M	58	RA	19	17	03:10	N	N	N	N	N
21	M	80	RT-PCR	9	7	01:15	P	N	N	N	N
22	M	50	RT-PCR	8	4	02:00	N	N	N	N	N
23	M	55	RT-PCR	13	7	01:35	P	N	N	N	N
24	M	38	RA	6	4	03:00	P	N	N	N	N

*RT-PCR = Reverse Transcription Polymerase Chain Reaction, RA = Rapid Antigen Test, M = Male, F = Female, P = Positive, N = Negative