SARS-CoV-2 neutralizing antibodies in patients with varying severity of acute COVID-19 illness

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Research Article

Keywords: SARS-CoV2, neutralizing antibodies, disease severity, prolonged shedding, persistence of antibodies

Posted Date: July 22nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-47016/v1

Abstract

In order to understand the kinetics, timing and persistence of SARS-CoV2 neutralizing antibodies (Nabs) we used a surrogate viral neutralization test to evaluate their levels in patients with varying severity of illness, in those with prolonged shedding and those with mild/asymptomatic illness at various time points. Patients with severe or moderate COVID-19 illness had earlier appearance of Nabs at higher levels compared to those with mild or asymptomatic illness. Furthermore, those who had prolonged shedding of the virus, had Nabs appearing faster and at higher levels than those who cleared the virus earlier. Although all individuals appeared to be antibody positive by end of week 5, the positivity rates declined thereafter, especially in those who had mild or asymptomatic illness.

Introduction

The SARS-CoV2 virus has resulted in over 14 million infections and half a million deaths in a period of just 7 months1. While many countries are under various degrees of lockdown there is a huge race to develop a safe and effective vaccine, and currently 21 vaccine candidates undergoing clinical trials2. The main aim of vaccination is to induce long lasting protection against infection with the SARS-CoV2 by inducing a robust virus specific neutralizing antibody and T cell response. Analysis of SARS-CoV2 specific neutralizing antibodies (NAbs from infected individuals showed that the majority of such antibodies target the receptor binding domain (RBD) and prevent binding to the host cell receptor ACE23. However, there have been recent concerns regarding decline of both total antibodies and NAbsto SARS-CoV-2 at 8 weeks since onset of illness, especially in those with mild illness4. This decline in antibody titres was seen more for the total IgG antibodies than the NAbs, suggesting that NAbs that are needed for subsequent protection could be long lasting. Following MERS and SARS infection, functional NAbs were shown to persist for over 1 year5 and up to at least 17 years6, although they were undetectable in significant proportion of individuals by 30 to 34 months7,8. Antibody responses to other seasonal coronaviruses also have shown to be short-lived and that individuals could be infected with coronaviruses such as NL63 within a 6 month period9. Therefore, in order to develop effective vaccines, it would be important to answer key questions such as if the appearance of NAbs led to less severe disease, stopped virus shedding and their persistence. In this study, we initially investigated the kinetics of SARS-CoV2 specific NAbs in a cohort of patients with varying severity of illness and then proceeded to further characterize the responses at different time points in relation to clinical disease severity.

Results

Determining specificity of the sVNT in measuring SARS-CoV2 Nabs in the Sri Lankan population

In order to determine the specificity of the sVNT in Sri Lankan individuals, we initially assessed the % of inhibition in 81 serum samples obtained from individuals who presented with a febrile illness to the outpatient department of the National Institute of Infectious Diseases (NIID), Sri Lanka in 2018. All these individuals had a % of inhibition less than the cut of value of ≤25%. We then assessed the specificity of the assay in 285 non-exposed individuals recruited from the Colombo Municipality area during the month of April. The percentage of inhibition in this population was also less than the cut-off value. Therefore , the specificity of this assay was found to be 100% as previously described 10.

Longitudinal changes in Nabs in patients with varying severity of clinical disease

In order to determine the longitudinal changes of SARS-CoV2 NAbs in patients with varying clinical disease severity, we assessed their levels in those with severe (n=6), moderate (n=5) and mild/asymptomatic illness (n=13) and also those who had mild illness but with prolonged shedding of the virus (n=21). The median duration of virus shedding in this whole cohort was 25 days (IQR 15 to 38 days) and therefore, those who had virus shedding for over 25 days and hospitalized for over 25 days were considered to have prolonged shedding of the virus. There were 10 individuals who had shedding for over 50 days. The patterns of virus shedding in those with mild but prolonged illness is shown in Fig 1A.

Three blood samples were obtained from these individuals during the course of illness and in the case of those with mild infection, 4 to 6 weeks after they were discharged from hospital.

In the longitudinal analysis of NAbs, they appeared earlier and faster, at higher levels in those who had severe and moderate pneumonia, followed by those who had prolonged shedding, while they appeared later, at lower levels in those who had mild/asymptomatic disease (Fig 1B). 4 individuals with mild illness had no detectable NAbs even 40 days since onset of illness, whereas all but 1/21 individuals had NAbs below this level.

The timing of the appearance of Nabs and their levels in relation to clinical disease severity

In order to further evaluate the appearance and the quantity of NAbs in relation to clinical disease severity, we assessed antibodies in blood samples at different time points from patients with severe pneumonia (n=10), moderate illness (n=19), mild illness (n=150) and prolonged shedding (n=82). The Nabs levels were determined in this larger cohort of individuals during the first 3 weeks and during week 4 to 8 of illness.

Again, those with moderate and severe illness had higher NAbs levels (median 63.16 and 48.9% of inhibition) during the 1st week, and in all subsequent time points compared to those with mild and

prolonged shedding (Fig 2A). After the 3rd week (4th to 8th week), although all patients with severe, moderate and prolonged shedding had a positive test result, 23/69 (33.3%) of those with mild/asymptomatic illness were negative (% of inhibition <25). Those with prolonged shedding, who had mild or asymptomatic illness had significantly higher ($p<0.0001$) NeutAb levels (median 76.4%, IQR 52.32 to 89.5% of inhibition) than those with mild/asymptomatic illness (median 47.9%, IQR 18.9 to 77.1% of inhibition) during week 4 to 8. This data further reinforces the longitudinal analysis of NAbs as shown in Fig 1B.

SARS-CoV2 Nab positivity at different time points and persistence

We then proceeded to assess the detection of NAbs at various time points in illness, irrespective of clinical disease severity and also to assess if NAbs persisted over 90 days since onset of illness. NAbs were measured by the sVTN on day 14 to 21 (n=98), day 22 to 28 (n=100), day 29 to 36 (n=132), day 37 to 42 (n=32), day 43 to 49 (n=16), day 50 to 70 (n=29) and >90 days (n=15). The positivity rates during day 14 to 21 was 79.8%, day 22 to 28 was 88.9%, day 29 to 36 was 100Therefore, all patients tested positive by the end of 5th week of illness (Fig 2B). However, the positivity rates declined thereafter, as the positivity was 90.6% at 37 to 42 days, 65.5% between 50 to 70 days of illness, and 53.3% in those with >90 days since onset of illness suggesting that the NAbs could be declining with time. All those who had Nabs below the cut-off value were those who had mild/asymptomatic illness, while those with moderate/severe illness and individuals with prolonged shedding were positive for Nabs after day 90 of illness.

Discussion

In this study, we show that the early appearance of SARS-CoV-2 NAbs at high levels was not associated with milder disease nor with early clearance of the virus. Early appearance of Nabs has previously shown to occur in those with severe disease compared to those with mild illness11, although the reasons for such occurrence is not clear. Higher initial viral loads were associated with progression to more severe disease in SARS12,13. Therefore, higher viral loads could drive a more robust antibody response. However, infants who were symptomatic had higher nasopharyngeal viral loads, but less severe illness compared to older children with more severe illness14, suggesting that higher viral loads were not necessarily associated with more severe illness. On the other hand, the early appearance of Nabs in patients with more severe disease could be due to the boosting of Nabs specific to previous coronaviruses. Therefore, early appearance of such cross-reactive antibody responses could have a potential to cause severe illness by antibody dependent enhancement15.

The relationship between the appearance of Nab with duration of virus shedding has not been previously studied. Surprisingly, those who had prolonged shedding had higher levels of NAbs than those who cleared the virus, and the NAbs appeared in such prolonged shedders earlier than in those who cleared

the virus earlier. In Sri Lanka, until recently, patients with COVID-19 were only discharged from hospital if they had 2 negative PCRs, 24 hours apart. Therefore, despite these prolonged shedders developing antibodies earlier than those who cleared the virus, and at higher titres, they still continued to shed the virus. Although the majority of such prolonged shedders had lower viral titires (Ct values >30), some individuals still had higher viral loads even after 30 days of illness. As many other countries do not keep patients in hospital until they become PCR negative, the relationship between early appearance of Nabs and yet persistence has not been documented previously and questions the role of Nab alone in viral clearance.

Although NAbs are thought to associate with protection, this has not been the case with infections such as dengue, which induce cross reactive antibodies as seen between different coronaviruses. Those with high NAbs for a particular dengue virus serotype were found to get re-infected with the same serotype16. In addition, the kinetics of NAbs levels in those with varying severity of dengue, was remarkably different based on the infecting dengue virus serotype17. Therefore, it is crucial to carry out further studies to identify the protective antibody responses for the SARS- CoV-2, their persistence and their ability to prevent re-infection.

All individuals appeared to be antibody positive by end of week 5, suggesting that the majority of COVID-19 individuals do develop Nabs at some time point during illness. However, the positivity rates declined thereafter. The decline in Nab antibodies in COVID-19 patients has been documented in recent reports11, which has implications in providing long lasting immunity through vaccination. Although we only tested Nabs in a small cohort of individuals with more 90 days since onset of illness, none of those with mild/asymptomatic illness had Nab above the cut-off value. Further studies are required to determine if such individuals have memory B cell responses and functional Nab even at low levels that would prevent re-infection.

In summary, we show that the early appearance of SARS-CoV-2 NAbs at high levels was not associated with milder disease nor with early clearance of the virus and that Nabs did not persist in those with mild/asymptomatic illness.

Methods

Patients

Patients were recruited from the National Institute of Infectious Diseases (NIID), Sri Lanka and the Theldeniya Covid-19 Management Centre in Kandy. Clinical disease severity was classified as mild, moderate and severe according to the WHO guidance of COVID-19 disease severity18. Accordingly, those who had a confirmed symptomatic SARS-CoV2 infection with no evidence of hypoxia or pneumonia were classified as having mild illness. Those with clinical signs of pneumonia with a respiratory rate of >30 breaths/minute, or with SpO2 <90% on room air were considered as having severe pneumonia18. Those with clinical and radiological signs of pneumonia, but who did not fulfill the criteria of severe disease were classified as having moderate illness.

Sera from 81 patients who presented to the outpatient department of the NIID, in 2018 for treatment for a febrile illness were used to determine the specificity of the assay.

Healthy individuals from the community

285 healthy individuals who were not exposed to individuals with COVID-19 were recruited from the Colombo Municipality Council area following informed written consent. Since the majority of the patients with COVID-19 who were admitted to NIID with acute illness were from this area, blood samples were obtained from them after 90 days since onset of illness to determine the persistence of Nabs.

Ethical approval:

Ethical approval was received by the Ethics Review Committee of Faculty of Medical Sciences, University of Sri Jayewardenepura. The study on humans were carried out in accordance with relevant guidelines and regulations (the Declaration of Helsinki).

RT-PCR for detection of SARS CoV-2:

Naso/Oro pharyngeal swabs or sputum samples of suspected SARS- CoV-2 patients were lysed and RNA was extracted using QIAmp® Viral RNA Mini Kit (Qiagen, USA, Cat: 52906) and used to detect the presence of N gene and ORF1ab gene of SARS-CoV2 with Da An Gene real time PCR kit (Da An Gene, China. Cat: DA-930) by real time RT PCR according to manufacturer's instructions in ABI 7500 real time PCR system (Applied Biosystems, USA).

Assay to measure Nab

As measuring SARS-CoV2 NAbs would require a BSL-3 facility and limit the number of samples that can be assessed, we adopted a recently developed surrogate virus neutralization test (sVNT), which measures the percentage of inhibition of binding of the RBD of the S protein to recombinant ACE210 (Genscript Biotech, USA). Inhibition percentage ≥ 25% in a sample was considered as positive for NAbs.

Declarations

Acknowledgements

We are grateful to the Centre for Dengue Research, UK Medical Research Council and the Foreign and Commonwealth Office for support. Serology work in the Wang Lab at Duke-NUS is supported by Singapore National Medical Research Council (STPRG-FY19-001 and COVID19RF-003).

Competing interests

The authors declare no competing interests.

References

1. *COVID-19 CORONAVIRUS OUTBREAK*, 2020).
2. Organization, W. H. Draft landscape of COVID-19 candidate vaccines. 7 (2020).
3. Kreer, C., Zehner, M., Weber, T., Ercanoglu, M.S., Gieselmann, L., Rohde, C., Halwe, , Korenkov, M., Schommers, P., Vanshylla, K., Di Cristanziano, V., Janicki, H., Brinker, R., Ashurov, A., Krähling, V., Kupke, A., Cohen-Dvashi, H., Koch, M., Eckert, J.M., Lederer, S., Pfeifer, N., Wolf, T., Vehreschild, M.J.G.T., Wendtner, C., Diskin, R., Gruell, H., Becker, S., Klein, F. Longitudinal isolation of potent near-germline SARS-CoV-2- neutralizing antibodies from COVID-19 patients. *Cell*, doi:https://doi.org/10.1016/j.cell.2020.06.044 (2020).
4. Long, Q. X. *et al.* Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nature medicine*, doi:10.1038/s41591-020-0965-6 (2020).
5. Choe, P. G. *et al.* MERS-CoV Antibody Responses 1 Year after Symptom Onset, South Korea, 2015. *Emerging infectious diseases* **23**, 1079-1084, doi:10.3201/eid2307.170310 (2017).
6. Anderson, D. E. *et al.* Lack of cross-neutralization by SARS patient sera towards SARS- CoV-2. *Emerg Microbes Infect* **9**, 900-902, doi:10.1080/22221751.2020.1761267 (2020).
7. Payne, D. C. *et al.* Persistence of Antibodies against Middle East Respiratory Syndrome Coronavirus. *Emerging infectious diseases* **22**, 1824-1826, doi:10.3201/eid2210.160706 (2016).
8. Cao, W. C., Liu, W., Zhang, P. H., Zhang, F. & Richardus, J. H. Disappearance of antibodies to SARS-associated coronavirus after recovery. *The New England journal of medicine* **357**, 1162-1163, doi:10.1056/NEJMc070348 (2007).
9. Kiyuka, K. *et al.* Human Coronavirus NL63 Molecular Epidemiology and Evolutionary Patterns in Rural Coastal Kenya. *The Journal of infectious diseases* **217**, 1728-1739, doi:10.1093/infdis/jiy098 (2018).
10. Tan CW, W., Qin X, Liu P, Chen MI-C, Tiu C, Hu Z, Chen VC-W, Young BE, Sia WR, Tan Y-J, Foo R, Yi Y, Lye DC, Anderson DE, Wang, L.-F A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction. *Nature biotechnology*, doi:10.21203/rs.3.rs-24574/v1 (2020).
11. Jeffrey Seow, C. G., Blair Merrick, Sam Acors, Kathryn J.A. Steel, Oliver Hemmings, Aoife O'Bryne, Neophytos Kouphou, Suzanne Pickering, Rui Galao, Gilberto Betancor, Harry D Wilson, Adrian W Signell, Helena Winstone, Claire Kerridge, Nigel Temperton, Luke Snell, Karen Bisnauthsing, Amelia Moore, Adrian Green, Lauren Martinez, Brielle Stokes, Johanna Honey, Alba Izquierdo-Barras, Gill Arbane, Amita Patel, Lorcan OConnell, Geraldine O Hara, Eithne MacMahon, Sam Douthwaite, Gaia Nebbia, Rahul Batra, Rocio Martinez-Nunez, Jonathan Edgeworth, Stuart J.D. Neil, Michael H. Malim, Katie Doores. (medRxiv, 2020).
12. Chu, C. M. *et al.* Initial viral load and the outcomes of SARS. *CMAJ* **171**, 1349-1352, doi:10.1503/cmaj.1040398 (2004).
13. Hung, I. F. *et al.* Viral loads in clinical specimens and SARS manifestations. *Emerging infectious diseases* **10**, 1550-1557, doi:10.3201/eid1009.040058 (2004).

14. Zachariah, *et al.* Symptomatic Infants have Higher Nasopharyngeal SARS-CoV-2 Viral Loads but Less Severe Disease than Older Children. *Clin Infect Dis*, doi:10.1093/cid/ciaa608 (2020).
15. Fierz, W. & Walz, B. Antibody Dependent Enhancement Due to Original Antigenic Sin and the Development of SARS. *Frontiers in immunology* **11**, 1120, doi:10.3389/fimmu.2020.01120 (2020).
16. Waggoner, J. J. *et al.* Homotypic Dengue Virus Reinfections in Nicaraguan Children. *The Journal of infectious diseases* **214**, 986-993, doi:10.1093/infdis/jiw099 (2016).
17. Wijesinghe, A. *et al.* Phenotype and functionality of follicular helper T cells in patients with acute dengue infection. *Journal of biomedical science* **27**, 50, doi:10.1186/s12929- 020-00641-2 (2020).
18. Organization, H. Clinical management of severe acute respiratory infection when novel coronavirus (2019-nCoV) infection is suspected: interim guidance. (WHO, 2020).

Figures

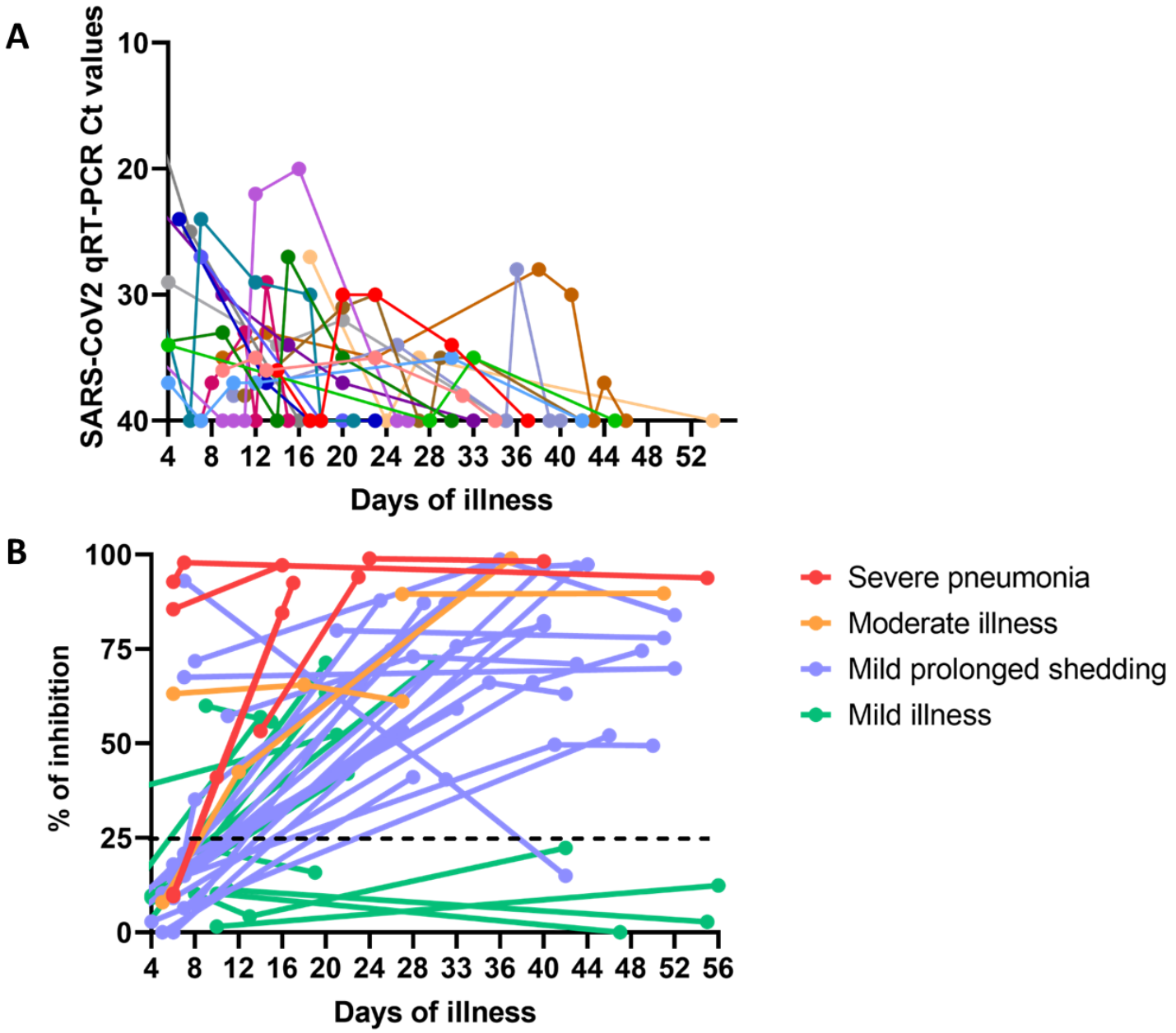

Figure 1

Longitudinal analysis of virus shedding and SARS-CoV-2 neutralizing antibodies in patients with COVID-19 Realtime qPCR was carried out in individuals with prolonged shedding (n=17) throughout the course of illness to determine the patterns and duration of virus shedding (A). SARS-CoV-2 NAbs were measured in those with severe (n=6), moderate (n=5) and mild/asymptomatic illness (n=13) and also those who had mild illness but with prolonged shedding of the virus (n=21). The black dotted line indicates the cut-off value of a positive result

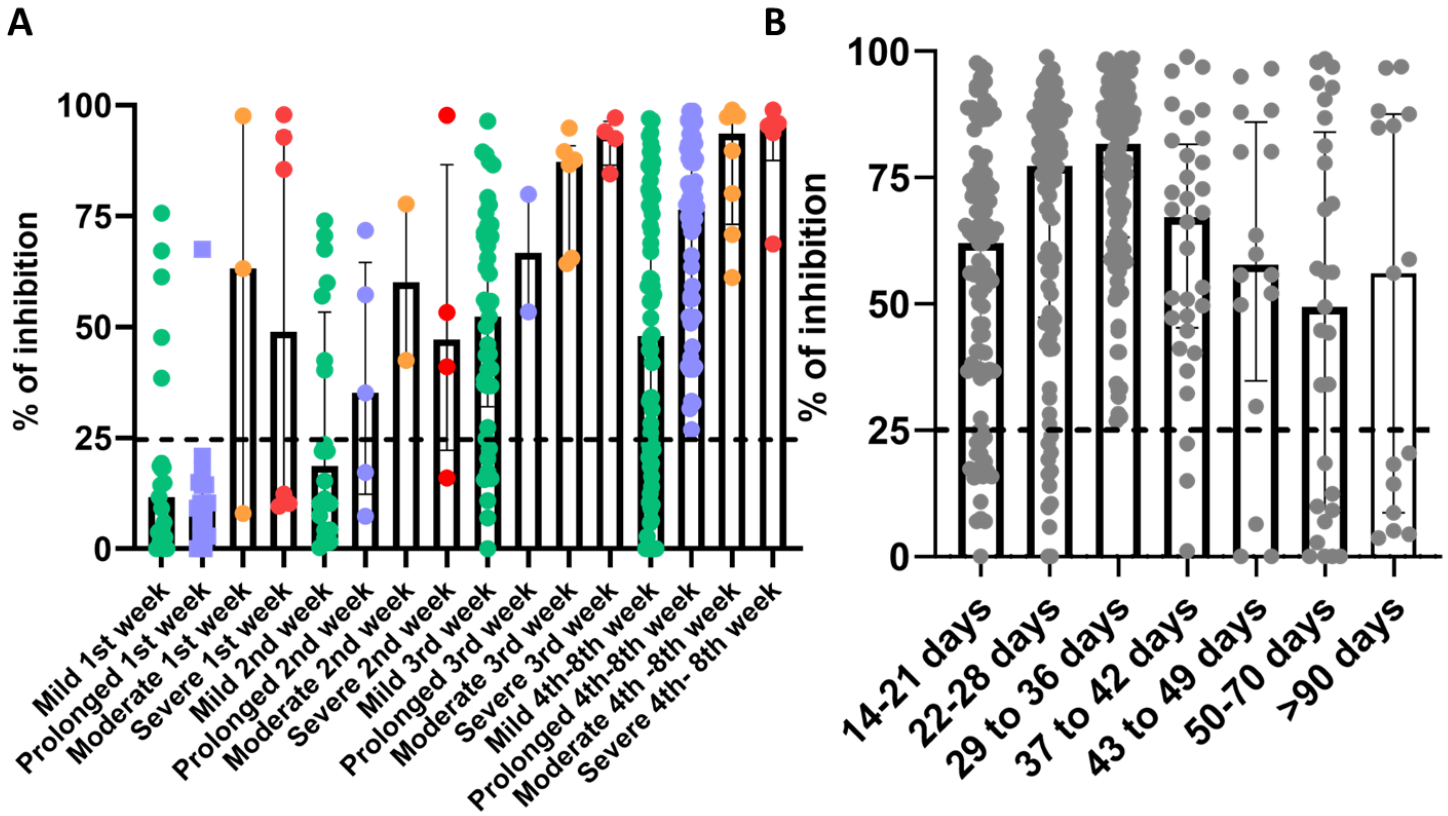

Figure 2

SARS-CoV-2 neutralizing antibody positivity during different weeks since onset of illness SARS-CoV-2 NAbs were measured at different time points from patients with severe pneumonia (n=10), moderate illness (n=19), mild illness (n=150) and prolonged shedding (n=82) (A) and also measured at different time points irrespective of disease severity. 14 to 21 (n=96), day 22 to 28 (n=99), day 29 to 36 (n=132), day 37 to 42 (n=32), day 43 to 49 (n=16), between ay 50 to 70 (n=29) and more than 90 day (n=15). The black dotted line indicates the cut-off value of a positive result