**Virologica Sinica**

**Supplementary Data**

**Characterization of two SARS-CoV-2 subgenomic RNA dynamics in severe COVID-19 patients**

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**Supplementary Fig S1** Scatterplots of the orf7a subgenomic RNA load in the three sample types with different duration of illness (n = 88) (**A**). Spearman’s rank correlation coefficient (r) and *P* value was attached for each sample type. Sample types were indicated with different colors. Generalized estimating equations were used to fit the dynamics of sgRNA load. **B** Violin plot of orf7a subgenomic RNA load versus the duration of treatment (days). Mann-Whitney U test showed no difference between cohorts with different days after treatment.

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**Supplementary Fig. S2** E sgRNA dynamic on the individual level (n = 14) through the illness duration. Each patient included 3–4 timepoints data and labeled with different color. Throat swabs (n = 12) and gut swabs (n = 3) were labeled with circle and asterisk, respectively. No significant difference was detected.

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**Supplementary Fig. S3** Comparison of the orf7a subgenomic RNA load between patients with and without four comorbidities 1–5 days after treatment (n = 88). Mann-Whitney U test showed no statistical significance between patients with and without such diseases.

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**Supplementary Fig. S4** Comparison of antibody titer (at day 10) against SARS-CoV-2 N, RBD and S protein between patients with positive and negative E sgRNA in the throat swab at day 1 (n = 18), which showed no significant difference between them using the *t*-test.

**Supplementary Table S1** Primer and probe sequence to quantify genomic E and subgenomic E and orf7a.

|  |  |
| --- | --- |
|  | Sequence |
| Genomic E |  |
| Forward primer | ACAGGTACGTTAATAGTTAATAGCGT |
| Probe | ACACTAGCCATCCTTACTGCGCTTCG |
| Reverse primer | ATATTGCAGCAGTACGCACACA |
| Subgenomic E | 　 |
| Forward primer | CGATCTCTTGTAGATCTGTTCTC |
| Probe | ACGAACTTATGTACTCATTCGTTTCGGAAG |
| Reverse primer | CAGTAAGGATGGCTAGTGTAACT |
| Subgenomic orf7a | 　 |
| Forward primer | TCGATCTCTTGTAGATCTGTTCTC |
| Probe | TTGGCACTGATAACACTCGCTACTTGT |
| Reverse primer | ACCTCTAACACACTCTTGGTAGT |

**Supplementary Table S2** Comparison of the number and frequency (%) of specimens that were positive for orf7a sgRNA with different duration and clinical outcomes.

|  |  |
| --- | --- |
|  | Subgenomic orf7a |
| Days after treatment | Standard Care | Lopinavir-Ritonavir | *P*\* |
| 1 | 17/24 (70.8) | 23/26 (88.5) | 0.22 |
| ≥ 5# | 9/15 (60.0) | 19/23 (82.6) | 0.24 |
|  | Outcome |
|  | Death | Recorvey | *P*\* |
| 1 | 20/21 (95.2) | 20/29 (69.0) | 0.05 |
| ≥ 5# | 11/13 (84.6) | 17/25 (68.0) | 0.71 |

\*Chi-square test.
#groups with days after treatment ≥ 5 were merged to one group for limited samples

**Supplementary Table S3** Meta data of the samples and patients involved on this study.

**Supplementary Table S4** Subgenomic E titer in the throat swabs and antibody raised 10 days later.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Patient\_id | N\_IgM | N\_IgG | RBD\_IgM | RBD\_IgG | S\_IgM | S\_IgG | Log2\_Subgenomic E (Day 1) | Log2\_Genomic E(Day1) |
| 6 | 0.1485 | 1.319 | 0.67175 | 1.07755 | 0.68825 | 1.0035 | 4 | 7.88 |
| 12 | 0.2572 | 0.83905 | 0.40295 | 0.825 | 0.66175 | 0.95915 | 2.68 | 8.8 |
| 18 | 0.48745 | 0.78825 | 0.3402 | 1.05535 | 0.3515 | 0.93375 | 0 | 5.13 |
| 19 | 0.60555 | 0.6908 | 1.04865 | 1.73565 | 0.77365 | 1.01995 | 0 | 6.3 |
| 29 | 0.9708 | 0.73165 | 0.23485 | 0.8824 | 0.26935 | 0.9962 | 7.64 | 11.96 |
| 32 | 0.2063 | 0.79195 | 0.30885 | 1.06655 | 0.28695 | 1.14095 | 2.58 | 7.22 |
| 36 | 0.44665 | 0.74825 | 0.7404 | 0.8119 | 1.0086 | 0.83845 | 3.46 | 7.96 |
| 38 | 0.20905 | 0.7622 | 0.7238 | 0.6746 | 1.15895 | 0.9532 | 0 | 2.26 |
| 40 | 0.7361 | 0.71375 | 0.2909 | 1.67475 | 0.8171 | 0.9952 | 0 | 2.42 |
| 44 | 0.21485 | 0.7358 | 0.31535 | 1.0903 | 0.1876 | 1.0849 | 0 | 0 |
| 48 | 0.7146 | 0.80705 | 0.4332 | 0.9924 | 0.5146 | 1.07105 | 0 | 4.27 |
| 50 | 0.35005 | 0.69255 | 0.47945 | 0.89605 | 0.28705 | 1.1686 | 1.85 | 8.56 |
| 68 | 0.19015 | 0.7489 | 0.64045 | 0.9423 | 0.3718 | 0.9677 | 4.25 | 10.65 |
| 70 | 0.19745 | 0.7404 | 0.49265 | 0.7491 | 0.2426 | 0.9731 | 0 | 6.5 |
| 75 | 0.30875 | 1.00245 | 0.23995 | 1.33385 | 0.50745 | 1.2094 | 0 | 5.22 |
| 86 | 0.2811 | 0.8092 | 0.281 | 0.80645 | 0.4343 | 1.1453 | 0 | 5.75 |
| 90 | 0.44375 | 0.73895 | 0.2719 | 0.7527 | 0.3323 | 0.94595 | 0 | 2.43 |
| 112 | 0.09495 | 0.3723 | 0.1344 | 0.43755 | 0.14705 | 0.8093 | 0 | 7.08 |