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Supplementary appendix

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Supplement to: Wu Y, Guo C, Tang L, et al. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *Lancet Gastroenterol Hepatol* 2020; published online March 19. [https://doi.org/10.1016/S2468-1253\(20\)30083-2](https://doi.org/10.1016/S2468-1253(20)30083-2).

Supplementary Appendix

Methods:

In the study, respiratory and fecal specimens were collected every 1–2 days depending on its availability until two sequential negative results were shown. Patients (41 out of 74, 55.4%) were defined as fecal sample positive because the SARS-CoV-2 viral RNA was detected by real-time PCR for at least one time. The diagnosis of real-time RT-PCR were performed in both our hospital and the local CDC. The majority of Real-time RT-PCR results shown in this study were performed in our hospital, while respiratory tract samples of suspected cases were required to be further tested in local CDC for official confirmation. The real-time RT-PCR was only performed to feces specimens after Feb 2nd in our hospital, so we don't know the status of SARS-CoV-2 viral RNA in patient before Feb 2nd for most patients.

In the Fifth Affiliated Hospital of Sun Yat-Sen University, extraction of nucleic acids from the respiratory and fecal samples was performed with the commercialized nucleic acid extraction kits (QIAamp Viral RNA Mini Kit, Catalog #: 52904, QIAGEN). The Real-time RT-PCR performed in The Fifth Affiliated Hospital of Sun Yat-Sen University was using the Novel Coronavirus (2019-nCoV) Real Time RT-PCR kit from LifeRiver Ltd. (Catalog #: RR-0479-02). Nucleocapsid gene (*N*), membrane gene (*E*) and RNA dependent RNA polymerase gene (*RdRp*) were the three targeted genes simultaneously amplified and tested. Detailed protocol is described at the website (1). The diagnosis of COVID-19 pneumonia performed in local CDC was based on the New Coronavirus Pneumonia Prevention and Control Program (6th edition) published by the National Health Commission of China (2). All reagents and protocols used are CFDA approved.

The numerical values in this study are presented as mean (\pm SD) for continuous variables, and the proportion of categorical variables are presented in number (n/N, %). Statistical analyses including Chi-square test, two-way Wilcox test and correlation analysis were performed using Prism 6.1 (GraphPad Software).

Reference:

1. http://www.liferiver.com.cn/productinfor/p16_400.html?&pageid=16&_id=400&r=0
2. <http://www.nhc.gov.cn/jkj/s3577/202003/4856d5b0458141fa9f376853224d41d7.shtml>

Tables:

Table S1. Clinical characteristics of patients with SARS-CoV-2 infection in this study.

	TS (+)/ FS (+) n=41	TS (+)/ FS (-) n=33	<i>P</i> -value	FS (+)≥14 days n=19	FS (+)<14 days n=22	<i>P</i> -value
Gender (F/M)	24/17	11/22	0.037*	13/6	11/11	0.35
Age	41.29±3.14	46.21±2.64	0.59	36.68±4.48	45.27±4.29	0.18
Severe	11(26.8%)	7(21.2%)	0.60	1(5.3%)	10(45.5%)	0.0048**
FS(+) lasting days	11.15±9.60	-	-	21.26±4.94	2.91±3.15	<0.0001***
Symptom						
Cough	24(58.5%)	13(39.4%)	0.16	5(26.3%)	12(54.5%)	0.17
Fever	27(65.9%)	18(54.5%)	0.35	11(57.9%)	16(72.7%)	0.51
Dyspnea	8(19.5%)	1(3.0%)	0.037*	3(15.8%)	5(22.7%)	1.0
Snivel	6(14.6%)	0(0%)	0.030*	4(21.1%)	2(9.1%)	0.39
Sore throat	6(14.6%)	0(0%)	0.030*	3(15.8%)	3(13.6%)	1.0
Diarrhea/Vomit/Stomach ache	11(26.8%)	12(36.4%)	0.45	7(36.8%)	4(18.2%)	0.29
Underlying disease						
Diabetes	3(7.3%)	3(9.1%)	1.0	1(5.3%)	2(9.1%)	1.0
Hypertension	5(12.2%)	7(21.2%)	0.35	3(15.8%)	2(9.1%)	1.0
Tumors	1(2.4%)	3(9.1%)	0.32	1(5.3%)	0(0%)	0.48
Bronchitis	0(0%)	1(3.0%)	0.45	0(0%)	0(0%)	1.0
Tuberculosis	1(2.4%)	0(0%)	1.0	0(0%)	1(4.5%)	1.0
Treatment						
Antibiotic	30(73%)	22(67%)	0.61	12(63%)	18(82%)	0.29
Antivirus	36(88%)	21(64%)	0.025*	17(89%)	19(86%)	1.0
Interferon	5(12%)	6(18%)	0.51	1(5%)	4(18.2%)	0.35
Globulin	23(56%)	17(52%)	0.82	7(37%)	16(73%)	0.030*
TCM	12(29%)	8(24%)	0.79	4(57%)	8(36%)	0.32
Chloroquine	25(61%)	20(61%)	1.0	12(63%)	13(59%)	1.0

The patients positive for viral RNA (n=41) were further divided into two groups: “FS(+) \geq 14 group” (patient1 - patient19) in which patient’s feces was positive for viral RNA \geq 14 days after respiratory swabs tested negative for viral RNA, and “FS(+) <14 group”(patient20 - patient41) in which patient’s feces was positive for viral RNA <14 days after respiratory swabs tested negative for viral RNA. 14 days cutoff time was used to create two subgroups because 14 days is the recommended time for self-quarantine at home after being discharged from the hospital according to the New Coronavirus Pneumonia Prevention and Control Program (6th edition) by the National Health Commission of China.

The numeric values (e.g., Age) in the table presents the Mean \pm SD value for continuous variables. The proportion of categorical variables in the table are presented in the form of n (n/N in %) for the number of identified number and the percentage in that group respectively for Gender, Symptom, Underlying disease and Treatment. The P-values were calculated separately for comparing between TS(+)/FS(+) group (patients with stool specimens SARS-CoV-2 viral RNA positive) or TS(+)/FS(-) group (patients with stool specimens SARS-CoV-2 viral RNA negative), and between “FS(+) <14 group” and “FS(+) \geq 14 group” using either Chi-square test or Wilcox-test. The number in bold indicates statistical significance. TS: throat swab sample; FS: fecal samples; F: Female; M: Male; FS(+) lasting days: Fecal sample viral RNA positive lasting days; TCM: Traditional Chinese Medicine. The significance of two group was analyzed by Wilcox tests or Chi-square test. *, P<0.05; **, P<0.01; ***, P<0.001.

Table S2. Summary of the fecal SARS-CoV-2 viral RNA C_t value in patients who were detected with fecal viral RNA positive by real-time RT-PCR.

	Patient	TS(+) (days)	FS(+) (days)	TS(-))/FS(+) (days)	<i>RdRp</i>	<i>N</i>	<i>E</i>
FS(+) \geq 14 group	1	9	42	33	28.564	36.117	25.578
	2	-	-	26	12.242	11.262	12.896
	3	18	39	21	39.103	-	36.815
	4	29	47	17	39.258	-	31.368
	5	12	36	24	14.344	29.825	14.893
	6	8	33	25	13.745	4.515	15.006
	7	20	40	20	30.440	28.210	26.610
	8	21	43	22	29.649	-	26.552
	9	14	38	24	28.173	37.347	24.390
	10	17	36	19	29.030	27.130	25.550
	11	11	28	17	32.522	-	28.759
	12	10	26	16	32.730	33.670	31.150
	13	12	33	21	32.451	-	28.674
	14	17	37	20	27.923	32.850	25.146
	15	17	48	31	34.017	-	31.215
	16	27	43	16	34.250	31.850	30.210
	17	-	-	19	38.394	-	39.700
	18	14	32	18	33.820	35.744	32.957
	19	-	-	15	17.944	25.853	25.699
	Mean \pm SD	16.00 \pm 6.07	37.56 \pm 6.26	21.26 \pm 4.94	28.42 \pm 8.46	27.33 \pm 10.07	26.47 \pm 6.95
FS(+) $<$ 14 group	20	31	31	0	14.689	29.641	17.127
	21	8	8	6	28.394	-	26.892
	22	-	-	2	32.531	-	34.713
	23	-	-	2	30.147	36.117	27.815
	24	15	17	6	33.128	-	29.476
	25	20	26	9	14.891	4.653	20.526
	26	24	33	6	35.607	-	31.877
	27	13	19	8	32.256	-	32.095
	28	15	23	5	39.070	-	-
	29	13	18	3	36.090	37.711	33.111
	30	14	17	5	32.479	8.308	30.301
	31	4	9	8	30.466	32.457	30.717
	32	-	-	0	38.139	-	-
	33	24	24	0	36.532	-	-
	34	22	22	0	36.481	-	32.482
	35	30	30	0	39.503	-	34.459
	36	18	18	0	34.181	-	33.256
	37	15	15	0	36.280	-	35.888
	38	17	17	0	11.648	-	12.605
	39	8	8	1	30.468	-	31.100
	40	-	-	3	36.143	-	36.027
	41	22	25	0	35.747	-	36.276
	Mean \pm SD	17.39 \pm 7.29	20.00 \pm 7.48	2.91 \pm 3.15	31.59 \pm 7.85	24.81 \pm 14.52	29.83 \pm 6.50
Total (n=41)	Mean \pm SD	16.74 \pm 6.68	28.26 \pm 11.22	11.15 \pm 9.60	30.33 \pm 8.12	26.85 \pm 11.42	28.42 \pm 6.79

The C_t values of the three targeted genes of the first detectable fecal sample in 43 patients were recorded and compared here. Interestingly, the average C_t values of all three targeted genes were lower in “FS(+) \geq 14 group” (RdRP/N/E: 28.37 \pm 9.44/26.87 \pm 10.63/26.89 \pm 8.7) comparing to “FS(+) $<$ 14 group” (RdRP/N/E: 32.23 \pm 2.69/28.04 \pm 13.34/30.29 \pm 1.925), while their C_t values did not reach statistical significance in our study (Wilcox-test p-values for *RdRp* gene, *N* gene and *E* gene were 0.479, 0.454 and 0.15 respectively). TS(+): days of throat swab positive since

symptom onset ; FS(+): days of fecal sample positive since symptom onset; TS(-)/FS(+) : days of fecal sample positive after throat sample turned negative.

Discussion: In many patients with feces positive for viral RNA, ACE2 receptors were found in higher abundance in gastrointestinal system when compared to patients with feces negative for viral RNA. ACE2 receptors are prerequisite for SARS-CoV-2 infection and potential viral shedding ¹. Our data suggest that patients no positivity for fecal viral RNA may be attributed to relative low expression of ACE2 in their intestinal tract, which needs to be experimental validated. Gut microbiome can also play an important role in regulating the viral transmission in the intestinal tract while its composition is individual dependent ².

Reference:

- 1 Xiao F, Tang M, Zheng X, Liu Y, Li X, Shan H. Evidence for gastrointestinal infection of SARS-CoV-2. *Gastroenterology* 2020; published online March 3. DOI:10.1053/j.gastro.2020.02.055.
- 2 Lima MT, Andrade AC dos SP, Oliveira GP, *et al.* Virus and microbiota relationships in humans and other mammals: An evolutionary view. *Hum Microbiome J* 2019; **11**: 100050.

Table S3. The summary of symptoms and medical treatments for all enrolled patients in the study.

Patient	Symptom onset	Admitted to hospital	Discharge from hospital	Cough	Fever	Dyspnea	Snivel	Sore throat	Diarrhea	Antibiotics	Antivirus	Interferon	TCM	Globulin	Chloroquine
1	1.28	1.31	3.14	1.28	-	-	-	-	-	-	-	-	-	-	-
2	-	1.30	3.6	-	-	-	-	-	-	-	2.12-3.1	-	2.22-2.25	-	-
3	1.24	1.30	3.6	-	-	1.24	-	-	2.1	1.31-2.6	1.31-2.6 / 2.26-3.2	-	-	1.31-2.1	2.6-2.15
4	1.1	2.1	3.5	1.1	-	1.31	-	-	-	2.1-2.5	2.1-2.5 / 2.26-3.5	-	2.1-2.4	2.4-2.7	2.6-2.10
5	1.25	1.27	3.1	-	1.25	-	-	-	1.31	-	1.27-29 / 2.27-3.1	-	-	-	1.29-2.8
6	1.29	1.30	3.5	1.29	2.3	-	-	-	1.31	1.30-2.6	2.27-3.5	-	-	2.5-2.9	1.31-2.9
7	1.23	1.30	3.5	1.23	-	-	-	1.30	-	2.1-2.5	1.30-2.4 / 2.27-3.5	-	-	-	2.6-2.14
8	1.22	1.28	3.10	1.22	1.26	-	1.22	-	-	1.28-2.11	1.28-2.1 / 2.19-3.2	-	-	1.28-2.12	-
9	1.24	1.27	3.1	1.24	1.24	-	1.24	-	-	-	2.19-3.1	-	-	1.28-2.11	-
10	1.28	1.30	3.14	1.28	1.28	-	1.28	1.28	2.2	1.30-2.21	1.30 / 2.1 / 2.5-2.7	-	-	2.10-2.21	-
11	1.26	1.27	2.27	1.26	-	-	-	-	-	1.27-2.11	1.27-2.11 / 2.24	-	-	-	1.29-1.30
12	1.31	2.2	2.27	1.30	1.30	-	-	-	-	2.2-2.13	2.2-2.6	-	-	-	2.6-2.12
13	1.28	1.30	3.1	1.30-1.31	-	-	-	1.28	1.31	-	2.27-3.1	-	-	-	1.30-2.11
14	1.28	1.29	3.1	-	1.28	-	-	-	2.19	1.30-2.8	1.30-2.6	-	-	-	2.6-2.13
15	1.23	1.24	3.15	12.24	1.23	-	-	-	-	1.24-28 / 2.3-2.10	1.28-2.6	-	-	2.3-2.20	2.6-2.12
16	1.24	1.26	3.1	1.24	1.25	-	1.25	-	1.29	-	1.26-1.27 / 2.10-2.12	1.26-1.28 / 2.8-2.22	1.26-1.27	-	-
17	-	2.13	3.5	-	2.13	-	-	-	-	2.13	2.13 / 2.28-3.5	-	-	-	2.13-2.18
18	2.2	2.3	3.6	-	2.2	-	-	-	-	2.3	2.3-2.6 / 2.27	-	2.3	-	2.6-2.18
19	-	2.14	3.6	-	-	-	-	-	-	-	2.29-3.1	-	-	-	2.14-2.19
20	1.17	1.17	2.18(Death)	1.11	1.17	1.18	-	-	-	1.7-1.28 / 2.12-2.18	1.17-1.23	-	-	1.17-20 / 2.7-2.13	-
21	2.4	2.5	3.13	-	2.5	-	-	-	-	2.5-2.28	2.6	-	2.5-2.6	2.5-2.19	-

														/ 2.24-2.25	
22	-	2.8	3.3	-	-	-	-	-	-	-	2.20-3.3	-	-	-	2.8-2.18
23	-	2.6	2.25	-	-	-	-	-	-	-	-	2.6-2.12	-	-	-
24	1.22	1.27	2.14	1.24	-	-	-	1.22	1.29	1.27-2.12	1.28-1.29	-	-	-	1.29-2.7
25	1.20	1.25	2.28	1.20	1.20	-	-	-	-	1.29-2.7	1.26-2.3	-	-	1.30-2.5	-
26	1.17	1.29	2.17	1.18	1.17	-	-	-	-	-	1.29-31 / 2.2-2.12	-	-	-	-
27	1.20	1.23	2.10	-	1.20	1.24	-	-	-	1.24-2.10	1.24-1.30	1.25-27	-	1.26-1.28	-
28	1.27	1.31	2.18	-	-	-	1.21	-	-	1.31-2.4	1.31-2.6	-	-	1.31-2.1	2.6-12
29	1.21	1.22	2.10	-	1.21	-	-	-	1.27	1.23-27 / 2.2--2.10	1.22-2.9	-	-	2.1	-
30	1.24	1.31	2.14	-	1.24	-	1.24	1.24	2.1	1.31-2.6	1.31-2.6	-	1.31-2.4	2.3-2.11	2.6-2.10
31	2.14	2.17	2.28	-	-	-	-	-	-	2.18-28	2.23-2.28	-	-	-	-
32	-	1.31	2.18	-	1.18	-	-	-	-	2.1-2.20	2.1-2.6	-	-	1.31-2.20	2.6-2.20
33	1.29	2.1	2.28	-	1.29	2.9	-	-	-	2.1-2.22	2.1-2.8 / 2.16-17	-	2.1-2.3	2.10-2.20	2.6
34	1.25	2.4	2.25	1.25	2.11	2.7	-	-	-	2.4-2.12	2.4-2.6	-	2.4-2.5	2.4-2.21	2.6-2.7
35	1.29	1.30	2.25	1.29	1.29	-	-	-	1.31	1.30-2.21	1.30-2.20	-	-	1.30-2.20	-
36	1.28	1.29	2.21	1.28	1.28	1.28	2.6	-	2.4	1.31-2.12	1.29-2.6	-	1.29-1.31	1.29-2.18	2.6-2.7
37	1.24	1.28	2.21	1.24	1.26	-	-	-	1.30	1.29-2.18	1.29-2.6	-	1.28	1.29-2.3	2.6-2.15
38	1.18	1.18	2.5	1.12	1.17	-	-	1.18	-	1.18-2.5	-	1.20-1.26	-	1.22-1.29	1.18-1.22
39	1.23	1.28	2.14	-	1.24	-	-	1.24	2.4	1.28-2.3	1.28-2.11	-	-	1.28-2.3	-
40	-	1.31	2.14	-	-	-	-	-	-	-	2.1-2.6 / 2.5-6	-	-	-	2.6-2.12
41	1.23	1.25	2.23	1.23	1.23	-	-	-	-	1.25-1.26 / 1.29-2.6	1.26-2.6	1.26	-	1.26-2.13	2.6-2.13
42	-	2.14	2.15	-	-	-	-	-	-	-	-	-	-	-	2.14-2.22
43	2.6	2.5	2.4	-	-	-	-	-	2.14	2.6-2.26	-	-	-	2.5-2.26	-
44	1.29	2.1	2.2	1.29	1.31	-	-	-	-	2.1-3 / 2.3-6	2.1-2.2 / 2.2-6	-	2.1-2.24	2.2-2.12	2.6-2.16
45	1.30	2.2	2.4	-	1.31	-	-	-	1.31	2.2-2.6	2.2-2.6 / 2.15-17	-	-	-	2.6-2.14
46	1.31	2.2	2.4	-	1.30	-	-	-	2.4	2.2-2.3 / 2.3-2.11	2.2-2.14	-	2.2-2.3	2.3-2.14	-
47	1.26	2.11	2.12	-	1.27	-	-	-	1.27	2.12-2.22	-	-	-	2.12-2.24	2.13-2.19

48	-	2.14	2.13	-	-	-	-	-	-	3.1-3.4	-	-	-	-	2.14-2.19
49	2.3	2.5	2.5	-	-	-	-	-	2.3	-	-	-	-	-	2.5-2.7
50	2.12	2.15	2.15	2.12	-	-	-	-	2.14	2.15-2.29	-	-	-	2.15-2.27	-
51	1.25	2.6	2.7	-	-	-	-	-	2.8	-	2.12-2.18	-	-	-	2.6-2.10
52	1.29	1.30	1.30	-	1.29	-	-	-	2.2	1.30-2.1	2.1-2.3 / 2.3-2.6	-	-	-	2.6-2.9
53	1.31	2.3	2.3	1.31	1.31	-	-	-	-	2.3-2.23	2.3-2.6	-	2.3-2.5	2.3-2.27	2.6-2.7
54	1.21	1.25	1.27	1.21	1.21	-	-	-	1.29	1.25-2.11	1.27-2.1	-	-	1.27-1.28 / 2.3-2.6	-
55	1.25	1.26	1.30	1.25	1.25	-	-	-	-	2.3-2.7	1.27-2.8	-	-	2.5-7	2.7-2.8
56	1.27	2.5	2.6	1.27	-	-	-	-	-	-	2.5-2.6 / 2.15-2.28	-	-	2.6-2.12	2.6-2.15
57	1.24	1.29	1.31	1.24	1.24	-	-	-	-	1.30-2.2	2.1-2.8	-	-	-	-
58	1.22	1.27	1.28	1.22	1.22	-	-	-	-	1.27-2.6	1.28-29	-	-	-	1.29-1.31
59	1.26	1.27	1.30	1.26	1.26	-	-	-	-	1.27-2.12	1.27-1.29	1.28-1.29	-	2.6-2.13	1.31-2.9
60	1.24	1.25	1.26	-	1.24	-	-	-	-	1.25-2.10	1.25-1.31	1.26	-	1.26-2.9	-
61	1.26	1.28	1.29	-	1.26	-	-	-	-	1.29-2.9	1.28-29	-	-	2.5-2.9	1.30-2.8
62	1.25	2.5	2.5	-	1.25	-	-	-	-	-	-	-	-	-	2.5-2.14
63	-	2.13	2.12	-	-	-	-	-	-	2.13	2.13	-	-	-	2.13-2.22
64	1.31	2.5	2.5	1.31	-	-	1.31	-	-	-	2.22-26	-	2.23-2.27	-	2.5-2.12
65	1.22	1.25	1.28	1.22	1.22	-	-	-	-	1.25-2.10	1.25-2.10	1.26-1.27	-	2.5-2.10	-
66	1.31	2.2	2.3	-	-	-	-	-	-	-	-	-	-	-	2.16-2.21
67	2.14	2.15	2.14	-	-	-	-	-	2.12	-	-	-	-	-	2.9-2.14
68	1.25	2.8	2.9	1.23	1.29	2.6	-	-	-	2.2-2.11	2.2-2.6	-	-	2.4-2.6	-
69	1.20	2.2	2.3	-	-	-	-	-	1.29	2.3-2.12	1.31-2.7	-	-	1.31-2.11	-
70	1.29	1.30	1.31	-	-	-	-	-	-	-	-	2.25	2.25-2.27	-	-
71	-	2.25	2.24	-	-	-	-	-	-	-	-	-	2.28-3.1	-	-
72	-	2.26	2.25	-	-	-	-	-	-	-	-	-	2.15-2.21	-	2.15-2.20
73	1.13	1.19	1.21	-	1.31	-	-	-	-	2.2-2.3	2.2-2.3	-	2.2-2.3	-	-
74	-	2.15	2.14	1.13	1.20	-	-	-	1.25	1.19-2.5	1.19-1.21	1.22-1.26	-	1.20-2.3	-

Total of 74 patient enrolled in the study were summarized in the table with information about the first date of symptom onset, date of admission to the hospital and the date of discharge from the hospital; the first day of typical symptoms shown; and the period of medical treatments. Antibiotic treatment includes medicine usage of Levofloxacin, Moxifloxacin, Cefprozil or Ceftriaxone. Antivirus treatment includes medicine usage of Lopinavir, Ritonavir, Umifenovir or Oseltamivir. The hyphen sign in the cell indicates the information is not applicable.

Contributors

YW, CG, LT, HS, GJ and XH had roles in the study design, clinical management, patient recruitment, data collection, data analysis, data interpretation, literature search, and writing of the manuscript. ZH, JZ, XD and HY had roles in the experiments, data collection, data analysis, and data interpretation. QX, YT, XQ, LK, XF, NM, and JL had roles in recruitment, data collection, and clinical management. All authors reviewed and approved the final version of the manuscript.