

1 **Longitudinal evaluation and decline of antibody responses in SARS-CoV-2 infection**

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28 **Abstract:**

29 Antibody (Ab) responses to SARS-CoV-2 can be detected in most infected individuals 10-15
30 days following the onset of COVID-19 symptoms. However, due to the recent emergence of
31 this virus in the human population it is not yet known how long these Ab responses will be
32 maintained or whether they will provide protection from re-infection. Using sequential serum
33 samples collected up to 94 days post onset of symptoms (POS) from 65 RT-qPCR confirmed
34 SARS-CoV-2-infected individuals, we show seroconversion in >95% of cases and neutralizing
35 antibody (nAb) responses when sampled beyond 8 days POS. We demonstrate that the
36 magnitude of the nAb response is dependent upon the disease severity, but this does not
37 affect the kinetics of the nAb response. Declining nAb titres were observed during the follow
38 up period. Whilst some individuals with high peak ID₅₀ (>10,000) maintained titres >1,000 at
39 >60 days POS, some with lower peak ID₅₀ had titres approaching baseline within the follow
40 up period. A similar decline in nAb titres was also observed in a cohort of seropositive
41 healthcare workers from Guy's and St Thomas' Hospitals. We suggest that this transient nAb
42 response is a feature shared by both a SARS-CoV-2 infection that causes low disease severity
43 and the circulating seasonal coronaviruses that are associated with common colds. This study
44 has important implications when considering widespread serological testing, Ab protection
45 against re-infection with SARS-CoV-2 and the durability of vaccine protection.

46 **Introduction:**

47 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a betacoronavirus
48 responsible for coronavirus disease-19 (COVID-19). Spike (S) is the virally encoded surface
49 glycoprotein facilitating angiotensin converting enzyme-2 (ACE-2) receptor binding on target
50 cells through its receptor binding domain (RBD). In a rapidly evolving field, researchers have
51 already shown that, in most cases, individuals with a confirmed PCR diagnosis of SARS-CoV-2
52 infection develop IgM, IgA and IgG against the virally encoded surface spike protein (S) and
53 nucleocapsid protein (N) within 1-2 weeks post onset of symptoms (POS) and remain elevated
54 following initial viral clearance.¹⁻⁷ S is the target for nAbs, and a number of highly potent
55 monoclonal antibodies (mAbs) have been isolated that predominantly target the RBD.^{8,9} A
56 wide range of SARS-CoV-2 neutralizing antibody (nAb) titres have been reported following
57 infection and these vary depending on the length of time from infection and the severity of
58 disease.⁴ Further knowledge on the magnitude, timing and longevity of nAb responses
59 following SARS-CoV-2 infection is vital for understanding the role nAbs might play in disease
60 clearance and protection from re-infection (also called renewed or second wave infections).
61 Further, as a huge emphasis has been placed on serological assays to determine
62 seroprevalence against SARS-CoV-2 in the community and estimating infection rates, it is
63 important to understand immune responses following infection to define parameters in
64 which Ab tests can provide meaningful data in the absence of PCR testing in population
65 studies.

66

67 Ab responses to other human coronaviruses have been reported to wane over time.¹⁰⁻¹³ In
68 particular, Ab responses targeting endemic human alpha- and betacoronaviruses can last for
69 as little as 12 weeks,¹⁴ whereas Abs to SARS-CoV and MERS can be detected in some
70 individuals 12-34 months after infection.^{11,15} Although several cross-sectional studies of nAb
71 responses arising from SARS-CoV-2 infection have been reported,^{4,7} there is currently a
72 paucity of information on the longevity of the nAb response using multiple sequential samples
73 from individuals in the convalescent phase beyond 30-40 days POS.^{3,5,16} This study uses
74 sequential samples from 65 individuals with PCR confirmed SARS-CoV-2 infection and 31
75 seropositive healthcare workers (HCW) up to 94 days POS to understand the kinetics of nAb
76 development and the magnitude and durability of the nAb response.

77

78 Here, we measured the Ab binding response to S, the receptor binding domain (RBD) and N,
79 as well as the neutralization potency against SARS-CoV-2 using an HIV-1 based pseudotype
80 assay. We show that IgM and IgA binding responses decline after 20-30 days POS. We
81 demonstrate that the magnitude of the nAb response is dependent upon the disease severity
82 but this does not impact on the time to ID₅₀ peak (serum dilution that inhibits 50% infection).
83 nAb titres peak on average at day 23 POS and then decrease 2- to 23-fold during an 18-65 day
84 follow up period. In individuals that only develop modest nAb titres following infection (100-
85 300 range), titres become undetectable (ID₅₀ <50) or are approaching baseline after ~50 days
86 highlighting the transient nature of the Ab response towards SARS-CoV-2 in some individuals.
87 In contrast, those with high peak ID₅₀ for neutralization maintain nAb titres in the 1000-3500
88 range at the final timepoint tested (>60 days POS). This study has important implications
89 when considering protection against re-infection with SARS-CoV-2 and the durability of
90 vaccine protection.

91

92 **Results:**

93 Cohort description:

94 The antibody response in 65 RT-qPCR confirmed SARS-CoV-2-infected individuals was studied
95 over sequential time points. The cohort consisted of 59 individuals admitted to, and 6
96 healthcare workers (HCW) at, Guy's and St Thomas' NHS Foundation Trust (GSTFT). The
97 cohort were 77.2% male with average age of 55.2 years (range 23-95 years). Ethnicity
98 information was not collected on this cohort. A severity score was assigned to patients based
99 on the maximal level of respiratory support they required during their period of
100 hospitalisation. The score, ranging from 0-5 (see methods), was devised to mitigate
101 underestimating disease severity in patients not for escalation above level one (ward-based)
102 care. This cohort included the full breadth of COVID-19 severity, from asymptomatic infection
103 to those requiring extra corporeal membrane oxygenation (ECMO) for severe respiratory
104 failure. Comorbidities included diabetes mellitus, hypertension, and obesity, with a full
105 summary in **Table S1**. Sequential serum samples were collected from individuals at time-
106 points between 1- and 94-days post onset of symptoms (POS) and were based upon
107 availability of discarded samples taken as part of routine clinical care, or as part of a HCW
108 study.

109

110 Antibody binding responses to SARS-CoV-2:

111 The IgG, IgM and IgA response against spike (S), the receptor binding domain (RBD) and
112 nucleocapsid (N) were measured by ELISA over multiple time points (**Figure 1 and S1**).⁶
113 Initially, the optical density at 1:50 serum dilution was measured for 300 samples from the 65
114 individuals (**Figure 1 and S1**). Only 2/65 individuals (3.1%) did not generate a detectable Ab
115 response against any of the antigens in the follow up period (**Table S2**). However, sera were
116 only available up until 2- and 8-days POS for these two individuals and as the mean time to
117 seroconversion against at least 1 antigen was 12.6 days POS, it is likely these individuals may
118 have seroconverted at a later time point after they were discharged from hospital. IgG
119 responses against S, RBD and N antigens were observed in 92.3%, 89.2% and 93.8% of
120 individuals respectively (**Table S2**). The frequency of individuals generating an IgM response
121 was similar to IgG, with 92.3%, 92.3% and 95.4% seropositive against S, RBD and N
122 respectively. The frequency of individuals with an IgA response to RBD and N was lower, with
123 only 72.3% and 84.6% seropositive respectively (**Table S2**) whereas the IgA to S frequency was
124 similar to the IgM and IgG.

125
126 A cumulative frequency analysis of positive IgG, IgA and IgM responses against S, RBD and N
127 across the cohort did not indicate a more rapid elicitation of IgM and IgA responses against a
128 particular antigen (**Figure 1A and S2A**) and may reflect the sporadic nature in which
129 sequential serum samples were collected. Therefore, a subset of donors from whom sera was
130 collected over sequential time points early in infection (<14 days POS) were analysed further
131 and different patterns of seroconversion were observed (**Figure S2B**). 51.6% (16/31) of
132 individuals showed synchronous seroconversion to IgG, IgM and IgA whilst some individuals
133 showed singular seroconversion to IgG (9.7%), IgM (9.7%) and IgA (9.7%). 58.1% (18/31) of
134 individuals showed synchronous seroconversion to S, RBD and N, whereas singular
135 seroconversion to N or S were both seen in 16.1% of individuals.

136
137 Longitudinal analysis across sequential samples highlighted the rapid decline in the IgM and
138 IgA response to all three antigens following the peak OD between 20- and 30-days POS for
139 IgM and IgA respectively (**Figure 1B and S1A**) as might be expected following an acute
140 infection. For some individuals sampled at time points >60 days POS, the IgM and IgA
141 responses were approaching baseline (**Figure 2B and S1A**). In contrast, the IgG OD (as

142 measured at 1:50 dilution) remained high in the majority of individuals, even up to 94 days
143 POS (**Figure 1B and S1A**). However, differences were apparent when patients were stratified
144 by disease severity and when half maximal binding (EC_{50}) was measured (see below).

145

146 Neutralizing antibody responses to SARS-CoV-2:

147 We next measured SARS-CoV-2 neutralization potency using HIV-1 (human immunodeficiency
148 virus-1) based virus particles, pseudotyped with SARS-CoV-2 S^{17,18} in a HeLa cell line stably
149 expressing the ACE2 receptor. Increased neutralization potency was observed with increasing
150 days POS (**Figure 2A**) with each individual reaching a peak neutralization titre (ranging from
151 98 to 32,000) after an average of 23.1 days POS (range 1-66 days) (**Figure S1B**). Only two
152 individuals (3.1%) did not develop a nAb response ($ID_{50} < 50$) which was consistent with their
153 lack of binding Abs at the time points tested (< 8 days POS). At peak neutralization, 7.7% had
154 low (50-200), 10.8% medium (201-500), 18.5% high (501-2000) and 60.0% potent (2001+)
155 neutralizing titres. For serum samples collected after 65 days POS, the percentage of donors
156 with potent nAbs ($ID_{50} > 2000$) had reduced to 16.7% (**Table S3**). Neutralization ID_{50} values
157 correlated well with IgG, IgM and IgA binding OD values to all three antigens, S, RBD and N
158 (**Figure S3**), and the best fit (r^2) was observed between ID_{50} and the OD for S IgA and S IgM.
159 The average time to detectable neutralization was 14.3 days POS (range 3-59 days). At earlier
160 time points POS, some individuals displayed neutralizing activity before an IgG response to S
161 and RBD was detectable by ELISA (**Figure S2C**). This highlights the capacity of S- and RBD-
162 specific IgM and IgA in acute infection to facilitate neutralization in the absence of measurable
163 IgG.¹⁹

164

165 To determine how disease severity impacts Ab titres, we compared the ID_{50} values between
166 individuals with 0-3 disease severity with those in the 4/5 group (**Figure 3**). Although the
167 magnitude of the nAb response at peak neutralization was significantly higher in the severity
168 4/5 group (**Figure 3A**), the time taken to measure detectable nAb titres (**Figure 3C**) and the
169 time of peak neutralization (**Figure 3B**) did not differ between the two groups suggesting
170 disease severity enhances the magnitude of the Ab response but does not alter the kinetics.
171 Comparison of the IgG, IgM and IgA OD values against S at peak neutralization showed
172 significantly higher IgA and IgM ODs in the severity 4/5 group but no significant difference
173 was observed for IgG to S (**Figure 3D-F**). This observation may further highlight a potential

174 role for IgA and IgM in neutralization.¹⁹ Within the severity 4/5 group, a proportion of patients
175 were treated with immunomodulation for a persistent hyperinflammatory state
176 characterized by fevers, markedly elevated CRP and ferritin, and multi-organ dysfunction.
177 Despite an initial working hypothesis that antibody responses may differ either as a cause or
178 consequence of this phenotype, no difference in ID₅₀ titres was observed between these
179 individuals and the remainder of the severity 4/5 cases (**Figure 3G**).

180

181 Longevity of the Ab response:

182 Following the peak in neutralization, a waning in ID₅₀ was detected in individuals sampled at
183 >40 days POS. Comparison of the ID₅₀ at peak neutralization and ID₅₀ at the final time point
184 collected showed a decrease in almost all cases (**Figure 4A**). For some individuals with
185 severity score 0, where the peak in neutralization was in the ID₅₀ range 100-300,
186 neutralization titres became undetectable (ID₅₀ <50) in the pseudotype neutralization assay
187 at subsequent time points (**Figure 4A and 2B**). For example, donors 52 and 54 both generated
188 a low nAb response (peak ID₅₀ of 174 and 434 respectively) but no neutralization could be
189 detected in our assay 39 and 34 days after the peak in ID₅₀ respectively (**Figure 2B**).

190

191 To gain a more quantitative assessment of the longevity of the IgG binding titres specific for
192 S, RBD and N, EC₅₀ values were measured at the peak of neutralization and compared to the
193 EC₅₀ at the final time point collected. EC₅₀ values correlated very well with ID₅₀ (**Figure 4E**).
194 Similar to neutralization potency, a decrease in EC₅₀ was observed within the follow up period
195 for S, RBD and N (**Figure 4B-D**). For those whose nAb titre decreased towards baseline, the
196 EC₅₀ for IgG to S and RBD also decreased in a similar manner. Finally, to determine whether
197 the reduction in IgG titres might plateau, EC₅₀ values for all time points for four representative
198 individuals were measured who had multiple samples collected in the convalescent phase
199 (**Figure 4F**). A steady decline in neutralization was accompanied by a decline in IgG binding to
200 all antigens within the time window studied. Further assessment of Ab binding and
201 neutralizing titres in samples collected >94 days POS will be essential to fully determine the
202 longevity of the nAb response.

203

204 Ab responses in a Healthcare worker cohort:

205 To gain further understanding of Ab responses in SARS-CoV-2 infection we next analysed
206 sequential serum samples from 31 seropositive (as determined by an IgG response to both N
207 and S)⁶ healthcare workers (HCW) from GSTFT. Ab responses in these individuals are likely to
208 be more akin to those who were never hospitalised. Sera were collected every 1-2 weeks from
209 March - June 2020 and any symptoms relating to COVID-19 recorded. Acute infection, as
210 determined by detectable SARS-CoV-2 RNA on RT-qPCR, was not measured routinely. 80.6%
211 (25/31) of seropositive individuals recorded COVID-19 compatible symptoms (including fever,
212 cough and anosmia) since 1st February 2020, 19.4% (6/31) reported none.

213

214 IgG and IgM binding to S, RBD and N by ELISA and neutralization titres were measured over
215 time using sequential samples (**Figure 5A and S4A**). Similar to the patient cohort, ID₅₀ values
216 correlated with the OD values for IgG and IgM against S and RBD (**Figure S4B**). However, in
217 contrast, the IgM and IgG responses to N in HCW correlated poorly ($r^2 = 0.030$ and 0.381
218 respectively) (**Figure S4B**). Comparison of the peak ID₅₀ between asymptomatic individuals,
219 and symptomatic HCWs showed a very similar mean peak ID₅₀. In contrast, both groups had
220 lower mean ID₅₀ values compared to hospitalized individuals in the 0-3 and 4/5 severity
221 groups (**Figure 5B**). Importantly, some asymptomatic individuals could generate
222 neutralization titres >1,000. Similar to the cohort with confirmed SARS-CoV-2 infection, a
223 decline in ID₅₀ was observed following peak neutralization. For many individuals with a peak
224 ID₅₀ in the 100-500 range, neutralization was approaching baseline after 50 days POS (**Figure**
225 **5C**). As the mean peak ID₅₀ was lower in the HCW cohort, the decline in nAb titres towards
226 baseline was more frequent compared to the patient cohort.

227

228 **Discussion:**

229 Here, we describe the Ab responses in sequential samples from multiple individuals following
230 SARS-CoV-2 infection in hospitalized patients and healthcare workers. We show that all PCR+
231 patients sampled >8 days POS developed nAbs with peak ID₅₀ in the range of 98-32,000. This
232 wide range in nAb titres against SARS-CoV-2 pseudotyped virus has been observed in other
233 cross-sectional cohorts.^{4,16} Although the average nAb titre was higher in those with more
234 severe disease, the average time to reach peak neutralization did not differ between the 0-3
235 and 4/5 severity groups. This suggests that disease severity enhances the magnitude of the
236 nAb response but to a lesser extent the kinetics of the nAb response. Importantly, some

237 seropositive individuals who were asymptomatic were able to generate nAb titres >1000.
238 Indeed, highly potent neutralizing monoclonal antibodies (mAbs) have been isolated from
239 asymptomatic patients.²⁰ It is not clear why nAb responses correlate with disease severity. A
240 higher viral load may lead to more severe disease and generate a stronger Ab response
241 through increased levels of viral antigen. Alternatively, Abs could have a causative role in
242 disease severity, although there is currently no evidence for antibody dependent
243 enhancement in COVID-19.²¹

244
245 Cross-sectional studies in SARS-CoV-2 infected individuals have shown lower mean ID₅₀ for
246 serum samples collected at later time points POS (23-52 days).⁷ Longitudinal Abs studies using
247 sequential samples have mostly been limited to 30 days POS.¹⁶ In two separate studies, IgG
248 binding to S was maintained up until 20-25 days³ and day 30 POS⁵. However, a decline in nAb
249 titres have been reported in a small subset of individuals followed sequentially for up to 43
250 days²². The sequential serum samples studied here allowed the measurement of Ab
251 responses up to 94 days POS enabling us to look further into the longevity of the nAb response
252 to SARS-CoV-2 infection in much greater detail than has hitherto been possible. A comparison
253 of the peak ID₅₀ value for each individual (mean 23.1 days POS) and ID₅₀ at their final timepoint
254 collected, showed a decline in neutralizing titres in both cohorts, regardless of disease
255 severity. This decrease was mirrored in the reduction in IgG binding titres (EC₅₀) to S and RBD
256 for the PCR+ cohort (**Figure 4B**). For some individuals with a peak ID₅₀ in the 100-300 range,
257 neutralizing titres were at, or below, the level of detection in the SARS-CoV-2 pseudotype
258 neutralization assay after only ~50 days from the measured peak of neutralization. This trend
259 was also seen in the HCW cohort, and reveals that in some individuals, SARS-CoV-2 infection
260 generates only a transient Ab response that rapidly declines. For those with peak ID₅₀ titres
261 >2,000, decline in nAb titres ranged from 2- to 23-fold over an 18-65 day period. It is not clear
262 whether this decline will continue on a downward trajectory or whether the IgG level will
263 plateau to a steady state. Although some nAb titres remain in the 1000-3500 range at the
264 final time point (ranging from 50-82 days POS), further follow up in these cohorts is required
265 to fully assess the longevity of the nAb response in these individuals. Importantly, class-
266 switched IgG memory B cells against S and RBD have been detected in blood of COVID-19
267 patients showing memory responses are generated during infection.^{8,23,24}

268

269 The rapid decline observed in IgM and IgA specific responses to S, RBD and N after 20-30 days
270 demonstrates the value of measuring longer lasting SARS-CoV-2 specific IgG in diagnostic
271 tests and seroprevalence studies. However, the waning IgG response should be considered
272 when conducting seroprevalence studies of individuals of unconfirmed PCR+ diagnosed
273 infection or in diagnosis of COVID-19 related syndromes such as PIMS-TS (inflammatory
274 multisystem syndrome temporally associated with SARS-CoV-2).²⁵ IgA and IgM could be used
275 as a marker of recent or acute SARS-CoV-2 infection and therefore may be more relevant in a
276 hospital setting. Although a strong correlation between ID₅₀ was observed between IgG, IgM
277 and IgA responses against S and RBD, there were still examples where high binding to S and
278 RBD was observed with very little neutralization and therefore care should be taken when
279 using ELISA (or other methods of detecting binding Abs) as a surrogate measurement for
280 neutralization.²⁶

281

282 The longevity of Ab responses to other coronaviruses have been studied previously.¹⁰⁻¹³ The
283 Ab response following SARS-CoV infection in a cohort of hospitalized patients was shown to
284 peak around day 30¹² (average titre 1:590) and a general waning of the binding IgG and nAb
285 followed during the 3-year follow up. Low nAb titres of 1:10 were detected in 17/18
286 individuals after 540 days.¹² In a second study, low nAb titres (mean titre, 1:28) could still be
287 detected up to 36 months post infection in 89% of individuals.¹⁵ In contrast to SARS-CoV-2
288 infection, SARS-CoV infection typically caused more severe disease and asymptomatic, low
289 severity disease were less common. Therefore, the difference in the longevity of the nAb
290 response observed here between SARS-CoV and SARS-CoV-2 infection may relate to the
291 different clinical manifestation of disease between the two viruses.²⁷ The more transient Ab
292 responses in the lower disease severity cases in our cohorts reflect more the immune
293 response to endemic seasonal coronaviruses (i.e. those associated with the common cold)
294 which have also been reported to be more transient.² For example, a recent report of 10
295 individuals studied over a 35-year period showed re-infections with endemic coronaviruses
296 were frequent 12 months after an initial infection.¹⁴ Further, individuals experimentally
297 infected with endemic alphacoronavirus 229E, generated high Ab titres after 2 weeks but
298 these rapidly declined in the following 11 weeks and by 1 year, the mean Ab titres had
299 reduced further but they were still higher than before the first virus challenge.¹⁰ Subsequent

300 virus challenge lead to reinfection (as determined by virus shedding) yet individuals showed
301 no cold symptoms.¹⁰

302

303 The nAb titre required for protection from re-infection in humans is not yet understood.
304 Neutralizing monoclonal antibodies (mAbs) isolated from SARS-CoV-2 infected individuals can
305 protect from disease in animal challenge models in a dose dependant manner.^{9,28,29} SARS-
306 CoV-2 infected rhesus macaques, who developed nAbs titres of ~100 (range 83-197), did not
307 show any clinical signs of illness when challenged 35 days after the first infection.³⁰ However,
308 virus was still detected in nasal swabs, albeit 5-logs lower than in primary infection, suggesting
309 immunologic control rather and sterilizing immunity. Similarly, a second study showed rhesus
310 macaques with nAb titres between 8-20 had no clinical signs of disease or detectable virus
311 following re-challenge 28 days after primary infection.³¹ Therefore, although nAb titres are
312 declining over a 2-3 month period in the two cohorts described here, individuals with high
313 peak ID₅₀S (>2,000) would likely have sufficient nAb titres to be protected from clinical illness
314 for some time if re-exposed to SARS-CoV-2.

315

316 Even though the role of nAbs in viral clearance in primary SARS-CoV-2 infection is not fully
317 understood, many current vaccine design efforts focus on eliciting a robust nAb response to
318 provide protection from infection. Vaccine challenge studies in macaques can give limited
319 insight into nAb titres required for protection from re-infection. Vaccine candidates tested
320 thus far in challenge studies have elicited modest nAb responses (ID₅₀ 5-250).³²⁻³⁵ For
321 example, a DNA vaccine encoding SARS-CoV-2 S generated nAb titres between 100-200 which
322 were accompanied by a lowering of the viral load by 3-logs. nAb titres in vaccinated animals
323 were shown to strongly correlate with viral load.³⁴ However, the role T-cell responses
324 generated through either infection³⁶ or vaccination play in controlling disease cannot be
325 discounted in these studies and defining further the correlates and longevity of vaccine
326 protection is needed. Taken together, despite the waning nAb titres in individuals, it is
327 possible that nAb titres will still be sufficient to provide protection from COVID-19 disease for
328 a period of time. However, sequential PCR testing and serology studies in individuals known
329 to have been SARS-CoV-2 infected will be critical for understanding the ability of nAbs to
330 protect from renewed infection in humans.

331

332 In summary, using sequential samples from SARS-CoV-2 infected individuals collected up to
333 94 days POS, we demonstrate declining nAb titres in the majority of individuals. For those
334 with a low nAb response, titres can return to base line over a relatively short period. Further
335 studies using sequential samples from these individuals is required to fully determine the
336 longevity of the nAb response and studies determining the nAb threshold for protection from
337 re-infection are needed.

338

339 **Methods:**

340 **Ethics**

341 Surplus serum from patient biochemistry samples taken as part of routine care were retrieved
342 at point of discard, aliquoted, stored and linked with a limited clinical dataset by the direct
343 care team, before anonymization. Work was undertaken in accordance with the UK Policy
344 Framework for Health and Social Care Research and approved by the Risk and Assurance
345 Committee at Guy's and St Thomas' NHS Foundation Trust (GSTFT). Serum was collected from
346 consenting healthcare workers with expedited approval from GSTFT Research &
347 Development office, Occupational Health department and Medical director.

348

349 **Patient and sample origin**

350 269 individual venous serum samples collected at St Thomas' Hospital, London from 59
351 patients diagnosed as SARS-CoV-2 positive via real-time RT-PCR, were obtained for serological
352 analysis. Samples ranged from 1 to 94 days after onset of self-reported symptoms or, in
353 asymptomatic cases, days after positive PCR result. Patient information is given in Table S1.

354

355 **Healthcare worker (HCW) cohort**

356 Sequential serum samples were collected every 1-2 weeks from healthcare workers at GSTFT
357 between 13th March and 10th June 2020. Seropositivity to SARS-CoV-2 was determined using
358 sera collected in April and early May 2020 using ELISA. Individuals were considered
359 seropositive if sera (diluted 1:50) gave an OD for IgG against both N and S that was 4-fold
360 above the negative control sera.⁶ Self-reported COVID-19 related symptoms were recorded
361 by participants and days post onset of symptoms in seropositive individuals was determined
362 using this information. For asymptomatic, seropositive individuals, days POS was defined as

363 the first timepoint SARS-CoV-2 Abs were detected. Six participants had confirmed PCR+
364 infection and were included with the PCR+ hospitalized patients in the initial analysis.

365

366 **COVID-19 severity classification**

367 Patients diagnosed with COVID-19 were classified as follows:

368 0 - asymptomatic OR no requirement for supplemental oxygen.

369 1 - requirement for supplemental oxygen ($FiO_2 < 0.4$) for at least 12 hrs.

370 2 - requirement for supplemental oxygen ($FiO_2 \geq 0.4$) for at least 12 hrs.

371 3 - requirement for non-invasive ventilation (NIV)/ continuous positive airways pressure
372 (CPAP) OR proning OR supplemental oxygen ($FiO_2 > 0.6$) for at least 12 hrs AND not a
373 candidate for escalation above level one (ward-based) care.

374 4 - requirement for intubation and mechanical ventilation OR supplemental oxygen (FiO_2
375 > 0.8) AND peripheral oxygen saturations $< 90\%$ (with no history of type 2 respiratory failure
376 (T2RF)) OR $< 85\%$ (with known T2RF) for at least 12 hrs.

377 5 - requirement for extracorporeal membrane oxygenation (ECMO).

378

379 **Protein expression**

380 N protein was obtained from Leo James and Jakub Luptak at LMB, Cambridge. The N protein
381 used is a truncated construct of the SARS-CoV-2 N protein comprising residues 48-365 (both
382 ordered domains with the native linker) with an N terminal uncleavable hexahistidine tag. N
383 was expressed in *E. Coli* using autoinducing media for 7h at 37°C and purified using
384 immobilised metal affinity chromatography (IMAC), size exclusion and heparin
385 chromatography.

386

387 S protein consists of a pre-fusion S ectodomain residues 1-1138 with proline substitutions at
388 amino acid positions 986 and 987, a GGGG substitution at the furin cleavage site (amino acids
389 682-685) and an N terminal T4 trimerisation domain followed by a Strep-tag II.⁸ The plasmid
390 was obtained from Philip Brouwer, Marit van Gils and Rogier Sanders at The University of
391 Amsterdam. The protein was expressed in 1 L HEK-293F cells (Invitrogen) grown in suspension
392 at a density of 1.5 million cells/mL. The culture was transfected with 325 µg of DNA using PEI-
393 Max (1 mg/mL, Polysciences) at a 1:3 ratio. Supernatant was harvested after 7 days and

394 purified using StrepTactinXT Superflow high capacity 50% suspension according to the
395 manufacturer's protocol by gravity flow (IBA Life Sciences).

396

397 The RBD plasmid was obtained from Florian Krammer at Mount Sinai University.¹ Here the
398 natural N-terminal signal peptide of S is fused to the RBD sequence (319 to 541) and joined
399 to a C-terminal hexahistidine tag. This protein was expressed in 500 mL HEK-293F cells
400 (Invitrogen) at a density of 1.5 million cells/mL. The culture was transfected with 1000 µg of
401 DNA using PEI-Max (1 mg/mL, Polysciences) at a 1:3 ratio. Supernatant was harvested after 7
402 days and purified using Ni-NTA agarose beads.

403

404 **ELISA protocol**

405 ELISA was carried out as previously described.⁶ All sera/plasma were heat-inactivated at 56°C
406 for 30 mins before use in the in-house ELISA. High-binding ELISA plates (Corning, 3690) were
407 coated with antigen (N, S or RBD) at 3 µg/mL (25 µL per well) in PBS, either overnight at 4°C
408 or 2 hr at 37°C. Wells were washed with PBS-T (PBS with 0.05% Tween-20) and then blocked
409 with 100 µL 5% milk in PBS-T for 1 hr at room temperature. Wells were emptied and sera
410 diluted at 1:50 in milk was added and incubated for 2 hr at room temperature. Control
411 reagents included CR3009 (2 µg/mL), CR3022 (0.2 µg/mL), negative control plasma (1:25
412 dilution), positive control plasma (1:50) and blank wells. Wells were washed with PBS-T.
413 Secondary antibody was added and incubated for 1 hr at room temperature. IgM was
414 detected using Goat-anti-human-IgM-HRP (1:1,000) (Sigma: A6907), IgG was detected using
415 Goat-anti-human-Fc-AP (1:1,000) (Jackson: 109-055-043-JIR) and IgA was detected Goat-anti-
416 human-IgA-HRP (1:1,000) (Sigma: A0295). Wells were washed with PBS-T and either AP
417 substrate (Sigma) was added and read at 405 nm (AP) or 1-step TMB substrate (Thermo
418 Scientific) was added and quenched with 0.5 M H₂SO₄ before reading at 450 nm (HRP).

419

420 EC₅₀ values were measured using a titration of serum starting at 1:50 and using a 5-fold
421 dilution series. Half-maximal binding (EC₅₀) was calculated using GraphPad Prism.

422

423 **Virus preparation**

424 Pseudotyped HIV virus incorporating the SARS-Cov2 spike protein was produced in a 10 cm
425 dish seeded the day prior with 3.5x10⁶ HEK293T/17 cells in 10 ml of complete Dulbecco's

426 Modified Eagle's Medium (DMEM-C) containing 10% (vol/vol) foetal bovine serum (FBS), 100
427 IU/ml penicillin and 100 µg/ml streptomycin. Cells were transfected using 35 µg of PEI-Max
428 (1 mg/mL, Polysciences) with: 1500 ng of HIV-luciferase plasmid, 1000 ng of HIV 8.91 gag/pol
429 plasmid and 900 ng of SARS-2 spike protein plasmid.^{17,18} The media was changed 18 hours
430 post-transfection and supernatant was harvested 48 hours post-transfection. Pseudotype
431 virus was filtered through a 0.45µm filter and stored at -80°C until required.

432

433 **Neutralization assays**

434 Serial dilutions of serum samples (heat inactivated at 56°C for 30mins) were prepared with
435 DMEM media and incubated with pseudotype virus for 1-hour at 37°C in 96-well plates. Next,
436 Hela cells stably expressing the ACE2 receptor (provided by Dr James Voss, The Scripps
437 Research Institute) were added and the plates were left for 72 hours. Infection level was
438 assessed in lysed cells with the Bright-Glo luciferase kit (Promega), using a Victor™ X3
439 multilabel reader (Perkin Elmer).

440

441 **Statistical analysis**

442 Analyses were performed using R (version 4.0.0) and GraphPad Prism (version 7.0.4). On
443 charts showing OD/ID₅₀ and days post-infection, the overall trend in the data was indicated
444 by lines generated using Loess regressions (span 1.5) with ribbons depicting the 95%
445 confidence intervals.

446

447

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462

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473

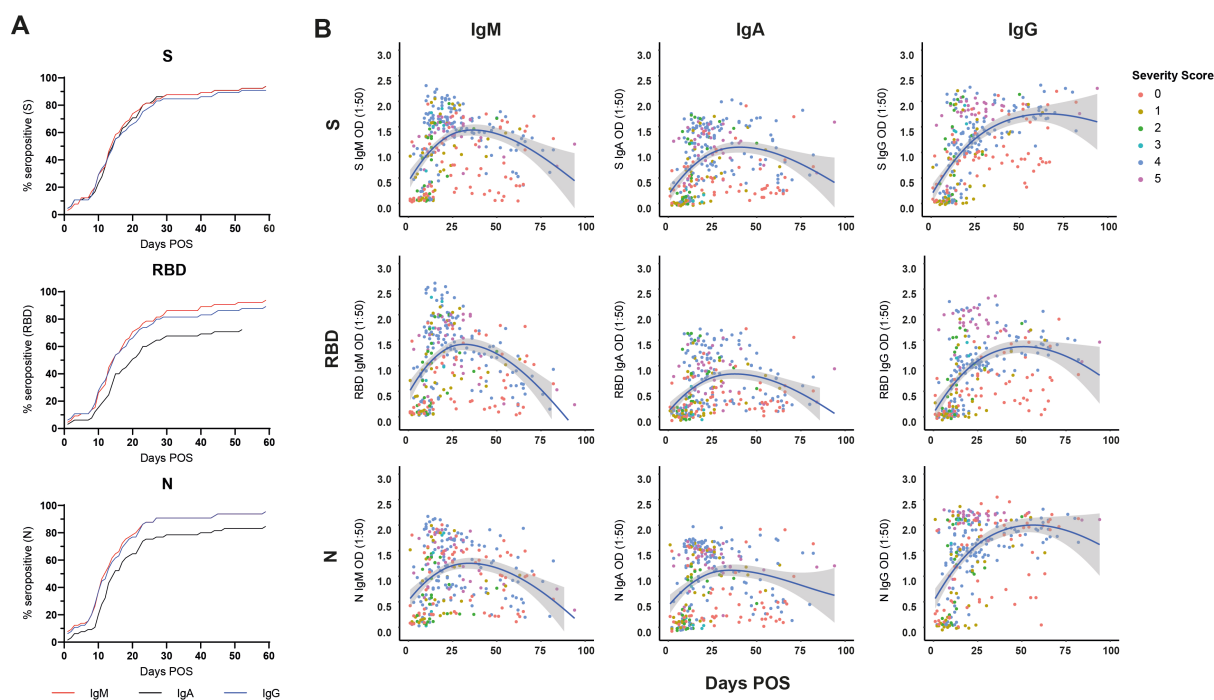
474 **Table 1: Cohort description.** Gender, severity, age, and outcome.

475

Gender	
Male	51 (78.5%)
Female	14 (21.5%)
Age	
Mean	55.2 years (23-95)
Severity	
0	14
1	10
2	7
3	2
4	25
5	7
Outcome	
HCW	6
Died	12
Discharged	41
Still in hospital	5
Transferred to local hospital	3

476

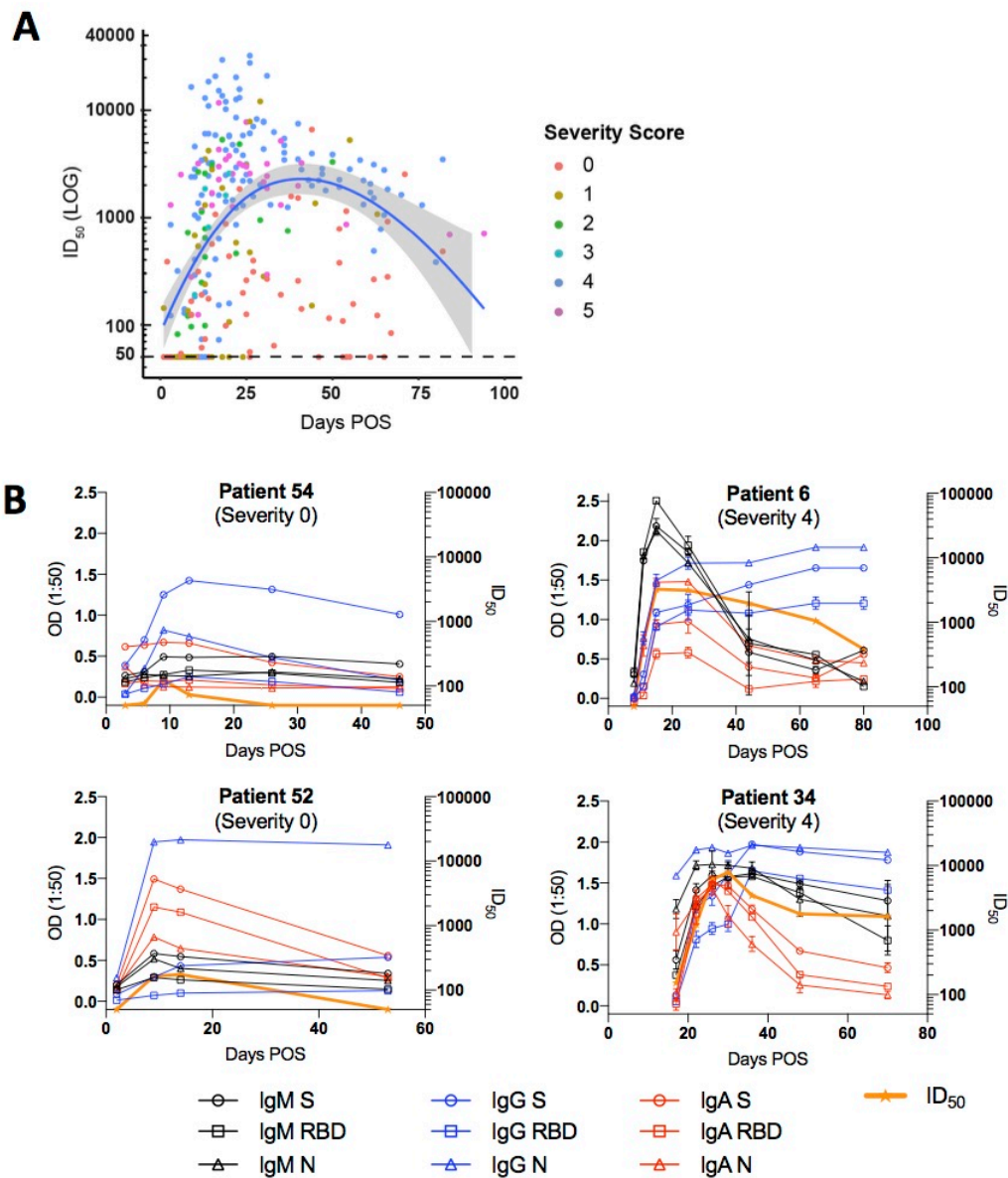
477 **Figure 1: Kinetics of antibody development against SARS-CoV-2 antigens over time.** A) A
478 cumulative frequency analysis describing the point of seroconversion for each person in the
479 cohort. Graph shows the percentage of individuals in the cohort that become IgM, IgA or IgG
480 positive to S, RBD and N each day. A serum is considered positive when the OD is 4-fold above
481 background. B) IgM, IgA and IgG OD values against S, RBD and N are plotted against the time
482 post onset of symptoms (POS) at which sera was collected. Coloured dots indicate disease
483 severity (0-5). The line shows the mean OD value expected from a Loess regression model,
484 the ribbon indicates the pointwise 95% confidence interval. OD = optical density.



485

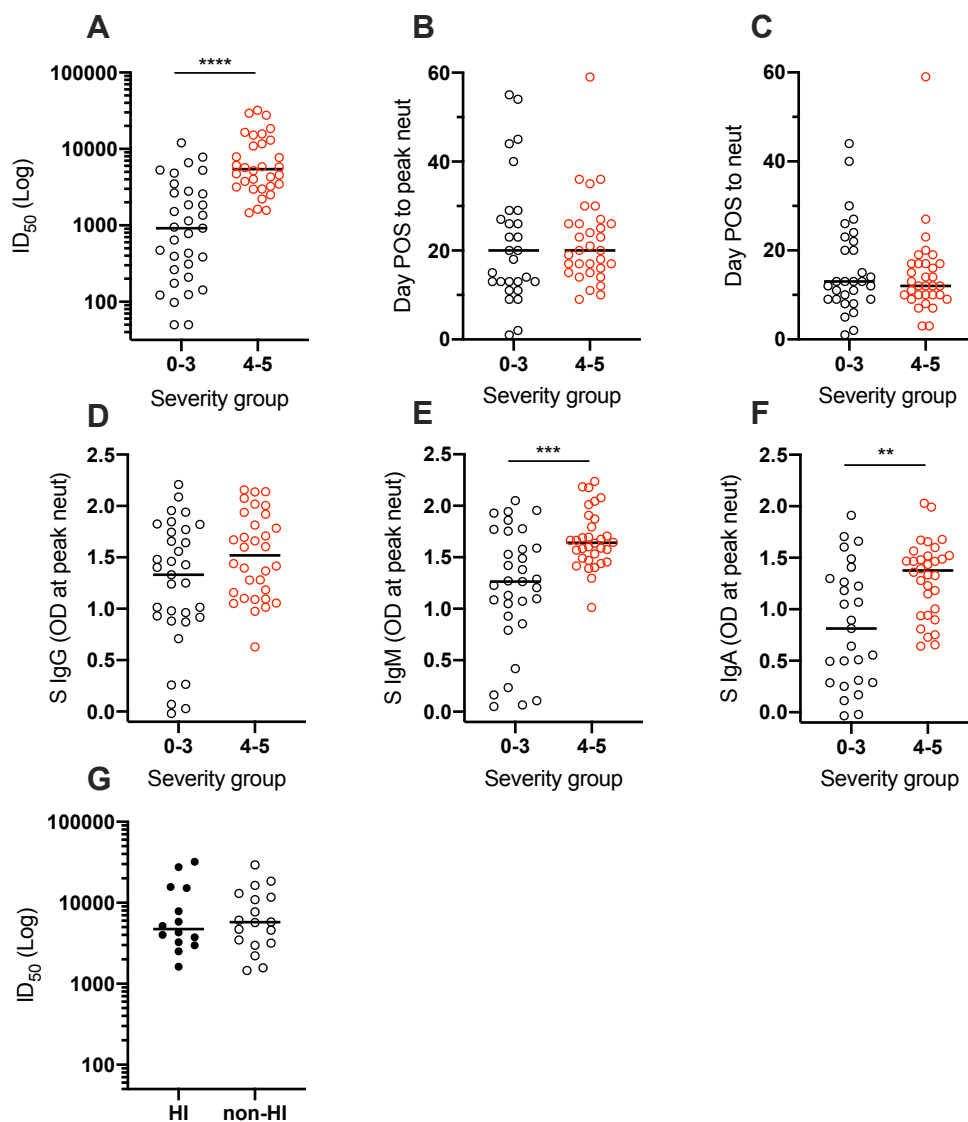
486

487 **Figure 2: Kinetics of neutralizing antibody responses in SARS-CoV-2 infection.** A) ID₅₀ values
 488 plotted against the days post onset of symptoms (POS) at which sera was collected. Coloured
 489 dots indicate disease severity (0-5). The line shows the mean ID₅₀ value expected from a Loess
 490 regression model, the ribbon indicates the pointwise 95% confidence interval. B) Example
 491 kinetics of Ab responses for four individuals during acute infection and the convalescent
 492 phase. Graphs show comparison between severity 0 (left) and severity 4 (right) rated disease.
 493



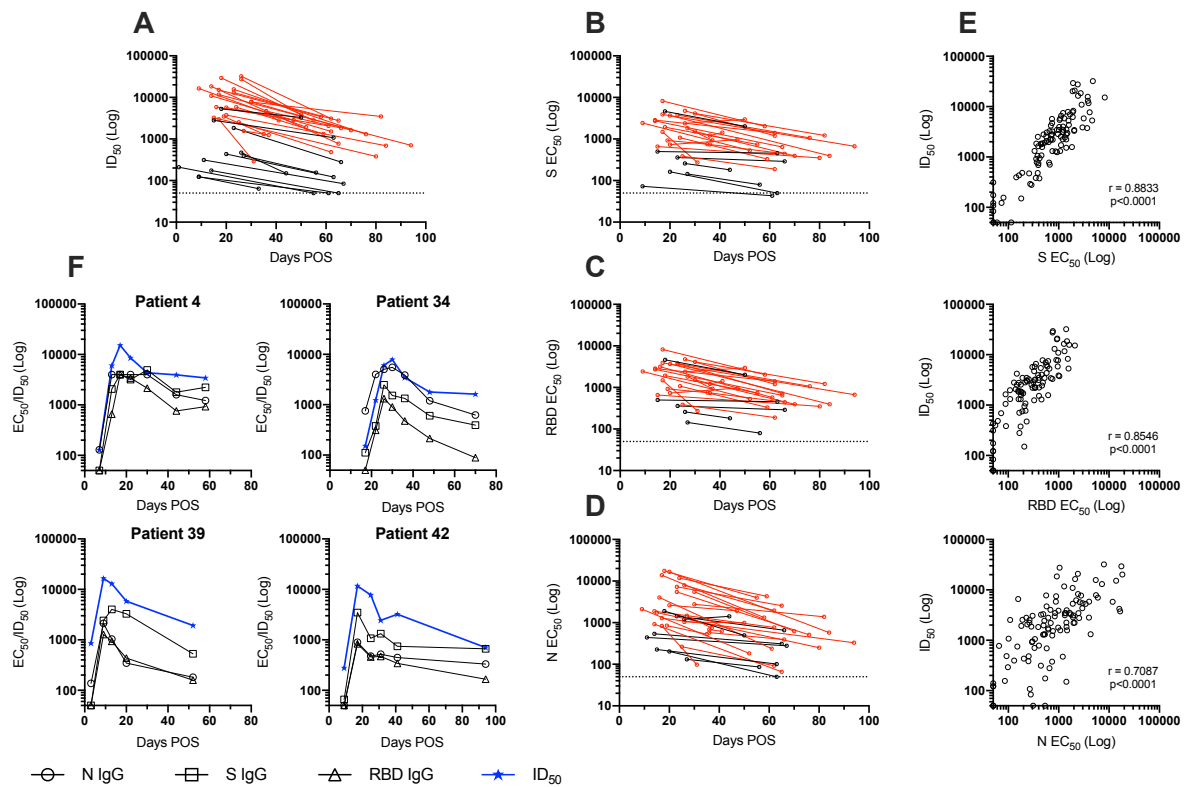
494

495 **Figure 3: Impact of disease severity of Ab responses to SARS-CoV-2 infection.** Comparison
496 for individuals with 0-3 or 4/5 disease severity for A) peak ID₅₀ of neutralization ($p < 0.0001$),
497 B) the time POS to reach peak ID₅₀ ($p = 0.674$), and C) the time POS to detect neutralizing
498 activity ($p = 0.9156$). Comparison in OD values for individuals with 0-3 or 4/5 disease severity
499 for D) IgG ($p = 0.0635$), E) IgM ($p = 0.0003$) and F) IgA ($p = 0.0018$) against S measured at peak
500 ID₅₀. G) Comparison of the peak ID₅₀ value for individuals who were treated for
501 hyperinflammation or not, and had 4/5 disease severity ($p > 0.999$). Statistical significance was
502 measured using a Mann-Whitney test.
503



504

505 **Figure 4: Longevity of the Ab response.** A) ID₅₀ at peak neutralization is plotted against the
 506 donor matched ID₅₀ at the last time point sera was collected. Only individuals where the peak
 507 ID₅₀ occurs before the last time point, and where the last time point is >30 days POS are
 508 included in this analysis. B-D) EC₅₀ values for IgG binding to S, RBD and N were calculated at
 509 time point with peak ID₅₀ and the final time point. EC₅₀ at peak neutralization is plotted with
 510 the donor matched EC₅₀ at the last time point sera was collected. Individuals with a disease
 511 severity 0-3 are shown in black and those with 4/5 are shown in red. E) Correlation of ID₅₀
 512 with IgG EC₅₀ against S ($r^2=0.8293$), RBD ($r^2=0.7128$) and N ($r^2=0.4856$) (Spearman correlation,
 513 r . A linear regression was used to calculate the goodness of fit, r^2). F) Change in IgG EC₅₀
 514 measured against S, RBD and N and ID₅₀ over time for 4 example patients (all severity 4).



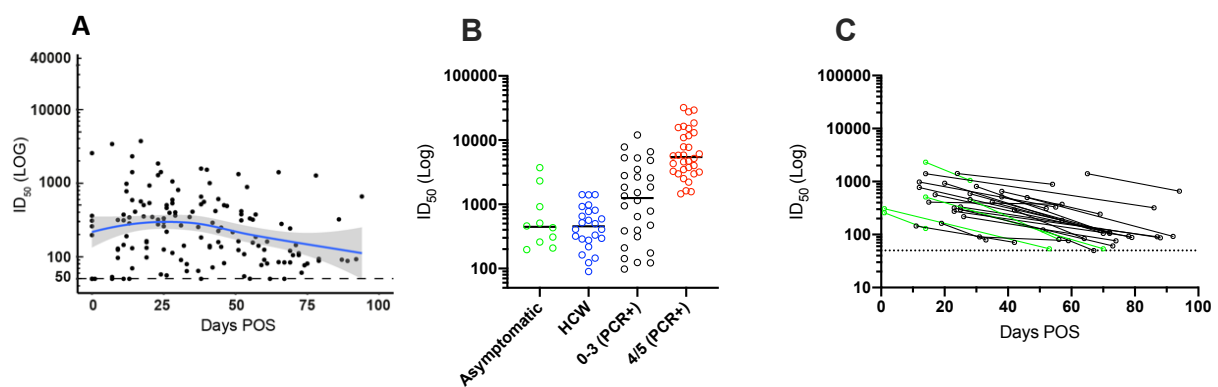
515

516

517 **Figure 5: Ab responses in a healthcare worker cohort.**

518 A) ID₅₀ values plotted against the time post onset of symptoms (POS) at which sera was
519 collected. The line shows the mean ID₅₀ value expected from a Loess regression model, the
520 ribbon indicates the pointwise 95% confidence interval. B) Comparison of the peak ID₅₀
521 between asymptomatic individuals (includes 7 HCW and 3 hospital patients), healthcare
522 workers (24 symptomatic HCW with no PCR test), and PCR+ individuals with either severity 0-
523 3 (n=28) or 4/5 (n=32). The 2 PCR+ individuals sampled at early time points (<8 days POS) and
524 did not seroconvert were not included in this analysis. C) ID₅₀ at peak neutralization is plotted
525 with the donor matched ID₅₀ at the last time point sera was collected. The dotted line
526 represents the cut-off for the pseudotype neutralization assay. Asymptomatic donors are
527 shown in green.

528



529

530 **References:**

- 531 1 Amanat, F. *et al.* A serological assay to detect SARS-CoV-2 seroconversion in humans.
532 *Nat Med*, doi:10.1038/s41591-020-0913-5 (2020).
- 533 2 Gorse, G. J., Donovan, M. M. & Patel, G. B. Antibodies to coronaviruses are higher in
534 older compared with younger adults and binding antibodies are more sensitive than
535 neutralizing antibodies in identifying coronavirus-associated illnesses. *J Med Virol* **92**,
536 512-517, doi:10.1002/jmv.25715 (2020).
- 537 3 Long, Q. X. *et al.* Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat*
538 *Med* **26**, 845-848, doi:10.1038/s41591-020-0897-1 (2020).
- 539 4 Luchsinger, L. L. *et al.* Serological Analysis of New York City COVID19 Convalescent
540 Plasma Donors. *medRxiv*, doi:10.1101/2020.06.08.20124792 (2020).
- 541 5 Okba, N. M. A. *et al.* Severe Acute Respiratory Syndrome Coronavirus 2-Specific
542 Antibody Responses in Coronavirus Disease Patients. *Emerg Infect Dis* **26**, 1478-1488,
543 doi:10.3201/eid2607.200841 (2020).
- 544 6 Pickering, P. *et al.* Comparative assessment of multiple COVID-19 serological
545 technologies supports continued evaluation of point-of-care lateral flow assays in
546 hospital and community healthcare settings. doi:10.1101/2020.06.02.20120345
547 (2020).
- 548 7 Prevost, J. *et al.* Cross-sectional evaluation of humoral responses against SARS-CoV-2
549 Spike. *bioRxiv*, doi:10.1101/2020.06.08.140244 (2020).
- 550 8 Brouwer, P. J. M. *et al.* Potent neutralizing antibodies from COVID-19 patients define
551 multiple targets of vulnerability. *Science*, doi:10.1126/science.abc5902 (2020).
- 552 9 Rogers, T. F. *et al.* Isolation of potent SARS-CoV-2 neutralizing antibodies and
553 protection from disease in a small animal model. *Science*,
554 doi:10.1126/science.abc7520 (2020).
- 555 10 Callow, K. A., Parry, H. F., Sergeant, M. & Tyrrell, D. A. The time course of the immune
556 response to experimental coronavirus infection of man. *Epidemiol Infect* **105**, 435-446,
557 doi:10.1017/s0950268800048019 (1990).
- 558 11 Kellam, P. & Barclay, W. The dynamics of humoral immune responses following SARS-
559 CoV-2 infection and the potential for reinfection. *J Gen Virol*,
560 doi:10.1099/jgv.0.001439 (2020).
- 561 12 Mo, H. *et al.* Longitudinal profile of antibodies against SARS-coronavirus in SARS
562 patients and their clinical significance. *Respirology* **11**, 49-53, doi:10.1111/j.1440-
563 1843.2006.00783.x (2006).
- 564 13 Moore, J. P. & Klasse, P. J. SARS-CoV-2 vaccines: 'Warp Speed' needs mind melds not
565 warped minds. *J Virol*, doi:10.1128/JVI.01083-20 (2020).
- 566 14 Edridge, A. *et al.* Coronavirus protective immunity is short-lasting. *medRxiv*,
567 doi:10.1101/2020.05.11.20086439 (2020).
- 568 15 Cao, W. C., Liu, W., Zhang, P. H., Zhang, F. & Richardus, J. H. Disappearance of
569 antibodies to SARS-associated coronavirus after recovery. *N Engl J Med* **357**, 1162-
570 1163, doi:10.1056/NEJMc070348 (2007).
- 571 16 Wu, F. *et al.* Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered
572 patient cohort and their implications. *medRxiv*, doi:10.1101/2020.03.30.20047365
573 (2020).
- 574 17 Grehan, K., Ferrara, F. & Temperton, N. An optimised method for the production of
575 MERS-CoV spike expressing viral pseudotypes. *MethodsX* **2**, 379-384,
576 doi:10.1016/j.mex.2015.09.003 (2015).

- 577 18 Thompson, C. *et al.* Neutralising antibodies to SARS coronavirus 2 in Scottish blood
578 donors - a pilot study of the value of serology to determine population exposure.
579 *medRxiv*, doi:10.1101/2020.04.13.20060467 (2020).
- 580 19 Sterlin, D. *et al.* IgA dominates the early neutralizing antibody response to SARS-CoV-
581 2. *medRxiv*, doi:10.1101/2020.06.10.20126532 (2020).
- 582 20 Robbiani, D. F. *et al.* Convergent antibody responses to SARS-CoV-2 in convalescent
583 individuals. *Nature*, doi:10.1038/s41586-020-2456-9 (2020).
- 584 21 Iwasaki, A. & Yang, Y. The potential danger of suboptimal antibody responses in
585 COVID-19. *Nat Rev Immunol* **20**, 339-341, doi:10.1038/s41577-020-0321-6 (2020).
- 586 22 Wang, X. *et al.* Neutralizing Antibodies Responses to SARS-CoV-2 in COVID-19
587 Inpatients and Convalescent Patients. *Clin Infect Dis*, doi:10.1093/cid/ciaa721 (2020).
- 588 23 Ju, B. *et al.* Human neutralizing antibodies elicited by SARS-CoV-2 infection. *Nature*,
589 doi:10.1038/s41586-020-2380-z (2020).
- 590 24 Seydoux, E. *et al.* Analysis of a SARS-CoV-2-Infected Individual Reveals Development
591 of Potent Neutralizing Antibodies with Limited Somatic Mutation. *Immunity*,
592 doi:10.1016/j.immuni.2020.06.001 (2020).
- 593 25 Whittaker, E. *et al.* Clinical Characteristics of 58 Children With a Pediatric
594 Inflammatory Multisystem Syndrome Temporally Associated With SARS-CoV-2. *JAMA*,
595 doi:10.1001/jama.2020.10369 (2020).
- 596 26 Premkumar, L. *et al.* The receptor binding domain of the viral spike protein is an
597 immunodominant and highly specific target of antibodies in SARS-CoV-2 patients. *Sci*
598 *Immunol* **5**, doi:10.1126/sciimmunol.abc8413 (2020).
- 599 27 Petersen, e. K., M.; Go, U.; Hamer, D.H.; Petrosillo, N.; Castelli, F.; Storgaard, M.; Al
600 Khalili, S.; Simonsen, L. Comparing SARS-CoV-2 with SARS-CoV and influenza
601 pandemics. *Lancet Infection*, doi:10.1016/S1473-3099(20)30484-9 (2020).
- 602 28 Shi, R. *et al.* A human neutralizing antibody targets the receptor binding site of SARS-
603 CoV-2. *Nature*, doi:10.1038/s41586-020-2381-y (2020).
- 604 29 Cao, Y. *et al.* Potent neutralizing antibodies against SARS-CoV-2 identified by high-
605 throughput single-cell sequencing of convalescent patients' B cells. *Cell*,
606 doi:10.1016/j.cell.2020.05.025 (2020).
- 607 30 Chandrashekar, A. *et al.* SARS-CoV-2 infection protects against rechallenge in rhesus
608 macaques. *Science*, doi:10.1126/science.abc4776 (2020).
- 609 31 Deng, W. *et al.* Primary exposure to SARS-CoV-2 protects against reinfection in rhesus
610 macaques. *Science*, doi:10.1126/science.abc5343 (2020).
- 611 32 Smith, T. R. F. *et al.* Immunogenicity of a DNA vaccine candidate for COVID-19. *Nat*
612 *Commun* **11**, 2601, doi:10.1038/s41467-020-16505-0 (2020).
- 613 33 van Doremalen, N. *et al.* ChAdOx1 nCoV-19 vaccination prevents SARS-CoV-2
614 pneumonia in rhesus macaques. *bioRxiv*, doi:10.1101/2020.05.13.093195 (2020).
- 615 34 Yu, J. *et al.* DNA vaccine protection against SARS-CoV-2 in rhesus macaques. *Science*,
616 doi:10.1126/science.abc6284 (2020).
- 617 35 Gao, Q. *et al.* Rapid development of an inactivated vaccine candidate for SARS-CoV-2.
618 *Science*, doi:10.1126/science.abc1932 (2020).
- 619 36 Sekine, T. *et al.* Robust T cell immunity in convalescent individuals with asymptomatic
620 or mild COVID-19. *medRxiv*, doi:10.1101/2020.06.29.174888 (2020).
- 621