Supplemental Information

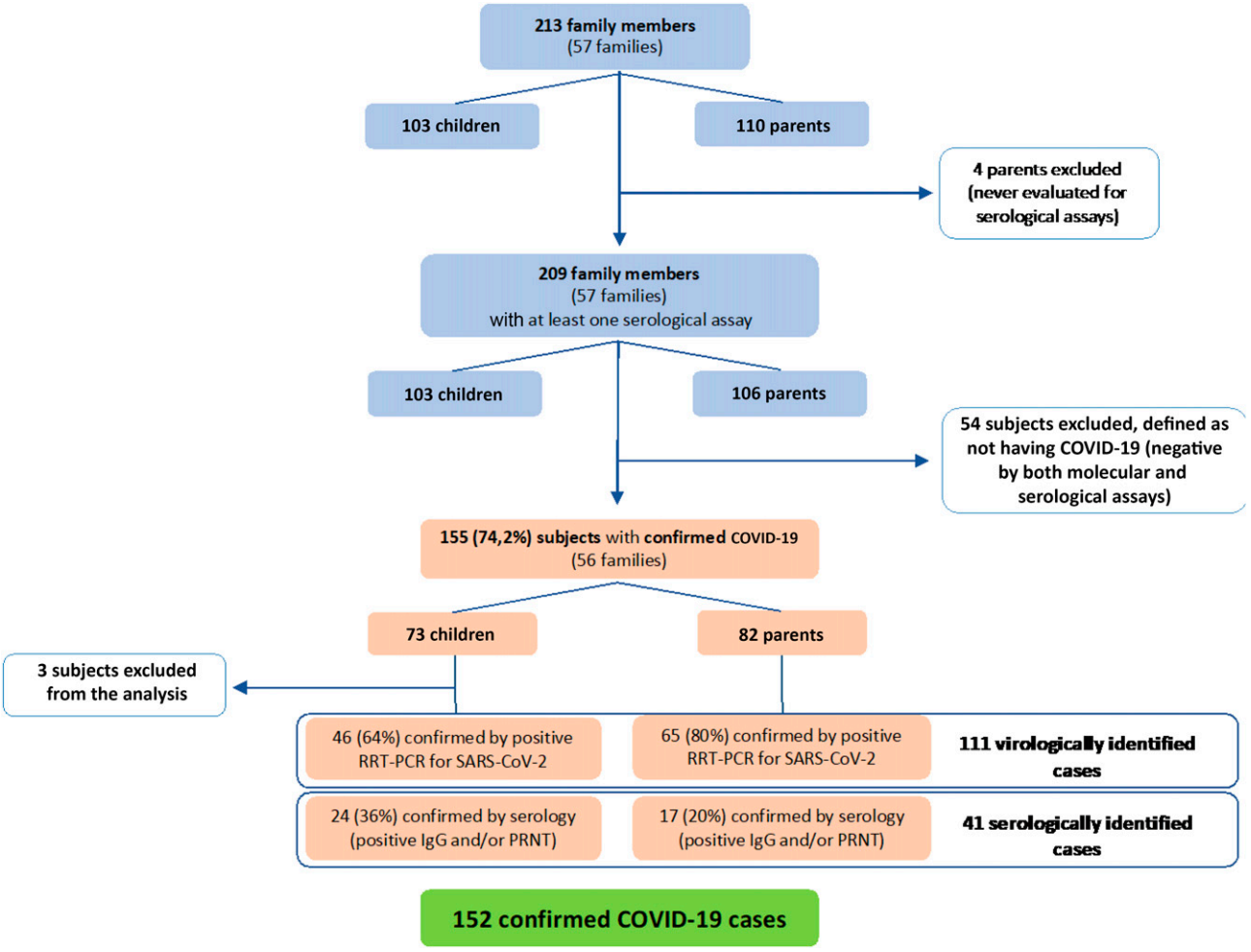
Serological Assays

Blood samples were collected in ethylenediamine tetraacetic acid-coated tubes to further separate cells and plasma by Ficoll procedure. Plasma and cellular samples were appropriately stored at −80°C and in liquid nitrogen, respectively, until use. A high-throughput method for PRNT was used for the quantification of nAbs in plasma samples as previously reported.[16] Samples were heat-inactivated by incubation at 56°C for 30 min, and 2-fold dilutions were prepared in Dulbecco modified Eagle medium. The dilutions, mixed to a 1:1 ratio with a virus solution containing ∼25 focus-forming units of SARS-CoV-2, were incubated for 1 hour at 37°C. Fifty microliters of the virus-serum mixtures were added to confluent monolayers of Vero E6 cells in 96-wells plates and incubated for 1 hour at 37°C in a 5% CO_2 incubator. The inoculum was removed, and 100 mL of overlay solution of minimum essential medium, 2% fetal bovine serum, penicillin (100 U/mL), streptomycin (100 U/mL) and 0.8% carboxy methyl cellulose were added to each well. After a 26-hour incubation, cells were fixed with a 4% paraformaldehyde solution. Visualization of plaques was obtained with an immunocytochemical staining method by using an anti–double-strand RNA monoclonal antibody (J2, 1:10 000; Sci-cons, Budapest, Hungary) for 1 hour, followed by 1 hour incubation with peroxidase-labeled goat antimouse antibodies (1:1000; DAKO, Santa Clara, CA, USA) and a 7-minute incubation with the True Blue (KPL, Milford, MA, USA) peroxidase substrate. Focus-forming units were counted after acquisition of pictures on a flatbed scanner. Biosafety level 3 laboratory setting was used for PRNT tests. The neutralization titer was defined as the reciprocal of the highest dilution resulting in a reduction of the control plaque count >50% ($PRNT_{50}$). Samples recording titers ≥1:10 were considered positive according to a previous validation conducted on a panel of archive samples collected in 2018 in Italy.[18]

Sera from the same donors were analyzed with the CLIA MAGLUMI 2019-nCoV IgM and IgG on the analytical system MAGLUMI 2000 Plus (New Industries Biomedical Engineering Co, Ltd, Shenzhen, China). IgG and IgM immunocomplexes are formed on addition of a recombinant antigen expressing the full-length spike and nucleocapsid proteins of SARS-CoV-2. According to the manufacturer's inserts (271 2019-nCoV IgM, V2.0, 2020-03 and 272 2019-nCoV IgG, V1.2, 2020-02), the 2019-nCoV IgM cutoff is 1.0 AU/mL, whereas the 2019-nCoV IgG cutoff is 1.1 AU/mL. The assay is intended for qualitative detection and differentiation of IgM and IgG antibodies. The combined sensitivity and specificity of IgG and IgM is declared to be 95.6% and 96.0%, respectively.

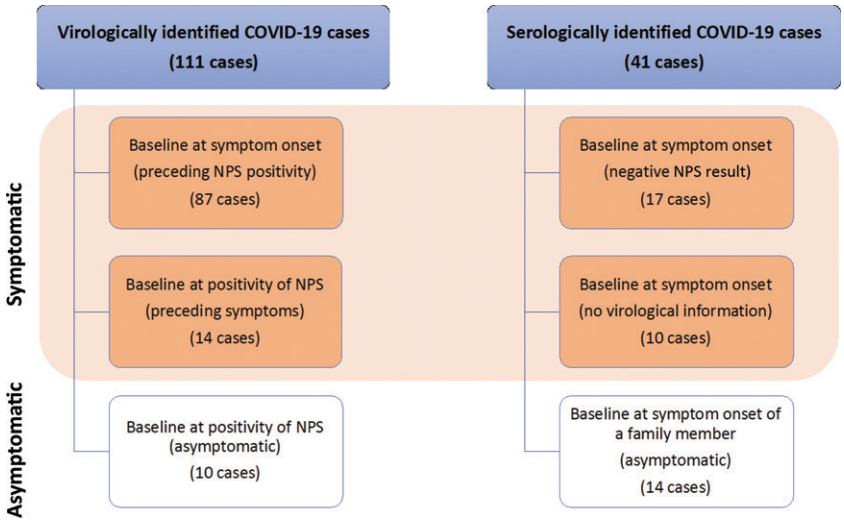
SARS-CoV-2 Viral Load Measurement

A selection of nasopharyngeal NPSs of enrolled subjects that had been originally screened at the Padova University Hospital were made available for quantification of the viral load. NPSs were collected by using flocked swabs in liquid-based collection and transport systems. Total nucleic acids were purified from 200 μl media and eluted in a final volume of 100 μl. Copies of SARS-CoV-2 were quantified by a homemade multiplex quantitative assay on the basis of 1-step ddPCR. The reaction mixture consisted of 5 μl of supermix (Bio-Rad, Hercules, CA, USA), 2 μl of reverse transcriptase, 2 μl of dithiothreitol final concentration 300 mM, forward and reverse primers of SARS-CoV-2 E gene to a final concentration of 400 nM each, and probe to a final concentration of 200 nM and 5 μl of nucleic acids were eluted from nasopharyngeal swab samples to a final volume of 20 μl. Housekeeping *GAPDH* was employed to verify the good quality of RNA extracted and amplified under the same conditions by using the *GAPDH* Kit (Applied Biosystems, Waltham, MA, USA).[19] Each well of the prepared mix was loaded into an 8-channel cartridge, and 70 μl of the Droplet Generation Oil for Probes (Bio-Rad) were added. Droplets were formed in the QX200TM Droplet Generator (Bio-Rad). Droplets in the oil suspension were transferred into a 96-well plate and placed into a Mastercycler (Eppendorf, Hamburg, Germany) with the following cycling parameters: 42°C to 50°C for 60 minutes; 95°C for 10 minutes; 95°C for 30 seconds, and 60°C for 1 minute; the last 2 passages were repeated for 40 cycles followed by 98°C for 10 minutes. The droplets were then read by the QX200TM Droplet Reader (Bio-Rad) and the results were analyzed with the QuantaSoft Analysis Software 1.7.4.0917 (Bio-Rad).[19] Wells with fewer than 10 000 droplets were discarded from the analysis. Each sample was run at least in duplicate. Results were expressed as SARS-CoV-2 copies per 5 μl.



SUPPLEMENTAL FIGURE 5

Flowchart of family clusters of COVID-19 observed from March 1, 2020, to the September 4, 2020, at the COVID-19 follow-up clinic of the Pediatric Department, Department of Women's and Children's Health, University of Padua. [a] Three children who tested positive for SARS-CoV-2 nAbs (PRNT) were further excluded from the analysis because they constituted peculiar cases if compared with the general cohort: in fact, 2 children presented with multisystem inflammatory syndrome in children 4 to 6 weeks after COVID-19 onset, and 1 newborn of a mother who tested positive for COVID-19 presented positive for SARS-CoV-2 nAbs (PRNT) detected 51 days after birth that could be related to maternal immunity and not seroconversion (SARS-CoV-2 molecular assay was never performed at birth). RRT-PCR, real time reverse-transcriptase polymerase chain reaction.



SUPPLEMENTAL FIGURE 6

Identification of cases and criteria for the definition of the baseline time, defined as the most likely onset of infection, for confirmed COVID-19 cases.

SUPPLEMENTAL TABLE 2 Descriptive Analysis of the 57 Families Observed at the Department of Women's and Children's Health of the University Hospital of Padua (Italy), Overall ($n = 209$) and Stratified by Familiar Status as Children or Older Siblings ($n = 103$) and Parents ($n = 106$)

	Overall			Children and Older Siblings			Parents		
	Positive for COVID-19 ($n = 155$)	Negative for COVID-19 ($n = 54$)	P^a	Positive for COVID-19 ($n = 73$)	Negative for COVID-19 ($n = 30$)	P^a	Positive for COVID-19 ($n = 82$)	Negative for COVID-19 ($n = 24$)	P^a
Female sex, *n* (%)	81 (52.3)	23 (42.6)	.22	36 (49.3)	12(40)	.39	45 (54.9)	11 (45.8)	.44
Mean age (SD)	25.8 (17.7)	23.4 (19.5)	.37	8.75 (6.3)	7.12(5.7)	.26	40.9(8.3)	43.7 (7.4)	0.13
Age class, *n* (%)									
<6 y	28 (18.1)	15 (27.8)	.28	28 (38.4)	15 (50)	.63	0 (0)	0 (0)	—
≥6 and <15 y	34 (21.9)	12 (22.2)	—	34 (46.6)	12 (40)	—	0 (0)	0 (0)	—
≥15 y	93 (60)	27 (50)	—	11 (15.1)	3 (10)	—	82 (100)	24 (100)	—
Symptomatic, *n* (%)	128 (82.6)	15 (27.8)	<.001	56 (76.7)	8 (26.7)	<.001	72 (87.8)	7 (29.2)	<.001
WHO classification, *n* (%)									
Asymptomatic	27 (17.4)	39 (72.2)	<.001	17 (23.3)	22 (73.3)	<.001	10 (12.2%)	17 (70.8)	<.001
Mild	118 (76.1)	15 (27.8)	—	53 (68.8)	7 (26.9)	—	65 (79.3)	7 (29.2)	—
Moderate	6 (3.9)	—	—	1 (1.3)	—	—	5 (6.1)	—	—
Severe	1 (0.6)	—	—	0 (0)	—	—	1 (1.2)	—	—
Critical	1 (0.6)	—	—	0 (0)	—	—	1 (1.2)	—	—
MIS-C	2 (1.3)	—	—	2 (2.6)	—	—	0 (0)	—	—
Pediatric comorbidities									
No	—	—	—	57 (78.1)	27 (90)	.26	—	—	—
Yes[b]	—	—	—	16 (21.9)	3 (10)	—	—	—	

MIS-C, multisystem inflammatory syndrome in children; WHO, World Health Organization; —, not applicable.

[a] Student's *t* test, χ^2 test, Fisher's exact test when appropriate.

[b] The following comorbidities were found among 16 children who tested positive for COVID-19: premature birth ($n = 1$), asthma ($n = 5$), allergy ($n = 1$), congenital heart disease ($n = 1$), rheumatic disease ($n = 1$), chronic neuropathy ($n = 1$), immunodeficiency ($n = 2$), cleft lip and palate ($n = 1$), kidney or ureteral disease ($n = 1$).

SUPPLEMENTAL TABLE 3 Serological Data of 283 Plasma Samples Obtained From 152 Confirmed COVID-19 Cases (38 Independent Samples, 245 Dependent Samples Obtained From 114 cases) Among Age Classes, Overall and Stratified by Time from Baseline

	<3 Years	≥3–<6 Years	≥6–<15 Years	≥15 Years	P[a]
All data, irrespective of onset					
n	30	25	58	170	—
GMT (95% CI)					
IgM (kAU/L)[b]	0.7 (0.6–0.9)	0.8 (0.6–1.1)	0.4 (0.4–0.5)	0.5 (0.4–0.5)	.0024
IgG (kAU/L)[b]	1.4 (0.7–2.5)	1.5 (0.8–2.8)	1.5 (1–2.3)	0.9 (0.7–1.2)	.1055
PRNT (end point titer)	298.6 (221.4–402.6)	155.6 (100.9–239.9)	96.7 (68.8–135.8)	47.8 (40.2–56.7)	<.0001
At 1–2 mo from onset					
n	14	8	14	57	—
GMT (95% CI)					
IgM (kAU/L)[b]	0.7 (0.6–0.9)	0.8 (0.5–1.2)	0.6 (0.5–0.8)	0.6 (0.5–0.7)	.4902
IgG (kAU/L)[b]	3.8 (2–7.3)	4.9 (2.4–9.8)	3.9 (1.8–8.1)	1.6 (1–2.7)	.0915
PRNT (end point titer)	275.8 (171.4–443.8)	95.1 (38.1–237.8)	152.3 (83.8–276.6)	62.2 (46.4–83.5)	<.0001
At 3–6 mo, from onset					
n	11	11	34	84	—
GMT (95% CI)					
IgM (kAU/L)[b]	0.7 (0.4–1.2)	0.8 (0.4–1.4)	0.4 (0.3–0.6)	0.5 (0.4–0.6)	.1481
IgG (kAU/L[b]	0.9 (0.5–1.7)	1.6 (0.7–3.7)	1.5 (1–2.4)	0.8 (0.6–1.2)	.1863
PRNT (end point titer)	340.8 (200.8–578.5)	193.3 (91–410.6)	74.2 (45.6–120.6)	42.9 (33.7–54.7)	<.0001
At 7–9 mo, from onset					
n	5	6	10	29	—
GMT (95% CI)					
IgM (kAU/L)[b]	0.7 (0.4–1.3)	0.7 (0.3–2)	0.3 (0.2–0.4)	0.3 (0.3–0.5)	.0203
IgG (kAU/L)[b]	0.1 (0.1–0.2)	0.3 (0.1–0.7)	0.4 (0.2–1.2)	0.4 (0.2–0.6)	.4997
PRNT (end point titer)	278.6 (90.7–855.6)	201.6 (95.1–427.3)	134.5 (68.5–264.3)	38.1 (24.2–60)	<.0001

—, not applicable.
[a] One-way analysis of variance.
[b] Missing data are handled in the analysis.

SUPPLEMENTAL TABLE 4 Temporal Distribution of Sample Collection Among Subjects Who Contributed to the Study With Either 1, 2, or 3 Plasma Samples

	First Sample		Second Sample			Third Sample	
Time From Baseline	1–2 mo	3–6 mo	1–2 mo	3–6 mo	7–9 mo	3–6 mo	7–9 mo
Subjects with only 1 sample (n = 38)	21	17	0	0	0	0	0
Subjects with only 2 samples (n = 97)	52	45	0	62[a]	35[b]	0	0
Subjects with 3 samples (n = 17)	17	0	3	14[a]	0	2	15[b]
Total No. samples per period	90	62	3	76	35	0	17
Total No. samples (n = 283)	152[c]	—	114[c]	—	—	17[c]	—

—, not applicable.
[a] Second samples included in subject-paired analyses of time window 1 (total of 76).
[b] Second and third samples included in subject-paired analyses of time window 2 (total of 50).
[c] Total No. samples represents the combined total across times from baseline for each sample group.

SUPPLEMENTAL TABLE 5 Distribution of Plasma Samples Across Age Classes and Baseline Intervals

	Age Classes, *n* (%)				
Baseline Intervals	<3 y (n = 30)	≥3–<6 y (n = 25)	≥6–<15 y (n = 58)	≥15 y (n = 170)	Total (n = 283)
1–2 mo	14 (15.1)	8 (8.6)	14 (15.1)	57 (61.3)	93 (100.0)
3–6 mo	11 (7.9)	11 (7.9)	34 (24.3)	84 (60.0)	140 (100.0)
7–9 mo	5 (10.0)	6 (12)	10 (20)	29 (58.0)	50 (100.0)

SUPPLEMENTAL TABLE 6 Estimators of Diagnostic Accuracy and Test Agreement of the MAGLUMI 2019-nCoV IgG With the PRNT Assay as Gold Standard Method

	Estimate	95% CI
Sensitivity	0.52	0.46–0.58
Specificity	0.85	0.65–1.0
Positive predictive value	0.99	0.96–1.0
Negative predictive value	0.08	0.04–0.13
Cohen's κ	0.08	0.02–0.13
Overall percent agreement	0.54	0.92–0.97
Positive percent agreement	0.52	0.46–0.58
Negative percent agreement	0.85	0.58–0.96

Estimates are calculated by using the contingency table, plotting 237 of 255 serological samples tested in the study.

SUPPLEMENTAL TABLE 7 Correlation Between SARS-CoV-2 Viral Load (Genome Copies). Detected by Means of ddPCR in NPSs Collected Within 4 Days From Symptom Onset and PRNT Titers Assessed 1–2 months Later, Overall and Stratified for Classes of Age

	NPSs Collected Within 4 Days From Symptom Onset		
	n	Pearson Coefficient	*P*
All ages	32	−0.00796	.9655
<15 y	13	0.67250	.0118
≥15 y	19	−0.29453	.2209