Supplementary Methods

Real-time Reverse Transcription Polymerase Chain Reaction (RT-qPCR)

Oro/nasopharyngeal swabs were collected and submitted to RNA extraction followed by real-time reverse transcription-PCR (RT-qPCR) testing for two genes of the nucleocapsid protein (N1 and N2) of the SARS-CoV-2 as described by the Center for Disease Control and Prevention.[1]

Definitions and other prespecified secondary outcomes

The institutional protocol recommends the use of non-invasive ventilation or high-flow nasal cannula for patients with peripheral oxygen saturation <93% with low-flow oxygen supplementation >5L/minute, and with partial pressure of arterial oxygen/ fraction of inspired oxygen ratio (PaO2/FiO2) of 200-300 or >24 respiratory movements/minute.

Evolution in an ordinal clinical scale during the first 28 days was assessed by the proportion of patients in each category of an ordinal scale during the first 28 days (1, patient not hospitalized; 2, hospitalized and not receiving supplemental oxygen; 3, hospitalized and receiving supplemental oxygen; 4, hospitalized and receiving oxygen supplementation administered by non-invasive ventilation or high flow nasal cannula; 5, hospitalized and receiving mechanical ventilation or extra-corporeal membrane oxygenation; and 6, death). Arterial partial pressure of oxygen/ fraction of inspired oxygen ratio (PaO2/FiO2 ratio during hospitalization (see Supplemental Material), evaluated as the proportion of patients assigned in an ordinal scale according to this ratio (400-301, 300-201, 200-101, ≤100). For PaO2/FiO2 evaluation the worst value of the day was considered. Peripheral oxygen saturation (SpO2)/Fio2 corrected for positive end expiratory pressure was used for days in which PaO2/FiO2 was not available.[2]

Other outcomes were days alive and free of supplemental oxygen support, defined as the number of days in which patients are alive and not receiving supplemental oxygen (non-survivors were assigned as 0 free-days); need of admission at an intensive care unit (ICU); occurrence of

documented deep venous thrombosis or pulmonary embolism; need of renal replacement therapy (RRT); need of prone positioning; and in-hospital mortality.

A set of laboratory parameters was also evaluated during the hospitalization, including C reactive protein, creatinine, D-dimers, leukocytes (total count and differential cell counts) and platelets.

Whole Genome Sequencing

All RT-qPCR positive clinical samples from patients attending at HCPA were stored at an institutional biobank. Sequencing libraries were prepared using the CleanPlex SARS-CoV-2 panel (Paragon Genomics. Hayward. United States) protocol for target enrichment and library preparation, following manufacturer instructions (https://www.paragongenomics.com/wp-content/uploads/2020/03/UG4001-01_-CleanPlex-SARS-CoV-2-Panel-User-Guide.pdf). The resulting libraries were sequenced in an Illumina MiSeq (Illumina. San Diego. US) equipment. Consensus sequences were generated by the QIASeq SARS-CoV-2 pipeline (QIAGEN CLC Genomics Workbench 21) with high quality (average coverage >350. <6% Ns. >29.8 Kb). The specimens were classified using the Phylogenetic Assignment of Named Global Outbreak Lineages (Pangolin) software tool (v3.1.5) and the sequences were deposited into the GISAID database (https://www.gisaid.org/).

Sample Size

The sample size was estimated based on the study of Ranzani et.[3] which showed that the median time from onset of symptoms to hospital admission in Brazilian hospitals was 9 days in a period when variants of concern were not present. We estimated that patients who would require advanced respiratory support would require it in a median of 3 days. Therefore, the median number of days from onset of symptoms was estimated to be 12 in COVID-19 patients with COVID-19 caused by non-Gamma. For a 1:1 sample. an alpha of 0.05 and a beta of 0.20. estimating that populational standard deviation of 6 days[3] and aiming to detect a difference of at least 3 days for advanced respiratory support in Gamma-infected patients. the total sample size is 72 patients. The final sample size was defined as 86 patients, admitting a 20% increase in the sample since multivariable analysis is planned for this outcome.

Complementary Statistical Procedures

The median number of days to the advanced respiratory support was evaluated by Mann-Whitney test. For this later analysis, the number of days for patients who have not required advanced respiratory support was considered undetermined. Secondary outcomes were analyzed using chi-square or Fisher's exact test. Mann-Whitney, log-rank and ordinal logistic regression, as defined in the statistical analysis plan.

For 28-day mortality from hospitalization, a Cox regression model was constructed including age (regardless of the P value) and variables with a P value ≤0.20 in the bivariate analysis, using forward stepwise selection. Variables with a P value ≤0.05 were maintained in the model. A gamma generalized estimating equations model with Bonferroni correction was used to simultaneously assess the parameters over time and between groups.

Supplementary Results

STable 1 Summary of SARS-CoV-2 lineages causing infection in patients included in the study.

Period	**Lineage**	**n (%)**
First: June to December 2020		42
	B.1.1.28	15 (37.5)
	B.1.1.161	10 (23.8)
	B.1.1	5 (11.9)
	B.1.1.33	3 (7.1)
	B.1.28	2 (4.8)
	P.2	2 (4.8)
	B.1.1.370	1 (2.4)
	B.1.1.409	1 (2.4)
	B.1.1.462	1 (2.4)
	B.1.91	1 (2.4)
	P.1	1 (2.4)
Second: February to May 2021		44
	P.1	38 (86.4)
	P.1.1	2 (2.3%)
	P.1.2	2 (2.3%)
	P.2	2 (2.3%)

STable 2 Description of samples of patients included in the study.

		Cycle Threshold			
N	**Date (M/Y)**	**N1**	**N2**	**Lineage**	**Average Coverage**
1	06/2020	18.81	20.44	B.1.1.161	1971.8
2	06/2020	24.33	25.33	B.1.91	411.3
3	06/2020	20.41	21.35	B.1.1.28	1011.7
4	06/2020	21.90	22.09	B.1.1.28	1643.3
5	07/2020	23.87	23.85	B.1.1.161	1287.2
6	07/2020	20.94	22.59	B.1.1.28	1424.4
7	07/2020	13.8	13.1	B.1.1.28	981.8
8	07/2020	15.67	16.32	B.1.1.161	1791.1
9	07/2020	18.47	18.70	B.1.1	1671.5
10	08/2020	22.43	22.71	B.1.28	1063.7
11	08/2020	21.51	21.87	B.1.28	1453.6
12	08/2020	19.96	19.3	B.1.1.409	1996.8
13	08/2020	22.07	21.29	B.1.1.462	1672.7
14	08/2020	23.27	23.5	B.1.1.28	1761.5
15	08/2020	23.64	23.23	B.1.1.161	1679.8
16	08/2020	21.14	20.33	B.1.1.161	2270.8
17	08/2020	19.7	19.08	B.1.1.161	2707.8

18	08/2020	22.83	23.9	B.1.1.33	879.1
19	08/2020	11.27	11.73	B.1.1.161	2818.6
20	09/2020	24.54	25.78	B.1.1	1275.4
21	09/2020	20.38	21.04	B.1.1.161	1484
22	09/2020	22.69	21.93	B.1.1.28	1279.7
23	09/2020	20.22	20.09	B.1.1.28	1385.1
24	09/2020	23.3	23.97	B.1.1.28	912.3
25	10/2020	25.69	18.12	B.1.1.161	1772.6
26	10/2020	20.64	18.9	B.1.1.33	1996.1
27	10/2020	21.36	20.12	B.1.1.370	2628
28	10/2020	18.09	19.22	B.1.1.161	2358
29	10/2020	24.3	24.39	B.1.1	1684.4
30	10/2020	19.24	18.45	B.1.1	2812
31	10/2020	24.1	25.6	B.1.1.33	1610.9
32	11/2020	22.31	22.71	B.1.1.28	1656.8
33	11/2020	17.69	16.81	B.1.1.28	2550.6
34	11/2020	23.99	22.86	P.2	1915.4
35	11/2020	22.37	22.53	B.1.1.28	1925.4
36	11/2020	20.64	19.68	B.1.1.28	3103.1
37	11/2020	17.42	17.13	B.1.1.28	4792.9

38	11/2020	18.86	18.71	B.1.1	2824.4
39	11/2020	21.41	22.33	P.1	1440.9
40	12/2020	22.17	25.94	B.1.1.28	3164.1
41	12/2020	19.32	20.34	P.2	3106.1
42	12/2020	25.11	24.18	B.1.1.28	2230.2
43	02/2021	20.53	20.27	P.1	1780.6
44	02/2021	18.85	19.45	P.2	2101.9
45	02/2021	14.75	15.35	P.1	1781.4
46	02/2021	16.52	17.35	P.1	2627
47	02/2021	17.68	18.58	P.1	1872.3
48	02/2021	19.37	22.23	P.1	1160.5
49	02/2021	24.83	25.87	P.1	353.5
50	02/2021	21.13	21.27	P.1.1	2058.2
51	02/2021	19.39	19.15	P.1.1	624.5
52	02/2021	20.28	20.14	P.1	1756.5
53	02/2021	19.37	19.38	P.1	1493.3
54	02/2021	12.8	19.15	P.1	2001.7
55	03/2021	17.33	18.43	P.1	1649.7
56	03/2021	18.99	19.59	P.1	1894.8
57	03/2021	17.8	18.02	P.1	2723.5

58	03/2021	20.29	20.2	P.1	2130.4
59	03/2021	21.39	21.06	P.1	1948.7
60	03/2021	19.76	20.79	P.1	1780.9
61	03/2021	19	19.75	P.2	2113.6
62	03/2021	20.08	20.87	P.1	2900.1
63	03/2021	25.12	25.34	P.1.2	1276.1
64	03/2021	15	16.1	P.1	2231
65	03/2021	15.19	14.28	P.1	1972.5
66	03/2021	23.73	23.59	P.1	1149.1
67	03/2021	24.17	32.97	P.1	1153.7
68	03/2021	15.38	15.12	P.1	1329.2
69	04/2021	21.74	21.24	P.1	1201.1
70	04/2021	20.48	20.11	P.1.2	1689.4
71	04/2021	18.08	19.39	P.1	1815.1
72	04/2021	23.06	23.03	P.1	634.3
73	04/2021	15.73	15.52	P.1	2981.6
74	04/2021	20.04	20.03	P.1	1697.7
75	04/2021	22.79	22.98	P.1	1695
76	04/2021	15.09	19.45	P.1	2141.8
77	04/2021	15.1	15.2	P.1	1984.3

78	04/2021	14.98	16.35	P.1	2061.5
79	04/2021	19.74	18.55	P.1	1895.7
80	04/2021	22.94	24.79	P.1	2040.3
81	04/2021	17.12	16.58	P.1	2620.8
82	04/2021	23.33	22.85	P.1	3517
83	04/2021	25.21	25.02	P.1	428.3
84	04/2021	22.54	21.81	P.1	2967.5
85	05/2021	24.77	23.79	P.1	536.3
86	05/2021	15.43	13.92	P.1	2126.1

Cycle threshold, Ct.

STable 3 Risk of Gamma-infected patients in comparison to non-Gamma-infected patients of receiving oxygen supplementation by non-invasive or invasive methods at the baseline and in four weeks of follow-up after hospitalization.

	Odds Ratio (95% Confidence Interval) for Gamma infections				
Ordinal Scale Category [a]	Baseline	Day 7	Day 14	Day 21	Day 28
1 or 2	reference	reference	reference	reference	reference
3 or 4 [b]	2.30 (0.91-5.86)	3.86 (0.75-20.01)	2.16 (0.66-7.02)	0.60 (0.16- 2.21)	0.762 (0.28-4.54)
5 or 6 [b]	2.30 (0.43-12.25)	10.91 (2.04-58.39)	3.51 (1.31-9.47)	5.25 (1.77-15.55)	4.35 (1.56-12.09)
P [c]	0.19	0.003	0.04	0.001	0.009

[a] Category 1, non-hospitalized without supplemental oxygen; 2, hospitalization without supplemental oxygen; 3, hospitalization with low-flow supplemental oxygen; 4, hospitalization with non-invasive ventilation or high-flow supplemental oxygen; 5, hospitalization with invasive mechanical ventilation and/or extracorporeal membrane oxygenation; 6, Death.

[b] The odds ratio refers to category 1 or 2 (reference).

[c] Obtained by ordinal logistic regression model.

STable 4 Risk of Gamma-infected patients in comparison to non-Gamma-infected patients of having a reduced pressure arterial oxygen/ fraction of inspired oxygen ratio (PaO2/FiO2) at the baseline and in four weeks of follow-up after hospitalization.

	Odds Ratio (95% Confidence Interval)				
PaO2/FiO2[a]	Baseline	Day 7	Day 14	Day 21	Day 28
>300	reference	reference	reference	reference	reference
300-201 [b]	0.90 (0.25-3.25)	6.98 (1.72-28.25)	3.26 (0.93-11.41)	1.33 (0.25-7.19)	[c]
200-101 [b]	1.85 (0.57-5.99)	4.07 (1.20-13.86)	4.08 (1.08-15.37)	1.67 (0.40- 6.88)	4.20 (0.78- 22.55)
≤100 [b]	3.74 (1.11-12.57)	11.40 (2.54-51.11)	3.26 (0.93- 11.41)	3.67 (1.04-12.94)	2.57 (0.84-7.86)
P. [c]	0.12	0.002	0.04	0.20	

[a] >300 corresponds to no Acute Distress Respiratory Syndrome (ARDS); 300-201, mild ARDS; 200-101, moderate ARDS; ≤100, severe ARDS.

[b] The odds ratio refers to category 1 or 2 (reference).

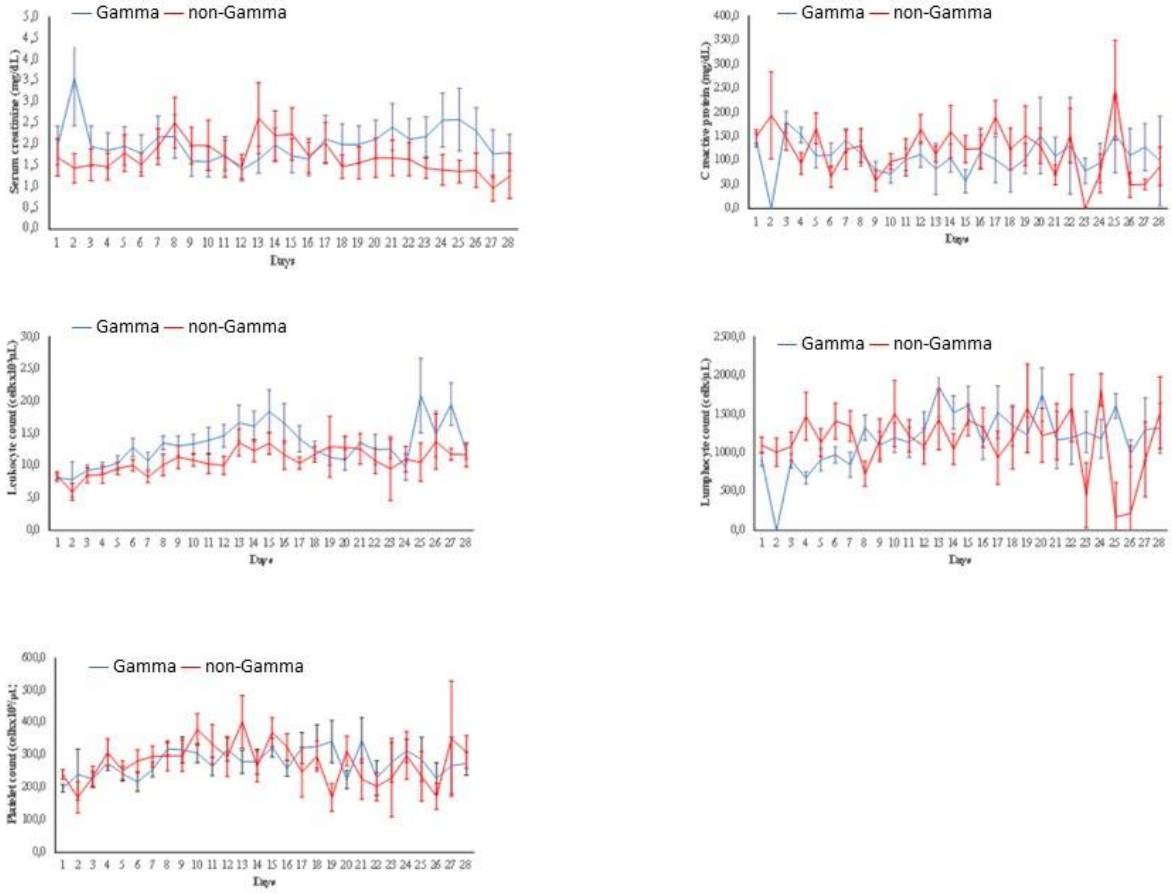
[c] The risk was not determined since there was no non-Gamma patient at this category on day 28.

[d] Obtained by ordinal logistic regression model.

STable 5 Other secondary outcomes.

Outcome	Gamma n (%)	non-Gamma n (%)	Relative Risk (95% CI)	P
Admission at ICU	29 (67.4)	20 (46.5)	1.45 (0.99-2.12)	0.08
Need of prone positioning	8 (18.6)	8 (18.6)	1.00 (0.41-2.42)	0.99
Need of RRT	14 (32.6)	11 (25.6)	1.27 (0.65-2.48)	0.64
Occurrence of thromboembolic event	7 (16.3)	6 (14.0)	1.17 (0.43-3.19)	0.99
In-hospital mortality. n (%)	12 (27.9)	8 (18.6)	1.28 (0.82-1.94)	0.44

CI, Confidence Interval; ICU, Intensive Care Unit; RRT, Renal Replacement Therapy.



SFigure 1 Laboratorial exams from baseline to day 28 of hospitalization.

Generalized estimating equations indicated no effect of variant on C reactive protein (P=0.35), creatinine (P=0.65), leukocyte count (P=0.34) and lymphocyte count (P=0.46) along the 28-day period.

STable 6 Baseline characteristics of all eligible patients from the first and second periods of the study. [a]

Characteristics	**Second period (n= 174)**	**First period (n= 259)**	**P**
Gender, male	89 (51.1)	136 (52.5)	0.86
Age, years	50.0 (40.0 – 58.0)	53.0 (43.0 – 60.0)	0.12
Charlson's Comorbidity Score	0 (0-1)	0 (0-2)	0.001
BMI, kg/m^2 [b]	31.2 (27.4 – 38.0)	31.0 (27.3 – 35.9)	0.44
BMI ≥30 kg/m^2 [b]	94 (58.4) [161]	140 (58.3) [240]	0.99
Time from onset of symptoms to hospital admission, days	7.0 (5.0 – 9.0)	7.0 (5.0 – 9.0)	0.52
PaO2/FiO2 at admission			<0.001
>300	55 (31.6)	156 (60.2)	
300-201	25 (14.4)	34 (13.1)	
200-101	47 (27.0)	48 (18.5)	
≤100	47 (27.0)	21 (8.1)	
Score on six-level ordinal scale			<0.001
2- hospitalization without supplemental oxygen	30 (17.2)	115 (44.4)	
3 - hospitalization with supplemental oxygen	82 (47.1)	108 (41.7)	
4 - hospitalization with non-invasive ventilation or high-flow supplemental oxygen	39 (22.4)	22 (8.5)	
5 - hospitalization with invasive mechanical ventilation and/or extracorporeal membrane oxygenation	23 (13.2)	14 (5.4)	

Data expressed as n (%), median (IQR) or mean ± SD. BMI, body mass index; PaO2/FiO2, partial pressure of arterial oxygen /fractional inspired oxygen; Ct, cycle threshold.

[a] Infections in patients from the second period are presumably caused by Gamma and from the first period are presumably caused by non-Gamma lineages.

[b] Thirteen (7.4%) patients in Gamma and 19 (7.3%) in non-Gamma.did not have BMI recorded.

STable 7 Outcomes of eligible patients from the first and second periods.[a]

Outcome	**Second (n= 174)**	**First (n= 259)**	**Relative Risk (95% CI)**	**P**
Advanced respiratory support	153 (87.9)	157 (60.6)	2.89 (1.92 – 4.34)	<0.001
Invasive respiratory support	121 (69.5)	89 (34.4)	2.42 (1.87 – 3.15)	<0.001
Death within 28 days from the onset of symptoms	31 (17.8)	21 (8.1)	1.59 (1.23 – 2.06)	0.004
Death within 28 days from hospitalization	39 (22.4)	30 (11.6)	1.52 (1.19 – 1.95)	0.004

CI, Confidence Interval.

[a] Infections in patients from the second period are presumably caused by Gamma and from the first period are presumably caused by non-Gamma lineages.

STable 8 Multivariate models for the advanced respiratory support, invasive ventilatory support, and 28-day mortality from the onset of symptoms and from hospitalization in patients from the first and second periods of the study.

Variable	**Adjusted Hazard Ratio (95% Confidence Interval)**	**P**
Model 1: Advanced respiratory support from onset of symptoms [a]		
Second period [b]	2.04 (1.60 – 2.59)	<0.001
Age	1.02 (1.00 – 1.03)	0.07
Sex, male	1.00 (0.79 – 1.26)	0.99
Charlson's Score	0.99 (0.93 – 1.06)	0.81
Body Mass Index	1.03 (1.02 – 1.05)	<0.001
Model 2: Invasive respiratory support from onset of symptoms [c]		
Second period [b]	2.72 (2.05 – 3.62)	<0.001
Age	1.03 (1.01 – 1.04)	<0.001
Sex, male	0.97 (0.73 – 1.28)	0.83
Charlson's Score	0.98 (0.91 – 1.07)	0.76
Body Mass Index	1.02 (1.00 – 1.03)	0.03
Model 3: 28-day mortality from onset of symptoms		
Second period [b]	2.62 (1.46 – 4.72)	0.001
Age	1.05 (1.02 – 1.08)	0.003
Sex, male	1.08 (0.61 – 1.93)	0.78
Charlson's Score	1.14 (1.01 – 1.28)	0.04
Body Mass Index	1.00 (0.97 – 1.04)	0.83
Model 4: 28-day mortality from hospital admission [d]		

Second period [b]	1.54 (0.93 – 2.57)	0.09
Age	1.03 (1.01 – 1.06)	0.008
Ordinal Scale	1.18 (1.08 – 1.29)	<0.001
Charlson`s Score	2.03 (1.59 – 2.60)	<0.001

[a] Advanced respiratory support was considered non-invasive ventilation, high-flow oxygen support, mechanical ventilation or extracorporeal membrane oxygenation.

[b] Infections in patients from the second period are presumably caused by Gamma.

[c] Invasive respiratory support was considered mechanical ventilation or extracorporeal membrane oxygenation.

[c] Forcing age into the model did not modify the effect of second period on the outcome.

References

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