

Title: Ophthalmologic evidence against the interpersonal transmission of 2019 novel coronavirus through conjunctiva

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Abstract

Background: The emerging 2019 novel coronavirus (2019-nCoV) has pushed several countries into state of emergency all over the world. The possible transmission of 2019-nCoV by conjunctiva is controversial and has substantial public health implications.

Methods: A retrospective cohort study was initiated to investigate the possible transmission of 2019-nCoV through aerosol contact with conjunctiva. We enrolled 67 cases of confirmed or suspected cases of novel coronavirus pneumonia (NCP) during 17–28 Jan 2020. Nasopharyngeal and conjunctival swabs were collected for real time RT-PCR analysis to detect 2019-nCoV.

Results: 63 patients were identified as laboratory-confirmed NCP and the remaining four were suspected NCP. Conjunctival swab samples from one NCP patient yielded positive PCR results and two NCP patients yielded probable positive PCR results. None of the three patients had ocular symptoms. The only one NCP patient with conjunctivitis as the first symptom had negative conjunctival sac 2019-nCoV test. Conjunctival swab samples from the four suspected cases of NCIP were negative.

Conclusion: 2019-nCoV can be detected in the conjunctival sac of patients with NCP. Through clinical analysis, viral transmission via the conjunctival route was not supported by the data. Good clinical protection can effectively cut off the transmission path.

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Key words: 2019-nCoV; NCP; conjunctiva

23 Introduction

24 The current outbreak of the 2019 novel coronavirus (2019-nCoV) originating in
 25 Wuhan, China has posed a threat to global public health and has been defined as a
 26 Public Health Emergency of International Concern (PHEIC) by the World Health
 27 Organization (WHO)¹. Thus far, tens of thousands of confirmed cases, including
 28 healthcare workers, have been identified in China, with the latest mortality rate
 29 calculated at approximately 2.52%². Generally, patients infected with 2019-nCoV
 30 develop respiratory illness, with the first symptoms of fever, cough and fatigue that
 31 quickly progress to pneumonia³. A number of patients were observed with
 32 extra-pulmonary manifestations at the onset of the illness, such as conjunctivitis, or
 33 even presented with asymptomatic infection^{4,5}. Additionally, the susceptible and
 34 potential routes of viral spread from patients or asymptomatic carriers to healthy
 35 person have yet to be fully understood⁶. These issues pose great challenges for disease
 36 diagnosis and outbreak control, and make the goal of improving our understanding
 37 one of the utmost urgencies.

38 2019-nCoV has been identified as one of a class of single-stranded enveloped
 39 RNA viruses, belonging to the beta-coronaviruses genus of the *Coronaviridae* family
 40 that has been isolated from several mammals, including humans⁷. Although the
 41 genome sequence of 2019-nCoV is distinct from severe acute respiratory syndrome
 42 coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus
 43 (MERS-CoV), they all have the potential to cause a severe acute respiratory disease
 44 and possess human-to-human transmissibility⁸. Similar to SARS-CoV, 2019-nCoV

gains entry into host cells through recognizing and binding to its potential host receptor, angiotensin-converting enzyme 2 (ACE2), which is distributed among various tissue and cell types, including the conjunctiva⁹⁻¹¹. A previous study indicates that healthcare workers suffer from higher risk of SARS infection in the case of unprotected eye contact with secretions¹². The SARS-CoV has been shown to spread through direct or indirect contact with mucous membranes in the eyes^{13,14}. Moreover, there are increasing reports suggesting that a few novel coronavirus pneumonia (NCP) cases began with conjunctivitis as the initial symptom following contact with confirmed patients without protective goggles^{4,15,16}. For these reasons, determining whether 2019-nCoV is capable of transmission through aerosol contact with conjunctiva is an important consideration that warrants for further exploration.

In view of the limited understanding and controversy surrounding knowledge of viral transmission through the conjunctival sac, we performed a retrospective cohort study designed to seek ophthalmologic evidence for the transmission of 2019-nCoV from patients via aerosol contact with the conjunctiva. The findings of this study may provide novel insights into spread routes and potential infection control measures for 2019-nCoV that may help battle this outbreak.

Methods

Case enrollment

From 17 Jan to 28 Jan 2020, we enrolled 67 patients with either confirmed or suspected cases of NCP in the Renmin Hospital of Wuhan University. Laboratory-confirmed cases were identified with the criteria of at least one positive

67 result from a respiratory specimen using viral isolation, next-generation sequencing,
68 or reverse transcription-polymerase chain reaction (RT-PCR) assays. Suspected cases
69 were recruited based on the criteria of fever or signs/symptoms of lower respiratory
70 illness (e.g. cough or shortness of breath), low or normal white-cell count or low
71 lymphocyte count, and no reduction in symptoms after antimicrobial treatment for 3
72 days. The history of epidemiologic exposure was also considered and incorporated¹⁷.

73 All patients invited to participate in the study provided consent for the
74 nasopharyngeal and conjunctival swab samples. The study was approved by the
75 Ethics Committees of Renmin Hospital of Wuhan University.

76 ***Study design***

77 Patient clinical details, including general information, clinical manifestations,
78 and extra ocular symptoms, were obtained through review of health records or the
79 implementation of questionnaire-styled interviews. General information was
80 documented, including age, gender, underlying chronic conditions, date of symptom
81 onset, date of hospital and ICU admission, and previous history of contact with
82 confirmed patients or potential exposure to 2019-nCoV. The clinical manifestations
83 included fever, cough, fatigue, shortness of breath, etc. Additionally, any ocular
84 symptoms at the very onset, such as conjunctivitis or other ophthalmic manifestations,
85 were solicited from all participants.

86 We took nasopharyngeal swabs and conjunctival swabs from all patients during
87 hospitalization. Further laboratory methods were performed on these swab samples
88 for the detection of 2019-nCoV utilizing real-time RT-PCR assays. The experimental

89 protocols are as follows: RNA extraction from conjunctival swabs and
 90 nasopharyngeal swabs was performed in preparation for subsequent one-step reverse
 91 transcription-coupled PCR reaction using a proprietary master mix containing a DNA
 92 binding dye and thermal cycler. The primers and probes for 2019-nCoV detection
 93 were selected according to the National Pathogen Resource Center (National Institute
 94 for Viral Disease Control and Prevention, China CDC), and the sequences were as
 95 follows: forward primer 5'-CCCTGTGGGTTTACACTTAA-3'; reverse primer
 96 5'-ACGATTGTGCATCAGCTGA-3'; and the probe
 97 5'-FAM-CCGTCTGCGGTATGTGGAAAGGTTATGG-BHQ1-3'. The conditions for
 98 the amplifications were 50°C for 15 min, 95°C for 3 min, followed by 45 cycles of
 99 95°C for 15 s and 60°C for 30 s¹⁸.

100 Results

101 Samples were collected from 67 patients initially suspected to have NCP over a
 102 period of 11 days (17–28 Jan 2020), of whom, 25 were male and 42 were female
 103 (Table 1). The mean age was 35.7 (SD 10.6) years (range 22–78 years). The majority
 104 were healthcare workers and female nurses. By Jan 28, 2020, 63 patients were
 105 identified as laboratory-confirmed NCP and the other four were suspected NCP. Of
 106 the four suspected NCP cases, there were two males and two females. Conjunctival
 107 swab samples from one NCP patient yielded positive PCR results, and swabs from
 108 two NCP patients yielded probable positive PCR results. None of the three patients
 109 had ocular symptoms. The only one NCP patient with conjunctivitis as the first
 110 symptom had negative conjunctival sac 2019-nCoV test. Conjunctival swabs samples

111 from the four suspected NCP cases were negative. The history of one positive case ,
112 two probable positive cases, and one case with the first symptom of the eye were as
113 follows:

114 Patient No. 8 was a male, 58 years old, and an emergency vehicle driver for the
115 pre-hospital emergency team. He was admitted to the hospital because of fever and
116 cough. The chest CT examination showed viral pneumonia. Both the nasopharyngeal
117 and conjunctival swabs tested positive for 2019-nCoV nucleic acid. There has been no
118 ocular discomfort. He had a history of unprotected contact with isolated patients.

119 Patient No. 24 was a male, 78 years old, and had a history of lung cancer with
120 brain metastasis, chronic obstructive pneumonia, and hypertension. He was admitted
121 to hospital for coughing, expectoration, and gasping. The results of 2019-nCoV RNA
122 testing revealed that the nasopharyngeal swab was positive and the conjunctival swab
123 was suspicious positive. There was no ocular discomfort. He had a history of contact
124 with an NCP recessive carrier. The recessive carrier was also diagnosed with NCP and
125 was hospitalized.

126 Patient No. 30 was a female, 29 years old, and pregnant at 36 weeks. She was a
127 physician. She was admitted to the hospital because of fever. The results of the
128 2019-nCoV RNA test revealed that the nasopharyngeal swab was positive and the
129 conjunctival swab was suspicious positive. She had no ocular complaint. She had a
130 history of contact with a confirmed NCP patient.

131 Patient No. 41 was a female, 48 years old, and an anesthesiologist. She was
132 admitted to hospital with fever, cough, redness of eyes, itching, and secretion. The

133 results of 2019-nCoV RNA testing revealed that the nasopharyngeal swab was
134 positive but the conjunctival swab was negative. The anesthesiologist presented with
135 conjunctivitis as the initial symptom. The ocular symptoms were mild. The discomfort
136 of the eye was relieved by itself without medication. She had a history of contact with
137 a confirmed NCP patient wearing a surgical mask but no protective goggles.

138 Discussion

139 Of the cases enrolled in this study, one was positive for the conjunctival sac
140 2019-nCoV test and two cases were suspicious positive. None of these three patients
141 had ocular symptoms. One anesthesiologist presented with conjunctivitis as the first
142 symptom but had a negative conjunctival sac 2019-nCoV test. There have been some
143 reports regarding conjunctival sac and coronavirus, and coronavirus has been isolated
144 from 7-month-old children with conjunctivitis and bronchitis¹⁹. Moreover, SARS
145 coronavirus was detected in the tears of SARS patients, although the detection rate of
146 coronavirus in the conjunctival sac was low²⁰. The results of this study revealed that
147 the incidence of conjunctivitis in patients with NCP is not high. At present, this kind
148 of conjunctivitis has no specific manifestation, and can present in one eye or two eyes.
149 In the early stage, it appears as common conjunctival hyperemia with fewer secretions.
150 It is watery and akin to thin mucus. Occasionally small pieces of conjunctival
151 hemorrhage are seen. The ocular symptoms of the patients were mild and tended to be
152 self-healing. There was great variation between the patients.

153 Our confirmed patient No. 41 was an anesthesiologist. She developed ocular
154 symptoms after performing intubation anesthesia for a patient, followed by fever and

155 cough. The patient was diagnosed with NCP, however during the anesthesia, the
156 anesthesiologist wore only an ordinary surgical mask, hats, and gloves, and did not
157 wear goggles, protective clothing, or other protective devices. Two surgeons (patient
158 No. 10 and patient No. 40) who operated on the NCP patient were later diagnosed
159 with NCP. The two surgeons had no ocular discomfort. General anesthesia involves
160 tracheal intubation and may increase the risk of viral infection. Our confirmed patient
161 No. 30 is a physician. She and her three colleagues (patient No. 28, patient No. 46,
162 and patient No. 65) were all infected from the same patient. The conjunctival swab
163 tests for the six infected doctors (patient No. 41, patient No. 10, patient No. 40,
164 patient No. 28, patient No. 46, and patient No. 65) were all negative, which did not
165 support the spread of the virus through temporary aerosol contact with the
166 conjunctiva.

167 This is a retrospective study of a small sample size, in which there is only one
168 time point. Negative results from conjunctival sac swabs could be influenced by the
169 sampling amount and time, as viral conjunctivitis is self-healing. There was a certain
170 false negative rate in the viral RNA test, and with this knowledge, we will continue to
171 pay close attention to the progress of the patients. Additionally, we should determine
172 whether 2019-nCoV is also present in convalescent patients as well as the infectivity
173 of the conjunctiva.

174 Our clinical observation and analysis can serve as a reminder that all medical
175 workers, not only ophthalmologists, must make solid efforts for protection when
176 treating patients. Specifically, doctors must wear masks, goggles, protective clothing,

177 and gloves. Following contact with patients, close attention must be paid to hand
178 disinfection as well as the disinfection of relevant inspection instruments and the
179 clinical environment. The above measures can serve to cut off transmission channels
180 and prevent cross infection in support of public health and safety.

181 In conclusion, 2019-nCoV can be detected in the conjunctival sac of patients
182 with NCP. Through our clinical analysis, the concept that the virus is transmitted
183 through the conjunctival route it is not supported. The prevention of and treatment for
184 new coronavirus infectious disease requires greater attention and activity. The
185 possibility of eye infection and the ocular route as a potential infection source should
186 be considered and further examined, and scientific protection should be carried out.

187 **Conflict of Interest Statement**

188 The authors declare no conflict of interest.

189 **Acknowledgement**

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241

242 Table 1. Signalment for 67 patients and viral detection results of nasopharyngeal and

243 conjunctival samples.

Patient No.	Sex	Age	2019-nCoV RNA test: nasopharyngeal swab	2019-nCoV RNA test: conjunctival swab
1	M	28	+	-
2	F	37	+	-
3	F	28	+	-
4	M	36	-	-
5	M	57	+	-
6	M	23	+	-
7	F	32	+	-
8	M	58	+	+
9	M	36	+	-
10	M	37	+	-
11	F	35	+	-
12	F	45	+	-
13	F	40	+	-
14	F	25	+	-
15	F	31	+	-
16	F	52	+	-
17	M	31	+	-
18	F	35	-	-
19	F	28	+	-
20	F	60	+	-

21	F	30	+	-
22	F	31	-	-
23	F	26	+	-
24	M	78	+	±
25	F	56	+	-
26	M	30	+	-
27	F	35	+	-
28	F	34	+	-
29	F	44	+	-
30	F	29	+	±
31	F	26	+	-
32	F	32	+	-
33	F	30	+	-
34	F	51	+	-
35	F	22	+	-
36	F	29	+	-
37	F	55	+	-
38	F	34	+	-
39	F	29	+	-
40	M	36	+	-
41	F	48	+	-
42	F	35	+	-

43	M	31	+	-
44	M	38	+	-
45	M	42	+	-
46	M	32	+	-
47	M	31	+	-
48	F	25	+	-
49	F	45	+	-
50	F	22	+	-
51	M	37	+	-
52	M	38	+	-
53	F	48	+	-
54	F	28	+	-
55	M	32	-	-
56	F	28	+	-
57	F	31	+	-
58	F	26	+	-
59	M	28	+	-
60	M	30	+	-
61	F	39	+	-
62	F	27	+	-
63	F	31	+	-
64	M	40	+	-

65	M	30	+	-
66	M	30	+	-
67	M	27	+	-

244 2019-nCoV, 2019 novel coronavirus; F, female; M, male; +, positive result; -, negative result;

245 ±, suspicious positive