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Supplemental information

Asymptomatic and symptomatic SARS-CoV-2 infections elicit polyfunctional antibodies

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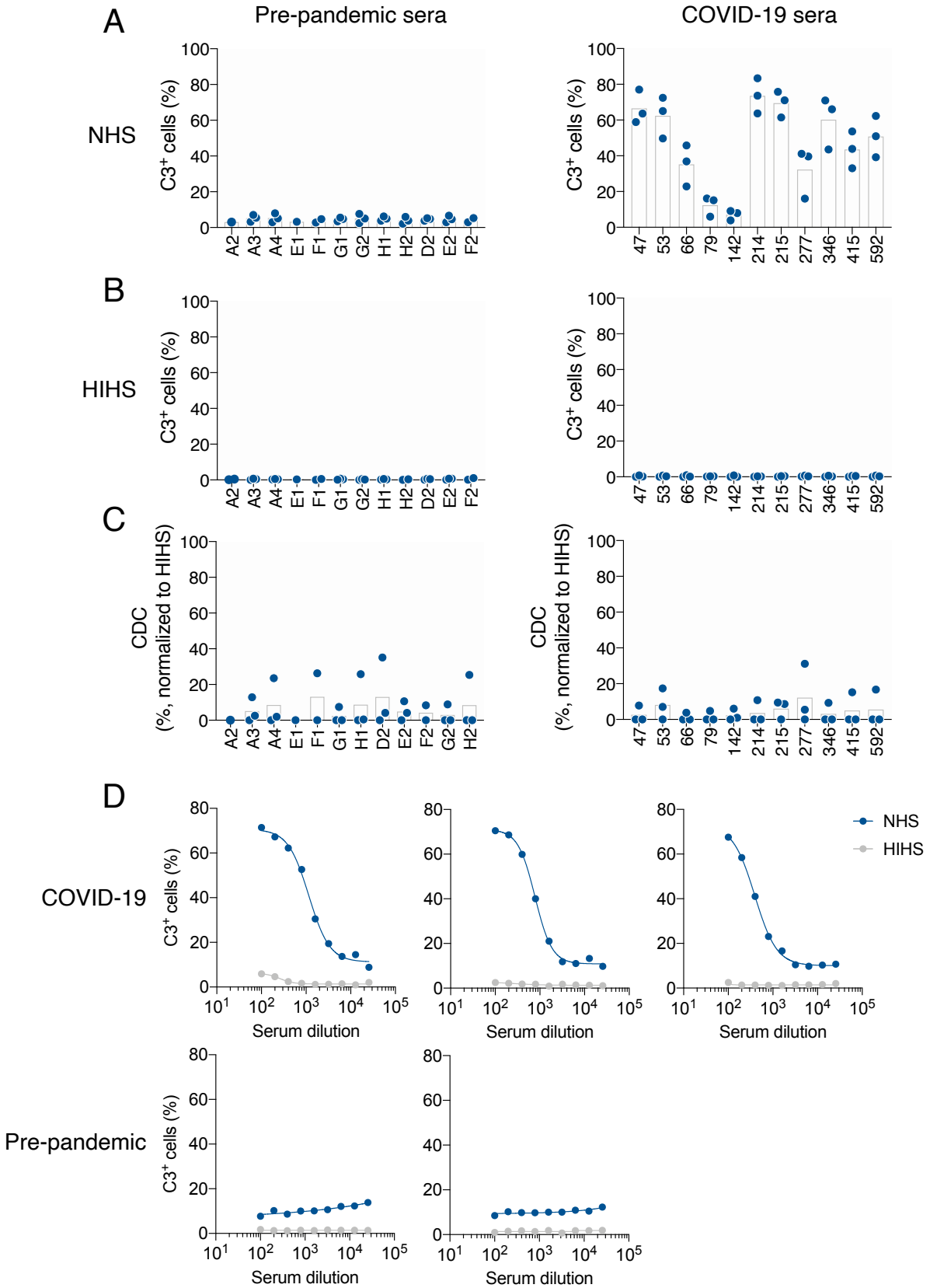


Figure S1. COVID-19 sera induce complement activation on SARS-CoV-2-infected cells. Related to Figure 1.

A. Percentage of C3+ cells among infected cells (Spike+) for each pre-pandemic (left) and COVID-19 (right) serum in presence of normal human serum (NHS) as a source of complement. Each dot represents an independent experiment (n=3).

B. Percentage of C3+ cells among infected cells (Spike+) for each pre-pandemic (left) and COVID-19 (right) serum in presence of heat-inactivated human serum (HIHS) as a control. Each dot represents an independent experiment (n=3).

C. Complement-dependent cytotoxicity (CDC) of infected A549-ACE2 cells was measured for each pre-pandemic (left) and COVID-19 (right) serum as the relative disappearance of Spike+ cells in the NHS condition compared to the HIHS condition. Each dot represents an independent experiment (n=3).

D. Percentage of C3+ cells among infected cells (Spike+) after incubation with increasing dilutions of 3 COVID-19 sera (top) and 2 pre-pandemic sera (bottom), and normal (NHS; blue) or heat-inactivated human serum (HIHS; grey). One representative experiment is shown.

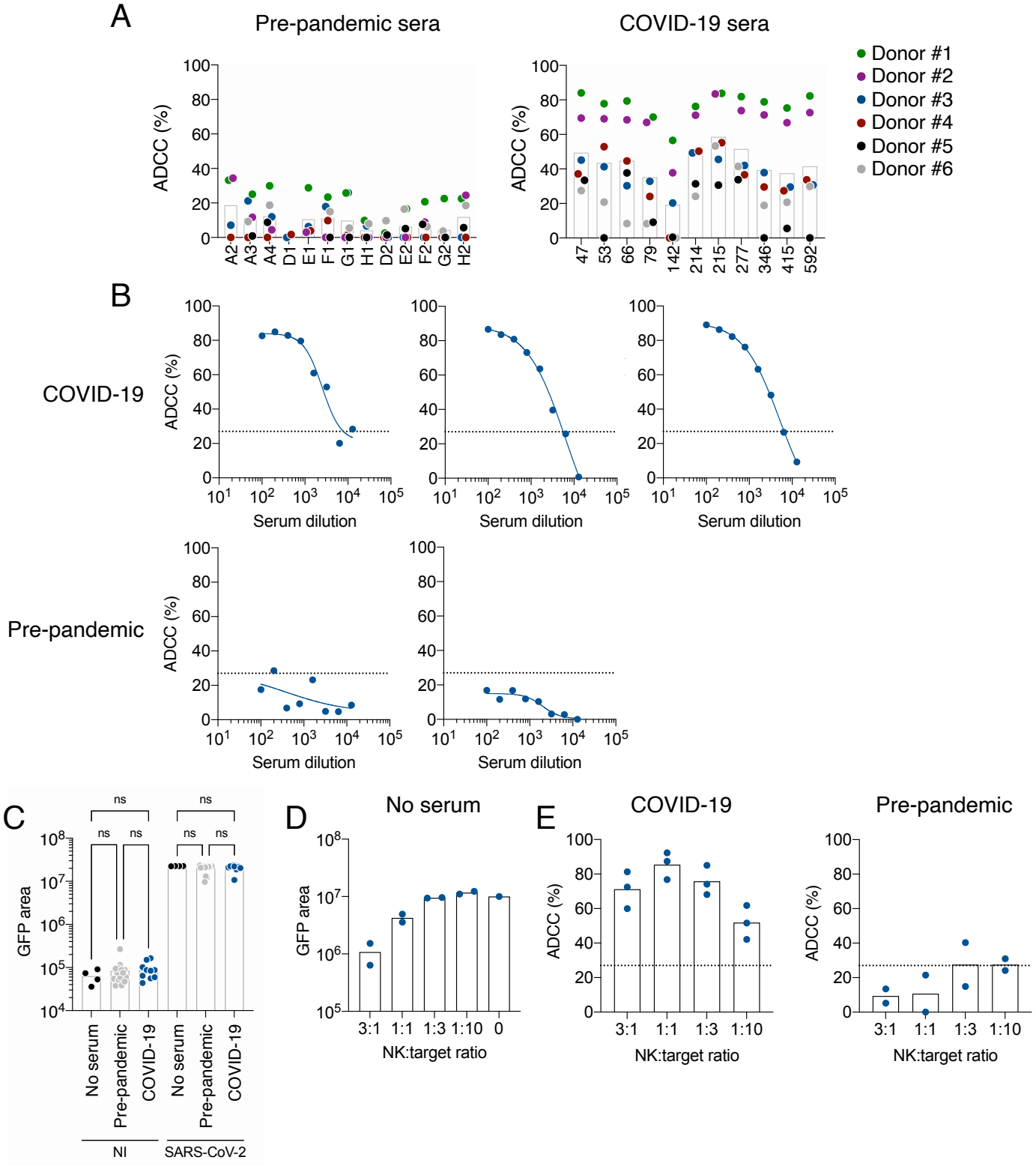


Figure S2. COVID-19 sera trigger antibody-dependent cellular cytotoxicity by NK cells. Related to Figure 2.

A. The percentage of ADCC of infected cells was measured for each pre-pandemic (left) and COVID-19 serum (right). Each dot represents a different donor of NK cells (n=2-6).

B. The percentage of ADCC of infected cells was measured with increasing dilutions of 3 COVID-19 sera (top) and 2 pre-pandemic sera (bottom). The mean of two donors of NK cells is shown.

C. U2OS-ACE2 cells infected or not with SARS-CoV-2 were incubated for 6h with pre-pandemic sera (n=15) or COVID-19 sera (n=10) (dilution 1:100). Cells incubated without serum were included as a control. The area of GFP is plotted. Each dot represents a different donor of serum. ns: not significant (Kruskal-Wallis test).

D. U2OS-ACE2 cells were infected with SARS-CoV-2 and co-cultured for 6h with NK cells at different NK cells:U2OS-ACE2 cells ratios. A control without NK cells (0) was included. The GFP area was then measured to detect any spontaneous killing of infected cells (GFP+) by NK cells. Each dot represents a different donor of NK cells (n=2).

E. U2OS-ACE2 cells were infected with. SARS-CoV-2 and co-cultured for 6h with NK cells at different NK cells:U2OS-ACE2 cells ratios and COVID-19 sera (n=3; left) or pre-pandemic sera (n=2; right). Each dot represents the mean ADCC for a donor of serum measured with NK cells from two different donors.

In B and E, the dashed line represents the threshold of positivity calculated with pre-pandemic sera.

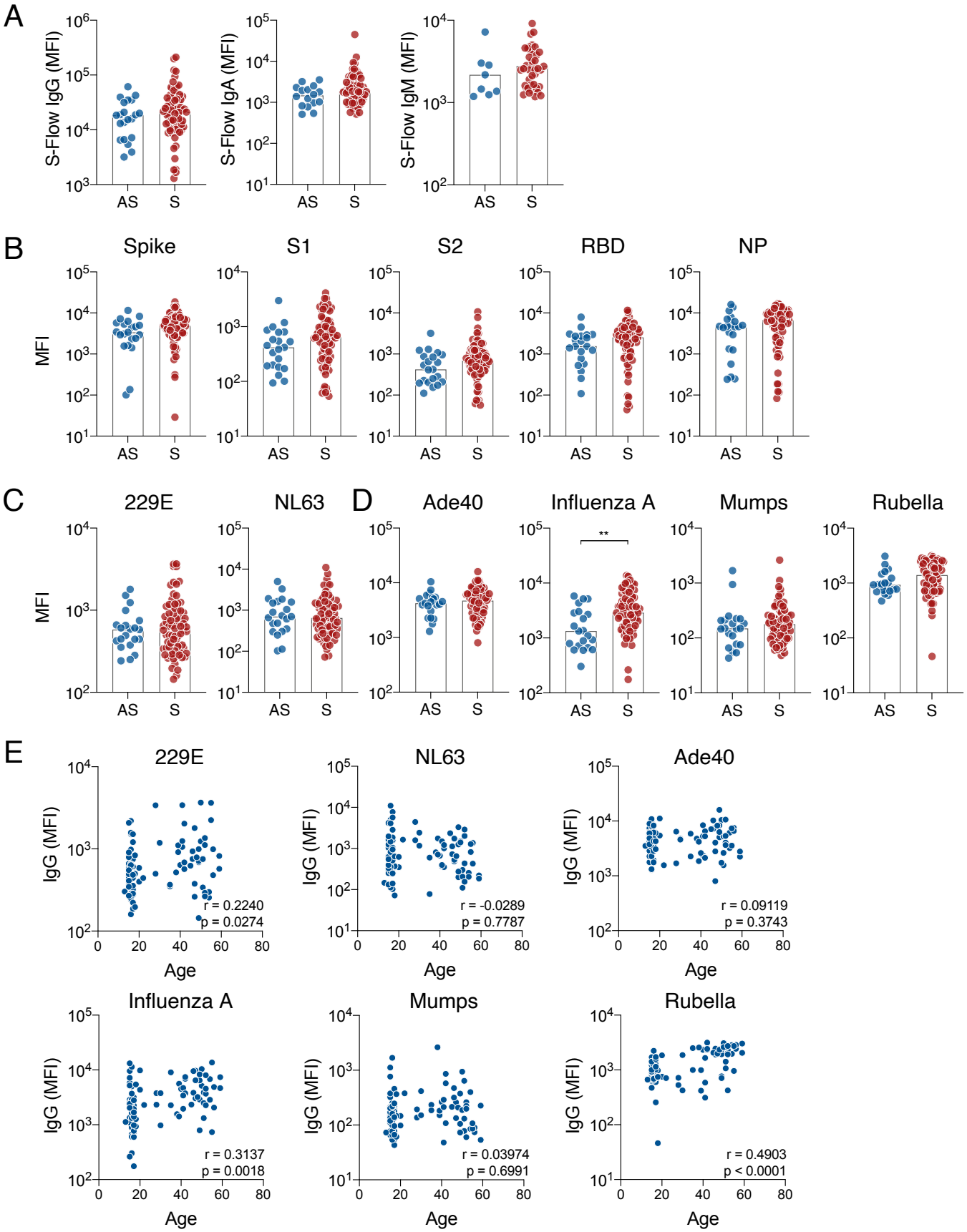


Figure S3. Antibody response against SARS-CoV-2, 229E and NL63 seasonal coronaviruses and control antigens. Related to Figure 3.

A. IgG (left), IgA (middle) and IgM (right) levels were quantified in asymptomatic (AS; blue; n=21) and mildly symptomatic (S; red; n=76) individuals using the flow-cytometry-based S-Flow assay. The median fluorescence intensity (MFI) of staining in S-Flow+ individuals is represented.

B-D. Asymptomatic (n=21; AS) and symptomatic (n=76; S) sera were analyzed by Luminex to measure the antibody response against SARS-CoV-2 viral antigens **(B)**, seasonal coronaviruses 229E and NL63 **(C)** and control antigens **(D)**. The median fluorescence intensities (MFI) are represented. The bar represents the median. **p<0.01 (Mann-Whitney test).

E. Correlation between the age and the antibody levels against the indicated antigens (n=97 individuals). A Spearman correlation test was performed and the correlation r and p-value are indicated.

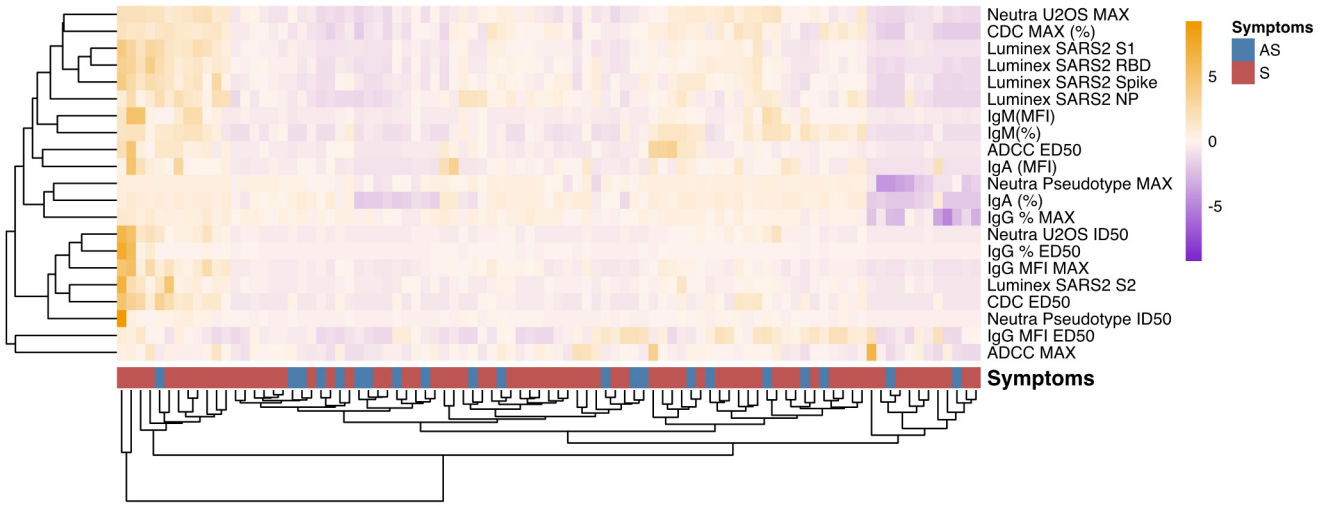


Figure S4. Hierarchical clustering of antibody features in asymptomatic and symptomatic individuals. Related to Figure 5.

Hierarchical clustering of asymptomatic and symptomatic patients based on serological features. Patients and antibody features were clustered using k-means clustering. Each column represents an individual patient. Each feature (line) was normalized with minimum value in purple and maximum value in orange. Patients were tagged as asymptomatic (blue, n=21) or symptomatic (red, n=70).

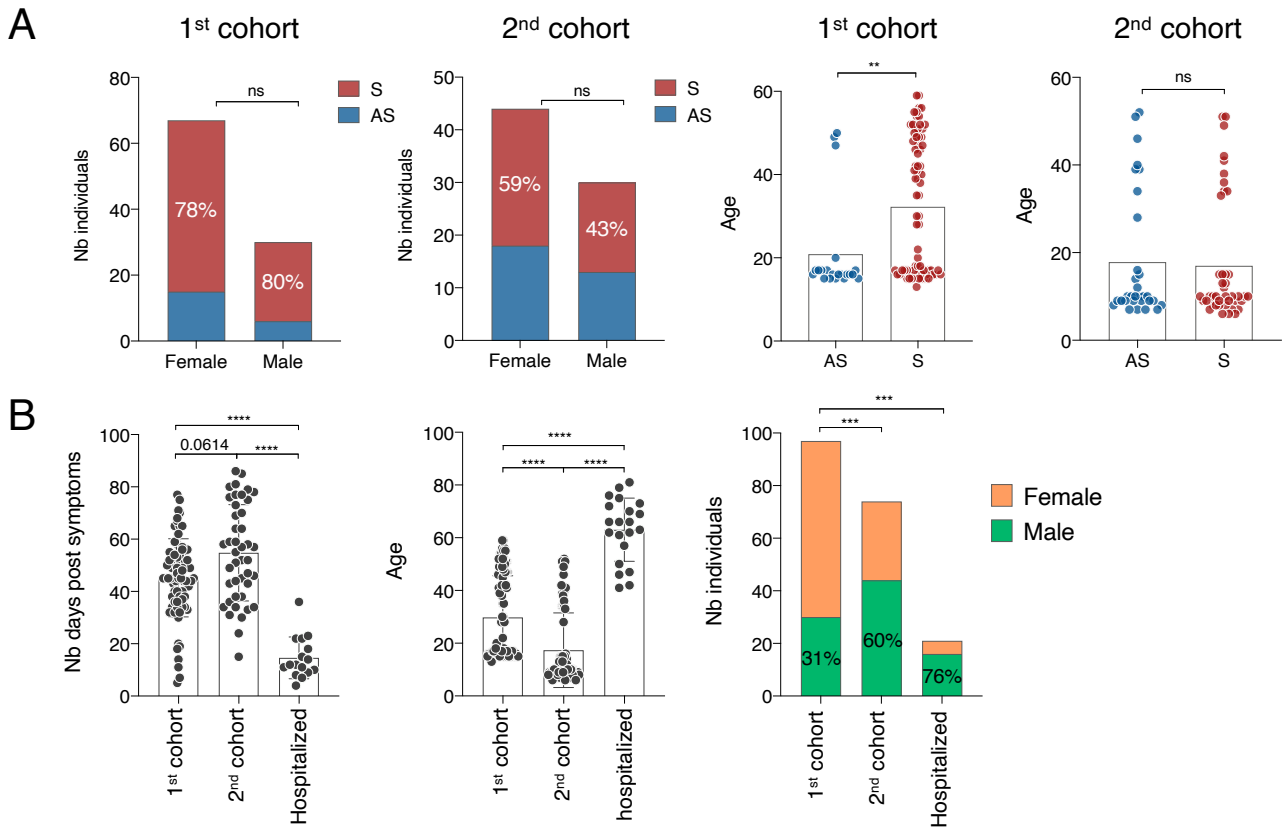
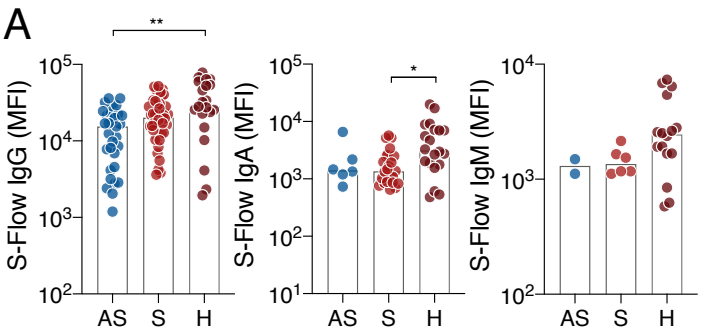


Figure S5. Age and gender composition of the different cohorts. Related to Figure 6.

A. The gender ratio and the age was compared between asymptomatic and symptomatic patients from the first and second cohorts. A Mann-Whitney test was performed. ns. not significant, **p<0.01.

B. The duration post-symptom onset, the age and the gender ratio were compared between the first cohort (AS and S), the second cohort (AS and S) and the hospitalized patients (H). A Kruskal-Wallis test was performed. ***p<0.001, ****p<0.0001.



B

Feature	%, in all individuals			MFI, in S-Flow+ individuals		
	IgG (%)	IgA (%)	IgM (%)	IgG (MFI)	IgA (MFI)	IgM (MFI)
AS vs. S p-value (Mann-Whitney test)	ns 0.066	*** 0.0007	* 0.0142	* 0.0177	ns 0.74	ns 0.5

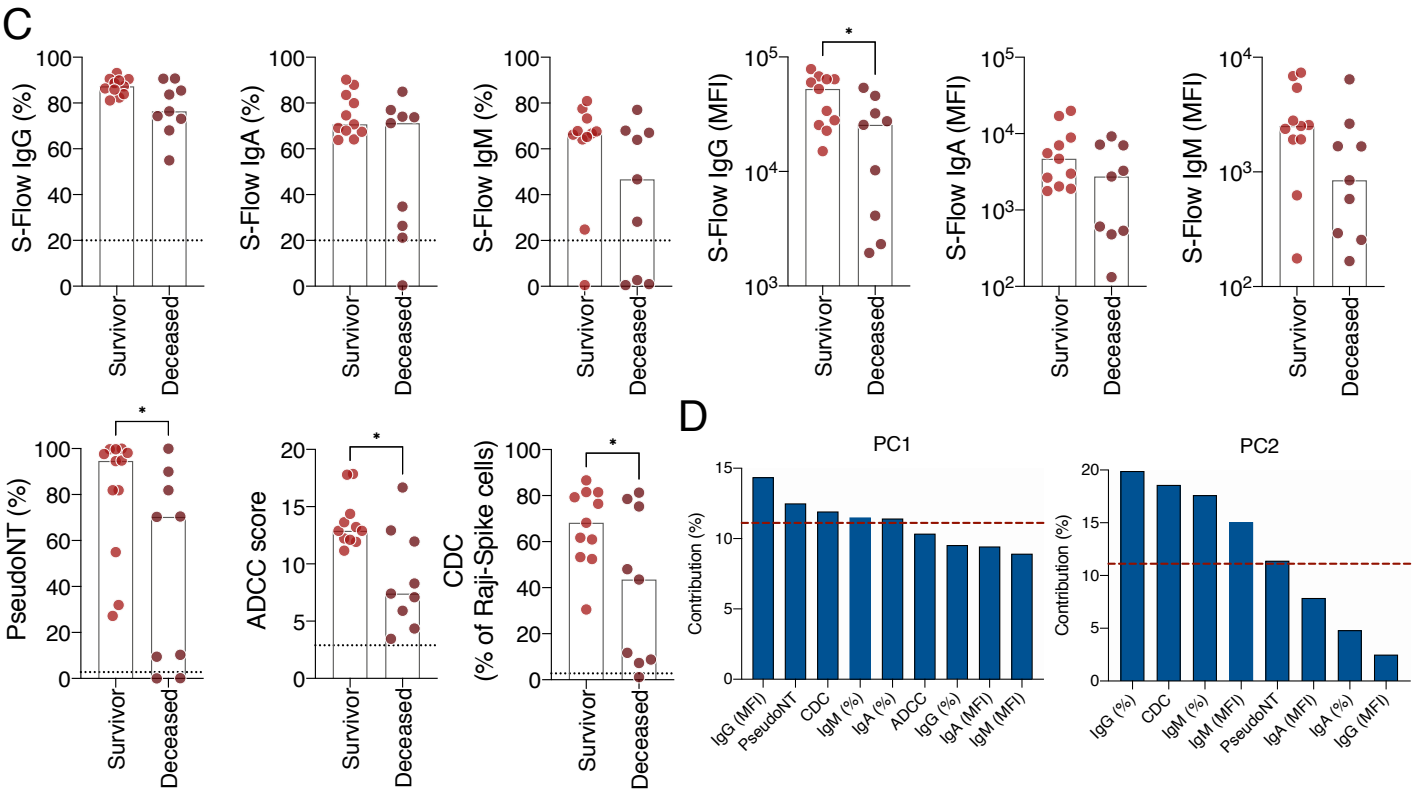


Figure S6. Antibody response in other cohorts of asymptomatic, symptomatic and hospitalized individuals. Related to Figure 6.

A. IgG (left), IgA (middle) and IgM (right) levels were quantified in asymptomatic (AS; blue; n=31), mildly symptomatic (S; red; n=43) and hospitalized (H; brown; n=21) individuals using the flow-cytometry-based S-Flow assay. The median fluorescence intensity (MFI) of staining in S-Flow+ individuals is represented.. The bar represents the median *p<0.05, **p<0.01 (Kruskal-Wallis test).

B. IgG, IgA and IgM levels in asymptomatic and symptomatic patients were compared using a Mann-Whitney test. Comparisons include the percentages of positive cells in all individuals (grey) and the MFI of staining in S-Flow+ individuals only (blue).

C. Antibody features were compared between hospitalized patients who survived (n=11) or died (n=9) from the infection. *p<0.05 (Mann-Whitney test).

D. Contribution percentages to first (left) and second (right) dimensions of the 9 antibody features included in the principal component analysis performed on asymptomatic (n=31), symptomatic (n=43), and hospitalized patients (n=21).

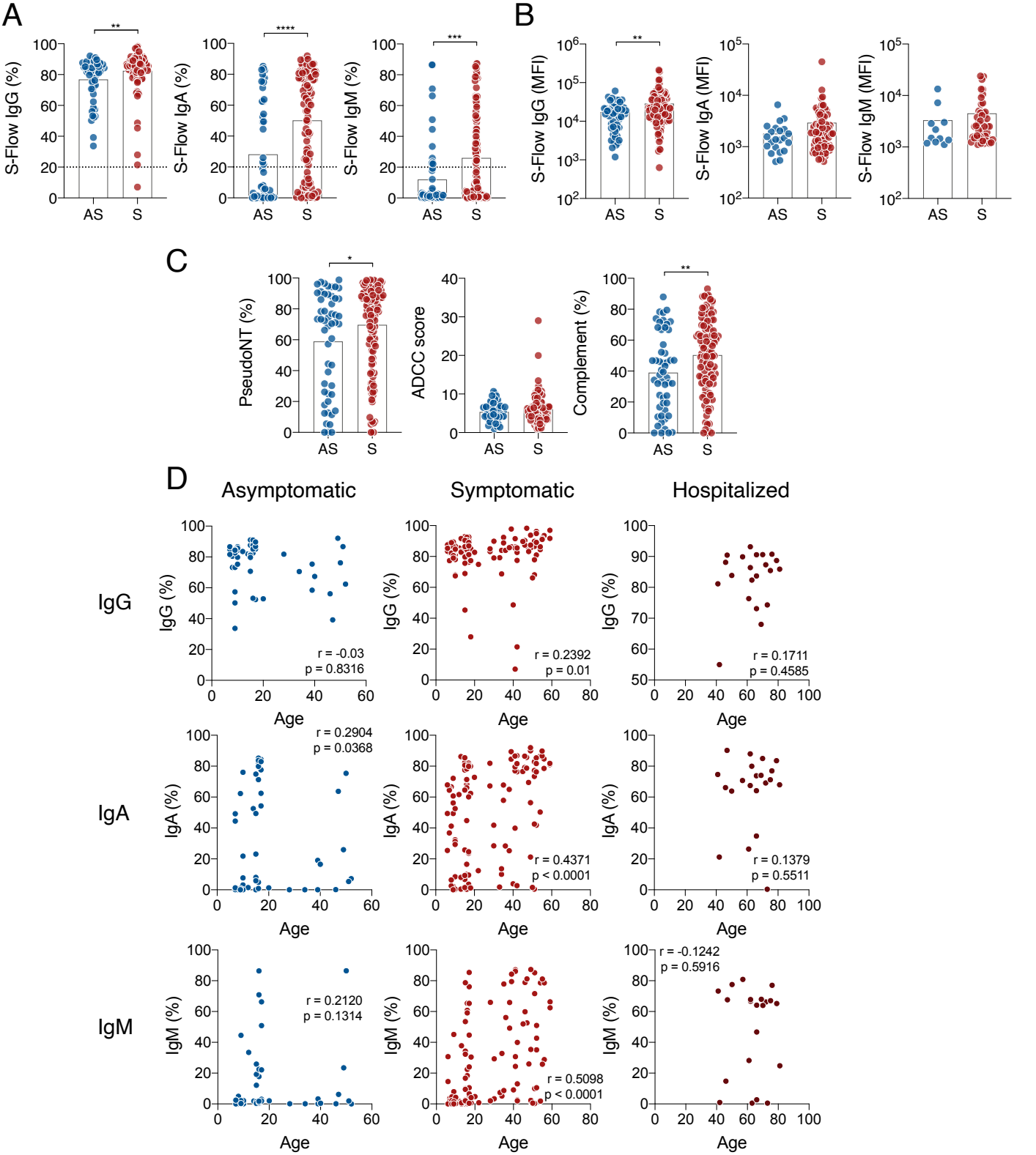


Figure S7. Pooled analysis of antibody responses in asymptomatic symptomatic individuals from the two cohorts. Related to Figure 6.

A. IgG (left), IgA (middle) and IgM (right) levels were quantified in asymptomatic (AS; blue; n=52) and mildly symptomatic (S; red; n=119) individuals from the two cohorts using the flow-cytometry-based S-Flow assay. The percentage of positive cells is represented. The dotted line represents the threshold of positivity measured with pre-pandemic sera.

B. IgG (left), IgA (middle) and IgM (right) levels were quantified in asymptomatic (AS; blue; n=52) and mildly symptomatic (S; red; n=119) individuals from the two cohorts using the flow-cytometry-based S-Flow assay. The median fluorescence intensity (MFI) of staining in S-Flow+ individuals is represented.

C. AS (n=52) and S (n=119) sera from the two cohorts were tested for their ability to neutralize Spike pseudoparticles (left), trigger ADCC in the Jurkat-CD16-NFAT-rLuc/Raji-Spike system (middle) or trigger CDC of Raji-Spike cells (right).

D. Correlation between the age of donors and IgG (top), IgA (middle) or IgM (bottom) levels in asymptomatic (left; n=52), symptomatic (center; n=119) or hospitalized (right; n=21) patients. A Spearman correlation test was performed and the correlation r and p-value are indicated.

In A-C, the bar indicates the median. Mann-Whitney tests were performed (*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001).

Table S1. Characteristics of the pre-pandemic individuals and donors of NK cells. Related to Figures 1-2.

	Pre-pandemic N=28	**NK cells donors N=6**
Gender (N (%))		
male	10 (35.7)	4 (66.6)
Age *(median (IQR))*	46 (35-52)	>18; <70
Sampling date (interval)	September 2014 - April 2019	July 2019 - September 2019

Table S2. Characteristics of patients enrolled in Crépy-en-Valois high school. Related to Figures 1-5.

	Asymptomatic N=21	**Symptomatic N=76**	**total N=97**
	N (%)	*N (%)*	*N (%)*
Gender			
male	6 (28.6)	24 (31.6)	30 (30.9)
Age *(median (IQR))*	16 (16-17)	30 (16.5-48.5)	18 (16-47)
Status			
teacher	0	17 (22.4)	17 (17.5)
staff (other than teacher)	0	8 (10.5)	8 (8.2)
parents	2 (9.5)	12 (15.8)	14 (14.4)
relatives	1 (4.8)	5 (6.6)	6 (6.2)
students	18 (85.7)	33 (43.4)	51 (52.6)
other	0	1 (1.3)	1 (1.0)
Hospitalization (outpatients)	0	8 (10.5)	8 (8.2)
Symptomes			
fever	0	40 (52.6)	40 (41.2)
cough	0	35 (46.0)	35 (36.1)
shortness of breath	0	21 (27.6)	21 (21.6)
loss of taste	0	28 (36.8)	28 (28.9)
loss of smell	0	30 (39.5)	30 (30.9)
muscle pain	0	37 (48.7)	37 (38.1)
sore throat	0	29 (38.2)	29 (29.9)
rhinorrhea	0	37 (48.7)	37 (38.1)
diarrhea	0	26 (34.2)	26 (26.8)
cephalgia	0	41 (53.9)	41 (42.3)
asthenia	0	41 (53.9)	41 (42.3)

Table S3. Characteristics of patients enrolled in Crépy-en-Valois primary schools. Related to Figure 6.

	Asymptomatic N=31	**Symptomatic N=43**	**total N=74**
	N (%)	*N (%)*	*N (%)*
Gender			
male	13 (41.9)	17 (39.5)	30 (40.5)
Age *(median (IQR))*	10 (9-28)	10 (9-15)	10 (9-16)
Status			
teacher	0	1 (2.3)	1 (1.3)
staff (other than teacher)	0	0	0
parents	8 (25.8)	9 (20.9)	17 (23.0)
relatives	4 (12.9)	7 (16.3)	11 (14.9)
students	19 (61.3)	26 (60.5)	45 (60.8)
other	0	0	0
Hospitalization	0	0	0
Symptomes			
fever	0	21 (48.8)	21 (28.4)
cough	0	16 (37.2)	16 (21.6)
shortness of breath	0	7 (16.3)	7 (9.5)
loss of taste	0	6 (13.9)	6 (8.1)
loss of smell	0	5(11.6)	5 (6.8)
muscle pain	0	10 (23.3)	10 (13.5)
sore throat	0	12 (27.9)	12 (16.2)
rhinorrhea	0	15 (34.9)	15 (20.3)
diarrhea	0	12 (27.9)	12 (16.3)
cephalgia	0	17 (39.5)	17 (23.0)
asthenia	0	22 (51.2)	22 (29.7)

Table S4. Characteristics of hospitalized patients. Related to Figures 6-7.

	Total N=21
	N (%)
Gender	
male	16 (76.2)
Age *(median (IQR))*	63 (55-72)
ICU admission	
yes	19 (90.5)
no	1 (4.8)
missing information	1 (4.8)
Treatment	
antivirals (remdesivir or lopinavir/ritonavir)	14 (66.7)
antibiotics	18 (85.7)
corticostreoids	11 (52.4)
antifungal agent	3 (14.3)
hydroxychloroquine	3 (14.3)
missing information	1 (4.8)
Outcome	
discharge alive	11 (52.4)
death	9 (42.8)
missing information	1 (4.8)
Symptomes	
fever	19 (90.5)
cough	14 (66.7)
cough with sputum	4 (19)
sore throat	1 (4.8)
wheezing	3 (14.3)
myalgia	6 (28.6)
arthralgia	3 (14.3)
fatigue or malaise	10 (47.6)
dyspnea	16 (76.2)
headache	2 (9.5)
altered consciousness or confusion	1 (4.8)
abdominal pain	2 (9.5)
nausea	4 (19)
diarrhea	4 (19)
skin rash	1 (4.8)
missing information	1 (4.8)
Comorbidities	
hypertension	9 (42.9)
chronic kidney disease	3 (14.3)
diabetes	4 (19)
chronic cardiac disease	6 (28.6)
chronic pulmonary disease	2 (9.5)
asthma	2 (9.5)
chronic neurological disorder	1 (4.8)
malignant neoplasm	1 (4.8)
chronic hematologic disease	1 (4.8)
rheumatologic disorder	1 (4.8)
missing information	1 (4.8)