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Retrospective Cohort Study**Clinical Significance of Anti-nucleocapsid-IgG Sero-positivity in SARS-CoV-2 Infection in Hospitalized Patients in North Dakota**

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Abstract**BACKGROUND**

During the peak of the coronavirus diseases 2019 (COVID-19) pandemic, clinicians actively studied the utility of various epidemiologic-clinical parameters to determine the prognosis for patients hospitalized with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Serum IgG antibody level, D-Dimer, C-reactive protein and neutrophil to lymphocyte ratio, *etc.* were studied to assess their association with the clinical course in hospitalized patients and predict who may be at increased risk for poor clinical outcome. However, the influence of SARS-CoV-2-anti-nucleocapsid-IgG antibody (IgG-N) sero-positivity on the clinical outcome of patients with COVID-19 is largely unknown.

AIM

To study the influence of SARS-CoV-2 anti-nucleocapsid-IgG seropositivity on clinical course and diseases severity in hospitalized COVID-19 patients.

METHODS

We conducted a retrospective study of adults admitted to a tertiary care community hospital in North Dakota with COVID-19. Included patients had severe COVID-19

disease or worse and so required supplemental oxygen on admission. They were serologically tested for SARS-CoV-2-anti-nucleocapsid-IgG (IgG-N). The IgG-N positive group were 26 patients and the IgG-N negative group had 33 patients. The groups received similar treatment for COVID-19 as approved by our healthcare system from Day 1 of admission until discharge or death. Measurable parameters for monitoring the patients' clinical course included the following: length of hospitalization (LOS), use of high flow nasal canula (HFNC), use of noninvasive bilevel positive pressure ventilation (BiPAP), admission into the intensive care unit (ICU), need for mechanical ventilation (VENT); and the patient outcome/discharge or death. Other variables included were age, gender and body-mass-index (BMI), and duration of symptoms before presentation (DOS). For each variable, the outcome was modeled as a function of SARS-CoV-2- IgG-N status (positive or negative) using a generalized linear model. For LOS-days, a negative binomial distribution was used as it had a better fit than a Poisson or Gaussian distribution as evidenced by a Pearson chi-square/df value closer to 1.0. All other outcomes utilized a binary logistic regression model.

RESULTS

After a thorough examination of patient data, it was found that admission rates to the Intensive Care Unit, as well as the usage of BiPAP, HFNC and VENT support, in conjunction with patient outcomes, were not significantly different across IgG-N status. However, the length of stay (LOS) variable when assessed by IgG-N status was found to be significant (t -value=2.16, p -value=0.0349). IgG-N negative patients had higher than average LOS in comparison to IgG-N positive patients (15.12 vs. 9.35 days). Even when removing the extreme value (an LOS of 158 days), IgG-N negative patients still had slightly higher than average stays (10.66 vs. 9.35 days) but the relationship was no longer significant. For patient outcome/ death, only age (numerical) was a significant predictor (F -value=4.66, p -value=0.0352). No other variables for any of the outcomes were significant predictors of clinical course or disease severity.

CONCLUSION

Our study demonstrated that IgG-N seroconversion had no significant association with clinical outcomes in hospitalized COVID-19 patients.

INTRODUCTION

⁹ SARS-CoV-2 is a novel virus which belongs to the family of *Coronaviridae*, the causative agent for coronavirus diseases 2019 (COVID-19).¹ SARS-CoV-2 emerged out of Wuhan in China and soon after, it spread to the entire world and thereby becoming a “Pandemic”.²⁻⁴ After two years of rapid spread and the virus claiming over five million lives, healthcare system continues to scramble to protect patients from the atypical pneumonia-like illness caused by COVID-19. ¹ Diagnosis of SARS-CoV-2 infection is primarily dependent on reverse-transcription polymerase chain reaction (RT-PCR) testing of nasopharyngeal swab samples with more recent progress into rapid antigen testing.^{5,6} Rapid community spread of the infection and development of herd immunity by community exposure has been a favorite topic of discussion by epidemiologists while the scientific community have successfully raced to design several effective vaccines for COVID-19.

Taking a dive into the pathophysiology of COVID-19, a strong comprehension of the role of the humoral immunity becomes very pertinent. It is known that infection with SARS-CoV-2, elicits an adaptive immune response by producing target specific antibodies which includes IgM, IgG and IgA.⁷⁻⁹ Among these antibodies, IgG has been of tremendous interest to the scientific community due to its role in long-term protection against the virus.¹⁰ After an infection with SARS-CoV-2, it takes about 10-14 days to produce IgG antibodies which peaks around the third week and continues to remain detectable for about 8-12 mo.^{11,12} ⁸ SARS-CoV-2 is a positive sense single stranded ³ RNA virus comprised of four different structural proteins. Those are the nucleocapsid protein (N protein), Spike protein (S-protein), matrix protein (M-protein) and the envelope protein (E-protein).¹³ Antibody against the S-protein (IgG-S) is believed to be

the neutralizing antibody that is the primary target of the vaccine trials. There have been reports showing a positive correlation between higher IgG-S levels and diseases severity.^{14,15} On the contrary, another study cited no association of IgG-S with patient outcomes such as need for maximal oxygen support, ICU admission, duration of hospitalization and death.¹⁶ Alternatively, it could be argued that the non-neutralizing antibodies against the nucleocapsid protein (IgG-N) leads to robust inflammation cascade and release of cytokines thus contributing to debilitating pulmonary injury. ⁵ It is believed that the cytokine storm plays a key role in the pathogenesis and COVID-19 prognosis.^{17,18} A report by Batra et.al studied the role of IgG-N in COVID-19 and based on the findings the authors recommended using IgG-N titers as a prognostic factor for the clinical course in patients. In this study, a higher IgG-N titer was associated with extended duration of stay in the hospital and increased rate of admission into the ICU.¹⁹ Another study demonstrated that the stronger IgG-N seroconversion response is associated with more diseases severity compared to the weak responders.²⁰ ¹ There is still a paucity of data about the functionality of IgG-N in the pathophysiology of COVID-19. Given this concept of targeting various structural components of this prolific virus, study of the seroconversion and IgG-kinetics has gained a lot of importance to the researchers. Literature on the long-term kinetics of IgG antibody levels and their corresponding neutralizing effectiveness is sparse.

Practicing in a tertiary care community-based teaching hospital in North Dakota, USA, we have had experience with the pre-vaccine phase of COVID-19 pandemic. Noncompliance with public mask usage and rapid community transmission led to a sharp rise in COVID-19 illness and hospitalizations in North Dakota. In the midst of a healthcare crisis, we decided to investigate whether a qualitative IgG-N could be used as a molecular marker to determine the prognosis in the hospitalized patients. Basing our hypothesis on a theory that a rampaging community spread of SARS-CoV-2 infection led to a measurable IgG-N seroconversion of our population, thus impacting outcomes from hospitalization due to COVID-19, we retrospectively analyzed the data provided by the single-center community hospital from which we practiced.

MATERIALS AND METHODS

Study population and data collection

All patients were admitted to the community hospital between December 1st 2020 and August 30th 2021. Fifty-nine patients were included in the study who were screened for IgG-N within 48 h of admission. We excluded all patients that had been admitted to the hospital with a non-COVID-19 diagnosis who incidentally tested positive for SARS-CoV-2 during screening. Patients with severe or critical COVID-19 illness as per the definition of National Institute of Health were included in the study. Those with mild and moderate illness were excluded from the study as most of them did not meet criteria for hospitalization. None of the included patients had been vaccinated against COVID-19. All the patients were confirmed positive for SARS-CoV-2 infection using RT-PCR from nasopharyngeal swab samples at admission. Both male and female patients aged 28 to 96 were included in the study. All patients were checked for the presence of SARS-CoV-2-IgG-N within 48 h of admission by using Abbott SARS-CoV-2-IgG assay, that uses a two-step chemiluminescent microparticle immunoassay method with acridinium-labeled anti-human IgG, performed at North Dakota state laboratory. Admission blood samples identified 26 patients positive for IgG-N against SARS-CoV-2 and 33 negatives. In October 2021, we started data acquisition, reviewing the electronic medical record of included patients.

Study design

As this retrospective cohort study investigated the study population from patient admission to outcome, a thorough review of the electronic medical record was performed to capture data. This data was inclusive of the following: age, gender, body mass index (BMI), duration of symptoms prior to hospitalizations (DOS), length of hospital stay measured in days (LOS), admission to intensive care unit (ICU), need for high flow nasal cannula (HFNC), bilevel positive airway pressure ventilation (BiPAP) or mechanical ventilation (VENT) for supplemental oxygen/support, as well as the final patient outcome - discharge or death. (Table-1)

Statistical analysis

Formatting:

Age, BMI and DOS were numerical variables. However, additional constructs split Age and BMI into two and three-group categories for some analyses. For example, BMI_2, patients with a BMI of <29.9 were put in one group, and those >30 in a second group. For BMI_3, patients with a BMI of <25 were put into one group, those between BMI of 25-29.9 in a second group, and those with BMI >30 in a third. For the variable labelled Age_2, patients <75 were put in one group, and those 75+ in a second group. For Age_3, patients with an age of <40 were put into the first group, those between 40-75 in a second, and those 75+ in a third group. It should be noted here that one patient had an extreme value for their LOS at-158 days. Models were run with both the patient included and excluded to determine the sensitivity of the models to this extreme value.

Correlation of Outcomes:

For each pair of outcomes (Death, ICU, BiPAP, VENT and HFNC), the phi coefficient (measure of association between binary variable, comparable to the Pearson coefficient for continuous normal variables) was calculated. (Table-2)

Outcomes by IgG status alone:

For each variable the outcome was modeled as a function of IgG-N status (positive or negative) using a generalized linear distribution. For LOS, it was determined that a negative binomial distribution had a better fit than a Poisson or Gaussian distribution, as evidence by a Pearson chi-square/df value closer to 1.0. The negative binomial distribution is also less sensitive to outliers. All other outcomes utilized a binary logistic regression model.

Outcomes by IgG-N status full model:

For any single models that were significant, a multiple regression model was utilized, accounting for the consequential effect (if any) of the defined confounding variables of age, sex, BMI, and duration of symptoms.

Outcomes by other factors:

LOS was modeled as a function of age using a negative binomial model. From there, age (categorical), BMI (numerical), and the interaction of BMI and age were each run with and without the extreme patient LOS-value noted in the previous section. The patient outcome / death was modeled as a function of LOS using a logistic model. Then, death was modeled as a function of age (numerical), and then age (categorical). The same was done for BMI. Finally, death was modeled as a function of sex. ICU, BiPAP, VENT, and HFNC were each modeled as a function of age (numerical), BMI (numerical), and sex separately.

Statistical analysis used SAS Studio V.3.8 (Cary, North Carolina, USA). The statistical review of the study was performed by a biomedical statistician.

RESULTS

We conducted a retrospective cohort study among fifty-nine adults aged between 28-96, admitted to the hospital with severe or critical COVID-19 illness between December 2020 and August 2021.

Correlation of Outcomes:

Unsurprisingly, most outcomes were strongly correlated. VENT and ICU rates were very strongly correlated (Phi Coeff=0.94). All but one patient who went on mechanical ventilation was also admitted to the ICU. In contrast, VENT and HFNC rates were only moderately correlated (Phi Coeff=0.43).

Outcomes by IgG status alone:

Patient outcome, ICU admissions, HFNC, BiPAP and VENT rates were not significantly different across IgG-N status (Figures 2-6). However, LOS by IgG-N status was found to be significant (t-value=2.16, p-value=0.0349) when including the extreme value (LOS>150 days). IgG-N negative patients had higher average LOS than IgG-N positive patients (15.12 vs. 9.35 days). However, when removing the extreme value (LOS of 150 days), IgG-N negative patients still had slightly higher average LOS (10.66 vs. 9.35

days), but the relationship was no longer significant. Furthermore, median LOS was lower in IgG-N negative patients (6.5 vs. 7.5 days).

Outcomes by IgG-N status full model:

Because LOS-days and IgG-N status was significant, at least when not removing the extreme value, the full model was considered which included age, BMI, and sex, and duration of symptoms. However, in the full model, IgG-N was not significant when controlling for the other variables. This remained true when using a model without the extreme value.

Outcomes by other factors:

For death, only age (numerical) was a significant predictor (F-value=5.07, p-value=0.0283). As age increased, the probability of having an endpoint of one (Death) increased (Figure 1). No other variables for any of the outcomes were significant.

DISCUSSION

SARS-CoV-2 infection causes an atypical pneumonia like respiratory illness known as COVID-19 characterized by fever, dyspnea, anosmia and a worsening hypoxia.^{21,22} Among those hospitalized with COVID-19, patients often required supplemental oxygen using HFNC, BiPAP and an increased admission into the ICU requiring mechanical ventilation depending on the severity of the respiratory failure and lung parenchymal involvement. The pathophysiology of COVID-19 is primarily an immune-mediated process with a variety of antibody signatures among which the IgG signatures were of interest to our study. A robust immune-mediated inflammatory cascade guides the pathophysiology of the COVID-19 illness.²²⁻²⁴ Different proinflammatory cytokines such as IL-6 and TNF- α have been correlated with diseases severity.²⁵

There is some data available to understand the humoral response to SARS-CoV-2 infection and role of various IgG subtypes in the body's line of defense. Much of it was inherited from the studies of SARS-CoV-1.²⁶ IgG antibodies directed towards the spike

protein (IgG-S) and that of the nucleocapsid protein (IgG-N) are the two important components of humoral immunity against SARS-CoV-2 infection. SARS-CoV-2 uses the spike protein to bind to the target cell through its receptor-binding domain and therefore is the target site for neutralizing antibody, IgG-S.²⁷ The role of IgG-S in early viral clearance is crucial for favorable clinical course and survival.²⁸⁻³⁰ IgG-S is considered the neutralizing antibody which may elicit a protection against SARS-CoV-2 by interfering with virion binding to host cell receptors, blocking cellular uptake and preventing endosomal processing of viral genome.^{13,27} However, the kinetics of the antibody response becomes more complex to understand with current available literature, which is conflicting. In one interesting study, there is a link between the IgG-S response and COVID-19 severity, but the antibody response has to develop in a specific time window to improve viral clearance and disease outcomes. A faster antibody response was associated with better survival (within the first 14 days of infection) and deceased patients showed a slower antibody response although they reached higher IgG titers later in the disease trajectory.³¹ Other studies have shown that, severely ill patients exhibit higher peak, faster and stronger antibody response compared to mildly symptomatic patients.^{13,32} Severely ill COVID-19 patients have been found to produce a unique serologic signature with increased IgG-S with afucosylated Fc glycans. The Fc modification of IgG-S triggers activation of natural killers cells and enhances production of IL-6 and TNF- α by primary monocytes that results in more severe disease.³³

The role of IgG-N in the pathogenesis and clinical course of COVID-19 remains largely unknown. As to our current knowledge, severe COVID-19 is characterized by a series of inflammatory signatures including a cytokine storm, inflammatory alveolar infiltrates and formation of vascular microthrombi.³³ During the peak of the pandemic, clinicians took their chance to use different inflammatory markers such as C-reactive protein, platelet count, D-dimer and Ferritin to name a few to monitor diseases progression and crisis planning, however data to support the specificity of these inflammatory signatures as reliable prognostic markers for COVID-19 is limited.^{34,35} As per one report

by Batra *et al*, showed that titers of IgG-N at the time of admission can be a prognostic factor in the clinical course of the diseases and was associated with increased incidence of hypoxemia, admission into the ICU and extended length of stay in the hospital.¹⁹ In our study, we hypothesized that the presence of IgG-N at the time of admission into the hospital could be used as a marker of impending diseases severity and determine hospital course. We pursued a qualitative measurement of IgG-N on all our patients. Some key parameters such as the degree of hypoxemia, mean length of hospitalization, ICU admission, need for mechanical ventilation and patient outcome as in-hospital datasets were examined in our study group. We enrolled a total of 59 patients who were admitted with hypoxia secondary to COVID-19, out of which 26 (44%) patients had IgG-N antibody at the time of admission into the hospital. Our goal was to investigate the role of IgG-N as a marker to anticipate the clinical course in hospitalized patients. Based on our results, we concluded that IgG-N might not be a reliable predictor of COVID-19 diseases severity.

Our data indicate that age was a single independent predictor of death following hospitalization, which is in support of reports published earlier.^{36,37} As age increased, the probability of death increased (Figure-1). Mortality rate was not significantly different in IgG-N positive group *vs* negative (Figure-2). We did not find any statistical difference with the need to use HFNC between the two groups (Figure-3). Many of our patient population had clinically progressive diseases with worsening respiratory failure requiring BiPAP or transfer to ICU to be intubated and placed on mechanical ventilation. After following the patient pool until discharge, we did not find any significant difference with the need to use BiPAP between the two groups with and without IgG-N at the time of admission (Figure-4). The admission rate into the ICU and need for mechanical ventilation was not statistically significant either (Figure-5,6). Although we saw an extended LOS among the IgG-N negative group, but after adjusting for the extreme outlier, the findings were no longer significant (Figure-7). Furthermore, median LOS was actually lower in the IgG-N negative group, showing that the extreme value was skewing the LOS average.

Our study had several limitations. We did not measure the IgG-N antibody titers in our study and so we cannot imply if high *vs* low antibody titers have any direct impact on the disease severity and mortality in COVID-19. Since every individual patient was enrolled into the study only when they were symptomatic enough to meet criteria for hospitalization, especially hypoxic with oxygen saturation <90%, it could be argued that they may be at different stage of the diseases course and different phase of the seroconversion. This could have confounded our findings since seroconversion and viral kinetics are time dependent phenomena. We did not standardize our patients based on their underlying comorbidities, which further could have influenced our results. More investigation using a larger sample size and different IgM/IgG subtypes is warranted to put more light in this area.

CONCLUSION

We have analyzed the presence or absence of IgG-N in patients admitted to the hospital with severe or critical COVID-19 illness and evaluated the effects of presence of IgG-N on clinical severity and outcome. Age happens to be the single independent risk factor for a worse outcome. Our analysis revealed no significant correlation between IgG-N status and degree of respiratory failure or mortality. The degree of respiratory failure was characterized by the utilization of high flow nasal canula, bilevel positive pressure ventilation and intubation with mechanical ventilation. IgG-N seroconversion had no significant effect on mean length of stay in the hospital. Further studies with large cohorts and risk-adjusted comorbidities are needed to demonstrate the more accurate role of IgG-N seroconversion on clinical outcome.

ARTICLE HIGHLIGHTS

Research background

During the peak of the COVID-19 pandemic, hospitals and clinicians had to adapt quickly to the rapid spread of the infection in the community. In the absence of adequate literature, clinicians hypothesized and studied the utility of various protein

markers to prognosticate their patients. We intended to study the correlation of anti-nucleocapsid-SARS-CoV-2 antibody (IgG-N) presence with the clinical outcome in severely ill hospitalized COVID-19 patients.

Research motivation

We were interested in characterizing a correlation between presence or absence of IgG-N and clinical outcome in hospitalized COVID-19 patients. We wanted to test the ability of IgG-N in predicting the severity of illness, maximal oxygen support needed and final outcome in order to mobilize staff, manage ICU beds and ventilators as a part of the crisis management planning.

Research objectives

To study the effect of SARS-CoV-2 anti-nucleocapsid-IgG on COVID-19 diseases severity and outcome. We studied the effect of presence or absence of IgG-N on mean length of stay in the hospital, maximal oxygen support needed and mortality.

Research methods

We conducted a retrospective cohort study on adults aged between 28-96, being admitted to the hospital with severe or critical COVID-19 illness. Blood sample was collected either at or within 48 h of admission to the hospital to check for SARS-CoV-2-IgG-N. A total of fifty nine patients were enrolled into the study. We utilized a binary logistic regression model to analyze the outcome data.

Research results

Our data demonstrated that the need for maximal oxygen support, mean length of stay in the hospital and mortality were not significantly different between the groups with or without IgG-N at the time of admission.

Research conclusions

We concluded that IgG-N seroconversion had no significant correlation with the need for maximal oxygen support as well as mortality during the course of hospitalization. Length of stay in the hospital was not significantly different across the IgG-N status.

Research perspectives

Our study demonstrated that presence of anti-nucleocapsid-IgG against SARS-CoV-2 infection had no impact on the clinical outcome or diseases severity in hospitalized COVID-19 patients. We did not find a correlation of statistical significance to use IgG-N as a biomarker to predict clinical outcome in COVID-19 patients admitted to a community hospital in North Dakota. Acknowledging the limitation of our study, we look forward to a future study with larger sample size and risk-adjusted comorbidities to investigate the association with better clarity.

ACKNOWLEDGEMENTS

We would like to acknowledge Ms. Shannon Yarbrough, for her contribution with medical library services. ² Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number U54GM128729.

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