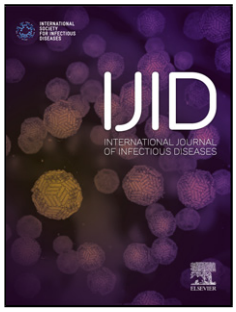
Journal Pre-proof



The effect of tocilizumab, anakinra, and prednisolone on antibody response to SARS-CoV-2 in patients with COVID-19: A prospective cohort study with multivariate analysis of factors affecting the antibody response

Seniha Başaran, Serap Şimşek-Yavuz, Sevim Meşe, Atahan Çağatay, Alpay Medetalibeyoğlu, Oral Öncül, Halit Özsüt, Ali Ağaçfidan, Ahmet Gül, Haluk Eraksoy

PII: S1201-9712(21)00249-6

DOI: https://doi.org/10.1016/j.ijid.2021.03.031

Reference: IJID 5233

To appear in: *International Journal of Infectious Diseases*

Received Date: 22 January 2021

Revised Date: 1 March 2021

Accepted Date: 10 March 2021

Please cite this article as: { doi: https://doi.org/

The effect of tocilizumab, anakinra, and prednisolone on antibody response to SARS-CoV-2 in patients with COVID-19: A prospective cohort study with multivariate analysis of factors affecting the antibody response

Seniha Başaran[a*], Serap Şimşek-Yavuz[a], Sevim Meşe[b], Atahan Çağatay[a], Alpay Medetalibeyoğlu[c], Oral Öncül[a], Halit Özsüt[a], Ali Ağaçfidan[b], Ahmet Gül[d], Haluk Eraksoy[a]

[a]Department of Infectious Diseases and Clinical Microbiology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey

[b]Department of Medical Microbiology, Division of Virology and Fundamental Immunology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey

[c]Department of Internal Medicine, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey

[d]Department of Rheumatology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey

senihabasaran@yahoo.com,

serapsimsekyavuz@gmail.com,

drsevimmese@gmail.com,

atayon@yahoo.com,

alibeyoglualpay@gmail.com,

oraloncul@gmail.com,

halitozsut@superonline.com,

aagacfidan@hotmail.com,

dr.agul001@gmail.com,

haluk.eraksoy@gmail.com

***Corresponding author**:

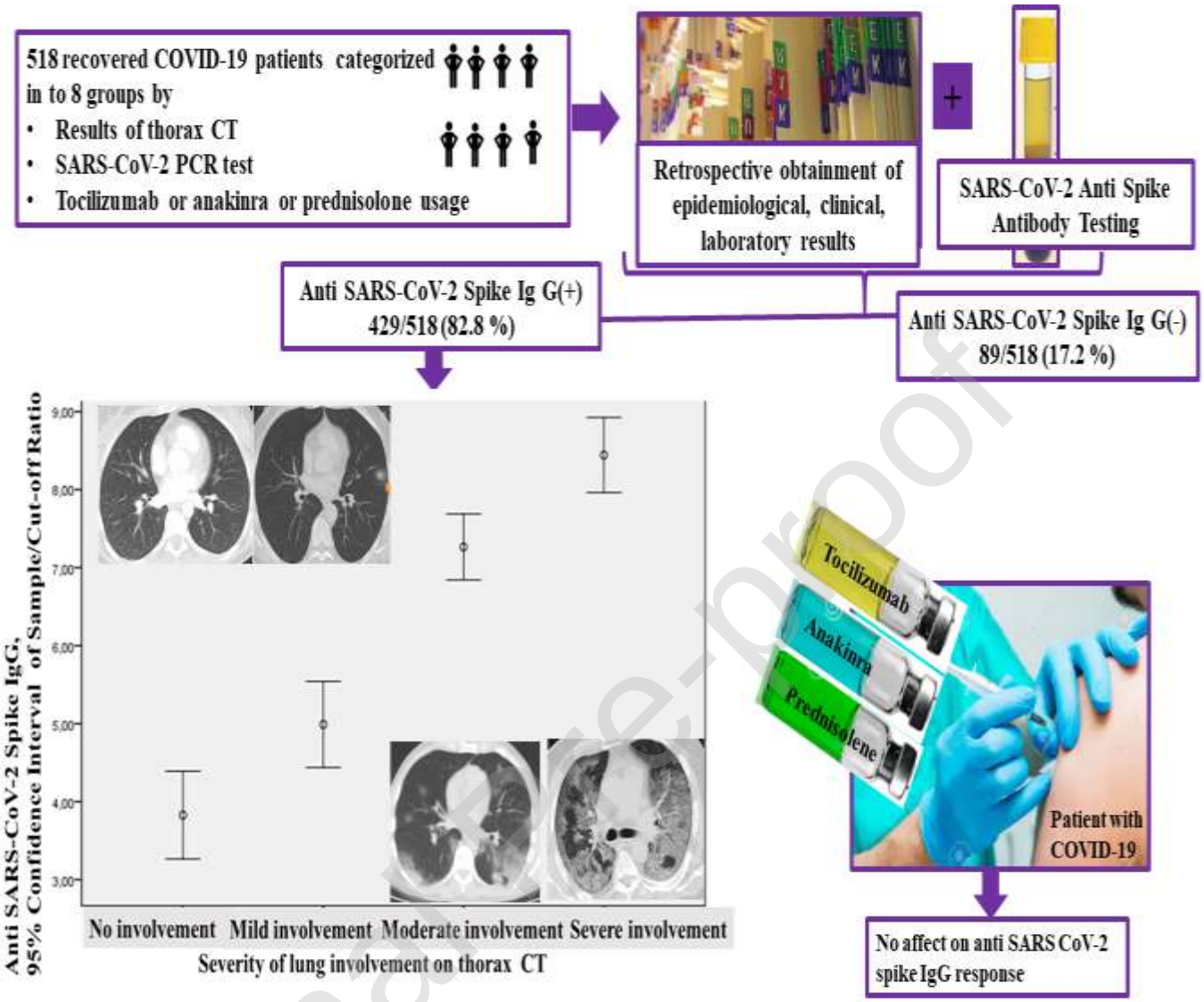
Seniha Başaran

Istanbul Faculty of Medicine , Department of Infectious Diseases and Clinical Microbiology ,

Fatih/Istanbul

Tel: +90 414 20 00 extention 35032 senihabasaran@yahoo.com

Graphical abstract



Highlights

- Tocilizumab, anakinra and prednisolone did not affect antibody response to SARS-CoV-2.
- Antibody response correlates with and could be predicted by involvement on thorax CT.

- Antibody level also correlates with the level of lung involvement on thorax CT.

Abstract

Objectives: Disease severity, previous medications, immunosuppresive agents could affect the antibody response against SARS-CoV-2. We aimed to analyze variables affecting the humoral response to SARS-CoV-2.

Methods: In this prospective cohort study, we included adult patients who recovered from COVID-19 and were admitted to COVID-19 follow-up unit. We defined 8 patient groups in accordance with the results of thorax CT, SARS-CoV-2 PCR test, and tocilizumab or anakinra use during active disease. Anti-S IgG antibodies were determined by ELISA in serum samples. Anti-S positive and negative cases were compared.

Results: A total of 518 patients were included in the study. SARS-CoV-2 IgG antibodies were positive in 82.8% of patients. SARS-CoV-2 PCR positivity, extent of lung involvement on CT, and time to antibody testing were independently associated with antibody positivity. Tocilizumab, anakinra or prednisolone use was not a factor affecting the antibody response. The rate of antibody response and sample/CO values among antibody positive patients showed a linear relationship with the extent of lung involvement on CT.

Conclusions: The use of tocilizumab, anakinra, and prednisolone for COVID-19 did not affect the antibody response against SARS-CoV-2. The main driver of antibody response among patients with COVID-19 was the extent of pulmonary involvement on CT.

Key words: tocilizumab, anakinra, prednisolone, SARS-CoV-2, antibody

Introduction

Protective immune response against SARS-CoV-2 requires syncronized function of viral protein-specific CD4+ and CD8+ T cells as well as B cells. COVID-19 patients with severe infection were more likely to have asynchronous immune responses. Either having a syncronized or asyncronized immune response, the vast majority of patients with PCR-confirmed SARS-CoV-2 infection produced antibodies against SARS-CoV-2 and those with the most severe disease had higher titers of anti-SARS-CoV-2 antibodies compared to asymptomatic or mild cases (Rydyznski Moderbacher et al., 2020, Wajnberg et al., 2020a, 2020b). However, many other factors including comorbidities, previous medications, and current treatments could also affect the antibody response to SARS-CoV-2 in COVID-19 in addition to the severity of infection. After the publication of some observational studies showing beneficial effects of anti-cytokine treatments for COVID-19, biologic agents blocking the activity of IL-6 such as tocilizumab and activity of IL-1, such as anakinra, have been widely used to treat COVID patients who developed findings of cytokine storm syndrome (Toniati et al., 2020). In addition, glucocorticoid treatment has become a standard of care for severe COVID-19 patients after demonstration of the beneficial effect of dexamethasone on mortality (RECOVERY Collaborative Group, 2020). All of these immunosupresive treatments could affect the antibody response against SARS-CoV-2 (Maeda et al., 2010, Roll et al., 2011, Dinarello, 2009).

In this study, we analyzed all potential factors which may affect the antibody response to SARS-CoV-2 in a cohort of patients with different levels of disease severity and SARS-CoV-2 PCR results, and specifically aimed to investigate whether or not this response was affected by tocilizumab, anakinra or by prednisolone treatments.

Methods

Study design and population

In this prospective cohort study, we included all consecutive adult patients who were diagnosed with probable or confirmed COVID-19 in our hospital, recovered from the disease, admitted to our COVID-19 Follow-up Unit between June, and September 2020 and fulfilled the below specific group criteria. The definitions of the World Health Organisation for COVID-19 case were used: A patient who meets the appropriate clinical criteria (acute onset of fever and cough; or acute onset of any three or more of the following signs or symptoms of fever, cough, general weakness/fatigue, headache, myalgia, sore throat, coryza, dyspnoea, anorexia/nausea/vomiting, diarrhoea, altered mental status) and residing or travel to an area with community transmission anytime within the 14 days prior to symptom onset and either with chest imaging showing findings were suggestive of COVID-19 disease and/or with a positive SARS-CoV-2 PCR test result (WHO, 2020). To include all patients with different disease severity and microbiologic result status, we defined 8 patient groups in accordance with the results of thorax computed tomography (CT), SARS-CoV-2 PCR test and use of tocilizumab or/and anakinra during the active phase of COVID-19 disease before the enrollment into the study: Group 1: Patients treated with at least 1 dose of tocilizumab and/or anakinra for COVID-19; Group 2: Patients with a positive SARS-CoV-2 PCR test, and had severe involvement on thorax CT; Group 3: Patients

with a positive SARS-CoV-2 PCR test, and had moderate involvement on thorax CT; Group 4: Patients with a negative SARS-CoV-2 PCR test, and severe involvement on thorax CT; Group 5: Patients with a negative SARS-CoV-2 PCR test, and had moderate involvement on thorax CT; Group 6: Patients with a positive SARS-CoV-2 PCR test, and mild involvement on thorax CT; Group 7: Patients with a negative SARS-CoV-2 PCR test, and had mild involvement on thorax CT; Group 8: Patients with a positive SARS-CoV-2 PCR test, and had no involvement on thorax CT.

The extent of lung involvement on thorax CT was graded as absent, minimal (<25%), moderate (25–50%), and severe (>50%) (Jalaber, 2020). Bilateral and multifocal ground-glass opacities predominating in the peripheral section of the lungs were defined as specific CT features of COVID-19 pneumonia. Tocilizumab, anakinra and prednisolone were used in case of hyperinflammatory response as follows: single or two 400 mg doses of tocilizumab, anakinra with a daily dose ranging between 2 x 100 mg SC to 3 x 200 mg IV, and at least 80 mg/day prednisolone for 5 days were used.

Laboratory examination

The laboratory diagnosis of COVID-19 was based on RT-qPCR, and anti-SARS-CoV-2 antibodies. Viral RNA extraction from respiratory specimens was done using a manual kit (Bioeksen the Co Ltd. R & D Technologies, Turkey). RT-qPCR process was performed on Rotor-Gene Q 5 Plex Real Time PCR device (Qiagen, Germany) using a national kit (Bioeksen the Co Ltd. R & D Technologies, Turkey). Anti-SARS-CoV-2 ELISA (IgG) kit (Euroimmun, Germany) was used to detect IgG antibodies developed against the S1 domain of the SARS-CoV-2 spike protein in serum samples.

Data collection and outcomes

Physical examination was performed, and blood samples were drawn from patients after the consents were obtained. The serum samples were stored at -20 ^{0}C until antibody testing. The epidemiological, clinical features, and admission laboratory tests and thorax CT results of patients were retrospectively obtained from the online database of the hospital.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) 21.0 (SPSS Inc., Chicago, IL, USA). Univariate analysis was done using Chi-square, Fisher's exact, Student's t, and Mann-Whitney *U* tests, where appropriate. For the multivariate logistic regression analysis, significant (with a p value of < 0.05) factors identified with univariate analysis were included to the model to determine the independent predictors of SARS-CoV-2 spike IgG antibody seroconversion. Colinear variables were excluded, collinearity between predictors was tested by correlation matrix. All factors included into the multivariate logistic regression analysis were shown in Table 1. Hosmer-Lemeshow goodness of fit statistics were used to assess the model fit. Kruskal-Wallis tests were conducted to compare the non-normally distributed parameters and ordinal variables between the groups, and the level of pulmonary involvement on thorax CT.

Results

A total of 518 patients (63.3% hospitalized, 36.7% outpatient) were included in the study. The mean age of the patients was 49.2 years, and 56.2% were male. While 442 of patients had pneumonia (85%), 76 had noncomplicated disease without pneumonia (15%); 192 out of 518 patients (37%) were categorized as having severe disease who required either oxygen support or had severe involvement on thorax CT. Nasopharengeal SARS-CoV-2 PCR test was positive in

60% of the patients. The mean time from hospital admission to antibody testing was 82 days, and 25, 50 and 75 percentiles of testing time were 42, 76 and 125 days, respectively. SARS-CoV-2 IgG antibodies were positive in 82.8% of patients, the rate was 90% and 72% among PCR positive and negative cases, respectively; and 94% of PCR positive patients with no antibody response had either no (52%) or minor (42%) involvement on thorax CT. The demographic, and clinical features of patients with and without antibody response are shown in Table 1.

In univariate analysis, solid organ transplantation (p=0.003), the use of immunosuppresive drugs before the COVID-19 diagnosis (p=0.01); presence of fever, low WBC, neutrophil and lymphocyte counts, high CRP and ferritin levels on admission; need for oxygen support and hospitalisation, severity of involvement on thorax CT, nasopharengeal SARS-CoV-2 PCR positivity, time from hospital admission to antibody testing, tocilizumab or anakinra or prednisolone use for COVID-19 were found to be related with antibody positivity (Table 1). In multivariate analysis, SARS-CoV-2 PCR positivity and extent of involvement on thorax CT and time from hospital admission to antibody testing were found independently associated with antibody positivity (Table 2).

The analysis of only the confirmed COVID-19 cases (with a positive result of SARS-CoV-2 PCR test) showed that older age (p=0.047), hospitalisation (p<0.001), presence of fever (p=0.014), and pneumonia (p=0.001), higher serum CRP (p<0.001), and ferritin levels (p<0.001), lower blood WBC (p=0.001), and PNL counts (p=0.032), moderate/severe lung involvement on thorax CT (p=0.001), the need for oxygen support (p=0.002), use of favipiravir (p=0.001) and tocilizumab and/or anakinra (p=0.012) were found as the factors associated with antibody response. Although serum D-dimer level was found higher, and blood lymphocyte count was found lower among patients with a positive SARS-CoV-2 spike IgG, the differences did not

reach to a statistical significance (p=0.064, and 0.07, respectively) (Table 1). Moderate/severe lung involvement on thorax CT was found as an independent factor of SARS-CoV-2 spike antibody positivity also among confirmed cases (OR 10.95, 95% CI 1.20-99.81, p=0.034) (Table 2).

Both the rate of antibody response and sample/Cut-off (S/CO) values among antibody positive patients showed a linear relationship between the extent of lung involvement on thorax CT (Figure 1) and both were significantly different between the groups ($p < 0.05$). S/CO level was found to be 2.21, 1.69 and 1.16 times higher in patients with severe, mild and moderate involvement compared to no lung involvement, respectively. Groups including severe COVID-19 cases (Group 1-3) showed significantly higher titer responses compared with the groups including milder cases (Group 5-8) ($p < 0.001$). The antibody concentrations among patients with a positive antibody result (429 patients) were significantly higher in patients who were treated with either tocilizumab, or anakinra or prednisolone ($p < 0.05$). However, S/CO levels of those patients were found to be similar with the S/CO levels of patients with severe disesase ($p > 0.05$) (Table 3).

Discussion

We evaluated the factors affecting antibody response against SARS-CoV-2 in a cohort of COVID-19 patients with different level of severity and PCR test results, and with and without tocilizumab and/or anakinra and/or prednisolone treatments in this study. We found that antibody response was independently associated with the level of lung involvement on thorax CT. 95%, 90%, 69% of patients with severe, moderate and mild lung involvement, respectively were positive for SARS-CoV-2 antibody. S/CO level was found to be 2.21, 1.69 and 1.16 times higher

in patients with severe, mild and moderate CT involvement than in patients with no involvement, respectively. When we limited the analysis to only confirmed cases, we also found the moderate/severe involvement of lung on the thorax CT as an independent factor affecting the antibody seroconversion. The extent of involvement on thorax CT was shown to be directly correlated with disease severity in COVID-19 in previous studies (Feng et al., 2020). There are also studies showing the correlations between the severity of infection and either involvement of lung on thorax CT or antibody responses to SARS-CoV-2 (Chen et al., 2020). The decrease of the number of CD4+ T cells and the increase of IL-6 levels were reported to be correlated with the volume of lung lesions in critically ill COVID-19 patients in one study (He et al., 2020). However, we found no other study showing a direct relationship between the extent of lung involvement on thorax CT and the rate and level of antibody response. Therefore, based on the findings of the current study, antibody responses and level of it could be predicted from the level of lung involvement on thorax CT, even in patients without a positive PCR test.

We also found that although antibody response rate was higher in patients who were treated with either tocilizumab, anakinra, or prednisolone compared with the patients who were not administered these drugs in univariate analysis. However, the use of those anticytokines and prednisolone for the treatment of COVID-19 were not found as independent factors affecting the antibody response rate. There could be so many reasons why we failed to obtain less SARS-CoV-2 antibody seroconversion in those COVID-19 patients who were treated with drugs affecting the immune system. First of all, it is well known that potential adverse effects of immunosupressive or immunomodulatory agents is related to both the average dose and the cumulative duration of use (Slade and Hepburn, 1983). In the case of COVID-19, those drugs were used no more than 1 or maximum of 2 weeks and as a result, this could be one of the

reasons why we found no adverse effect of those drugs on antibody response to SARS-CoV-2. Our finding of lower antibody response among COVID-19 patients who were on long term immunosuppressive drugs also supports that idea.

IL-6 works as a B cell differentiation factor, which induces activated B cells to produce immunoglobulin (Muraguchi et al., 1988). T cell-dependent antibody response against virus infection is impaired in IL-6 deficient mice (Kopf et al., 1994). But we found that tocilizumab, an IL-6 receptor blocker did not affect the antibody response in patients with COVID-19. It was also reported in another study that tocilizumab did not impair the viral specific antibody responses in COVID-19 (Masiá et al., 2020a). Antibody response to influenza vaccine in rheumatoid arthritis patients was not hampered by tocilizumab (Mori et al., 2012). IL-1 could affect antibody response as a result of its effects on T cells, and IL-6 production (Dinarello, 2009). However, we found that either anakinra alone, or combined with tocilizumab did not affect antibody responses in patients with COVID-19. We found no other study evaluating the effect of anakinra on antibody response in COVID-19 patients. In one study, anakinra showed no significant difference in anti-pneumococcal antibody responses (Quartier et al., 2011). One explanation of our result may be that hyperactivation of both innate, and acquired immunity develops because of continous and high level pathogen and danger associated molecular pattern signals in severe COVID-19, and targeting only IL-1 or IL-6 inhibitors could not be adequate to control or in suppression of dysregulated immune responses including humoral response. The failure of recent randomized clinical trials of tocilizumab in treatment of patients with severe COVID-19 (Stone et al., 2020) and the positive effect of personalized treatment decisions on early mortality in COVID-19 patients (Garcia-Vidal et al., 2020) also support that idea. This was also the case in previous sepsis trials. Based on the assumption that sepsis mortality was driven

by excessive inflammation, many trials had been performed with a variety of anti-inflammatory agents and none of them showed benefit, strongly suggesting that inhibition of specific components of the inflammatory response does not improve survival in all-comers with sepsis selected based on severity of disease. The key to success of either anti-inflammatory or immunostimulatory type of immunomodulation is to identify the patients who may benefit from a particular intervention. Considering the complexity of the host response during COVID-19 and the diversity of pathophysiologic mechanisms at play, it is unlikely that the current"one-target" and "one-size-fits-all" strategy will control all part of the dysregulated immune system (Van der Poll and Wiersinga, 2020). Another explanation may be that tocilizumab and/or anakinra are not targeting B cell specifically, antibody production may not depend simply on IL-1, or IL-6. IL-6 was not required for influenza virus specific antibody responses by tonsillar mononuclear cells in an *in vitro* study (Costelloe et al., 1993). Lastly, inflammatory environment in severe patients with cytokine storm results in exhaustion of lymphocytes and lymphopenia, and improvement of inflammatory status may be associated with better immune functions against SARS-CoV-2 both in humoral, and cellular immunity (Riva et al., 2020).

Although glucocorticoids cause no significant acute changes on B cells (Olnes et al., 2016), the number of circulating B lymphocytes and levels of IgG may be reduced with short-term glucocorticoid administration (Slade and Hepburn, 1983). In our study, prednisolone used for COVID-19 treatment was not found to be associated with the antibody response. In a study, hospitalized COVID-19 patients received corticosteroids and/or tocilizumab, no detrimental effect was found on antibody responses. Although differences in antibody response even favored patients receiving corticosteroids, such differences vanished after adjustment (Masiá et al., 2020b). It was same in our study, patients treated with either tocilizumab, anakinra, or

prednisolone had higher antibody response than patients who did not take these medications, but none were found as an independent factor for antibody response in multivariate analysis. As a result, we may conclude that, tocilizumab, anakinra and prednisolone, even in combination, do not negatively impact the humoral immune response against SARS-CoV-2.

We found that seroconversion in COVID-19 cases were related to the duration between hospital admission, and antibody testing (OR 1.02; 95% CI 1.01-1.03; p <0.001). It was shown that SARS-CoV-2 IgG antibodies develop over a period of 7–50 days from the symptom onset, with a median of 24 days (Wajnberg et al., 2020a). Although the mean time from hospital admission to antibody testing was 82 days in our study, antibody testing was done before 42 days in 25% of our patients, and as a result some of them could not produce adequate antibody in a given time. As 76% of our patients with a negative antibody response have mild disease either with no involvement or mild involvement on thorax CT, another explanation of time dependence of seroconversion could be the longer duration of antibody response among asymptomatic and mild cases. It was also shown that seroconversion in mild COVID-19 cases might take longer time to mount (Wajnberg et al., 2020a, 2020b). Another explanation for this could be due to a mild and transient response, antibodies become negative in a short time among mild or asymptomatic cases (Poland et al., 2020). In the convalescent phase, IgG titres in symptomatic individuals remained significantly higher than those in asymptomatic individuals (Adams et al., 2020). Notably, in one study IgG titres declined during the convalescent phase in both symptomatic and asymptomatic individuals, with 13% and 40% of symptomatic and asymptomatic individuals becoming IgG seronegative within 2–3 months following infection (Long et al., 2020). Finally, some of our patients might not have COVID-19 at all as the rate of antibody positivity of our patients was found as 72% without a PCR positivity. However, 31 out

of 89 antibody negative patients were confirmed COVID-19 cases with a positive PCR test. Our analysis of the only confirmed COVID-19 cases, showed that the time from hospital admission to antibody testing was not found as a factor affecting the antibody response. This could be related to the very early sampling and false negativity of PCR testing among PCR negative cases, which could be the result of delayed antibody response among those cases. As we did not plan to assess the kinetics of the antibody response we cannot make a final comment on this issue.

Our study has some limitations. Although we included the patients prospectively during convalescent phase, the clinical and laboratory data of active COVID-19 were obtained from hospital database retrospectively, as a result we could not get some of the data including the duration of symptoms, total duration of prednisolone use, and the time from the symptoms to thorax CT evaluation. As SARS-CoV-2 RNA PCR was negative in 40% of our patients, some of them might not have COVID-19 at all. But we included also the thorax CT results for our analysis, and 72% of our patients with a negative PCR test had a positive antibody test result, which shows that selection of patient is appropriate.

Our study has some strengths. We analyzed factors affecting antibody response in a high number of patients with different disease severity, and PCR result status. For the first time, we showed a direct association between antibody response rate and S/CO ratio and extent of lung involvement on CT. We also showed that anakinra treatment does not affect the antibody response in patients with severe COVID-19.

Conclusion

Although tocilizumab, anakinra and prednisolones have some effects on T cells, B cells, and antibody response, their use during the course of COVID-19 was not found to be associated with a negative effect on antibody response.

The main driver of the antibody response to SARS-CoV-2 and level of it among patients with COVID-19 was found to be the extent of lung involvement on thorax CT, which is correlated well with the severity of infection, and as a result we could predict the antibody response to SARS-CoV-2 and levels of it by thorax CT.

Authors' contributions

SB, SŞY and SM conceived of and designed the study. SB and SŞY analysed the results, and wrote the manuscript. All authors reviewed and approved the final version for submission.

Funding

This work was supported by the Scientific Research Projects Coordination Unit of Istanbul University (Project number: 36884).

Conflict of interest

No conflict of interest was declared by the authors.

Acknowledgements

The authors thank Yunus Çatma for his help in data collection.

References

1. Adams ER, Ainsworth M, Anand R, Andersson MI, Auckland K, Baillie JK, et al. Antibody testing for COVID-19: a report from the National COVID Scientific Advisory Panel. medRxiv 2020. https://doi.org/ 10.1101/2020.04.15.20066407 (preprint).
2. Chen Y, Zuiani A, Fischinger S, Mullur J, Atyeo C, Travers M, et al. Quick COVID-19 healers sustain anti-SARS-CoV-2 antibody production. Cell 2020; 183(6):1496-507.e16. https://doi.org/10.1016/j.cell.2020.10.051.
3. Costelloe KE, Smith SH, Callard RE. Interleukin 6 is not required for antigen-specific antibody responses by human B cells. Eur J Immunol 1993; 23(4):984-7. https://doi.org/10.1002/eji.1830230436.
4. Dinarello CA. Immunological and inflammatory functions of the Interleukin-1 family. Annu Rev Immunol 2009; 27:519-50. https://doi.org/10.1146/annurev.immunol.021908.132612.
5. Feng Z, Yu Q, Yao S, Luo L, Zhou W, Mao X, et al. Early prediction of disease progression in COVID-19 pneumonia patients with chest CT and clinical characteristics. Nat Commun 2020; 11(1):4968. https://doi.org/10.1038/s41467-020-18786-x.

6. Garcia-Vidal C, Moreno-García E , Hernández-Meneses M, Puerta-Alcalde P, Chumbita M, Garcia-Pouton N, et al. Personalized therapy approach for hospitalized patients with COVID-19. Clin Infect Dis 2020; ciaa964. https://doi.org/10.1093/cid/ciaa964
7. He S, Zhou C, Lu D, Yang H, Xu H, Wu G, et al. Relationship between chest CT manifestations and immune response in COVID-19 patients. Int J Infect Dis 2020; 98:125-9. https://doi.org/10.1016/j.ijid.2020.06.059.
8. Jalaber C, Lapotre T, Morcet-Delattre T, Ribet F, Jouneau S, Lederlin M. Chest CT in COVID-19 pneumonia: A review of current knowledge. Diagn Interv Imaging 2020; 101(7-8):431-7. https://doi.org/10.1016/j.diii.2020.06.001.
9. Kopf M, Baumann H, Freer G, Freudenberg M, Lamers M, Kishimoto T, et al. Impaired immune and acute-phase responses in interleukin-6-deficient mice. Nature 1994; 368(6469):339-42. https://doi.org/10.1038/368339a0.
10. Long QX, Tang XJ, Shi QL, Li Q, Deng HJ, Yuan J, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med 2020; 26(8):1200-4. https://doi.org/10.1038/s41591-020-0965-6.
11. Maeda K, Mehta H, Drevets DA, Coggeshall KM. IL-6 increases B-cell IgG production in a feed-forward proinflammatory mechanism to skew hematopoiesis and elevate myeloid production. Blood 2010; 115(23):4699-706. https://doi.org/10.1182/blood-2009-07-230631.

12. Masiá M, Fernández-González M, Padilla S, Ortega P, García JA, Agulló V, et al. Impact of interleukin-6 blockade with tocilizumab on SARS-CoV-2 viral kinetics and antibody responses in patients with COVID-19: a prospective cohort study. EBioMedicine 2020a; 60:102999. https://doi.org/10.1016/j.ebiom.2020.102999.

13. Masiá M, Fernández-González M, García JA, Padilla S, Gutiérrez F. Lack of detrimental effect of corticosteroids on antibody responses to SARS-CoV-2 and viral clearance in patients hospitalized with COVID-19. J Infect 2020b; S0163-4453(20)30676-9. https://doi.org/10.1016/j.jinf.2020.10.018.

14. Mori S, Ueki Y, Hirakata N, Oribe M, Hidaka T, Oishi K. Impact of tocilizumab therapy on antibody response to influenza vaccine in patients with rheumatoid arthritis. Ann Rheum Dis 2012; 71(12):2006-10. http://dx.doi.org/10.1136/annrheumdis-2012-201950.

15. Muraguchi A, Hirano T, Tang B, Matsuda T, Horii Y, Nakajima K, at al. The essential role of B cell stimulatory factor 2 (BSF-2/IL-6) for the terminal differentiation of B cells. J Exp Med 1988; 167(2):332-44. https://doi.org/10.1084/jem.167.2.332.

16. Olnes MJ, Kotliarov Y, Biancotto A, Cheung F, Chen J, Shi R, et al; CHI Consortium. Effects of systemically administered hydrocortisone on the human immunome. Sci Rep 2016; 6:23002. https://doi.org/10.1038/srep23002.

17. Poland GA, Ovsyannikova IG, Kennedy RB. SARS-CoV-2 immunity: review and applications to phase 3 vaccine candidates. Lancet 2020; 396(10262):1595-606.

https://doi.org/10.1016/S0140-6736(20)32137-1.

18. Quartier P, Allantaz F, Cimaz R, Pillet P, Messiaen C, Bardin C, et al. A multicentre, randomised, double-blind, placebo-controlled trial with the interleukin-1 receptor antagonist anakinra in patients with systemic-onset juvenile idiopathic arthritis (ANAJIS trial). Ann Rheum Dis 2011; 70(5):747-54. http://dx.doi.org/10.1136/ard.2010.134254.
19. RECOVERY Collaborative Group, Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, et al. Dexamethasone in hospitalized patients with Covid-19 - preliminary report. N Engl J Med 2020; NEJMoa2021436. http://doi.org/10.1056/NEJMoa2021436.
20. Riva G, Nasillo V, Tagliafico E, Trenti T, Luppi M. COVID-19: room for treating T cell exhaustion? Crit Care 2020; 24(1):229. https://doi.org/10.1186/s13054-020-02960-0.
21. Roll P, Muhammad K, Schumann M, Kleinert S, Einsele H, Dorner T, et al. Invivoeffects of the anti–interleukin-6 receptor inhibito rtocilizumab on the B cell compartment. Arthritis Rheum 2011; 63(5):1255-64. https://doi.org/10.1002/art.30242.
22. Rydyznski Moderbacher C, Ramirez SI, Dan JM, Grifoni A, Hastie KM, Weiskopf D, et al. Antigen-specific adaptive immunity to SARS-CoV-2 in acute COVID-19 and associations with age and disease severity. Cell 2020; 183(4):996-1012.e19. https://doi.org/10.1016/j.cell.2020.09.038.
23. Slade JD, Hepburn B. Prednisone-induced alterations of circulating human lymphocyte subsets. J Lab Clin Med 1983; 101(3):479-87.

24. Stone JH, Frigault MJ, Serling-Boyd NJ, Fernandes AD, Harvey L, Foulkes AS, et al; BACC Bay Tocilizumab Trial Investigators. Efficacy of tocilizumab in patients hospitalized with Covid-19. N Engl J Med 2020; 383(24):2333-44. https://doi.org/10.1056/NEJMoa2028836.

25. Toniati P, Piva S, Cattalini M, Garrafa E, Regola F, Castelli F, et al. Tocilizumab for the treatment of severe COVID-19 pneumonia with hyperinflammatory syndrome and acute respiratory failure: A single center study of 100 patients in Brescia, Italy. Autoimmun Rev 2020; 19(7):102568. https://doi.org/10.1016/j.autrev.2020.102568.

26. Van der Poll T, Wiersinga WJ. Sepsis and septic shock. In: Bennett JE, Dolin R, Blaser MJ, editors. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, Philadelphia: Elsevier; 2020, p.1005-8.

27. Wajnberg A, Mansour M, Leven E, Bouvier NM, Patel G, Firpo-Betancourt A, et al. Humoral response and PCR positivity in patients with COVID-19 in the New York City region, USA: an observational study. Lancet Microbe 2020a; 1(7):e283-9. https://doi.org/10.1016/S2666-5247(20)30120-8.

28. Wajnberg A, Amanat F, Firpo A, Altman DR, Bailey MJ, Mansour M, et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. Science 2020b; 370(6521):1227-30. https://doi.org/10.1126/science.abd7728.

29. World Health Organization (WHO). WHO COVID-19 case definition.2020 https://www.who.int/publications/i/item/WHO-2019-nCoV-Surveillance_Case_Definition-2020.2

Table 1. Comparison of patients with and without a positive SARS-CoV-2 antibody

Characteristics	**Probable +Confirmed COVID-19 cases (n=518)**		p value	**Confirmed COVID-19 cases (n=311)**		p value
	Patients with positive anti SARS CoV-2 IgG (n=429)	**Patients with negative anti SARS CoV-2 IgG (n=89)**		**Patients with positive anti SARS CoV-2 IgG (n=280)**	**Patients with negative anti SARS CoV-2 IgG (n=31)**	
Age, mean±SD	48.99±13.69	49.75±16.33	0.647[a]	48.15±14.44	43.32±12.21	**0.047[a]**
Gender, female, n (%)	184 (42.9)	43 (48.3)	0.348[a]	123 (43.9)	18 (58.1)	0.134[a]
Hypertension, n (%)	98 (22.8)	25 (28.1)	0.290	58 (20.7)	3 (9.7)	0.230
Diabetes mellitus, n (%)	73 (17.0)	16 (17.9)	0.827	44 (15.7)	2 (6.4)	0.282
Hypothyroidism, n (%)	23 (5.3)	6(6.7)	0.610	18 (6.4)	3 (9.7)	0.453
Rheumotological diseases, n (%)	21 (4.9)	8(9.0)	0.282	13 (4.6)	2 (6.4)	0.814
Asthma, n (%)	19 (4.4)	6 (6.7)	0.354	14 (5.0)	2 (6.4)	0.666
Cancer, n (%)	15 (3.5)	3 (3.4)	1.000	9 (3.2)	1 (3.2)	1.000
Ischemic heart disease, n (%)	13 (3.0)	5 (5.6)	0.213	7 (2.5)	0	1.000
Chronic obstructive pulmonary disease, n (%)	9 (2.1)	3 (3.4)	0.442	5 (1.8)	0	1.000
Previous tuberculosis, n (%)	7(1.6)	4 (4.5)	0.102	6 (2.1)	1 (3.2)	0.524
Solid organ transplantation, n (%)	4 (0.9)	6 (6.7)	**0.003[a]**	2 (0.7)	0	1.000
Congestive heart failure, n (%)	3 (0.7)	3 (3.4)	0.066	3 (1.1)	0	1.000
Chronic renal failure, n (%)	4 (0.9)	1 (1.1)	1.000	1 (0.4)	0	1.000
Previous medications						
Immunosupressives [b]	**14 (3.3)**	**8 (8.9)**	**0.015[a]**	9	1	0.656
Clinical and laboratory findings on admission for COVID-19						
Body temperature, mean±SD	37.4± 1.0	37.0 ±0.89	**0.001[a]**	37.4±0.7	36.9±07	**0.014[a]**
Peripheral arterial oxygen saturation (SpO_2), mean±SD	95.6±3.9	96.1±4.5	**0.007**	95.9±4.0	98.2±1.1	**<0.001**
Blood leucocyte count, /mm^3, mean±SD	6211±5827	8904±4004	**<0.001**	5821±2280	8405±5179	**0.001**
Blood neutrophil count /mm^3, mean±SD	3941±2105	6305±3978	**<0.001[a]**	3780±2011	5771±5575	**0.032[a]**
Blood lymphocyte count, /mm^3, mean±SD	1396±651	1780±985	**0.001[a]**	1415±644	1742±815	0.073
Serum CRP level, mg/L, mean±SD	48.46±61.94	46.17±68.54	**0.038[a]**	45.29±65.53	13.56±28.92	**<0.001[a]**
Serum ferritin level, mg/dL, mean±SD	453.4±715.5	256.1±441.7	**<0.001[a]**	420.9±732.0	84.5±105.2	**<0.001[a]**
Serum D-dimer level, mg/dL, mean±SD	1012±1817	1060±1174	0.771	942±1635	768±964	0.064
Hospitalized for COVID-19, n (%)	282 (65.7)	46 (51.7)	**0.012[a]**	169 (60.4)	6 (19.4)	**<0.001[a]**
Presence of pneumonia, n (%)	371 (86.5)	71 (79.8)	0.104	220 (78.6)	13 (41.9)	**<0.001[a]**
Need for nasal O_2 support, n (%)	144 (33.6)	16 (18.0)	**0.010[a]**	82 (29.3)	0	**0.002[a]**
Need for high flow nasal oxygen, n (%)	25 (5.8)	5 (5.6)	0.728	15 (5.4)	0	0.347
Need for mechanical ventilation, n (%)	13 (3.0)	2 (2.2)	0.672	6 (2.1)	0	0.599
Need for intensive care unit support, n (%)	23 (5.4)	7 (7.9)	0.485	13 (4.6)	0	0.393
SARS-CoV-2 PCR positivity, n (%)	280 (65.3)	31 (34.8)	**<0.001[a]**			
Extent of lung involvement on Thorax CT, n (%)			**<0.001[a]**			**<0.001[a]**
None, n (%)	58 (13.5)	18 (20.2)		58 (20.7)	18 (58.1)	
Mild, n (%)	112 (26.1)	50 (56.2)		73 (26.1)	12 (38.7)	
Moderate, n (%)	138 (32.2)	15 (16.8)		79 (28.2)	1 (3.2)	
Severe, n (%)	121 (28.2)	6 (6.7)		70 (25.0)	0	

Table 1. Comparison of patients with and without a positive Sars-CoV-2 antibody (continued)

Characteristics	Probable+Confirmed Cases (n=518)		p value	Confirmed Cases (n=311)		p value
	Patients with positive anti SARS CoV-2 IgG (n=429)	Patients with negative anti SARS CoV-2 IgG (n=89)		Patients with positive anti SARS CoV-2 IgG (n=280)	Patients with negative anti SARS CoV-2 IgG (n=31)	
Patient groups			**<0.001**			**<0.001**
Group 1, n (%)	77 (17.9)	3 (3.4)		45	0	
Group 2, n (%)	38 (8.9)	0 (0)		38	0	
Group 3, n (%)	66 (15.4)	1 (1.1)		66	1	
Group 4, n (%)	26 (6.1)	5 (5.6)		-	-	
Group 5, n (%)	52 (12.1)	12 (13.5)		-	-	
Group 6, n (%)	73 (17.0)	12 (13.5)		73	12	
Group 7, n (%)	39 (9.1)	38 (42.7)		-	-	
Group 8, n (%)	58 (13.5)	18 (20.2)		58	18	
Severe disease (either needing supplemental oxygen or severe involvement on thorax CT), n (%)	176 (41.0)	16 (17.9)	**<0.001**	99 (35.4)	0	**<0.001**
Anti-SARS-CoV-2 treatments						
Favipiravir ±others, n (%)	138 (32.2)	20 (22.5)	0.070	94 (33.6)	2 (6.5)	**0.001[a]**
Hydroxychloroquine ± others, n (%)	414 (96.5)	85 (95.5)	0.656	269 (96.1)	31 (100)	1.000
Tocilizumab or anakinra for COVID-19			**0.006**			0.120
Tocilizumab, n (%)	55 (12.8)	1 (1.1)		32 (11.4)	0	
Anakinra, n (%)	10 (2.3)	1 (1.1)		7 (2.5)	0	
Tocilizumab+Anakinra, n (%)	12 (2.8)	1 (1.1)		6 (2.1)	0	
Tocilizumab and/or anakinra for COVID-19, n (%)	77 (17.9)	3 (3.4)	**<0.001[a]**	45 (16.1)	0	**0.012[a]**
Prednisolone for COVID-19, n (%)	21 (4.9)	0 (0)	**0.034[a]**	11 (3.9)	0	0.610
Convalescent plasma for COVID-19, n (%)	5 (1.2)	0 (0)	0.594	3 (1.1)	0	1.000
IVIG for COVID-19, n (%)	8 (0.7)	1 (1.1)	1.000	5 (1.8)	0	1.000
Time from hospital admission to antibody testing, /mm^3, mean±SD	86.90±43.44	62.15±37.20	**<0.001[a]**	80.84±42.41	77.64±42.29	0.712
Blood lymphocyte count at the antibody testing, /mm^3, mean±SD	2144±750	2053±588	0.484	2118±695	2154±533	0.601

SARS CoV-2: severe acute respiratory syndrome coronavirus-2, IgG: immunoglobulin G, CT: computed tomography, PCR: polymerase chain reaction, COVID-19: Coronavirus disease 2019, IVIG: intravenous immunoglobulin

[a]Entered into multivariate analysis, [b]Including prednisolones and/or monoclonal immunosuppressive antibodies and/or other immunosupressives

Table 2. Multivariate analysis of factors affecting SARS-CoV-2 spike antibody response

Characteristics	Probable+Confirmed COVID-19 Cases (n=518)			Confirmed COVID-19 Cases (n=311)		
	p value	OR	95% CI	p value	OR	95% CI
SARS-CoV-2 PCR test positivity	<0.001	5.639	2.806-11.331	NA	NA	NA
Moderate/Severe lung involvement on thorax CT	<0.001	8.193	3.824-17.552	0.034	10.95	1.20- 99. 81
Time from hospital admission to antibody testing, /mm^3, mean±SD	<0.001	1.015	1.007-1.023	-	-	-
Previous immunosuppressive drug usage	0.027	0.213	0.054-0.835	-	-	-

SARS CoV-2: severe acute respiratory syndrome coronavirus-2, PCR: polymerase chain reaction, CT: computed tomography

Table 3. Comparison of antibody level among patients with a positive antibody result, and severe disease.

Feature	(n)	S/CO level (median±SD)	p value
Patients with a positive SARS-CoV-2 antibody result (n=429)			
Need for oxygen support	No (285)	5.55±2.82	**<0.001**
	Yes (144)	8.40±2.77	
SARS-CoV-2 PCR test positivity	No (149)	6.71±3.04	0.388
	Yes (280)	6.44±3.15	
Level of lung involvement on thorax	None (58)	3.82±2.14	**<0.001**
CT	Mild (112)	4.99±2.94	
	Moderate (138)	7.26±2.50	
	Severe (121)	8.44±2.67	
Patient treated with either	No (352)	6.15±3.12	**<0.001**
tocilizumab, or anakinra	Yes (77)	8.30±2.39	
Patient treated with	No (417)	6.57±3.11	0.171
tocilizumab+ anakinra	Yes (12)	7.76±3.12	
Patient treated with tocilizumab	No (374)	6.24±3.11	**<0.001**
	Yes (55)	8.55 ±2.26	
Patient treated with anakinra	No (419)	6.51±3.73	0.145
	Yes (10)	7.71±1.63	
Patient treated with corticosteroid	No (405)	6.39±3.08	**0.001**
for COVID-19	Yes (21)	8.73±2.77	
Patient treated with corticosteroid	No (416)	6.52 ±3.09	0.553
before COVID-19 diagnosis	Yes (10)	5.97±3.66	
Patients with a positive SARS-CoV-2 antibody result and severe disease (n=176)			
Patient treated with either	No (104)	8.22±2.88	0.644
tocilizumab, or anakinra	Yes (72)	8.44±2.33	
Patient treated with tocilizumab	No (123)	8.18±2.82	0.473
	Yes (53)	8.60±2.28	
Patient treated with anakinra	No (168)	8.33±2.71	0.733
	Yes (8)	7.99±1.53	
Patient treated with	No (165)	8.33±2.65	0.898
tocilizumab+anakinra	Yes (11)	7.99±3.05	
Patient treated with corticosteroid	No (156)	8.18±2.66	0.223
for COVID-19	Yes (19)	9.19±2.49	
Patient treated with corticosteroid	No (170)	8.38±2.59	**0.032**
before COVID-19 diagnosis	Yes (5)	5.04±3.12	

SARS CoV-2: Severe acute respiratory syndrome coronavirus-2, PCR: polymerase chain reaction, CT: computed tomography, COVID-19: Coronavirus disease 2019

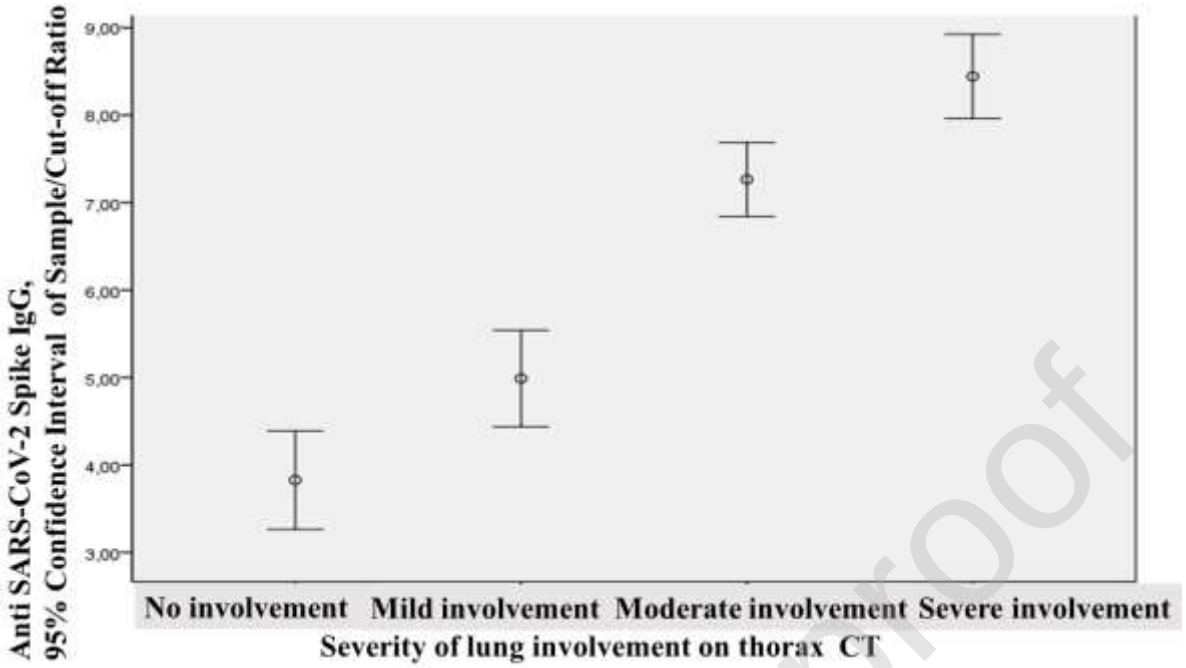


Figure caption. Distribution of sample/cut-off ratios of patients according to lung involvement on thorax CT.