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Observational Study

SARS-CoV-2 in inflammatory bowel diseases population: Antibodies, disease and correlation with therapy

SARS-CoV-2 IN IBD COHORT

Clara Benedetta Conti, Elsa Mainardi, Sara Soro, Sophie Testa, Annalisa De Silvestri, Andrea Drago, Fabrizio Cereatti, Roberto Grassia

Abstract

BACKGROUND

Guidelines recommend to hold IBD biologic therapy during COVID-19.

AIM

To investigate SARS-CoV-2 antibodies positivity in IBD cohort, COVID-19 disease severity and to evaluate the correlation with clinical/therapeutic variables.

METHODS

Prospective observational cohort study. IBD patient were tested for SARS-CoV-2 IgG. Data on COVID-19 disease, demographic/therapeutic and clinical features of IBD population were collected. IgG >=7 were set for SARS-CoV-2 antibodies positivity. Throat swab was performed in case of IgG positivity. Correlations between antibodies positivity or COVID-19 symptoms and therapeutic/clinical data were assessed.

RESULTS

103 IBD patients were enrolled. 18.4% had IgG>= 7. At multivariate analysis antibodies positivity correlated only with IBD treatment. For IgG >= 7 OR was 1.44 and 0.16 for AZA and mesalazine, respectively, *vs* biologic drugs (P = 0.0157 between them).

63% of patients with IgG positivity reported COVID-19 related symptoms. All but one patient with COVID-19 symptoms did not require to hold IBD treatment nor hospitalization. IBD treatment and BMI correlated with COVID-19 disease development with symptoms.

CONCLUSION

IBD population does not have higher risk of severe COVID-19. The RR of having SARS-CoV2 antibodies and symptoms was higher for patients under AZA, then biologic therapy and lastly mesalazine. None of the patients under biologic therapy developed severe COVID-19.

Key Words: inflammatory bowel disease; SARS-CoV-2; COVID-19; biologic treatment; SARS-CoV-2 antibody; inflammatory bowel disease therapy

Conti CB, Mainardi E, Soro S, Testa S, De Silvestri A, Drago A, Cereatti F, Grassia R. SARS-CoV-2 IN IBD POPULATION: ANTIBODIES, DISEASE AND CORRELATION WITH THERAPY. *World J Gastrointest Endosc* 2021; In press

Core Tip: Guidelines recommend to hold IBD biologic therapy during COVID-19. IBD patients were prospectively tested for SARS-CoV-2 IgG. 103 IBD patients were enrolled. We found that 18.4% of them had IgG positivity and 63% developed COVID-19 disease with symptoms. However, all but one patient with symptoms did not require to hold IBD treatment nor hospitalization. The relative risk of having SARS-CoV2 antibodies and COVID-19 symptoms was higher for patients under AZA, then biologic therapy and lastly mesalazine. None of the patients under biologic therapy developed severe COVID-19. Therefore, IBD population does not seem to have high risk of severe COVID-19, particularly if under biological treatment or mesalazine.

INTRODUCTION

A new β-coronavirus (SARS-CoV-2) spread in November 2019 in China and then worldwide, becoming pandemic. The related disease, known as COVID-19, mainly involves the respiratory system. The elderly and patients affected by chronic diseases seem to be at higher risk to develop severe pneumonia and acute distress syndrome, eventually (1). In this scenario, the patients affected by inflammatory bowel diseases (IBD) appeared to be as an at-risk population for severe COVID-19, considering also the possible gastrointestinal system involvement (2-6). Indeed, it seems that the high expression of ACE2 in the intestinal tract, above all in the absorptive enterocytes of ileum, colon and in the epithelial cells of the esophagus, makes these parts highly susceptible to SARS-CoV-2 infection. Mucosal damage was observed in the esophagus stomach, duodenum, and rectum by histological examinations, as plasma cells and lymphocytes infiltrated the lamina propria. Noteworthy, approximately 3% of COVID-19 cases have only digestive symptoms. Moreover, the detection of SARS-CoV-2 in the stool suggested that the virus could replicate in the digestive tract (6).

Initial indication from IBD center in Wuhan, China, was to discontinue all biological and immunosuppressive treatments. They reported that, among 318 registered IBD patients, none developed COVID-19 (7). Nevertheless, scientific societies suggested that IBD patients should continue the ongoing treatment to avoid relapse, including the biological therapies (1). However, regarding IBD patients affected by COVID-19, guidelines suggested to handle the treatments with more caution. In particular, AGA guidelines divided them in three different categories: IBD patients without SARS-CoV-2 infection; IBD patients with SARS-CoV-2 infection but no symptoms of COVID-19; IBD patients with COVID-19 symptoms. The first category should continue all treatments; the second one should hold thiopurines, methotrexate, tofacitinib and delaying biological therapies for 2 wk, monitoring symptoms of COVID-19. The third category is recommended to hold thiopurines, methotrexate, tofacitinib and biological therapy during the illness (1). Since the scientific community had to develop new guidelines in a

short time with a new and unknown disease, the recommendations carry a low grade of evidence. In an Italian cohort of 522 IBD patients, none was hospitalized for SARS-CoV-2 infection and 16% of the patients were under biologic treatment. However, 11% of the patients were children, a population with an unclear susceptibility to the virus (8). Moreover, some interesting observational studies report COVID-19 prevalence and symptoms/outcome in IBD cohorts (9-10). However, little is known about the possible role of IBD treatments in the development of severe COVID-19 disease. Importantly, it remains unclear weather IBD patients are at higher or lower risk of severe COVID-19.

Systemic inflammation is a crucial target for the treatment of COVID-19 pneumonia, as the severity of the respiratory disease seems to be linked to the up-regulation of inflammatory cytokines, by creating a "cytokine storm", producing IL-6, IL-1, TNF, INF-γ. The exaggerated synthesis of IL-6 can lead to an acute severe systemic inflammatory response. It should be noted that cytokine blockers and Jak-inhibitors were considered for clinical therapy of COVID-19 ARDS. (11-13). Interestingly, TNF inhibition has also been suggested in selected patients with high IL-6 levels. Indeed, when TNF is blocked, there is a serical decrease of IL-6 and IL-1 within 12 h in the patients with active rheumatoid arthritis. A reduction of adhesion molecules and vascular endothelial growth factor was observed as well (14). Nevertheless, no definitive treatment has been approved. Therefore, many hypothesis but few certainties are present. In particular, COVID-19 outcome in patients with IBD immunomodulant/immunosuppressive treatments remains under debate.

The present study aims to investigate the prevalence of SARS-CoV-2 antibody positivity and COVID-19 disease severity in IBD cohort, in both symptomatic and asymptomatic patients and to evaluate the correlation with clinical/therapeutic variables.

MATERIALS AND METHODS

2.1 Study Design

Prospective cohort study. The informed consent for the study was obtained from all the patients in accordance with the World Medical Association's 2008 Declaration of

Helsinki: Ethical Principles for Medical Research Involving Human Subjects. The privacy rights of patients were always observed. All authors had access to the study data, reviewed and approved the final manuscript.

2.2 Patients

Cohort of patients affected by Inflammatory bowel diseases (CD or UC). From April 22th to May 31st 2020 each IBD patient followed-up at ASST Cremona was offered to participate to the study. The patients were consecutively enrolled.

2.3 Data Collection

Each IBD patient was asked about his/her recent clinical history (respiratory and gastrointestinal symptoms) from the beginning of COVID-19 pandemic in Europe (21th February) by filling a questionnaire and all the information were validated with the doctor who conducted the interview. Data collected in the questionnaire are summarized in Appendix 1.

Age, sex, BMI, IBD type, treatments and clinical activity and other comorbidities were anonymously collected in a Database. Charlson Comorbidity Index (CCI) was calculated for each patient.

2.4 Antibody testing

A single blood test was performed to each patient to search for Immunoglobuline IgG anti SARS-Cov-2. The LIAISON® SARS-CoV-2 S1/S2 IgG test [DiasorinS.p.A, Saluggia (VC) – Italy] was used according to manufacturers' instructions. S1 and S2 are subunits of the Spike protein and are responsible for binding (S1) & fusion (S2) of virus to cell. The Spike Proteins is the target of neutralizing antibodies. They are defined as antibodies that protect cells from pathogens or infectious particles by neutralizing their biological effects. The manufacturer reports a positive agreement of 94.4% (CI 95% 88.8-97.2) with the plaque reduction neutralization test. The IgG test has diagnostic specificity of 98.5% (95%CI: 97.5-99.2) in blood donors and 98.9% in presumably SARS-Cov-2 negative diagnostic routine samples. The IgG values are considered as negative when <12.0 kAU/L; equivocal from 12 kAU/L to 15.0 kAU/L; positive when ≥15.0 kAU/L. Applying a cut off >15 kAU/L the reported test's sensitivity is time-

dependently: 25% (14.6-39.4) <= 5 days after RT-PCR-confirmed diagnosis, 90.4 % (79.4-95.8) from day 5 to day 15, and 97.4% (86.8-99.5) after > 15 days from PCR diagnosis (15). However, a study of Plebani group found that 6.2 kAU/L was the appropriated cut off for Diasorin method to reach a sensitivity of 97.1% and a specificity of 88.9% (16). Moreover, in our hospital, all health care workers (HCW) were tested for serology immediately after the first two months of pandemic (between April and May 2020). Among the HCW who were previously confirmed ill, only the 85% of them resulted having IgG value > 15, whereas 14% of them had values between 7 and 15 (data from National Institute of Heath, 2020)..

Thus, in the present study we decided to perform the analysis using both 15 and 7 as cut offs, considering 7 as the most reliable value.

2.5 Swab throat test

All patients who resulted positive to SARS-CoV-2 IgG were tested with SARS-CoV-2 swab throat test during the same week, using the Allplex 2019-nCoV assay (Arrow Diagnostics S.r.l., Genova, Italy) a single-tube assay able to detect the three target genes (E gene, RdRP gene and N gene) as in the WHO recommended protocols.

2.6 Statistical analysis

Categorical variables were described as count and percentage and compared between groups with chi square test; continuous variables were described as mean and standard deviation or median and inter-quartile range if not normally distributed (Shapiro-Wilks test) and compared with independent t- test or Mann-Whithney.

Through uni and multivariate logistic regression models were assessed:

- association between age, sex, BMI, IBD type, IBD treatments, IBD clinical activity, Charlson Comorbidity Index (CCI) and SARS-CoV-2 IgG positivity
- association between age, sex, BMI, IBD type, IBD treatments, IBD clinical activity, Charlson Comorbidity Index (CCI) and presence of COVID-19 symptoms

The analysis was performed using SARS-CoV-2 IgG value cut off of > 7 kAU/L (15-16).

RESULTS

103 IBD consecutive patients were enrolled. 54 had Crohn's Disease (CD) and 49 Ulcerative Colitis (UC). 36 (35%) patients were treated with biologic treatment, 14 (13.6%) with azathioprine (AZA) and 53 (51.4%) with mesalazine. Demographic, clinical and therapeutic characteristics of the cohort are summarized in Table 1.

Survey's results are summarized in Table 2

3.1 *Prevalence of SARS-CoV-2 IgG positivity in IBD cohort*

19 out of 103 patients (18.4%) had SARS-CoV-2 IgG positivity, with value > 7. Among them: 10 were under biological treatment, 5 under AZA and 4 under mesalazine. 12 out of 19 (63%) reported symptoms related to COVID-19 disease. Among them, 2 were treated with mesalazine, 4 with AZA and 6 with biologic treatment. Among the 7 out 19 patients without history of COVID-19 related symptoms, but positive for antibodies, 2 were treated with mesalazine, one with AZA and 4 with biologic therapy.

All but one patient, who had pneumonia and was under AZA treatment, did not require hospitalization.

Data regarding the patients with IgG > 7 are summarized in Table 3

3.2 Swab throat test

All the patients with IgG > 7 were tested with swab throat test. All of them resulted negative. The patient with history of COVID-19 pneumonia had tested positive before the enrollment and after tested negative.

3.3 Correlation between SARS-CoV-2 IgG positivity and clinical/therapeutic variables in IBD cohort

SARS-CoV-2 IgG value >= 7 correlated at multivariate analysis only with IBD treatment. In detail, stratifying the population for treatment, the relative risk (RR) of having SARS-COV-2 IgG >= 7 was higher for patients treated with AZA and lower with mesalazine: Odds Ratio (OR) 1.44 [95% C.I. 0.27-7.56] and 0.16 [95% C.I. 0.03-0.71], for AZA and mesalazine, respectively, *vs* biologic drug (P = 0.0157 between them). The RR for patients under mesalazine was lower than for those under biologic therapy, P = 0.016.

3.4 Correlation between the presence of COVID-19 related symptoms and clinical/therapeutic variables in IBD cohort

The presence of COVID-19 related symptoms resulted correlated at multivariate analysis with Body Mass Index (BMI), $P = 0.05$ and with IBD therapy. The RR of having symptoms was strongly higher for patients treated with AZA and lower with mesalazine *vs* biologic drug: Odds Ratio (OR) 7.47 [95% C.I. 1.22-45.73] and 0.52 [95% C.I. 0.17-1.72, $P = 0.03$], for AZA and mesalazine, respectively ($P = 0.004$ between them).

DISCUSSION

The use of SARS-Cov-2 antibodies to monitor the immunity against COVID-19 remains a matter of debate in the general population. However, the presence of SARS-CoV-2 IgG antibodies certify the previous or recent infection (17). In our hospital, all health care workers (HCW) were tested for serology immediately after the first two months of pandemic, in the same week of the start of our study on IBD cohort. 364 out of 1600 operators were diagnosed as affected by COVID-19 between 21th February and April the 22th and all of them tested positive for SARS-CoV-2 swab throat test. Among the HCWs who were previously confirmed ill, the 99% resulted having IgG3 value > 7. Interestingly, 20% of operators who did not report symptoms suggestive for COVID-19 resulted having SARS-CoV-2 antibodies >= 7. (data from National Institute of Health, 2020). This observation confirms the presence of an unknown number of asymptomatic infected people (18). The available studies on the serum concentration of IgG after COVID-19 infection revealed conflicting results and the duration of antibodies rises is currently unknown, but is estimated around nine months (data from National Institute of Health, 2021). There is a possible decrease of IgG title after the first two wk of infection and it is unclear whether the test is able to detect lower antibody levels in milder and asymptomatic COVID-19 disease (17-20). Plebani group tried to harmonize the thresholds to allow a larger agreement on IgG anti Sars-Cov2 antibodies determination. They found 6.2 KAU/L as the cut off for Diasorin method to reach a sensitivity of 97.1% and a specificity of 88.9% for the diagnosis of SARS-CoV-2 infection

(16). Our data are thus in line with this latter observation. The COVID-19 symptoms occurred in IBD patients at least one month before the interview. During the time between the symptoms and the enrollment, they lived the complete lock down, established in Italy from 09 March to 18th May. They tested all negative at the swab test performed at the enrollment. This is in line with the overall sensitivity of the test, ranging from 56 to 83%: 66.7% in the first week of the infection and lower in the following wk observation that the SARS-CoV-2 positivity in the swab (21).

Prevalence of patients with SARS-CoV-2 IgG positivity in our cohort was 18.4%. This means that those patients got infected with SARS-CoV-2 virus in the previous period, but only 63% of them developed the disease, reporting symptoms. Moreover, only one patient required hospitalization for pneumonia. The patients with history of COVID-19 related symptoms mainly had mild respiratory symptoms or minor manifestations. None but one patient (5%) required hospitalization, but without the need of intensive care unit. Conversely, in the general population, during both the first and the second wave of the pandemic, 10% of people required hospitalization in intensive care unit (data from the National Institute of Health, 2021). Half of the IBD patients that resulted positive to antibody test remained asymptomatic and in 48% of cases they developed only mild symptoms. We can thus conclude that the IBD population does not seem at higher risk to develop severe COVID-19 disease in comparison with the general population, confirming the observation of Bezzio *et al* (9). Only the patient with pneumonia hold the IBD treatment. This happened because, due to the mildness of the disease, the patients informed the general practitioner but not the IBD center about the symptoms. These data, even if do not confirm the AGA guidelines strategy, gave us the opportunity to evaluate the cohort (1). The results obtained are encouraging, as it seems that IBD patients with COVID-19 ongoing disease with symptoms could continue any treatments both avoiding IBD relapse and without a significant higher risk of developing severe COVID-19 requiring hospitalization. Differently from Bezzio *et al* (10), nobody died in our cohort; moreover, nor age neither active IBD were significantly associated with a COVID-19 worse prognosis.

SARS-coV-2 serology resulted associated only with the ongoing IBD treatment. Among the patients having a positive serology there was a prevalence of biologic therapy. The presence of COVID-19 disease was associated with both IBD therapy and BMI. The patients who reported previous symptoms were treated with mesalazine in 2 cases, with AZA in 4 and with biological treatment in 6; the only patient with pneumonia was treated with AZA. The calculated relative risk of being infected was higher for patients treated with AZA, then for patients treated with biologic drugs and the lowest risk was found for patients treated with mesalazine. We decided to separate the different treatments in the analysis, as the AZA and the biologic therapy have a different mechanism of action: AZA is an immunosuppressive agent, whereas the biologic therapies are known as immunomodulating agents. None of the patients treated with biologic therapy developed a severe COVID-19 disease. Our results show that the use of biologic therapy does not seem to expose the patients to higher risk of severe COVID-19 disease, even when the infection is present. We did not perform a sub-analysis of the different type of biologic treatment for the small sample size. However, we report that the 80% of patients was treated with anti-TNF agents. More studies are needed to confirm whether it is appropriate to continue biological drugs for IBD patients who are affected with Sars-cov-2. The other variable associated with the presence of COVID-19 related symptoms was the BMI. This data is supported by the literature, as obesity is a factor associated with bad prognosis in the patients with COVID-19 pneumonia (22). Interestingly, nor the old age neither the comorbidities or the type of IBD were associated with the antibody positivity or the development of COVID-19 symptoms in our study. This could be explained by the fact that these variables were associated in literature to death or very bad outcome, and none of our patients reported such complication (23).

All the 103 patients of the study had been clinically followed up for ten months after the beginning of the study. None of them hold the IBD treatments or developed new symptoms of COVID-19 until April 2021. After this period of time all our IBD patients had been received the vaccine against COVID-19.

The main limitation of the study is the small sample. Therefore, further studies with larger populations are needed to confirm our observations.

CONCLUSION

We investigated both the SARS-CoV-2 IgG positivity in symptomatic and asymptomatic IBD patients and the relationship between IBD therapy and COVID-19 disease severity. The results are interesting and seem encouraging for the patients treated with biologic therapy, since they don't seem to carry a high risk of developing severe COVID-19. However, further and larger studies are needed to confirm these observations

ARTICLE HIGHLIGHTS

Research background

Guidelines recommend to hold IBD biologic therapy during COVID-19. It is still not clear if the IBD patients carry a high risk of developing severe COVID-19.

Research motivation

IBD patients could carry a high risk of relapse or worsening of the intestinal disease in holding the therapy.

Research objectives

Investigate the prevalence of SARS-CoV-2 antibodies positivity and COVID-19 disease severity in IBD patients. Evaluate the correlation with clinical/therapeutic variables.

Research methods

Prospective cohort study.

Patients with IBD were consecutively enrolled from April 22th to May 31st 2020.

Age, sex, BMI, IBD type, treatments and clinical activity and other comorbidities were anonymously collected in a Database. Charlson Comorbidity Index (CCI) was calculated for each patient.

A single blood test was performed to each patient to search for Immunoglobuline IgG anti SARS-Cov-2. The LIAISON® SARS-CoV-2 S1/S2 IgG test [DiasorinS.p.A, Saluggia (VC) – Italy] was used according to manufacturers' instructions. The analysis was performed using SARS-CoV-2 IgG value cut off of > 7 kAU/L

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Categorical variables were described as count and percentage and compared between groups with chi square test; continuous variables were described as mean and standard deviation or median and inter-quartile range if not normally distributed (Shapiro-Wilks test) and compared with independent t- test or Mann-Whithney.

Through uni and multivariate logistic regression models were assessed: association between age, sex, BMI, IBD type, IBD treatments, IBD clinical activity, Charlson Comorbidity Index (CCI) and SARS-CoV-2 IgG positivity or the presence of COVID-19 symptoms

Research results

103 IBD consecutive patients were enrolled: 54 with Crohn's Disease (CD) and 49 Ulcerative Colitis (UC). 36 (35%) patients were treated with biologic treatment, 14 (13.6%) with azathioprine (AZA) and 53 (51.4%) with mesalazine.

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SARS-CoV-2 IgG value >= 7 correlated at multivariate analysis only with IBD treatment. The relative risk (RR) of having SARS-COV-2 IgG >= 7 was higher for patients treated with AZA and lower with mesalazine: Odds Ratio (OR) 1.44 [95% C.I. 0.27-7.56] and 0.16 [95% C.I. 0.03-0.71], for AZA and mesalazine, respectively, *vs* biologic drug (*P* = 0.0157 between them). The RR for patients under mesalazine was lower than for those under biologic therapy, *P* = 0.016.

The presence of COVID-19 related symptoms resulted correlated at multivariate analysis with Body Mass Index (BMI), *P* = 0.05 and with IBD therapy. The RR of having symptoms was strongly higher for patients treated with AZA and lower with mesalazine *vs* biologic drug: Odds Ratio (OR) 7.47 [95% C.I. 1.22-45.73] and 0.52 [95% C.I. 0.17-1.72, *P* = 0.03], for AZA and mesalazine, respectively (*P* = 0.004 between them).

Research conclusions

The patients treated with biologic therapy don't seem to carry a high risk of developing severe COVID-19.

Research perspectives

The patients treated with biologic therapy don't seem to carry a high risk of developing severe COVID-19. Therefore, further and larger studies are needed to confirm these observations and to understand if the strategy to hold the IBD treatment during COVID-19 disease could be modified

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