

THE LANCET Microbe

Supplementary appendix 2

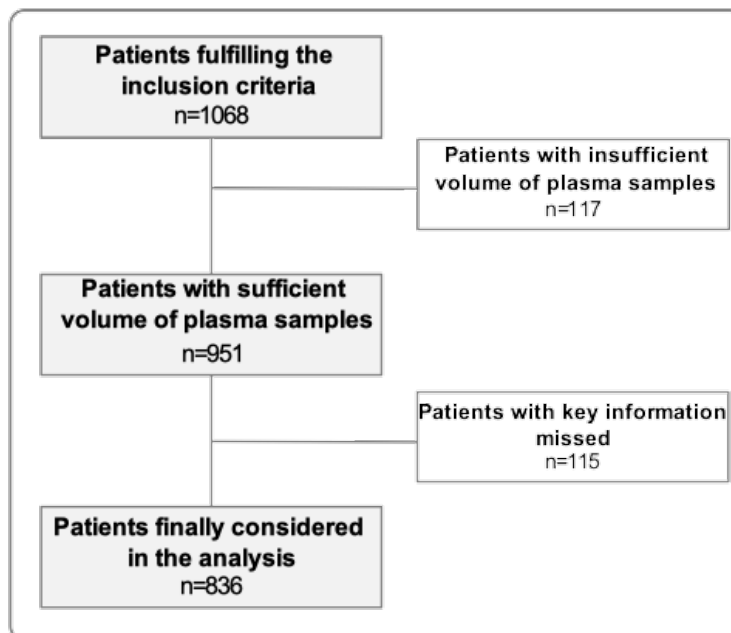
This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

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5	Hospital Punta de Europa, Algeciras, Spain
6	Hospital de Mataró, Mataró, Spain
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8	Hospital Universitario Marqués de Valdecilla, Santander, Spain
9	Hospital Universitario Lucus Augustí, Lugo, Spain
10	Hospital Universitario Príncipe de Asturias, Alcalá de Henares, Spain
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13	Complejo Hospitalario Universitario de Ourense, Ourense, Spain
14	Complejo Asistencial de Salamanca, Salamanca, Spain
15	Hospital General Universitario Gregorio Marañón, Madrid, Spain
16	Hospital Clínico Universitario de Valencia, Valencia, Spain
17	Hospital Universitario Arnau de Vilanova y Santa María, Lleida, Spain
18	Hospital Universitario de Torrejón, Torrejón de Ardoz, Spain
19	Hospital Universitario de León, León, Spain
20	Hospital General de Segovia, Segovia, Spain
21	Hospital Nuestra Señora de Gracia, Zaragoza, Spain
22	Hospital Universitario de la Paz, Madrid, Spain
23	Hospital Universitario Nuestra Señora de Valme, Sevilla, Spain



Supplementary file 1. Flow chart depicting numbers of patients included and excluded from the study.

Supplementary file 2. Methodology to profile anti-SARS-CoV-2 S antibodies in plasma and plasma's inhibition activity of the binding between the SARS-CoV-2 S protein and ACE2

Immunoassay for antibody quantification: a specific immunoassay was developed to quantify anti-SARS-CoV-2 S IgG and IgM antibodies in plasma. The plasmid pαH coding for the S protein ectodomain (residues 1-1208) of the SARS-CoV-2 2019-nCoV (GenBank: MN908947) was kindly provided by Dr. Jason McLellan (the University of Texas at Austin-USA) [1]. Mutagenesis was carried out to obtain a HexaPro construct that allowed a high-yield production of a stabilized prefusion spike protein [2]. The following substitutions were included at the ectodomain: glycine at residue 614 (D614G), a "GSAS" substitution at the furin cleavage site (residues 682–685), and proline at residues 817, 892, 899, 942, 986, and 987. For trimerization and purification, the C-terminal end of the S protein ectodomain was fused to the T4 fibrin trimerization motif (foldon), an HRV3C protease cleavage site, and an 8XHisTag. The expression vector coding for the SARS-CoV-2 S protein ectodomain was used to transiently transfect FreeStyle 293F cells (Thermo Fisher, Waltham, MA, USA) using polyethylenimine. The S protein domain was purified from filtered cell supernatants using Ni-NTA resins (Sigma Aldrich, San Luis, MO, USA) and subjected to an additional purification step by size-exclusion chromatography using a Superose 6 10/300 column (GE Healthcare, Chicago, IL, USA).

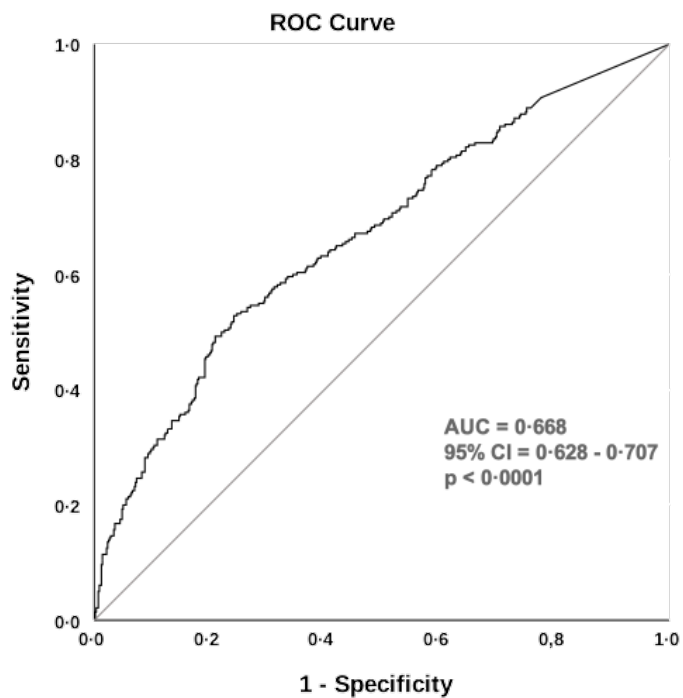
Antibody titration: Antibody titers against the S protein were determined by incubating serial dilutions of serum samples (starting at a 1:50 dilution) with the purified S protein ectodomain. Ninety-six well plates were coated with 200 ng per well of the S protein ectodomain. The following day, serum samples were added, and the binding to the S protein was determined by successive incubations with a secondary peroxidase-conjugated anti-human IgG or IgM (Jackson ImmunoResearch, West Grove, PA, USA) antibody and the OPD substrate (Sigma Aldrich, San Luis, MO, USA). One phase exponential decay least-squares fit curves, and the area under the curve (AUC) was calculated by using GraphPad Prism 8.0 (GraphPad Software, Inc., San Diego, CA, USA) and the following parameters: Baseline (cut-off value for each plate) Y= the average of the optical density values of blank wells (no S protein) plus three standard deviations; ignore peaks that are less than 10% of the distance from minimum to maximum Y; all peaks must go above the baseline. The AUC is expressed as X units times the Y units.

ACE2 binding inhibition assay: An ELISA binding inhibition assay of soluble ACE2 receptor to the SARS-CoV-2 S protein was performed as follows: Fifty nanograms per well of the S protein ectodomain were captured by a chimeric version of a monoclonal anti-Foldon antibody [3] previously coated in 96 well plates. Next, a 1:10 dilution of the different serum samples was added, and plates were incubated for 45 min at room temperature (RT). Then, one µg of the cell receptor ACE2 complexed with StrepTactin-peroxidase (Bio-Rad; Halle, Germany) was added to each well,

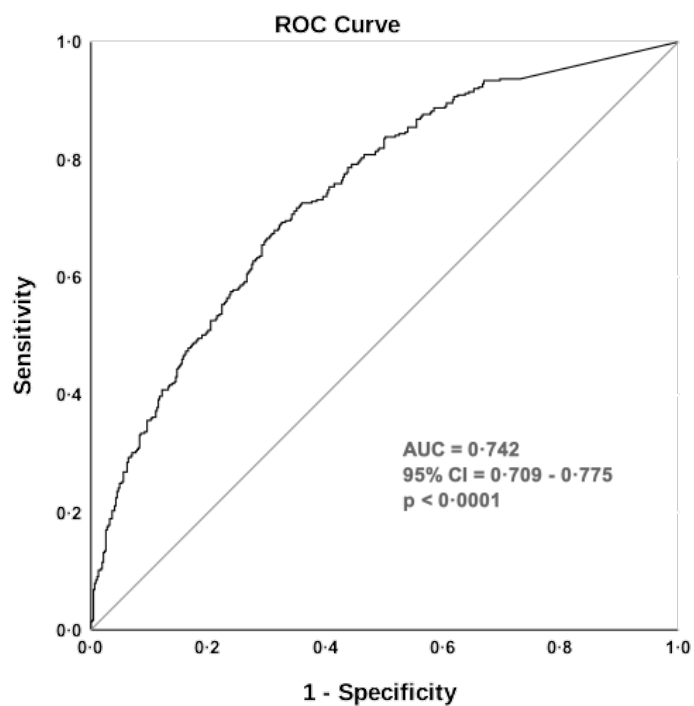
incubated for 15 min at RT, revealed with the OPD substrate (Sigma Aldrich, San Luis, MO, USA), and the OD₄₉₃ was measured in a spectrophotometer. The assay background was determined in parallel plates coated with a locked closed conformation of the S protein unable to bind the ACE2 receptor. A pool of sera from individuals negative for anti-S antibodies, collected in 2016, was used as a control. After subtraction of the background, the percentage of inhibition was calculated as $[1 - (\text{OD}_{493} \text{ test serum} / \text{OD}_{493} \text{ control serum})] \times 100 \%$. The percentage of inhibition relative to ACE2 in which the StrepTag was removed was further calculated as $(\% \text{ inhibition of serum samples} / \% \text{ inhibition of ACE2}) \times 100 \%$.

References:

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2. Hsieh C-L, Goldsmith JA, Schaub JM, et al. Structure-based design of prefusion-stabilized SARS-CoV-2 spikes. *Science* **2020**; 369:1501–1505.
3. Battles MB, Mas V, Olmedillas E, Cano O, Vazquez M, Rodriguez L, et al. Structure and immunogenicity of pre-fusion-stabilized human metapneumovirus F glycoprotein. *Nat Commun* **2017**; 8(1):1528.



Supplementary file 3. Receiver operating characteristic curve to evaluate accuracy of the [SARS-CoV-2 N1 RNA] in plasma to identify non survivors in the first 90 days following admission to the ICU. Cut-off point: 2747 copies/ml, sensitivity 0.529, specificity: 0.757, Youden index: 0.286



Supplementary file 4. Receiver operating characteristic curve to evaluate accuracy of the [SARS-CoV-2 N1 RNA] in plasma to identify N-Antigenemia. Cut-off point: 1010 copies/ml, sensitivity 0.693, specificity: 0.675, Youden index: 0.368.

Secondary infections	All patients	VIR-N1-Zero	VIR-N1-Low	VIR-N1-Storm
Secondary infections [n (%)]	435 (32·20)	47 (32·20)	208 (52·10)	180 (65·00)
Lungs	287 (66·00)	28 (60·90)	132 (63·50)	127 (71·30)
Gastrointestinal system	4 (0·90)	0 (0·00)	3 (1·40)	1 (0·60)
Urinary tract	52 (12·00)	5 (10·60)	30 (14·40)	17 (9·40)
Skin and soft tissue	3 (0·70)	0 (0·00)	2 (1·00)	1 (0·60)
Central nervous system	1 (0·20)	0 (0·00)	0 (0·00)	1 (0·60)
Bloodstream	85 (19·50)	13 (27·70)	41 (10·70)	31 (17·20)
Unknown	3 (0·70)	1 (2·10)	0 (0·00)	2 (1·10)

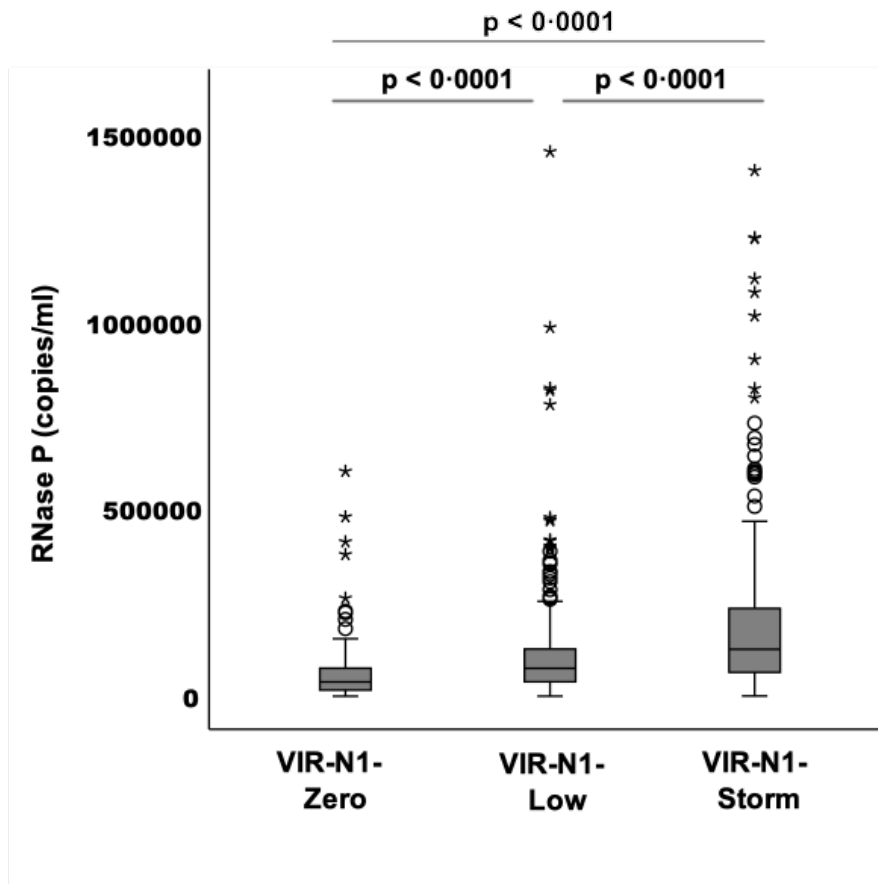
Supplementary file 5. Secondary infections in VIR-N1-Zero, VIR-N1-Low and VIR-N1-Storm patients. Statistics: categorical variables are represented as [n, (%)].

Causes of 90-day mortality	All patients	VIR-N1-Zero	VIR-N1-Low	VIR-N1-Storm
90-day mortality [n (%)]	280 (33·50)	26 (17·30)	106 (26·30)	148 (52·30)
Multi organ failure	121 (43·20)	8 (30·80)	49 (46·20)	64 (43·80)
Respiratory failure	123 (43·90)	7 (26·90)	46 (43·40)	70 (47·90)
Heart failure	1 (0·40)	0 (0·00)	0 (0·00)	1 (0·70)
Septic shock	22 (7·90)	8 (30·80)	6 (5·70)	8 (5·50)
Other causes	11 (3·90)	3 (11·50)	5 (4·70)	3 (2·10)
Unknown	2 (0·40)	0 (0·00)	0 (0·00)	2 (1·40)

Supplementary file 6. Causes of mortality in VIR-N1-Zero, VIR-N1-Low and VIR-N1-Storm patients. Statistics: categorical variables are represented as [n, (%)].

Measurements at ICU admission	All patients	VIR-N1-Zero (1)	VIR-N1-Low (2)	VIR-N1-Storm (3)	p value (1 vs. 2)	p value (1 vs. 3)	p value (2 vs. 3)
No. (%)	836	150 (17.94)	403 (48.20)	283 (33.85)	-	-	-
Temperature (°C) [median (Q1,Q3)]	36.70 (36.00,37.40)	36.60 (36.00,37.20)	36.70 (36.10,37.40)	36.80 (36.00,37.50)	n.s	n.s	n.s
Glucose (mg/dL) [median (Q1,Q3)]	155.00 (126.00,208.75)	144.00 (120.00,182.70)	154.00 (124.00,200.45)	166.00 (130.40,223.00)	0.57	0.0049	0.038
Bilirubin (mg/dL) [median (Q1,Q3)]	0.53 (0.39,0.71)	0.60 (0.40,0.71)	0.54 (0.40,0.71)	0.50 (0.36,0.71)	n.s	n.s	n.s
Creatinine (mg/dL) [median (Q1,Q3)]	0.85 (0.70,1.12)	0.81 (0.67,1.06)	0.80 (0.66,1.06)	0.93 (0.74,1.25)	1.000	0.0024	< 0.0001
Platelets (cells x 10 ³ /mL) [median (Q1,Q3)]	233.00 (178.00,308.75)	284.50 (209.25,375.25)	233.00 (181.00,305.00)	210.00 (162.00,279.00)	< 0.0001	< 0.0001	0.0079
GPT (U/L) [median (Q1,Q3)]	39.00 (25.00,64.00)	37.50 (22.88,70.00)	41.00 (26.00,64.00)	37.00 (24.00,60.00)	n.s	n.s	n.s
C-reactive protein (mg/L)[median (Q1,Q3)]	111.00 (56.00,210.00)	70.00 (28.58,174.83)	107.00 (58.50,199.40)	131.75 (64.48,233.23)	0.0097	< 0.0001	0.012
Hematocrit (%) [median (Q1,Q3)]	40.00 (37.00,43.48)	40.40 (36.60,44.00)	40.20 (37.20,40.30)	39.60 (36.48,43.48)	n.s	n.s	n.s
WBC (cells x 10 ³ /mL) [median (Q1,Q3)]	9.40 (6.82,12.98)	10 (7.50,13.66)	8.92 (6.79,12.12)	9.73 (6.47,13.34)	n.s	n.s	n.s
Lymphocytes (cells x 10 ³ /mL) [median (Q1,Q3)]	0.66 (0.42,0.95)	0.80 (0.50,1.20)	0.68 (0.45,0.92)	0.60 (0.39,0.90)	0.28	0.0026	0.068
Neutrophils (cells x 10 ³ /mL) [median (Q1,Q3)]	8.22 (5.80,11.51)	8.95 (6.30,12.22)	7.68 (5.60,10.70)	8.86 (5.78,12.11)	0.026	1.000	0.035
Monocytes (cells x 10 ³ /mL) [median (Q1,Q3)]	0.38 (0.21,0.57)	0.40 (0.30,0.70)	0.39 (0.20,0.58)	0.33 (0.20,0.50)	0.031	< 0.0001	0.055
Anti-SARS-CoV-2 S IgM (AUC) [median (Q1,Q3)]	41.54 (1.97,153.90)	67.83 (11.03,271.15)	55.57 (5.26,197.20)	9.81 (0.00,75.03)	0.43	< 0.0001	< 0.0001
Anti-SARS-CoV-2 S IgG (AUC) [median (Q1,Q3)]	177.00 (18.30,892.50)	787.25 (114.20,2028.50)	268.80 (39.94,1012.00)	39.17 (1.44,252.05)	0.0018	< 0.0001	< 0.0001
Inhibition of S-ACE2 binding > 50% [n (%)]	404 (52.70)	114 (76.00)	198 (55.30)	92 (35.50)	< 0.0001	< 0.0001	< 0.0001
RNase P (copies/mL) [median (Q1,Q3)]	78611.19 (37953.57,144050.50)	38304.96 (15692.01,75370.32)	74674.03 (38678.28,127591.27)	125620.79 (63143.87,235809.30)	< 0.0001	< 0.0001	< 0.0001

Supplementary file 7. Laboratory parameters. Statistics: Continuous variables are represented as [median (quartile 1, quartile 3 (Q1, Q3))] and categorical variables as absolute count [(n, (%)]. P-values were assessed by using the Kruskal–Wallis test with Bonferroni post hoc test adjustment, and Chi-squared tests for categorical variables. Significant differences (p < 0.05) are shown in bold. Missing data were present for temperature (40), glucose (28), GPT (94), C-reactive protein (57), hematocrit (52), WBC (28), lymphocytes (47), neutrophils (70), monocytes (74), anti-SARS-CoV-2 S IgM (1), anti-SARS-CoV-2 S IgG (1) and inhibition of S-ACE2 binding > 50% (69). Abbreviations: p-value, level of significance; n.s, not statistically significant; GPT, glutamic-pyruvic transaminase; WBC, white blood cells.



Supplementary file 8. Levels of RNase P mRNA in plasma (copies/ml).

Measurements at ICU admission [median (Q1,Q3)]	No.	All patients (836)	VIR-N1-Zero (150) 1	VIR-N1-Low (403) 2	VIR-N1-Storm (283) 3	Healthy Controls (20) 0	p-value (0 vs. 1)	p-value (0 vs. 2)	p-value (0 vs. 3)	p-value (1 vs. 2)	p-value (1 vs. 3)	p-value (2 vs. 3)
ICAM-1 (pg/ml)	831	425180·50 (346895·00,528326·00)	408121·00 (331647·00,516557·50)	417817·00 (343761·25,524101·50)	440320·50 (362610·75,537795·25)	293497·00 (256013·25,343634·50)	< 0·0001	< 0·0001	< 0·0001	1·000	0·58	0·39
Lipocalin -2 (pg/ml)	831	98533·00 (74418·00,135066·00)	98188·00 (72977·00,131069·50)	95300·50 (73353·75,132686·50)	105324·50 (79896·25,144261·75)	91579·50 (85111·25,110544·50)	n·s	n·s	n·s	n·s	n·s	n·s
Myeloperoxidase (pg/ml)	831	176813·00 (106385·00,305477·00)	130545·00 (91966·00,227232·50)	159731·50 (94263·00,271109·25)	223763·00 (138683·25,385361·75)	31955·50 (21451·25,46467·75)	< 0·0001	< 0·0001	< 0·0001	0·38	< 0·0001	< 0·0001
VCAM-1 (pg/ml)	831	1245995·00 (959614·00,1611304·00)	1083354·00 (808838·50,1449106·00)	1205079·50 (932743·00,1531594·25)	1410407·00 (1045792·00,1786010·75)	686543·50 (574934·75,757602·75)	< 0·0001	< 0·0001	< 0·0001	0·092	< 0·0001	< 0·0001
D-dimer (pg/ml)	817	1940664·00 (1069870·00,4160595·50)	1469475·00 (923574·75,3454350·25)	1894675·00 (1008786·25,4039864·50)	2407209·00 (1279762·50,4755883·00)	267184·50 (189371·75,416976·00)	< 0·0001	< 0·0001	< 0·0001	0·52	0·0038	0·12
E-selectin (pg/ml)	831	27809·00 (20429·00,37945·00)	25993·00 (18858·00,39072·50)	27795·50 (20972·50,37430·75)	29012·50 (20751·75,41107·00)	22180·00 (18214·25,28538·25)	n·s	n·s	n·s	n·s	n·s	n·s
Ferritin (pg/ml)	831	1189776·00 (628530·00,2082095·00)	926904·0 (476101·00,1728296·50)	1182345·00 (660826·00,2028756·50)	1374003·00 (676819·75,2403001·75)	67128·50 (38397·75,94993·25)	< 0·0001	< 0·0001	< 0·0001	0·048	0·0005	0·36
Rantes (pg/ml)	806	23228·00 (13020·50,40708·50)	24058·00 (12242·50,42716·00)	23272·00 (12865·00,42306·00)	22410·50 (13407·50,39582·75)	16707·00 (12685·50,25515·75)	n·s	n·s	n·s	n·s	n·s	n·s
CD27 (pg/ml)	810	7862·50 (5616·75,11519·75)	7769·00 (5380·00,11488·50)	8089·00 (5659·25,11490·75)	7761·00 (5669·50,11770·50)	3983·00 (3524·50,4336·50)	< 0·0001	< 0·0001	< 0·0001	1·0000	1·0000	1·0000
Fas (pg/ml)	810	14184·00 (10704·25,18964·25)	14363·00 (10409·00,18460·00)	13910·00 (10706·25,18687·00)	14829·00 (10947·00,19978·00)	8530·00 (7116·25,11378·75)	< 0·0001	< 0·0001	< 0·0001	1·0000	1·0000	1·0000
SP-D (pg/ml)	830	18006·50 (9635·00,35265·75)	14888·00 (8731·00,31361·00)	17444·50 (9144·25,33170·25)	20577·00 (11216·00,41867·50)	6136·00 (4704·25,9382·00)	0·0001	< 0·0001	< 0·0001	1·000	0·11	0·29
PTX3 (pg/ml)	809	29693·00 (13164·50,64399·50)	15671·00 (8279·00,32889·00)	27487·00 (12085·50,59533·25)	45964·50 (21353·50,93351·50)	1400·00 (766·50,2195·00)	< 0·0001	< 0·0001	< 0·0001	< 0·0001	< 0·0001	< 0·0001
IL-10 (pg/ml)	829	13·30 (7·50,23·85)	6·73 (4·03,14·45)	11·90 (7·38,19·50)	20·80 (12·50,33·45)	1·29 (1·11,1·81)	< 0·0001	< 0·0001	< 0·0001	< 0·0001	< 0·0001	< 0·0001
IL-17A (pg/ml)	829	0·12 (0·00,0·85)	0·00 (0·00,0·56)	0·15 (0·00,0·93)	0·23 (0·00,0·97)	0·04 (0·00,0·32)	1·0000	0·63	0·39	0·054	0·015	1·000
GM-CSF (pg/ml)	829	1·17 (0·72,1·80)	0·95 (0·63,1·45)	1·19 (0·75,1·79)	1·31 (0·75,1·94)	0·83 (0·61,1·17)	1·0000	0·13	0·025	0·025	0·0003	0·51
IL-7 (pg/ml)	829	6·71 (4·22,10·95)	6·91 (4·22,10·70)	6·34 (4·04,10·50)	6·98 (4·33,12·00)	2·59 (1·91,3·36)	< 0·0001	< 0·0001	< 0·0001	1·0000	1·0000	1·0000
CXCL10 (pg/ml)	828	1613·00 (965·25,2516·75)	783·00 (356·00,1266·50)	1482·50 (945·00,2236·25)	2345·00 (1648·00,3198·00)	112·50 (69·53,160·75)	0·0023	< 0·0001	< 0·0001	< 0·0001	< 0·0001	< 0·0001
Angiopoietin-2 (pg/ml)	830	1202·50 (784·00,1810·00)	1165·00 (775·50,1728·50)	1157·00 (753·50,1766·25)	1278·00 (819·00,1994·00)	825·00 (703·25,1054·25)	0·093	0·070	0·0086	1·000	0·67	0·24
IL-1RA (pg/ml)	824	935·00 (596·25,1835·25)	755·00 (472·50,1457·50)	861·50 (589·00,1609·50)	1261·00 (718·00,2755·00)	460·50 (181·75,1235·75)	0·38	0·028	< 0·0001	0·18	< 0·0001	< 0·0001
IL-6 (pg/ml)	830	49·95 (17·35,174·50)	23·60 (7·75,70·95)	43·80 (15·20,163·25)	85·80 (30·85,311·00)	1·65 (1·18,2·07)	< 0·0001	< 0·0001	< 0·0001	0·0013	< 0·0001	< 0·0001
CCL2 (pg/ml)	829	422·00 (242·50,746·00)	260·00 (166·00,471·50)	394·00 (239·50,644·25)	572·50 (317·50,1046·75)	139·00 (110·00,163·50)	0·0004	< 0·0001	< 0·0001	< 0·0001	< 0·0001	< 0·0001
IL-12p70 (pg/ml)	829	0·64 (0·36,0·96)	0·61 (0·38,0·84)	0·62 (0·36,0·95)	0·66 (0·36,1·02)	0·88 (0·67,1·07)	n·s	n·s	n·s	n·s	n·s	n·s
IL-2 (pg/ml)	829	0·23 (0·04,0·56)	0·09 (0·00,0·27)	0·23 (0·03,0·54)	0·35 (0·09,0·73)	0·12 (0·04,0·25)	1·0000	0·37	0·019	0·0003	< 0·0001	0·0062
IL-4 (pg/ml)	829	0·15 (0·05,0·29)	0·17 (0·09,0·26)	0·16 (0·05,0·31)	0·14 (0·04,0·26)	0·28 (0·19,0·52)	0·017	0·0049	0·0011	1·000	0·73	1·0000
IL-15 (pg/ml)	829	6·33 (4·95,8·31)	5·38 (4·33,7·11)	6·13 (4·84,7·82)	7·40 (5·59,9·08)	2·25 (2·02,2·50)	< 0·0001	< 0·0001	< 0·0001	0·020	< 0·0001	< 0·0001

Granzyme B (pg/ml)	829	26.70 (18.15,42.50)	21.00 (13.05,28.90)	26.75 (18.00,41.70)	30.15 (21.60,54.23)	13.35 (8.82,20.05)	0.098	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0026
IFN- γ (pg/ml)	828	1.93 (0.86,5.96)	0.75 (0.36,2.19)	1.84 (0.96,5.81)	3.26 (1.47,9.20)	0.60 (0.50,0.89)	0.50	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
TNF- α (pg/ml)	828	11.40 (8.66,15.58)	10.00 (0.74,13.00)	11.00 (8.42,15.23)	12.60 (9.67,17.45)	6.11 (5.33,7.03)	< 0.0001	< 0.0001	< 0.0001	0.038	< 0.0001	0.0011
Endothelin-1 (pg/ml)	806	2.33 (1.60,3.46)	1.86 (1.30,2.84)	2.23 (1.51,3.30)	2.82 (1.87,4.11)	1.12 (0.52,1.28)	0.0002	< 0.0001	< 0.0001	0.035	< 0.0001	< 0.0001
Granzyme A (pg/ml)	806	72.25 (55.63,95.20)	71.10 (52.40,91.90)	69.70 (55.88,94.33)	76.50 (56.80,101.00)	99.75 (85.33,120.75)	0.0004	0.0007	0.0038	1.0000	0.69	1.0000
IFN- α (pg/ml)	806	1.24 (0.45,4.42)	0.73 (0.26,1.76)	1.24 (0.46,3.66)	1.95 (0.62,5.90)	0.00 (0.00,0.19)	< 0.0001	< 0.0001	< 0.0001	0.010	< 0.0001	0.012
IL-8 (pg/ml)	806	18.30 (11.76,27.00)	13.30 (8.91,20.80)	17.20 (11.85,24.10)	22.20 (15.40,34.40)	3.72 (2.86,6.07)	< 0.0001	< 0.0001	< 0.0001	0.0019	< 0.0001	< 0.0001
CTLA-4 (pg/ml)	806	6.62 (4.32,10.70)	5.73 (3.74,9.62)	6.51 (4.15,10.58)	7.24 (4.81,12.10)	3.56 (2.70,9.79)	0.39	0.053	0.0077	0.58	0.016	0.40
EGF (pg/ml)	807	34.70 (14.10,79.20)	37.10 (12.60,90.50)	32.50 (14.00,79.50)	37.50 (14.95,76.65)	64.45 (46.18,92.65)	0.073	0.030	0.045	1.0000	1.0000	1.0000
TREM-1 (pg/ml)	806	608.00 (426.50,821.00)	552.00 (361.00,759.00)	577.00 (401.25,799.50)	672.00 (503.00,859.50)	322.00 (271.00,386.00)	< 0.0004	< 0.0001	< 0.0001	1.000	0.0001	0.0005
uPA (pg/ml)	806	913.00 (719.75,1136.75)	829.00 (657.50,1062.50)	890.00 (698.50,1116.00)	996.00 (799.00,1232.00)	942.50 (815.50,1177.00)	0.39	1.0000	1.000	0.32	< 0.0001	0.0006
PD-L1 (pg/ml)	830	211.00 (161.00,290.00)	174.00 (127.00,240.50)	199.00 (155.00,264.00)	249.00 (197.00,328.25)	71.75 (59.38,105.50)	0.0008	< 0.0001	< 0.0001	0.014	< 0.0001	< 0.0001
G-CSF (pg/ml)	829	48.70 (28.75,87.50)	39.10 (21.75,61.10)	47.80 (27.40,84.20)	55.20 (38.50,102.00)	11.10 (6.54,17.83)	< 0.0001	< 0.0001	< 0.0001	0.019	< 0.0001	0.0006
IFN- β (pg/ml)	799	0.07 (0.00,1.47)	0.09 (0.00,1.56)	0.14 (0.00,1.55)	0.01 (0.00,1.32)	0.00 (0.00,0.25)	n.s	n.s	n.s	n.s	n.s	n.s
IL-1 β (pg/ml)	828	0.22 (0.11,0.40)	0.21 (0.12,0.39)	0.22 (0.10,0.40)	0.24 (0.12,0.40)	0.56 (0.09,2.53)	n.s	n.s	n.s	n.s	n.s	n.s

Supplementary file 9. Biomarkers levels in VIR-N1-Zero, VIR-N1-Low, VIR-N1-Storm patients and healthy controls. Statistics: Continuous variables are represented as [median, (quartile 1, quartile 3 (Q1, Q3))], differences were assessed by using the Kruskal–Wallis test with Bonferroni post hoc test adjustment. Significant differences ($p < 0.05$) are shown in bold. N.s means “not significant” in the Kruskal–Wallis test.