Study title	**Gastrointestinal symptoms of Sars-Cov-2 infection: a prospective multicenter study.**
Study code:	**GI-COVID19**
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INTRODUCTION

The coronavirus disease 2019 (COVID-19) initially developed at the beginning of December 2019 in Whuan, Hubei province of China has spread all over the world. Beside the most common symptoms at onset of illness including fever, fatigue, dry cough, myalgia and dyspnoea, there are less common symptoms such as headache abdominal pain, diarrhoea, nausea and vomiting. The proportion of patients complaining gastrointestinal symptoms is variable between 3,4% and 17,0%. Interestingly, SARS-CoV2 RNA has been reported to be detectable in 50% of patients' stool samples and in these patients around the 50% had diarrhoea.

SARS-CoV2 transmission has been reported to be through droplets. However, mounting evidence indicates that SARS-CoV2 has a tropism for the gastrointestinal tract and is excreted with faeces. Accordingly, a faecal-oral route of transmission of the virus has been recently postulated. Indeed, SARS-CoV2 binds to host ACE 2 receptors (ACE2) to entry into cells which are abundantly expressed by intestinal epithelial cells and regulate intestinal inflammation. Taken together, this evidence could provide a rational basis for the development of gastrointestinal symptoms reported by COVID19 infected patients.

The characterization of gastrointestinal symptoms in patients with COVID-19 would help to better define the clinical features of the infection, assess the predictive value of these symptoms as alarm features of the infection in COVID19 case without typical pneumonia symptoms.

The long-term consequences of COVID-19 infection on the gastrointestinal tract remain uninvestigated. It is extensively known that acute infection gastro-enteritis represents the strongest known risk factor for irritable bowel syndrome (IBS) development, a condition known as post-infection (PI) IBS (PI-IBS). Acute infectious gastro-enteritis is defined as a positive stool culture, a validated molecular biology analysis, or by the presence of 2 symptoms among fever, vomiting, or diarrhoea. Few studies evaluated the incidence of PI-IBS after viral infection compared with those reporting after bacterial infection, thus, it would be interesting to evaluate the development of PI-IBS after COVID19.

STUDY AIMS

Primary aim: to evaluate the prevalence and prognosis of gastrointestinal symptoms in patients admitted to hospital for COVID19 disease

Secondary aims

a) to evaluate long term consequences of COVID-19 on gastrointestinal symptoms
b) to evaluate long term consequences of COVID-19 on the development of PI IBS
c) to evaluate long term consequences of COVID-19 on the development of PI dyspepsia
d) to assess the clinical and laboratory predictors (risk factors) of PI gastrointestinal symptom development

STUDY DESIGN

This is an observational prospective multicenter international study that will include all inpatients Units from different countries. The estimated duration of the study will be 1 year. Expected starting date will be 17th April 2020.

The study cohort will be composed by consecutively enrolled COVID19 confirmed inpatients (case group) and COVID19 negative patients hospitalized for other reasons (controls group) referred to the participants centres from April 17th 2020. From the beginning of the study (first patient enrolled in the centre), the enrolment will last for 1 month.

Inclusion criteria:

- Signed informed consent
- Age ≥18 years and ≤85 years
- Consecutive COVID19 confirmed inpatients (COVID +ve)
- Consecutive hospitalized COVID19 negative patients (COVID –ve)
- Ability to conform to study protocol

Exclusion criteria:

- Patients under mechanical ventilation.
- Patients unable to report required data.
- Current diagnosis of cancer

Confirmed COVID19 cases

The definitions of COVID19 state are according to the WHO document released in March 2020.

A person with laboratory confirmation of COVID-19 infection, irrespective of clinical signs and symptoms. Patients categorized as suspect or probable COVID19 cases, as defined below, will be excluded from enrolment.

- Suspect case: A patient with acute respiratory illness (fever and at least one sign/symptom of respiratory disease, e.g., cough, shortness of breath), AND a history of travel to or residence in a location reporting community transmission of COVID-19 disease during the 14 days prior to symptom onset OR a patient with any acute respiratory illness AND having been in contact with a confirmed or probable COVID-19 case (see definition of contact) in the last 14 days prior to symptom onset OR a patient with severe acute respiratory illness (fever and at least one sign/symptom of respiratory disease, e.g., cough, shortness of breath; AND requiring hospitalization) AND in the absence of an alternative diagnosis that fully explains the clinical presentation.

- Probable case: A suspect case for whom testing for the COVID-19 virus is inconclusive. Inconclusive being the result of the test reported by the laboratory OR a suspect case for whom testing could not be performed for any reason.

COVID19 negative patients (controls)

Consecutive COVID19 negative in-patients admitted for any clinical condition other than a primary gastrointestinal disease fulfilling the inclusion criteria.

PROCEDURES

All patients will be clinically evaluated at admission according to standard clinical practice. All data present in the inpatient medical record will be used for the study, in particular:

- Demographics and clinical parameters: age, sex, weight, height, body mass index measurement, physical activity, alcohol intake, country of residence, ethnicity will be recorded.
- Past medical history and chronic medication intake.
- Routine blood examination.
- Results of oro-pharyngeal and nasal swab for the diagnosis of COVID19 infection.
- Results of imaging examinations.
- Therapies administered during hospitalization.
- Patients will be asked for the presence of symptoms COVID19 related (fever, cough, dyspnoea, etc) and of gastrointestinal symptoms with a validated symptom questionnaire (Gastrointestinal Symptoms Rating Scale- GSRS).

Patient's follow up will start at discharge and will continue for 12 months.

After enrolment, all patients will be contacted telephonically at 1 month after discharge for reassessment of GSRS. Subsequently, patients will be contacted every 6 months for a total of 12 months. At telephonic evaluation at 6 and 12 months after discharge patients will undergo the GSRS questionnaire, the Rome IV Diagnostic Questionnaire for Functional Gastrointestinal Disorders in

Adults (R4DQ) and the hospital anxiety and depression scale (HADS) and thus the onset of PI-IBS will be recorded.

SAMPLE SIZE

Based on preliminary data suggesting a prevalence of gastrointestinal symptoms ranging from 3.4% to 17%, we plan to include about 1000 patients with COVID19. Since we aim to compare the presence of post-infective gastrointestinal symptoms of patients with and without COVID19, we plan to include about 1000 patients without COVID19.

DATA COLLECTION AND MANAGEMENT

Data flow

Data will be collected in custom e-CRFs on REDCap platform. System backups for data stored and records retention for the study data will be consistent with standard procedures.

Electronic Case Report Forms

eCRFs are to be completed using RedCAP system. Sites will receive training and a have access to a manual for appropriate eCRF completion. eCRFs will be submitted electronically and should be handled in accordance with instructions. All eCRFs should be completed by designated, trained site staff. eCRFs should be reviewed and electronically signed and dated by the investigator or a designee.

Safety of Electronic data

Data will be collected in an ad hoc electronic record storage. The creation and the management of electronic record storage will be evaluated preliminarily with institution information technology staff. Data will be collected in REDCap platform. Redcap platform is an online platform maintained in a server that is propriety of the institution. Access and data transfer from and to REDCap platform are managed under https protocol and 128-bit encryption wit SSL certificate (a secure connection with encrypted data transfer). The access at the site is allowed only for the investigators and require a user-specific password of at least 8 characters including number and special character. REDcap comes

with a system of user privileges, so only the account of the principal investigator has the authorization to explore the entire dataset. The sub-investigators and data manager have limited authorizations, and can see only data that they entered personally. REDCap provider and the principal investigator guarantee that creation and management of username and password are compliant with 'codice in materia di protezione dei dati personali, allegato B. Disciplinare tecnico in materia di misure minime di sicurezza – punti 1-11' law for protection of personal data from Garante della Privacy.

The access of REDCap platform is regulated by 'Codice in materia di protezione dei dati personali, allegato B. Disciplinare tecnico in materia di misure minime di sicurezza, punti 7, 12-15' law for protection of personal data from Garante della Privacy. Administrative procedures on applications (e.g. user creation and suspension, study configuration, query creation, data mining) are pertinence of the principal investigator and his information technology support team. REDCap platform, to manage identity of people involved in the study, generates a unique identification number associated at every eCRF of the subject, that allow the investigators to maintain association with name and surname of the subject locally.

Data collected on REDCap, even if they do not allow to associate data with a specific subject or to recognize the subject, are registered in double-key SSL encryption, and mantained with AES 128 encryption. As regulated by 'Codice in materia di protezione dei dati personali, allegato B. Disciplinare tecnico in materia di misure minime di sicurezza, punto 18' law for protection of personal data from Garante della Privacy, REDCap and the principal investigator guarantee the weekly backup of data in a password-protected storage.

Data will be accessed only by principal investigator and his support team (sub investigators), and they will have confidentiality obligation.

STATISTICAL ANALYSIS

All registered data will be reported in an excel database. For the primary aim of the study all laboratory test, demographics, past medical history, the presence of gastrointestinal symptoms at admission and at follow-up made 1 month after discharge will be reported with descriptive statistics. Parametric variables will be compared by Student T-test, while non-parametric variables will be analysed by Fischer, Chi-square or Mann-Whitney tests. The continuous variables will be reported as

median with interquartile range (IQR), while the categorical variables as numbers and percentages. Patients without COVID19 will be used as controls. For the secondary aim of the study, after follow-up visits, data of the Rome IV Diagnostic Questionnaire for Functional Gastrointestinal Disorders in Adults (R4DQ), of the HADS questionnaire and the onset of PI gastrointestinal symptoms will be reported. In addition, data recorded at admission and used for the primary aim of the study will be tested as predictors of PI gastrointestinal symptoms onset occurring within a specific time frame using the Logistic Regression or Cox regression method. Subsequently to confirm their independent predictive value, the variables with $p < 0.1$ will be studied in a multivariate logistic or Cox model; the comparison groups will be studied using the Student's t-test and the Chi-square test. Those with a p-value < 0.05 will be considered statistically significant associations. The incidence of events will be calculated using the Kaplan-Meier method and compared through the log-rank test. The above analyses will be performed using STATA13 or other statistical analysis software.