Manuscript Doi: 10.1093/ecco-jcc/jjaa237

Seroprevalence of SARS-CoV2 in IBD patients treated with biological therapy

Roberto Berte'[1], Stefano Mazza[1], Marta Rachele Stefanucci[1], Daniele Noviello[1], Stefania Costa[1], Clorinda Ciafardini[1], Erika Mileti[2], Marina Mapelli[2], Sebastiano Pasqualato[2], Sergio Pinto[6,7], Agnese Favale[6,7], Maurizio Vecchi[1,3], Markus F. Neurath[4,5] , Raja Atreya[4,5], Massimo Claudio Fantini[6,7], Federica Facciotti[2], Flavio Caprioli[1,3]

[1] Gastroenterology and Endoscopy Unit, Fondazione IRCCS Cà Granda, Ospedale Maggiore Policlinico, 20135 Milan, Italy

[2] European Institute of Oncology IRCCS, Department of Experimental Oncology, 20139 Milan, Italy

[3] Department of Pathophysiology and Transplantation, Università degli Studi di Milano, 20135 Milan Italy

[4]Deutsches Zentrum fuer Immuntherapie (DZI), FAU Erlangen-Nuremberg and Universitätsklinikum Erlangen, Ulmenweg 18, 91054 Germany.

[5]Department of Internal Medicine 1, FAU Erlangen-Nuremberg and Universitätsklinikum Erlangen, Ulmenweg 18, 91054 Erlangen, Germany.

[6]Gastroenterology Unit, Duilio Casula Hospital, AOU Cagliari, Italy.

[7]Department of Medical Science and Public Health, University of Cagliari, Italy.

Correspondence to: Prof. Flavio Caprioli, Gastroenterology and Endoscopy Unit, Fondazione IRCCS, Cà Granda, Ospedale Maggiore Policlinico, 20135 Milan, Italy. flavio.caprioli@unimi.it

Conflict of Interest: Authors declare no conflict of interest.

Background and aims: A similar course of COVID-19 in patients with inflammatory bowel diseases (IBD) and in the general population has been reported. However, disease prevalence in IBD patients is presently unknown. In this prospective observational study we aimed at determining SARS-CoV2 infection prevalence in IBD patients treated with biological therapy.

Methods: 354 sera from IBD patients under biological therapy recruited from three different locations in Italy and Germany were evaluated for antibody presence by RBD ELISA. Control groups were i) age-matched healthy subjects tested in the same time period in Milan, Italy; ii) healthy subjects collected in the pre-COVID era; iii) IBD patients under biological therapy collected in the pre-COVID era.

Results: 8 out of 354 patients tested positive for the anti-RBD-SARS-CoV2 IgG antibody (prevalence 2.3%). IgG positive patients' percentage recruited from Milan was significantly higher than those recruited from other locations (prevalence 5.4% vs. 0.4% $p < 0.005$). IgG positive patients reported a significantly higher incidence of fever, anosmia and ageusia, and were more likely to have entered in close contact with COVID-19 positive subjects before the study enrolment.

Conclusions: Seroprevalence of SARS-CoV2 in IBD patients treated with biological therapy reflects values measured in the local general population. Specific symptoms and contact history with SARS-CoV2-infected individuals strongly increase the likelihood of SARS-CoV2 seropositivity.

Keywords: IBD, COVID-19,seroconversion, anti-SARS-CoV2 antibodies

Introduction

Infection by the novel SARS-CoV2 betacoronavirus [1] induces the immune system activation, finalized to the clearance of infected cells [2]. The impact of COVID-19 on IBD patients, especially those on immunosuppressive therapy, is not fully understood [3]. Recent reports described a rate of serious disease and death comparable to general population [4, 5], but the real prevalence and clinical manifestations of infection among IBD patients remain largely unknown.

Humoral immune response against SARS-CoV2 proteins, including the receptor binding domain (RBD) of the spike (S) protein, leads the production of antibodies of different classes [6, 7]. Thus, serological tests represent a useful tool to identify patients who contracted the infection [8].

Here, we prospectively collected data regarding the prevalence of SARS-CoV2 infection in patients treated with intravenous (i.v.) or subcutaneous biological therapy (e.g. infliximab, IFX; adalimumab, ADA; golimumab, GOL, vedolizumab, VDZ; ustekinumab, UST) in different geographical areas of Italy (Milan and Cagliari) and in Germany (Erlangen) through the detection of anti-SARS-CoV2 specific IgG and IgA by an home-made validated ELISA assay [9-11]. Risk factors and clinical variables linked with SARS-CoV2 seropositivity were investigated, and kinetic of SARS-CoV2 circulating antibodies over time was assessed.

Results.

From April to June 2020, sera from 354 IBD patients under biological therapy (Table 1) were prospectively collected. 129 patients were recruited from Milan, Italy, 48 from Cagliari, Italy, and 177 from Erlangen, Germany. Sera from a control group of 129 otherwise healthy subjects matched for

age and sex were collected in the same period in Milan. Additional controls were sera from a cohort of 50 IBD patients under biological therapy and from a group of 63 healthy subjects, both collected in Milan during the pre-COVID era (2018).

Overall, 8 out of 354 patients tested positive for anti-RBD IgG antibodies (prevalence 2.3%, 95% CI 0.8-3.8%, Figure 1). The percentage of IgG-positive patients recruited from Milan was significantly higher than those recruited from other locations (prevalence 5.4% [95%CI 3.1-7.7%] vs. 0.4% [95%CI 0-1.1%] $p < 0.005$). No differences were observed in the percentage of IgG-positive IBD patients in Milan and the general population recruited in the same time period in the same area (5.4% [95%CI 3.1-7.7%] vs 7.0% [95%CI 4.4-9.6%], p=ns). The percentage of IgG-positive patients in Milan was significantly higher than that in the pre-COVID era, both from IBD patients and the general population (5.4% [95%CI 3.1-7.7%] vs 0%, $p = 0.01$). No false positive results were observed for IgG antibodies in patients treated with biological therapy in the pre-COVID era.

12 out of 354 IBD patients tested positive for anti-RBD IgA (prevalence 3.4%, 95%CI 1.6-5.2%, Supplementary Figure 1). No differences were observed in IgA serum prevalence between patients recruited from Milan and from other locations Similarly, no differences in anti-RBD SARS-CoV2 IgA seroprevalence was found between IBD patients and general population of Milan (prevalence 3.1% [95%CI 1.4-4.8] vs 5.4%,[95%CI 2.1-7.7]; p=ns).

Demographic and clinical variables associated with anti-RBD SARS-CoV2 seropositivity in patients enrolled in Milan and Cagliari indicated that a close contact with a COVID-19 positive individual and a COVID-19 infected relative were significantly associated with IgG seropositivity at univariate analysis ($p<0.01$ and $p<0.0001$, respectively, Table 2 and Supplementary Table 2). The presence of a COVID-19 infected relative was identified as an independent predictor of IgG seropositivity at multivariate analysis (RR 52.4, 95%CI 1.5-1769.2; p=0.027). Concomitant antiTNF therapy was associated with a significantly reduced seroprevalence of IgG at univariate, but not at multivariate, analysis. Positive history of fever and anosmia/ageusia in the last two months were significantly associated with IgG

seropositivity at univariate analysis (p<0.0001 for all variables) (Table 3). Notably, history of anosmia/ageusia was confirmed as an independent predictor of IgG seropositivity at multivariable analysis (RR 54.5, 95%CI 2.1-1434.9; p=0.016)

Predictors of IgA seropositivity were similar to IgG ones, including close/family contacts with a COVID-19 infected individual, and positive history for fever and anosmia/ageusia, even if these factors were not confirmed at multivariate analysis (Supplementary Tables 1 and 2)

To evaluate the kinetics of antibody titers, IgG and/or IgA seropositive patients were retested 8 weeks following the first measurement. RBD IgG and IgA antibody titers were declining in the majority of individuals (mean OD from 0.949±0.200 to 0.668±0.203 for IgG and from 0.915±0.210 to 0.735±0.185 for IgA, p<0.05), in line with previous reports in paucisymptomatic convalescent individuals [9, 12, 13] (Figure 2, Supplementary Figure 2).

SARS-CoV2 infection had a benign course in the totality of patients (Table 4). In no patient biological treatment for IBD was interrupted, and the majority of patients remained in clinical remission two months following enrolment. In one 70 year old UC female patient, receiving maintenance vedolizumab treatment for 24 months, a clinical relapse of the disease was observed following infection, successfully treated with dose optimization. Active infection of IgG-positive patients was confirmed SARS-CoV2 nasopharingeal swab testing.

Discussion

The current work is, to our knowledge, the first report on SARS-CoV2 seroprevalence in IBD patients treated with biological therapy. Previous reports suggested, on the basis of symptom reporting, an extremely low SARS-CoV2 infection rate in IBD patients [14]. We observed that SARS-CoV2 IgG seroprevalence in IBD closely reflects values measured in background populations, whose prevalence was of 7% in Milan. This result is in line with an Italian nationwide study indicating SARS-CoV2 seroprevalence of 7.5% and 0.3% in Lombardy and in Sardinia, respectively [15], and of 0.9%

among blood donors in Germany [16]. Thus, our data confute the hypothesis that IBD patients under biological therapy may be protected from SARS-CoV2 infection. An explanation may reside in the sharing of risk factors for SARS-CoV2 infection between IBD patients and general population, i.e. a history of close contacts with a COVID-19 affected individual or a COVID-19 affected relative, the latter emerging as the sole independent predictor for SARS-CoV2 IgG seropositivity in our multivariate analysis. Consistently, a SARS-CoV2 seroprevalence of 41.7% has been reported in subjects with COVID-19 affected relatives in a Italian nationwide serological study [15].

IBD patients who tested positive with the serological test were confirmed by nasopharyngeal swab within 24 hours. Moreover, we did not report any false positive among almost 400 subjects tested, and an interference of circulating levels of biological therapies over serological test results could be excluded by the absence of positive tests in sera collected from IBD patients in the preCOVID era.

To note, biological therapy did not prevent the mounting of efficient humoral responses in infected IBD patients, including those treated with anti-integrin molecules, for which an interference with IgA humoral responses following administration of oral vaccines has been previously reported [17]. Confirming recent results [4, 5] , our data provide further reassurances over the benign course of COVID-19 in IBD patients under biological therapy.

The present study has several strengths, including a population cohort numerically relevant (almost 400 IBD patients treated with intravenous biologicals), and recruited from areas with different exposure to the SARS-CoV2 virus. Moreover, anti-SARS-COV2 seroprevalence was evaluated in highly homogeneous adult CD and UC patients. Additionally, different control populations were included in the study. Finally, the in-house ELISA test used has extremely high performance values (specificity 95.2% and sensitivity 97,64 for IgG, 99,8% and 71,4% for IgA) [9]. Conversely, a previous report [14] tested a mixed population of pediatric and adult IBD patients with a poorly perfoming not CE approved lateral flow rapid assay [18].

A possible limitation given by sparse-data bias [19] was nonetheless overcome by the association strengths between the identified risk variables and the magnitude of the OR.

In conclusion, our results demonstrate that seroprevalence of SARS-CoV2 in IBD patients treated with biological therapy reflects background values of the general population. Specific symptoms and contact history with SARS-CoV2-infected individuals strongly increase the likelihood of SARS-CoV2 seropositivity.

The data underlying this article are available in the article and in its online supplementary material.

References

1. Wu, F., et al., *A new coronavirus associated with human respiratory disease in China.* Nature, 2020. **579**(7798): p. 265-269.
2. Tay, M.Z., et al., *The trinity of COVID-19: immunity, inflammation and intervention.* Nat Rev Immunol, 2020. **20**(6): p. 363-374.
3. Rahier JF, M.F., Abreu C, Armuzzi A, Ben-Horin S, Chowers Y, et al., *Second European evidence-based consensus on the prevention, diagnosis and management of opportunistic infections in inflammatory bowel disease. .* J Crohns Colitis, 2014. **8**(6): p. 443–68.
4. Brenner, E.J., et al., *Corticosteroids, But Not TNF Antagonists, Are Associated With Adverse COVID-19 Outcomes in Patients With Inflammatory Bowel Diseases: Results From an International Registry.* Gastroenterology, 2020. **159**(2): p. 481-491 e3.
5. Bezzio C, S.S., Variola A, Allocca M, Massari A, Gerardi V, et al., *Outcomes of COVID-19 in 79 patients with IBD in Italy: an IG-IBD study.* Gut, 2020. **69**(7): p. 1213-1217.
6. Long, Q.X., et al., *Antibody responses to SARS-CoV-2 in patients with COVID-19.* Nat Med, 2020. **26**(6): p. 845-848.
7. Seydoux, E., et al., *Analysis of a SARS-CoV-2-Infected Individual Reveals Development of Potent Neutralizing Antibodies with Limited Somatic Mutation.* Immunity, 2020. **53**(1): p. 98-105 e5.
8. Caini, S., et al., *Meta-analysis of diagnostic performance of serological tests for SARS-CoV-2 antibodies up to 25 April 2020 and public health implications.* Euro Surveill, 2020. **25**(23).
9. Bruni M, C.V., Diaz-Basabe A, Lattanzi G, Mileti E, Monzani S, Pirovano L, Rizzelli F, Visintin C, et al., *Persistence of anti-SARS-CoV-2 antibodies in non-hospitalized COVID-19 convalescent health care workers* Journal of Clinical Medicine, 2020(9(10):E3188).
10. Stadlbauer, D., et al., *SARS-CoV-2 Seroconversion in Humans: A Detailed Protocol for a Serological Assay, Antigen Production, and Test Setup.* Curr Protoc Microbiol, 2020. **57**(1): p. e100.
11. Amanat, F., et al., *A serological assay to detect SARS-CoV-2 seroconversion in humans.* Nat Med, 2020.
12. Seow J, G.C., Merrick B, Acors S., *Longitudinal evaluation and decline of antibody responses in SARS-COV2 infection.* 2020.
13. Long, Q.X., et al., *Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections.* Nat Med, 2020.

14. Norsa, L., et al., *Asymptomatic SARS-CoV-2 infection in patients with inflammatory bowel disease under biologic treatment.* Gastroenterology, 2020.
15. LL, S., *Primi risultati dell'indagine di sieroprevalenza SARS-CoV-2. .* 2020.
16. Fisher B, K.C., Vollmer T, *SARS-CoV2-2 IgG seroprevalence in blood donors located in three different federal states, Germany, March to June 2020.* Eurosurveiallnce, 2020. **25**(28).
17. Wyant T, L.T., Sankoh S, Wang Y, Paolino J, Pasetti M, Feagan BG, Parikh A, *Vedolizumab affects antibody responses to immunisation selectively in the gastrointestinal tract: randomised controlled trial results* Gut, 2015. **64**(1): p. 77-83.
18. Cassaniti I, N.F., Giardina F, Salinaro F, Sachs M, Perlini S, et al., *Performance of VivaDiag COVID-19 IgM/IgG Rapid Test is inadequate for diagnosis of COVID-19 in acute patients referring to emergency room department.* Journal of Medical Virology, 2020. **10.1002**.
19. Greenland S, S.J., Finkle WD, *Problems due to small samples and sparse data in conditional logistic regression analysis.* Am J Epidemiol 2000. **1**(151(5)): p. 531-9.

Table 1: Demographical characteristics of the study populations

	IBD overall N=354	IBD Milano N=129	IBD Cagliari N=48	IBD Erlangen N=177	Healthy controls N=129
Age (median, IQR)	43 (31-57)	47 (33-62)	44 (33-62)	39 (29-55)	45 (35-60)
Sex (Male, %)	220 (62.5)	88 (68)	33 (69)	99 (57)	84 (65)
IBD subtype					
CD	216 (61)	82 (63.6)	19 (39.6)	115 (65)	
UC	132 (37.3)	47 (36.4)	29 (60.4)	56 (31.6)	
IBDU	6 (1.7)	-	-	6 (3.4)	
Biological therapy					
AntiTNF	197 (55.6)	76 (58.9)	32 (66.7)	89 (50.3)	
Vedolizumab	95 (26.8)	47 (36.4)	16 (33.3)	32 (18.1)	
Ustekinumab	61 (17.5)	6 (4.7)	--	56 (31.46)	

Table 2. Clinical and demographical variables associated with presence of SARS-CoV2 RBD IgG antibodies.

Clinical parameter	Overall n=177	IgG anti-RBD pos n=7	IgG anti-RBD neg n=170	P value (univariate)	P value (multivariate)
Male, n (%)	121 (68.4)	5 (71.4)	114 (68.9)	0.859	-
Age, median (IQR)	46 (33-62)	56 (29-65)	46 (33-62)	0.988	-
Current smoker, n (%)	32 (18.1)	1 (14.3)	31 (18.2)	0.79	-
Geographical origin, n (%)					
Milano	129 (72.9)	7 (100)	122 (71.8)	0.192	-
Cagliari	48 (27.1)	-	48 (28.2)		
Comorbidities, n (%)	47 (26.6)	1 (14.3)	46 (27.1)	0.453	-
Cardiopulmonary	30 (16.9)	-	30 (17.6)		
Hepatlc	3 (1.7)	-	3 (1.7)		
Renal	4 (2.6)	1 (14.3)	3 (1.7)		
Multiple	10 (5.6)	-	10 (5.9)		
IBD subtype				0.465	-
CD, n (%)	101 (57.1)	3 (43)	98 (57.6)		
L1	27 (15.3)	-	24 (14.1)		
L2	10 (5.6)	-	10 (5.9)		
L3	61 (34.5)	3(43)	56 (32.9)		
L4	2 (1.1)	-	2 (1.2)		
PERIANAL	37 (20.9)	1(14.3)	36 (21.2)		
UC, n (%)	76 (42.9)	4 (57)	72 (42.4)		
E1	1 (0.6)	-	1 (0.6)		
E2	33 (18.6)	2 (28.6)	31 (18.2)		
E3	42 (23.7)	2 (28.6)	40 (23.5)		
Age at diagnosis, median (IQR)	30 (21-47)	24 (17-49)	30 (21-47)	0.329	-
Disease length, median (IQR)	11 (5-18)	17 (6-32)	11 (5-18)	0.352	-
Current biological therapy, n (%)					
Anti-TNF	108 (61)	1 (14.3)	107 (62.9)	0.01*	0.299
Vedolizumab	63 (35.5)	4 (57)	59 (34.7)	0.224	-
OTHERS	6 (3.4)	2 (28.6)	4 (2.4)	0.02*	0.592
Concomitant immunosuppressants, n (%)	23 (13)	-	23 (13.5)	0.597	-
Concomitant steroids, n (%)	20 (11.3)	-	20 (11.8)	0.335	-
HBI score, median (IQR)	1 (1-3)	3 (3-6)	1 (1-3)	0.062	-
Mayo Score, median (IQR)	3 (1-5)	3(1-5)	3 (1-5)	0.884	-

Table 3. COVID-19-related symptoms and risk factors associated with presence of SARS-CoV2 RBD IgG antibodies

Clinical parameter	Overall n=177	IgG anti-RBD pos n=7	IgG/IgA anti-RBD neg n=170	P value (univariate)	P value (multivariate)
COVID-19-related symptom, n (%)					
Fever	35 (19.8)	6 (85.7)	29 (17.1)	<0.0001	0.152
Dyspnea	9 (5.1)	-	9 (5.3)	-	
Cough	54 (30.5)	3 (42.8)	51 (30)	0.438	
Arthromyalgia	61 (34.5)	4 (57.1)	57 (33.5)	0.235	
Fatigue	73 (41.2)	5 (71.4)	68 (40)	0.126	
Headache	45 (25.4)	4 (57.1)	41 (46.1)	0.07	
Conjunctivitis	13 (7.3)	1 (14.3)	12 (7.1)	0.419	
Vomiting	22 (12.4)	2 (28.6)	20 (11.7)	0.211	
Diarrhea	74 (41.8)	4 (57.1)	70 (41.2)	0.454	
Anosmia/ageusia	7 (4)	5 (71.4)	2 (1.2)	<0.0001	0.016
Contacts, n (%)					
COVID-19 positive close contact	21 (11.9)	4 (57.1)	17 (10)	0.004	
COVID-19 positive infected relative	14 (7.9)	5 (71.4)	9 (5.3)	<0.0001	0.027

Table 4. Clinical details and outcomes of SARS-CoV2 IgG positive individuals. AM=arthromyalgia

Patient #	Sex	Age	IBD subtype	Mayo score	HBI score	Biological therapy	Symptoms	IgG-T1	IgG-T2	Clinical outcome
1	M	31	CD	-	3	IFX	*Fever, Fatigue Anosmia. Ageusia*	1.172	0.242	Unchanged
2	M	59	CD	-	6	VDZ		0.501	0.602	
							Fever, AM, Fatigue, Cephalea, Vomiting, Diarrhea, Anosmia,Ageusia			Unchanged
3	M	29	UC	1	-	VDZ		0.592	0.398	
4	F	70	UC	3	-	VDZ		1.408	1.581	Unchanged
5	M	29	CD	-	3	UST	*Fever, Cough, AM, Cephalea, Fatigue, Diarrhea*	0.791	0.421	Relapse[§]
6	F	56	UC	2	-	UST		1.275	0.465	Unchanged
7	M	65	UC	6	-	VDZ	*Fever, Cough, AM, Fatigue, Vomiting, Anosmia, Ageusia*	1.802	1.55	Unchanged
8	M	32	CD	-	1	IFX		0.869	0,7036758 0.703	Unchanged
							Cephalea, Diarrhea			
							Fever, Cephalea, Anosmia, Ageusia			Unchanged
							Fever, Cough AM, Fatigue, Diarrhea, Anosmia, Ageusia			
							Fever			

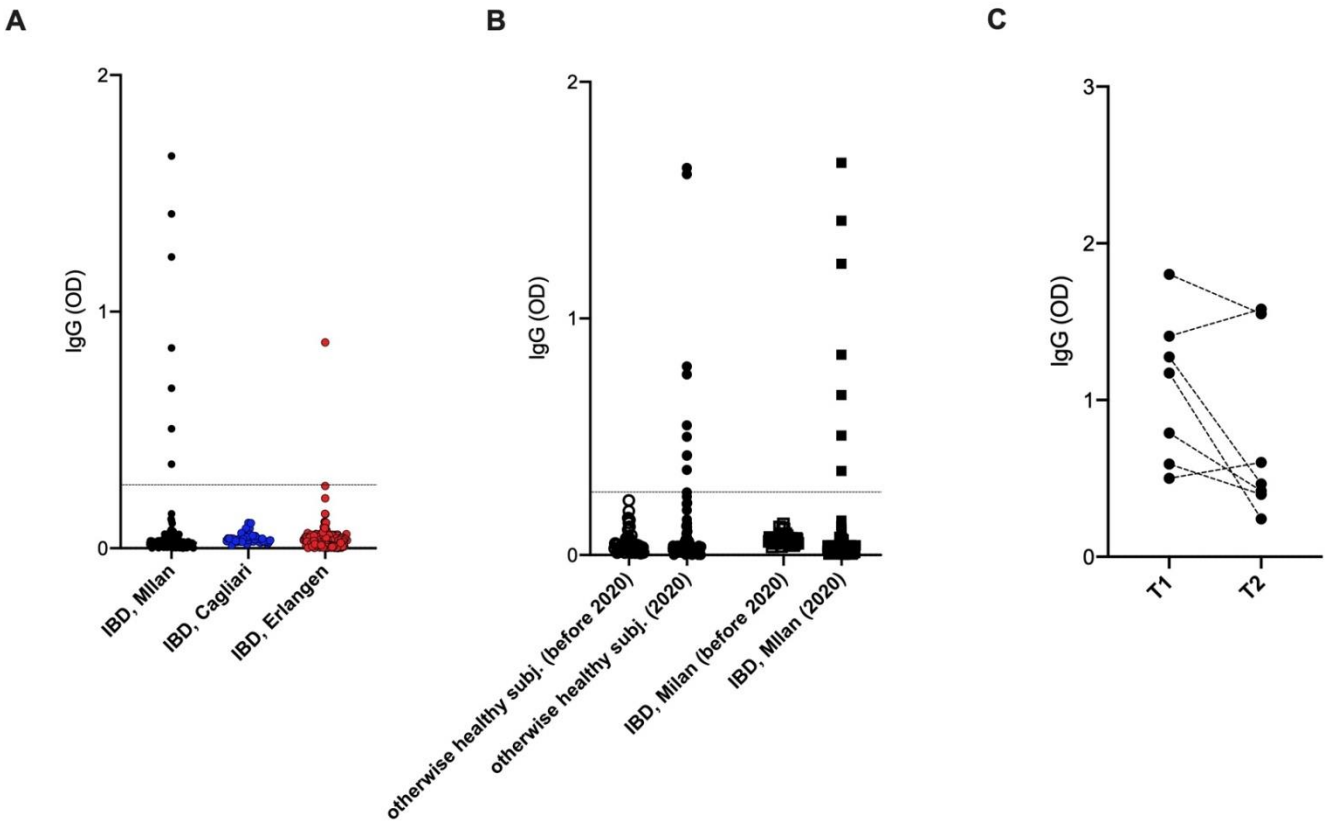

Figure 1: *Evaluation of IgG antibody titers in IBD patients.(* A) anti-RBD IgG antibodies in IBD patients enrolled in Milan (black dots), Cagliari (blue dots) and Erlangen (red dots). (B) anti-RBD IgG antibodies in sera of otherwise healthy subjects collected before 2020 (open circles) or between April and June 2020 (closed circles), in IBD patients collected before 2020 (open squares) or between April and June 2020 (closed squares). (C) Longitudinal variation of serum anti-RBD IgG antibodies in COVID-19+ IBD patients.