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Longitudinal clinico-serological analysis of anti-nucleocapsid and anti-receptor binding domain of spike protein antibodies against SARS-CoV-2

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Keywords: SARS-CoV-2 nucleocapsid, receptor binding domain, neutralizing antibody response, IgG antibody response on 240 days

ABSTRACT

Objectives: Monitoring the antibody responses to SARS-CoV-2 infection and its correlation to clinical spectrum of disease is critical in understanding the disease progression and protection against re-infection. We assessed the nucleocapsid (N) and receptor-binding-domain of spike (SRBD) protein specific IgG and neutralizing antibody (NAb) responses in COVID-19 patients up to 8 months and its correlation with diverse disease spectrum.

Methods: During the first wave of the SARS-CoV-2 pandemic, from 284 COVID-19 patients, 608 samples were collected up to 8 months post infection. The patients were categorized as asymptomatic, symptomatic and severe. The N and SRBD IgG and NAb titers were evaluated and correlated with clinical data.

Results: A steep increase in antigen specific antibody titers was observed till 40 days post onset of the disease (POD), followed by a partial decline till 240 days. Severe disease was associated with a stronger SRBD IgG response and higher NAb titers. The persistence of antibody response was observed in 76% against N, 80% against SRBD and 80% for NAb of cases up to 8 months POD.

Conclusion: RBD and N protein specific IgG persisted till 240 days POD which correlated with NAb response, irrespective of individual’s symptomatic status indicating overall robust protection against re-infection.

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Patients
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Statement
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and
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and
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prone,
it
in
this
study,
patients.

In
this
study,
a
total
of
608
serum/plasma
samples
were
collected
from
284
COVID-19
patients
during
the
first
wave
of
SARS-CoV-2
from
designated
COVID-19
hospitals
in
Pune
Municipal
Corporation
(PMC)
and
Pimpri-
Chinchwad
Municipal
Corporation
(PCMC),
Maharashtra,
India.
RT-PCR
done
from
the
throat/
nares
swabs
collected
from
a
subset
of
these
patients
revealed
that
the
circulating
SARS-CoV-2
strains
were
from
the
G
(D614G)
clade
(Potdar,
et
al.,
2020) (Potdar
et
al.,
2021).
After
the
discharge
of
the
patients,
follow
up
blood
samples
were
collected
till
a
maximum
period
of
eight
months
from
the
onset
date
of
illness
at
PMC/PCMC
clinic
by
the
trained
staff.
None
of
the
recovered
patients
were
re-infected
within
the
8-month
period
of
follow
up.
Among
qRT-PCR
confirmed
positive
cases,
the
participants
were
classified
into
three
categories
based
on
their
clinical
symptoms.
Asymptomatic
cases
were
patients
who
did
not
develop
any
symptoms
throughout
the
course
of
the
disease.
Symptomatic
cases
were
patients
with
fever,
fatigue,
body
ache,
diarrhoea,
abdominal
pain,
dyspnoea
and
respiratory
symptoms
like
runny
nose,
cough,
sore
throat,
and
nasal
discharge,
whereas
patients
with
any
of
the
following
3
criteria
with
or
without
the
above
symptoms
were
included
in
severe
cases:
Respiratory
distress
with
breathing
difficulty
(≥30
breaths/min)
and
Oxygen
saturation
with
≤90%
at
rest
or
chest
imaging
with
>50%
observed
lesions
(Ministry
of
Health
and
Family
Welfare.,
2020) (Xiang
et
al.,
2020).
The
study
included
208
asymptomatic,
60
asymptomatic
and
16
severe
symptomatic
patients.
Of
the
284
patients,
132
patients
provided
multiple
samples
(56
patients
provided
2,
23
patients
provided
3,
19
patients
provided
4,
21
patients
provided
5,
3
patients
provided
6,
6
patients
provided
7,
3
patients
provided
8
and
1
patient
provided
10
samples)
while
152
patients
provided
one
sample.
Case
histories
of
all
the
patients
were
documented
from
hospitals.
Hematological
parameters
like
Hb,
platelet
count,
total
RBC
count,
total
differential
WBC
counts
were
recorded
for
125
of
the
284
patients.

SRBD protein specific SARS-CoV-2 IgG capture ELISA
Anti-SARS-CoV-2 SRBD protein IgG in human serum specimens
was
tested
by
coating
the
recombinant
SRBD
protein
on
the
to
the
microware
wells
followed
by
post-coating
procedures.
Serum
samples
were
diluted
in
the
ratio
of
1:50
with
sample
diluent.
Fifty
microlitres
each
of
the
diluted
samples,
positive
and
negative
controls
were
distributed
respectively.
ELISA
plate
was
incubated
at
37°C
for
1
hour
followed
by
washing
with
wash
buffer
5
times.
Fifty
microlitres
of
ready
to
use
anti-human
IgG
HRP
was
added
to
each
well
and
was
incubated
at
37°C
for
30
minutes.
After
washing,
100μL
of
liquid
3.3,5,5-Tetramethylbenzidine
(TMB)
substrate
was
added
and
incubated
at
room
temperature
in
the
dark
for
10
minutes.
The
reaction
was
stopped
by
adding
100μL
stop
solution
(1N
H2SO4)
after
10
minutes.
The
absorbance
was
measured
at
450nm.
If
OD
value
of
sample
tested
exceeds
0.2
and
sample
OD/ negative
control
OD
(sample
ratio)
>2.7,
the
sample
was
considered
positive
(Supplementary
Figure
1).

SARS-CoV-2 recombinant N protein IgG capture ELISA
As
described
for
S-RBD
ELISA,
similar
procedures
and
criteria
were
followed
for
dilution
of
the
samples,
testing
protocol
and
interpretation
for
recombinant
N
protein
ELISA
(Supplementary
Figure
1).

Plaque reduction neutralization test (PRNT)
Total
298
samples
were
tested
for
PRNT
(subset
of
samples
tested
for
N
and
SRBD
protein
specific
IgG
by
ELISAs);
performed
as
described
elsewhere
(Deshpande,
et
al.,
2020).

Statistics
Descriptive
statistics
were
calculated
for
continuous
variables,
counts
and
percentages
for
categorical
variables.
Mann–Whitney
U-tests and Kruskal-Wallis test were performed to compare the differences between groups. Pearson’s correlation was drawn to evaluate correlation between two methods. Geometric mean titers were calculated for ELISA ratios and NAb titers. The analysis was performed on GraphPad Prism 9. Cox proportional hazards was evaluated for the association between neutrophil to lymphocyte ratio and antibody responses and Receiver Operating Characteristic (ROC) curves for SRBD and N IgG ELISAs against PRNT were plotted on IBM SPSS 26.

Results

Demographics and Clinical findings

For total of 284 patients, the median age of the study population was 38 years with interquartile range (IQR) of 26–51, of which (158) 55.6% of them were males and (126) 44.3% were females. About 21.12% and 78.88% patients were asymptomatic and symptomatic with mild to severe symptoms respectively. Of the 224 symptomatic patients, 71.4% patients presented with severe symptoms. Majority of the older males (62.50%) showed severe symptoms. All the hematological parameters were recorded for 125 patients. Further analyses revealed that patients with severe disease condition had higher neutrophil (78.4%) and lower lymphocyte counts (15.4%). The most common symptoms observed were cold/cough/sore throat/nasal discharge (72.76%), fever (66.96%), body ache (28.57%) and breathing problems / Dyspnoea (14.73%), while comparatively less common were fatigue (11.16%) and diarrhoea (4.55%)(Table 1).

The SARS-CoV-2 N and SRBD protein-specific IgG antibody response (n = 608) and NAB activities (n = 298) were investigated and correlated in acute (0–21 days POD) and convalescent (post 21 days POD) samples. A marked increase in the mean antibody titers from acute to convalescent samples was observed for both the protein specific IgG antibodies as well as for NABs (Figures 1A, 1B, 1C). We observed SARS-CoV-2 SRBD specific IgG ELISA seropositivity in the majority of (78.94%) of COVID-19 samples (185/291 acute, 295/317 convalescent) (Figure 1A). However, N specific IgG antibody levels were detected relatively lower (74.83%) in all the samples (170/291 acute, 285/317 convalescent) (Figure 1B) compared to SRBD IgG antibody levels.

Additionally, SARS-CoV-2 NAB assessment data indicated the neutralizing activity among 75.83% COVID-19 samples tested (66/100 acute, 160/198 convalescent) (Figure 1C). Quantitatively, SRBD IgG levels showed positive correlation with N IgG levels (r = 0.6848, p < 0.0001) (Figure 1D). The SARS-CoV-2 NAB titers correlated more with SRBD IgG levels (r = 0.7911, p < 0.0001) (Figure 1E) than with respective N IgG levels. (r = 0.7207, p < 0.0001) (Figure 1E).

We examined and compared the IgG antibody levels against SRBD, N protein and the NAB titers at different time points post virus detection (Figure 2). From days 7 POD, there was a sharp rise in the average N and SRBD antibody ratios which continued to rise until day 40 after the onset of symptoms. Further, SRBD and N IgG levels showed a gradual decline till day 120. Both the N and SRBD IgG levels remained relatively stable from 120 to 240 days POD (p < 0.05, p = ns). Similar trend was observed for NAB response, with increase in average titers till 45 days POD and a decline till 120 days POD (p < 0.05, p = ns) (Supplementary Figure: 3). This was followed by a quiescent phase till 240 days POD. This persistence of immune response is of immense importance as it can provide an estimate of level of protection post natural infection.

Percent seropositivity was calculated for N IgG and SRBD IgG to observe the trends of antibody response in 284 patients till 240 days POD (Figure 3). During the early phases (0–7days) of infection, IgG antibodies were detected as early as day 3 and day 4 against SRBD protein and N protein of SARS CoV-2 respectively. During the first week POD, approximately one third of individuals showed ap-
Figure 1. Humoral immune response assessed in 608 samples collected from 284 COVID-19 patients. Antibody responses to SARS-CoV-2 against N and SRBD proteins (A-B); neutralizing antibody activity (C) in acute and convalescent samples; Correlation of SRBD IgG with N IgG antibody levels (D) ($r = 0.6848, p < 0.0001$); Correlation of SARS-CoV-2 N IgG antibody levels and neutralizing antibody titers (E) ($r = 0.7207, p < 0.0001$); Correlation of SARS-CoV-2 SRBD IgG antibody levels and neutralization antibody titers (F) ($r = 0.7911, p < 0.0001$).

Figure 2. Longitudinal responses to SARS-CoV-2 IgG and NAbs till 240 days POD. The longitudinal trends of N (blue) and SRBD (red) specific IgG and neutralizing antibodies (green) with trend line showing geometric mean titers of each parameter for 608 samples from 284 patients collected over a period of 240 days POD.
pearance of IgG antibodies against SRBD protein (35.71%) versus N protein (28.57%) of SARS-CoV-2. Thereafter, the seropositivity rate for both the antigens increased simultaneously until week 4 and reached 98.07% for antibodies detected against SRBD and N proteins. From week 5 up to 12 weeks the positivity remained relatively constant (93.44 - 97.73%) and this period denoted the timeframe of maximum seropositivity for antibodies against N and RBD proteins. At the beginning of week 13 to end of week 17, the N protein IgG levels substantially dropped to 81.39% and that of RBD declined slightly to 88.37%. From week 18-27 both SRBD and N protein specific seropositivity stagnated to 80.64% and 77.41%. The seropositivity remained near constant for both antibodies till week 35.

Among 284 patients, the average IgG concentration (P/N ratio) against SRBD and N among symptomatic cases (SRBD ratio = 10.30; N ratio = 7.69) and asymptomatic cases (SRBD ratio = 10.02; N ratio = 8.66) was not significant (Figure 4). The average IgG ratio in severe symptomatic cases for N antigen (N ratio = 6.98) showed comparable, albeit slightly lower, ratio as compared to the symptomatic and asymptomatic cases, but showed higher values (p < 0.05) of average SRBD IgG ratio (SRBD ratio = 16.79). The average RBD and N IgG ratios showed very little variation among male (SRBD ratio = 10.90; N ratio = 8.08) and female (SRBD ratio = 10.06; N ratio = 7.89) patients as well (data not shown). However there was a slight variance between the proteinspecific IgG immune response mounted when comparing individuals on either side of age 50 years. Patients above the age of 50 years (SRBD ratio = 11.97; N ratio = 8.63) showed a slightly higher antibody levels as compared to those below the age of 50 years (SRBD ratio = 9.56; N ratio = 7.32).

Considering neutrophil to lymphocyte ratio (NLR) is linked to innate immunity (Zhang et al., 2020) and is an early warning signal of severe COVID-19 (Xia et al., 2020), we analyzed NLR data against IgG response among asymptomatic, symptomatic and severe symptomatic patients (n = 125). Both N and SRBD IgG ratios were high with increased NLR in severe symptomatic patients (Figure 5A, 5B). Some symptomatic patients exhibited high NLR but the cumulative NLR of this category was within range. Also, the IgG ratios for both N and SRBD for symptomatic patients were not associated with increase in the NLR.

We then performed a time-dependent covariate Cox regression analysis of antibody responses (adjusted for sex and stratified for NLR at the time of sampling) on subsequent sampling (Figure 6). The development of SARS-CoV-2 RBD IgG antibodies was positively associated with high NLR in regression analysis with a hazard ratio (HR) for time of last sampling 1.061 (p < 0.001). The responses to the N protein (HR = 0.931) were not linked to high hazard ratio.

The levels of N and SRBD specific IgG antibody were also evaluated for correlations with all the hematological parameters in asymptomatic, symptomatic and severe symptomatic patients. With increase in POD, the increase of N and SRBD IgG positively correlated with the increase in WBC and platelet counts in severe patients, the correlation coefficient (r) was 0.53 and 0.38 for N and 0.52 and 0.39 for SRBD IgG respectively. Negative correlation was observed between RBC counts and N (r = -0.47) as well as with SRBD (r = -0.50). However, no correlation with changes in Hb was observed in severe patients. The changes in N and SRBD specific IgG antibodies showed no significant correlations with any of the hematological parameters in asymptomatic and symptomatic patients (Supplementary Figure: 2).

**Discussion**

In SARS-CoV-2 infection, waning of immunity and probability of re-infection is a major concern and there are reports affirming that antibody titers decline more quickly in asymptomatic or mild symptomatic cases than severe cases (Yamayoshi et al., 2021). Studies showing longevity of antibody response in SARS CoV-2 in a large sample size are very scarce with contrasting results (Hartley et al., 2020; Choe et al., 2021b). Here, we provide a comprehensive analysis of antibody dynamics and persistence of antibody response by evaluating protein specific IgG levels in 284 individuals with varied disease severity up to 240 days POD.

The antibodies against SRBD IgG appeared slightly earlier and remained substantially more persistent than the N protein specific IgG throughout the course of the study (240 POD). Notably, the antibody levels tend to decrease with increased interval between days post onset of symptoms until they reached a constant value. Although seropositivity reaches its maximum by week 4, average relative IgG titers against both N and SRBD antigens continue to increase till week 6 followed by a steady decline in average relative titers during weeks 7-17. After this point, the average titers of anti-SRBD & anti-N IgG antibodies remained constant till week 35.
Figure 4. Levels of IgG antibodies and titers of NAbS against SARS-CoV-2 of asymptomatic (blue), symptomatic (red) and severe symptomatic (green) cases among 284 patients collected from 0-240 days POD. Antibody levels are expressed as ratio values (A, B) and NAb titers are plotted (C). The median and quartiles were represented in the violin plots. (∗ p < 0.05, ∗∗ p < 0.01, ∗∗∗ p < 0.001, ∗∗∗∗ p < 0.0001)

Figure 5. Immune response across diverse disease severity with respect to neutrophil to lymphocyte ratio (NLR) and IgG antibody levels (N = 125). Association between A) N IgG ratio and NLR (cut-off = 3.04); B) SRBD IgG ratio and neutrophil to lymphocyte ratio for 125 patients. (Asymptomatic = 42, Symptomatic = 68 and severe symptomatic = 15 cases.)

Figure 6. Associations of immune response and disease severity correlogram of COVID-19 patients: A) Asymptomatic cases, B) Symptomatic cases and C) Severe symptomatic cases. Spearman rank order correlation values (r) are shown from red (-1.0) to blue (1.0); r values are indicated by color and square size.
It is worth mentioning that various factors may predict an enhanced initial antibody response against SARS-CoV-2 and the persistence of antibodies over time (Terpos et al., 2021). Of the measured SARS-CoV-2 antibodies, the IgG response against the SRBD domain was associated with NAbs (r = 0.79) independent of other factors such as sex or age. This indicated that SRBD specific antibodies in the patient sera meant improved patient survival rate supporting the concept that these antibodies are a major contributor to the protective effect of humoral immunity in COVID-19. This finding may have implications in the anticipated protection against re-infection over time. Additionally, the SRBD specific IgG response showed comparatively higher average levels of antibody titers in patients with severe disease, than the asymptomatic and symptomatic cases. A previous study reported that the severe disease was associated with more robust serological responses including early seroconversion (<day 16) and higher IgG levels (Zhang et al., 2020), which is in agreement with our study observation. Our findings in turn indicate higher titers of NABs among severe cases. Conversely, the overall quantitative IgG response against N protein did not differ significantly based on the disease severity, demonstrating that it is independent of the severity of the disease. Differing from other studies (Hibino et al., 2021|Long et al., 2020), we witnessed that there was no early waning in IgG titers in asymptomatic or symptomatic cases. This difference in observations could probably be attributed to the variable genetic makeup and individual overall immune status. Also, the differences in detection methods and disease severity in different cohorts may be responsible for such variations ( Bölke et al., 2020).

In addition, contradictory to some studies (Santis et al., 2020|Marklund et al., 2020|Lee et al., 2020), not all patients, irrespective of their symptomatic status, had developed an IgG response to SARS-CoV-2. Of the sequential samples received, 7 patients failed to mount a response against N protein, 1 failed to mount a response against SRBD protein, 5 patients failed to mount a NAB response, 2 failed to mount a response altogether against both antigens and 2 patients did not mount any IgG or NAB response at any given time point. These patients or a subset of these patients can be considered as ‘non-responders’ and further studies are needed for confirmation as these patients hold the key to complete disease elimination following the global vaccination drives.

Though the global studies show upwards of 45% COVID-19 cases as being asymptomatic, the number was much lower in our study due to the selection bias of hospitals to admit patients mainly based on their severity of symptoms. Based on clinical data, mild to severe symptoms were observed more among the older male population in the study. The recent report indicated that the NLR was identified as a powerful predictive and prognostic factor for severe COVID-19 and systematic inflammatory response ( Liu et al., 2020|Yang et al., 2020). Though the number of severe symptomatic cases was less compared to symptomatic cases, in our observations, more severe cases presented with high NLR and high IgG antibody levels. The changes in N-IgG and SRBD IgG antibody response showed no significant correlations with Hb, total RBC count, total WBC count and platelet count in asymptomatic and symptomatic patients. Furthermore, the high disease severity was associated with an increase in the neutrophils and a decrease of lymphocyte count. Our observations were corroborated by other studies as well and a decrease of lymphocyte count may be attributed to SARS-CoV-2 induced syncytia formation leading to lymphocyte loss in the patients with COVID-19 (Aschenbrenner et al., 2021).

The limitations of this study include unavailability of data on virus titers during SARS-CoV-2 infection which would have emphasized a better correlation between disease severity and immune response. Due to limited availability of clinical data, we could only analyze NLR but other immunological markers of cell mediated immunity also need to be studied in order to understand the diverse behaviour of immune responses. Nevertheless, this study tried to elucidate the understanding of varied antibody dynamics among infected patients along with disease severity and its clinical correlation with NLR which is very important clinical marker used for early screening of critical illness of patients. Current pandemic status necessitates the assessment of the antibody response for a prolonged period in COVID-19 patients to establish a link between the presence of antibodies and the level of protection against re-infection.

In conclusion, our study demonstrated the persistence of N and SRBD IgG response up to 8 months irrespective of the disease spectrum along with the strong longitudinal responses elicited against SRBD protein and correlated with the NAB, which in turn predicts the protective immunity. This data may help in vaccination strategies for those who were previously infected with COVID-19 and public health decisions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2021.09.024.

References

Aschenbrenner AC, Mouktaroudi M, Krämer B, Oestreich M, Antonakos N, Nuesch-Germano M, et al. Disease severity-specific neutrophil signatures in blood transcriptomes stratify COVID-19 patients. Genome Med 2021;13:7. doi:10.1186/s13073-020-00823-5.

 Bölke E, Matuschek C, Fischer JC. Loss of Anti-SARS-CoV-2 Antibodies in Mild COVID-19. N Engl J Med 2020;383:1094–9. doi:10.1056/NEJM20200701.

Brochet E, Derrey B, Touzé A, Belouazad S, Dubusson J, Schmit JL, et al. Anti-spike, Anti-nucleocapid and Neutralizing Antibodies in SARS-CoV-2 Inpatients and Asymptomatic Individuals. Front Microbiol 2020;11:2468.

Choe PG, Kang CK, Suh HJ, Jung J, Song K-H, Bang JH, et al. Waning Antibody Responses in Asymptomatic and Symptomatic SARS-CoV-2 Infection. Emerg Infect Dis 2021a;27:327–38. doi:10.3201/eid2701.203515.

Choe PG, Kim K-H, Kang CK, Suh HJ, Kang E, Lee SY, et al. Antibody Responses 8 Months After Asymptomatic or Mild SARS-CoV-2 Infection. Emerg Infect Dis 2021b;27:928–31. doi:10.3201/eid2703.200453.

Ciaccio M, Agnello L. Biochemical biomarkers alterations in Coronavirus Disease 2019 (COVID-19). Diagnosis 2020;7:365–72. doi:10.1515/dx-2020-0057.

Definitions and Descriptions C. WHO-2019-nCoV-Surveillance_Case-Definition-2020-2-eng 2020:2020.

Deshpande G, Sankal G, Tilekar B, Yadav P, Gurav Y, Gaikwad S, et al. Neutralizing antibody responses to SARS-CoV-2 in COVID-19 patients. Indian J Med Res 2020;152:82–7. doi:10.4103/ijmrm.IJMR_2382_20.

Dohado C, Santano R, Jiménez A, Vidal M, Chu J, Rodrigo Menezes N, et al. Immuno- genicity and crossreactivity of antibodies to the nucleocapid protein of SARS-CoV-2: utility and limitations in seroprevalence and immunity studies. Transl Res 2021. doi:10.1016/j.trsl.2021.02.006.
Hartley GE, Edwards ESJ, Aoi PM, Varese N, Stojanovic S, McMahon J, et al. Rapid generation of durable B cell memory to SARS-CoV-2 spike and nucleocapsid proteins in COVID-19 and convalescence. Sci Immunol 2020;5:eaaz8891. doi:10.1126/sciimmunol.aba9391.

Hibino S, Hayashida K, Ahn AC, Hayashida Y. COVID-19 seropositivity changes in asymptomatic individuals during the second and third waves of COVID-19 in Tokyo. MedRxiv 2021. doi:10.1101/2020.09.21.20198796. https://doi.org/10.1016/j.jinf.2020.09.21.20198796.

Kontou PI, Brailiou GG, Dimou NL, Nikolopoulos G, Bagos PG. Antibody tests in detecting SARS-CoV-2 infection: a meta-analysis. MedRxiv 2020. doi:10.1016/j.jinf.2020.04.22.20074914. https://doi.org/10.10110/2021.09.21.20198796.

Lee Y-L, Liao C-H, Liu P-Y, Cheng C-Y, Chung M-Y, Liu C-E, et al. Dynamics of anti-SARS-CoV-2 IgM and IgG antibodies among COVID-19 patients. J Infect 2020;81:e55–8. doi:10.1016/j.jinf.2020.04.019.

Liu J, Liu Y, Xiang P, Pu L, Xiong H, Li C, et al. Neutrophil-to-lymphocyte ratio predicts critical illness patients with 2019 coronavirus disease in the early stage. J Transl Med 2020;18:206. doi:10.1186/s12967-020-02374-0.

Long Q-X, Tang X-J, Shi Q-L, Li Q, Deng H-J, Yuan J, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med 2020;26:1200–4. doi:10.1038/s41591-020-0956-5.

Marklund E, Leach S, Axelsson H, Nystroem K, Norder H, Bemark M, et al. Serum-IgG responses to SARS-CoV-2 after mild and severe COVID-19 infection and analysis of IgG non-responders. PLoS One 2020;15.

Ministry of Health and Family Welfare. Clinical Management Protocol 2020. Oran DP, Topol EJ. Prevalence of Asymptomatic SARS-CoV-2 Infection. Ann Intern Med 2020;173:362–7. doi:10.7326/M20-3012.

Poddar V, Cherian SS, Deshpande GR, Ullas PT, Yadav PD, Choudhary ML, et al. Genomic analysis of SARS-CoV-2 strains among Indians returning from Italy, Iran & China, & Italian tourists in India. Indian J Med Res 2020;151:255–60. doi:10.4103/ijmr.IJMR_1058_20.

Poddar V, Vipat V, Ramdas B, Jadhav S, Pawar-Patil J, Walmbeem A, et al. Phylogenetic classification of the whole-genome sequences of SARS-CoV-2 from India & evolutionary analysis of the spike protein (S) gene. Indian J Med Res 2021;153:166–74. doi:10.4103/ijmr.IJMR_3418_20.

Pourbagheri-Sigaroodi A, Barchash D, Fateh F, Aboolahemsi H. Laboratory findings in COVID-19 diagnosis and prognosis. Clin Chim Acta 2020;510:475–82. doi:10.1016/j.cca.2020.08.019.

Santis LV-D, Pérez-Camacho I, Sobrino B, González GE, Ruiz-Mesa JD, Plata A, et al. Clinical and immunoserological status 12 weeks after infection with COVID-19: prospective observational study. MedRxiv 2020;2020.10.06.20206600. https://doi.org/10.1016/j.jinf.2020.09.21.20198796.