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1 **Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-**  
2 **label non-randomized clinical trial**

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24

25

26 *Abstract*

## 27 Background

28 Chloroquine and hydroxychloroquine have been found to be efficient on SARS-CoV-2, and  
29 reported to be efficient in Chinese COV-19 patients. We evaluate the role of  
30 hydroxychloroquine on respiratory viral loads.

## 31 Patients and methods

32 French Confirmed COVID-19 patients were included in a single arm protocol from early  
33 March to March 16<sup>th</sup>, to receive 600mg of hydroxychloroquine daily and their viral load in  
34 nasopharyngeal swabs was tested daily in a hospital setting. Depending on their clinical  
35 presentation, azithromycin was added to the treatment. Untreated patients from another center  
36 and cases refusing the protocol were included as negative controls. Presence and absence of  
37 virus at Day6-post inclusion was considered the end point.

## 38 Results

39 Six patients were asymptomatic, 22 had upper respiratory tract infection symptoms and eight  
40 had lower respiratory tract infection symptoms.

41 Twenty cases were treated in this study and showed a significant reduction of the viral  
42 carriage at D6-post inclusion compared to controls, and much lower average carrying duration  
43 than reported of untreated patients in the literature. Azithromycin added to  
44 hydroxychloroquine was significantly more efficient for virus elimination.

## 45 Conclusion

46 Despite its small sample size our survey shows that hydroxychloroquine treatment is  
47 significantly associated with viral load reduction/disappearance in COVID-19 patients and its  
48 effect is reinforced by azithromycin.

49

50 *Key words:* 2019-nCoV; SARS-CoV-2; COVID-19; hydroxychloroquine; azithromycin;

51 clinical trial

52

## 53        1. Introduction

54        In late December 2019, an outbreak of an emerging disease (COVID-19) due to a novel  
55        coronavirus (named SARS-CoV-2 latter) started in Wuhan, China and rapidly spread in China  
56        and outside [1,2]. The WHO declared the epidemic of COVID-19 as a pandemic on March  
57        12<sup>th</sup> 2020 [3]. According to a recent Chinese stud, about 80% of patients present with mild  
58        disease and the overall case-fatality rate is about 2.3% but reaches 8.0% in patients aged 70 to  
59        79 years and 14.8% in those aged  $\geq 80$  years [4]. However, there is probably an important  
60        number of asymptomatic carriers in the population, and thus the mortality rate is probably  
61        overestimated. France is now facing the COVID-19 wave with more than 4500 cases, as of  
62        March 14<sup>th</sup> 2020 [5]. Thus, there is an urgent need for an effective treatment to treat  
63        symptomatic patients but also to decrease the duration of virus carriage in order to limit the  
64        transmission in the community. Among candidate drugs to treat COVID-19, repositioning of  
65        old drugs for use as antiviral treatment is an interesting strategy because knowledge on safety  
66        profile, side effects, posology and drug interactions are well known [6,7].

67        A recent paper reported an inhibitor effect of remdesivir (a new antiviral drug) and  
68        chloroquine (an old antimalarial drug) on the growth of SARS-CoV-2 *in vitro*, [8] and an  
69        early clinical trial conducted in COVID-19 Chinese patients, showed that chloroquine had a  
70        significant effect, both in terms of clinical outcome and viral clearance, when comparing to  
71        controls groups [9,10]. Chinese experts recommend that patients diagnosed as mild, moderate  
72        and severe cases of COVID-19 pneumonia and without contraindications to chloroquine, be  
73        treated with 500 mg chloroquine twice a day for ten days [11].

74        Hydroxychloroquine (an analogue of chloroquine) has been demonstrated to have an anti-  
75        SARS-CoV activity *in vitro* [12]. Hydroxychloroquine clinical safety profile is better than  
76        that of chloroquine (during long-term use) and allows higher daily dose [13] and has fewer

77 concerns about drug-drug interactions [14]. Our team has a very comprehensive experience in  
78 successfully treating patients with chronic diseases due to intracellular bacteria (Q fever due  
79 to *Coxiella burnetii* and Whipple's disease due to *Tropheryma whipplei*) with long-term  
80 hydroxychloroquine treatment (600 mg/day for 12 to 18 months) since more than 20 years.  
81 [15,16] We therefore started to conduct a clinical trial aiming at assessing the effect of  
82 hydroxychloroquine on SARS-CoV-2-infected patients after approval by the French Ministry  
83 of Health. In this report we describe our early results, focusing on virological data in patients  
84 receiving hydroxychloroquine as compared to a control group.

85

## 86 **2. Study population and Methods**

### 87 *Setting*

88 This ongoing study is coordinated by The Méditerranée Infection University Hospital Institute  
89 in Marseille. Patients who were proposed a treatment with hydroxychloroquine were recruited  
90 and managed in Marseille centre. Controls without hydroxychloroquine treatment were  
91 recruited in Marseille, Nice, Avignon and Briançon centers, all located in South France.

### 92 *Patients*

93 Hospitalized patients with confirmed COVID-19 were included in this study if they fulfilled  
94 two primary criteria: i) age >12 years; ii) PCR documented SARS-CoV-2 carriage in  
95 nasopharyngeal sample at admission whatever their clinical status.

96 Patients were excluded if they had a known allergy to hydroxychloroquine or chloroquine or  
97 had another known contraindication to treatment with the study drug, including retinopathy,  
98 G6PD deficiency and QT prolongation. Breastfeeding and pregnant patients were excluded  
99 based on their declaration and pregnancy test results when required.

100 *Informed consent*

101 Before being included in the study, patients meeting inclusion criteria had to give their consent  
102 to participate to the study. Written informed signed consent was obtained from adult  
103 participants ( $\geq 18$  years) or from parents or legal guardians for minors ( $<18$  years). An  
104 information document that clearly indicates the risks and the benefits associated with the  
105 participation to the study was given to each patient. Patients received information about their  
106 clinical status during care regardless of whether they participate in the study or not. Regarding  
107 patient identification, a study number was assigned sequentially to included participants,  
108 according to the range of patient numbers allocated to each study centre. The study was  
109 conducted in accordance with the International Council for Harmonisation of Technical  
110 Requirements for Pharmaceuticals for Human Use (ICH) guidelines of good clinical practice,  
111 the Helsinki Declaration, and applicable standard operating procedures.

112 The protocol, appendices and any other relevant documentation were submitted to the French  
113 National Agency for Drug Safety (ANSM) (2020-000890-25) and to the French Ethic  
114 Committee (CPP Ile de France) (20.02.28.99113) for reviewing and approved on 5<sup>th</sup> and 6<sup>th</sup>  
115 March, 2020, respectively. This trial is registered with EU Clinical Trials Register, number  
116 2020-000890-25.

117 *Procedure*

118 Patients were seen at baseline for enrolment, initial data collection and treatment at day-0, and  
119 again for daily follow-up during 14 days. Each day, patients received a standardized clinical  
120 examination and when possible, a nasopharyngeal sample was collected. All clinical data were  
121 collected using standardized questionnaires. All patients in Marseille center were proposed oral  
122 hydroxychloroquine sulfate 200 mg, three times per day during ten days (in this preliminary  
123 phase ,we did not enrolled children in the treatment group based in data indicating that children  
124 develop mild symptoms of COVID-19 [4]). Patients who refused the treatment or had an



125 exclusion criteria, served as controls in Marseille centre. Patients in other centers did not receive  
126 hydroxychloroquine and served as controls. Symptomatic treatment and antibiotics as a  
127 measure to prevent bacterial super-infection was provided by investigators based on clinical  
128 judgment. Hydroxychloroquine was provided by the National Pharmacy of France on  
129 nominative demand.

### 130 *Clinical classification*

131 Patients were grouped into three categories: asymptomatic, upper respiratory tract infection  
132 (URTI) when presenting with rhinitis, pharyngitis, or isolated low-grade fever and myalgia, and  
133 lower respiratory tract infections (LRTI) when presenting with symptoms of pneumonia or  
134 bronchitis.

### 135 *PCR assay*

136 SARS-CoV-2 RNA was assessed by real-time reverse transcription-PCR [17].

### 137 *Hydroxychloroquine dosage*

138 Native hydroxychloroquine has been dosed from patients' serum samples by UHPLC-UV using  
139 a previously described protocol [18]. The peak of the chromatogram at 1.05 min of retention  
140 corresponds to hydroxychloroquine metabolite. The serum concentration of this metabolite is  
141 deduced from UV absorption, as for hydroxychloroquine concentration. Considering both  
142 concentrations provides an estimation of initial serum hydroxychloroquine concentration.

143

144

145

### 146 *Culture*

147 For all patients, 500  $\mu$ L of the liquid collected from the nasopharyngeal swab were passed  
148 through 0.22- $\mu$ m pore sized centrifugal filter (Merck millipore, Darmstadt, Germany), then  
149 were inoculated in wells of 96-well culture microplates, of which 4 wells contained Vero E6  
150 cells (ATCC CRL-1586) in Minimum Essential Medium culture medium with 4% fetal calf  
151 serum and 1% glutamine. After centrifugation at 4,000 g, microplates were incubated at 37°C.  
152 Plates were observed daily for evidence of cytopathogenic effect. Presumptive detection of  
153 virus in supernatant was done using SU5000 SEM (Hitachi) then confirmed by specific RT-  
154 PCR.

### 155 *Outcome*

156 The primary endpoint was virological clearance at day-6 post-inclusion. Secondary outcomes  
157 were virological clearance overtime during the study period, clinical follow-up (body  
158 temperature, respiratory rate, long of stay at hospital and mortality), and occurrence of side-  
159 effects.

### 160 *Statistics*

161 Assuming a 50% efficacy of hydroxychloroquine in reducing the viral load at day 7, a 85%  
162 power, a type I error rate of 5% and 10% loss to follow-up, we calculated that a total of 48  
163 COVID-19 patients (ie, 24 cases in the hydroxychloroquine group and 24 in the control group)  
164 would be required for the analysis (Fleiss with CC). Statistical differences were evaluated by  
165 Pearson's chi-square or Fisher's exact tests as categorical variables, as appropriate. Means of  
166 quantitative data were compared using Student's t-test. Analyses were performed in Stata  
167 version 14.2.

168

169 **3. Results** (detailed results are available in supplementary Table 1)

170 *Demographics and clinical presentation*

171 We enrolled 36 out of 42 patients meeting the inclusion criteria in this study that had at least  
172 six days of follow-up at the time of the present analysis. A total of 26 patients received  
173 hydroxychloroquine and 16 were control patients. Six hydroxychloroquine-treated patients  
174 were lost in follow-up during the survey because of early cessation of treatment. Reasons are  
175 as follows: three patients were transferred to intensive care unit, including one transferred on  
176 day2 post-inclusion who was PCR-positive on day1, one transferred on day3 post-inclusion  
177 who was PCR-positive on days1-2 and one transferred on day4 post-inclusion who was PCR-  
178 positive on day1 and day3; one patient died on day3 post inclusion and was PCR-negative on  
179 day2; one patient decided to leave the hospital on day3 post-inclusion and was PCR-negative  
180 on days1-2; finally, one patient stopped the treatment on day3 post-inclusion because of nausea  
181 and was PCR-positive on days1-2-3. The results presented here are therefore those of 36  
182 patients (20 hydroxychloroquine-treated patients and 16 control patients). None of the control  
183 patients was lost in follow-up. Basic demographics and clinical status are presented in Table 1.  
184 Overall, 15 patients were male (41.7%), with a mean age of 45.1 years. The proportion of  
185 asymptomatic patients was 16.7%, that of patients with URTI symptoms was 61.1% and that  
186 of patients with LRTI symptoms was 22.2%). All patients with LRTI symptoms, had confirmed  
187 pneumonia by CTScan. Hydroxychloroquine-treated patients were older than control patients  
188 (51.2 years vs. 37.3 years). No significant difference was observed between  
189 hydroxychloroquine-treated patients and control patients with regard to gender, clinical status  
190 and duration of symptoms prior to inclusion (Table 1). Among hydroxychloroquine-treated  
191 patients six patients received azithromycin (500mg on day1 followed by 250mg per day, the  
192 next four days) to prevent bacterial super-infection under daily electrocardiogram control.  
193 Clinical follow-up and occurrence of side-effects will be described in a further paper at the end  
194 of the trial.

195 *Hydroxychloroquine dosage*

196 Mean hydroxychloroquine serum concentration was  $0.46 \mu\text{g/ml} \pm 0.2$  (N=20).

197 *Effect of hydroxychloroquine on viral load*

198 The proportion of patients that had negative PCR results in nasopharyngeal samples  
199 significantly differed between treated patients and controls at days 3-4-5 and 6 post-inclusion  
200 (Table 2). At day6 post-inclusion, 70% of hydroxychloroquine-treated patients were  
201 virologically cured comparing with 12.5% in the control group ( $p= 0.001$ ).

202 When comparing the effect of hydroxychloroquine treatment as a single drug and the effect of  
203 hydroxychloroquine and azithromyc in combination, the proportion of patients that had  
204 negative PCR results in nasopharyngeal samples was significantly different between the two  
205 groups at days 3-4-5 and 6 post-inclusion (Table 3). At day6 post-inclusion, 100% of patients  
206 treated with hydroxychloroquine and azithromycin combination were virologically cured  
207 comparing with 57.1% in patients treated with hydroxychloroquine only, and 12.5% in the  
208 control group ( $p<0.001$ ). These results are summarized in Figures 1 and 2. Drug effect was  
209 significantly higher in patients with symptoms of URTI and LRTI, as compared to  
210 asymptomatic patients with  $p<0.05$  (data not show).

211 Of note, one patient who was still PCR-positive at day6-post inclusion under  
212 hydroxychloroquine treatment only, received azithromycin in addition to hydroxychloroquine  
213 at day8-post inclusion and cured her infection at day-9 post infection. In contrast, one of the  
214 patients under hydroxychloroquine and azithromycin combination who tested negative at  
215 day6 post-inclusion was tested positive at low titer at day8 post-inclusion.

216 *Cultures*

217 We could isolate SARS-CoV-2 in 19 out of 25 clinical samples from patients.

218

219 **4. Discussion**

220 For ethical reasons and because our first results are so significant and evident we decide to  
221 share our findings with the medical community, given the urgent need for an effective drug  
222 against SARS-CoV-2 in the current pandemic context.

223 We show here that hydroxychloroquine is efficient in clearing viral nasopharyngeal carriage  
224 of SARS-CoV-2 in COVID-19 patients in only three to six days, in most patients. A  
225 significant difference was observed between hydroxychloroquine-treated patients and controls  
226 starting even on day3 post-inclusion. These results are of great importance because a recent  
227 paper has shown that the mean duration of viral shedding in patients suffering from COVID-  
228 19 in China was 20 days (even 37 days for the longest duration) [19]

229 Very recently, a Chinese team published results of a study demonstrating that chloroquine and  
230 hydroxychloroquine inhibit SARS-CoV-2 *in vitro* with hydroxychloroquine  
231 ( $EC_{50}=0.72\% \mu M$ ) found to be more potent than chloroquine ( $EC_{50}=5.47\% \mu M$ ) [14]. These  
232 *in vitro* results corroborate our clinical results. The target values indicated in this paper [14]  
233 were reached in our experiments. The safer dose-dependent toxicity profile of  
234 hydroxychloroquine in humans, compared to that of chloroquine [13] allows using clinical  
235 doses of hydroxychloroquine that will be over its  $EC_{50}$  observed *in vitro* [14].

236 Our preliminary results also suggest a synergistic effect of the combination of  
237 hydroxychloroquine and azithromycin. Azithromycin has been shown to be active *in vitro*  
238 against Zika and Ebola viruses [20-22] and to prevent severe respiratory tract infections when  
239 administrated to patients suffering viral infection [23]. This finding should be further explored  
240 to know whether a combination is more effective especially in severe cases. Speculated  
241 potential risk of severe QT prolongation induced by the association of the two drugs has not

242 been established yet but should be considered. As for each treatment, the cost benefits of the  
243 risk should be evaluated individually. Further studies on this combination are needed, since  
244 such combination may both act as an antiviral therapy against SARS-CoV-2 and prevent  
245 bacterial super-infections.

246 The cause of failure for hydroxychloroquine treatment should be investigated by testing the  
247 isolated SARS-CoV-2 strains of the non-respondents and analyzing their genome, and by  
248 analyzing the host factors that may be associated with the metabolism of hydroxychloroquine.  
249 The existence of hydroxychloroquine failure in two patients (mother and son) is more  
250 suggestive of the last mechanism of resistance.

251 Such results are promising and open the possibility of an international strategy to decision-  
252 makers to fight this emerging viral infection in real-time even if other strategies and research  
253 including vaccine development could be also effective, but only in the future. We therefore  
254 recommend that COVID-19 patients be treated with hydroxychloroquine and azithromycin to  
255 cure their infection and to limit the transmission of the virus to other people in order to curb  
256 the spread of COVID-19 in the world. Further works are also warranted to determine if these  
257 compounds could be useful as chemoprophylaxis to prevent the transmission of the virus,  
258 especially for healthcare workers. Our study has some limitations including a small sample  
259 size, limited long-term outcome follow-up, and dropout of six patients from the study,  
260 however in the current context, we believe that our results should be shared with the scientific  
261 community.

262

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267

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273 **Competing Interests:** N/A

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275

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367 **Titles for figures**

368 **Figure 1.** Percentage of patients with PCR-positive nasopharyngeal samples from inclusion to  
369 day6 post-inclusion in COVID-19 patients treated with hydroxychloroquine and in COVID-  
370 19 control patients.

371 **Figure 2.** Percentage of patients with PCR-positive nasopharyngeal samples from inclusion to  
372 day6 post-inclusion in COVID-19 patients treated with hydroxychloroquine only, in COVID-  
373 19 patients treated with hydroxychloroquine and azithomycin combination, and in COVID-19  
374 control patients.

Table 1 Characteristics of the study population.

	Age (years)			Male gender		Clinical status				Time between onset of symptoms and inclusion (days)		
	Mean $\pm$ SD	t	p-value	n (%)	p-value	Asymptomatic	URTI	LRTI	p-value	Mean $\pm$ SD	t	p-value
Hydroxychloroquine treated patients (N=20)	51.2 $\pm$ 18.7	-1.95	0.06	9 (45.0)	0.65	2 (10.0)	12 (60.0)	6 (30.0)	0.30	4.1 $\pm$ 2.6	-0.15	0.88
Control patients (N=16)	37.3 $\pm$ 24.0			6 (37.5)		4 (25.0)	10 (62.5)	2 (12.5)		3.9 $\pm$ 2.8		
All patients (36)	45.1 $\pm$ 22.0			15 (41.7)		6 (16.7)	22 (61.1)	8 (22.2)		4.0 $\pm$ 2.6		

URTI: upper tract respiratory infection, LRTI: lower tract respiratory infection

Table 2. Proportion of patients with virological cure (negative nasopharyngeal PCR) by day, in COVID-19 patients treated with hydroxychloroquine and in COVID-19 control patients.

	Day3 post inclusion			Day4 post inclusion			Day5 post inclusion			Day6 post inclusion		
	Number of negative patients/total number of patients	%	p-value	Number of negative patients/total number of patients	%	p-value	Number of negative patients/total number of patients	%	p-value	Number of negative patients/total number of patients	%	p-value
Hydroxychloroquine treated patients (N=20)	10/20	50.0	0.005	12/20	60.0	0.04	13/20	65.0	0.006	14/20	70.0	0.001
Control patients (N=16)	1/16	6.3		4/16	25.0		3/16	18.8		2/16	12.5	

<sup>a</sup>control patients from centers other than Marseille did not underwent daily sampling, but were sampled every other day in most cases, they were considered positive for PCR when actually positive the day(s) before and the day(s) after the day(s) with missing data.

Table 3. Proportion of patients with virological cure (negative nasopharyngeal PCR) by day, in COVID-19 patients treated with hydroxychloroquine only, in COVID-19 patients treated with hydroxychloroquine and azithromycin combination, and in COVID-19 control patients.

	Day3 post inclusion			Day4 post inclusion			Day5 post inclusion			Day6 post inclusion		
	Number of negative patients/total number of patients	%	p-value	Number of negative patients/total number of patients	%	p-value	Number of negative patients/total number of patients	%	p-value	Number of negative patients/total number of patients	%	p-value
Control patients	1/16	6.3	0.002	4/16	25.0	0.05	3/16	18.8	0.002	2/16	12.5	<0.001
Hydroxychloroquine treatment only	5/14	35.7		7/14	50.0		7/14	50.0		8/14	57.1	
Hydroxychloroquine and azithromycin combined treatment	5/6	83.3		5/6	83.3		6/6	100		6/6	100	

Supplementary Table 1.

Patient	Age (years)	Sex	Clinical status	Time between onset of symptoms and inclusion (days)	Hydroxychloroquine treatment	Hydroxychloroquine serum concentration µg/ml (day of dosage)	Azithromycin treatment	D0	D1	D2	D3	D4	D5	D6
1	10	M	Asymptomatic	-	No	-	No	31	NEG	NEG	NEG	NEG	NEG	NEG
2	12	F	Asymptomatic	-	No	-	No	26	ND	33	34	NEG	34	NEG
3	14	F	Asymptomatic	-	No	-	No	26	31	23	22	27	NEG	26
4	10	M	Asymptomatic	-	No	-	No	24	NEG	33	33	NEG	NEG	32
5	20	M	URTI	4	No	-	No	24	24	24	27	NEG	31	29
6	65	F	URTI	2	No	-	No	POS	ND	POS	ND	POS	ND	POS
7	46	M	URTI	Unknown	No	-	No	28	ND	ND	ND	26	ND	30
8	69	M	LRTI	2	No	-	No	POS	ND	POS	ND	POS	POS	POS
9	62	F	LRTI	10	No	-	No	POS	ND	POS	ND	POS	ND	POS
10	66	F	URTI	0	No	-	No	POS	ND	POS	ND	ND	ND	POS
11	75	F	URTI	3	No	-	No	POS	ND	POS	ND	POS	ND	ND
12	23	F	URTI	5	No	-	No	ND	ND	POS	ND	POS	ND	ND
13	45	F	URTI	Unknown	No	-	No	POS	ND	POS	ND	POS	ND	POS
14	16	M	URTI	2	No	-	No	POS	ND	POS	ND	ND	POS	ND
15	42	F	URTI	5	No	-	No	ND	ND	ND	POS	ND	POS	ND
16	23	F	URTI	6	No	-	No	POS	ND	ND	ND	ND	POS	ND
17	44	F	URTI	6	Yes	0.519 (D6)	No	30	ND	29	26	32	26	31
18	54	M	Asymptomatic	-	Yes	0.462 (D6)	No	29	NEG	NEG	NEG	NEG	NEG	NEG
19	25	M	URTI	3	Yes	0.419 (D6)	No	23	25	28	25	NEG	NEG	NEG
20	59	F	Asymptomatic	-	Yes	0.288 (D4)	No	30	NEG	NEG	NEG	NEG	NEG	NEG
21	49	F	URTI	1	Yes	0.621 (D6)	No	34	27	19	16	34	24	22
22	24	F	URTI	10	Yes	0.723 (D6)	No	28	NEG	32	34	NEG	NEG	NEG
23	81	F	LRTI	2	Yes	0.591 (D6)	No	22	21	30	NEG	32	28	NEG
24	85	F	LRTI	1	Yes	0.619 (D6)	No	17	21	23	21	26	24	24
25	40	M	URTI	3	Yes	0.418 (D6)	No	22	ND	28	21	15	20	17
26	53	M	URTI	5	Yes	0.515 (D6)	No	27	28	32	31	NEG	NEG	NEG
27	63	F	URTI	8	Yes	0.319 (D4)	No	34	NEG	30	NEG	NEG	NEG	NEG
28	42	F	URTI	1	Yes	0.453 (D6)	No	19	16	17	17	19	20	31
29	87	F	URTI	5	Yes	0.557 (D6)	No	25	30	NEG	NEG	NEG	ND	ND
30	33	M	URTI	2	Yes	0.194 (D2)	No	15	23	26	26	NF	32	32
31	53	F	LTRI	7	Yes	1.076 (D6)	Yes	28	31	34	NEG	34	NEG	NEG
32	48	M	URTI	2	Yes	0.57 (D6)	Yes	23	29	29	NEG	NEG	NEG	NEG
33	50	F	LRTI	5	Yes	0.827 (D6)	Yes	30	27	NEG	NEG	NEG	NEG	NEG
34	20	M	URTI	2	Yes	0.381 (D6)	Yes	27	31	29	NEG	NEG	NEG	NEG
35	54	M	LRTI	6	Yes	0.366 (D4)	Yes	24	ND	ND	29	NEG	NEG	NEG
36	60	M	LRTI	4	Yes	0.319 (D4)	Yes	29	31	31	NEG	NEG	NEG	NEG

URTI: upper tract respiratory infection, LRTI: lower tract respiratory infection, POS: positive PCR, NEG: negative PCR (CT value  $\geq 35$ ), ND: PCR not done





