

AWARD NUMBER: W81XWH-19-1-0287

TITLE: Prebiotic Inulin to Limit Antimicrobial-Resistant Infections During Critical Illness: A Phase II Clinical Trial

PRINCIPAL INVESTIGATOR: Dr. Daniel Freedberg

CONTRACTING ORGANIZATION: Columbia University, New York, NY

REPORT DATE: July 2022

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release; Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE July 2022		2. REPORT TYPE Annual Report		3. DATES COVERED 01Jul2021-30Jun2022	
4. TITLE AND SUBTITLE Prebiotic Inulin to Limit Antimicrobial-Resistant Infections During Critical Illness: A Phase II Clinical Trial				5a. CONTRACT NUMBER W81XWH-19-1-0287	
				5b. GRANT NUMBER PR181960	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Daniel Freedberg, PI Elissa Lynch, Research RN E-Mail: def2004@cumc.columbia.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Trustees of Columbia University in the City of New York, 630 W 168th Street, Fl 4, New York, NY 10032-3725				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Antibiotic resistance is an emerging healthcare crisis and critically ill patients are among the hardest hit; over half of all infections in intensive care unit (ICU) patients are due to multi-drug resistant (MDR) bacteria, and systemic infection with these bacteria significantly increases risk of death. Active duty military personnel are particularly susceptible to acquisition of MDR organisms due to traumatic injury and because they work in regions where the prevalence of antimicrobial resistance is high. The normal commensal gut bacteria protect against MDR organism (MDRO) colonization, but these protective bacteria are typically lost in the ICU as a result of antibiotics and other interventions. The proposed research hypothesizes that treatments aimed at preserving the gut bacteria that ferment fiber into short-chain fatty acids (SCFAs) will limit the proliferation of antimicrobial resistance within the gut bacteria and ultimately prevent infections with MDROs. Specifically, we will test inulin, a prebiotic fiber made from root vegetables that is the preferred nutrient source for SCFA-producing bacteria. 90 ICU patients will be randomized 1:1:1 to receive placebo or one of two inulin doses for 7 days. The overall aims of the trial are to assess the safety and feasibility of inulin administration in the ICU patients, and its ability to limit the proliferation of antibiotic resistant MDROs.					
15. SUBJECT TERMS antimicrobial resistance, antibiotics, intensive care unit, critical illness, sepsis, prebiotics, inulin, fiber, microbiome, colonization, infection, infection control					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	224	19b. TELEPHONE NUMBER (include area code)

Table of contents

Table of contents	Page 3
1. Introduction	Page 4
2. Keywords	Page 4
3. Accomplishments	Page 5
4. Impact	Page 10
5. Changes/problems	Page 10
6. Products	Page 10
7. Participants and other collaborating organizations	Page 10
8. Special reporting requirements	Page 11
9. Appendices	Page 12

1. Introduction

Antibiotic resistance is an emerging healthcare crisis and critically ill patients are among the hardest hit; over half of all infections in intensive care unit (ICU) patients are due to multi-drug resistant (MDR) bacteria, and systemic infection with these bacteria significantly increases risk for death. Active duty military personnel are particularly susceptible to acquisition of MDR organisms due to traumatic injury and because they work in regions where the prevalence of antimicrobial resistance is high. The normal commensal gut bacteria protect against MDR organism (MDRO) colonization, but these protective bacteria are typically lost in the ICU as a result of antibiotics and other interventions. The proposed research hypothesizes that treatments aimed at preserving the gut bacteria that ferment fiber into short-chain fatty acids (SCFAs) will limit the proliferation of gut antimicrobial resistance and ultimately prevent infections with MDROs. Specifically, we will test inulin, a prebiotic fiber made from root vegetables that is the preferred nutrient source for SCFA-producing bacteria. 90 adult ICU patients with sepsis will be randomized 1:1:1 to receive placebo or one of two inulin doses for 7 days. The overall aims of the trial are to assess the safety and feasibility of inulin administration in the ICU patients, and its ability to limit the proliferation of antibiotic resistant MDROs.

2. Keywords

antimicrobial resistance, antibiotics, intensive care unit, critical illness, sepsis, prebiotics, inulin, fiber, microbiome, colonization, infection, infection control

3. Accomplishments: Year 3 Quarter 4 (annual report ending June 2022)

3.1 Major goals

The primary overall goal during this project quarter and project year (ending in funding month 36) was to continue enrolling patients. 62 patients have been enrolled to date (see enrollment table below). Detailed reporting of the activities towards these goals is below with our original statement of work (SOW) timeline shown. Enrollment has been slowed because of COVID-19, and we anticipate that we will request a no-cost extension during the final year of the award.

	Original SOW Timeline (months)	Accomplished ?
Major Task 1: Finalize Protocols and Approvals for the Study		
<i>Task goal: regulatory and safety compliance is finalized, study is in the "on deck" position</i>		
Obtain FDA approval of IND	Completed	Yes
Refine eligibility criteria, exclusion criteria, screening protocol	1-3	Yes
Finalize consent form & human subjects protocol	1-3	Yes
Obtain CUMC IRB approval of final study documents including study consent and HIPAA forms	1-3	Yes
Military 2nd level IRB review (ORP/HRPO)	1-6	Yes
Submit amendments, AEs and protocol deviations	As Needed	Yes
IRB report for continuing review	Annually	Yes
DSMO review and implementation of revisions prior to enrollment of first subject	1-3	Yes
Milestone Achieved: Local IRB approval at CUMC, data and safety review with revisions implemented, and HRPO approval for all protocols		
Major Task 2: Coordinate Study Staff for Clinical Trials		
<i>Task goal: concurrent with major task 1, hiring of necessary personnel to implement study, finalization of study protocols</i>		
Systems access and additional training for Coordinator(s)	1-6	Yes
Finalize study assessment protocols and implementation	1-6	Yes
Coordinate for space allocation for new staff	1-6	Yes
Coordinate for independent evaluators hiring and trainings	1-8	Yes
Milestone Achieved: Trained personnel and independent evaluators throughout the trial		
Major Task 3: Participant Recruitment, Therapy, Participant Evaluation		
<i>Task goal: Initiate Phase 2 Trial</i>		
Flow chart for all study steps, web data collection and database requirements	6	Yes
Finalize assessment measurements	1-4	Yes
First participant enrolled	7-12	Yes
Milestone Achieved: 1st participant consented, screened and enrolled, study begins		
N=10 subjects completed Day 7 assessment Triggers first DSMO/DSMB AE review	6-9	Yes

N=30 subjects completed Day 7 assessment	6-12	Yes
N=60 subjects completed Day 7 assessment	18-21	Yes
N=90 subjects completed Day 7 assessment	28-30	No
Milestone Achieved: enrollment for the trial is complete		
Complete 3-month telephone follow-up assessment	33	No
Complete 6-month telephone follow-up assessment	36	No
Milestone Achieved: report crude 6-month follow-up data		

3.2. Accomplishments under these goals

3.2.1. Major activities

Major study activities during the period ending June 30, 2022 are bulleted below.

Regulatory activities

- There were no significant regulatory changes made.

Personnel, hiring, and on-boarding

- There were no changes made to the key study personnel.

Study intervention and supplies

- There were no changes in the study intervention.
- Study supplies continue to be ordered as needed and include but are not limited to: simple cherry syrup, an anti-static weighing canoe, sample collection tubes and bags, distilled water, amber plastic bottles with caps, pipettes, Copan FLOQ swabs, and sterile foam tipped applicators. For the microbiome core lab, Anne-Catrin Uhlemann continues to obtain culture plates for *C. Difficile*, MRSA, VRE, and culture media for Gram negatives including ESBL agar as needed.

Study protocol and logistics.

- There were no major logistical changes.
- We continue to utilize an E-consent form is captured within the REDCap database and a PDF version is included in the appendices of this report. The IRB approved the E-Consent form and process on June 19, 2020.
- Elissa Lynch continues to meet regularly with the nursing staff on all active study units to discuss study logistics and answer any questions.

Protocol implementation

- COVID has been relatively quiet in the ICUs since the spring, and enrollment has accelerated.
- On June 29 2022, we enrolled patient 62 out of 90 who provided us with Day 3 samples (69% of target).
- Since the start of the study, 64 out of 231 (28%) patients or surrogates approached for the study have been enrolled; 2 patients were enrolled who did not provide Day 3 samples and will be excluded from the primary analyses.
- The following table cumulatively lists the patients screened for the study.

Patient 6	1/23/20	11	4	Day 14
Patient 7	2/4/20	1	2	Day 3
Patient 8	2/4/20	13	5	Day 30
Patient 9	2/28/20	12	5	Day 30
Patient 10	6/23/20	8	4	Day 14
Patient 11	7/29/20	14	5	Day 30
Patient 12	8/6/20	13	4	Day 14
Patient 13	8/26/20	14	4	Day 14
Patient 14	9/8/20	12	4	Day 14
Patient 15	9/8/20	13	4	Day 14
Patient 16	9/10/20	11	5	Day 30
Patient 17	9/14/20	13	4	Day 14
Patient 18	9/14/20	12	4	Day 14
Patient 19	9/22/20	2	2	Day 3
Patient 20	9/25/20	14	5	Day 30
Patient 21	10/12/20	6	4	Day 14
Patient 22	10/19/20	3	1	Day 0
Patient 23	10/22/20	14	5	Day 30
Patient 24	10/22/20	14	5	Day 30
Patient 25	11/6/20	13	3	Day 7
Patient 26	11/13/20	13	5	Day 30
Patient 27	11/13/20	12	3	Day 7
Patient 28	11/20/20	12	4	Day 14
Patient 29	1/21/21	14	3	Day 7
Patient 30	1/22/21	11	2	Day 3
Patient 31	2/9/21	11	2	Day 3
Patient 32	3/21/21	11	3	Day 7
Patient 33	3/24/21	5	3	Day 3
Patient 34	4/7/21	13	4	Day 14
Patient 35	4/21/21	11	4	Day 14
Patient 36	5/10/21	10	4	Day 14
Patient 37	5/11/21	14	4	Day 14
Patient 38	5/25/21	2	1	Day 0
Patient 39	5/27/21	14	5	Day 30
Patient 40	5/27/21	14	5	Day 30
Patient 41	6/16/21	5	2	Day 3
Patient 42	6/18/21	13	3	Day 7
Patient 43	6/21/21	13	5	Day 30
Patient 44	6/23/21	12	4	Day 14
Patient 45	6/25/21	14	5	Day 30
Patient 46	7/28/21	12	5	Day 30
Patient 47	8/11/21	14	3	Day 7
Patient 48	8/12/21	14	3	Day 7
Patient 49	8/17/21	14	4	Day 14
Patient 50	8/26/21	13	4	Day 14
Patient 51	9/24/21	13	5	Day 30
Patient 52	9/30/21	14	4	Day 14
Patient 53	10/5/21	11	5	Day 30
Patient 54	10/7/21	14	4	Day 14

Patient 55	10/29/21	5	2	Day 3
Patient 56	12/10/21	3	4	Day 14
Patient 57	2/15/22	13	4	Day 14
Patient 58	3/24/22	14	4	Day 14
Patient 59	3/30/22	9	4	Day 14
Patient 60	4/22/21	14	5	Day 30
Patient 61	4/25/22	6	2	Day 3
Patient 62	5/20/22	14	3	Day 7
Patient 63	5/9/22	9	4	Day 14
Patient 64	6/29/22	11	4	Day 14

Summary sample data is as follows:

Sample type	Number of samples
Rectal swabs	243
Oral swabs	243
Whole stools	109
Urine	144
Residual blood	236
Total samples	975

3.2.3. Significant results

Trial findings are anticipated in year 4 of the award.

3.2.4. Other achievements

Nothing to report.

3.3. Opportunities for training and professional development

Nothing to report.

3.4. Dissemination of results to communities of interest

Nothing to report.

3.5. Plans for the next reporting period

The new primary goal for the next reporting period (Y4Q1) is to continue enrolling. We would like to continue to enroll at a rate of 1 patient per week to complete enrollment by Feb 2023.

4. Impact

Nothing to report. It is anticipated that the main impact of the trial will be after the final results have been published and disseminated, towards the conclusion of the award.

5. Changes/problems

Nothing to report.

6. Products

It is anticipated that the main products of the trial will be after the trial has been completed, towards the conclusion of the award.

7. Participants and other collaborating organizations

7.1. Individuals who have worked on the project

In addition to the individuals listed below, the following individuals have contributed to the project at less than 1 annualized calendar month of effort: Zhezhen Jin (Co-I, 0.5 Cal Mos), David Chong (Co-I, 0.4 Cal Mos).

Name:	Daniel Freedberg
Project role:	PI
Researcher identifier:	ORCID 0000-0003-2023-2899
Nearest person-month worked:	2.4
Contribution to project:	Dr. Freedberg has overseen all of the research activities described in this report and in the original SOW, including all of the study set-up activities that have taken place during this quarter.
Funding support:	ACG Clinical Research Award

Name:	Julian Abrams
Project role:	Co-I
Researcher identifier:	ORCID 0000-0002-6021-9376
Nearest person-month worked:	1.8
Contribution to project:	Dr. Abrams meets with Dr. Freedberg weekly to strategize regarding the study, and helps with study conduct and oversight and with planning of analyses and assays.
Funding support:	R01 CA238433, U54 CA163004

Name:	Anne-Catrin Uhlemann
Project role:	Co-I
Researcher identifier:	ORCID 0000-0002-9798-4768
Nearest person-month worked:	1.2
Contribution to project:	Dr. Uhlemann, who heads Columbia's microbiome core facility, will oversee all of the microbial cultures and analyses described under this award.
Funding support:	R01 AI116939, R35 HL135800, U01 AI131339, R21 HL145319, R01 CA238433, U54 DK104309

Name:	Elissa Lynch
Project role:	Research Nurse
Researcher identifier:	None
Nearest person-month worked:	12
Contribution to project:	RN Lynch works full-time with Dr. Freedberg on the set-up and conduct of the study, gathering of samples and data, and arranging sample assays and analyses.
Funding support:	None.

7.2. Change in active other support for key personnel

Dr. Freedberg received a clinical research award from the American College of Gastroenterology related to irritable bowel syndrome after COVID-19.

7.3. Other organizations involved as partners

Nothing to report.

8. Special reporting requirements

The U.S. Army quad chart is included as the first item in the appendices.

9. Appendices

9.1	U.S. Army Medical Research Acquisition Activity Quad Chart
9.2	Current working version of the study protocol (v5)
9.3	Approved Columbia IRB #AAAS2576 (approval date 3/17/20)
9.4	Approved Columbia consent form (English)
9.5	Approved Columbia consent form (Spanish)
9.6	Approved Columbia E-consent form (REDCap PDF)
9.7	Case Report Forms (print version of a REDCap-based system)
9.8	Current c.v. of Dr. Freedberg (PI)
9.9	Peer-reviewed research published by key study personnel during this reporting period (April 2022-June 2022)
9.10	Columbia IRB Approval Letter

Appendix 9.1

Prebiotic inulin to limit antimicrobial-resistant infections

Contract W81XWH1910287
PR181960



PI: Daniel Freedberg

Org: Columbia University

Award Amount: 1,890,421.00

Study Aims

Aim 1: To determine the feasibility, tolerability, and safety of inulin in the ICU.

Primary outcome: Feasibility, assessed as enrollment rate, protocol adherence, and intervention fidelity.

Aim 2: To determine the impact of inulin on gut colonization with antibiotic-resistant pathogens.

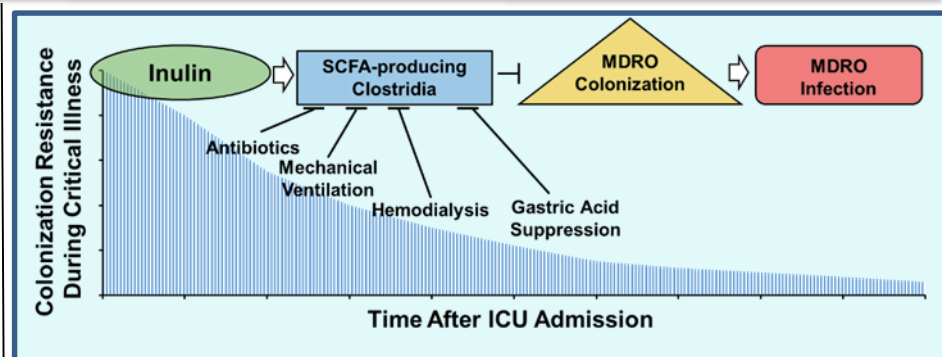
Primary outcome: gut antibiotic resistance colonization status, assessed based on culture from serial rectal swabs.

Aim 2A: To determine the impact of inulin on ICU-acquired antibiotic-resistant infections.

Primary outcome: composite of death and ICU-acquired infections.

Approach

To accomplish these Aims, we will conduct a Phase 2 double-blind clinical trial. ICU adults meeting sepsis criteria will be randomized to receive inulin 32 g/day, inulin 16 g/day, or placebo for 7 days and will donate samples and data on ICU days 0, 3, 7, 14, and 30.



Schematic depicting the scientific rationale for the proposed trial.

Gastrointestinal colonization resistance gradually declines during critical illness. Multidrug-resistant organisms (MDROs) colonize the gut and are disseminated into the ICU room, and ultimately inoculated into the patient to cause systemic infection. Inulin, a soluble prebiotic fiber, promotes the growth of short chain fatty acid (SCFA)-producing bacteria which decreases MDRO colonization and downstream infection. Data from healthy volunteers supports the beneficial effects of inulin while data from our own ICU demonstrates the importance of SCFA-producing bacteria in gastrointestinal colonization resistance.

Timeline and Cost

Activities (AY)	Y1	Y2	Y3	Y4	Y5
Aim 1: Feasibility, tolerability, safety					
Aim 2: Colonization					
Aim 2A: Infection					
Budget (k)	\$462	\$443	\$402	\$254	\$329

Goals/Milestones

Y1: AY19-20 Goal – Protocol finalization

☒ Implement protocol and complete enrollment of 30 patients

Y2: AY20-21 Goals – Ongoing enrollment

☒ Enroll 45 patients

Y3: AY21-22 Goal – Ongoing enrollment

☐ Enroll 60 patients

Y4: AY22-23 Goal – Ongoing enrollment and beginning assays

☐ Enroll 90 patients

☐ Lock data and begin assays

Y5: AY23-24 Goal – Assays and data analysis

☐ Finalize and lock trial primary results

☐ Data analysis and dissemination

Comments/Challenges/Issues/Concerns

- No-cost extension requested for loss of enrollment with COVID.

Updated: July 2021

Appendix 9.2

CLINICAL TRIAL PROTOCOL

Prebiotic Inulin to Limit Antimicrobial-Resistant Infections During Critical Illness: A Phase 2 Clinical Trial

Draft Date: February 24, 2020

Version 5

Columbia IRB: AAAS2576
FDA IND: 137359
DoD PRMRP: PR181960
DoD HRPO: Log Number E00684.1a
ClinicalTrials.gov: NCT03865706

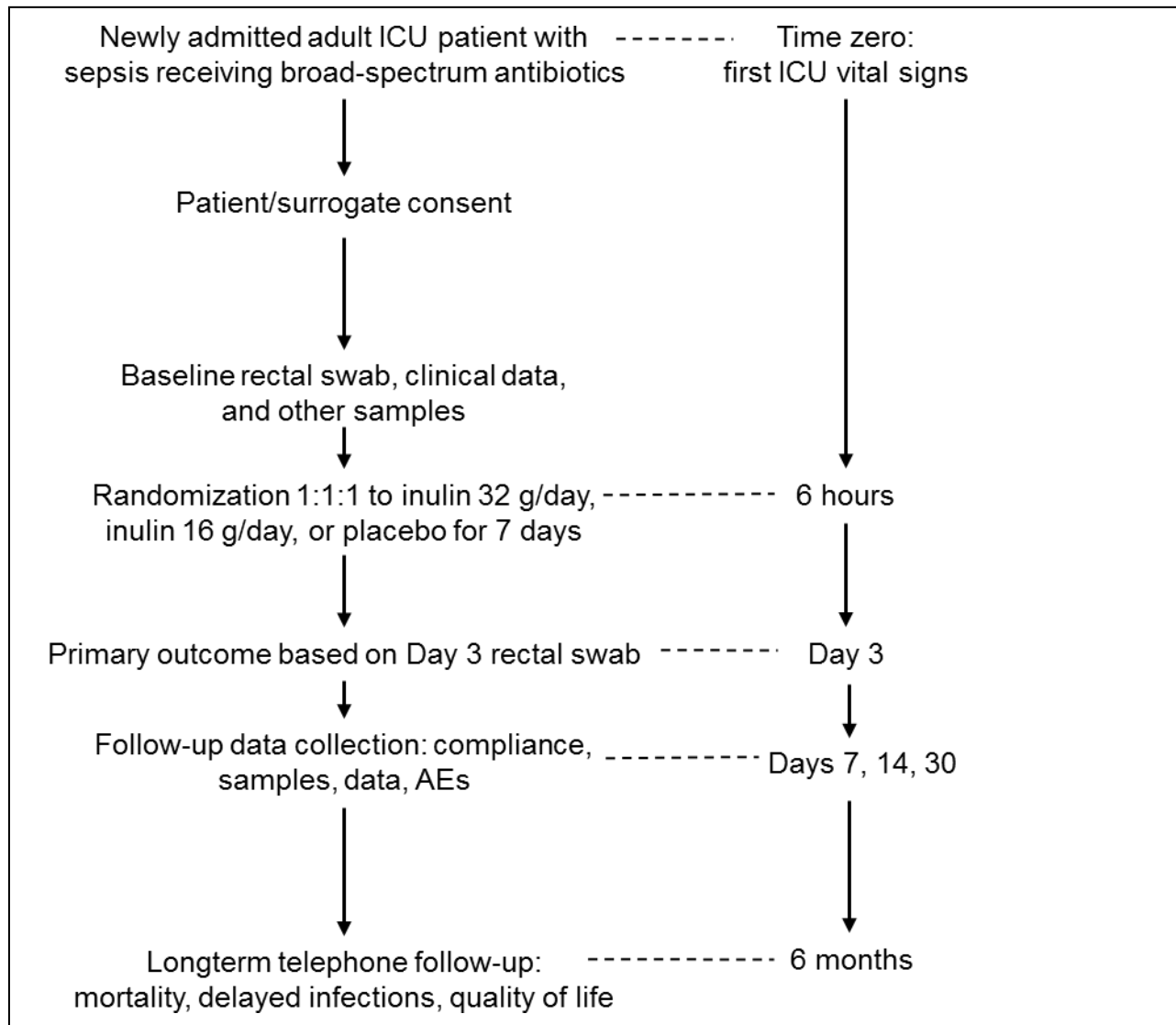
1. Table of Contents

1. Table of contents	Page 2
2. Protocol synopsis	Page 3
2.1. Study diagram	Page 4
3. Administrative information	Page 5
4. Funding	Page 5
5. Background and rationale	Page 5
6. Previous data	Page 6
7. Study objectives	Page 10
8. Study design	Page 11
9. Intervention	Page 11
10. Outcomes	Page 13
11. Study population	Page 16
12. Enrollment strategy	Page 17
13. Study assessments	Page 19
14. Data collection and management	Page 23
15. Safety and adverse events	Page 23
16. Termination and completion	Page 25
17. Statistical methods	Page 25
18. Publication policy	Page 27
Appendices	
Appendix A: References	Page 28
Appendix B: Abbreviations and terms	Page 36

2. Protocol synopsis

Title	Prebiotic Inulin to Limit Antimicrobial-Resistant Infections During Critical Illness: A Phase 2 Clinical Trial
Short title	Inulin in the ICU
Design	Randomized, double-blind, placebo-controlled trial in the ICU
Outcomes	<i>Primary outcome:</i> Change in relative abundance of SCFA producers through ICU Day 3 <i>Secondary outcome:</i> MDRO colonization status on ICU Day 3 <i>Additional outcomes:</i> 1. Feasibility 2. Rates of pre-specified AEs 3. Antibiotic resistance gene fraction on ICU Day 3 4. Culture-proven MDR infections through ICU Day 30 5. Fecal SCFA levels 6. Proportion of goal calories through ICU Days 3 and 7 7. Length of ICU stay 8. Length of hospital stay 9. Mortality through ICU Day 90 10. Self-assessed quality of life at 6 months 11. Times series data including ICU Day 7 outcomes
Intervention	1:1:1 allocation to inulin 32 g/day, inulin 16 g/day, or placebo dissolved in 250 cc sterile water and given in 2 daily doses for 7 days or until death/hospital discharge
Sample size	90 critically ill patients meeting Sepsis-3 criteria
Eligibility criteria	<i>Inclusion criteria:</i> 1. Medical ICU 2. Age ≥ 18 years old 3. Sepsis, defined according to the Sepsis-3 (2016) consensus as a known or suspected infection with a SOFA score of ≥ 2 points above baseline 4. Broad-spectrum antibiotics, received within the last 24 hours or ordered and pending administration 5. Able to complete enrollment within 24 hours of ICU admission for administration of the intervention within 48 hours of ICU admission <i>Exclusion criteria:</i> 1. Inability to receive oral or enteric fluids 2. Inulin allergy 3. Hyponatremia (serum sodium ≤ 128 mEq/L) 4. Immunosuppression, defined as CD4 count < 200 or absolute neutrophil count < 500 or reasonable expectation of either during the study intervention period 5. Surgery involving the intestinal lumen within 30 days or known intestinal strictures 6. Do Not Resuscitate (DNR) or Do Not Intubate (DNI) status, or “no escalation of care” orders 7. Lack capacity for consent and no appropriate Legally Authorized Representative (LAR).

2.1 Study diagram



3. Administrative information

Primary Investigator	Daniel E. Freedberg def2004@cumc.columbia.edu Columbia University Medical Center Division of Digestive and Liver Diseases 622 West 168 th Street, P&S Building 3-401, New York, NY 10032 Research office: (212) 342-3302, Cell: (857) 998-9370
Co-Investigators	<i>All at Columbia University Medical Center</i> Julian Abrams: ja660@cumc.columbia.edu David Chong: dhc5@cumc.columbia.edu Zhezhen Jin: zj7@columbia.edu Anne-Catrin Uhlemann: au2110@@cumc.columbia.edu
Data and Safety Monitoring Officer	Daniel Brodie hdb5@cumc.columbia.edu Columbia University Medical Center Vivian and Seymour Milstein Family Heart Center 173 Fort Washington Avenue, 4-005A, New York, NY 10032 Phone: (212)305-9817
Other Key Personnel	Howard Andrews, Columbia University Data Coordinating Center, hfa1@columbia.edu Research Nurse to be hired

4. Funding

This project is funded by a Department of Defense Peer-Reviewed Medical Research Program Clinical Trial Award (PR181960) recommended for funding on February 20, 2019.

4.1 Role of funding bodies

The study will be designed and conducted, and the results analyzed, presented, and published by the investigators independent of the funding bodies.

5. Background and rationale for study

5.1 Background

Multidrug resistant (MDR) bacterial infections are a leading cause of morbidity, death, and healthcare-associated costs.¹⁻³ Nowhere is this more important than in the intensive care unit (ICU), where over half of all infections are MDR⁴ and where sepsis from MDR bacteria confers 10-20% absolute increased mortality.⁵⁻⁷

This project hypothesizes that antibiotic resistance in the ICU is a problem of the human gastrointestinal microbiome, which is the critical reservoir for the bacteria and plasmids that encode antibiotic resistance genes.⁸⁻¹⁰ The normal gut microbiota prevents colonization and subsequent infection with MDR organisms through competition for scarce resources and other mechanisms.¹¹ In the setting of critical illness, normal colonization resistance is lost and there are no currently available therapies to restore it.¹²⁻¹⁵

Specific gut bacteria contribute to colonization resistance. ICU patients have low levels of butyrate and other short chain fatty acids (SCFAs) which are derived from dietary fiber by anaerobic bacteria primarily within the Clostridial Clusters IV and XIVa (e.g., *Faecalibacterium prausnitzii*). SCFAs moderate colonic inflammation,¹⁶ enlarge the pool of regulatory T cells,¹⁷ and

contribute to the thickness of the colonic mucus layer and pathogen resistance.¹⁸ During critical illness, levels of SCFA-producing bacteria and SCFAs are low, and these levels further decline with antibiotic treatment. Loss of SCFA-producing bacteria correlates with an increase in colonization with vancomycin-resistant *Enterococcus* and other antibiotic-resistant pathogens. We believe that preservation of SCFA-producing bacteria during critical illness is of major importance and can prevent the proliferation of colonizing organisms with antimicrobial resistance.

The prebiotic fiber **inulin** is a vegetable-derived polysaccharide that restores gastrointestinal colonization resistance against MDROs. SCFAs cannot be easily given to humans due to oral intolerance and rapid metabolism,^{19,20} but inulin promotes growth of SCFA producers to raise SCFA levels. In animals, inulin-type fibers improve survival after pathogen challenge^{21,22} even in the face of antibiotics.^{18,23-25} In a randomized trial of 30 outpatients, inulin increased fecal levels of SCFA producers by 35-90%, with a 3-fold decrease of serum markers of bacterial translocation (e.g., lipopolysaccharide) relative to placebo.²⁶ Inulin is safe in ambulatory patients²⁷⁻³⁵ with biologic effects seen at 16 g/day.²⁶ In the ICU, inulin-type fibers have been given up to 36 g/day without adverse events³⁶ and in our ICU cohort we have observed 30 g/day dietary fiber without intolerance. This trial will test whether inulin supplementation has similar effects in the ICU as it does in animal models and as it appears to in healthy volunteers: will it result in higher levels of SCFA-producing Clostridia, decreased gastrointestinal antibiotic resistance and lower risk for MDR infections?

5.2 Trial purpose and design

The purpose of this Phase 2 trial is to determine the feasibility and safety of inulin for the prevention of antibiotic resistant infections or colonization in critically ill patients. We will perform a double-blind, randomized controlled trial of inulin in the ICU with three arms (placebo, inulin 8 g twice daily, and inulin 16 g twice daily). Subjects will be treated with inulin or placebo for 7 days with bedside follow-up for 30 days or hospital discharge and with long-term telephone follow-up for 6 months.

6. Previous data

Our observational ICU data supports the importance of SCFA producers in the ICU. In animals, inulin protects against sepsis. Inulin increases SCFA producers in ambulatory studies and inulin-like fibers are safe in the ICU setting.

6.1 Evidence supporting the importance of SCFA producers in the ICU

We have assembled two large prospective cohorts now totaling over 500 ICU patients who have contributed rectal swabs and other data.³⁷⁻³⁹ Results from these cohorts confirm the importance of SCFA producers in protecting against pathogen colonization and infection in the ICU.

- **Cohort #1.**⁴⁰ A single rectal swab was performed on medical ICU admission for consecutive patients. Swabs were cultured for VRE and residual DNA was extracted for 16S rRNA gene sequencing; patients were then followed for up to 30 days for death or culture-proven infections. In 9 months, **301 patients** were enrolled of whom 91 (30%) were VRE colonized. Thirty-day mortality was 25% and an overlapping 21% patients developed culture-proven MDR infections.

Cohort #1 demonstrated that the bacteria causing infections in the ICU are present as gut colonizers at the time of admission. We tested whether colonizing MDROs predicted subsequent death or culture-proven infection. Presence of the colonizing organism at ICU admission was almost always associated with subsequent organism-specific infection, even after adjusting for

acute severity illness as captured by the Simplified Acute Physiology Score-3 (SAPS-3)^{41,42} and other relevant clinical variables. This was true for VRE (aHR 5.85, 95% CI 2.25-15.2, 16 infections), *C. difficile* (aHR 19.0, 95% CI 4.40-81.8, 9 infections), *Pseudomonas* (aHR 2.37, 95% CI 1.04-5.43, 28 infections), and *Klebsiella spp.* (aHR 3.81, 95% CI 1.10-13.2, 28 infections). To interrogate the process by which MDRO colonization may lead to infection, we also examined MDRO domination defined as $\geq 30\%$ 16S reads corresponding to a potential pathogen.^{43,44} Because this cohort contained VRE culture data, we were able to test whether patients who had *Enterococcus* domination were in fact colonized by VRE. *Enterococcus* domination (15% of the cohort) and VRE colonization (30%) both

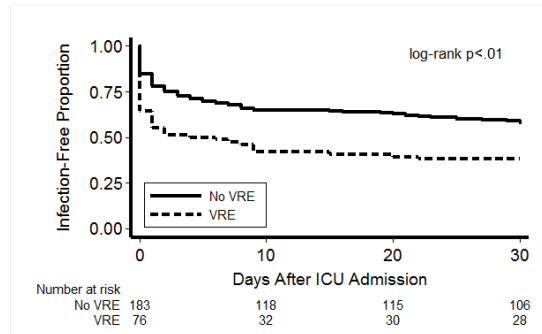


Figure 1. VRE colonization/domination was associated with death or culture-proven infection in the ICU. VRE colonization or domination was assessed at the time of ICU admission and patients were followed for all-cause culture-proven infection.

predicted *all-cause* infection (**Figure 1**). When 30-day mortality was examined as a stand-alone outcome, VRE colonization/domination predicted 40% increased mortality, after adjusting for SAPS-3 score and other factors.

Next, we sought to determine the relationship between SCFA producers and VRE based on culture at the time of ICU admission. Only VRE was cultured within this cohort, so we did not have the ability to assess colonization with other MDROs using culture (e.g., Gram negative bacteria). Based on the 16S sequencing results, SCFA producers were classified as absent (<.01% relative abundance), low (.01% to 1%), or high (>1%) after Haak *et al.*⁴⁵ High levels of SCFA producers were associated with lower prevalence of VRE colonization based on culture: 40% when SCFA producers were absent, 27% when they were low, and 22% when they were high ($p < .01$). Results were similar for *Enterococcus* domination.

- **Cohort #2.**^{37,39} This cohort was designed to examine *dynamic* changes in the ICU microbiome, focusing on SCFA producers and MDROs. Swabs were performed in duplicate at ICU admission and 72 hours later (± 4 hours). Swabs were then (1) cultured for MRSA, VRE, and MDR Gram negatives and (2) 16S sequenced. In 3 years, **230 patients** out of a target of 300 have been enrolled with 126 sequenced to date.

Cohort #2 demonstrated that SCFA-producing bacteria associate with preserved colonization resistance. Within cohort #2, we first performed an unbiased analysis using LEfSe⁴⁶ to ask which bacterial taxa changed most within individuals, comparing their admission and Day 3 samples. 14 taxa were identified as having significantly changed at a false-discovery adjusted $p < .05$. Ten taxa decreased over these first 72 hours of which 6 were SCFA producers. These changes were substantial in magnitude (e.g., 8-fold for *F. prausnitzii*).

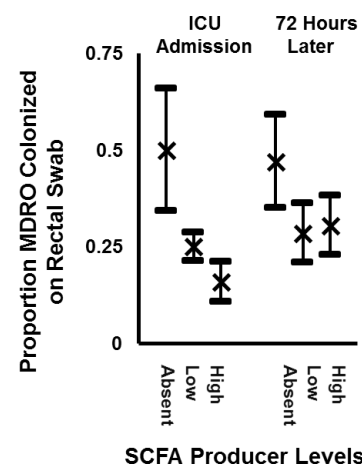


Figure 2. Higher levels of SCFA-producing bacteria associated with lower colonizing MDROs, both at the time of ICU admission and 72 hours later. Lines are 95% confidence estimates.

We wanted to know how SCFA producers associated with MDRO colonization. SCFA producers were again classified as absent, low, or high based on sequencing. MDROs were classified as present vs absent based on culture results. At both ICU admission and 72 hours later, there was a stepwise relationship

where higher SCFA producers was associated with decreased MDRO colonization (**Figure 2**, previous page). Comparing high to absent SCFA levels, the difference in rates of MDRO colonization was over 25%.

Last, we sought to retrospectively assess the impact of dietary fiber on SCFA producers and MDROs. Observed fiber intake was low overall with a mean of 13.4 g over 72 hours, but some patients did receive large amounts of fiber (maximum 30 g/24 hours). There was no intolerance of fiber based on documentation of abdominal distension, diarrhea, or nausea/vomiting. Fiber intake was classified into tertiles and SCFA producers were examined as a continuous measure based on 16S sequencing. At ICU admission, baseline relative abundance of SCFA producers was similar across fiber intake groups. After 72 hours, higher fiber intake was associated with higher levels of SCFA producers (**Figure 3**). Higher fiber was also associated with less *Enterococcus* (median abundance .08% high

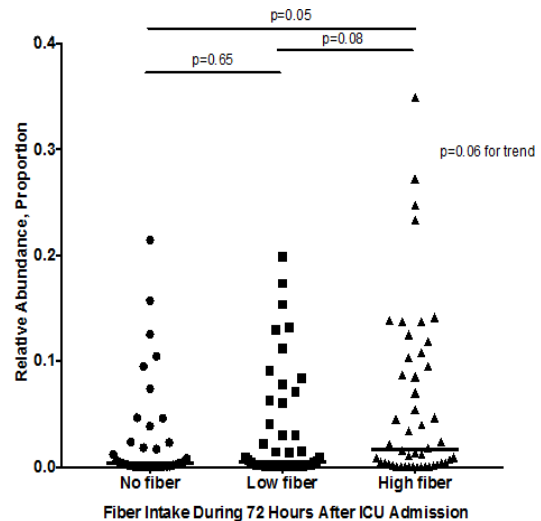


Figure 3. Higher fiber intake associated with higher levels of SCFA producers in the ICU. Fiber intake was classified into tertiles, with relative abundance of SCFA producers classified based on 16S sequencing.

fiber vs .35% no fiber, $p=.03$). This data is observational so there were baseline patient differences between those who did and did not receive high dietary fiber. To account for this, we built a multivariable model adjusted for acute severity of illness, receipt of antibiotics, and total caloric intake. In this model, there was a 0.30% median increase in SCFA producers after 72 hours per additional 10g of fiber intake ($p<0.01$). This suggests that dietary fiber has the potential to impact levels of SCFA producers, even during critical illness and with receipt of broad-spectrum antibiotics (78% of the cohort).

6.2 Inulin in animal models

Animal models show protective effects of inulin or similar compounds, which appear to be mediated by an increase in SCFA producers.^{47,48} Importantly, these studies demonstrate that inulin is likely to be effective even when given concurrently with antibiotics. Animal models include cecal ligation and puncture (CLP),²¹ lipopolysaccharide (LPS) injection,²² challenge with *Citrobacter rodentium*¹⁸ or pathogenic *Escherichia coli* strains,⁴⁷ and *Salmonella typhimurium* challenge with²³ or without antibiotics.^{24,25} All models have their strengths and weaknesses, so it is remarkable that results align well across them. Inulin and similar fructo-oligosaccharides (FOS) seem to confer protective effects through multiple mechanisms all of which likely depend on SCFA fermenters. First, they maintain the protective colonic mucus layer. Inulin fermenters activate carbohydrate metabolism pathways and switch fuel utilization from dietary fiber to colonic mucus when inulin-like fibers are unavailable, causing thinning of the colonic mucus layer.^{18,23} Second, the bacteria nourished by inulin have direct antagonistic effects on pathogens²⁵ by making protonated SCFAs which diffuse across Gram negative cell walls to delay pathogen growth.²⁴ Luminal acidification by SCFAs also drives broad microbiome shifts towards relatively pH-tolerant, Gram positive obligate anaerobes.^{49,50} Third, SCFA producers induce host immunity. When SCFA-producing anaerobe levels increase during sepsis, there is induction of T regulatory cells (Tregs), reduction in activated dendritic cells, and downstream decreased DNA binding of NF- κ B for an overall reduction in pro-inflammatory cytokines and reduced T cell anergy.²² In these animal studies, the protective effect is large, with dramatic differences in outcomes including weight loss, colitis severity, and mortality.

6.3. Inulin in the ambulatory setting

Inulin has been studied in over 400 ambulatory patients where it has shown excellent safety and tolerability.²⁷⁻³⁵ No serious adverse events have been reported in trials using doses up to 34 g/day inulin⁵¹ or 55 g/day mixed fiber,¹⁶ with self-resolving bloating the main side effect. Three trials specifically examined outcomes related to the ability of inulin to prevent colonization or infection. First, Dewulf *et al.* randomized 30 patients to inulin 16 g/day vs placebo; the inulin group had a doubling of *F. prausnitzii* and other SCFA-producers and concurrent decreases in serum CRP and LPS concentration.²⁶ Second, Lecerf *et al.* randomized 60 volunteers 1:1:1 to placebo, a combination xylo-oligosaccharide (XOS), or inulin-XOS.⁵² The inulin group had the highest post-treatment SCFA levels and butyrate levels, the lowest circulating LPS, and decreased gene expression for the pro-inflammatory cytokines IL-1 β , TNF α , and IL-12. Third, Miguez *et al.* found that an inulin-like fructo-oligosaccharides (FOS) preserved gut microbiome diversity after broad-spectrum antibiotics, mitigated loss of SCFA producers, and increased butyrate levels 3-fold.⁵³ **Table 1** lists selected studies of inulin in the ambulatory setting, focusing on those with results most relevant to this project.

Table 1. Selected ambulatory trials testing inulin alone or in combination

Author	Subjects	Intervention	Effect	AEs
Kruse ⁵¹	8 healthy volunteers	Inulin 34 g/day	Increased <i>Bifidobacteria</i> , unchanged SCFA levels	Self-resolving bloating and flatulence.
Dewulf ²⁶	30 obese women	inulin 16 g/day (50% FOS enriched) x 3 months	Increased <i>Bifidobacteria</i> and <i>F. prausnitzii</i> and decreased LPS.	Self-resolving bloating and flatulence.
Lecerf ⁵²	60 healthy volunteers	inulin 3g/day plus 1g/day XOS	Increased fecal SCFAs, decreased LPS, attenuated LPS-induced serum changes in IL-1b and IL-13.	Self-resolving bloating and flatulence, small decrease in general well-being.
Azpiroz ²⁷	36 with IBS	inulin 8g/day x 4 weeks	Increased <i>Bifidobacteria</i> .	None reported.
Canfora ²⁹	44 obese pre-diabetic adults	inulin 15g/day x 12 weeks	Increased <i>Bifidobacteria</i> .	None reported.
Garcia-Peris ³⁰	31 adult women with gynecological cancer	inulin 12g/day x 4 weeks (50% FOS enriched)	Increased in <i>Lactobacillus</i> and <i>Bifidobacteria</i> .	Self-resolving bloating.
Holscher ³¹	29 healthy adults	inulin 7.5g/day x 21 days (agave-derived)	Increased <i>Bifidobacteria</i> .	None reported.
Petry ³²	32 obese female adults	inulin 20 g/day x 4 weeks	Increased <i>Bifidobacteria</i> .	None reported.
Rahat-Rozenbloom ⁵⁴	25 healthy obese or lean adults	inulin 24 g/day x 3 days	Increased serum SCFAs.	None reported.
Ramirez-Farias ³³	12 healthy adults	inulin 10g/day x 16 days	Increased <i>Bifidobacteria</i> and <i>F. prausnitzii</i> , no change in fecal SCFAs.	None reported.
Vandeputte ³⁵	42 healthy adults	inulin 12g/day x 4 weeks	Increased <i>Bifidobacteria</i> .	None reported.

6.4 Inulin-like fibers in the ICU

Inulin has not been studied in the ICU but data can be extrapolated from ICU trials of similar fructo-oligosaccharides (FOS). To date, there are no reports of serious adverse effects or major intolerance with over 1,000 patients studied.⁵⁵ Doses have been used up to 36 g/day for 5 weeks.³⁶ This emerging safety data is robust enough that consideration of supplemental fiber has

been incorporated into relevant ICU nutrition guidelines.⁵⁶ Data on the effects of inulin-like FOS on infections in the ICU is scarce but promising. Three double-blind, randomized ICU trials tested a commercial synbiotic containing *Lactobacillus spp.* and mixed soluble prebiotic fiber including some inulin (up to 20 g/day total).⁵⁷⁻⁵⁹ In these studies, the overall rates of ICU-acquired infections were 16-27% lower in the group receiving prebiotic fiber, although MDR infections were not separately examined. In an open-label trial of mixed fiber up to 24 g/day in 30 critically ill adults, the median hospital length of stay was 5 days shorter in the fiber group and the overall rate of complications, including feeding-related complications was lower.⁶⁰ This provides important reassurance given the concerns occasionally raised regarding prebiotics in patients with altered small bowel or colonic motility.^{61,62} **Table 2** lists selected studies of fiber supplementation in the ICU, focusing on those with results most relevant to this project

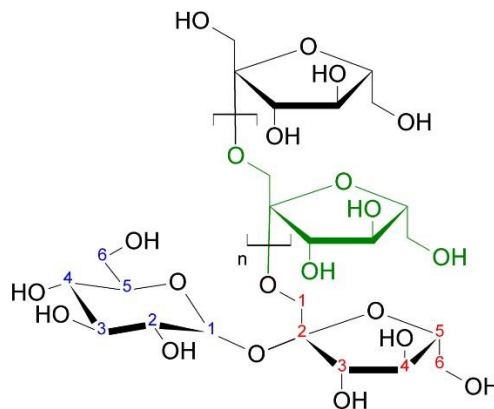


Table 2. Selected ICU-based trials testing inulin or similar fibers, ordered by decreasing amounts of fiber.

Author	Subjects	Intervention	Effect	AEs
O'Keefe ³⁶	13 ICU patients (most with pancreatitis)	32 g/day wheat dextrin up to 5 weeks	Higher fecal SCFA producers and SCFAs.	None reported.
Karakan ⁶⁰	30 ICU patients with pancreatitis	24 g/day mixed soluble and insoluble fiber (open label)	Shorter hospital stays and lower complications.	Self-resolving bloating.
Knight ⁵⁸	259 ICU patients on ventilation	20 g/day mixed fiber synbiotic including 5 g/day inulin	Trend towards lower rates of VAP.	None reported.
Kotzampassi ⁵⁷	65 ICU patients with SIRS	20 g/day mixed fiber synbiotic including 5 g/day inulin	Fewer infections and less diarrhea.	None reported.
Majid ⁶³	22 adults in the ICU	inulin 7g/day	No difference in <i>Bifidobacteria</i> and lower <i>F. prausnitzii</i> .	None reported.
Spapen ⁶⁴	25 ICU patients with septic shock	22 g/L guar fiber enteral feeding, dose individualized	Less diarrhea.	None reported.
Spindler-Vesel ⁵⁹	113 ICU trauma patients	17 g/day average mixed soluble fiber-synbiotic	Fewer infections.	Higher gastric residuals.

6.5 Summary of previous experience with inulin

Our prospective ICU cohort data provides real-world data supporting the hypothesis that inulin, by nourishing SCFA producers, will lead to decreased pathogen colonization and infection in the ICU. Animal studies show that inulin confers protection across various pathogen challenge models and sepsis models including LPS injection and cecal ligation-and-puncture. Ambulatory studies of inulin demonstrate safety and generally show increases in SCFA producers related to inulin. Few ICU studies have tested inulin, but there is ample ICU experience with inulin-like fibers that provides reassurance that high doses of fiber are safe even in the context of sepsis and hypotension in the ICU.

7. Study objectives

The short-term goal of the study is to test inulin for the prevention of MDRO colonization and infection in the intensive care unit. The long-term goal of this research is to develop antibiotic-free strategies that prevent or ameliorate ICU-acquired infections.

8. Study Design

This will be a double-blind, randomized, placebo-controlled trial with two doses.

9. Intervention

Block randomization (blocks of six) will be performed with 1:1:1 random computer allocation of subjects into the three study arms. In the two inulin arms, chicory root-derived inulin (Cargill Inc., Wayzata, MN) will be diluted in 250 ml of sterile water and given at 8 or 16 g twice daily (**Figure 4**). The placebo arm will receive the same volume of sterile water twice daily, with the intervention given for 7 days or until death or hospital discharge. Prior studies suggest that these doses are likely to be safe and sufficient to confer benefit,^{26,36} even in the setting of critical illness.^{57,58,63,65} Subjects, investigators, and the treating ICU team will be blinded to the intervention arm, with blinding managed by the CUMC Research Pharmacy which will provide labeled, de-identified, semi-opaque fluid bags containing inulin/placebo in accordance with the U.S. Code of Federal Regulations governing labelling

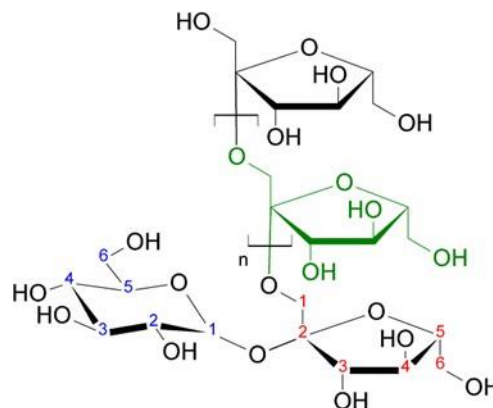


Figure 4. Chemical structure of inulin. Inulin is a naturally occurring polysaccharide derived from chicory root, agave, garlic, and other vegetable sources; it is a long chain carbohydrate of fructose polymers with $\beta(1,2)$ glycosidic linkages which are indigestible by human enzymes but fermented by colonic anaerobic bacteria into short-chain fatty acids (chemical formula: $C_6nH_{10n+2}O_{5n+1}$).

of new investigational drugs (21CFR 312.6). The intervention will be taken orally or given with enteric feeding by nursing staff overseen by the study team, with the first dose administered within 6 hours of ICU admission. The time course for the intervention and remaining study assessments is shown below in **Table 3**.

Table 3. Time course of intervention and main assessments during the study.

Type of Assessment	Inulin ----->			Monitoring ----->			
	Day 0	Day 3	Day 7	Day 14	Day 30	3 months	6 months
Rectal swab	X	X	X	X	X		
Whole stool	X	X	X	X	X		
Clinical data	X	X	X	X	X	X	X
Telephone follow-up						X	X

9.1. Storage, accountability, and dispensing

The study medication will be delivered to the CUMC Research Pharmacy as a powder via Swanson/Cargill protected from moisture and light and accessible only to authorized individuals. The medication will be stored at 20-25°C in powder form and reconstituted before dispensation. Medication labels will be blinded. The Research Pharmacy will maintain a record of the inventory and disposition of all medication and placebo. This receipt record will include from whom the medication was received, to whom it was distributed, the date, quantity, and lot number. The study medication has the following characteristics (**Table 4**).

Table 4: Study medication characteristics.

Product name:	Inulin powder
Manufacturer:	Cargill Inc., 15407 McGinty Rd W, Wayzata MN 55391-2365
Distributor:	Swanson Health, 4075 40 th Ave SW, Fargo, ND 58104-3912
Appearance:	White powder
Flavor:	Neutral to sweet
Bulk density:	0.6 g/mL
Solubility:	>100 g/L
Moisture:	3.41%
Dry stability:	4 years

9.2. Rationale for the selection of duration and dose.

9.2.1. Rationale for duration. Inulin will be given for 7 days with the primary outcomes adjudicated after 3 days. The Day 7 data will be used to test whether an effect can be extended or whether a delayed effect is present. In our cohort data, 84/126 (67%) new infections occurred within 3 days and 97/126 (77%) within 7 days.³⁸ This shows that any ICU-based intervention seeking to prevent infections must work quickly if it is to be effective. Meanwhile, our preliminary cohort data suggests that fiber can have an impact even within this short timeframe (Figure 3). A final assessment will be made on the day of hospital discharge in subjects who are to be discharged prior to Day 14.

9.2.2. Rationale for dose. In the ambulatory setting, inulin impacts SCFA producer levels at doses of 12 to 16 g/day, with biologic effects relevant for ICU patients (e.g., decreases in serum LPS and CRP).^{26,66} Approximately 16 g/day therefore represents the minimum dose at which an effect *might* be seen; this was selected for the low dose arm of the study. However, patients in these ambulatory studies were not critically ill and did not receive antibiotics. It is possible that inulin will have lesser effects in the ICU compared to the ambulatory setting, and logical to test higher doses. To select a maximum study dose, we referenced our own experience with dietary fiber in the prospective cohort and published tolerability data from ICU studies of inulin-like prebiotic fiber. In our prospective ICU cohort, 30 g/day was the maximum observed dose of dietary fiber, and was not associated with any intolerance. In published literature, O’Keefe *et al.* reported tolerance up to a maximum of 36 g/day wheat dextran in the ICU.³⁶ We therefore selected 32 g/day for the high dose study arm because it lies between these values and is exactly double a dose with proven biologic activity.

9.3. Regulatory

No subjects will be enrolled until after review and approval by the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of Research Protections (ORP), Human Research Protection Office (HRPO). The PI holds FDA IND 137359 (FDA date: September 4, 2018) for inulin for the prevention of MDRO colonization/infection in the ICU or other at-risk settings. Safety and adverse effects will be overseen by the PI in conjunction with Daniel Brodie, the Director of Medical Intensive Care Units at our institution, who will serve as the Data Safety Monitoring Officer (DSMO) and will convene an appropriate Data Safety Monitoring Board (DSMB) for the study. Before initiating research, the study will be registered on clinicaltrials.gov.

9.4. Documentation of administration of the intervention

The study team will work with the treating ICU team to ensure that the study drug is delivered in a timely manner. The time and dose of each medication administration will be documented at each study assessment by referring to the documentation of medication administration within the

electronic medical record (EMR). Patients will be enrolled with a goal of administering the intervention within 24 hours of ICU admission and it will be considered a protocol deviation if the intervention is administered more than 48 hours from ICU admission.

10. Outcomes

Primary and secondary outcomes focus on the comparison of baseline to Day 3 samples. This project will also gather samples at ICU Days 7, 14, and 30. These additional samples will be used to construct longitudinal time series data and test for differences between groups that may be slower to emerge (e.g., baseline vs Day 7 comparisons).

10.1. Primary outcome: Relative abundance of SCFA producers through ICU Day 3. The within-individual change in the relative abundance of SCFA producers will be determined by 16S rRNA gene sequencing of rectal swabs and compared between intervention groups. From extracted rectal swab DNA, polymerase chain reaction (PCR) will be performed on a Bio-Rad T100 Thermal Cycler (Bio-Rad, Hercules, CA) to amplify the V4 hypervariable region of the 16S ribosomal RNA gene with primers derived from Klindworth *et al.*⁶⁷ appended with overhang sequences for compatibility with Illumina index and sequencing adapters (Illumina, San Diego, CA). The relative abundance of SCFA producers levels will be determined as the sum total across the bacterial species known to ferment the SCFA butyrate and subtracted within each individual to obtain the change (Day 3 minus baseline).^{39,45,68-70}

10.2. Secondary outcome: MDRO colonization status on ICU Day 3. MDRO colonization status will be based on Day 3 rectal swab cultures, classified categorically and compared between intervention groups. MDROs will be defined as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), and MDR Gram negative bacteria⁷¹ (defined as Gram negatives with *in vitro* non-susceptibility for third-generation cephalosporins).⁷² Based on culture results, Day 3 MDRO colonization status will be classified categorically and compared between groups.

10.3. Additional outcomes:

10.3.1. *Feasibility.* Descriptive demographic and clinical data will be compared across study groups to assess adequacy of randomization. Summary statistics (count data) will be recorded for patients across all 3 intervention arms to assess feasibility as (1) the enrollment rate (enrolled)/(enrolled+refusals) over each 12 month period; (2) protocol adherence (proportion of randomized subjects who receive 1 or more doses of the allocated intervention); and (3) fidelity of the intervention (defined as the proportion of high/low inulin allocated vs received and also categorically as receipt of 90% or more allocated doses of inulin across groups). Feasibility goals are as stated in Aim 1.

10.3.2. *Rates of pre-specified AEs.* There will be active ascertainment of AEs in the following categories, which will be compared between intervention groups. This will be done through the end of the intervention period (ICU Day 7) and also through Day 30/discharge to assess for delayed AEs.

- Stool frequency, measured by subject or nursing report by the number of spontaneous stools over the 24 hours preceding each assessment;
- Stool consistency, measured by subject or nursing report as an ordinal variable (0-4) over the 24 hours preceding each assessment;

- Abdominal bloating, measured by subject or nursing report as an ordinal variable (0-3) over the 24 hours preceding each assessment.
- Electrolyte abnormalities, measured by examining for differences in median values, minimum/maximum values, and proportions of values outside the normal range. This will be done for glucose, calcium, magnesium, phosphate, potassium, and sodium.
- Nausea, vomiting, obstruction, and abdominal pain. These will be recorded as present or absent based on bedside assessment of the subject or based on the nursing impression.
- Culture-proven infections, which will be considered both an AE and an outcome (see 10.3.4).

10.3.3. *Antibiotic resistance gene fraction on ICU Day 3.* DNA will be extracted from rectal swabs (MoBio PowerFecal, Carlsbad, CA) and assessed using multiplex quantitative PCR (Qiagen Cat. No. 330261, Valencia, CA) for 87 common antibiotic resistance genes.⁷³ Within-individual differences in multiplex qPCR results will be compared from baseline to Day 3 both as summed changes and within relevant gene categories (e.g., β -lactamase resistance genes) and compared between intervention groups.

10.3.4. *Culture-proven MDR infections through ICU Day 30.* Antibiotic-resistant infections will be defined as those showing all of the following: (1) site-specific symptoms or signs of infection, (2) initiation of targeted antimicrobials by the treating ICU team, and (3) molecular diagnostics or cultures showing an MDRO as adapted from the CDC and NHSN⁷⁴ and previously used by our group (**Table 4**).⁴⁰ Rates of infections will be compared between intervention groups as a sum total, and separately for 3 distinct periods: the intervention period of Day 0 to 7, the Day 7 to 30 period, and the Day 30 to 6 month period.

Table 4. Criteria used to adjudicate culture-proven MDR infections for. Criteria based on CDC/NHSN guidelines.

Criteria	Operationalization	Example
1. Site-specific symptoms or signs of infection.	• Pulmonary: documentation of new or worsening cough <u>or</u> imaging consistent with an infection • Urinary: fever, dysuria, increased urinary frequency, flank pain, mental status changes, or hypotension <u>and</u> clinical exclusion of other causes for infection. • Bloodstream: clinical evidence of a bloodstream infection (fever, chills, or hypotension) without alternative sources for infection • Stool: documentation of new loose stools or frequent stools ≥ 3 per 24 hours • Wound: appropriate surgical site incision or wound with discharge, warmth, or erythema.	Cough and a chest X-ray showing new infiltrate.
2. Positive cultures or other diagnostics with an MDRO.	• Pulmonary: quantitative or semi-quantitative sputum or bronchoscopic cultures • Urinary: $\geq 10^5$ CFU/mL growth. • Bloodstream: growth of a recognized bloodstream pathogen from blood but not another site <u>or</u> growth of a NHSN commensal from blood but not another site.	Sputum culture growing $>10^5$ CFU/ml of <i>K. pneumoniae</i> resistant to ceftriaxone.

- Stool: positive PCR for the *C. difficile* toxin B gene (Cepheid or BioFire) or positive toxin B EIA
- Wound: growth of aseptically obtained organisms.

3. Initiation of targeted antibiotics within 24 hours.	Antibiotics showing non-susceptibility using standard CLSI breakpoints or appropriate empiric antibiotics initiated at the time of clinical suspicion/testing and continued for ≥ 3 days <u>or</u> initiated within 24 hours following the availability of diagnostic testing showing a culture-proven infection.	Initiation of ceftriaxone when the X-ray was ordered; later changed to meropenem.
---	--	---

10.3.5. *Fecal SCFA levels.* The SCFAs butyrate, acetate, and propionate will be measured from whole stools on a 6490 triple quadrupole mass spectrometer and compared between intervention groups using the first stool sample produced after the Day 3 assessment. Patients who fail to produce a stool from Day 3 to 7 will be excluded from this analysis.

10.3.6. *Proportion of goal calories through ICU Days 3 and 7.* Proportion of goal calories consumed will be defined by manually extracting data from electronic nursing flow sheets. Intake during the intervention be calculated by multiplying enteral or oral feeding caloric content by the flow rate or percentage of meal consumption. A patient-specific calorie target will be calculated by a registered clinical dietician using the Mifflin-St. Jeor equation and the Penn State 2003b and 2010 equations, taking into consideration age, body mass index, ventilation status, and comorbidities.⁷⁵ The proportion of goal calories consumed will then be calculated as observed nutritional intake divided by calorie target. Day 3 and Day 7 proportions will be compared between intervention groups.

10.3.7. *Length of ICU stay.* Length of ICU stay through Day 30 will be compared between treatment groups, with Fine and Grey methods used to adjust for death as a competing risk. Length of ICU stay will be measured from the first to the last set of recorded ICU vital signs.

10.3.8. *Length of hospital stay.* Similarly, length of hospital stay through Day 30 will be compared between treatment groups, again adjusting for death as a competing risk. Length of hospital stay will be measured from the first set of recorded ICU vital signs to the last set of hospital recorded vital signs.

10.3.9. *Mortality through ICU Day 90.* Death data will be extracted from the hospital EMR which immediately captures in-hospital death and receives monthly mortality updates from the National social security death index. Kaplan-Meier methods will be used to compared 30- and 90-day mortality between treatment groups.

10.3.10. *Self-assessed quality of life at 6 months.* Subjects or their caregivers will complete the EQ-5D-5L⁷⁶⁻⁷⁸ and Katz ADL questionnaires^{79,80} to test their quality of life at 3- and 6 months post ICU admission. Composite data will be compared between intervention groups.

10.3.11. *Time series data including Day 7 outcomes.* Time series data will be built and visualized for all relevant outcomes. We will explore the possibility of a delayed effect by testing for differences between groups from baseline to Day 7. If there is an observed effect, the Day 14 and Day 30 data will allow us to assess its durability.

11. Study population

11.1. Study setting

Patients will be enrolled from medical ICUs affiliated with Columbia University Medical Center. These ICUs include but are not limited to the following locations, all of which are in the general vicinity of Columbia University's medical campus:

ICU short designation	Full name	Hospital building, floor location	Street address
MICU-A	Medical ICU-A	Milstein, 4 th floor	177 Fort Washington Ave, NY, NY
MICU-B	Medical ICU-B	Milstein, 4 th floor	177 Fort Washington Ave, NY, NY
NICU	Neurological ICU	Milstein, 8 th floor	177 Fort Washington Ave, NY, NY
AICU	Allen ICU	Allen Pavilion, 2 nd floor	41 Broadway, NY, NY

The study will enroll 90 consecutive adult ICU patients who meet criteria for sepsis, are receiving appropriate antibiotics, and can complete a bedside assessment within 4 hours of ICU admission and receive the trial intervention within 6 hours of ICU admission. A replacement strategy will be used. If subjects are randomized but fail to receive the intervention or complete the Day 3 assessment, an additional subject will be enrolled to take their place with a final target enrollment of 30:30:30 patients who complete a minimum of 3 days of assessments. Specific inclusion and exclusion criteria are as follows:

11.2. Inclusion criteria

- Medical ICU. Subjects will be newly admitted to one of two CUMC medical ICUs during the study enrollment period;
- Age. Subjects will be eighteen or more years old at the time of ICU admission;
- Sepsis. Subjects will meet criteria for sepsis with life-threatening organ dysfunction caused by infection (Sepsis-3 third international consensus definition, 2016).⁸¹ According to this definition, life-threatening organ dysfunction is operationalized as an increase in the Sequential [Sepsis-Related] Organ Failure Assessment (SOFA)⁸² score of ≥ 2 points above baseline. Sepsis-3 criteria also require patients to have a known or suspected infection, defined as sampling of blood or any other body fluid for infection with concurrent administration of appropriate antibiotics (see Table 4);⁸¹
- Appropriate antibiotics. Subjects will receive appropriate antibiotics, which will be operationalized as (1) among subjects with positive culture data, the antibiotics within classes where the organism is susceptible based on standard Clinical and Laboratory Standards Institute (CLSI) cut-offs⁷²; or (2) among subjects receiving empiric antibiotics (i.e., with negative cultures), antibiotics within classes with significant anaerobic activity. This will include β -lactam/ β -lactamase inhibitor combination antibiotics, cephalosporins (generation 2 or greater), fluoroquinolones, lincosamides (clindamycin), metronidazole, and monobactams (a.g., meropenem). Vancomycin monotherapy is intentionally excluded. Antibiotics must be received within 24 hours before ICU admission or be actively ordered at the time of bedside screening;

- Prompt intervention. Subjects will complete study enrollment within 4 hours of ICU admission so that the intervention can be administered within 6 hours of ICU admission. The first set of recorded ICU vital signs will define the time of ICU admission.

11.3. Exclusion criteria

- Inability to receive oral or enteric fluids. Patients will be excluded if they are *nil per os* or are unable to take fluid by mouth or delivered via enteric nasal or oral tube because they would be unable to receive the study intervention;
- Inulin allergy. Patients will be excluded for safety reasons if they have a known chicory allergy. Serious chicory allergies are exceedingly rare although mild respiratory symptoms and IgE-mediated food allergies have been reported;⁸³
- Hyponatremia. Patients will be excluded for hyponatremia with serum sodium ≤ 128 mEq/L because the intervention will be delivered in 500 ml of free water daily. If subjects develop hyponatremia (serum sodium serum sodium 125-128 mEq/L) after enrollment and randomization which persists at the time the subject is due to receive the study intervention, the study team will confer with the treating ICU team regarding whether the intervention should be deferred or further concentrated in order to minimize free water. If subjects develop severe hyponatremia (serum sodium serum sodium ≤ 124 mEq/L) after enrollment and randomization, the intervention will be withheld until 2 consecutive serum sodium values are within normal limits;
- Immunosuppression. Patients will be excluded if they have immunosuppression, as CD4 count < 200 or absolute neutrophil count < 500 or reasonable expectation of either during the study intervention period. We believe that the inulin works by modifying the gut bacteria. Excluding patients who lack the ability to mount an immune response improves trial homogeneity;
- Surgery involving the intestinal lumen. Patients will be excluded for surgeries involving the intestinal lumen within 30 days. The most common side effects from inulin are subjective bloating and abdominal distension.^{26,28,30,52} Although this bloating appears to be mild and self-limited, patients with recent bowel surgeries may be at greatest risk for such symptoms and will therefore be excluded;
- DNR or DNI status. Such patients have limited treatment goals and will be excluded to minimize heterogeneity;
- No LAR. Patients will be excluded if they lack capacity for consent and have no appropriate Legally Authorized Representative (LAR) to act as surrogate decision-maker.

12. Enrollment strategy

Patients will be identified at the time of ICU bed allocation and screened for inclusion/exclusion criteria. After consultation with the treating ICU team, subjects who meet screening criteria will have the eligibility evaluation completed at the ICU bedside. This will be done after first obtaining agreement from the medical ICU attending of record that the patient/surrogate can be approached for the study. We anticipate that $> 50\%$ of patients will lack decision-making capacity and will accept consent from a surrogate in these situations, as previously described by our group.³⁷ Issues related to altered capacity, privacy and time for decision-making, consent of LARs, and ongoing consent are discussed in detail in the **Human Subjects and Recruitment and Safety**

Procedures section. We expect that patients will usually be initially enrolled with surrogate consent. Patients who are on study will then be reassessed for capacity at each interaction, with the final determination of capacity made by the treating ICU team. When subjects regain capacity, they will make their own determination of whether or not to continue in the study. If subjects do not regain capacity by the time of hospital discharge, surrogate consent will be accepted as final. This approach, which we have used before,³⁷⁻³⁹ preserves complete patient autonomy while recognizing that the target population for the study is unlikely to have decision-making capacity at the time of ICU admission.

Compliance statement regarding consent

This research will meet the requirements of **10 U.S. Code 980** for experimental human subjects research that “(1) the informed consent of the subject is obtained in advance; or (2) in the case of research intended to be beneficial to the subject, the informed consent may be obtained from a legal representative of the subject.”

Retention

Potential loss of patients after randomization will be from death/discharge prior to ICU Day 3, or withdrawal of consent (e.g., after initial surrogate consent). In our ongoing ICU cohort studies, there has been 11.3% mortality within 3 days and a 0% Day 3 discharge rate. Additional patients will be enrolled for patients who do not complete the Day 3 assessment due to death or discharge. Thus, we expect to enroll 102 patients to obtain 90 who are able to donate a Day 3 sample and therefore can included in the intent-to-treat analysis.

12.1. Randomization and blinding

Randomization and data entry will take place through a secure, web-based system managed by the CUMC Data Coordinating Center (DCC) headed by Howard Andrews. This password-protected system is based on the Research Electronic Data Capture (REDCap) platform. The Research Nurse will use paper clinical research forms (CRFs) to document the initial screening process. When this information is entered into REDCap, the system will automatically generate a subject identifier and randomization code using 1:1:1 randomization in randomly permuted blocks of 6. This randomization code will be electronically communicated to the research pharmacy along with a separate key which will allow them to identify the random assignment. The Columbia Research Pharmacy will prepare the study intervention in indistinguishable semi- opaque fluid bags. Although inulin is readily soluble at the study doses, the opacity of these bags will eliminate any possibility that a small amount of precipitant might reveal the study assignment to the ICU nurses who deliver the intervention. Five drops of non-caloric flavoring syrup (McKesson Supply, Yonkers, NY, Product #570727) will be placed into the reconstituted inulin/placebo to prevent any possibility of accidental unblinding based on taste in patients who are alert. The patient, treating ICU team including the ICU nurse, and study team will remain blinded to each assignment until the completion of the trial, with the DCC preparing unblinded safety reports for the DSMO at pre-specified intervals.

12.1.1. Clinical need for unblinding

It seems unlikely that clinical need for unblinding will arise. If this occurs, the PI will coordinate with the DCC to facilitate unblinding for the treating ICU team. In any case of unblinding, data collection and follow-up will be maintained and the patient analyzed per-protocol.

12.2. Delivery of the intervention

The treating medical team will order the study intervention as a non-formulary medication to be delivered by ICU nurses, as they would any other medication, and the study team will work to ensure prompt delivery of the intervention with a goal of having it delivered within 24 hours from

the first set of ICU vital signs.

Delivery of the intervention will be managed by ICU nurses who are not part of the study team by encouraging the subject to drink the liquid formulation or, in subjects who are not taking oral medications, by attaching the intervention to the patient's oral or nasogastric tube. The time of dosing will be recorded electronically within the patient EMR and documented by study staff on the CRFs and within REDCap.

12.3. Premature cessation of the intervention

Efforts will be made to ensure that all subjects who are randomized receive the study intervention. The initial medication order will specify that the study intervention is to be given q12h for a total of 14 doses. The study intervention will be stopped under the following conditions:

- Death;
- Discharge from the hospital;
- Request to withdraw from the study by the patient or surrogate. This request can be made at any time, without providing a reason. Consent to continue data collection and retain samples already collected will be sought in these situations;
- Definite clinical indication or contraindication becomes apparent. If the treating ICU team feels at any time that the patient needs or should not have fiber supplementation, the intervention will be immediately halted. The patients will remain in the study for follow-up and the intervention will be held as long as the indication/contraindication remains. In general, if the indication/contraindication resolves within the 7 day timeframe of the intervention, the intervention will be resumed. If the intervention is withheld for hyponatremia, the intervention will be resumed only once 2 consecutive serum sodium values are within normal limits;
- Adverse or serious adverse event related to the study intervention. Such events will constitute unanticipated problems. The patient will remain in the study for follow-up, but no further interventions will be administered.

13. Study assessments

The total study duration will be no more than 30 days of bedside assessments and no more than 6 months of telephone follow-up, both measured from the first recorded set of ICU vital signs. Samples will be gathered and bedside assessments made at the following intervals after ICU admission or until hospital discharge/death: baseline, 3 days, 7 days, 14 days, and 30 days. During study assessments, data will be combined from the following sources: (1) samples collected by the study team, (2) bedside assessments, and (3) data from the EMR.

13.1 Sample collection and storage

Sample collection is focused around **rectal swabs** because the timing of swabs (as opposed to whole stools) can be precisely dictated and because rectal colonization with MDR bacteria best reflects overall MDR colonization status⁸⁴⁻⁸⁸ and risk for subsequent infection.⁸⁹ Samples are described below.

- Rectal swabs. At each study assessment, deep flocked nylon swabs of the rectum will be performed in duplicate to allow independent processing of swabs for 16S rRNA gene sequencing/multiplex qPCR and for bacterial culture of MDROs. Swabs will be inserted 5 cm rectally with patients in the left lateral decubitus position and rotated 5 times, with adequate

sampling verified by looking for fecal staining. Swabs to be sequenced will be flash-frozen at -80°C for long-term cold storage⁹⁰ and batched extraction;⁹¹ swabs to be cultured will be transported on soy broth with 20% glycerol (Becton Dickinson, Franklin Lakes, NJ) for culture or, when culture cannot be performed immediately, for storage overnight at 4° C and culture the following morning.⁹²

- Whole stools. Nursing staff will collect the first spontaneous whole stool subsequent to each study assessment. 4 x 8 ml aliquots of stool will be flash-frozen and stored at -80 ° C.
- Oral swabs. Two cotton-tipped oral swabs will be used to gather a composite of the oral flora by brushing the following consecutive locations x 10 each: left cheek, right cheek, hard palate, tongue, floor of the mouth, and maxillary gingivae (same as the Human Microbiome Project).⁹³ Swabs will be flash frozen at -80 ° C.
- Urine. ICU nurses will withdraw 2 x 8 ml of urine using standard technique from the urinary catheter sampling port. In patients who lack a urinary catheter, clean catch urine samples will be accepted and aliquoted as above.
- Blood. Residual blood from clinical samples drawn immediately prior to each study assessment will be retrieved by the study team. For each subject, one 6 mL serum-separator and one 8 mL EDTA tube will be retrieved. Buffy coat will be separated by centrifuge and with 1-2 mL aliquots generated for plasma/buffy coat and stored at -80 ° C. This strategy spares subjects the need for an additional blood draw.

Oral swabs, urine, and blood will be stored for future testing. The rationale for collecting these additional samples is that they are non-invasive, can be easily collected, and will provide opportunities for future studies seeking to explore additional markers or mechanisms (e.g., urinary 3-indoxyl sulfate for gut microbiome disruption⁹⁴ or micro-RNAs in sepsis).⁹⁵ All sample storage will be in the PI's -80°C freezer unit (P&S-8) except for the fresh rectal swab used for culture which will be stored within the Uhlemann lab (P&S-9). Assays performed from rectal swabs and whole stools (SCFAs) are described below.

13.2. Sample assays

The assays described below will be performed from rectal swabs or whole stools (for fecal SCFA levels). Residual sample material will be retained in -80°C storage for the duration of the study.

- 16S rRNA gene sequencing. 16S rRNA gene sequencing will be performed to determine the **primary outcome**. From extracted rectal swab DNA, polymerase chain reaction (PCR) will be performed on a Bio-Rad T100 Thermal Cycler (Bio-Rad, Hercules, CA) to amplify the V4 hypervariable region of the 16S ribosomal RNA gene with primers derived from Klindworth *et al.*⁶⁷ appended with overhang sequences for compatibility with Illumina index and sequencing adapters (Illumina, San Diego, CA). Samples will be purified with Agencourt AMPure XP beads (Beckman Coulter, Jersey City, NY) and quantified using the Quant-iT broad range dsDNA Assay Kit (Thermo Fisher Scientific, Fair Lawn, NJ). Libraries will be normalized, pooled, and denatured in preparation for cluster generation and sequencing, which will be performed on the Illumina MiSeq 300PE.
- Bacterial culture. Aerobic culture will be performed to determine the **secondary outcome**. Culture has been selected for the primary outcome because culture identifies viable bacteria, remains the most common clinical method for testing for MDRO colonization in

the ICU, and best predicts longterm risk for organism-specific infection.⁹⁶⁻⁹⁹ Additional analyses will utilize alternative methods for classifying MDROs. Cultures will identify MRSA, VRE, and MDR Gram negatives, which cause 94% of all MDR bacterial infections in the ICU.⁶ Rectal swabs will be gently vortexed to dissociate DNA from the swabs into the broth media. Media will then be split into equal aliquots and plated under a laminar flow hood for MRSA (Spectra-MRSA, Remel), VRE (Spectra-VRE, Remel), and ESBL or *Klebsiella pneumoniae* carbapenemase (KPC)-producing bacteria (Uhlemann lab). In addition, we will plate cultures on Gram-negative selective agar (MacConkey II agar, Becton Dickinson) directly and at three serial dilutions for semi-quantitation (10^{-2} , 10^{-4} , and 10^{-6}). While the main analysis will be based on a categorical classification of MDROs (present vs absent), an exploratory analysis will be conducted using semi-quantitative culture data. Preliminary data show that these dilutions will yield a countable number of colonies. After 18-24 hours of aerobic growth, selective plates will be classified as positive vs negative for growth according to the media characteristics. The colony-forming units (CFUs) on the non-selective plates for Gram positives/negatives will be counted to provide relative quantification of each MDRO based on the total CFU counts from the non-selective plates. If multiple non-selective plates show growth, the plate with 10-100 colonies will be used for CFU counting with results normalized to the 10^{-2} plate. From these plates, organisms will be identified using the VITEK 2 system (bioMerieux) and AST-N010/020 cards with confirmatory testing as needed. Resistant isolates will be stored for whole genome sequencing analyses.

- Quantitative PCR. Quantitative PCR for resistance genes will be performed as an additional outcome. DNA will be extracted from rectal swabs (MoBio PowerFecal, Carlsbad, CA) and assessed using multiplex quantitative PCR (Qiagen Cat. No. 330261, Valencia, CA) for 87 common antibiotic resistance genes.⁷³ In this assay, 250 ng of template genomic DNA will be added to each reaction on a 96-well plate which will be run on a StepOnePlus RT-PCR machine using the following cycling conditions: initial denaturation for 10 min at 95° C, cycling 45 x 15 sec at 95° C denaturing followed by 2 min at 60° C annealing and extension. Controls will detect the presence of bacterial DNA, PCR inhibitors and background (no template control (NTC)). PCR cycle thresholds (C_T) of <35 will be considered positive for the presence of any given gene. Adequate reactions will be determined by C_T values of <29 for pan-bacterial reference genes (e.g., *16S rRNA*, *gyrA*, *recA*, *rpoB*)¹⁰⁰ and positive PCR controls (PPC) C_T values of <24. Linearity and sensitivity for this PCR have been determined using synthetic templates over a 6-log serial dilution ranging from 1 to 1 million copies, with results verified across multiple antibiotic resistance genes using pyrosequencing.⁷³
- Fecal SCFA levels. Fecal SCFAs levels will be assayed as an additional outcome. Whole stools will be aliquoted and diluted with 80% ethanol to be agitated at 4°C. After centrifugation, 20 µl of supernatant from the homogenized samples will be mixed with 20 µl of 200 mM N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (1-EDC HCl) in 5% pyridine and 40 µl of 100 mM 2-Nitrophenylhydrazine (2-NPH) in 80% acetonitrile (ACN) with 50 mM HCl. After a brief incubation, ACN will be added to the solution which will be injected onto a 6490 triple quadrupole mass spectrometer (Agilent, Santa Clara, CA) and tested for concentrations of the SCFAs butyrate, acetate, and propionate.

13.3. Bedside assessments including data gathered from the EMR

During each study assessment, a study-specific Case Report Form (CRF) will be used to record the following data: origin for admission (classified as from the hospital floor, operating room, emergency room, or outside ICU transfer based on the most recent location prior to CUMC ICU

admission); the Glasgow Coma Scale (GCS);¹⁰¹ the use of life support (classified as present vs absent) including the use of mechanical ventilation, hemodialysis, or additional life-support devices such as extra-corporeal membrane oxygen (ECMO) or an intra-atrial balloon pump (IABP); and the presence or absence of intravenous lines or catheters including a central venous catheter, urinary catheter, arterial catheter, nasal or oral gastric tube, or rectal tube. Using duplicate data entry, anonymized CRF data will be transferred into the secure REDCap database. Additional clinical variables will be entered directly into REDCap from the EMR. These variables will include laboratory analyses, all of the component variables of the Sequential Organ Failure Assessment (SOFA) score,⁸² measures related to nutritional intake, and ICU interventions including administration of vasopressors, proton pump inhibitors, and antibiotics (with the name of the antibiotic and route of administration recorded).

13.3.1. Tolerability

Patients who are alert will be asked regarding abdominal bloating which will be graded on a 4-point Likert scale (0=none, 1=a little, 2=a moderate amount, 3=a lot/severe). When patients are not alert, this will be graded as “unable.” ICU nurses will be asked to grade the average consistency of stools within each preceding 24 hours on a 5-point scale (0=like water, 1=like porridge, 2=loose but with chunks, 3=formed, soft, 4=formed, hard). This information will also be recorded from the EMR into REDCap as a separate entry. When subjects have no stools within the preceding 24 hours, this will be recorded as “no stools.” The frequency of stools within the preceding 24 hours will be recorded as an integer at each study assessment. Again, this will be done based both on the nurse’s in-person report and separately based on EMR documentation.

ICU nurses will be additionally asked regarding the presence or absence of the following symptoms during the 24 hour period prior to each study assessment: nausea, vomiting, bowel obstruction, and abdominal pain. This data will be recorded categorically on the CRFs/REDCap and will appear on DSMO reports.

13.3.2. Nutritional intake

Information related to nutritional intake will be gathered from the EMR including goal calories, actual calories consumed, and the amount of dietary fiber consumed in grams. All ICU patients at our institution receive a complete clinical nutritional assessment with calculation of goal calories based on the Penn State Equation¹⁰² with the modified equation used for individuals >60 years old or those with BMI >30 as described by Frankenfeld *et al.* in 2009.^{103,104} Actual calories and grams of fiber consumed will be based on the specific components of the tube feeds received or, among patients on an *ad lib* diet, the food consumed. The nutritional components of all CUMC diets are completely specified in terms of calories, fat, protein, carbohydrate, and fiber content. To calculate calories and fiber consumed, the study team will refer to the proportion of each meal consumed as recorded by ICU nurses and to the volume of tube feeds delivered which is captured hourly in the EMR. In those receiving tube feedings, the interval number of tube feeding interruptions will be recorded at each assessment or recorded as 0 in those receiving *ad lib* diets.

13.3.3. Long-term telephone follow-up

Telephone interviews will be conducted with subjects or their surrogates 3 months after ICU admission and again 6 months after ICU admission. In the event that subjects are still hospitalized, these interviews will be conducted at the bedside. Vital status will be ascertained and, to minimize loss of follow-up information, we will interview subjects or surrogates to determine (1) discharge location type (classified as home, short-term rehabilitation, or long-term rehabilitation including skilled care facilities), (2) hospital admission to non-CUMC facilities after ICU discharge, (3) physician office visits after ICU discharge, and (4) infections or other adverse events after ICU discharge. If there are non-CUMC hospital admissions or other healthcare

interactions, permission will be requested to obtain relevant records to determine the nature of infections and to assess for potential medium- to long-term AEs related to the study intervention. Among surviving subjects, the EQ-5D-5L (EuroQol 2009) will be used to determine health status across the domains of mobility, self-care, activity levels, pain, and anxiety/depression.^{76,77} Use of this highly validated instrument will facilitate standardization and generalizability of results and will provide preliminary data for post-ICU discharge monitoring for later phase trials. This instrument can be applied via telephone in 5 minutes and was recommended in 2017 for post-ICU assessment of overall well-being after a three-round modified Delphi process involving clinical researchers from over 16 countries, patients/caregivers, clinicians, and research funders.⁷⁸ EQ-5D-5L responses have the potential to vary depending on whether answers are provided on patients versus surrogates,¹⁰⁵ and therefore we will additionally assess post-ICU disability using Katz' Index of Independence in ADLs (Activities of Daily Living).⁷⁹ Although focused on functional status rather than well-being, Katz' ADLs has been validated as an important patient-centered outcome across dozens of populations and unlike the EQ-5D-5L has high agreement between patients and caregivers.⁸⁰

14. Data collection and management

The Columbia DCC will oversee use of a custom-designed, REDCap-based clinical trial data management system. REDCap is a widely used web-based relational data system developed at Vanderbilt University with NIH support to collect research information in 21CRFPart11, FISMA and HIPAA-compliant environments. REDCap functionality that will be used in this trial include randomization, subject scheduling, *ad hoc* reporting, data verification and audit trails. Paper CRFs will be used to collect bedside data, while readily available selected electronic data (e.g., laboratory values) will either be directly “pulled” from Columbia clinical servers and merged into the REDCap database or manually entered into REDCap. REDCap ‘Events’ will be specified corresponding to the study assessment timepoints: baseline, Days 3, 7, 14, 30, 3 months, and 6 months. Data of each type will be associated in the system with the appropriate time period. REDCap “Anytime” functionality will be used to capture adverse events and other incidents that can occur at any time, i.e. events that are not associated with fixed time periods. The REDCap system has built-in data verification aspects that will be utilized for the trial including minimum and maximum ranges and other logical checks, and automatic checks for completion of individual items and forms.

14.1. Data security within REDCap

The servers on which DCC REDCap project data are stored are located in a physically and electronically secure environment maintained by institutional IT staff on the CUMC campus. The DCC confirms project data access privileges on a monthly basis, and REDCap requires strong passwords, which must be changed periodically. In this trial, to ensure data security, explicit identifiers needed for recruitment and scheduling (names, addresses, phone numbers, email addresses, medical record numbers) will be maintained separately and will *not* be stored in the REDCap database.

14.2. Record retention

All electronic and paper records will be retained for a minimum of 6 years from the trial end date in accordance with New York State law and meeting or exceeding Federal minimums. Consent forms, CRFs, and other relevant paper documents will be moved to secure longterm paper storage once the trial has been closed and the data locked. Record retention will be prolonged beyond 6 years as necessary if required by the funding agency.

15. Safety and adverse events (AEs)

Adverse health events, laboratory irregularities, and even death are expected in ICU patients with sepsis and it would be difficult or impossible to accurately document and label all adverse health events. This is a common problem faced by ICU trials. The solution recommended by academic critical care research groups (e.g., Cook *et al.*) is to pre-specify AEs of interest for careful monitoring. In keeping with this approach, this study will produce detailed reports of these AEs for the DSMC at standard intervals while only unanticipated problems that also meet criteria for serious adverse events (SAEs) will be reported to the DSMC in real time.¹⁰⁶ AEs will be reported for all individuals who have received one or more doses of the intervention. This study has pre-specified AEs in the following categories.

- Bloating: graded 0-3 based on patient report: patient mean and maximum values will be reported;
- Stool consistency and frequency: graded 0-4 for consistency and with an integer denoting frequency over 24 hours based on nursing report. For each patient, mean and maximum values will be reported;
- Electrolyte imbalances: For each patient, the minimum and maximum values for serum calcium, magnesium, phosphate, potassium, and sodium will be reported. This will be done for the 7 days of the intervention period and separately from Day 7 through Day 30/discharge;
- Gastrointestinal AEs: the gastrointestinal AEs of nausea, vomiting, bowel obstruction, and abdominal pain will be recorded as present or absent for each assessment and separated from other AEs to improve early pattern recognition by the DSMO;
- Infections: Infections are both an AE and an outcome and will be adjudicated as previously described. For each infection, the date of the infection, suspected site, organism, and antibiotics received will be recorded. Infections will be further classified as MDR vs non-MDR using the criteria previously described;
- Death: death will be gathered from the hospital EMR, which interfaces with the social security death index.

15.1. Serious adverse events

Federal Regulation 62 Oct. 7, 1997 defines SAEs as those events occurring at any dose which:

- Result in death;
- Are life-threatening, meaning the patient was at risk for death at the time of the event;
- Require hospitalization or prolong hospitalization;
- Results in significant disability or incapacity;
- Is a congenital abnormality/birth defect;
- Or does not meet these criteria but is thought by the investigator to be unusual and potentially serious.

SAEs in this trial will be AEs meeting the above Fed. Reg. 62 definition.

15.2. Adjudicating and reporting adverse events as unanticipated problems

Unanticipated problems (UPs) will be any unforeseen medical event thought likely related to the study intervention. Such events will be monitored for in real time. UPs that do not meet criteria for seriousness (same as SAE criteria) will be reported to the DSMO and IRB at pre-specified intervals. UPs that meet criteria for seriousness will be reported within 24 hours from our notification of the event. This reporting will include:

- Contact of the DSMO;
- Contact of the Columbia IRB;
- Fax or email scan containing when the event occurred, the name of the reporter, callback information, the protocol number, and a description of the event;
- Availability of the PI to provide follow-up information regarding the event.

The proposed research is pending review by the Columbia University IRB (IRB #AAAS2576) and will be reviewed by the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of Research Protections (ORP), Human Research Protection Office (HRPO) prior to implementation. Prior to initiation of research, the study will be registered on clinicaltrials.gov.

Safety and adverse effects will be overseen by the PI in conjunction with the Data and Safety Monitoring Officer (DSMO). **Daniel Brodie**, the Director of the Medical Intensive Care Units at our institution will serve as the trial DSMO and will convene an appropriate Data and Safety Monitoring Committee (DSMC) to oversee interim study results. The primary responsibility of the DSMC will be to review interim data in terms of safety and make recommendations whether the study should be changed, halted, or terminated. Full details of the DSMC procedures and processes as well as additional details regarding AE and SAE reporting are in the DSMP.

15.2.1. Compliance statement regarding research monitoring

The DSMB under Dr. Brodie will function as a **Research Monitor** and is responsible to oversee the safety of the research and report observations/findings to the IRB or a designated institutional official. The Research Monitor will review all unanticipated problems involving risks to subjects or others associated with the protocol and provide an independent report of the event to the IRB. The Research Monitor may discuss the research protocol with the investigators; shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report; and shall have the responsibility to promptly report their observations and findings to the IRB or other designated official and the HRPO.

15.3. Unblinding for adverse events

All possible efforts will be made to retain blinding for the duration of the trial. However, if a treating clinician feels that blinding must be removed in order to assess a possible adverse reaction quickly and correctly, the PI will facilitate this process by working through the DCC.

16. Study termination and completion

The study may be terminated at any time for safety concerns by the PI, DSMC, IRB, or other appropriate regulatory bodies. Otherwise the study will continue until 90 patients are enrolled who complete the Day 3 assessment and will be considered complete when all study assessments and analyses have been concluded.

17. Statistical methods

17.1. Overall plan for statistical analysis

After enrollment is complete, the REDCap dataset will undergo DCC cross-checks for quality and accuracy. The PI will review blinded data to identify errors which, if they exist, will be rectified by reference to paper CRFs or to the EMR. After quality checks are complete, the REDCap dataset will be closed and locked. The biostatistician will then perform an intent-to-treat analysis to determine the primary and secondary outcomes. The DSMO will have complete access both to the raw data and to the analytic code used by the biostatistician and will nominate a qualified monitor who will review this work for rigor. Once this is complete, the primary and secondary outcomes will be finalized. At this point, the locked data will be made available to the PI and other investigators for additional analyses. For all analyses, $\alpha < 0.05$ will be considered statistically significant and two-sided testing will be performed. When death is a competing risk, Fine and Gray regression analyses will be performed.

17.2. Determination of the primary and secondary outcomes

Primary outcome: within-individual change in relative abundance of SCFA producers. 16S rRNA gene sequencing data will be processed using the QIIME platform with appropriate rarification and filtering.¹⁰⁷ The primary analysis will be intent-to-treat, restricted to the subjects who complete the Day 3 assessment and contribute data. Within-individual change in SCFA producers will be calculated as Day 3 minus baseline relative abundance and an ANOVA or Kruskal-Wallis (KW) test (if skewed data) will be used to assess for differences across the three groups. If there is no significant difference between the low- and high-dose inulin arms, these arms will be combined for comparison against placebo in the final analysis using a two-sample t-test or rank-sum test. Although the primary analysis will be with the Day 3 samples, unadjusted and adjusted longitudinal data analysis will be performed using the generalized estimating equation approach with working independence correlation structure.

Secondary outcome: MDRO colonization. Subjects who culture positive from rectal swabs from Day 3 for MRSA, VRE, or a Gram negative with non-susceptibility to a third generation cephalosporin will be classified as MDRO colonized and all other subjects as not MDRO colonized. The proportion of patients who are MDRO colonized on Day 3 will be compared between intervention groups using a chi-squared test or Fisher's test. For this analysis, patients in the inulin arms will be combined if testing shows no significant difference between these inulin arms. Because baseline colonization status is likely to be an important predictor of Day 3 colonization status, we will test whether baseline MDRO colonization rates differ between intervention groups. If such a difference is observed, we will reanalyze Day 3 MDRO colonization with stratification according to baseline MDRO colonization (baseline MDRO colonized vs not colonized). Exploratory analyses will examine Day 7 MDRO colonization status and semi-quantitative culture data using ANOVA or Kruskal-Wallis testing. The difference in rates of Day 3 MDRO colonization will be taken as the effect size estimate for future trials.

17.3. Power calculation and sample size

Primary outcome: within-individual change in relative abundance of SCFA producers. The study is powered to detect a difference in SCFA producer levels that is equal to or greater than the observed difference in SCFA producer levels from our prospective cohort (6.6% for high vs 3.7% low dietary fiber). The rationale for this is that inulin must be at least as impactful as mixed dietary fiber to be a viable therapeutic. The proposed sample size of 30:30:30 will yield 80% power to detect a minimum difference of 0.73 standard deviations (SD) comparing any one inulin arm to the placebo arm using a two-sided t-test with 0.05 significance level and assuming equal variance. If the inulin arms have similar changes in SCFA producer levels, they will be combined and the study would have 80% power to detect a minimum 0.63 SD for inulin compared to placebo at the .05 significance level. This sample size will also have 80% power to detect 0.33 SD differences among the 3 means versus the alternative of equal means using an F-test at the .05 significance level.

Secondary outcome: MDRO colonization. Power calculations were performed using the observed 42% rate of MDRO colonization in our prospective ICU cohort and 1:1:1 allocation to the intervention. Because this is an early phase study conducted with the expectation that promising results will be followed up by definitive larger studies, sample size calculations are based on one-sided testing with alpha .10 using a t-test based on the binomial distribution. Under these assumptions, a sample size of 90 patients will yield 80% power to detect a 20.4% or less rate of MDRO colonization on Day 7 with inulin, assuming that the two inulin arms are combined. If the inulin arms are not combined, there will be 80% power for a 17.3% or less rate of Day 7 MDRO colonization comparing any one inulin intervention arm vs placebo. The two-sided 95% confidence interval for the effect size estimate within the combined inulin arms would be 7.1 to 26.6% for an estimate centered on 15% and 10.8 to 32.3% for an estimate centered on 20%.

17.4. Interim analyses

Interim analyses will be performed for safety, and the DSMC will have the ability to halt the trial at any time. Full DSMP reports will be produced after 10, 20, 30, and 45 patients are randomized and at least semi-annually based on the date of enrollment of the first patient. Toxicity monitoring will be performed using a frequentist approach and targeting an excess rate of toxic death of no more than 5%. Toxicity will be monitored for each arm at the same 4 time points. Sequential boundaries will be calculated using the Pocock method. If the number of toxicities differs in any one study by 3, 4, 5, or 7 patients corresponding to the assessments above, enrollment will be halted and toxicities will be further evaluated to assess if it is appropriate to terminate the study. This stopping boundary yields a probability of early stopping of at most 0.05 when the dose-limiting toxicity is equal to 0.05. Toxic death will be adjudicated by the DSMC. There are no plans for futility monitoring because of the relatively small size of the proposed trial, non-invasiveness of the samples collected, and the potential for longitudinal study results to add significant mechanistic understanding to MDRO colonization in the ICU.

18. Publication policy

Study results will be disseminated as widely as possible by publication and presentation. The PI is ultimately responsible for publications arising from the study. Funding sources will be recognized.

Appendix A: References

1. Neidell MJ, Cohen B, Furuya Y, Hill J, Jeon CY, Glied S, Larson EL. Costs of healthcare- and community-associated infections with antimicrobial-resistant versus antimicrobial-susceptible organisms. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2012;55:807-815.
2. National Nosocomial Infections Surveillance S. National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. *American journal of infection control* 2004;32:470-485.
3. Slayton RB, Toth D, Lee BY, Tanner W, Bartsch SM, Khader K, Wong K, Brown K, McKinnell JA, Ray W, Miller LG, Rubin M, Kim DS, Adler F, Cao C, Avery L, Stone NT, Kallen A, Samore M, Huang SS, Fridkin S, Jernigan JA. Vital Signs: Estimated Effects of a Coordinated Approach for Action to Reduce Antibiotic-Resistant Infections in Health Care Facilities - United States. *MMWR. Morbidity and mortality weekly report* 2015;64:826-831.
4. Garrouste-Orgeas M, Timsit JF, Tafflet M, Misset B, Zahar JR, Soufir L, Lazard T, Jamali S, Mourvillier B, Cohen Y, De Lassence A, Azoulay E, Cheval C, Descorps-Declere A, Adrie C, Costa de Beauregard MA, Carlet J, Group OS. Excess risk of death from intensive care unit-acquired nosocomial bloodstream infections: a reappraisal. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2006;42:1118-1126.
5. Blot S, Koulenti D, Dimopoulos G, Martin C, Komnos A, Krueger WA, Spina G, Armaganidis A, Rello J, Investigators E-VS. Prevalence, risk factors, and mortality for ventilator-associated pneumonia in middle-aged, old, and very old critically ill patients*. *Crit Care Med* 2014;42:601-609.
6. Tabah A, Koulenti D, Laupland K, Misset B, Valles J, Bruzzi de Carvalho F, Paiva JA, Cakar N, Ma X, Eggimann P, Antonelli M, Bonten MJ, Csomos A, Krueger WA, Mikstacki A, Lipman J, Depuydt P, Vesin A, Garrouste-Orgeas M, Zahar JR, Blot S, Carlet J, Brun-Buisson C, Martin C, Rello J, Dimopoulos G, Timsit JF. Characteristics and determinants of outcome of hospital-acquired bloodstream infections in intensive care units: the EURO-BACT International Cohort Study. *Intensive Care Med* 2012;38:1930-1945.
7. Kollef KE, Schramm GE, Wills AR, Reichley RM, Micek ST, Kollef MH. Predictors of 30-day mortality and hospital costs in patients with ventilator-associated pneumonia attributed to potentially antibiotic-resistant gram-negative bacteria. *Chest* 2008;134:281-287.
8. Sommer MO, Dantas G, Church GM. Functional characterization of the antibiotic resistance reservoir in the human microflora. *Science* 2009;325:1128-1131.
9. Donskey CJ. Antibiotic regimens and intestinal colonization with antibiotic-resistant gram-negative bacilli. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2006;43 Suppl 2:S62-69.
10. Forsberg KJ, Reyes A, Wang B, Selleck EM, Sommer MO, Dantas G. The shared antibiotic resistome of soil bacteria and human pathogens. *Science* 2012;337:1107-1111.
11. Britton RA, Young VB. Role of the intestinal microbiota in resistance to colonization by *Clostridium difficile*. *Gastroenterology* 2014;146:1547-1553.
12. Zaborin A, Smith D, Garfield K, Quensen J, Shakhsher B, Kade M, Tirrell M, Tiedje J, Gilbert JA, Zaborina O, Alverdy JC. Membership and behavior of ultra-low-diversity pathogen communities present in the gut of humans during prolonged critical illness. *MBio* 2014;5:e01361-01314.
13. Shimizu K, Ogura H, Hamasaki T, Goto M, Tasaki O, Asahara T, Nomoto K, Morotomi M, Matsushima A, Kuwagata Y, Sugimoto H. Altered gut flora are associated with septic

- complications and death in critically ill patients with systemic inflammatory response syndrome. *Dig Dis Sci* 2011;56:1171-1177.
14. Filius PM, Gyssens IC, Kershof IM, Roovers PJ, Ott A, Vulto AG, Verbrugh HA, Endtz HP. Colonization and resistance dynamics of gram-negative bacteria in patients during and after hospitalization. *Antimicrob Agents Chemother* 2005;49:2879-2886.
 15. Lankelma JM, van Vught LA, Belzer C, Schultz MJ, van der Poll T, de Vos WM, Wiersinga WJ. Critically ill patients demonstrate large interpersonal variation in intestinal microbiota dysregulation: a pilot study. *Intensive Care Med* 2017;43:59-68.
 16. O'Keefe SJ, Li JV, Lahti L, Ou J, Carbonero F, Mohammed K, Posma JM, Kinross J, Wahl E, Ruder E, Vippera K, Naidoo V, Mtshali L, Tims S, Puylaert PG, DeLany J, Krasinskas A, Benefiel AC, Kaseb HO, Newton K, Nicholson JK, de Vos WM, Gaskins HR, Zoetendal EG. Fat, fibre and cancer risk in African Americans and rural Africans. *Nat Commun* 2015;6:6342.
 17. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, Fukuda S, Saito T, Narushima S, Hase K, Kim S, Fritz JV, Wilmes P, Ueha S, Matsushima K, Ohno H, Ollé B, Sakaguchi S, Taniguchi T, Morita H, Hattori M, Honda K. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* 2013;500:232-236.
 18. Desai MS, Seekatz AM, Koropatkin NM, Kamada N, Hickey CA, Wolter M, Pudlo NA, Kitamoto S, Terrapon N, Muller A, Young VB, Henrissat B, Wilmes P, Stappenbeck TS, Nunez G, Martens EC. A Dietary Fiber-Deprived Gut Microbiota Degrades the Colonic Mucus Barrier and Enhances Pathogen Susceptibility. *Cell* 2016;167:1339-1353 e1321.
 19. Topping DL, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol Rev* 2001;81:1031-1064.
 20. Lewis SJ, Heaton KW. Increasing butyrate concentration in the distal colon by accelerating intestinal transit. *Gut* 1997;41:245-251.
 21. Morowitz MJ, Di Caro V, Pang D, Cummings J, Firek B, Rogers MB, Ranganathan S, Clark RSB, Aneja RK. Dietary Supplementation With Nonfermentable Fiber Alters the Gut Microbiota and Confers Protection in Murine Models of Sepsis. *Crit Care Med* 2017;45:e516-e523.
 22. Di Caro V, Cummings JL, Alcamo AM, Piganelli JD, Clark RSB, Morowitz MJ, Aneja RK. Dietary Cellulose Supplementation Modulates the Immune Response in a Murine Endotoxemia Model. *Shock* 2018;
 23. Schroeder BO, Birchenough GMH, Stahlman M, Arike L, Johansson MEV, Hansson GC, Backhed F. Bifidobacteria or Fiber Protects against Diet-Induced Microbiota-Mediated Colonic Mucus Deterioration. *Cell Host Microbe* 2018;23:27-40 e27.
 24. Jacobson A, Lam L, Rajendram M, Tamburini F, Honeycutt J, Pham T, Van Treuren W, Pruss K, Stabler SR, Lugo K, Bouley DM, Vilches-Moure JG, Smith M, Sonnenburg JL, Bhatt AS, Huang KC, Monack D. A Gut Commensal-Produced Metabolite Mediates Colonization Resistance to Salmonella Infection. *Cell Host Microbe* 2018;
 25. Brugiroux S, Beutler M, Pfann C, Garzetti D, Ruscheweyh HJ, Ring D, Diehl M, Herp S, Lotscher Y, Hussain S, Bunk B, Pukall R, Huson DH, Munch PC, McHardy AC, McCoy KD, Macpherson AJ, Loy A, Clavel T, Berry D, Stecher B. Genome-guided design of a defined mouse microbiota that confers colonization resistance against Salmonella enterica serovar Typhimurium. *Nat Microbiol* 2016;2:16215.
 26. Dewulf EM, Cani PD, Claus SP, Fuentes S, Puylaert PG, Neyrinck AM, Bindels LB, de Vos WM, Gibson GR, Thissen JP, Delzenne NM. Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut* 2013;62:1112-1121.

27. Azpiroz F, Molne L, Mendez S, Nieto A, Manichanh C, Mego M, Accarino A, Santos J, Sailer M, Theis S, Guarner F. Effect of Chicory-derived Inulin on Abdominal Sensations and Bowel Motor Function. *J Clin Gastroenterol* 2017;51:619-625.
28. Bonnema AL, Kolberg LW, Thomas W, Slavin JL. Gastrointestinal tolerance of chicory inulin products. *J Am Diet Assoc* 2010;110:865-868.
29. Canfora EE, van der Beek CM, Hermes GDA, Goossens GH, Jocken JWE, Holst JJ, van Eijk HM, Venema K, Smidt H, Zoetendal EG, Dejong CHC, Lenaerts K, Blaak EE. Supplementation of Diet With Galacto-oligosaccharides Increases Bifidobacteria, but Not Insulin Sensitivity, in Obese Prediabetic Individuals. *Gastroenterology* 2017;153:87-97 e83.
30. Garcia-Peris P, Velasco C, Lozano MA, Moreno Y, Paron L, de la Cuerda C, Breton I, Camblor M, Garcia-Hernandez J, Guarner F, Hernandez M. Effect of a mixture of inulin and fructo-oligosaccharide on Lactobacillus and Bifidobacterium intestinal microbiota of patients receiving radiotherapy: a randomised, double-blind, placebo-controlled trial. *Nutr Hosp* 2012;27:1908-1915.
31. Holscher HD, Bauer LL, Gourineni V, Pelkman CL, Fahey GC, Jr., Swanson KS. Agave Inulin Supplementation Affects the Fecal Microbiota of Healthy Adults Participating in a Randomized, Double-Blind, Placebo-Controlled, Crossover Trial. *J Nutr* 2015;145:2025-2032.
32. Petry N, Egli I, Chassard C, Lacroix C, Hurrell R. Inulin modifies the bifidobacteria population, fecal lactate concentration, and fecal pH but does not influence iron absorption in women with low iron status. *Am J Clin Nutr* 2012;96:325-331.
33. Ramirez-Farias C, Slezak K, Fuller Z, Duncan A, Holtrop G, Louis P. Effect of inulin on the human gut microbiota: stimulation of Bifidobacterium adolescentis and Faecalibacterium prausnitzii. *Br J Nutr* 2009;101:541-550.
34. Tovar AR, Caamano Mdel C, Garcia-Padilla S, Garcia OP, Duarte MA, Rosado JL. The inclusion of a partial meal replacement with or without inulin to a calorie restricted diet contributes to reach recommended intakes of micronutrients and decrease plasma triglycerides: a randomized clinical trial in obese Mexican women. *Nutr J* 2012;11:44.
35. Vandeputte D, Falony G, Vieira-Silva S, Wang J, Sailer M, Theis S, Verbeke K, Raes J. Prebiotic inulin-type fructans induce specific changes in the human gut microbiota. *Gut* 2017;
36. O'Keefe SJ, Ou J, Delany JP, Curry S, Zoetendal E, Gaskins HR, Gunn S. Effect of fiber supplementation on the microbiota in critically ill patients. *World J Gastrointest Pathophysiol* 2011;2:138-145.
37. Terry MA, Freedberg DE, Morris MC. An Alternative Consent Process for Minimal Risk Research in the ICU. *Crit Care Med* 2017;
38. Freedberg DE, Zhou MJ, Cohen ME, Annavajhala MK, Khan S, Moscoso DI, Brooks C, Whittier S, Chong DH, Uhlemann AC, Abrams JA. Pathogen colonization of the gastrointestinal microbiome at intensive care unit admission and risk for subsequent death or infection. *Intensive Care Med* 2018;
39. Livanos AE, Snider EJ, Whittier S, Chong DH, Wang TC, Abrams JA, Freedberg DE. Rapid gastrointestinal loss of Clostridial Clusters IV and XIVa in the ICU associates with an expansion of gut pathogens. *PLoS One* 2018;13:e0200322.
40. Freedberg DE, Zhou MJ, Cohen ME, Annavajhala M, Khan S, Moscoso DI, Brooks CA, Whittier S, Chong DH, Uhlemann AC, Abrams JA. Pathogen colonization of the gastrointestinal microbiome at intensive care unit admission and risk for subsequent death or infection. *Intensive Care Medicine*, Accepted for publication June 4, 2018
41. Metnitz PG, Moreno RP, Almeida E, Jordan B, Bauer P, Campos RA, Iapichino G, Edbrooke D, Capuzzo M, Le Gall JR, Investigators S. SAPS 3--From evaluation of the

- patient to evaluation of the intensive care unit. Part 1: Objectives, methods and cohort description. *Intensive Care Med* 2005;31:1336-1344.
42. Moreno RP, Metnitz PG, Almeida E, Jordan B, Bauer P, Campos RA, Iapichino G, Edbrooke D, Capuzzo M, Le Gall JR, Investigators S. SAPS 3--From evaluation of the patient to evaluation of the intensive care unit. Part 2: Development of a prognostic model for hospital mortality at ICU admission. *Intensive Care Med* 2005;31:1345-1355.
 43. Taur Y, Jenq RR, Perales MA, Littmann ER, Morjaria S, Ling L, No D, Gobourne A, Viale A, Dahi PB, Ponce DM, Barker JN, Giralt S, van den Brink M, Pamer EG. The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. *Blood* 2014;124:1174-1182.
 44. Taur Y, Xavier JB, Lipuma L, Ubeda C, Goldberg J, Gobourne A, Lee YJ, Dubin KA, Socci ND, Viale A, Perales MA, Jenq RR, van den Brink MR, Pamer EG. Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2012;55:905-914.
 45. Haak BW, Littmann ER, Chaubard JL, Pickard AJ, Fontana E, Adhi F, Gyaltsen Y, Ling L, Morjaria SM, Peled JU, van den Brink MR, Geyer AI, Cross JR, Pamer EG, Taur Y. Impact of gut colonization with butyrate-producing microbiota on respiratory viral infection following allo-HCT. *Blood* 2018;131:2978-2986.
 46. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C. Metagenomic biomarker discovery and explanation. *Genome Biol* 2011;12:R60.
 47. Fukuda S, Toh H, Hase K, Oshima K, Nakanishi Y, Yoshimura K, Tobe T, Clarke JM, Topping DL, Suzuki T, Taylor TD, Itoh K, Kikuchi J, Morita H, Hattori M, Ohno H. Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature* 2011;469:543-547.
 48. Rivera-Chavez F, Zhang LF, Faber F, Lopez CA, Byndloss MX, Olsan EE, Xu G, Velazquez EM, Lebrilla CB, Winter SE, Baumler AJ. Depletion of Butyrate-Producing Clostridia from the Gut Microbiota Drives an Aerobic Luminal Expansion of Salmonella. *Cell Host Microbe* 2016;19:443-454.
 49. Duncan SH, Louis P, Thomson JM, Flint HJ. The role of pH in determining the species composition of the human colonic microbiota. *Environ Microbiol* 2009;11:2112-2122.
 50. Walker AW, Duncan SH, McWilliam Leitch EC, Child MW, Flint HJ. pH and peptide supply can radically alter bacterial populations and short-chain fatty acid ratios within microbial communities from the human colon. *Appl Environ Microbiol* 2005;71:3692-3700.
 51. Kruse HP, Kleessen B, Blaut M. Effects of inulin on faecal bifidobacteria in human subjects. *Br J Nutr* 1999;82:375-382.
 52. Lecerf JM, Depeint F, Clerc E, Dugenet Y, Niamba CN, Rhazi L, Cayzele A, Abdelnour G, Jaruga A, Younes H, Jacobs H, Lambrey G, Abdelnour AM, Pouillart PR. Xylo-oligosaccharide (XOS) in combination with inulin modulates both the intestinal environment and immune status in healthy subjects, while XOS alone only shows prebiotic properties. *Br J Nutr* 2012;108:1847-1858.
 53. Miguez B, Gomez B, Parajo JC, Alonso JL. Potential of fructooligosaccharides and xylooligosaccharides as substrates to counteract the undesirable effects of several antibiotics on elder faecal microbiota: a first in vitro approach. *J Agric Food Chem* 2018;
 54. Rahat-Rozenbloom S, Fernandes J, Cheng J, Gloor GB, Wolever TM. The acute effects of inulin and resistant starch on postprandial serum short-chain fatty acids and second-meal glycemic response in lean and overweight humans. *Eur J Clin Nutr* 2017;71:227-233.
 55. Manzanares W, Lemieux M, Langlois PL, Wischmeyer PE. Probiotic and synbiotic therapy in critical illness: a systematic review and meta-analysis. *Crit Care* 2016;19:262.

56. McClave SA, Taylor BE, Martindale RG, Warren MM, Johnson DR, Braunschweig C, McCarthy MS, Davanos E, Rice TW, Cresci GA, Gervasio JM, Sacks GS, Roberts PR, Compher C, Society of Critical Care M, American Society for P, Enteral N. Guidelines for the Provision and Assessment of Nutrition Support Therapy in the Adult Critically Ill Patient: Society of Critical Care Medicine (SCCM) and American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.). *JPEN J Parenter Enteral Nutr* 2016;40:159-211.
57. Kotzampassi K, Giamarellos-Bourboulis EJ, Voudouris A, Kazamias P, Eleftheriadis E. Benefits of a synbiotic formula (Synbiotic 2000Forte) in critically ill trauma patients: early results of a randomized controlled trial. *World J Surg* 2006;30:1848-1855.
58. Knight DJ, Gardiner D, Banks A, Snape SE, Weston VC, Bengmark S, Girling KJ. Effect of synbiotic therapy on the incidence of ventilator associated pneumonia in critically ill patients: a randomised, double-blind, placebo-controlled trial. *Intensive Care Med* 2009;35:854-861.
59. Spindler-Vesel A, Bengmark S, Vovk I, Cerovic O, Kompan L. Synbiotics, prebiotics, glutamine, or peptide in early enteral nutrition: a randomized study in trauma patients. *JPEN J Parenter Enteral Nutr* 2007;31:119-126.
60. Karakan T, Ergun M, Dogan I, Cindoruk M, Unal S. Comparison of early enteral nutrition in severe acute pancreatitis with prebiotic fiber supplementation versus standard enteral solution: a prospective randomized double-blind study. *World J Gastroenterol* 2007;13:2733-2737.
61. Besselink MG, van Santvoort HC, Buskens E, Boermeester MA, van Goor H, Timmerman HM, Nieuwenhuijs VB, Bollen TL, van Ramshorst B, Witteman BJ, Rosman C, Ploeg RJ, Brink MA, Schaapherder AF, Dejong CH, Wahab PJ, van Laarhoven CJ, van der Harst E, van Eijck CH, Cuesta MA, Akkermans LM, Gooszen HG, Dutch Acute Pancreatitis Study G. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. *Lancet* 2008;371:651-659.
62. McIvor AC, Meguid MM, Curtas S, Warren J, Kaplan DS. Intestinal obstruction from cecal bezoar; a complication of fiber-containing tube feedings. *Nutrition* 1990;6:115-117.
63. Majid HA, Cole J, Emery PW, Whelan K. Additional oligofructose/inulin does not increase faecal bifidobacteria in critically ill patients receiving enteral nutrition: a randomised controlled trial. *Clin Nutr* 2014;33:966-972.
64. Spapen H, Diltoer M, Van Malderen C, Opdenacker G, Suys E, Huyghens L. Soluble fiber reduces the incidence of diarrhea in septic patients receiving total enteral nutrition: a prospective, double-blind, randomized, and controlled trial. *Clin Nutr* 2001;20:301-305.
65. Dilli D, Aydin B, Zenciroglu A, Ozyazici E, Beken S, Okumus N. Treatment outcomes of infants with cyanotic congenital heart disease treated with synbiotics. *Pediatrics* 2013;132:e932-938.
66. Vandeputte D, Falony G, Vieira-Silva S, Wang J, Sailer M, Theis S, Verbeke K, Raes J. Prebiotic inulin-type fructans induce specific changes in the human gut microbiota. *Gut* 2017;66:1968-1974.
67. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glockner FO. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res* 2013;41:e1.
68. Van den Abbeele P, Belzer C, Goossens M, Kleerebezem M, De Vos WM, Thas O, De Weirtd R, Kerckhof FM, Van de Wiele T. Butyrate-producing *Clostridium* cluster XIVa species specifically colonize mucins in an in vitro gut model. *ISME J* 2013;7:949-961.
69. Vital M, Howe AC, Tiedje JM. Revealing the bacterial butyrate synthesis pathways by analyzing (meta)genomic data. *MBio* 2014;5:e00889.
70. Tye H, Yu CH, Simms LA, de Zoete MR, Kim ML, Zakrzewski M, Penington JS, Harapas CR, Souza-Fonseca-Guimaraes F, Wockner LF, Preaudet A, Mielke LA, Wilcox SA, Ogura Y, Corr SC, Kanojia K, Kouremenos KA, De Souza DP, McConville MJ, Flavell

- RA, Gerlic M, Kile BT, Papenfuss AT, Putoczki TL, Radford-Smith GL, Masters SL. NLRP1 restricts butyrate producing commensals to exacerbate inflammatory bowel disease. *Nat Commun* 2018;9:3728.
71. Siegel JD, Rhinehart E, Jackson M, Chiarello L, Healthcare Infection Control Practices Advisory C. Management of multidrug-resistant organisms in health care settings, 2006. *American journal of infection control* 2007;35:S165-193.
72. (CLSI) CaLSI. Performance standards for antimicrobial susceptibility testing. CLSI Supplement M100. 2017(27th Ed.).
73. Fosbrink M, Wilt G, Chen L, Kreisworth B, Devgan V. Identification of antibiotic resistance genes in *Klebsiella pneumoniae* isolates and metagenomic samples using real-time PCR arrays. Sample to Insight, accessed on-line at <https://www.slideshare.net/QIAGENscience/identification-of-antibiotic-resistance-genes-in-klebsiella-pneumoniae-isolates-and-metagenomic-samples-using-realtime-pcr-arrays>. April 6, 2016.;
74. Magill SS, Hellinger W, Cohen J, Kay R, Bailey C, Boland B, Carey D, de Guzman J, Dominguez K, Edwards J, Goraczewski L, Horan T, Miller M, Phelps M, Saltford R, Seibert J, Smith B, Starling P, Viergutz B, Walsh K, Rathore M, Guzman N, Fridkin S. Prevalence of healthcare-associated infections in acute care hospitals in Jacksonville, Florida. *Infection control and hospital epidemiology* 2012;33:283-291.
75. Gottschlich MM, DeLegge MH, Guenter P, American Society for P, Enteral N. *The A.S.P.E.N. nutrition support core curriculum : a case-based approach : the adult patient*. Silver Spring, MD: American Society for Parenteral and Enteral Nutrition; 2007.
76. Janssen MF, Pickard AS, Golicki D, Gudex C, Niewada M, Scalone L, Swinburn P, Busschbach J. Measurement properties of the EQ-5D-5L compared to the EQ-5D-3L across eight patient groups: a multi-country study. *Qual Life Res* 2013;22:1717-1727.
77. Herdman M, Gudex C, Lloyd A, Janssen M, Kind P, Parkin D, Bonnel G, Badia X. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). *Qual Life Res* 2011;20:1727-1736.
78. Needham DM, Sepulveda KA, Dinglas VD, Chessare CM, Aronson Friedman L, Bingham Iii CO, Turnbull AE. Core Outcome Measures for Clinical Research in Acute Respiratory Failure Survivors: An International Modified Delphi Consensus Study. *Am J Respir Crit Care Med* 2017;
79. Katz S, Downs TD, Cash HR, Grotz RC. Progress in development of the index of ADL. *Gerontologist* 1970;10:20-30.
80. Schandl A, Bottai M, Holdar U, Hellgren E, Sackey P. Early prediction of new-onset physical disability after intensive care unit stay: a preliminary instrument. *Crit Care* 2014;18:455.
81. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, Hotchkiss RS, Levy MM, Marshall JC, Martin GS, Opal SM, Rubenfeld GD, van der Poll T, Vincent JL, Angus DC. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* 2016;315:801-810.
82. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonca A, Bruining H, Reinhart CK, Suter PM, Thijs LG. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med* 1996;22:707-710.
83. Cadot P, Kochuyt AM, van Ree R, Ceuppens JL. Oral allergy syndrome to chicory associated with birch pollen allergy. *Int Arch Allergy Immunol* 2003;131:19-24.
84. Thurlow CJ, Prabaker K, Lin MY, Lolans K, Weinstein RA, Hayden MK, Centers for Disease C, Prevention Epicenters P. Anatomic sites of patient colonization and

- environmental contamination with *Klebsiella pneumoniae* carbapenemase-producing Enterobacteriaceae at long-term acute care hospitals. *Infection control and hospital epidemiology* 2013;34:56-61.
85. Ray AJ, Pultz NJ, Bhalla A, Aron DC, Donskey CJ. Coexistence of vancomycin-resistant enterococci and *Staphylococcus aureus* in the intestinal tracts of hospitalized patients. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2003;37:875-881.
 86. Klotz M, Zimmermann S, Oppen S, Heeg K, Mutters R. Possible risk for re-colonization with methicillin-resistant *Staphylococcus aureus* (MRSA) by faecal transmission. *International journal of hygiene and environmental health* 2005;208:401-405.
 87. Acton DS, Plat-Sinnige MJ, van Wamel W, de Groot N, van Belkum A. Intestinal carriage of *Staphylococcus aureus*: how does its frequency compare with that of nasal carriage and what is its clinical impact? *Eur J Clin Microbiol Infect Dis* 2009;28:115-127.
 88. Bhalla A, Aron DC, Donskey CJ. *Staphylococcus aureus* intestinal colonization is associated with increased frequency of *S. aureus* on skin of hospitalized patients. *BMC Infect Dis* 2007;7:105.
 89. Dangerfield B, Chung A, Webb B, Seville MT. Predictive value of methicillin-resistant *Staphylococcus aureus* (MRSA) nasal swab PCR assay for MRSA pneumonia. *Antimicrob Agents Chemother* 2014;58:859-864.
 90. Sinha R, Chen J, Amir A, Vogtmann E, Shi J, Inman KS, Flores-Munguia R, Sampson JN, Knight R, Chia N. Collecting Fecal Samples for Microbiome Analyses in Epidemiology Studies. *Cancer Epidemiol Biomarkers Prev* 2015;
 91. Walker AW, Martin JC, Scott P, Parkhill J, Flint HJ, Scott KP. 16S rRNA gene-based profiling of the human infant gut microbiota is strongly influenced by sample processing and PCR primer choice. *Microbiome* 2015;3:26.
 92. Bonten MJ, Nathan C, Weinstein RA. Recovery of nosocomial fecal flora from frozen stool specimens and rectal swabs: comparison of preservatives for epidemiological studies. *Diagnostic microbiology and infectious disease* 1997;27:103-106.
 93. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. *Nature* 2007;449:804-810.
 94. Farowski F, Els G, Tsakmaklis A, Higgins PG, Kahlert CR, Stein-Thoeringer CK, Bobardt JS, Dettmer-Wilde K, Oefner PJ, Vehreschild JJ, Vehreschild M. Assessment of urinary 3-indoxyl sulfate as a marker for gut microbiota diversity and abundance of Clostridiales. *Gut Microbes* 2018;1-9.
 95. Seeley JJ, Baker RG, Mohamed G, Bruns T, Hayden MS, Deshmukh SD, Freedberg DE, Ghosh S. Induction of innate immune memory via microRNA targeting of chromatin remodelling factors. *Nature* 2018;559:114-119.
 96. Ziakas PD, Thapa R, Rice LB, Mylonakis E. Trends and significance of VRE colonization in the ICU: a meta-analysis of published studies. *PLoS One* 2013;8:e75658.
 97. Garrouste-Orgeas M, Timsit JF, Kallel H, Ben Ali A, Dumay MF, Paoli B, Misset B, Carlet J. Colonization with methicillin-resistant *Staphylococcus aureus* in ICU patients: morbidity, mortality, and glycopeptide use. *Infect Control Hosp Epidemiol* 2001;22:687-692.
 98. Loo VG, Bourgault AM, Poirier L, Lamothe F, Michaud S, Turgeon N, Toye B, Beaudoin A, Frost EH, Gilca R, Brassard P, Dendukuri N, Beliveau C, Oughton M, Brukner I, Dascal A. Host and pathogen factors for *Clostridium difficile* infection and colonization. *N Engl J Med* 2011;365:1693-1703.
 99. Frencken JF, Wittekamp BHJ, Plantinga NL, Spitoni C, van de Groep K, Cremer OL, Bonten MJM. Associations Between Enteral Colonization With Gram-Negative Bacteria and Intensive Care Unit-Acquired Infections and Colonization of the Respiratory Tract. *Clin Infect Dis* 2017;

100. Rocha DJ, Santos CS, Pacheco LG. Bacterial reference genes for gene expression studies by RT-qPCR: survey and analysis. *Antonie Van Leeuwenhoek* 2015;108:685-693.
101. Teasdale G, Jennett B. Assessment of coma and impaired consciousness. A practical scale. *Lancet* 1974;2:81-84.
102. Frankenfield DC, Rowe WA, Smith JS, Cooney RN. Validation of several established equations for resting metabolic rate in obese and nonobese people. *J Am Diet Assoc* 2003;103:1152-1159.
103. Frankenfield DC, Coleman A, Alam S, Cooney RN. Analysis of estimation methods for resting metabolic rate in critically ill adults. *JPEN J Parenter Enteral Nutr* 2009;33:27-36.
104. Frankenfield DC, Ashcraft CM, Galvan DA. Prediction of resting metabolic rate in critically ill patients at the extremes of body mass index. *JPEN J Parenter Enteral Nutr* 2013;37:361-367.
105. Dinglas VD, Chessare CM, Davis WE, Parker A, Friedman LA, Colantuoni E, Bingham CO, Turnbull AE, Needham DM. Perspectives of survivors, families and researchers on key outcomes for research in acute respiratory failure. *Thorax* 2017;
106. Cook D, Lauzier F, Rocha MG, Sayles MJ, Finfer S. Serious adverse events in academic critical care research. *CMAJ* 2008;178:1181-1184.
107. Franzosa EA, Sirota-Madi A, Avila-Pacheco J, Fornelos N, Haiser HJ, Reinker S, Vatanen T, Hall AB, Mallick H, McIver LJ, Sauk JS, Wilson RG, Stevens BW, Scott JM, Pierce K, Deik AA, Bullock K, Imhann F, Porter JA, Zhernakova A, Fu J, Weersma RK, Wijmenga C, Clish CB, Vlamakis H, Huttenhower C, Xavier RJ. Gut microbiome structure and metabolic activity in inflammatory bowel disease. *Nat Microbiol* 2018;

Appendix B: Abbreviations and terms

ADLs	activities of daily living
AE	adverse event
ANOVA	analysis of variance
APACHE	acute physiology and chronic health evaluation
ASPEN	American Society for Parenteral and Enteral Nutrition
BMI	body mass index
CDC	Centers for Disease Control and Prevention
CDI	<i>Clostridium difficile</i> infection
CFU	colony-forming units
CLP	cecal ligation and puncture
CRF	case report form
CUMC	Columbia University Medical Center
DNI	Do not intubate
DNR	Do not resuscitate
DCC	Data Coordinating Center
DSMC	data and safety monitoring committee
DSMO	data and safety monitoring officer
ECMO	extra-corporeal membrane oxygen
EDTA	ethylenediaminetetraacetic acid
EMR	electronic medical record
EQ-5D-5L	EuroQol 5-dimension 5-level questionnaire
ER	emergency room
ESBL	extended-spectrum beta-lactamase
FDA	Food and Drug Administration
GCS	Glasgow coma scale
GI	gastrointestinal
GMP	good manufacturing practices
GRAS	generally recognized as safe
HRPO	human research protection office
IABP	intra-atrial balloon pump
ICD	International classification of diseases
ICU	intensive care unit
IND	investigational new drug
IQR	interquartile range
IRB	institutional review board
KPC	<i>Klebsiella pneumonia</i> carbapenemase
LAR	legally authorized representative
LDA	least discriminant analysis
LEfSe	linear discriminant analysis effect size

LPS	lipo-polysaccharide
MDR	multidrug resistant
MDRO	multidrug resistant organism
MRSA	methicillin-resistant <i>Staphylococcus aureus</i>
NHSN	National Health and Safety Network
OR	operating room
ORP	Office of Research Protections
OTU	operational taxonomic unit
PCR	polymerase chain reaction
PI	primary investigator
PICRUST	phylogenetic investigation of communities by reconstruction of unobserved states
PPIs	proton pump inhibitors
PRMRP	peer-reviewed military research projects
QIIME	Quantitative Insights Into Microbial Ecology
qPCR	quantitative polymerase chain reaction
REDCap	Research Electronic Data Capture
rRNA	ribosomal ribonucleic acid
RT-PCR	real-time polymerase chain reaction
SAE	serious adverse event
SCFA	short-chain fatty acids
SD	standard deviation
SAPS3	simplified acute physiology score-3
SIRS	systemic inflammatory response syndrome
SOFA	sequential organ failure assessment
TNF	tumor necrosis factor
USAMRMC	U.S. Army Medical Research and Materiel Command
VRE	vancomycin-resistant <i>Enterococcus</i>
WGS	whole genome sequencing

Appendix 9.3

Columbia University Human Subjects Protocol Data Sheet

General Information

Protocol:	AAAS2576(M00Y04)	Protocol Status:	Approved
Effective Date:	02/23/2022	Expiration Date:	02/22/2023
Originating Department Code:			MED Medicine (751800X)
Principal Investigator:			Freedberg, Daniel (def2004)
From what Columbia campus does this research originate:			Medical Center
Title:	Inulin the ICU		
Protocol Version #:	1	Abbreviated Title:	Inulin in the ICU
Was this protocol previously assigned a number by an IRB:			No

Is the purpose of this submission to obtain a "Not Human Subjects Research" determination?

No

Renewal Information

Enrollment status:

Open to enrollment or ongoing review of records/specimens

Provide any additional information necessary to explain the study status:

Since the last renewal:

Have there been any changes in the relevant literature that would affect the study design or procedures?

No

Have there been any interim findings associated with this study?

No

Have there been any publications resulting from this study?

No

Have any participants been enrolled using the Short Form process?

No

Is there a Data Monitoring Committee (DMC), Data Safety Monitoring Board (DSMB), or other monitoring entity for this study?

Yes

Is the most recent report attached as part of this submission?

Yes

Is an annual Progress Report required by the funding organization or coordinating center for this study?

Yes

Is the most recent report attached as part of this submission?

Yes

Does this submission include a modification?

No

Has the consent form been revised in this submission?

No

Does this submission include a report of a protocol violation?

No



Attributes

Special review type: Check all that apply or check "None of the Above" box.

- ☐ Review for 45 CFR 46.118 Determination (involvement of human subjects is anticipated but is not yet defined)
- ☐ Funding review for Administrative IRB approval (such as for Center or Training Grants)
- ☒ None of the above

IRB of record information: Will a Columbia IRB be the IRB that is responsible for providing review, approval, and oversight for this study?

Yes

Select the most appropriate response:

Columbia will be the IRB of record for the study procedures conducted by Columbia researchers (Note: this response will apply to most submissions).

Is this research part of a multicenter study?

No

Please indicate if any of the following University resources are utilized:

- ☐ Cancer Center Clinical Protocol Data Management Compliance Core (CPDM)
- ☐ CTSA-Irving Institute Clinical Research Resource (CRR)
- ☐ CTSA- Irving Institute Columbia Community Partnership for Health (CCPH)
- ☒ None of the above

Background

Abbreviated Submission:

The IRB has an abbreviated submission process for multicenter studies supported by industry or NIH cooperative groups (e.g., ACTG, HVTN, NCI oncology group studies, etc.), and other studies that have a complete stand-alone protocol. The process requires completion of all Rascal fields that provide information regarding local implementation of the study. However, entering study information into all of the relevant Rascal fields is not required, as the Columbia IRBs will rely on the attached stand-alone (e.g., sponsor's) protocol for review of the overall objectives.

If you select the Abbreviated Submission checkbox and a section is not covered by the attached stand-alone protocol, you will need to go back and provide this information in your submission.

Study Purpose and Rationale:

Provide pertinent background description with references that are related to the need to conduct this study. If this is a clinical trial, the background should include both preclinical and clinical data. Be brief and to the point.

- ☐ Abbreviated Submission - This information is included in an attached stand-alone protocol. Proceed to the next question

Note to the IRB on the inulin-COVID substudy In light of the COVID-19 pandemic, we are modifying this study protocol to create a COVID substudy. The following changes will be implemented for the COVID inulin substudy. Newly hospitalized (within 72 hours) patients 18 years old or more will be considered for enrollment if they test COVID+ and are receiving intravenous broad-spectrum antibiotics. Exclusion criteria will remain the

same. Because of safety concerns and restrictions to direct access to patients, patients will be approached for consent remotely by telephone or WebEx so that study personnel only enters the room after the patient has given verbal consent. After verbal consent is obtained in the patient's room, the patient will sign standard print consent forms which will be placed in a clear ziplock plastic bag with the signature page upright. Before it is taken from the room, the bag will be wiped with bleach wipes to decontaminate it, soaking the exterior surfaces for >30 seconds on each side. Outside the room, this bag will then be placed into a second ziplock bag. If a surrogate gives consent and is on quarantine, faxed consent forms will be used to avoid face-to-face contact with the surrogate. After consent is obtained, samples will be collected and placed directly into freezer-safe containers containing EPA-approved viral inactivation media. Samples will be triple bagged for transport to the freezer facility by appropriately trained personnel. Other than as described above, the study approach to enrollment, intervention, and assessments will be identical to the main protocol. The main protocol and COVID substudy will be separately analyzed when the studies are complete.

Study Purpose and Rationale Multidrug resistant (MDR) infections are the leading cause of death in the intensive care unit (ICU) with mortality as high as 9 for every 100 patients admitted.¹ The bacteria and plasmids that encode antibiotic resistance reside in the human gut and prevent MDRO colonization. With antibiotics and other treatments received during critical illness, this protection is lost. New data show that there are specific "good" bacteria that combat colonization. Of these, the best data is for short chain fatty acid (SCFA)-producing colonic anaerobes. To understand the relationship between SCFA producers and colonization in the ICU, we have built two observational cohorts totaling over 500 Columbia ICU patients.²⁻⁴ In these patients, levels of SCFA-producing bacteria declined 5- to 10-fold during the 72 hours after ICU admission, with a 22% concurrent rise in prevalence of MDROs⁴ and a 50% increased risk for subsequent death or infection.³ The prebiotic fiber inulin is a vegetable-derived polysaccharide that appears to restore gastrointestinal colonization resistance against MDROs in animals and ambulatory humans. Inulin is safe in ambulatory patients⁵⁻¹³ with biologic effects seen at 16 g/day.¹⁴ In the ICU, inulin-type soluble fibers have been given up to 36 g/day without adverse events.¹⁵ This protocol hypothesizes that inulin supplementation in ICU patients will result in higher levels of SCFA-producing bacteria, decreased gut MDRO colonization, and lower risk for ICU-acquired MDR infections. To test this hypothesis, we will conduct a double-blind randomized clinical trial of inulin in 90 newly admitted medical ICU adults who meet sepsis criteria. Enrolled adults will receive 1:1:1 inulin 16 g/day, inulin 32 g/day, or placebo in divided doses for 7 days in the ICU and will provide serial rectal swabs, fecal samples, and clinical data for up to 30 days of active follow-up.

REFERENCES
1. Vincent JL, Rello J, Marshall J, et al. International study of the prevalence and outcomes of infection in intensive care units. JAMA 2009;302:2323-9.
2. Terry MA, Freedberg DE, Morris MC. An Alternative Consent Process for Minimal Risk Research in the ICU. Crit Care Med 2017.
3. Freedberg DE, Zhou MJ, Cohen ME, et al. Pathogen colonization of the gastrointestinal microbiome at intensive care unit admission and risk for subsequent death or infection. Intensive Care Med 2018.
4. Livanos AE, Snider EJ, Whittier S, et al. Rapid gastrointestinal loss of Clostridial Clusters IV and XIVA in the ICU associates with an expansion of gut pathogens. PLoS One

2018;13:e0200322.5. Azpiroz F, Molne L, Mendez S, et al. Effect of Chicory-derived Inulin on Abdominal Sensations and Bowel Motor Function. *J Clin Gastroenterol* 2017;51:619-25.6. Bonnema AL, Kolberg LW, Thomas W, et al. Gastrointestinal tolerance of chicory inulin products. *J Am Diet Assoc* 2010;110:865-8.7. Canfora EE, van der Beek CM, Hermes GDA, et al. Supplementation of Diet With Galacto-oligosaccharides Increases Bifidobacteria, but Not Insulin Sensitivity, in Obese Prediabetic Individuals. *Gastroenterology* 2017;153:87-97 e3.8. Garcia-Peris P, Velasco C, Lozano MA, et al. Effect of a mixture of inulin and fructo-oligosaccharide on Lactobacillus and Bifidobacterium intestinal microbiota of patients receiving radiotherapy: a randomised, double-blind, placebo-controlled trial. *Nutr Hosp* 2012;27:1908-15.9. Holscher HD, Bauer LL, Gourineni V, et al. Agave Inulin Supplementation Affects the Fecal Microbiota of Healthy Adults Participating in a Randomized, Double-Blind, Placebo-Controlled, Crossover Trial. *J Nutr* 2015;145:2025-32.10. Petry N, Egli I, Chassard C, et al. Inulin modifies the bifidobacteria population, fecal lactate concentration, and fecal pH but does not influence iron absorption in women with low iron status. *Am J Clin Nutr* 2012;96:325-31.11. Ramirez-Farias C, Slezak K, Fuller Z, et al. Effect of inulin on the human gut microbiota: stimulation of Bifidobacterium adolescentis and Faecalibacterium prausnitzii. *Br J Nutr* 2009;101:541-50.12. Tovar AR, Caamano Mdel C, Garcia-Padilla S, et al. The inclusion of a partial meal replacement with or without inulin to a calorie restricted diet contributes to reach recommended intakes of micronutrients and decrease plasma triglycerides: a randomized clinical trial in obese Mexican women. *Nutr J* 2012;11:44.13. Vandeputte D, Falony G, Vieira-Silva S, et al. Prebiotic inulin-type fructans induce specific changes in the human gut microbiota. *Gut* 2017.14. Dewulf EM, Cani PD, Claus SP, et al. Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut* 2013;62:1112-21.15. O'Keefe SJ, Ou J, Delany JP, et al. Effect of fiber supplementation on the microbiota in critically ill patients. *World J Gastrointest Pathophysiol* 2011;2:138-45.

Study Design:

Describe the methodology that will be used in this study, covering such factors as retrospective vs. prospective data collection, interventional vs. non-interventional, randomized vs. non-randomized, observational, experimental, ethnography, etc.

[] Abbreviated Submission - This information is included in an attached stand-alone protocol. Proceed to the next question

This will be a double-blind, randomized, placebo-controlled trial with two doses. In general, eligible subjects will be newly admitted medical ICU patients 18 years old with sepsis as defined by Sepsis-3 criteria who are receiving broad-spectrum antibiotics at the time of enrollment. Block randomization (blocks of 4) will be performed with 1:1:1 random computer allocation of subjects to inulin vs placebo. **Population** A total of 90 patients will be enrolled who complete both the baseline and Day 3 samples. If there are participant who are randomized but do not complete the Day 3 sample (e.g., because they die or decline to continue in the study) an additional participant will be enrolled to take their place. Participants will meet the following inclusion criteria: (1) hospitalized in a Columbia medical ICU, (2) 18 or more years old, (3) with sepsis, defined according to Sepsis-3 criteria, (4) on broad spectrum antibiotics, either ordered or received within the preceding 24 hours, (5) able to complete enrollment within 4 hours of ICU admission. The following are the exclusion

criteria: (1) inability to receive oral or enteric fluids, (2) inulin allergy (exceedingly rare), (3) hyponatremia with serum sodium < 128 mEq/L because the intervention comes with a small amount of free water, (4) immunosuppression, defined as CD4 count <200 cells/mm³, neutrophil count <500 cells/mm³, or reasonable expectation that either CD4 count or neutrophils will fall below these levels during the 7 day study intervention period, (5) surgery on the intestinal lumen within 30 days, because such patients may have poor fiber tolerance, (6) DNR/DNI status, (7) lack of capacity and no legally authorized representative.

COVID sub-study: Newly hospitalized (within 72 hours) patients 18 years old or more will be considered for enrollment if they test COVID+ and are receiving intravenous broad-spectrum antibiotics. Exclusion criteria will remain the same. Intervention

In the active study arms, chicory root-derived inulin (Cargill Inc., Wayzata, MN) will be diluted in 250 ml of sterile water and given at 16 g or 32 g/day in 2 divided daily doses. The placebo arm will receive the same volume of sterile water twice daily. The intervention will be given for 7 days or until death or hospital discharge (continued on the floor, if necessary). Subjects, investigators and the treating ICU team will be blinded to the intervention arm. The intervention will be taken orally or given with enteric feeding by nursing staff overseen by the study team, with the first dose administered within 6 hours of ICU admission. **Intervention Dose** In the ambulatory setting, inulin impacts SCFA producer levels at doses of 12 to 16 g/day, with corresponding biologic effects relevant for ICU patients (e.g., decreases in serum LPS and CRP).^{14,17} Approximately 16 g/day therefore represents the minimum dose at which an effect *might* be seen; this was selected for the low dose arm of the study. However, patients in these ambulatory studies were not critically ill and did not receive antibiotics. It is possible that inulin will have lesser effects in the ICU compared to the ambulatory setting, and logical to test higher doses. To select a maximum study dose, we referenced our own experience with dietary fiber in the prospective cohort and published tolerability data from ICU studies of inulin-like prebiotic fiber. In our prospective ICU cohort, 30 g/day was the maximum observed dose of dietary fiber, and was not associated with any intolerance. In published literature, O'Keefe *et al.* reported tolerance up to a maximum of 36 g/day wheat dextran in the ICU.¹⁵ We therefore selected 32 g/day for the high dose study arm because it lies between these values and is exactly double a dose with proven biologic activity.

Assessments Samples will be gathered and bedside assessments made at day 0/baseline, 3 days, 7 days, 14 days, and 30 days. Primary analyses will use the baseline and Day 3 assessments. The additional samples will (1) test whether effects can be extended through 7 days (Day 7 samples), (2) test durability of effects (Day 14 and 30 samples), and (3) allow visualization of time-series data. At each time point, the following samples and data will be gathered: rectal swabs, spontaneous whole stools, urine, residual blood, and clinical data. At 3 and 6 months after ICU discharge, a telephone follow-up phone call will be made to gather clinical information and assess long-term outcomes.

Outcomes The study will compare the inulin vs placebo groups to accomplish 3 aims: (1) to determine the impact of inulin on SCFA producers in the ICU, (2) to determine the impact of inulin on gut colonization with MDR bacteria in the ICU, and (3) to determine the impact of inulin on delivery of nutrition in the ICU.

REFERENCES:

14. Dewulf EM, Cani PD, Claus SP, et al. Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut* 2013;62:1112-21.
15. O'Keefe SJ, Ou J, Delany JP, et al. Effect of fiber supplementation on the microbiota in critically ill patients. *World J Gastrointest Pathophysiol* 2011;2:138-45.
17. Vandeputte D, Falony G, Vieira-Silva S, et al. Prebiotic inulin-type fructans induce specific changes in the human gut microbiota. *Gut* 2017;66:1968-74.

Statistical Procedures:

Provide sufficient details so that the adequacy of the statistical procedures can be evaluated including power calculations to justify the number of participants to be enrolled into the study. Definitions of subject terms such as enrolled and accrued as used for Rascal submissions can be found in the Subjects section.

[] Abbreviated Submission - This information is included in an attached stand-alone protocol. Proceed to the next question

The overall aims are (1) understand the effects of inulin on the “good” or commensal bacteria and the overall dynamics of the microbiome in the ICU, (2) understand the effects of inulin on “bad” bacteria focusing on MDR organisms, and (3) understand the impact (if any) of inulin on the delivery of nutrition in the ICU. **Primary outcome.** The primary outcome will be determined by testing for a difference in ICU Day 3 SCFA producer levels, comparing any one inulin group to the placebo group. SCFA producer levels will be the proportion of reads assigned to producers of the SCFA butyrate as measured by whole genome sequencing of Day 3 rectal swabs. (This refers to sequencing of the bacterial genome, and no human sequencing will be done as part of this study.) Under this Aim, comprehensive “multi-omic” analyses will be conducted from rectal swabs, urine, blood, and stool to understand the dynamics of the microbiome, the effects of inulin, and the effects of other ICU interventions (e.g., antibiotics, opioids). A modified intention-to-treat approach will be used with subjects who receive 1 or more doses of the intervention analyzed under this approach. The study has been powered to detect a difference in SCFA producer levels that is similar to the observed difference in SCFA producer levels from our prospective ICU cohort. The rationale for this is that inulin must be at least as impactful as mixed dietary fiber to be a viable therapeutic. The proposed sample size of 30:30:30 will yield 80% power to detect a minimum difference of 0.32 standard deviations (SD) of within-individual change in SCFA producer levels, using an F-test with 0.05 significance level. In addition, the sample size will yield 80% power to detect a difference of 0.63 SD comparing any one inulin arm to the placebo arm using a two-sided t-test with 0.05 significance level and assuming equal variance. If the inulin arms have similar changes in SCFA producer levels, they will be combined and the study would have 80% power to detect a minimum 0.55 SD for inulin compared to placebo at the .05 significance level. **COVID sub-study: The main protocol and COVID substudy will be separately analyzed when the studies are complete. Additional outcomes.** Aim 2 of the study will test for a difference in ICU Day 3 MDRO colonization status, comparing any one inulin group to the placebo group. MDRO colonization will be classified categorically (colonized vs not colonized) based on the results of culture for VRE, MRSA, and MDR Gram negatives. VRE and MRSA will be defined as present if they grow on selective (i.e., antibiotic-impregnated) media. MDR Gram negatives will be defined as present if they grow on MacConkey agar and show non-susceptibility to ceftriaxone using standard cut-offs. Aim 3 of the study will test for a difference in the ICU Day 3 proportion of goal

calories consumed, comparing any one inulin group to the placebo group. Goal calories consumed will be defined as the observed calories delivered divided by the patient-specific caloric requirement estimates which will be generated by a dietician using the Mifflin St. Jeor equation with the Penn State modification.

Exempt and Expedited

Is the purpose of this submission to obtain an exemption determination, in accordance with 45CFR46.101(b):

No

Is the purpose of this submission to seek expedited review, as per the federal categories referenced in 45CFR46.110?

No

Funding

Is there any external funding or support that is applied for or awarded, or are you the recipient of a gift, for this project?

Yes

Award Type	Funding Source Name	Name of awarding agency	Status	Award # or Application Date	Federal/State /Local Government Direct or Subcontract	What is the award covering?	Rascal PT Number
Federal/State/Local Government	DoD PRMRP CTA	Department of Defense	Awarded/Received	PR181960	Direct Recipient: No Subcontract Sites	Entire Protocol	PT-AA07879

Locations

Location Type	Facility Name	Domestic or International	Geographic Location	Local IRB Ethics Approval	Local Site Approval
NewYork-Presbyterian Hospital @ Columbia	Allen Pavilion ICU (Allen 2RE, 5141 Broadway)				
Columbia/CUMC	Mailman School, Rosenstein 7: Columbia Data Coordinating Center				
NewYork-Presbyterian Hospital @ Columbia	Milstein 3 MICA, MICB: enrollment of subjects				

Location Type	Facility Name	Domestic or International	Geographic Location	Local IRB Ethics Approval	Local Site Approval
NewYork-Presbyterian Hospital @ Columbia	Neurological ICU (Milstein 8th floor Garden South)				
Columbia/CUMC	P&S 3-401: analysis of data in PI's office				
Columbia/CUMC	P&S 8: storage of samples in PI's freezer unit				
Columbia/CUMC	P&S 9: processing of samples in Department of Medicine Microbiome Core Lab				

Personnel

UNI/Phone	Name	Role	Department	Edit/View	Obtaining Informed Consent
def2004 857-998-9370	Freedberg, Daniel	Principal Investigator	MED Digestive & Liver Diseases (751870X)	Edit	Y
Roles and Experience: Dr. Freedberg is the PI on the study and has extensive human subjects experience, including several years of experience enrolling ICU patients.					
el2888 412-779-8369	Lynch, Elissa	Other Engaged Personnel	MED Digestive & Liver Diseases (751870X)	Edit	Y
Roles and Experience: Clinical Research Nurse working with the PI and former NYP-Columbia RN.					
ep2681 212-305-4837	Poptanich, Erin	Non-Engaged Personnel	PAT Pathology (753800X)	View	N
Roles and Experience: CALM Lab member who will be helping with retrieval of left over blood samples.					
jn355 212-305-4837	Netterwald, Jane	Non-Engaged Personnel	PAT Pathology (753800X)	View	N
Roles and Experience: CALM Lab member who will be helping with retrieval of left over blood samples.					
rmw2126 443-857-0421	Wahab, Romina	Investigator	MED Pulmonary (751960X)	View	N
Roles and Experience: Dr. Wahab is an ICU physician with over 5 years in independent practice. She is permanently based at the Allen ICU (AICU) so we are adding her to the study personnel so that we have an on-site person at AICU if any issues arise and Dr. Freedberg cannot come up.					

Training and COI

The PI must ensure that each individual that is added as personnel has met the training requirements for this study (<http://www.cumc.columbia.edu/dept/irb/education/index.html>). For help identifying which research compliance trainings you may be required to take, visit the [Research Compliance Training Finder](#).

UNI	Name	COI	HIPAA	HSP (CITI)	Research with Minors (CITI)	FDA-Regulated Research (CITI)	S-I	CRC	Good Clinical Practice (GCP)	GCP - Third-party tracking	GCP Refresher	Genetic Research Consent



def2004	Freedberg, Daniel	08/24/2021	02/28/2011	01/06/2020	05/02/2014	01/06/2020	03/23/2020		01/06/2020			
el2888	Lynch, Elissa	09/20/2021	08/07/2019	08/07/2019		08/16/2019	08/07/2019	04/29/2020				
ep2681	Poptanich, Erin	10/12/2021	12/18/2017	01/11/2021	12/18/2017	12/18/2017	12/19/2017	12/20/2017	01/11/2021			
jn355	Netterwald, Jane	08/26/2021	04/02/2013	11/07/2019	11/07/2019	11/07/2019		08/21/2014	12/11/2019			
rmw2126	Wahab, Romina	05/24/2021	05/25/2009	12/03/2019	07/24/2020	07/24/2020			10/28/2020			

Departmental Approvers

Electronic Signature: Daniel Freedberg (751870X) -
Principal Investigator

Date: 02/09/2022

Privacy & Data Security

Indicate the methods by which data/research records will be maintained or stored (select all that apply):

☐ Hardcopy (i.e., paper)

☒ Electronic

Where will the data be stored?

Y

☐ On a System

☒ On an Endpoint

Identify what type of endpoint will be used (select all that apply):

☒ Desktop Computer

☐ Laptop Computer

☐ Mobile Device

☐ Other

Does this study involve the receipt or collection of Sensitive Data?

Yes

If any Sensitive Data is lost or stolen as part of your research protocol, you must inform both the IRB and the appropriate IT Security Office (CUMC IT Security if at CUMC; CUIT if at any other University campus).

What type of Sensitive Data will be obtained or collected? Select all that apply:

☐ Personally Identifiable Information (PII), including Social Security Numbers (SSN)

Will Social Security Numbers (SSNs) be collected for any purpose?

☒ Protected Health Information (PHI), including a Limited Data Set (LDS)

If any PHI is lost or stolen, you must inform both the IRB and the Office of HIPAA Compliance.

Indicate plans for secure storage of electronic sensitive data: check all that apply

- ☐ Sensitive data will not be stored in electronic format
- ☐ Sensitive data will be stored on a multi-user system
- ☒ Sensitive data will be stored on an encrypted endpoint

By Selecting an Endpoint Device and approving this protocol for submission to the IRB, the PI is attesting that the device and any removable media that may be used have been or will be registered and/or will be maintained in compliance with the University's Information Security Charter and all related policies. It is important that this information is updated, during the course of the study, as new devices are added.

Provide a description of how the confidentiality of study data will be ensured, addressing concerns or protections that specifically relate to the data storage elements identified above (e.g. hard copy, electronic, system, and/or endpoint):

Study data will be stored electronically or on paper CRFs in a coded form, and only the PI and the research study coordinator will have access to the linking file that provides the code. This password-protected linking file will contain MRNs (the only directly identifiable data that we will retrieve) and random alphanumeric identifiers. The main data file will not contain direct identifiers. Both files will be stored on encrypted Columbia endpoints.

If your project is not NIH funded, has a Certificate of Confidentiality (CoC) been requested for this research?

No

Provide a description of the protections in place to safeguard participants' privacy while information is being collected:

Information will be collected from the electronic medical record or by direct visualization of the participant. This information will be recorded electronically, and the safeguards for the security of this information are described above under the Data Security section. The only situation in which information will be collected by asking the participant questions is at the 3 and 6 month telephone follow-up interviews regarding overall health and functional status. These phone calls will be made from a locked private research office and we will begin by asking "are you in a place where you can speak privately?" in order to minimize the potential for loss of privacy while describing current health status. We recognize that privacy could potentially be compromised by compelled disclosure of research notes or by mandatory reporting.

Procedures

Is this project a clinical trial?

Yes

Is this project a clinical trial that requires registration with www.clinicaltrials.gov?

Yes

Has this study been registered with www.clinicaltrials.gov?

Yes

Please provide the registration number:

NCT03865706

Is this project associated with, or an extension of, an existing Rascal protocol?

No

Do study procedures involve any of the following?

Analysis of existing data and/or prospective record review

No

Audio and/or video recording of research subjects

No

Behavioral Intervention?

No

Biological specimens (collection or use of)

Yes

Cancer-related research

No

Drugs or Biologics

Yes

Future use of data and/or specimens

Yes

Genetic research

No

Home Visits

No

Human embryos or human embryonic stem cells

No

Imaging procedures or radiation

No

Medical Devices

No

Surgical procedures that would not otherwise be conducted or are beyond standard of care

No

Will any of the following qualitative research methods be used?

Survey/interview/questionnaire

Yes

NOTE: You must attach a PDF version of the survey(s)/interview(s)/questionnaire(s) to this protocol prior to submission.

Systematic observation of public or group behavior

No

Program evaluation

No

Will any of the following tests or evaluations be used?

Cognitive testing

No

Educational testing

No

Non-invasive physical measurements

No

Taste testing

No

Is there an external protocol that describes ALL procedures in this study?

No

Please describe ALL study procedures in detail.

NOTE: Be sure to detail all of the procedures above to which a "yes" response was selected. Also detail any additional procedures that may or may not fall into the categories listed above.

Study enrollment

Newly admitted ICU patients will be identified at the time of ICU bed allocation through the assistance of ICU personnel, a strategy we have used in past studies. This allows rapid identification of potential subjects so that they can be quickly reached for the intervention. After consultation with the treating ICU team, subjects who are thought by the medical team to be eligible will have the final, formal eligibility evaluation completed at the bedside. Subjects will only be approached after consent has been obtained from the attending physician of record (i.e., the ICU attending who is on duty). We anticipate that >50% of patients will lack decision-making capacity and will accept consent from a Legally Authorized Representative (LAR) acting as the patient's surrogate in these situations. This is a common approach in ICU trials and our other trials take the same approach.

Study assessments

Samples will be gathered and bedside assessments made at day 0/baseline, 3 days, 7 days, 14 days, and 30 days. Primary analyses will use the baseline and Day 3 assessments. The additional samples will (1) test whether effects can be extended through 7 days (Day 7 samples), (2) test durability of effects (Day 14 and 30 samples), and (3) allow visualization of time-series data. At each time point, the following samples and data will be gathered: rectal swabs, spontaneous whole stools, urine, residual blood, and clinical data. At 3 and 6 months after ICU discharge, a telephone follow-up phone call will be made to gather clinical information and assess long-term outcomes. Biologic samples are described below. For the clinical data gathered, case report forms for each study assessment will be completed which document adverse events, long-term origin (e.g., nursing home), short-term origin (e.g., hospital floor), the Glasgow Coma Scale (GCS), the use of life support devices including mechanical ventilation, hemodialysis, or additional life-support devices such as extra-corporeal membrane oxygen (ECMO) or an intra-atrial balloon pump (IABP); and the presence or absence of intravenous lines or catheters including a central venous catheter, urinary catheter, arterial catheter, nasal or oral gastric tube, or rectal tube. Using duplicate data entry, anonymized CRF data will be transferred into a secure REDCap database custom-designed for the trial by the CUMC Data Coordinating Center. Additional variables will be entered directly into REDCap including the component variables of the SOFA score and measures related to nutritional intake. At 3 and 6 months, telephone interviews will be conducted with subjects or their surrogates to ascertain vital status (1) discharge location type (home, short-term rehabilitation, or long-term rehabilitation), (2) hospital admission or physician office visits after ICU discharge, (3) infections or other adverse events after ICU discharge. If there are non-CUMC healthcare interactions, permission will be requested to obtain relevant records. Among surviving subjects, the EQ-5D-5L (EuroQol 2009) and Katz' Index of Independence in ADLs (Activities of Daily Living) will be used to determine function.

COVID sub-study:

Samples will be collected and placed directly into freezer-safe containers containing EPA-approved viral inactivation media. Samples will be triple bagged for transport to the freezer facility by appropriately trained personnel.

Biological Specimens

Add an individual entry for each human specimen type that will be collected or utilized for the proposed study. For each specimen type, indicate the source or sources from which you will obtain the specimens.

The use of specimens for research purposes may require that informed consent (or a waiver, if applicable) and HIPAA Authorization (or a waiver, if applicable) be obtained from subjects.

Type:

Stool

Source:

☒ From Columbia and/or NYP Subjects/Patients or Repositories managed by Columbia

Select all that apply to this specimen type:

☐ Specimens will be prospectively collected specifically for this research.

☒ Residual specimens from clinical care that would otherwise be discarded have been or will be collected.

☐ Specimens to be analyzed will be (or have been) collected from a commercial source.

☐ Specimens to be analyzed will be (or have been) collected under a separate CU IRB-approved protocol; this could be an approved research repository protocol.

☐ From Non-Columbia/NYP Subjects/Patients or Repositories not managed by Columbia

Description of Specimen and Method of Obtaining:

At each study assessment, the study team will work with nurses to gather the first stool produced after each study assessment. In ICU patients, these stools are routinely gathered using a bedpan and then discarded. The study team will intercept the stools prior to discard and scoop out an 8 g aliquot.

Indicate the manner in which the specimens will be labeled:

☐ Specimens will be labeled with direct identifiers

☒ Specimens will be labeled with a code and the research team has the key and can link specimens to direct identifiers. This code would be considered an indirect identifier

☐ The identifiers will be removed prior to the receipt of the specimens by Columbia researchers and no link will remain

☐ Specimens were originally collected without identifiers

If specimens are collected or received at any point in time as with direct or indirect identifiers by the current researchers, then the specimens are considered to be identifiable, and the requirements for Informed Consent (or a waiver, if applicable) and HIPAA Authorization (or a waiver, if applicable) apply. The necessary information will need to be included in the respective sections of this Rascal submission.

Type:

Blood

Source:

☒ From Columbia and/or NYP Subjects/Patients or Repositories managed by Columbia

Select all that apply to this specimen type:

☐ Specimens will be prospectively collected specifically for this research.

☒ Residual specimens from clinical care that would otherwise be discarded have been or will be collected.

☐ Specimens to be analyzed will be (or have been) collected from a commercial source.

☐ Specimens to be analyzed will be (or have been) collected under a separate CU IRB-approved protocol; this could be an approved research repository protocol.

☐ From Non-Columbia/NYP Subjects/Patients or Repositories not managed by Columbia

Description of Specimen and Method of Obtaining:

At each study assessment, the study team will work with the CALM laboratory to obtain leftover clinical blood samples for future analyses. We will collect no more than 6 mL of residual blood from a yellow-top tube and no more than 4 mL of residual blood from a lavender-top (EDTA) tube. There will be no blood draws as part of this study.

Indicate the manner in which the specimens will be labeled:

☐ Specimens will be labeled with direct identifiers

☒ Specimens will be labeled with a code and the research team has the key and can link specimens to direct identifiers. This code would be considered an indirect identifier

☐ The identifiers will be removed prior to the receipt of the specimens by Columbia researchers and no link will remain

☐ Specimens were originally collected without identifiers

If specimens are collected or received at any point in time as with direct or indirect identifiers by the current researchers, then the specimens are considered to be identifiable, and the requirements for Informed Consent (or a waiver, if applicable) and HIPAA Authorization (or a waiver, if applicable) apply. The necessary information will need to be included in the respective sections of this Rascal submission.

Type:

Urine

Source:

☒ From Columbia and/or NYP Subjects/Patients or Repositories managed by Columbia

Select all that apply to this specimen type:

☒ Specimens will be prospectively collected specifically for this research.

☐ Residual specimens from clinical care that would otherwise be discarded have been or will be collected.

☐ Specimens to be analyzed will be (or have been) collected from a commercial source.

☐ Specimens to be analyzed will be (or have been) collected under a separate CU IRB-approved protocol; this could be an approved research repository protocol.

☐ From Non-Columbia/NYP Subjects/Patients or Repositories not managed by Columbia

Description of Specimen and Method of Obtaining:

At each study assessment, 15 mL of freely voided or catheterized urine will be gathered.

Indicate the manner in which the specimens will be labeled:

☐ Specimens will be labeled with direct identifiers

☒ Specimens will be labeled with a code and the research team has the key and can link specimens to direct identifiers. This code would be considered an indirect identifier

☐ The identifiers will be removed prior to the receipt of the specimens by Columbia researchers and no link will remain

☐ Specimens were originally collected without identifiers

If specimens are collected or received at any point in time as with direct or indirect identifiers by the current researchers, then the specimens are considered to be identifiable, and the requirements for Informed Consent (or a waiver, if applicable) and HIPAA Authorization (or a waiver, if applicable) apply. The necessary information will need to be included in the respective sections of this Rascal submission.

Type:

Other

Rectal swab

The Anatomic Pathology approval request form and guidance on requirements to attach the form can be found on the HRPO website, in the "Biospecimen Research" section of the Human Research Policy Guide page: <https://research.columbia.edu/human-research-policy-guide>

Source:

☒ From Columbia and/or NYP Subjects/Patients or Repositories managed by Columbia

Select all that apply to this specimen type:

☒ Specimens will be prospectively collected specifically for this research.

☐ Residual specimens from clinical care that would otherwise be discarded have been or will be collected.

☐ Specimens to be analyzed will be (or have been) collected from a commercial source.

☐ Specimens to be analyzed will be (or have been) collected under a separate CU IRB-approved protocol; this could be an approved research repository protocol.

☐ From Non-Columbia/NYP Subjects/Patients or Repositories not managed by Columbia

Description of Specimen and Method of Obtaining:

IRB-AAAS2576

At each study assessment, duplicate flocked nylon swabs (Copan Diagnostics) will be performed with the patient in the left lateral decubitus position. These soft swabs are gently rubbed against the anal canal to gather fecal debris.

Indicate the manner in which the specimens will be labeled:

- ☐ Specimens will be labeled with direct identifiers
- ☒ Specimens will be labeled with a code and the research team has the key and can link specimens to direct identifiers. This code would be considered an indirect identifier
- ☐ The identifiers will be removed prior to the receipt of the specimens by Columbia researchers and no link will remain
- ☐ Specimens were originally collected without identifiers

If specimens are collected or received at any point in time as with direct or indirect identifiers by the current researchers, then the specimens are considered to be identifiable, and the requirements for Informed Consent (or a waiver, if applicable) and HIPAA Authorization (or a waiver, if applicable) apply. The necessary information will need to be included in the respective sections of this Rascal submission.

Drugs/Biologics

On the General Information page you have indicated that the protocol version associated with the use of this drug/biologic is as follows: 1

Please note that a Protocol Version # is required for protocols using a drug or biologic, and you will not be allowed to submit this protocol until the Protocol Version # field is complete. Please ensure that the Protocol Version # is completely and accurately reported on the General Information page.

List each drug or biologic that will be administered as the object of the protocol or is being used because it is relevant to the aims of the research protocol. This applies whether the drug/biologic is not yet FDA-approved (i.e., is investigational), is FDA approved and used in accordance with its labeling, or is an approved product that is being used in an investigational manner (i.e., off-label use is being studied).

Note that the questions apply only to drugs used in clinical investigations. Emergency use of a drug that is not yet FDA-approved is not a clinical investigation, and a submission in Rascal may not be required. Please contact the IRB for assistance if emergency use of a drug or biologic that is not yet FDA-approved is being considered: (212) 305-5883.

Name:

Chicory root-derived inulin in 250 mL sterile water

Dose:

16g/day or 32g/day in 2 divided doses

Study phase:

Phase 2

Manufacturer Information

Name: Cargill, Inc.

Address: 15407 McGinty Road West, Wayzata, MN 55391

Contact information: Cargill Inc. - 800-227-4455

Route of administration:

Oral

Is the drug/biologic FDA-approved and used in accordance with its labeling?

No

Select a category:

Not FDA-approved

Does the Use of the drug/biologic require an Investigational New Drug (IND) or Biological IND (BB-IND) application?

Yes

Select status of IND:

Accepted (e.g. IND# issued)

Provide IND#:

137359

Select type of IND holder:

Columbia University Faculty

Please note that the following documents will be required prior to final approval of this protocol:

- Most recent protocol submitted to the FDA (required before IRB review)
- FDA Form 1571
- FDA Form 1572
- FDA acknowledgment letter or FDA "Safe to Proceed" documentation, including the conditional approval letter if one has been received
- Form of Notice by CU Faculty IND/IDE Holder

Please describe how the responsibilities of the IND holder, who is considered per FDA regulations to be the sponsor, will be met. Elements to be included are described in the Help Text. If all elements are in an attached stand-alone protocol, please so indicate by selecting that statement below, in which case, entry in the text box is not required.

☒ Plans to address Sponsor requirements - IRB approval, monitoring, compliance with the IRB-approved protocol, evaluating safety and efficacy, recordkeeping, submitting amendments, IND Safety Reports and Annual Reports to the FDA, and submitting required reports to the IRB - are in the attached stand-alone protocol.

IND/BB-IND holder name:

Julian Abrams and Daniel Freedberg

IND/BB-IND holder address:

Columbia University Medical Center, 630 WEST 168TH STREET, P&S 3-401, New York, NY 10032

IND/BB-IND holder contact info:

Daniel Freedberg 212-342-3302

Will the drug/biologic be dispensed by the CUMC Research Pharmacy, which is responsible for the storage, handling, accountability, and dispensing of investigational drugs to research investigators? CUMC Research Pharmacy policy: <https://researchpharmacy.cumc.columbia.edu/policies.html>

Yes, I confirm the drug will be dispensed by the Research Pharmacy

Future Use

For what materials do you anticipate future research use? Select all that apply.

☒ Data

☒ Biological Specimens

Please indicate how data and/or specimens will be retained for future use:

IRB-AAAS2576

Page 16 of 29

[x]Some or all data and/or specimens, as applicable, will be retained by Columbia researchers for future use.

How are the materials intended to be used for research in the future?

Current PI will retain the materials and there is no intent to create a repository or share with other CU researchers. Note: Information provided in original consent forms will be considered when an addition of future uses is submitted via modification.

What future uses are anticipated?

Spin-off studies using samples and data are likely, to appropriately follow-up on the findings of this trial. All future use of samples and data will involve de-identified materials - only the PI will have access to identifiers.

How will the data and/or specimens, as applicable, be labeled during storage for future uses.

[x]In the same manner as during collection (e.g., with direct identifiers, coded, de-identified, anonymous)

[]In a different manner than during collection. Select all that apply:

Describe the physical storage for the specimens/data, including location.

[x]In the same manner as during collection

[]In a different manner than during collection

Describe who will have access to the stored data and/or specimens.

Only the PI will have access to data/specimens at this time. He will share them with appropriate future researchers depending on the results of the trial.

[x]Some or all data/specimens will be released to a non-Columbia entity for future use and Columbia researchers will not have direct control.

Indicate to whom the data/specimens will be released

[]Sponsor

[]Non-Columbia repository

[x]Other

Describe

Collaborative researchers.

Describe plans for release of data and/or specimens.

We anticipate the possibility of future research collaborations outside of Columbia, depending on the results of the trial and the availability of expertise.

Recruitment And Consent

Recruitment:

Will you obtain information or biospecimens for purposes of screening or determining eligibility?

Yes

Select one of the following:

The investigator will obtain identifiable private information or identifiable biospecimens by accessing records or stored identifiable biospecimens.

Describe plans for screening and/or determining eligibility of prospective subjects:

During screening, investigators will work with the ICU team and look at the EMR of ICU patients in order to determine eligibility. No data will be retained during this screening process.

Describe how participants will be recruited:

The ICUs at Columbia are "open" units which means that all of the patients in the ICU are under the care of the ICU

attending at that time. Within these units, we will identify potentially eligible patients at the time of ICU admission by working with unit coordinators who are aware of new admissions at the time of bed assignment (i.e., slightly before the patient is actually transferred into that ICU bed). At this time there are plans to do this on weekdays only and during standard business hours. After prescreening patients for eligibility against the inclusion/exclusion criteria through the unit coordinators, we will ask the ICU attending if he or she feels that the patient is appropriate for the study and, if so, if they would provide the patient/surrogate with information about the study (default) or, if not, if they would ask the patient/surrogate if we can approach them and, if yes, document this. We will always check with the ICU attending before approaching the patient. We anticipate that >50% of patients will lack decision-making capacity and we will consent from a surrogate in these situations, with the surrogate providing written (signed) informed consent. Surrogates who are not available at the ICU bedside immediately will be contacted by telephone and either asked to meet at the bedside or sent a facsimile copy of the consent form to review and return if they are not in the area. The surrogate (or the subject if he or she regains capacity) will have the ability to withdraw at any time. Issues related to altered capacity, privacy and time for decision-making, consent of LARs, and ongoing consent are discussed in detail below. Once ICU permission has been obtained and eligibility criteria have been met, the patient or surrogate will be approached regarding the study at the earliest possible moment that is appropriate clinically and preserves privacy.

COVID sub-study:

Because of safety concerns and restrictions to direct access to patients, patients will be approached for consent remotely by telephone or WebEx so that study personnel only enters the room after the patient has given verbal consent. After verbal consent is obtained in the patient's room, the patient will sign standard print consent forms which will be placed in a clear ziplock plastic bag with the signature page upright. Before it is taken from the room, the bag will be wiped with bleach wipes to decontaminate it, soaking the exterior surfaces for >30 seconds on each side. Outside the room, this bag will then be placed into a second ziplock bag. If a surrogate gives consent and is on quarantine, faxed consent forms will be used to avoid face-to-face contact with the surrogate. When patients are enrolled, the date and time of enrollment will be entered into the research record and documented on the consent form.

Select all methods by which participants will be recruited:

- ☐ Study does not involve recruitment procedures
- ☒ Person to Person
- ☐ Radio
- ☐ Newspapers
- ☐ Direct Mail
- ☐ Website
- ☐ Email
- ☐ Television
- ☐ Telephone
- ☐ Flyer/Handout
- ☐ Newsletter/Magazine/Journal
- ☐ ResearchMatch
- ☐ CUMC RecruitMe

Additional Study Information: Please add a description of your study as you would like it to be displayed on the RecruitMe website.

Informed Consent Process:

Informed Consent Process, Waiver or Exemption: Select all that apply

☒ Informed consent with written documentation will be obtained from the research participant or appropriate representative.

Documentation of informed consent is applicable to:

The study in its entirety

Identify the portion of the study (e.g., prospective portion, focus groups, substudy 2) or subject population for which documentation of consent will be obtained::

Subjects with capacity or LARs who are physically present at the bedside.

Documentation of participation will be obtained from::

☒ Adult participants

☐ Parent/Guardian providing permission for a child's involvement

☒ Legally Authorized Representatives (LARs)

Describe how participants' written consent will be obtained:

Study personnel will concisely describe the study and what it involves. The consent form will be given to subjects or surrogates in a manner such that they have the opportunity to review it carefully and discuss the information provided.

For any illiterate subjects or surrogates, the study will be briefly described and the consent form will be read verbatim. The information presented will be easily understandable to laypeople regardless of education level but will be sufficiently detailed such that the entire study and its aims are comprehensible.

☐ Informed consent will be obtained but a waiver of written documentation of consent (i.e., agreement to participate in the research without a signature on a consent document) is requested.

☐ A waiver of some or all elements of informed consent (45 CFR 46.116) is requested.

☐ Planned Emergency Research with an exception from informed consent as per 21 CFR 50.24.

☐ This is exempt research.

Subject Language

Enrollment of non-English speaking subjects is expected.

Languages anticipated:

Spanish

As you plan on enrolling non-English speaking subjects, administrative IRB approval of the translated documents (e.g., consent, recruitment materials, questionnaires) in the above selected languages are required. Please see the IRB's policy on the Enrollment of Non-English Speaking Subjects in Research for further details

(<http://www.cumc.columbia.edu/dept/irb/policies/documents/Nonenglishspeakingsubjects.Revised.FINALDRAFT.111909.website.doc>).

Capacity to Provide Consent:

Do you anticipate using surrogate consent or is research being done in a population where capacity to consent may be questionable?

Yes

Who will be enrolled?

Those with and/or without capacity to provide their own consent (surrogate consent is proposed)

Describe the process that will be in place to identify an appropriate surrogate to provide consent:

In patients who lack capacity to give informed consent, the LAR who is acting as the patient's surrogate will be determined in accordance with New York State law as defined in the Family Health Care Decision Act. In brief, this law states that the procedure to determine the LAR who may act as surrogate is in the following order of priority: First, an individual who is designated as the representative or agent through a healthcare proxy that is properly executed. Second, if a designated surrogate does not exist, reasonably willing and competent people in the following order of priority: (A) the spouse (if not legally separated) or the domestic partner; (B) a son or daughter 18 years of age or older; (C) a parent; (D) a brother or sister 18 years of age or older; (E) a close friend (meaning a person 18 years of age or older who has maintained such regular contact with the subject as to be familiar with the subject's activities, health and beliefs). In practice, identification of surrogates is common in the ICU where patients frequently lack capacity and consent must be obtained for invasive procedures such as the intubation and mechanical ventilation or the placement of central venous lines.

Describe the plan to assess capacity both at the time of enrollment and, if applicable, throughout each subject's participation:

Assessment of capacity. Capacity will be assessed by the treating ICU team rather than study personnel, and study personnel will reference the ICU's determination of capacity. If there is any question of a possible change in decision-making capacity since the ICU team's assessment, the study team will directly confer with the ICU. The ICU's standard approach for assessment of capacity is that the patient (1) is able to communicate; (2) can understand their treatments and alternatives relating to their hospital care and participation in the proposed study or intervention (the patient must be able to repeat back in his/her own words an explanation of treatment plan or study, the risks involved, and the alternative to not participate); (3) can grasp that there are no consequences to declining the study; (4) has ability to reason (can express their goals and values in participating in a study that seeks to reduce risk for infections in patients like themselves).

Ongoing consent. When subjects have decision-making capacity, their willingness to participate in the study will be reassessed at each study visit by asking the question, "Do you wish to continue in the study?" Subjects who answer "No" will be immediately withdrawn from the study. When subjects do not have decision-making capacity, their surrogates will be provided with the investigators' telephone contact information at the time of initial consent and instructed that they should call at any time to withdraw subjects from the study.

Ongoing assessment of capacity. All subjects with surrogate consent will be assessed for capacity throughout the rest of their hospital stay at each study visit. If these subjects regain capacity, they will consent or decline to continue to participate at that time. Again, the ICU team's determination of capacity

will be utilized, and the ICU team will be directly consulted if there is any question regarding a change in capacity. If subjects with surrogate consent never regain capacity by the time of discharge because of death or neurological disability, then surrogate consent will be accepted as final.

Research Aims & Abstracts

Research Question(s)/Hypothesis(es):

The overall aim of this study is to perform a randomized, double-blind trial of inulin versus placebo for the purpose of testing whether inulin prevents gastrointestinal colonization of multidrug-resistant bacteria. The specific hypotheses that will be tested are: (1) Inulin increases SCFA producers and fecal SCFA levels during critical illness; (2) Inulin decreases gut MDRO colonization and infection during critical illness; and (3) Inulin does not diminish caloric intake during critical illness and is well tolerated.

Scientific Abstract:

The gastrointestinal microbiome checks the expansion of pathogens through a process known as colonization resistance. Loss of colonization resistance permits the expansion of pathogens including vancomycin-resistant *Enterobacteriaceae* (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), and multidrug resistant (MDR) Gram negative bacteria and leads to subsequent infections with these and other difficult-to-treat MDR organisms. Nowhere is this more important than in the intensive care unit (ICU), where antibiotic-resistant infections are the leading cause of morbidity and mortality. Currently, there are no strategies designed to preserve colonization resistance in the ICU or similar at-risk settings, and this trial will test the use of a vegetable derived soluble fiber (inulin) for this purpose. Dietary fiber is the most important driver of gut microbiome composition in healthy individuals and is the primary fuel substrate for commensal colonic anaerobes. Fiber-metabolizing gastrointestinal bacteria generate short-chain fatty acids (SCFAs) which moderate colonic inflammation, contribute to the thickness of the colonic mucus layer, and directly resist enteric pathogens. Our preliminary data from critically ill adults suggest that depletion of fiber-metabolizing bacteria from the gut results in loss of colonization resistance. Ironically, standard practice is to minimize prebiotic fiber in the ICU based on the assumption that fiber will be poorly tolerated. We hypothesize that inulin will be well tolerated and will favorably alter gut microbiome colonization resistance even in the setting of antibiotics to decrease MDR pathogen colonization in the ICU. The *short-term objective* of this research is to test inulin in the ICU by randomization of 120 critically ill adults to 16 or 32 g/day inulin or placebo. The *long-term objective* of the research is to contribute to knowledge which will lead to the development of successful, gut microbiome-based strategies to prevent pathogen colonization and infection in the ICU. The results of these Aims will provide data in support of definitive subsequent trials. We believe that fiber is a novel and readily accessible therapy that can rapidly be translated into an intervention to reduce pathogen colonization and the spread of antibiotic resistant infections in the ICU.

Lay Abstract:

Trillions of bacteria coexist with humans, primarily within our gastrointestinal tracts. Normally,

"good" gut bacteria have a symbiotic relationship with us and one of their main functions is to keep down disease-causing bacteria. This is particularly important in the intensive care unit (ICU), where normal bacteria are often depleted by antibiotics and where infections by disease-causing bacteria are very common and deadly. Prebiotic fiber is a major component of all plant-based foods and is the main fuel for gut bacteria. Studies show that, after antibiotics, how much fiber you eat is the most important controller of the types and amounts of bacteria that live inside you. Fiber is natural and is well tolerated and safe even in very sick patients. This study will test one type of fiber called inulin, which exists in large amounts in unprocessed chicory root, garlic, onions, and other vegetables. We already know that inulin promotes good bacteria in healthy volunteers. The goal of the study is to understand whether inulin will also promote good bacteria even in ICU patients who are getting antibiotics and many other powerful medical interventions. In this setting, are the good bacteria increased by inulin and do the bad bacteria diminish? Beyond testing inulin, we want to understand how bacteria interact in the ICU to cause health or disease so that in the future we can develop therapies (either inulin or other therapies) that will prevent ICU infections.

Risks, Benefits & Monitoring

Abbreviated Submission:

The IRB has an abbreviated submission process for multicenter studies supported by industry or NIH cooperative groups (e.g., ACTG, HVTN, NCI oncology group studies, etc.), and other studies that have a complete stand-alone protocol. The process requires completion of all Rascal fields that provide information regarding local implementation of the study. However, entering study information into all of the relevant Rascal fields is not required, as the Columbia IRBs will rely on the attached stand-alone (e.g., sponsor's) protocol for review of the overall objectives. .

If you select the Abbreviated Submission checkbox and a section is not covered by the attached stand-alone protocol, you will need to go back and provide this information in your submission.

Potential Risks:

Provide information regarding all risks to participants that are directly related to participation in this protocol, including any potential for a breach of confidentiality. Risks associated with any of the items described in the Procedures section of this submission should be outlined here if they are not captured in a stand-alone protocol. Risks of procedures that individuals would be exposed to regardless of whether they choose to participate in this research need not be detailed in this section, unless evaluation of those risks is the focus of this research. When applicable, the likelihood of certain risks should be explained and data on risks that have been encountered in past studies should be provided.

☐ Abbreviated Submission - This information is included in an attached stand-alone protocol. Proceed to the next question

Foreseeable risks fall into three categories: (1) risks related to the study intervention, (2) risks related to the gathering of study samples, and (3) risks related to the privacy and confidentiality of data. (1) Risks related to the study intervention. Inulin has been studied extensively in ambulatory patients or healthy volunteers where it has been safe. The main reported adverse effect is self-resolving bloating. A literature review identified a total of 12 published ICU-based trials of fiber tested alone or in combination including 1,295 subjects. Several studies tested inulin in combination with other fibers or in combination with probiotics (living bacteria) but no ICU studies

tested inulin alone. The ICU trials of mixed fiber did not test fiber for the purpose of preventing pathogen colonization, but they did monitor carefully for adverse effects. In these trials, there were no adverse effects from fiber other than self-resolving abdominal distension and higher residual gastric volumes. Based on these data, the primary anticipated risk from fiber is bloating/abdominal distension which is expected to resolve without intervention. Tolerability will be carefully monitored as part of this study and will be an important consideration for future trials.

(2) Risks related to study samples. All of the study samples are minimally invasive. Specific risks are as follows: · *Flocked rectal swabs.* There is the potential risk for discomfort and embarrassment when the rectal swab is performed, for rectal irritation or minor trauma in the setting of preexisting peri-rectal pathology (e.g., hemorrhoids), or for problems related to turning the subject in subjects who lack the ability to turn themselves. · *Whole stools.* There is the potential risk for embarrassment related to fecal testing. · *Urine.* There is the potential risk for discomfort related to manipulation of urinary catheters or for embarrassment related to clean-catch voiding of urine. · *Venous blood.* Residual blood will be used so the only risk related to the blood sample is loss of privacy. (3) Risks related to the privacy and confidentiality of data. There is the potential for loss of privacy as a consequence for taking part in this study, both related to the collection/labelling of samples and related to the collection and storage of clinical data. Measures are in place to minimize these risks.

Potential Benefits:

Provide information regarding any anticipated benefits of participating in this research. There should be a rational description of why such benefits are expected based on current knowledge. If there is unlikely to be direct benefit to participants/subjects, describe benefits to society. Please note that elements of participation such as compensation, access to medical care, receiving study results, etc. are not considered benefits of research participation.

[] Abbreviated Submission - This information is included in an attached stand-alone protocol. Proceed to the next question

This study was not designed to directly benefit participants. Future ICU patients may benefit from the knowledge gained. There is no financial compensation for participating in the study.

Alternatives:

If this research involves an intervention that presents greater than minimal risk to participants, describe available alternative interventions and provide data to support their efficacy and/or availability. Note, participants always have the option not to participate in research.

[] Abbreviated Submission - This information is included in an attached stand-alone protocol. Proceed to the next question

There is no obligation to participate in this study and subjects/surrogates who do initially choose to participate can withdraw at any time. Participation or non-participation in the study will not alter patient care in any way, aside from receipt or non-receipt of the study intervention. We consider all ICU patients to be vulnerable because they are all critically ill. Accordingly, procedures are in place to minimize influence and coercion. Study staff will stress throughout the informed consent process that (1) the research team is not involved with the subject's care in any way and (2) that the subject or surrogate may choose not to participate or may withdraw from the study at any time without explanation.

Data and Safety Monitoring:

Describe how data and safety will be monitored locally and, if this is a multi-center study, how data and safety
IRB-AAAS2576

will be monitored across sites as well.

[] Abbreviated Submission - This information is included in an attached stand-alone protocol. Proceed to the next question

Daniel Brodie, the Director of Medical Intensive Care Units at our institution, will serve as the Data Safety Monitoring Officer (DSMO) and will oversee the study Data Safety Monitoring Board (DSMB). Before initiating research, the study will be registered on clinicaltrials.gov. Specific Adverse Events (AEs) have been pre-defined to ensure rigorous ascertainment (Aim 3). DSMB reports will be generated through REDCap, a secure, web-based data management system after 10, 20, 30, and 45 patients are enrolled. Toxicity monitoring will be performed using a frequentist approach at the same intervals. A complete Data and Safety Monitoring Plan (DSMP) is attached.

Subjects

Unless otherwise noted, the information entered in this section should reflect the number of subjects enrolled or accrued under the purview of Columbia researchers, whether at Columbia or elsewhere.

Target enrollment:

90

Number enrolled to date:

57

Number enrolled since the last renewal or, if this is the first renewal, since the initial approval:

25

Number anticipated to be enrolled in the next approval period:

15

Does this study involve screening/assessment procedures to determine subject eligibility?

No

Of the number of subjects enrolled, or the number accrued for interventional studies with a screening process:

How many remain on the study?

0

How many are off study?

57

How many completed the study?

42

Have any withdrawn of their own initiative?

No

Have any been removed by PI?

No

Have any been lost to follow-up?

No

Have any died while on study?

Yes

How many?

15

Please explain, including whether death was related to participation in this study:

The study patient population is acutely ill in the ICU. We consider any death within 30 days of enrolling in the

study to be an "on study death." The 15 deaths that occurred on study are consistent with general ICU mortality and were unrelated to the study intervention.

Have any subject complaints been received?

No

Is this a multi-center study?

No

Does this study have one or more components that apply to a subset of the overall study population (e.g. Phase 1/2, sub-studies)?

No

Of the number enrolled, or the number accrued for interventional studies with a screening process, indicate:

Population Gender

Females	Males	Non Specific
42%	58%	0%

Population Age

0-7	8-17	18-65	>65	Non Specific
0%	0%	49%	51%	0%

Population Race

American Indian/Alaskan Native	Asian	Native Hawaiian or Other Pacific Islander	Black or African American	White	More than One Race	Non-Specific
0%	0%	0%	0%	0%	0%	100%

Population Ethnicity

Hispanic or Latino	Not Hispanic or Latino	Non-Specific
0%	0%	100%

Vulnerable Populations as per 45 CFR 46:

Will children/minors be enrolled

No

Will pregnant women/fetuses/neonates be targeted for enrollment?

No

Will prisoners be targeted for enrollment?

No

Other Vulnerable Populations:

☒ Individuals lacking capacity to provide consent

Are the procedures this population will be exposed to minimal risk?

Yes

Please ensure that your plan for assessing capacity to provide consent is described on the Informed Consent page.

☐ CU/NYPH Employees/Residents/Fellows/Interns/Students

☐ Economically disadvantaged

☐ Educationally disadvantaged

☒ Non-English speaking

Please ensure that your plan to enroll subjects in their primary language is described on the Informed Consent page.

[x]Other Vulnerable populations

This is ICU research-based, and we believe that the ICU population is inherently vulnerable by virtue of being critically ill and totally dependent on care providers. Procedures are in place to make sure that this inherent vulnerability is not a major factor in the decision by a patient or surrogate regarding whether to participate.

[]None of the Populations listed above will be targeted for Enrollment

Subject Population Justification:

The ICU population has been selected for this study because the ultimate aim of the study is to prevent the difficult-to-treat, antibiotic-resistant infections that arise most often in ICU patients.

Does this study involve compensation or reimbursement to subjects?

No

Attached Attestation

Principal Investigator	Date Created
Daniel Freedberg (def2004)	02/09/2022

Attached HIPAA Forms

Number	Type	Title	Status
AAAO7053	A	ICU Inulin Study Spanish	Approve
AAAO3920	A	Inulin in the ICU	Approve
AAAO6281	D	screening for inulin study	Approve

Attached Consent Forms

Number	Copied From	Form Type	Title	Active/Inactive	Initiator
AACM5350	AABS4300	Consent	ICU inulin study - Consent Form	Inactive	Elissa Lynch (el2888)

Documents

Archived	Document Identifier	Document Type	File Name	Active	Stamped	Date Attached	Created By
No	O:\dim2115's Profile\Project Files\Dr.Freedberg's projects\I	Certificate of Translation	Certification of Translation for Inulin CF Spanish July 19 2019.pdf	Y	No	07/19/2019	Dagmara Moscoso (dim2115)
No	Spanish Translation Certificate	Certificate of Translation	Certification of Translation personnel update August 26 2019.pdf	Y	No	08/26/2019	Dagmara Moscoso (dim2115)

Archived	Document Identifier	Document Type	File Name	Active	Stamped	Date Attached	Created By
No	Spanish CF personnel update	Consent Form/Addendum	AAAS2576 ICF Spanish personnel update August 26 2019.pdf	Y	No	08/26/2019	Dagmara Moscoso (dim2115)
No	E-consent REDCap PDF	Consent Form/Addendum	AAAS2576_econsent_form.pdf	Y	No	05/29/2020	Elissa Lynch (el2888)
No	Consent form with modified format	Consent Form/Addendum	Inulin Consent Form 3.1.19.pdf	Y	No	03/01/2019	Daniel Freedberg (def2004)
No	updated CF July 10 2019	Consent Form/Addendum	Inulin Consent Form 7.10.19 clean copy.pdf	Y	No	07/11/2019	Dagmara Moscoso (dim2115)
No	English consent for Y1M5	Consent Form/Addendum	Inulin Consent Form 8.2.19 clean copy.pdf	Y	No	08/02/2019	Daniel Freedberg (def2004)
No	Inulin Consent Form English 2.20.20	Consent Form/Addendum	Inulin Consent Form English 2.20.20.pdf	Y	No	02/20/2020	Elissa Lynch (el2888)
No	Inulin Consent Form Spanish 2.20.20	Consent Form/Addendum	Inulin Consent Form Spanish 2.20.20.pdf	Y	No	02/20/2020	Elissa Lynch (el2888)
No	Inulin e-consent screenshots	Consent Form/Addendum	Inulin e-consent screenshots.pdf	Y	No	06/09/2020	Elissa Lynch (el2888)
No	Inulin COVID sub-study consent form	Consent Form/Addendum	Inulin-COVID sub-study consent form.pdf	Y	No	03/24/2020	Daniel Freedberg (def2004)
No	Inulin COVID sub-study Spanish consent form	Consent Form/Addendum	spanish consent form.pdf	Y	No	04/20/2020	Elissa Lynch (el2888)
No	Updated CF tracked changes July 10 2019	Consent Form/Addendum (tracked)	Inulin Consent Form 7.10.19_tracked changes.docx	Y	No	07/11/2019	Dagmara Moscoso (dim2115)
No	CTO IND form signed by D Landry	CTO Form of Notice	CTO IND form signed by D Landry.pdf	Y	No	03/12/2020	Amy Jansen (aj2870)
No	FDA-1571_Abrams_signed	FDA Form/Documentation	FDA-1571_Abrams_signed.pdf	Y	No	03/20/2019	Brenna Posner (bp2538)
No	FDA-1572_completed	FDA Form/Documentation	FDA-1572_completed.pdf	Y	No	03/20/2019	Brenna Posner (bp2538)
No	DoD Award Application Project Narrative	Funding/Grant Application/Subcontract	1-Project Narrative.pdf	Y	No	01/14/2019	Daniel Freedberg (def2004)
No	1-Project Narrative	Funding/Grant Application/Subcontract	1-Project Narrative.pdf	Y	No	03/20/2019	Brenna Posner (bp2538)
No	10-Study Personnel and Organization	Funding/Grant Application/Subcontract	10-Study Personnel and Organization.pdf	Y	No	03/20/2019	Brenna Posner (bp2538)
No	11-Data Management Plan	Funding/Grant Application/Subcontract	11-Data Management Plan.pdf	Y	No	03/20/2019	Brenna Posner (bp2538)
No	12-Transition Plan	Funding/Grant Application/Subcontract	12-Transition Plan.pdf	Y	No	03/20/2019	Brenna Posner (bp2538)
No	13-Regulatory Strategy	Funding/Grant Application/Subcontract	13-Regulatory Strategy.pdf	Y	No	03/20/2019	Brenna Posner (bp2538)
No	14-Representations	Funding/Grant Application/Subcontract	14-Representations.pdf	Y	No	03/20/2019	Brenna Posner (bp2538)
No	2-Supporting Documentation	Funding/Grant Application/Subcontract	2-Supporting Documentation.pdf	Y	No	03/20/2019	Brenna Posner (bp2538)
No	3-Technical and Lay Abstracts	Funding/Grant Application/Subcontract	3-Technical and Lay Abstracts.pdf	Y	No	03/20/2019	Brenna Posner (bp2538)
No	4-Statement of Work	Funding/Grant Application/Subcontract	4-Statement of Work.pdf	Y	No	03/20/2019	Brenna Posner (bp2538)

Archived	Document Identifier	Document Type	File Name	Active	Stamped	Date Attached	Created By
No	5-Impact Statement	Funding/Grant Application/Subcontract	5-Impact Statement.pdf	Y	No	03/20/2019	Brenna Posner (bp2538)
No	6-Military Relevance	Funding/Grant Application/Subcontract	6-Military Relevance.pdf	Y	No	03/20/2019	Brenna Posner (bp2538)
No	7-Intervention with DSMP	Funding/Grant Application/Subcontract	7-Intervention with DSMP.pdf	Y	No	03/20/2019	Brenna Posner (bp2538)
No	8-Human Subjects and Consent Form	Funding/Grant Application/Subcontract	8-Human Subjects and Consent Form.pdf	Y	No	03/20/2019	Brenna Posner (bp2538)
No	9-Surveys and Questionnaires	Funding/Grant Application/Subcontract	9-Surveys and Questionnaires.pdf	Y	No	03/20/2019	Brenna Posner (bp2538)
No	IND Approval from FDA and Communications	Investigator Brochure/Packaging Insert/Device Manual	IND - all documents.pdf	Y	No	01/14/2019	Daniel Freedberg (def2004)
No	Inulin COVID sub-study protocol with tracked changes	Other	Inulin COVID sub-study tracked changes.pdf	Y	No	03/24/2020	Daniel Freedberg (def2004)
No	verbal phone script for surrogates	Other	Verbal script for phone consent.pdf	Y	No	03/01/2019	Daniel Freedberg (def2004)
No	Department of Defense Annual Report (for renewal process)	Other	W81XWH Annual Report (modified).pdf	Y	No	02/09/2022	Elissa Lynch (el2888)
No	Department of Defense Report (for annual renewal process)	Other	W81XWH Year 1 Quarter 4 (Annual) Report.pdf	Y	No	03/04/2021	Elissa Lynch (el2888)
No	Pathology approval for residual blood	Pathology Approval Form	Path19-54_AAAS2576_Freedberg.pdf	Y	No	03/01/2019	Daniel Freedberg (def2004)
No	DSMP included in DoD Application	Safety/Monitoring Report	7-Intervention with DSMP.pdf	Y	No	01/14/2019	Daniel Freedberg (def2004)
No	DSMB_deaths	Safety/Monitoring Report	DSMB_deaths.pdf	Y	No	02/18/2022	Yaritza Collazo (yr111)
No	DSMB_June_2021	Safety/Monitoring Report	DSMB_June_2021.pdf	Y	No	02/18/2022	Yaritza Collazo (yr111)
No	DSMB Report (for annual renewal process)	Safety/Monitoring Report	DSMP Report 1.pdf	Y	No	03/04/2021	Elissa Lynch (el2888)
No	Inulin Trial Protocol v5 February 24, 2020	Standalone/Sponsor's Protocol	Trial Protocol_2.24.20.pdf	Y	No	02/24/2020	Elissa Lynch (el2888)
No	Inulin Trial Protocol v3 April 8, 2019	Standalone/Sponsor's Protocol	Trial Protocol_4.8.19.pdf	Y	No	07/24/2019	Daniel Freedberg (def2004)
No	Trial Protocol_3.25.20_highlighted_changes	Standalone/Sponsor's Protocol (tracked)	Trial Protocol_3.25.20_highlighted_changes.docx	Y	No	03/25/2020	Amy Jansen (aj2870)
No	3 month telephone questionnaire and script	Study Material/Instrument	3 Month Assessment.pdf	Y	No	02/26/2019	Daniel Freedberg (def2004)
No	6 month telephone assessment and script	Study Material/Instrument	6 Month Assessment.pdf	Y	No	02/26/2019	Daniel Freedberg (def2004)
No	Spanish Consent Form	Translated Study Material	Inulin CF Spanish July 19 2019.pdf	Y	No	07/19/2019	Dagmara Moscoso (dim2115)

Tasks

Section	Task	Required for submission	Completed	Created By	Date Created
Recruitment Consent	For "Describe how participants' written consent will be obtained": describe how consent will be obtained, including: - by whom (i.e., list one or more titles, roles, or names which are consistent with the information provided in the Personnel section when applicable), - when (e.g. same day, prior to screening procedures, etc.). If a protocol does not qualify for a same day consent process, it should be clear when, in advance of screening procedures, consent will be obtained.	Yes	No	Yaritza Collazo (yr111)	2022-02-18 09:14:12.0

Appendix 9.4

Columbia University Consent Form

Principal Investigator: Daniel E. Freedberg

Attached to Protocol: IRB-AAAS2576

Protocol Title: Inulin for the Prevention of Antibiotic-Resistant Infections in the ICU

Contact Information	Title	Contact Information
Elissa Lynch	Research Nurse	Phone: 212-304-5737 Cell: 412-779-8369 Email: el2888@cumc.columbia.edu
Daniel Freedberg	Principal Investigator	Phone: 212-342-3302 Cell: 857-998-9370 Email: def2004@cumc.columbia.edu

KEY INFORMATION

What is being asked of me?	This form seeks your consent to participate in a research study.
Why is this research being done?	This research tests whether inulin, a fiber supplement made from chicory root, encourages the growth of “good” bacteria to prevent infections in the ICU.
What does it involve?	Two thirds of the patients in this study will receive inulin and one third will receive placebo. All of the patients will have non-invasive assessments where samples and information will be gathered.
How long does it last?	While you are in the hospital, with telephone calls at 3 months and 6 months.
Risks?	Inulin causes bloating in some studies but has no known serious side effects.
Benefits?	Inulin may breed “good” bacteria to help to fight infections but this is not proven.
Alternatives?	There is no obligation to participate. The care you get here will be identical whether you do or do not participate.

Purpose of this form

The purpose of this form is to give you information so that you can decide if you want to take part in the study while in the intensive care unit (ICU). Many ICU patients are not capable of making decisions on their own, and this form is written to address both the patient or the patient’s surrogate decision maker as “you.”

Why am I being asked about this study?

Because you are hospitalized in the ICU, have a known or suspected infection, and are on antibiotic.

Why is this study being done?

Infection is the number one killer in the ICU. This study is being done to test **inulin** as an investigational new drug (not FDA approved) to prevent infections. Patients are “randomized” so that 2 out of 3 patients get the drug but no one knows who will get the drug and who will not.

What happens to me in this study?

If you choose to participate, you will receive either inulin 32 g/day, inulin 16 g/day, or placebo. Two thirds of the patients get inulin and 1/3 of the patients get placebo. No one, including you and the ICU team, will know whether you are getting inulin or placebo.

We will perform a total of 5 assessments: now and again in 3, 7, 14, and 30 days as long as you are hospitalized here. If you are about to be discharged, we will perform an assessment before you leave. No assessments are done once you go home but after 3 and 6 months we will call you to check how you are doing. This call has 13 questions and lasts 5-10 minutes. At each assessment, we look at your medical chart, and take the following samples:

Sample type	What it means for me
Rectal swabs	Two soft nylon swabs will be gently inserted into the anal area to gather bacteria.
Whole stools	A stool that you make will be taken.



Columbia University IRB

IRB-AAAS2576 (Y04M00)

IRB Approval Date: 02/23/2022

For use until: 02/22/2023

Oral swab	Two soft cotton swabs will be brushed against your mouth to gather bacteria.
Urine	A tablespoon of urine will be taken after you urinate or from a catheter, if you have one.
Residual blood samples	About 2 tablespoons of blood will be taken from blood that was already drawn for normal tests. Most of this blood is thrown away, and we will use the "leftover" blood if there is any. You will <i>not</i> have a blood draw as part of this study.

Risks

Inulin comes from dried chicory (a root vegetable). It is a common food additive and is slightly sweet (the placebo in this study is also slightly sweetened to match). A common side effect of inulin is bloating, which goes away on its own. Some studies have reported that inulin worsens diarrhea whereas other studies have reported that inulin improves diarrhea. Inulin has been used in the ICU with other fibers before and appears to be safe, but it is possible that we will discover new effects of inulin which could be good or bad.

With the samples gathered, there is risk of discomfort, emotional embarrassment, and inconvenience. For the rectal swab, we will ask you to turn onto your side and we will ask that your family leave the room for a moment to preserve your privacy.

With both the samples and the health information gathered, there is always a risk of a breach of confidentiality if your data is lost. This is discussed below, and safeguards are in place to make sure that this doesn't happen.

Benefits

Our hope is that inulin encourages the growth of good bacteria and therefore may help to prevent infections. However, this study was not designed to directly benefit subjects. Future ICU patients may benefit from the knowledge that we gain by doing this study.

Alternative procedures

The alternative is not to participate. All of the other care that you receive will be identical regardless of whether or not you take part in the study.

Confidentiality

Any information collected during this study that can identify you by name will be kept confidential. There will be no human genetic sequencing as part of this study. We will do everything we can to keep your data secure, however, complete confidentiality cannot be promised. Despite all our efforts, unanticipated problems such as a stolen computer can occur, although this is unlikely. To make your data more secure, health information and biological samples will all be assigned a code number and separated from your name or other information that could identify you. The file that links you to the code number will be kept on an encrypted, password-protected computer, and only the principal investigator and the study team will have access to this file.

Additionally, the following agencies will be able to look at and copy your research records: Authorities from Columbia University Medical Center and NewYork-Presbyterian Hospital, the Office of Human Research Protections ("OHRP"), an independent research monitor, the FDA, and the Department of Defense (DoD) Office for Human Research Protections (OHRP). Your participation in this research study will be documented in your electronic medical record in Epic and can be viewed by authorized personnel from Columbia University Irving Medical Center, Weill Cornell Medical Center and NYP affiliate institutions who are involved in your health care. A description of this clinical trial will be available on <http://www.ClinicalTrials.gov>, as required by U.S. law. This website will not include information that can identify you. At most, the website will include a summary of the study results. You can search this website at any time.

Your authorization to use and share your health information does not have an expiration (ending) date. Once your health information has been disclosed to a third party (for example, a pharmaceutical company participating in a study), federal privacy laws may no longer protect it from further disclosure.

You may change your mind and revoke (take back) this consent and authorization at any time and for any reason. To revoke this consent and authorization, you must contact the Principal Investigator Daniel Freedberg at def2004@cumc.columbia.edu. However, if you revoke your consent and authorization, you will not be allowed to continue taking part in the Research. Also, even if you revoke this consent and authorization, the Researchers and the Sponsor (if applicable) may continue to use and disclose the information they have already collected.

What happens to my samples?

We will retain your samples and data indefinitely. Because our research study is funded by the U.S. Department of Defense (DoD), we are required to submit some data in coded form to one or more databases managed by the Federal government. Some of these databases permit public unrestricted access to the data. The data may be the combined or individual data of many people. Any data that is submitted will not be labeled with your name or other information that could be used to easily identify you. However, it is possible that your information, when combined with information from other public sources, could be used to identify you. We believe that this is unlikely to happen.

If you agree that we may retain and use your samples and/or data indefinitely, they will be stored at CUMC either with the researchers on this study or in a central storage facility called a repository in identifiable form in a coded manner. This means that your samples and/or data will be identified by a unique code number that is linked to your name. The key to the code will be stored securely on an encrypted electronic device.

Also with your permission, your samples and/or data may be used by other Columbia researchers or researchers at other institutions, including commercial companies. If they are given to researchers who are not on this study, they will only be given in de-identified form. This means that your name and other identifying information have been removed from your samples and/or data or that your samples and/or data are coded and the researchers who will use them will not have the key that links your name to the code number.

Any future testing or research using your samples and/or data may lead to the development and use of information, products, tests and/or treatments having commercial value. You will not receive any compensation that may result from these tests or treatments.

Research related injuries

Taking part in this research study may result in injury or harm to you. In the event of an injury resulting from your participation in this study, you should seek appropriate medical care and inform the study doctor. In the event of an emergency you should go to an emergency room. If you are injured or harmed as a result of participating in the study and receive medical care through the NewYork-Presbyterian Hospital (NYPH), a Columbia doctor, or any other health provider, you will be sent a bill for whatever medical care you receive. All or part of your bill may be paid by your health insurance. The study sponsor will not pay for any medical expenses, even if these medical expenses result from participation in the study. Columbia University and NewYork-Presbyterian Hospital (NYPH) are not offering to provide you the drug/device after the termination of the study or to pay you for pain, worry, lost income, the cost of your medical care or non-medical care costs that might occur as a result of your taking part in this study. However, you do not waive any of your legal rights in signing this form.

Compensation

There is no compensation. If your samples result in some commercial profit, you will not share in this profit.

Additional Costs

There are no costs for taking part in this study.

Voluntary Participation

Taking part in this study is your choice. You can decide not to take part in or stop being in the study at any time. Your choice will not affect the treatment you receive from doctors and staff at Columbia University Medical Center and NewYork-Presbyterian Hospital.

Will results be returned to me?

The tests for this study will be performed at the end of the study, long after you have left the ICU. None of these test results are likely to have an impact on your future healthcare, and there are no plans to return any test results to you.

Whom Do I Call if I Have Questions?

If you have any questions about your rights as a subject, you may contact the Principal Investigator of the



Columbia University IRB

IRB-AAAS2576 (Y04M00)
IRB Approval Date: 02/23/2022
For use until: 02/22/2023

study Dr. Freedberg at 212-342-3302 (research office) or 212-305-1021 (clinical office) if you have any questions or concerns. You may also contact the institutional review board (IRB) at the following location. Human Research Protection Office, Institutional Review Board, Columbia University Medical Center, 154 Haven Avenue, 1st Floor, New York, NY 10032, Telephone: (212) 305-5883, Email: irboffice@columbia.edu.

An IRB is a committee organized to protect the rights and welfare of human subjects involved in research. More information about taking part in a research study can be found on the Columbia University IRB website at: <http://www.cumc.columbia.edu/dept/rb>.

Statement of Consent

I have read this consent form and the research study has been explained to me. I agree to be in the research study described above. A copy of this consent form will be provided to me after I sign it. By signing this consent form, I have not given up any of the legal rights that I would have if I were not a participant in the study.

Participant Signature Lines

Study Participant

Print Name _____ Signature _____
Date _____

Legally Authorized Representative

Print Name _____ Signature _____
Date _____

Research Signature Lines

Principal Investigator

Print Name _____ Signature _____
Date _____

Study Coordinator

Print Name _____ Signature _____
Date _____



Columbia University IRB

IRB-AAAS2576 (Y04M00)

IRB Approval Date: 02/23/2022

For use until: 02/22/2023

Appendix 9.5

Formulario de Consentimiento de la Universidad de Columbia

Investigador Principal: Daniel E. Freedberg

Adjunto al Protocolo: IRB-AAAS2576

Título del Protocolo: Inulina para la prevención de las infecciones resistentes a los antibióticos en la Unidad de Cuidados Intensivos

Información de Contacto	Título	Información de Contacto
Elissa Lynch	Enfermera de Investigación	Teléfono: 212-304-5737 Móvil: 412-779-8369 Correo electrónico: el2888@cumc.columbia.edu
Daniel Freedberg	Investigador Principal	Teléfono: 212-342-3302 Móvil: 857-998-9370 Correo electrónico: def2004@cumc.columbia.edu

INFORMACIÓN CLAVE

¿Qué me están pidiendo?	Este formulario busca su consentimiento para participar en un estudio de investigación.
¿Por qué se está realizando esta investigación?	Esta investigación comprueba si la inulina, un suplemento de fibras fabricado de la raíz de achicoria, estimula el crecimiento de bacterias “buenas” para prevenir las infecciones en la Unidad de Cuidados Intensivos (ICU por sus siglas en inglés).
¿En qué consiste?	Las dos terceras partes de los pacientes en este estudio recibirán la inulina y una tercera parte recibirá el placebo. Todos los pacientes tendrán evaluaciones no invasivas donde se recogerán muestras e información.
¿Qué tiempo durará el estudio?	Mientras usted permanezca en el hospital, con llamadas telefónicas a los 3 meses y 6 meses.
¿Riesgos?	En algunos estudios se ha observado que la inulina causa distensión abdominal, pero no se conocen efectos secundarios graves.
¿Beneficios?	La inulina puede hacer crecer las bacterias “buenas” para ayudar a luchar contra las infecciones, pero no se ha comprobado.
¿Alternativas?	No hay obligación de participar. La atención que usted recibe aquí será igual si participa o no participa.

Propósito de este formulario

El propósito de este formulario es brindarle a usted la información para que pueda decidir si quiere participar en el estudio mientras está en la Unidad de Cuidados Intensivos (ICU). Muchos pacientes en la Unidad de Cuidados Intensivos (ICU) no pueden tomar sus propias decisiones y este formulario está dirigido tanto para el paciente como para el sustituto del paciente para tomar decisiones como “usted”.

¿Por qué me piden estar en este estudio?

Porque usted está hospitalizado en la Unidad de Cuidados Intensivos (ICU), tiene una infección o se sospecha la presencia de una infección y está recibiendo antibióticos.

¿Por qué se lleva a cabo este estudio?

La infección es la causa de muerte número uno en la Unidad de Cuidados Intensivos (ICU). El estudio se realiza para poner a prueba la **inulina** como un nuevo medicamento bajo investigación (no aprobado por la Administración de Alimentos y Medicamentos, FDA por sus siglas en inglés) para prevenir las infecciones. Los pacientes son asignados de manera “aleatoria” para que 2 de 3 pacientes reciban el medicamento, pero nadie sabe quién recibe el medicamento y quién no lo recibe.

¿Qué me sucede en este estudio?

Si elige participar, usted recibirá la inulina 32 g/día, inulina 16 g/día o placebo. Las dos terceras partes (2/3) de los pacientes recibirán la inulina y la tercera parte (1/3) de los pacientes recibirá el placebo. Nadie, incluyéndolo a usted y al grupo de la Unidad de Cuidados Intensivos (ICU), sabrá si usted está recibiendo inulina o placebo.

Realizaremos un total de 5 evaluaciones: ahora y otra vez a los 3, 7, 14 y 30 días, siempre y cuando usted esté hospitalizado aquí. Si usted está a punto de ser dado de alta, realizaremos una evaluación antes de irse. No se harán las evaluaciones una vez que se vaya a su casa, pero después de los 3 y 6 meses lo llamaremos para saber cómo está. En esta llamada le haremos 13 preguntas y durará 5-10 minutos. En cada evaluación, revisaremos su expediente médico y obtendremos las siguientes muestras:

Tipo de muestra	Qué significa para mí
Pasar un hisopo rectal	Dos hisopos suaves de nylon se insertarán suavemente dentro del área anal para recoger bacterias.
Heces fecales	Se recogerán las heces que usted haga.
Pasar un hisopo bucal	Dos hisopos suaves de algodón se frotarán en la mejilla para recoger bacterias.
Orina	Se recogerá una cucharada de orina después que usted orine o de un catéter, si tiene uno colocado.
Muestras de sangre residual	Se obtendrán unas 2 cucharadas de la sangre que ya fue extraída para análisis habituales. La mayoría de esta sangre se desecha y nosotros usaremos la sangre que “sobre”, si hay alguna. A usted no se le sacará sangre como parte de este estudio.

Riesgos

La inulina proviene de la achicoria seca (la raíz de un vegetal). Este es un aditivo común de los alimentos y es ligeramente dulce (el placebo en este estudio también está ligeramente endulzado para que se parezcan). Un efecto secundario común de la inulina es la distensión (hinchazón) abdominal, que desaparece sola. Algunos estudios han reportado que la inulina empeora la diarrea, sin embargo, otros estudios han reportado que mejora la diarrea. La inulina se ha usado antes en la Unidad de Cuidados Intensivos (ICU) con otras fibra y parece ser segura (inocua, no hace daño), pero es posible que se descubra algún efecto nuevo de la inulina que pueda ser bueno o malo.

Con las muestras recogidas, existe el riesgo de molestia, sentimientos de vergüenza e inconvenientes. Para colocar el hisopo rectal, le pediremos que se voltee de lado y que su familia salga de la habitación por un momento para preservar su privacidad.

Con las muestras y la información de salud recogidas, existe siempre el riesgo de violación de la privacidad si se pierden sus datos. Esto se discute posteriormente y se han implementado salvaguardas para garantizar que esto no suceda.

Beneficios

Esperamos que la inulina estimule el crecimiento de las bacterias buenas y por lo tanto, pueda ayudar a prevenir las infecciones. Sin embargo, este estudio no fue diseñado para beneficiar directamente a los sujetos. Los pacientes futuros de la Unidad de Cuidados Intensivos (ICU) pueden beneficiarse del conocimiento que hemos obtenido por realizar este estudio.

Procedimientos alternativos

La alternativa es no participar. Todos los otros cuidados que usted recibe serán idénticos, independientemente de si usted participa o no en el estudio.

Confidencialidad

Toda información recopilada durante este estudio que pueda identificarlo por su nombre se mantendrá confidencial. No habrá secuenciación genética humana como parte de este estudio. Haremos todo lo posible para mantener sus datos seguros. Sin embargo, no podemos prometer completa confidencialidad. A pesar de todo nuestro esfuerzo, pueden ocurrir problemas imprevistos, tales como el robo de una computadora, aunque esto es muy poco probable. Para hacer más seguros sus datos, a toda la información de salud y a las muestras biológicas le asignarán un número de código y estarán separadas de su nombre o de cualquier otra información que pueda identificarle. El archivo que lo vincula a usted con el número de código se mantendrá en una computadora codificada y protegida por contraseña y solamente el investigador principal y el personal del estudio tendrán acceso a este archivo.

Además, las agencias siguientes podrán mirar y copiar sus expedientes de la investigación: las autoridades del Centro Médico de la Universidad de Columbia y del Hospital New York Presbyterian, la Oficina para la Protección de Humanos en la Investigación ("OHRP", por sus siglas en inglés), un monitor independiente de investigación, la FDA y el Department of Defense (DoD) Office for Human Research Protections (OHRP). Su participación en este estudio de investigación será documentada en su expediente médico electrónico. Este expediente puede ser visto por el personal autorizado del Centro Médico Irving de la Universidad de Columbia (*Columbia University Irving Medical Center*), Centro Médico Weill Cornell (*Weill Cornell Medical Center*), Hospital New York Presbyterian y sus instituciones afiliadas, porque estas instituciones comparten el sistema de expediente médico electrónico. Los monitores del estudio y otros que supervisan el estudio pueden también necesitar el acceso a este expediente. Una descripción de este estudio clínico estará disponible en <http://www.ClinicalTrials.gov>, como lo exigen las leyes de los Estados Unidos. El sitio web no incluirá información que pueda identificarlo. A lo sumo, el sitio web puede incluir un resumen de los resultados. Usted puede buscar este sitio web en cualquier momento.

Su autorización para usar y compartir su información de salud no tiene fecha de vencimiento. Una vez que su información de salud se haya revelado a una tercera parte (por ejemplo, una compañía farmacéutica que participa en un estudio), las leyes federales de la privacidad posiblemente no la protejan de otras revelaciones. Usted puede cambiar de opinión y revocar (cancelar) este consentimiento y autorización en cualquier momento y por cualquier motivo. Para cancelar este consentimiento y autorización, debe comunicarse con el Investigador Principal, el Dr. Daniel Freedberg en def2004@cumc.columbia.edu. Sin embargo, si usted cancela su consentimiento y autorización, no se le permitirá continuar participando en la investigación. Además, aunque usted cancele este consentimiento y autorización, los investigadores y el patrocinador (si corresponde) pueden continuar usando y revelando la información que ya han recopilado.

¿Qué sucede con mis muestras?

Retendremos sus muestras y datos indefinidamente. Ya que nuestro estudio de investigación está financiado por el Departamento de Defensa (DoD por sus siglas en inglés) de los Estados Unidos, nos exigen presentar algunos datos en forma codificada a una o más bases de datos administradas por el gobierno federal. alguna de estas bases de datos permite el acceso no restringido del público a los datos. Los datos pueden ser datos combinados o individuales de muchas personas. Todo dato que es presentado no será rotulado con su nombre ni otra información pueda ser usada para identificarlo a usted fácilmente. Sin embargo, es posible que su información, cuando se combine con la información de otras fuentes públicas, se pueda usar para identificarlo a usted. Creemos que esto es muy poco probable que ocurra.

Si usted acepta que nosotros podamos retener y usar sus muestras y/o datos indefinidamente, ellos serán almacenados en el Centro Médico de la Universidad de Columbia (CUMC por sus siglas en inglés) o bien con los investigadores en este estudio o en un centro de almacenamiento central, llamado repositorio, de forma identificable de una manera codificada. Esto significa que sus muestras y/o datos serán identificados por un número de código único que es vinculado a su nombre. La clave del código será almacenada de manera segura en un dispositivo electrónico codificado.

También con su permiso, sus muestras y/o sus datos pueden ser usados por otros investigadores de Columbia o investigadores de otras instituciones, incluyendo compañías comerciales. Si son entregados a investigadores que no forman parte de este estudio, se entregarán solamente de forma no identificada. Esto quiere decir que su nombre y otra información de identificación han sido retirados de sus muestras y/o datos o que sus muestras y/o datos están codificados y los investigadores que los usen no tendrán la clave que vincula su nombre al número de código.

Cualquier prueba o investigación usando sus muestras y/o datos puede dar lugar al desarrollo y uso de información, productos, pruebas y/o tratamientos que tienen valor comercial. Usted no recibirá ninguna compensación que pueda resultar de estas pruebas ni tratamientos.

Lesiones relacionadas con la investigación

Participar en este estudio de investigación puede resultar en lesión o daño a usted. En caso de una lesión que sea resultado de su participación en este estudio, debe buscar atención médica apropiada e informarlo al médico del estudio. En caso de una emergencia debe ir a una sala de emergencia. Si usted se lesiona o se daña como resultado de participar en este estudio y recibe cuidado médico a través del Hospital New York Presbyterian (NYPH por sus siglas en inglés), un médico de Columbia o cualquier otro proveedor de salud, se le enviará una factura por cualquier cuidado médico que reciba. Su seguro de salud puede pagar toda o parte de la factura. El patrocinador del estudio no pagará ningún gasto médico, aun cuando estos gastos médicos sean resultado de su participación en el estudio. La Universidad Columbia y el Hospital New York Presbyterian (NYPH) no están ofreciendo proporcionarle a usted el medicamento/dispositivo después de terminar el estudio ni pagar por su dolor, preocupación, pérdida de ingresos, costo de su cuidado médico ni costos no médicos en los que usted pudiera incurrir a consecuencia de su participación en este estudio. Sin embargo, usted no renuncia a ninguno de sus derechos legales al firmar este formulario.

Compensación

No hay compensación. Si sus muestras dan lugar a algún beneficio comercial, no se compartirá con usted este beneficio.

Costos adicionales

No hay costos por participar en este estudio.

Participación voluntaria

La participación en este estudio es su elección. Puede decidir no participar o dejar de participar en el estudio en cualquier momento. Su elección no afectará el tratamiento que recibe de los médicos ni del personal en el Centro Médico de la Universidad de Columbia y en el Hospital New York Presbyterian.

¿Me entregarán los resultados?

Las pruebas para este estudio se realizarán al final del estudio, mucho después de que usted haya salido de la Unidad de Cuidados Intensivos (ICU). Es posible que ninguno de los resultados de estas pruebas tenga un impacto en su cuidado de salud en el futuro y no hay planes de entregarle ningún resultado de las pruebas.

¿A quién puedo llamar si tengo preguntas?

Si tiene preguntas sobre sus derechos como un sujeto, puede contactar con el Investigador Principal del estudio, el Dr. Freedberg al 212-342-3302 (oficina de investigación) o al 212-305-1021 (consultorio de la clínica) si tiene alguna pregunta o preocupación. También puede contactar con la Comisión Institucional de Revisión (IRB por sus siglas en inglés) en el siguiente lugar: Human Research Protection Office, Institutional Review Board, Columbia University Medical Center, a [154 Haven Avenue, 1st Floor, New York, NY 10032](https://www.columbia.edu/154HavenAvenue), [teléfono: \(212\) 305-5883](tel:2123055883), [correo electrónico: irboffice@columbia.edu](mailto:irboffice@columbia.edu).

Una IRB es un comité organizado para proteger los derechos y el bienestar de los sujetos humanos que participan en investigación. Más información sobre la participación en un estudio de investigación puede encontrarla en el sitio web de la IRB de la Universidad de Columbia en: <http://www.cumc.columbia.edu/dept/rb>.

Declaración de Consentimiento

He leído este formulario de consentimiento y me han explicado el estudio de investigación. Acepto participar en el estudio de investigación descrito anteriormente. Me proporcionarán una copia de este formulario de consentimiento después de firmarlo. Al firmar este formulario de consentimiento, no renuncio a ninguno de los derechos legales que tendría si no fuera un participante en el estudio.

Líneas de firmas del participante

Participante del estudio

Nombre en letras de imprenta_____Firma_____

Fecha_____

Representante legalmente autorizado

Nombre en letras de imprenta_____Firma_____

Fecha_____

Líneas de firmas del miembro de la investigación

Investigador Principal

Nombre en letras de imprenta_____Firma_____

Fecha_____

Coordinadora del Estudio

Nombre en letras de imprenta_____Firma_____

Fecha_____

Appendix 9.6

Inulin in the ICU e-consent form

Please read through thoroughly. Thank you!

Study ID

(entered by research staff)

Date/Time

Study Participant's First Name

(el nombre de participante del estudio)

Study Participant's Last Name

(el apellido de participante del estudio)

Consent form (English)

[Attachment: "Inulin Consent Form English.pdf"]

Consent form (Spanish)

[Attachment: "Inulin Consent Form Spanish.pdf"]

HIPAA form (English)

[Attachment: "HIPAA English.pdf"]

HIPAA form (Spanish)

[Attachment: "HIPAA Spanish.pdf"]

Are you a legally authorized representative?

☐ Yes
☐ No

First Name

(Please enter your first name)

Last Name

(Please enter your last name)

Email address

(Please enter your email address to receive a copy of this consent.)

Signature

(Please sign your name with your finger or mouse.)

Appendix 9.7

B2. SOFA SCORE WORKSHEET

Study ID: 4 digits followed by a letter

(4 digit number followed by A, or B if a new subject must be substituted because original subject with this number did not complete Day 3 visit, or C if another substitution must be made. Numbers are assigned consecutively as subjects are randomized, beginning with 1001, 1002 etc. Only randomized subjects are given a Study ID.)

Baseline SOFA will be calculated for patients who have ANY data within the CUMC EMR during the previous 12 months that allows us to calculate a baseline. For most patients, this will be the discharge information from a previous hospitalization, or the information from an outpatient medical visit. The timepoint pertaining to this visit/hospitalization will then be selected and the baseline SOFA will be assessed using the 24 hour period preceding that timepoint. If data is missing, it will be assumed to be normal. The WORST data point over the 24 hour period should always be selected, excluding spurious values.

SOFA Assessment Date

SOFA Assessment Time

Oxygen Flow-Related Data (used to calculate FiO2 delivered, below, for SOFA scoring)

1. Select type of oxygen.

- ☐ 1. room air
☐ 2. nasal canula
☐ 3. nonrebreather (NRB) mask
☐ 4. ventilator including CPAP

1b. Nonrebreather (NRB) mask, Select flow rate

(15-Jan)

1a. Nasal canula, Select flow rate

(15-Jan)

1c. Ventilator including CPAP, Select flow rate

(Enter a number between 20 and 100 in 5 unit increments (e.g. 20, 25, 30....))

2. PaO2 from arterial blood gas .

(Take this value from the arterial blood gas (ABG) measurement with or without lactate. Enter 20-100; if no ABG was not performed during the preceding 24 hours, enter -1)

FiO2 delivered (calculated from above)

3. Platelets -Choose lowest value in 24 hours .

4. Bilirubin in mg/dL - Choose highest value in 24 hours.

5. Glasgow Coma Scale (GCS): This is the assumed GCS in sedated patients. That is, what score would the patient have if he or she was NOT sedated? Within each category, choose the LOWEST value.

5a. GCS Motor - Best motor response.

- ☐ 1 no response
☐ 2 extension to pain
☐ 3 flexion to pain
☐ 4 withdrawal from pain
☐ 5 localizes to pain
☐ 6 obeys commands
(Most patients will score 1, 5, or 6.)

5b. GCS Verbal - Best verbal response

- ☐ 1 no response
☐ 2 incomprehensible
☐ 3 inappropriate
☐ 4 confused
☐ 5 oriented
(Intubated patients will score a 1 (no response).)

5c. GCS Eye Response -Best eye response

- ☐ 1 not opening
☐ 2 to pain
☐ 3 to verbal commands
☐ 4 spontaneously

6. Mean arterial pressure (MAP)

(0 - 150. If missing, enter -1 and provide systolic/diastolic blood pressure below)

6a. Systolic blood pressure.

(Enter 1-250)

6b. Diastolic blood pressure

(Enter 1-200)

7. Use of Vasopressors (if yes, provide values below: higher values are worse; single bolus doses should be ignored; all values should be entered with 1 decimal value, e.g, 2.5, 50.6; if a value is not recorded/not available, enter the negative value -1.0).

☐ No ☐ Yes

7a. Dopamine in mcg/kg/min

(.1 to 100.0; enter -1.0 if not recorded)

7b. Dobutamine in mcg/kg/min

(.1 to 100.0; enter -1.0 if not recorded)

7c. Epinephrine in mcg/kg/min

(.1 to 100.0; enter -1.0 if not recorded)

7d. Norepinephrine (levophed) in mcg/kg/min

(.1 to 100.0; enter -1.0 if not recorded.)

Phenylephrine (neosynephrine) in mcg/kg/min

(.1 to 100.0; enter -1.0 if not recorded)

Vasopressin

☐ Yes ☐ No

8. Creatinine in mg/dL - Higher values are worse.

(1.0 to 30.0; enter -1.0 if not recorded)

9. Urine output (UOP) - Urine output during the preceding 24 hours.

(0 -5000; enter -1 if not recorded)

C Intervention Assignment

This form should be filled out only for subjects who have met all eligibility criteria (Form B1) and who have consented to participate in the study.

1. SCREENING ID (from SCREENING database)

(Example: SCN001, SCN002 etc)

2. Go to the research pharmacy to facilitate administration of the intervention.

☐ Done

3. Enter a clinical note documenting that the patient is participating in a research study.

☐ Done
(This note should include the study IRB number, the time of enrollment, and who gave consent (patient vs surrogate).)

E1. ICU ADMISSION ASSESSMENT

First set of ICU vital signs :

Date

((mm/dd/yyyy))

Time (military)

((military))

Baseline assessment

1. Which sex category best describes the patient?

☐ Male ☐ Female

2. Age in years at the time of ICU admission.

3. Which ethnicity best describes you?

☐ Hispanic
☐ Non-Hispanic

4. Which race best describes you?

☐ White
☐ Hispanic
☐ Black or African American
☐ Asian
☐ Native Hawaiian or Pacific Islander
☐ American Indian or Alaska Native
☐ None of the above

5. Before being hospitalized in the ICU, where were you most recently?

☐ Came from home via the ER
☐ Came from home via the OR (rare)
☐ Hospital floor
☐ Another hospital's ICU
((Surrogate to answer if subject unable.))

6. Which best describes your home environment?

☐ Home, totally independent
☐ Home, with some help
☐ Assisted living facility
((Surrogate to answer if subject unable.))

EMR data, taken from the ICU admission notes

7. Baseline chronic medical comorbidities: CHECK ALL THAT APPLY.

All baseline medical comorbidities should be indicated here, based on the comorbidities listed in the most recent relevant electronic notes. These comorbidities represent the patient's baseline problems (i.e., chronic conditions) and not why he or she was sent to the ICU. Use standardized language.

- ☐ MI/heart attack
- ☐ Congestive heart failure
- ☐ Peripheral vascular disease
- ☐ CVA/stroke or TIA
- ☐ Dementia
- ☐ COPD
- ☐ Peptic ulcer disease
- ☐ Mild liver disease
- ☐ Moderate liver disease (cirrhosis or portal 11,hypertension)
- ☐ Severe liver disease (cirrhosis or portal 13,hypertension and variceal bleeding)
- ☐ Diabetes uncomplicated
- ☐ Diabetes severe - with end organ damage such as retinopathy, neuropathy, nephropathy
- ☐ Hemiplegia
- ☐ Moderate CKD - creatinine > 3 chronically
- ☐ Severe CKD - on dialysis, s/p renal transplant, or with uremia
- ☐ Solid malignant tumor - localized
- ☐ Solid malignant tumor - metastatic
- ☐ Leukemia
- ☐ Lymphoma
- ☐ AIDS - controlled HIV does NOT count
- ☐ Other

Other--List other comorbidities, if any.

8. Primary reason for admission (CHECK ONE)

- ☐ Cardiac arrest
- ☐ GI bleeding
- ☐ Heart failure
- ☐ Liver failure
- ☐ Neurologic including stroke
- ☐ Renal failure
- ☐ Respiratory failure including ECMO
- ☐ Sepsis
- ☐ Toxic ingestion
- ☐ Other

If 'Other' primary reason reason for admission, explain in no more than 2 words, taken based on the admission notes.

E2. ICU FOLLOW-UP ASSESSMENT

Assessment Date _____

If this form could not be completed at this study assessment, please indicate why, skip the remainder of the questions and submit the form; then complete Form H: Patient Disposition Record

- ☐ Death
☐ Discharge
☐ Refusal

1. Presence of lines or catheters.

Done by visual inspection at the bedside at the time of assessment.

Urinary catheter (foley) ☐ Yes ☐ No

Femoral central venous line. ☐ Yes ☐ No

Neck central venous line. ☐ Yes ☐ No

Arterial line. ☐ Yes ☐ No

2. Specific AEs of interest

For all of these, ask the subject first, then ask the nurse if he cannot answer.

2A. During the last 24 hours, has there been bloating or abdominal distension ? ☐ none
☐ a little
☐ a moderate amount
☐ a lot or severe

2B. During the last 24 hours, how many stools have been produced _____

2C. During the last 24 hours, what has been the loosest stool consistency ? ☐ like water,
☐ like porridge,
☐ loose with chunks,
☐ formed but soft,
☐ formed and hard

EMR data

For each assessment ,this data should be taken for the 24 hour period preceding the relevant assessment. E.g., if the Day 3 assessment was performed at 13:00 on April 23, this data would represent the 24 hour time period from 13:00 April 22 to 13:00 April 23.

1. Arterial pH from arterial blood gas.

If none performed, leave blank and check the indicator below

(6.60 - 8.00 (2 decimals). From the arterial blood gas (ABG) measurement with or without lactate. Take the LOWEST value.)

1a. Indicate if no Arterial pH from arterial blood gas within past 24 hours.

☐ No pH from ABG within 24 hours.

2. Lactate from the arterial blood gas or direct lactate measurement--take the LOWEST value.

(two decimal places)

2a. No available lactate from the arterial blood gas or direct lactate measurement with 24 hours .

☐ No lactate from ABG within 24 hours

3. ECMO within the last 24 hours?

☐ Yes ☐ No

4. CVVH within the last 24 hours?

☐ Yes ☐ No

5. Albumin - Choose the LOWEST value in 24 hours.

6. Hematocrit - Choose the LOWEST value in 24 hours.

7. White blood cell count - Choose the HIGHEST value in 24 hours.

(.1 to 100 with one decimal)

8. Electrolytes - For each of the following, enter both the LOWEST and the HIGHEST values during the preceding 24 hours

Glucose from BMP in mg/dL - LOWEST

(integer 10 to 1000)

Glucose from BMP in mg/dL -HIGHEST

(integer 10- - 1000)

Calcium from BMP in mg/dL - LOWEST

(number 5 to 15 with one decimal)

Calcium from BMP in mg/dL - HIGHEST

(5 to 15 with one decimal)

Magnesium in mg/dL - LOWEST

(0 to 10 with one decimal)

Magnesium in mg/dL - HIGHEST

(0 to 10 with one decimal)

Phosphorus in mg/dL - LOWEST

(number 1 to 10 with one decimal)

Phosphorous in mg/dL - HIGHEST

(1 to 10 with 1 decimal)

Potassium from BMP in mmol/L - LOWEST

(number 1 to 10 with one decimal)

Potassium from BMP in mmol/L - HIGHEST

(1 to 10 with 1 decimal)

Sodium from BMP in mmol/L - LOWEST

(integer 50-200)

Sodium from BMP in mmol/L - HIGHEST

(Integer 50 -200)

11. VENOUS lactate during the preceding 24 hours

(0.00 TO 20.00)

EMR data representing ICU interventions

Taken for the 24 hour period preceding the first set of ICU vitals or for the relevant study period if this is a follow-up assessment. E.g., if this was the Day 3 assessment this data would represent the interventions from baseline to Day 3. If this was the Day 7 assessment, it would represent the interventions from Day 3 to Day 7.

1. Receipt of a proton pump inhibitor

☐ Yes ☐ No

(PPIs are esomeprazole, omeprazole, pantoprazole, rabeprazole, lansoprazole, dexlansoprazole. Medications received should be determined based on the doses given in the nursing flowsheets not based on orders.)

2. Receipt of opioids.

☐ yes ☐ No

(Common opioids are fentanyl, morphine, dilaudid, Percocet/oxycodone. Medications received should be determined based on the doses given in the nursing flowsheets not based on orders.)

6. Receipt of antibiotics.

	Yes	No
Aminoglycosides (gentamicin)	☐	☐
B-lactams (e.g., amoxicillin)	☐	☐
Cephalosporins (cefazolin or ceftriaxone)	☐	☐
Daptomycin	☐	☐
Extended-spectrum B-lactams (piperacillin-tazobactam/zosyn)	☐	☐
Fluoroquinolones (levofloxacin)	☐	☐
Lincosamides (clindamycin)	☐	☐
Linezolid	☐	☐
Macrolides (azithromycin)	☐	☐
Metronidazole	☐	☐
Monobactams (meropenem, ertapenem)	☐	☐
Polymixin	☐	☐
Sulfonamides (Bactrim)	☐	☐
Tetracyclines (doxycycline)	☐	☐
Vancomycin	☐	☐

G1. SAMPLE COLLECTION: RECTAL SWAB, ORAL SWAB, AND URINE

Form Completed

☐ Yes ☐ No

Reason

☐ death ☐ discharge

☐ refusal

(Fill out Form H--Disposition)

RECTAL SWABS- Two pre-moistened flocked Copan diagnostics swabs; one is flash frozen at -80C and the other is brought on soy broth with 20% glycerol to the ACU lab.

ORAL SWABS- Two EpiCentre cotton-tipped swabs, flash frozen.

URINE SAMPLES- 2 X 8ML urine aliquots, flash frozen

Sample Date (mm/dd/yyyy)

Sample Time (military)

Rectal

☐ Yes/collected ☐ No/not collected

Oral

☐ Yes/collected ☐ No/not collected

Urine

☐ Yes/collected ☐ No/not collected

G2. SAMPLE COLLECTION: WHOLE STOOLS

Work with the nurses to collect 4 X 8mL aliquots of whole stool from each period. The times recorded should represent the date/time WHEN THE STOOL WAS PRODUCED as best as we can tell. For stools, stool 1 refers to the stool produced from after the intervention has been received until Day 3, stool 2 from Day 3 until Day 7, etc. Record the stool collection information at the end of the appropriate time period, e.g. record baseline to Day 3 stool collection at Day 3 in REDCap, etc.

Form Completed

☐ Yes ☐ No

Reason

☐ death ☐ discharge
☐ refusal
(Complete form H: Disposition)

Stool Collection Date

Stool Collection Time

((military))

G3. RESIDUAL BLOOD SAMPLES

Residual Blood Form Completed

☐ Yes ☐ No

Reason

☐ death ☐ discharge

☐ refusal

(FILL OUT FORM H: DISPOSITION)

Residual Blood Date

Residual Blood Time

((military))

K. POST-DISCHARGE ASSESSMENT

Post-Discharge Assessment Completed

☐ Yes ☐ No

Reason

☐ death ☐ discharge
☐ refusal
(Fill out Disposition--Form H)

Post-Discharge Assessment Date

Post-Discharge Assessment Time

Who is answering the questions?

☐ Subject ☐ Surrogate
☐ Caregiver ☐ Relative

Is the patient living?

☐ Yes ☐ No

If no, confirm that date of death has been entered
and the remaining forms can be closed

In what kind of a place is the patient living?

☐ Home, totally independent
☐ Home, with some help
☐ Assisted living facility

On a scale from 0 to 100 where 0 is the worst health
imaginable and 100 is the best, how would you
describe your health TODAY?

Has the patient been hospitalized since discharge
from the index admission at CUMC?

☐ Yes ☐ No

If yes, enter the best approximation of the date of
the start of the first hospitalization following
discharge.

Katz Index of Independence

Bathing

- ☐ Bathes self completely or needs help bathing only a single part of the body such as the back
☐ Needs help with bathing more than one part of the body

Dressing

- ☐ Gets clothes from closets and drawers and puts them on, may have help tying shoes
☐ Needs help with dressing

Toileting

- ☐ Goes to the toilet, gets on and off, and arranges clothes
☐ Needs help transferring to the toilet, cleaning, or using bedpan

Transferring

- ☐ Moves in and out of bed or chair unassisted. Mechanical transferring aides are acceptable.
- ☐ Needs help in moving from bed to chair or in completing transfer

Continence

- ☐ Exercises complete self control over urination and defecation
- ☐ Is partially or wholly incontinent of bowel or bladder

Feeding

- ☐ Gets food from plate into mouth without help. Food prep may be done by another person.
- ☐ Needs partial or total help with feeding or requires parenteral feeding

L. POST-DISCHARGE HEALTH QUESTIONNAIRE

Post Discharge Health Form Date

Post Discharge Health Form Time

Mobility

- ☐ I have no problems in walking about
- ☐ I have slight problems in walking about
- ☐ I have moderate problems in walking about
- ☐ I have severe problems in walking about
- ☐ I am unable to walk about

Self-Care

- ☐ I have no problems washing or dressing myself
- ☐ I have slight problems washing or dressing myself
- ☐ I have moderate problems washing or dressing myself
- ☐ I have severe problems washing or dressing myself
- ☐ I am unable to wash or dress myself

Usual Activities (ex: work, study, housework, family, or leisure activities)

- ☐ I have no problems doing my usual activities
- ☐ I have slight problems doing my usual activities
- ☐ I have moderate problems doing my usual activities
- ☐ I have severe problems doing my usual activities
- ☐ I am unable to do my usual activities

Pain/Discomfort

- ☐ I have no pain or discomfort
- ☐ I have slight pain or discomfort
- ☐ I have moderate pain or discomfort
- ☐ I have severe pain or discomfort
- ☐ I have extreme pain or discomfort

Anxiety/Depression

- ☐ I am not anxious or depressed
- ☐ I am slightly anxious or depressed
- ☐ I am moderately anxious or depressed
- ☐ I am severely anxious or depressed
- ☐ I am extremely anxious or depressed

F. INTERVENTION ADMINISTRATION RECORD

Date _____

Time _____
((military))

Dose

Date (mm/dd/yyyy) _____

Time (military) from start of dose _____

Check if complete dose administered ☐ complete ☐ incomplete

if incomplete, how many mL was administered _____

J. CULTURE-PROVEN INFECTIONS

Infection Date

Suspected Site

- ☐ Blood
- ☐ Urine
- ☐ Respiratory
- ☐ Wound or soft tissue
- ☐ Stool
- ☐ Other

Suspected Site

Organism

- ☐ Escherichia coli
- ☐ Clostridium difficile
- ☐ MRSA
- ☐ MSSA
- ☐ Klebsiella pneumonia
- ☐ Psuedomonas aeruginosa
- ☐ Serratia marescens
- ☐ Stenotrophomonas
- ☐ VRE
- ☐ Other

If 'Other', please specify organism

Antibiotic Resistance

- ☐ Amikacin
- ☐ Ampicillin-Sulbactam
- ☐ Aztreonam
- ☐ Cefazolin
- ☐ Cefepime
- ☐ Cefoxitin
- ☐ Cetazadime
- ☐ Ceftriaxone
- ☐ Ertapenem
- ☐ Gentamycin
- ☐ Levofloxacin
- ☐ Meropenem
- ☐ Nitrofurantoin
- ☐ Piperacillin-Tazobactam
- ☐ Tetracycline
- ☐ Tobramycin
- ☐ Bactrim
- ☐ Tigecycline

Comments

I. AE-UP RECORD

Date of UP Onset

Description of unanticipated problem

(100 characters or less)

Date of UP End

UP Severity

- ☐ Mild
- ☐ Moderate
- ☐ Severe
- ☐ Life threatening

UP Relatedness to study intervention

- ☐ Possibly
- ☐ Likely related
- ☐ Definitely related

Action on UP

- ☐ None
- ☐ Intervention altered
- ☐ Intervention discontinued
- ☐ Medical intervention
- ☐ Surgical intervention

H. PATIENT DISPOSITION RECORD

Completed ☐ Yes ☐ No

Reason ☐ death ☐ discharge
☐ refusal

Date _____

Time _____

ICU discharge date _____

Hospital discharge date _____

Withdrawal date (if withdrew) _____

Did the patient die before study completion? ☐ No ☐ Yes

Death date _____

Enter a proximate cause of death in 1-2 words _____

Last study evaluation date
This represents the date on which the final set of
samples were taken. _____

Appendix 9.8

Daniel E. Freedberg, M.D., M.S.
Division of Digestive and Liver Diseases
Columbia University Medical Center
630 West 168th Street, 3-418
New York, NY 10032
Cell: (857) 998-9370

Positions

July 2020 – present	Herbert Irving Assistant Professor of Medicine and Epidemiology, Columbia University Medical Center
July 2018 – June 2020	Assistant Professor of Epidemiology, Mailman School of Public Health, Columbia University Medical Center
July 2015 – June 2020	Assistant Professor of Medicine, Division of Digestive and Liver Diseases, Columbia University Medical Center
July 2014 – June 2015	Instructor of Medicine, Division of Digestive and Liver Diseases, Columbia University Medical Center

Training

July 2011 – June 2014	Fellow in Gastroenterology and Hepatology, Division of Digestive and Liver Diseases, Columbia University Medical Center
July 2009 – June 2011	Resident in Medicine, Columbia University Medical Center
July 2008 – June 2009	Intern, Columbia University Medical Center

Education

July 2012 – May 2014	M.S., Patient-Oriented Research, Mailman School of Public Health, Columbia University
Sept 2004 – June 2008	M.D., Harvard Medical School
Sept 2001 – May 2003	Certificate in pre-medical studies, Columbia University
Sept 1993 – June 1997	B.A., History major, Northwestern University

Licensure

New York State License, Internal Medicine (exp. Sept 2016)
Board certified in Internal Medicine, American Board of Internal Medicine, 2011 (exp. 2021)
Board certified in Gastroenterology, 2014 (exp. 2024)

Previous work experience

Simon & Schuster, New York, Aug. 1998 – Sept. 2001
Acquired, edited, and published general interest, serious nonfiction books, specializing in history. Editorial Assistant (until Aug. 1999), Assistant Editor (until June 2000), and Associate Editor (until Sept. 2001).

Awards & honors

2019	Selected as an AGA Future Leader
2018	Selected for Irving Institute “Reach for the R01” competitive grant workshop
2017	Ewig Clinical Scholar Award for outstanding teaching for medical students, house staff, and fellows (annually for three years)
2017	John N. Loeb annual lecture on hospital-associated <i>C. difficile</i> infection
2017	EPIC scholarship award, National Institute for General Medical Sciences
2016	One of top ten most influential articles of the year, IDweek 2016 (for the article on risk for <i>C. difficile</i> related to the prior bed occupant, <i>JAMA Int Med</i> 2016)
2016	Selected for AGA Institute-AASLD Annual Academic Skills Workshop

- 2012 Arnold P. Gold Foundation Humanism and Excellence in Teaching Award
- 2011 Best Graduating Resident in Medicine, Columbia-Presbyterian Hospital
- 1997 History Department Thesis Honors, Northwestern University

Professional organizations and service

- 2020 – 2021 American Gastroenterological Association (AGA), Nominating Committee
This committee provides overall direction for the AGA and selects the AGA governing board (AGA president, etc.). I was chosen as the early career research member.
- 2020 – 2022 American Gastroenterological Association (AGA), Microbiome and Microbial Therapy (MMT) Nominating Committee
This committee helps the AGA to organize content related to the microbiome and to select the next MMT chair.
- 2019 – present Scientific Advisory Council, Peggy Lillis Foundation
This charitable foundation is dedicated to publication and advocacy related to *Clostridioides difficile* infection.
- 2016 – 2019 Executive Committee, Society of Practitioners of Columbia-University Medical Center
This voluntary society meets weekly with the hospital administration. The society provides a mechanism that allows doctors to help determine what exemplary patient care means at Columbia.

Society memberships

- 2011 – present American Gastroenterological Association
- 2011 – present American College of Gastroenterology
- 2011 – present American Society of Gastrointestinal Endoscopy
- 2011 – present New York Society for Gastrointestinal Endoscopy
- 2008 – present American Medical Association
- 2004 – present Massachusetts Medical Society

Editorial activities

- Associate editor ACG Case Reports Journal (2013-2014), one of eight founding associate editors
- Editorial board World Journal of Gastroenterology (2013-2016)
- Manuscript review (selected) American Journal of Infection Control
- Annals of Internal Medicine (highest quality reviewer)
- Annals of the New York Academy of Sciences
- BMC Gastroenterology
- British Medical Journal, Gastroenterology
- Cancer Prevention Research
- Clinical Gastroenterology and Hepatology
- Digestive Diseases and Sciences
- Expert Opinion on Drug Safety
- Gastroenterology
- Gut
- JAMA Internal Medicine
- Journal of Gastroenterology and Hepatology
- PLoS One
- Practical Gastroenterology

Scientific Reports
Therapeutic Advances in Endoscopy

Grant Review

2019	Study Section, Department of Defense PRMRP (Peer-Reviewed Medical Research Program)
2019	Study Section, NIH CRFS (Clinical Research and Field Studies of Infectious Diseases)
2018	Irving Institute (Columbia CTSA) KL2 Reviewer

Clinical work

Attending Physician, Division of Digestive Diseases, Columbia University (since July 2014)

- Active general gastroenterology clinical practice with 1 office session and 1 endoscopy sessions per week generating 2,000-2,500 work RVUs/year since FY14
- 1-2 weeks attending on General GI (consult) service annually
- 1 mos attending on General Medicine 2 (primary) service every other year

Ongoing Research Support

Irving Scholars Award, Columbia Irving Institute for Clinical and Translational Research
(Freedberg, PI) July 2020 – June 2023 Project

title: Leveraging gut colonization resistance to prevent multidrug resistant infections in the intensive care unit

Goal: The purpose of this intensive care unit-based translational research is to (1) identify 1:1 bacterial interactions that prevent gut colonization with multidrug-resistant organisms, (2) risk-stratify patients by testing whether the novel microbiome-based biomarker urine 3-indoxyl sulfate predicts loss of colonization resistance, and (3) elucidate how colonization with multidrug-resistant organisms leads to infection by determining the relationship between gut colonization and systemic infection at the strain level.

Role: PI

Digestive Diseases Research Center Pilot (Freedberg, PI) Jan 2020 – Dec 2020 Project

title: Preventing multidrug-resistant organism enteric colonization in the intensive care unit

Goal: This project award will use previously collected fecal samples from ICU patients to understand the bacteria-bacteria interactions that prevent or facilitate gut colonization with multidrug-resistant organisms and how this process is shaped by antibiotics.

Role: PI.

Clinical Trial Award (Freedberg, PI) July 2019 – June 2023 Department of Defense (DoD) Peer-Reviewed Medical Research Program (PRMRP) Project title: Prebiotic inulin to limit antimicrobial-resistant infections during critical illness: a phase II clinical trial

Goal: The purpose of this research is to perform a double-blind, randomized trial of inulin supplementation for the purpose of preventing multidrug-resistant organism colonization and infection in critically ill patients meeting sepsis criteria.

Role: PI.

NIH K23 DK111847 (Freedberg, PI) Feb 2017 – Dec 2020

Project title: Loss of Gastrointestinal Colonization Resistance and Antibiotic-Resistant Infections in the Intensive Care Unit.

Goal: This 4 year mentored research award has enabled me to expand our ICU cohort to study dynamic changes within the gastrointestinal microbiome, with a new focus on antibiotic resistance.

Primary mentor: Timothy Wang.

Role: Mentored PI.

Completed Research Support

Irving Institute/CTO Pilot (Freedberg, PI)

July 2018 – June 2019 Project

title: Dietary fiber to prevent loss of gastrointestinal colonization resistance and pathogen colonization in the intensive care unit.

Goal: This pilot award offered through Columbia's CTSA (NCATS UL1 TR001873) funds enrollment for a prospective clinical trial comparing high- versus low-fiber feeds in the ICU.

Role: PI.

Feldstein Medical Foundation (Freedberg, PI)

July 2018 – June 2019

Project title: Dietary fiber to prevent pathogen colonization in the intensive care unit.

Goal: This medical foundation pilot award funds additional analyses for the ongoing ICU-based fiber study.

Role: PI.

Research Scholar Award (Freedberg, PI)

July 2015 – June 2018

American Gastroenterological Association (AGA)

Project title: Clinical and gut microbiome factors associated with ICU-acquired infections.

Goal: This 3 year mentored research award allowed me to build an ICU cohort with collection of biospecimens and data for the purpose of studying ICU-acquired infections.

Primary mentor: Timothy Wang.

Role: Mentored PI.

NIH KL2 TR000081 (Ginsberg, PI)

July 2014 – June 2016

Clinical and Translational Sciences Award

Project title: Adverse effects of proton pump inhibitors on the gastrointestinal health of children.

Goal: This 2 year mentored research award included didactic training and enabled me to build a cohort of children who were studied to assess the effects of acid suppression medications on gut health.

Primary mentor: Timothy Wang.

Role: Mentored awardee.

Clinical Research Award (Abrams, PI)

July 2014 – Dec 2015

American College of Gastroenterology (ACG)

Project title: Proton pump inhibitors and *Clostridium difficile* infection in children.

Goal: This project specific award enabled us to build a large retrospective cohort related to CDI.

Role: Co-Investigator

NIH UL1 RR024156 (Ginsberg, PI)

July 2012 – June 2014

Fundamentals of Patient-Oriented Clinical Research

Goal: This training grant from the Mailman School of Public Health facilitates biostatistical and epidemiological training in clinical research and enabled me to obtain a Master's degree in biostatistics and other additional training during GI fellowship.

Role: Training award recipient.

NIH T32 DK083256-04 (Wang, PI) July 2012 – June 2014
Multidisciplinary Training in Translational Gastrointestinal and Liver Research
Goal: This training grant facilitates the career development of basic, clinical, and translational researchers and funds from this grant were combined with NIH UL1 RR024156 to enable me to obtain post-graduate training in epidemiology and biostatistics at the Mailman School.
Role: Training award recipient.

Student/training grants

National Kidney Foundation Resident-Investigator Grant, Fall 2008
American Federation of Aging Research Geriatric Scholars Program, Summer 2005
PASTEUR Fellowship (Patient-Associated Science: Training, Education, Understanding, and Research), Harvard Medical School, Summer 2005
Louis E. Kirstein Fellowship, Harvard Medical School, 2004 – 2006

Publications

Original, peer-reviewed research

1. Blackett JW, Faye A, Phipps M, Li J, Lebwohl B, **Freedberg DE**. Prevalence and Risk Factors for Inappropriate Continuation of Proton Pump Inhibitors After Discharge from the Intensive Care Unit, Mayo Clinical Proceedings, *Accepted July 23, 2020 (print to come)*.
2. Anderson MR, Geleris J, Anderson DR, Zucker J, Nobel YR, **Freedberg DE**. Small-Saunders J, Rajagopalan KN, Greendyk R, Chae S, Natarajan K, Roh D, Edwin E, Gallagher D, Podolanczuk A, Barr RG, Ferrante AW, Baldwin MR. Body mass index and risk of intubation or death in SARS-CoV-2 infection: a retrospective cohort study. *Annals of Internal Medicine, Accepted July 17, 2020 (print to come)*.
3. Varma S, Faye AS, Kannan A, Lawlor G, Verma A, Axelrad J, **Freedberg DE**. Patients with more severe IBD get *Clostridioides difficile* rather than *Clostridioides difficile* increasing the severity of IBD. *Digestive Diseases and Sciences, Accepted July 13, 2020 (print to come)*.
4. Munck C, Sheth RU, Cuaresma C, Weidler J, Stump SL, Zachariah P, Chong DH, Uhlemann AC, Abrams JA, Wang HH, **Freedberg DE**. The effect of short-course antibiotics on the resistance profile of colonizing gut bacteria in the ICU: a prospective cohort study. *Critical Care, Accepted June 4, 2020 (print to come)*.
5. Zachariah P, Rozenberg FD, Stump S, Moscoso D, Krishnamurthy G, Saiman L, Uhlemann AC, **Freedberg DE**. Evolution of the environmental microbiota of a new neonatal intensive care unit and implications for infection prevention and control. *Infection Control & Hospital Epidemiology, Accepted May 23, 2020 (print to come)*.
6. **Freedberg DE**, Conigliaro J, Wang, TC, Tracey KJ, Callahan MV, Abrams JA on behalf of the Famotidine Research Group. Famotidine use is associated with improved clinical outcomes in hospitalized COVID-19 patients: A propensity score matched retrospective cohort study. *Gastroenterology* 2020, Epub ahead of print. PMID: 32446698 PMCID: PMC7242191
7. Nobel YR, Phipps M, Zucker J, Lebwohl B, Wang TC, Sobieszczyk ME, **Freedberg DE**. Gastrointestinal symptoms and COVID-19: case-control study from the United States. *Gastroenterology* 2020, Epub ahead of print. PMID: 3229447 PMCID: PMC7152871
8. **Freedberg DE**, Messina M, Lynch E, Tess M, Miracle E, Chong DH, Wahab R, Abrams JA, Wang HH, Munck C. Impact of fiber-based enteral nutrition on the gut microbiome of ICU patients receiving broad-spectrum antibiotics: a randomized pilot trial. *Critical Care Explorations, Accepted May 18, 2020, print pending*.

9. Munck C, Sheth R, **Freedberg DE**, Harris Wang HH. Recording mobile DNA in the gut microbiota using an *Escherichia coli* CRISPR-Cas spacer acquisition platform. *Nature Communications* 2020, Epub ahead of print. PMID: 31911609 PMCID: PMC6946703
10. Fu Y, Moscoso DI, Porter J, Krishnareddy S, Abrams JA, Seres D, Chong DH, **Freedberg DE**. The effect of dietary fiber intake on short chain fatty acid-producing bacteria during critical illness: a prospective cohort study. *Journal of Parenteral and Enteral Nutrition* 2020, 44(3): 463-471. PMID: 31385326 PMCID: PMC7002227
11. Hung K, Knotts RM, Faye AS, Pont AR, Lebwohl B, Abrams JA, **Freedberg DE**. Factors associated with adherence to *Helicobacter pylori* testing during hospitalization for bleeding peptic ulcer disease. *Clinical Gastroenterology and Hepatology* 2020, 18(5): 1091-1098. PMID: 31352090
12. Wang Y, Schluger A, Li J, Gommez-Simmonds A, Salmasian H, **Freedberg DE**. Does addition of intravenous metronidazole to oral vancomycin improve outcomes in *Clostridioides difficile* infection? *Clinical Infectious Diseases* 2019, Epub ahead of print. PMID: 31714955
13. Faye AS, Hung K, Cheng K, Blackett JW, McKenney AS, Pont AR, Li J, Lawlor G, Lebwohl B, **Freedberg DE**. Minor hematochezia decreases use of venous thromboembolism prophylaxis in patients with inflammatory bowel disease. *Inflammatory Bowel Disease* 2019, Epub ahead of print. PMID: 31689354
14. La Carpia F, Wojczyk B, Annavajhala M, Rebbaa A, Culp-Hill R, D'Alessandro A, **Freedberg DE**, Uhlemann AC, Hod EA. Transfusional iron overload and intravenous iron infusions modify the mouse gut microbiota similarly to dietary iron. *NPJ Biofilms and Microbiomes* 2019, 5: 26. PMID: 31583109 PMCID: PMC6760189
15. Horvath A, Rainer F, Bashir M, Leber B, Schmerboeck B, Klymiuk I, Groselj-Strele A, Durdevic M, **Freedberg DE**, Abrams JA, Fickert P, Stiegler P, Stadlbauer V. Biomarkers for oralization during long-term proton pump inhibitor therapy predict survival in cirrhosis. *Scientific Reports* 2019, 9(1): 12000. PMID: 31427717 PMCID: PMC6700098
16. Snider EJ, Compres G, **Freedberg DE**, Khiabani H, Nobel YR, Stump S, Uhlemann AC, Lightdale CJ, Abrams JA. Alterations to the esophageal microbiome associated with progression from Barrett's esophagus to esophageal adenocarcinoma. *Cancer Epidemiology, Biomarkers & Prevention* 2019, 28(10): 1687-1693. PMID: 31466948 PMCID: PMC6774849
17. Macesic N, Khan S, Giddins M, **Freedberg DE**, Whittier S, Green D, Furuya EY, Verna E, Annavajhala M, Gomez-Simmonds A, and Uhlemann AC. *Escherichia coli* harboring mcr-1 in a cluster of liver transplant recipients: detection through active surveillance and whole genome sequencing. *Antimicrobial Agents and Chemotherapy* 2019, 63(6): e02680-18. PMID: 30962343 PMCID: PMC6535534
18. Zachariah P, **Freedberg DE**. Vancomycin use in surrounding patients during critical illness and risk for persistent colonization with vancomycin-resistant *Enterococcus*. *Journal of Hospital Infection* 2019, 102(3): 343-346. PMID 30641098 PMCID: PMC6589391
19. Axelrad J, **Freedberg DE**, Whittier S, Greendyke W, Lebwohl B, Green D. Impact of gastrointestinal panel implementation on healthcare utilization and outcomes. *Journal of Clinical Microbiology* 2019; Epub Jan 16 ahead of print. PMID: 30651393 PMCID: pending
Selected for oral presentation at DDW 2019
20. Wang Y, Li J, Zachariah P, Abrams JA, **Freedberg DE**. Relationship between remote cholecystectomy and incident *Clostridioides difficile* infection. *Clinical Microbiology and Infection* 2019, 25(8): 994-999. PMID: 30583059 PMCID: PMC6589130
21. Nobel Y, Snider E, Compres G, Khiabani H, **Freedberg DE**, Lightdale CJ, Toussaint NC, Abrams JA. Increasing dietary fiber intake is associated with a distinct esophageal microbiome. *Clinical and Translational Gastroenterol*, Accepted for publication October 2, 2018. PMID: 30356041 PMCID: PMC6200756

22. Mahadev S, Jin Z, Lebwohl B, Rosenberg R, Garcia-Carrasquillo RJ, Ramirez I, **Freedberg DE**. Trainee colonoscopy quality is influenced by the independent and unobserved performance characteristics of supervising physicians. *Endoscopy International Open* 2019 Jan 7; (1): E74-E82. PMID: 30746431 PMCID: PMC6368225 *Selected for oral presentation at DDW 2017*
23. Livanos AE, Snider EJ, Whittier S, Chong DH, Wang TC, Abrams JA, **Freedberg DE**. Rapid gastrointestinal loss of Clostridial Clusters IV and XIVa in the ICU associates with an expansion of gut pathogens. *PLoS One* 2018 Aug 1, 13(8): e0200322. PMID: 30067768 PMCID: PMC6070193 *Selected for oral presentation at CUMC Medicine Grand Rounds 2017*
24. Zhou MZ, Li J, Salmasian S, Zachariah P, Yang YX, **Freedberg DE**. The local hospital milieu and healthcare-associated VRE acquisition. *J Hosp Infect* 2019; 101(1): 69-75. PMID: 30026006 PMCID: PMC6309650
25. Axelrad JE, Lebwohl B, Cuasmasa E, Cadwell K, Green PHR, **Freedberg DE**. Gut colonization with vancomycin-resistant *Enterococcus* and risk for subsequent enteric infection. *Gut Pathog* 2018 Jul 9; 10:28. PMID: 30002733 PMCID: PMC6038175.
26. **Freedberg DE**, Zhou MJ, Cohen ME, Annavaiahala M, Khan S, Moscoso DI, Brooks CA, Whittier S, Chong DH, Uhlemann AC, Abrams JA. Pathogen colonization of the gastrointestinal microbiome at intensive care unit admission and risk for subsequent death or infection. *Intensive Care Med* 2018; 44(8): 1203-1211. PMID: 29936583 PMCID: PMC6309661 *Selected for oral presentation at DDW 2018*
27. Seeley JJ, Baker RG, Mohamed G, Bruns T, Hayden MS, Deshmukh SD, **Freedberg DE**, Ghosh S. Induction of innate immune memory via microRNA targeting of chromatin remodelling factors. *Nature* 2018; 559(7712): 114-119. PMID: 29950719 PMCID: PMC6044474.
28. Snider EJ, Compres G, **Freedberg DE**, Giddins MJ, Khiabani H, Lightdale CJ, Nobel YR, Toussaint NC, Uhlemann AC, Abrams JA. Barrett's esophagus is associated with a distinct oral microbiome. *Clin Transl Gastroenterol* 2018; 9(3): 135. PMID: 29491399 PMCID: PMC5862155 *Selected for oral presentation at DDW 2017*
29. Cohen ME, Salmasian H, MD, Li J, Liu J, Zachariah P, Wright JD, **Freedberg DE**. Surgical antibiotic prophylaxis is not associated with risk for post-operative antibiotic-resistant infections. *J Am Coll Surg* 2017; 225(5): 631-638. PMID: 29030239 PMCID: PMC5673556
30. Terry MA, **Freedberg DE**, Morris MC. An alternative consent process for minimal risk research in the intensive care unit. *Crit Care Med* 2017; 45(9): 1450-1456. PMID: 28617695 PMCID: PMC5661876
31. Cohen ME, Hathway J, Salmasian H, Liu J, Terry M, Abrams JA, **Freedberg DE**. Prophylaxis for stress ulcers with proton pump inhibitors is not associated with increased risk of bloodstream infections in the intensive care unit. *Clin Gastroenterol and Hepatol* 2017; 15(7): 1030-1036. PMID: 28110095 PMCID: PMC5474142
32. **Freedberg DE**, Salmasian H, Cohen B, Abrams JA, Larson EL. Receipt of antibiotics in hospitalized patients and risk for *Clostridium difficile* infection in subsequent patients who occupy the same bed. *JAMA Int Med* 2016; 176(12): 1801-1808. PMID: 27723860 PMCID: PMC5138095 *Selected as one of the top 10 most influential articles of the year at IDweek 2016 (annual meeting of the IDSA/SHEA)*
33. Faleck DM, Salmasian H, Furuya EY, Larson EL, Abrams JA, **Freedberg DE**. Proton pump inhibitors do not affect risk for *Clostridium difficile* infection in the intensive care unit. *Am J Gastroenterol* 2016; 111(11): 1641-1648. PMID: 27575714 PMCID: PMC5096970
34. Jackson MA, Goodrich JK, Maxan ME, **Freedberg DE**, Abrams JA, Poole AC, Sutter JL, Welter D, Ley RE, Bell JT, Spector TD, Steves CJ. Proton pump inhibitors alter the composition of the gut microbiota. *Gut* 2016; 65(5): 749-756. PMID: 26719299 PMCID: PMC4853574

35. Lebwohl B, Luchsinger JA, **Freedberg DE**, Green PH, Ludvigsson JF, Risk of dementia in patients with celiac disease: a population-based cohort study. *J Alzheimers Dis* 2015; 49(1): 179-185. PMID: 26444775
36. **Freedberg DE**, Toussaint NC, Chen SP, Ratner AJ, Whittier S, Wang TC, Wang HH, Abrams JA. Proton pump inhibitors alter specific taxa in the human fecal microbiome: a crossover trial. *Gastroenterology* 2015; 149(4): 883-885.e9. PMID: 26164495 PMCID: PMC4584196
37. **Freedberg DE**, Lamou  -Smith ES, Lightdale JR, Jin Z, Yang Y, Abrams JA. Use of acid suppression medication is associated with risk for *C. difficile* infection in infants and children: a population-based study. *Clin Infect Dis* 2015; 61(6): 912-917. PMID: 26060292 PMCID: PMC4551005
38. **Freedberg DE**, Haynes K, Denburg MR, Zemel BS, Leonard MB, Abrams JA, Yang Y. Use of proton pump inhibitors is associated with fractures in young adults: a population-based study. *Osteoporos Int* 2015; 26(10): 2501-2507. PMID: 25986385 PMCID: PMC4575851
39. Latorre M, Lagana SM, **Freedberg DE**, Lebwohl B, Bhagat G, Lewis SK, Green PH. Endoscopic biopsy technique in the diagnosis of celiac disease: One bite or two? *Gastrointest Endosc* 2015; 81(5): 1228-1233. PMID: 25599173
40. **Freedberg DE**, Salmasian H, Abrams JA, Green RA. Orders for intravenous proton pump inhibitors decrease after implementation of an electronic alert. *JAMA Int Med* 2015; 175(3): 452-454. PMID: 25599173 PMCID: PMC5022559
41. Hillyer GC, Schmitt KM, **Freedberg DE**, Rosenberg RR, Kramer R, Neugut AI. Colorectal cancer screening among the uninsured in Northern Manhattan, 1998-2010: Experience with guaiac-based fecal occult blood testing and fecal immunochemical test. *Am J Prev Med* 2014; 47(2): 182-187. PMID: 24951037 PMCID: PMC4127996
42. Sharaiha RZ, **Freedberg DE**, Abrams JA, Wang YC. Cost-Effectiveness of Chemoprevention with Proton Pump Inhibitors in Barrett's Esophagus. *Dig Dis Sci* 2014; 59(6): 1222-1230. PMID: 24795040
43. Salmasian H, **Freedberg DE**, Friedman C. Deriving comorbidities from medical records using natural language processing. *J Am Med Inform Assoc* 2013; 20(e2): e239-e242. PMID: 24177145 PMCID: PMC3861932
44. **Freedberg DE**, Salmasian H, Friedman C, Abrams JA. Proton pump inhibitors and risk for recurrent *Clostridium difficile* infection among inpatients. *Am J Gastroenterol* 2013; 108(11): 1794-1801. PMID: 24060760 PMCID: PMC3966060.
45. Salmasian H, **Freedberg DE**, Abrams JA, Friedman C. An automated tool for detecting medication overuse based on electronic health records. *Pharmacoepidemiol Drug Saf* 2013; 22(2): 183-189. PMID: 23233423 PMCID: PMC3566345
46. **Freedberg DE**, Dave JK, Kurth T, Gaziano JM, Bludau JHA. Cognitive impairment over the age of 85: hospitalization and mortality. *Arch Gerontol Geriatr* 2008; 46(2): 137-145. PMID: 17498822
47. **Freedberg DE**, Rigas SH, Russak J, Gai W, Kaplow M, Osman I, Turner F, Randerson-Moor JA, Houghton A, Busam K, Bishop DT, Bastian BC, Newton-Bishop JA, Polsky D. Frequent p16-independent inactivation of p14ARF in human melanoma. *J Natl Cancer Inst* 2008; 100(11): 784-795. PMID: 18505964 PMCID: PMC4410798.

Reviews

1. Choy A, **Freedberg DE**. Impact of microbiome-based interventions on gastrointestinal pathogen colonization in the intensive care unit. *Therapeutic Advances in Gastroenterology* 2020; *Accepted for publication June 14, 2020, print pending*.
2. Gupta A...**Freedberg DE**...Landry DW. Extrapulmonary manifestations of COVID-19. *Nature Medicine* 2020; *Accepted for publication June 6, 2020, print pending*.
3. Lukovic E, Moitra VK, **Freedberg DE**. Microbiome: implications for perioperative and critical care. *Current Opinion in Anaesthesiology* 2019; 32(3): 412-420. PMID: 30925514

4. **Freedberg DE**, Kim L, Yang YX. The risks and benefits of long-term use of proton pump inhibitors: expert review and best practice advice from the American Gastroenterological Association. *Gastroenterology* 2017; 152(4): 706-715. PMID: 28257716.
5. Snider E, **Freedberg DE**, Abrams JA. Potential role of the microbiome in Barrett's esophagus and esophageal adenocarcinoma. *Dig Dis Sci* 2016; 61(8): 2217-2225. PMID: 27068172
6. Latorre M, Krishnareddy S, **Freedberg DE**. Microbiome as mediator: do systemic infections start in the gut? *World J Gastro* 2015; 21(37): 10487-10492. PMID: 26457009 PMCID: PMC4588071
7. **Freedberg DE**, Lebwohl B, Abrams JA. The impact of proton pump inhibitors on the human gastrointestinal microbiome. *Clinics in Laboratory Medicine* 2014; 34(4): 771-785. PMID: 25439276 PMCID: PMC4254461

Editorials, letters, and case reports

1. Krigel A, Kong XF, **Freedberg DE**. The light at the end of the tunnel. *Gastroenterology* 2019; S0016-5085 [Epub ahead of print]. PMID 30610863
2. Bell JS, **Freedberg DE**. More data on PPI use and kidney disease. *GI and Hepatology News: The official newspaper of the AGA*, Nov. 8, 2017.
3. **Freedberg DE**, Abrams JA, Yang YX. Proton pump inhibitors and myocardial infarction: the current perspective. *Gastroenterology* 2015; 149(4): 830-833. PMID: 26277262
4. **Freedberg DE**, Abrams JA, Wang TC. Prevention of gastric cancer with antibiotics: can it be done without eradicating *Helicobacter pylori*? *J Natl Cancer Inst* 2014; 106(7). PMID: 24925352
5. **Freedberg DE**, Bhadelia N, Poneros JM, Oster MW. Visceral varicella in a patient with chronic lymphocytic leukemia on fludarabine: a case report. *Clin Lymphoma Myeloma Leuk* 2013; 13(1): 90-92. PMID: 23103084
6. **Freedberg DE**, Bao F, Stein JA. Rectal leiomyosarcoma after pelvic irradiation. *Clinical Gastroenterol Hepatol* 2013; 11(7): A28. PMID: 22902282
7. **Freedberg DE**. Proton pump inhibitors: a balanced view. *Practical Gastroenterology* 2013; 37(12): 44.
8. **Freedberg DE**, Husain S, Swaminath A. Severe inflammatory bowel disease-associated pyoderma gangrenosum. *Journal Gastroenterol Hepatol* 2013; 28(11): 1691. PMID: 24147454
9. **Freedberg DE**, Garcia-Carrasquillo RJ, Schwartz B, Rosenberg RM. A technique for skin-level gastrostomy tube placement after gastrostomy tube dislodgement. *Gastrointest Endosc* 2013; 78(6): 963-964. PMID: 24237952
10. **Freedberg DE**, Abrams JA. *Clostridium difficile* infection in the community: are proton pump inhibitors to blame? *World J Gastroenterol* 2013; 19(40): 6710-6713. PMID: 24187445 PMCID: PMC3812469
11. **Freedberg DE**, Abrams JA. Recent therapeutic advances in gastroenterology and hepatology. *Adv Ther* 2013; 30(10): 855-857. PMID: 24170588 PMCID: PMC3898138
12. **Freedberg DE**, Abrams JA. Does confounding explain the association between PPIs and *Clostridium difficile*-related diarrhea? *Am J Gastroenterol* 2013; 108(2): 278-279. PMID: 23381076
13. **Freedberg DE**. To eGFR or not to eGFR: here is an intern's answer. *Kidney Int* 2009; 76(2): 129-130. PMID: 19564852
14. **Freedberg DE**. Lessons from long-lived physicians. *J Am Geriatr Soc* 2006; 54(8): 1293. PMID: 16914002

Abstracts (selected)

1. Blackett WJ, Faye AS, Phipps M, Li J, Lebwohl BL, **Freedberg DE**. Inappropriate discharge on proton pump inhibitors after intensive care unit hospitalization. *Digestive*

- Disease Week, May 6, 2020 (WebEx due to COVID-19). *Selected for oral presentation and featured in AGA Today newsletter.*
2. Kuo S, Dettmer K, Annavajhala M, Uhlemann AC, Abrams JA, Oefner P, **Freedberg DE**. Urine 3-indoxyl sulfate, a gut microbiome-derived biomarker, is associated with mortality after intensive care unit admission. Digestive Disease Week, May 5, 2020 (WebEx due to COVID-19). *Selected for oral presentation.*
 3. Wang Y, Salmasian H, Li J, **Freedberg DE**. Validation of peripheral eosinopenia as a novel risk factor for death in hospitalized patients with *Clostridioides difficile* infection. Digestive Disease Week, May 4, 2020 (WebEx due to COVID-19). *Selected for oral presentation.*
 4. Zachariah P, Krishnamurthy G, Annavajhala MK, Moscoso DI, Rozenberg FD, Saiman L, Uhlemann AC, **Freedberg DE**. Evolution of the environmental microbiome of a new neonatal intensive care unit. Pediatric Academy of Science (PAS) 2019 Meeting, April 27, 2019, Baltimore, MD.
 5. Fu Y, Moscoso DI, Porter J, Krishnareddy S, Abrams JA, Seres D, Chong DH, **Freedberg DE**. The effect of dietary fiber intake on short chain fatty acid-producing bacteria during critical illness: a prospective cohort study. Digestive Disease Week, May 19, 2019, San Diego, CA (selected for poster highlights tour). *Also presented at (1) AGA Gut Microbiota for Health Summit conference, March 23, 2019 in Miami, FL (winner: \$500 travel award) and (2) CUMC Medical Student Research Day, March 21, 2019 (winner: best poster award).*
 6. Wang Y, Schluger A, Li J, Gomez-Simmonds A, **Freedberg DE**. Does addition of metronidazole to oral vancomycin improve outcomes in *Clostridioides difficile* infection? Digestive Disease Week, May 20, 2019, San Diego, CA. *Selected for oral presentation at Columbia Resident Research Day Grand Rounds, March 27, 2019.*
 7. Faye AS, Hung K, Blackett JW, Pont AR, Lawlor G, Lichtiger S, Lebwohl B, **Freedberg DE**. Hematochezia decreases use of venous thromboembolism prophylaxis in patients with inflammatory bowel disease. Digestive Disease Week, May 20, 2019, San Diego, CA.
 8. Wang Y, Li J, Zachariah P, Abrams JA, **Freedberg DE**. Relationship between remote history of cholecystectomy and risk for incident *Clostridium difficile* infection. American College of Gastroenterology, October 8, 2018, Philadelphia, PA.
 9. Hung K, Abdelmessih R, Faye AS, Pont AR, **Freedberg DE**. Adherence to *Helicobacter pylori* testing after hospitalization for bleeding peptic ulcer disease. American College of Gastroenterology, October 8, 2018, Philadelphia, PA. *Awarded first place honors among posters at the 42nd annual meeting of the New York Society of Gastrointestinal Endoscopy, February 6, 2019.*
 10. Zhou MZ, Li J, Salmasian S, Zachariah P, Yang YX, **Freedberg DE**. The local hospital milieu and healthcare-associated VRE acquisition. IDweek 2018, October 5, 2018, San Francisco, CA.
 11. Cohen ME, Salmasian H, MD, Li J, Liu J, Zachariah P, Wright JD, **Freedberg DE**. Surgical Antibiotic Prophylaxis is not Associated with Risk for Post-Operative Antibiotic-Resistant Infections. IDweek 2017, October 7, 2017, San Diego, CA.
 12. Mahadev S, Lebwohl B, **Freedberg DE**. Trainee colonoscopy quality is influenced by the independent performance characteristics of supervising physicians. Digestive Disease Week, May 6-9, 2017. *Selected for oral presentation.*
 13. Livanos AE, Snider EJ, Terry ME, Abrams JA, Chong DH, **Freedberg DE**. Loss of Short Chain Fatty Acid-Producing Clostridia Occurs Rapidly After ICU Admission and Associates with Receipt of Antibiotics. Digestive Disease Week, May 6-9, 2017. *Additionally selected for presentation at CUMC Medicine Grand Rounds (Clinician-Scientist Research Pathway Presentation Day, Nov 8, 2017).*

14. Mahadev S, **Freedberg DE**. Impact of trainee experience on the decision to perform endoscopy on weekends. Digestive Disease Week, May 6-9, 2017.
15. Snider EJ, Nobel YR, Compres G, **Freedberg DE**, Toussaint NC, Abrams JA. Oral Microbiome Alterations in Patients with Barrett's Esophagus. Digestive Disease Week, May 6-9, 2017. *Selected for oral presentation.*
16. **Freedberg DE**, Salmasian H, Wang TC, Abrams JA. Specific hospital rooms are associated with increased risk for *Clostridium difficile* infection. Digestive Disease Week, May 6-9, 2017.
17. **Freedberg DE**, Salmasian H, Cohen B, Abrams JA, Larson EL. Receipt of antibiotics in hospitalized patients increases risk for *Clostridium difficile* infection in future patients who occupy the same bed. IDWeek 2016, October 29, 2016, New Orleans, LA.
18. Hathway J, Larson EL, Abrams JA, **Freedberg DE**. Exposure to antibiotics in the intensive care unit is associated with increased risk for bacteremia from enteric organisms. IDWeek 2016, October 29, 2016, New Orleans, LA.
19. Jang S, Uhde, Indart AM, **Freedberg DE**, Alaedini A. Markers of celiac disease and immune reactivity to gluten in response to the use of proton pump inhibitors. Women in Science at Columbia Symposium, April 23, 2016. *Selected for oral presentation.*
20. Laszkowska M, Terry M, Whittier S, Abrams JA, **Freedberg DE**. Timing and Risk Factors for Gastrointestinal Acquisition of Multi-Drug Resistant Organisms in the Intensive Care Unit. Digestive Disease Week 2016, San Diego, CA. *Additionally selected for oral presentation at Columbia University Medical Center Grand Rounds on Resident Research Day, April 6, 2016.*
21. Mahadev S, Lebwohl B, Ramirez I, Garcia-Carrasquillo RJ, **Freedberg DE**. Transition to a GI Hospitalist System Is Associated With Expedited Upper Endoscopy. Digestive Disease Week 2016, San Diego, CA.
22. **Freedberg DE**, Toussaint NC, Chen SP, Ratner AJ, Whittier S, Wang TC, Wang HH, Abrams JA. Proton Pump Inhibitors Alter Specific Taxa in the Human Fecal Microbiome: Results of a Crossover Trial. Digestive Disease Week 2015, Washington, DC.
23. **Freedberg DE**, Haynes K, Denburg MR, Zemel BS, Leonard MB, Abrams JA, Yang Y. Use of Proton Pump inhibitors is Associated with Non-Osteoporotic Fractures: A Population-Based Study. Digestive Disease Week 2015, Washington, DC.
24. Margolin EJ, Hur L, Wang HH, Toussaint NC, Abrams JA, **Freedberg DE**. Impact of diet on the relationship between obesity and the colonic microbiome. Annual Eastern-Atlantic Student Research Conference 2015, Miami, FL.
25. Faleck DM, Salmasian H, Furuya Y, Abrams JA, **Freedberg DE**. Risk factors for intensive care unit-onset *Clostridium difficile* infection. American College of Gastroenterology Annual Scientific Meeting 2014, Philadelphia, PA.
26. **Freedberg DE**, Salmasian H, Abrams JA, Green RA. Intravenous proton pump inhibitor orders can be decreased with an electronic alert. American College of Gastroenterology Annual Scientific Meeting 2013, San Diego, CA.
27. **Freedberg DE**, Salmasian H, Friedman CF, Abrams JA. Proton pump inhibitor use and risk for *Clostridium difficile*-associated diarrhea among inpatients. Digestive Disease Week 2013, Orlando, FL.
28. Salmasian H, **Freedberg DE**, Abrams JA, Friedman C. An automated tool for detecting medication overuse. American Medical Informatics Association Annual Symposium 2012, Chicago, IL. *Selected for conference highlights poster session.*
29. **Freedberg DE**, Dave JK, Kurth T, Gaziano J, Bludau JHA. Cognitive impairment in the oldest old: hospitalization and mortality. American Geriatrics Society 2006 Annual Meeting, Chicago, IL. Journal of General Internal Medicine 2006; 21(4): S29-30.
30. **Freedberg DE**, Yates LB, Kurth T, Gaziano J. Self-assessment of health in nonagenarian men. American Geriatrics Society 2006 Annual Meeting, Chicago, IL. Journal of the

American Geriatrics Society 2006; 54(4): S196. *Selected for the Presidential Poster Session.*

Invited oral presentations and talks (regional, national, or international)

1. Famotidine for COVID-19 (Yes, Famotidine). New York Society of Gastrointestinal Endoscopy Research Seminar: COVID-19, Data from the Epicenter (keynote address). June 2, 2020 (given via zoom). Similar talk given May 6, 2020 for Columbia Oncology Grand Rounds; May 20, 2020 for the Mailman School of Public Health COVID-19 Virtual Symposium; June 9, 2020 for Columbia ID Grand Rounds.
2. Problems in large, pragmatic randomized trials in the intensive care unit. Otsuka Pharmaceuticals, Princeton, NJ, March 18, 2020 (given via WebEx).
3. Can the Gut Microbiome be Changed to Decrease Risk for Infection in the Intensive Care Unit? University of Pennsylvania School of Medicine Gastroenterology Grand Rounds, Philadelphia, PA, January 15, 2020.
4. Can the Gut Microbiome be Changed to Alter Risk for Infection? Cornell-Weill School of Medicine GI Grand Rounds, New York, NY, September 26, 2019.
5. AGA Roadmap for a Pathway for Successfully Transitioning Early-Stage Clinical-Translational Investigators to Independence. Presented with Ryan Ungaro. American Gastroenterological Association (AGA) Joint Committee Meeting, Washington, DC, September 21, 2019.
6. Causes and consequences of gut pathogen colonization in the intensive care unit. Microbiome Science, Berchtesgaden, Germany, September 21, 2018.
7. Gastrointestinal bacterial pathogen colonization and risk for subsequent infection in the intensive care unit. Digestive Diseases Week (annual meeting of the AGA), Washington, DC, June 5, 2018.
8. Longterm proton pump inhibitors: balancing the risks and benefits. Gastroenterology Grand Rounds, Icahn School of Medicine at Mt. Sinai, New York, NY, January 5, 2018.
9. Longterm proton pump inhibitors: balancing the risks and benefits. Gastroenterology Grand Rounds, Sidney Kimmel School of Medicine, Thomas Jefferson University, Philadelphia, PA, November 15, 2017.
10. Longterm proton pump inhibitors: balancing the risks and benefits. Quarterly Meeting of the Massachusetts Gastroenterology Association, Boston, MA, September 27, 2017.
11. The Role of the Microbiome in Gastrointestinal Diseases. Harvard Medical School Board Review in Gastroenterology & Hepatology, Boston, MA (recorded), June 27, 2017.
12. Infectious complications of chronic use of proton pump inhibitors. AGA clinical symposium, Digestive Diseases Week (annual meeting of the AGA), San Diego, CA, May 21, 2016.
13. Effect of proton pump inhibitors on the human colonic microbiome. Lucille Castori Workshop. Memorial Sloan Kettering Cancer Center, New York, NY, July 16, 2014.
14. Proton pump inhibitors and fracture risk in children and young adults within The Health Improvement Network (THIN): a nested case-control study. THIN Annual Research Symposium. University of Pennsylvania, Philadelphia, PA, June 4, 2014.
15. Research questions related to proton pump inhibitors. Gastroenterology Grand Rounds. Methodist Hospital, Brooklyn, NY, November 21, 2013.

Invited oral presentations and talks (local)

1. Can nutritional therapy be used to change the gut microbiome to decrease risk for systemic infection? Columbia Medical Nutrition Service Grand Rounds, May 20, 2020 (given via zoom).
2. Can the Microbiome be Changed to Decrease Risk for Infections? CUMC Division of Infectious Disease Grand Rounds, New York, NY, February 21, 2019.
3. Can Fiber Improve Outcomes Related to the Gut Microbiome in the ICU? CUMC Critical Care Grand Rounds, New York, NY, December 21, 2018.
4. How the hospital environment shapes risk for *Clostridium difficile* infection. Mailman School of Public Health, New York, NY, June 23, 2017.

5. *Clostridium difficile* infection and toxic megacolon in the intensive care unit. Neurocritical Care Grand Rounds, Columbia University Medical Center, New York, NY, June 1, 2017.
6. How the hospital environment shapes risk for *Clostridium difficile* infection. Medicine Grand Rounds (John N. Loeb annual lecture), Columbia University Medical Center, New York, NY, April 19, 2017.
7. Gut colonization with antibiotic-resistant bacteria in the intensive care unit. Critical Care Grand Rounds, Columbia University Medical Center, New York, NY, February 2, 2017.
8. Balancing the risks and benefits of long-term proton pump inhibitors. Columbia-Cornell 33rd Annual Postgraduate Update in Gastroenterology, Hepatology, and Nutrition, Columbia University Medical Center, New York, NY, December 3, 2016.
9. How the hospital environment shapes risk for *Clostridium difficile* infection. Columbia Microbiome Working Group, Columbia University Medical Center, New York, NY, November 3, 2016.
10. High yield topics in gastroenterology. 22nd Annual Update & Intensive Board Review of Internal Medicine 2016, Columbia University Medical Center, New York, NY, June 9, 2016.
11. *Clostridium difficile* infection and the gastrointestinal microbiome in the intensive care unit. Neurocritical Care Grand Rounds, Columbia University Medical Center, New York, NY, April 21, 2016.
12. Loss of Gastrointestinal Colonization Resistance in the Intensive Care Unit. Critical Care Grand Rounds, Columbia University Medical Center, New York, NY, April 14, 2016.
13. Loss of gastrointestinal colonization resistance leads to antibiotic-resistant infections in the intensive care unit. Clinical-Epidemiological Research Seminar Series. Columbia University Medical Center, New York, NY, October 1, 2015.
14. Effect of proton pump inhibitors on the fecal microbiome in children: a prospective study. Pediatric Gastroenterology Grand Rounds. Columbia University Medical Center, New York, NY, August 22, 2014.
15. Clinical and microbiome factors associated with intensive care unit-onset *Clostridium difficile* infection. Clinical-Epidemiological Research Seminar Series. Columbia University Medical Center, New York, NY, June 5, 2014.
16. Upper gastrointestinal bleeding, proton pump inhibitors, and the gastrointestinal microbiome. Medicine Rounds. Allen Hospital, New York, NY, April 22, 2014.
17. Adverse effects of proton pump inhibitors. Gastroenterology Grand Rounds. Columbia University Medical Center, New York, NY, October 1, 2012.

Appendix 9.9



Type II Achalasia Is Increasing in Prevalence

Margaret J. Zhou¹ · Afrin Kamal² · Daniel E. Freedberg¹ · David Markowitz¹ · John O. Clarke² · Daniela Jodorkovsky¹

Received: 3 July 2020 / Accepted: 8 October 2020 / Published online: 22 October 2020
© Springer Science+Business Media, LLC, part of Springer Nature 2020

Abstract

Background Three manometric subtypes of achalasia were defined in the Chicago Classification approximately 10 years ago: type I (aperistalsis), type II (pan-pressurization), and type III (spastic). Since the widespread use of this classification scheme, the evolving prevalence of these subtypes has not been elucidated. We aim to determine the prevalence of each subtype a decade after the adoption of the Chicago Classification.

Methods This is a retrospective cohort analysis of patients diagnosed with achalasia on high-resolution manometry (HRM) at two major academic medical centers between 2015 and 2018. Patients were excluded if they had a diagnosis of another esophageal motility disorder, previously treated achalasia, or foregut surgery. Demographic data, manometric subtype, and esophageal dilatation grade on endoscopy were obtained. Prevalence of achalasia subtypes was compared with a published historical control population (2004–2007). Fischer’s exact and *t* tests were used for analysis.

Results Of 147 patients in the contemporary cohort and 99 in the historical control cohort, the prevalence of type I achalasia was 8% versus 21%, type II 63% versus 50%, and type III 29% versus 29%, respectively ($p=0.01$). The mean age in our population was 58 years compared to 57 years in the historical control, and the proportion of men 48% versus 47%, respectively ($p=0.78$). Mean endoscopic dilatation grade in the contemporary cohort was 1.5 for type I patients, 0.9 for type II, and 0.4 for type III, compared with 1.5, 0.6, and 0.4, respectively. Overall mean dilatation grade was 0.8 in our cohort versus 0.7 in the historical control ($p=0.58$).

Conclusion The prevalence of type II achalasia was significantly greater and prevalence of type I significantly less in our patient population compared to our predefined historical control. Other characteristics such as age and sex did not appear to contribute to these differences. Histopathological evidence has suggested that type II achalasia may be an earlier form of type I; thus, the increased prevalence of type II achalasia may be related to earlier detection of the disease. The adoption of HRM, widespread use of the Chicago Classification, and increased disease awareness in the past decade may be contributing to these changes in epidemiology.

Keywords Achalasia · High-resolution esophageal manometry · Dysphagia

Introduction

Achalasia is a rare esophageal motility disorder characterized by impaired relaxation of the lower esophageal sphincter (LES) and absent or abnormal peristalsis. In recent years, the annual reported incidence of achalasia has ranged from 1.6 to 2.8 per 100,000 individuals with a prevalence of about 10.8 cases per 100,000 individuals [1, 2]. Despite the relatively low incidence, however, the diagnosis and treatment of achalasia remains an important problem as the chronicity of the disease significantly impairs quality of life [1, 3]. The current gold standard for diagnosis of achalasia is esophageal manometry. In the last decade, use of “conventional”

✉ Daniela Jodorkovsky
Dj2470@cumc.columbia.edu

¹ Division of Digestive and Liver Disease, Department of Medicine, Columbia University Medical Center, New York, NY, USA

² Division of Gastroenterology and Hepatology, Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA

manometry probes has given way to the widespread use of high-resolution manometry (HRM), which incorporates pressure sensors at 1–2 cm intervals spanning the esophageal length and allows for the presentation of data in an esophageal pressure topography format. This has led to the characterization of three distinct manometric subtypes of achalasia [4]. The Chicago Classification, a classification system devised to apply standardized interpretation of HRM metrics, defines these subtypes as: type I—elevated integrated relaxation pressure (IRP) and 100% aperistalsis, type II—elevated IRP with 100% aperistalsis and pan-esophageal pressurization in at least 20% of swallows, and type III—elevated IRP with no normal peristalsis and premature (spastic) distal esophageal contractions in at least 20% of swallows [4, 5]. These subtypes appear to have clinically different phenotypes and response to treatment; type II patients are significantly more likely to respond to any therapy compared to type I or type III [5, 6].

The use of HRM probes has increased the diagnostic sensitivity for achalasia, and thus has likely led to increased detection, perhaps at an earlier stage in the disease course [7, 8]. While type II achalasia has represented the most common subtype in a variety of studies, it is unclear whether the prevalence of the subtypes has changed over time [6, 7]. Since the initial proposal of the Chicago Classification in 2008, a few studies have demonstrated that the detection rate of achalasia is increasing [9, 10]. However, none have examined the evolving prevalence of the three different achalasia subtypes. The aim of this study is to determine the prevalence of each achalasia subtype at two academic medical centers approximately 10 years following the adoption of the Chicago Classification, as compared to a historical control cohort.

Methods

Study Design

This was a retrospective cohort analysis of treatment-naïve patients diagnosed with achalasia on HRM at two tertiary referral academic medical centers, Columbia University Medical Center and Stanford University Medical Center, between 2015 and 2018. A three-year window was chosen to match the historical control cohort from the 2008 study by Pandolfino et al. [7] All patients referred for esophageal manometry testing in that time frame were reviewed. Patients were included only if they had met criteria for types I, II, or III achalasia as defined by the Chicago Classification described in the introduction. Patients were excluded if they had previously been treated for achalasia or had prior foregut surgery (e.g., fundoplication). Patients with esophagogastric junction outlet obstruction (EGJO) with normal peristalsis were

excluded due to the heterogeneity of the condition and low rate of progression to achalasia. Demographic data including age, sex, date of diagnosis, the degree of esophageal dilatation on endoscopy (if available), and manometric achalasia subtype were abstracted from chart review. Dilatation grade was determined from review of endoscopy reports and scored based on the same methods described by Pandolfino et al. used with the historical control [4], with a score of 0 (normal) assigned if there was no mention of dilatation or retained secretions, 1 (mild) if the endoscopist described possible dilatation, and 2 (severe) if there was obvious dilatation or presence of retained secretions or food. This scoring system used the assumption that absence of dilatation or retained secretions in endoscopy reports reflected true absence of dilatation [7]. This study was approved by the Institutional Review Board of Columbia University Irving Medical Center and Stanford University Medical Center.

Historical Control Cohort

The historical control cohort population included 99 patients who underwent HRM between 2004 and 2007 at Northwestern Memorial Hospital who had impaired EGJ relaxation and were subsequently diagnosed with one of the three achalasia subtypes. Patients were excluded if they had previously undergone therapy for achalasia, had functional obstruction, or had esophageal cancer. Clinical variables were obtained via chart review, including demographics, symptoms at presentation, treatment type, and treatment response. Manometric characteristics and clinical variables were then compared between achalasia subtypes.

Statistical Analysis

Continuous data were reported as mean and standard deviation after assumptions of normality were tested. *t* tests were used to compare normal data. For categorical data, Chi-squared tests were used or Fisher's exact tests when expected cell counts were less than 5. The primary outcome of the study was the prevalence of the three achalasia subtypes, comparing the contemporary to the historical control cohort, and was calculated as a Chi-squared test on a 2×3 table. Stratified *t* tests were performed to compare age and dilatation grade among each achalasia subtype. All statistical tests were two-sided with an alpha of 0.05 as a cut-off to denote statistical significance. Analysis was conducted using Stata version 15 (StataCorp, College Station, TX).

Results

Nine hundred and twenty-two consecutive patients at Columbia University Medical Center and 2203 patients at Stanford University Medical Center underwent HRM testing and were reviewed. Of the combined cohort, 2978 were excluded due to having a diagnosis other than achalasia, prior treatment of achalasia, or history of foregut surgery. Our study cohort therefore consisted of 52 patients from Columbia University Medical Center and 95 patients from Stanford University Medical Center for a total of 147 patients (Table 1). The historical control cohort included 99 patients. The mean age in the contemporary cohort was 58 years compared to 57 years in the historical control, with the proportion of men in our cohort similar to that of the historical control (48.3% vs. 46.5%, respectively; $p=0.78$), as seen in Table 1. Type I achalasia was diagnosed in 8.2% of the current cohort compared to 21.2% in the historical control, type II achalasia in 62.6% compared to 49.5% in the historical control, and type III achalasia in 29.2% versus 29.3% in the historical control population ($p=0.01$) (Fig. 1). Comparing the historical control cohort to each individual institution contemporary cohort yielded similar results (versus Columbia $p=0.018$, versus Stanford, $p=0.05$).

In the contemporary cohort, 128 patients had endoscopy dilatation grade data available for analysis. Fifty-five patients (37.4%) had esophageal dilatation grade 1 or 2 on endoscopy, including 8 patients with type I achalasia (66.7% of the type I patients), 38 patients with type II

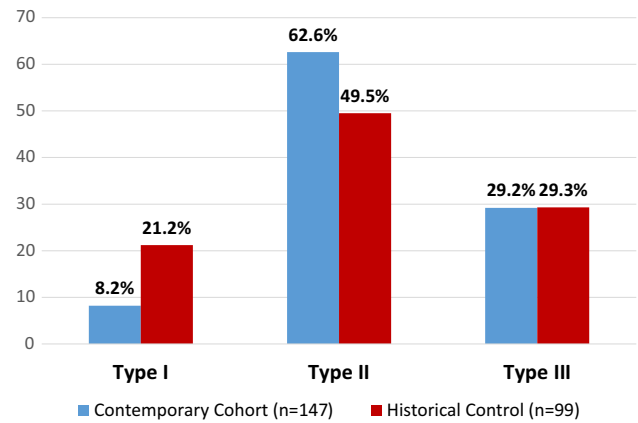


Fig. 1 Achalasia subtypes in the contemporary cohort and historical control

(41.3% of the type II patients), and 9 patients with type III (20.9% of the type III patients). Mean dilatation grade was 1.5 for type I patients, 0.9 for type II, and 0.4 for type III, compared with 1.5, 0.6, and 0.4 in the historical control, respectively. Overall mean dilatation grade was 0.79 in our cohort versus 0.73 in the historical control ($p=0.58$). When stratifying the analysis for dilatation grade by achalasia subtype, mean dilatation grade for the contemporary cohort compared to the historical control cohort was 1.5 for both groups among patients with type I achalasia ($p=1.00$), 0.9 versus 0.6, respectively, among patients with type II achalasia ($p=0.02$), and 0.4 for both groups among patients with type III achalasia ($p=0.89$).

Table 1 Patient characteristics in the contemporary cohort and historical control

	Contemporary cohort (n = 147)	Historical control (n = 99)	p value
Achalasia type			0.01
Type I	12 (8.2%)	21 (21.2%)	
Type II	92 (62.6%)	49 (49.5%)	
Type III	43 (29.2%)	29 (29.3%)	
Sex, male	71 (48.3%)	46 (46.5%)	
Sex, female			0.78
Type I	7 (58.3%)	12 (57.1%)	
Type II	47 (51.1%)	15 (30.6%)	
Type III	17 (39.5%)	19 (65.5%)	
Age, mean (SD)	57.6 (15.7)	57.0 (SD not provided)	0.88
Type I	52.5 (17.6)	58.0 (16.9)	0.16
Type II	57.2 (15.9)	53.4 (19.6)	0.10
Type III	59.8 (14.5)	63.5 (15.6)	0.18
Dilatation grade, mean (SD)	0.8 (0.1)	0.7 (SD not provided)	0.58
Type I	1.5 (0.8)	1.5 (0.7)	1.0
Type II	0.9 (0.9)	0.6 (0.7)	0.02
Type III	0.4 (0.8)	0.4 (0.6)	0.89

Bold value indicates statistical significance $p < 0.05$

Comparing the contemporary Columbia and Stanford populations, age was similar with mean age of 59 at Columbia compared to 57 at Stanford ($p=0.38$) and proportion of men 40.1% vs. 52.6%, respectively ($p=0.16$). There was no statistical difference in the proportion of achalasia subtypes between the Columbia and Stanford cohort. The Columbia cohort was comprised of 5.8% type I, 69.2% type II, and 25% type III whereas the Stanford cohort comprised of 9.5% type I, 59% type II, and 31.5% type III ($p=0.5$). Mean dilatation grade was greater at Stanford with a mean dilatation grade for all subtypes of 0.92 compared to 0.57 ($p=0.04$), and this trend was also seen comparing dilatation grade within each achalasia subtype, although did not reach significance.

Discussion

The aim of our study was to define the epidemiology of the manometric subtypes of achalasia. Recent studies have demonstrated that the overall incidence and prevalence of achalasia does appear to be increasing. Samo et al. found that the prevalence of achalasia in central Chicago has increased nearly threefold from 2004 to 2014, which they attributed to increased awareness of the disease and advances in diagnostic technology and criteria [9]. Indeed, a recent study confirmed earlier findings that HRM increases the diagnostic sensitivity to detect achalasia. Roman et al. randomized 247 patients with dysphagia to HRM or conventional manometry and found that a quarter of the patients were diagnosed with achalasia in the HRM arm compared to only 12% in the conventional arm [10]. However, less is known about whether changes in epidemiology are uniform across all three manometric subtypes of achalasia or if the incidence of the subtypes is also evolving. Differences may exist due to underlying variations in the pathophysiology between the subtypes.

Histopathological evidence has suggested that type II achalasia may be a precursor of type I [11]. Pathologic review of specimens from patients with achalasia has demonstrated that achalasia likely results from a cytotoxic T cell mediated immune response to an unidentified antigen—possibly a viral infection—leading to loss of myenteric neurons [12–14]. In a recent study by Sodikoff et al., full-thickness biopsies obtained from patients undergoing laparoscopic esophagomyotomy demonstrated a greater proportion of specimens with complete absence of ganglion cells and cell loss from patients with type I achalasia compared to patients with type II [12]. In contrast, type III achalasia appears to arise from a non-cytotoxic immune response and changes in gene expression that lead to changes in post-ganglionic neuronal function, rather than neuron loss [5]. Thus type III is considered a somewhat distinct disease entity.

If type II is a precursor to type I, the increased prevalence of type II achalasia and decreased prevalence of type I demonstrated in our study could indicate earlier detection of the disease. Earlier detection is likely multifactorial and may be secondary to the widespread adoption of HRM probes, routine use of a standardized classification scheme, the Chicago Classification, and greater availability of another novel treatment option—per-oral endoscopic myotomy (POEM)—thus all enhancing disease awareness over the last decade. Since data on the incidence of the specific achalasia subtypes has not been provided in prior studies, it is unknown whether the increased incidence and prevalence of achalasia reported by Samo et al. and Duffield et al. may be predominantly due to an increase in type II specifically.

One unexpected finding in our study that argues against the theory that increased type II achalasia indicates earlier detection is that we did not find a significant difference in esophageal dilatation grade between the contemporary and historical control cohorts. One would expect a cohort of “earlier” stages of achalasia to have on average a smaller esophageal caliber. In fact, the mean dilatation grade for patients with type II achalasia in the contemporary cohort was statistically greater than that of the historical control cohort, though the clinical significance of a difference between 0.6 and 0.9 dilatation grade is doubtful. However, our analysis of endoscopic esophageal dilatation had limitations; first, assessment of esophageal dilatation grade is a subjective imprecise measure that is endoscopist-dependent. Second, given the retrospective methodology, we assumed that lack of mention of dilation or retained secretions scored a 0—as done in the original study by Pandolfino et al. [7]—when in reality the endoscopist may not have included all of their findings in the report. Finally, not all patients had endoscopy reports available to review.

To our knowledge, this is the first study that has directly compared the prevalence of the three achalasia subtypes between contemporary cohorts and a historical control cohort prior to the widespread adoption of the Chicago Classification and use of HRM. The strength of our study is that it consisted of a large cohort of patients with achalasia at two different tertiary care academic centers in two regions of the USA. In addition, our results are supported by a plausible physiologic explanation based on the natural history of achalasia. However, our study is limited due to its retrospective design with lack of important clinical information like duration of symptoms and opiate use. Our historical control cohort was taken from published data and not raw data so these important clinical details were also not available. Ideally, we would have used our own institution as a historical control cohort, but neither institution had active databases of manometry studies during the years in question. We therefore acknowledge referral bias as another limitation. Finally, both the historical control cohort and our

contemporary cohort comprised of patients presenting to tertiary care medical centers, which may affect the generalizability of our results.

In conclusion, we found that in a contemporary cohort of patients diagnosed with achalasia by HRM, the prevalence of type II achalasia is increasing and type I achalasia is decreasing compared to a historical control cohort from a decade prior, without changes in age of diagnosis and gender. Our findings may indicate that the adoption of the Chicago Classification and use of HRM have contributed to the earlier detection of achalasia. Additional studies will be needed to determine whether earlier detection and earlier therapeutic intervention ultimately change the natural history of this chronic disease.

Funding No grant support or financial arrangements pertaining to this research is noted.

Compliance with Ethical Standards

Conflict of interest The authors do not have any relevant conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study formal consent is not required.

References

1. Sadowski DC, Ackah F, Jiang B, Svenson LW. Achalasia: incidence, prevalence and survival. A population-based study. *Neurogastroenterol Motil.* 2010;22:e256–e261.
2. Duffield JA, Hamer PW, Heddl R, Holloway RH, Myers JC, Thompson SK. Incidence of achalasia in South Australia based on esophageal manometry findings. *Clin Gastroenterol Hepatol.* 2017;15:360–365.
3. Nenshi R, Takata J, Steglenko S, et al. The cost of achalasia: quantifying the effect of symptomatic disease on patient cost burden, treatment time, and work productivity. *Surg Innov.* 2010;17:291–294.
4. Pandolfino JE, Ghosh SK, Rice J, Clarke JO, Kwiatek MA, Kahrilas PJ. Classifying esophageal motility by pressure topography characteristics: a study of 400 patients and 75 controls. *Am J Gastroenterol.* 2008;103:27–37.
5. Kahrilas PJ, Bredenoord AJ, Fox M, et al. The Chicago Classification of esophageal motility disorders, v3.0. *Neurogastroenterol Motil.* 2015;27:160–174.
6. Rohof WO, Salvador R, Annese V, et al. Outcomes of treatment for achalasia depend on manometric subtype. *Gastroenterology.* 2013;144:718–725. (**quiz e713–714**).
7. Pandolfino JE, Kwiatek MA, Nealis T, Bulsiewicz W, Post J, Kahrilas PJ. Achalasia: a new clinically relevant classification by high-resolution manometry. *Gastroenterology.* 2008;135:1526–1533.
8. Ghosh SK, Pandolfino JE, Rice J, Clarke JO, Kwiatek M, Kahrilas PJ. Impaired deglutitive EGJ relaxation in clinical esophageal manometry: a quantitative analysis of 400 patients and 75 controls. *Am J Physiol Gastrointest Liver Physiol.* 2007;293:G878–G885.
9. Samo S, Carlson DA, Gregory DL, Gawe SH, Pandolfino JE, Kahrilas PJ. Incidence and prevalence of achalasia in central Chicago, 2004–2014, since the widespread use of high-resolution manometry. *Clin Gastroenterol Hepatol.* 2017;15:366–373.
10. Roman S, Huot L, Zerbib F, et al. High-resolution manometry improves the diagnosis of esophageal motility disorders in patients with dysphagia: a randomized multicenter study. *Am J Gastroenterol.* 2016;111:372–380.
11. Kahrilas PJ, Boeckxstaens G. The spectrum of achalasia: lessons from studies of pathophysiology and high-resolution manometry. *Gastroenterology.* 2013;145:954–965.
12. Sodikoff JB, Lo AA, Shetuni BB, Kahrilas PJ, Yang GY, Pandolfino JE. Histopathologic patterns among achalasia subtypes. *Neurogastroenterol Motil.* 2016;28:139–145.
13. Hoshino M, Omura N, Yano F, Tsuboi K, Kashiwagi H, Yanaga K. Immunohistochemical study of the muscularis externa of the esophagus in achalasia patients. *Dis Esophagus.* 2013;26:14–21.
14. Raymond L, Lach B, Shamji FM. Inflammatory aetiology of primary oesophageal achalasia: an immunohistochemical and ultrastructural study of Auerbach's plexus. *Histopathology.* 1999;35:445–453.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Prevalence of *Clostridioides difficile* and Other Gastrointestinal Pathogens in Patients with COVID-19

Monika Laszkowska^{1,2} · Judith Kim¹ · Adam S. Faye^{1,3} · Andrew M. Joelson¹ · Myles Ingram⁴ · Han Truong⁴ · Elisabeth R. Silver⁴ · Benjamin May⁵ · William G. Greendyke⁶ · Jason Zucker⁶ · Benjamin Lebwohl^{1,7} · Chin Hur^{1,4,5} · Daniel E. Freedberg¹

Received: 29 August 2020 / Accepted: 4 December 2020

© The Author(s), under exclusive licence to Springer Science+Business Media, LLC part of Springer Nature 2021

Abstract

Background Gastrointestinal symptoms are common in patients with COVID-19, but prevalence of co-infection with enteric pathogens is unknown.

Aims This study assessed the prevalence of enteric infections among hospitalized patients with COVID-19.

Methods We evaluated 4973 hospitalized patients ≥ 18 years of age tested for COVID-19 from March 11 through April 28, 2020, at two academic hospitals. The primary exposure was a positive COVID-19 test. The primary outcome was detection of a gastrointestinal pathogen by PCR stool testing.

Results Among 4973 hospitalized individuals, 311 were tested for gastrointestinal infections (204 COVID-19 positive, 107 COVID-19 negative). Patients with COVID-19 were less likely to test positive compared to patients without COVID-19 (10% vs 22%, $p < 0.01$). This trend was driven by lower rates of non-*C. difficile* infections (11% vs 22% in COVID-19 positive vs. negative, respectively, $p = 0.04$), but not *C. difficile* infection (5.1% vs. 8.2%, $p = 0.33$). On multivariable analysis, infection with COVID-19 remained significantly associated with lower odds of concurrent GI infection (aOR 0.49, 95% CI 0.24–0.97), again driven by reduced non-*C. difficile* infection. Testing for both *C. difficile* and non-*C. difficile* enteric infection decreased dramatically during the pandemic.

Conclusions Pathogens aside from *C. difficile* do not appear to be a significant contributor to diarrhea in COVID-19 positive patients.

Keywords COVID-19 · SARS-CoV-2 · *Clostridioides difficile* · Enteric infection · Diarrhea

Abbreviations

C. difficile Clostridioides difficile
GI Gastrointestinal

COVID-19 Coronavirus Disease 2019
SARS-CoV-2 Severe Acute Respiratory Syndrome
Coronavirus 2

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1007/s10620-020-06760-y>.

✉ Monika Laszkowska
ml3228@caa.columbia.edu

¹ Department of Medicine, Division of Digestive and Liver Diseases, New York Presbyterian Columbia University Medical Center, New York, NY, USA

² Department of Medicine, Gastroenterology, Hepatology, and Nutrition Service, Memorial Sloan Kettering Cancer Center, New York, NY, USA

³ Department of Medicine, Henry D. Janowitz Division of Gastroenterology, Mount Sinai Hospital, New York, NY, USA

⁴ Department of Medicine, Division of General Medicine, Columbia University Irving Medical Center, New York, NY, USA

⁵ Herbert Irving Comprehensive Cancer Center, Columbia University Irving Medical Center, New York, NY, USA

⁶ Department of Medicine, Division of Infectious Diseases, Columbia University Irving Medical Center, New York, NY, USA

⁷ Department of Epidemiology, Mailman School of Public Health, Columbia University Irving Medical Center, New York, NY, USA

Introduction

The 2019 coronavirus disease (COVID-19) has become a global pandemic with cases spreading rapidly throughout the world. While fever and cough are the most common symptoms associated with this disease, gastrointestinal manifestations have also been noted in 32 to 61% of individuals [1–8]. Diarrhea, in particular, has been reported in 4 to 34% of individuals in cohorts from China and 12 to 34% in cohorts from the USA [2–8].

A likely mechanism for the prevalence of gastrointestinal symptoms in patients with COVID-19 is the presence of Angiotensin Converting Enzyme 2 (ACE2) receptors on gut epithelial cells, a well-described target for viral binding and entry [9, 10]. Nonetheless, it is also possible that intestinal inflammation due to COVID-19 could predispose individuals to co-infection with other gastrointestinal pathogens. Thus far, only one prior study from Wuhan, China, evaluated whether co-infection with enteric pathogens plays a prominent role in this presentation, finding no evidence of enteric pathogens by stool culture in 74 patients with COVID-19 and gastrointestinal symptoms [8].

Highly sensitive and specific stool polymerase chain reaction-based assays have improved the ability to diagnose gastrointestinal infections, including those caused by *Clostridioides difficile* (*C. difficile*), and are routinely used at our institution [11, 12]. Such infections, particularly *C. difficile*, can cause a significant burden to infected individuals thus making recognition and treatment key [13, 14]. This retrospective cohort study aimed to evaluate the prevalence of co-infection with gastrointestinal pathogens among admitted patients with and without COVID-19 infection. In addition, we aimed to compare institution-wide patterns in gastrointestinal infection testing and rates of positive cases before and after the start of the COVID-19 pandemic in New York City.

Methods

Study Design

This retrospective study evaluated 4973 hospitalized patients ≥ 18 years of age who underwent testing for COVID-19 from March 11, 2020, (when institution-based testing began) through April 28, 2020, at two affiliated academic medical centers (Columbia University Irving Medical Center and Allen Hospital). The primary exposure of interest was a positive COVID-19 test, defined as a positive result on real-time reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assay of nasopharyngeal swab specimens testing for the presence of severe acute respiratory syndrome

coronavirus 2 (SARS-CoV-2). The primary outcome of interest was the detection of a gastrointestinal (GI) pathogen (*C. difficile* and non-*C. difficile*) during the index hospitalization by PCR test of the stool. Data regarding duration of GI symptoms with COVID-19 is limited, but one study from China found that COVID-19 positive patients with GI symptoms alone had on average 16 days from initial symptom onset to hospital admission and 31 days from symptom onset to viral clearance [15]. Therefore, stool tests performed either 2 weeks before or at any time after SARS-CoV-2 testing were included in order to capture an adequate time period during which patients may have had symptoms related to COVID-19. The institutional laboratory policy during this period was that only unformed stools would be tested for enteric pathogens (i.e., implying that all tested patients did indeed have diarrhea). It was at the discretion of the provider caring for the patient whether to order a *C. difficile* PCR stool test, a gastrointestinal pathogen PCR panel stool test, or both tests. Secondary variables of interest were age, sex, body mass index (BMI), and race/ethnicity. Stool studies performed from February 1, 2020 to April 17, 2020 were used to determine trends in testing patterns before and during the pandemic.

Gastrointestinal Pathogen PCR Panel Stool Test

Gastrointestinal pathogens were identified using the FilmArray gastrointestinal pathogen PCR panel (GI PCR; BioFire Diagnostics, Salt Lake City, UT). This assay allows for simultaneous detection and identification of nucleic acids from multiple bacteria, viruses, and parasites directly from spontaneously voided stool samples. The assay identifies 20 pathogens including Adenovirus, Astrovirus, *Campylobacter*, *Cryptosporidium*, *Cyclospora*, *Entamoeba histolytica*, Enteroaggregative *E. coli*, Enteropathogenic *E. coli*, Enterotoxigenic *E. coli*, *Giardia*, Norovirus, *Plesiomonas shigelloides*, Rotavirus, *Salmonella* species, Sapovirus, Shigalike toxin-producing *E. coli* STX/ST2, Shigella/Enteroinvasive *E. coli*, *Vibrio cholerae*, *Vibrio* species, and *Yersinia enterocolitica*.

Clostridioides difficile Testing

C. difficile PCR testing was done via PCR for the toxin B gene (Xpert *C. difficile*, Cepheid, Sunnyvale, CA). At our institution, this test is ordered separately from and/or in addition to the GI PCR test panel at the discretion of the ordering provider. There is an institutional electronic “hard stop” that prohibits stool PCR testing in patients who have received laxatives within the previous 24 h.

Statistical Analysis

Continuous variables were expressed as medians or interquartile ranges, with Wilcoxon rank-sum test used to assess differences in distributions between the groups. Categorical variables were summarized as counts and percentages, with Chi-square or Fisher's exact tests used for comparison. A multivariable model was built to examine whether COVID-19 was independently associated with gastrointestinal infection after adjusting for all included variables. A modified Charlson comorbidity index was utilized, which incorporated weighted values of all components of the index (myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, dementia, chronic obstructive pulmonary disease, connective tissue disease, peptic ulcer disease, diabetes mellitus, chronic kidney disease, hemiplegia, leukemia, lymphoma, solid tumor, liver disease, and AIDS) but excluded age since it was included separately in our multivariable model. An alpha of 0.05 was considered statistically significant. Statistical calculations were performed in STATA 16 (Stata Corp, College Station, TX). This study was approved by the Institutional Review Board of Columbia University Medical Center.

Results

We identified 4973 individuals requiring inpatient admission who were tested for COVID-19 between March 11, 2020 and April 28, 2020 at two affiliated academic centers in New York City. 2870 (58%) were COVID-19 positive and 2103 (42%) tested negative. Among these individuals, 311 were also tested for gastrointestinal infections in either the 2 weeks prior or at any time after their SARS-CoV-2 PCR, including 204 COVID-19 positive and 107 COVID-19 negative patients. 261 patients were tested for *C. difficile* infection and 195 patients were tested for non-*C. difficile* infection, with 145 patients being tested for both.

Of the 311 patients with stool studies, there was no difference in age or sex between COVID-19 positive and negative individuals (see Table 1). Significantly more non-Hispanic whites were tested in the COVID-19 negative group (31%, compared to 16% in positive patients), while more Hispanics were tested among the COVID-19 positive cohort (35%, compared to 30% in negatives; see Table 1). More of the COVID-19 positive patients were also overweight or obese with a BMI ≥ 25 (69% vs. 48%, $p < 0.01$). There was a significantly higher proportion of COVID-19 negative patients with a modified Charlson comorbidity index ≥ 2 than COVID-19 positive patients (68% vs. 34% respectively, $p < 0.01$).

COVID-19 positive patients had a significantly longer median length of stay than COVID negative patients (14.6

vs. 7.7, respectively, $p < 0.01$) and were more likely to be tested for *C. difficile* later during hospitalization (73% tested > 72 h after admission, compared to 52% in COVID-19 negative patients; $p < 0.01$; see Table 1). However, there was minimal difference in the timing of testing for non-*C. difficile* enteric pathogens, comparing those with versus those without COVID-19 ($p = 0.06$, Table 1). Among all 4,973 patients, COVID-19 positive patients were tested more often for *C. difficile* compared to COVID-19 negative patients, but there was no significant difference in rates of testing for other GI pathogens (Supplementary Table 1).

COVID-19 positive patients were less likely to have any GI infection overall compared to COVID-19 negative patients (10% vs 22%, $p < 0.01$, Table 2). This trend persisted when evaluating only non-*C. difficile* infection (11% vs 22%, $p = 0.04$). However, no significant difference was seen between the two groups in testing positive for *C. difficile* infection. Of the non-*C. difficile* pathogens, enteropathogenic *E. coli* and enteroaggregative *E. coli* were the most common, found in 2.7% and 2.9% of patients with a GI PCR test, respectively. Seven patients had multiple GI pathogens detected on the GI PCR panel, including five patients with two pathogens detected and two patients with three pathogens detected. Of these, four were COVID-19 positive and three were negative. One additional patient had *C. difficile* infection as well as a second pathogen detected on the GI PCR panel. There were no significant differences in the rates or types of gastrointestinal pathogens detected in individuals with severe COVID-19 (resulting in intubation and/or death) and those with a milder course of COVID-19 (Supplementary Tables 2 and 3).

Of individuals with stool studies during the study period, those with COVID-19 were less likely to have a GI infection on univariable analysis (69% vs 47%, $p < 0.01$, Table 3). On multivariable analysis, infection with COVID-19 remained significantly associated with lower odds of GI infection in tested patients after adjusting for age, sex, BMI, and race/ethnicity (aOR 0.49, 95% CI 0.24–0.97, $p = 0.04$, Table 4).

We also evaluated the trend in GI PCR and *C. difficile* testing before and after the beginning of the COVID-19 pandemic. COVID-19 testing became widely available at our institution on March 11, 2020. As shown in Fig. 1, as of March 14, testing for both *C. difficile* and non-*C. difficile* enteric infection decreased dramatically, with the lowest volume of testing observed at the end of March. In April, *C. difficile* testing increased, approaching pre-pandemic rates, whereas GI PCR testing rates remained lower than pre-pandemic rates.

Table 1 Characteristics of COVID-19+ and COVID-19 – patients with stool studies

Variables	All <i>n</i> = 311	COVID+ <i>n</i> (%) <i>n</i> = 204	COVID- <i>n</i> (%) <i>n</i> = 107	<i>p</i> Value
Age, years				
Mean (SD)	62.5 (16.5)	62.9 (15.6)	61.7 (19.3)	0.57
Median (IQR)	64.7 (53.2–74.6)	63.9 (53.6–73.2)	65.9 (50.3–75.5)	0.98
Distribution <i>n</i> (%)				
18–39	36 (12)	20 (10)	16 (15)	0.25
40–59	75 (24)	51 (25)	24 (22)	
60–74	125 (40)	88 (43)	37 (35)	
75+	75 (24)	45 (22)	30 (28)	
Male Sex <i>n</i> (%)	187 (60)	125 (61)	62 (58)	0.57
Race/ethnicity <i>n</i> (%)				
Non-Hispanic White	66 (21)	33 (16)	33 (31)	0.01
Hispanic	103 (33)	71 (35)	32 (30)	
Black	80 (26)	51 (25)	29 (27)	
Asian	5 (2)	4 (2)	1 (1)	
Other	57 (18)	45 (22)	12 (11)	
BMI <i>n</i> (%)				
< 25	95 (30)	50 (25)	45 (42)	< 0.01
25–30	90 (30)	67 (33)	23 (22)	
> 30	102 (33)	74 (36)	28 (26)	
Unknown	24 (7)	13 (6)	11 (10)	
Modified Charlson comorbidity Score				
0	90 (29)	68 (33)	22 (21)	< 0.01
1	59 (19)	47 (23)	12 (11)	
2+	162 (52)	89 (34)	73 (68)	
Time of stool studies relative to SARS-CoV-2 test				
<i>C. difficile</i> (<i>n</i> = 261)				
2 weeks before	13 (5)	7 (4)	6 (7)	0.51
30 days after	239 (92)	157 (92)	82 (91)	
> 30 days after	9 (3)	7 (4)	2 (2)	
<i>Other GI pathogens</i> (<i>n</i> = 195)				
2 weeks before	15 (8)	5 (4)	10 (13)	0.03
30 days after	180 (92)	112 (96)	68 (87)	
> 30 days after	0 (0)	-	-	
Time of stool studies relative to Admission				
<i>C. difficile</i>				
Before admission	7 (3)	3 (2)	4 (5)	< 0.01
Within 72 h of admission	82 (31)	45 (25)	37 (43)	
> 72 h post-admission	172 (66)	128 (73)	44 (52)	
<i>Other GI pathogens</i>				
Before admission	7 (4)	2 (2)	5 (6)	0.06
Within 72 h of admission	94 (48)	52 (44)	42 (54)	
> 72 h post-admission	94 (48)	63 (54)	31 (40)	
Length of stay [†] —Median (IQR)	12.5 (6.2–25.1)	14.6 (8.3–26.3)	7.7 (4.7–17.1)	< 0.01

[†]Patients still currently admitted were censored at the conclusion of the study on 4/28/20

Table 2 GI pathogen detection rates in COVID-19+ and COVID-19—patients with stool studies

Variables	All	COVID + <i>n</i> (%)	COVID- <i>n</i> (%)	P value
<i>Any GI pathogen, n = 311</i>				
Positive	45 (14.5)	21 (10.3)	24 (22.4)	<0.01*
Negative	266 (85.5)	183 (89.7)	83 (77.6)	
<i>C. difficile, n = 261</i>				
Positive	16 (6.1)	9 (5.1)	7 (8.2)	0.33
Negative	245 (93.9)	167 (94.9)	78 (91.8)	
<i>Other GI pathogens, n = 195</i>				
Positive	30 (15.4)	13 (11.1)	17 (21.8)	0.04*
Negative	165 (84.6)	104 (88.9)	61 (78.2)	
<i>Other GI pathogens, n = 195</i>				
Adenovirus	0 (0)	0 (0)	0 (0)	
Astrovirus	0 (0)	0 (0)	0 (0)	
<i>Campylobacter</i>	2 (0.6)	1 (0.5)	1 (0.9)	
<i>Cryptosporidium</i>	1 (0.3)	1 (0.5)	0 (0)	
<i>Cyclospora</i>	0 (0)	0 (0)	0 (0)	
<i>Entamoeba histolytica</i>	0 (0)	0 (0)	0 (0)	
Enteroaggregative <i>E. coli</i>	8 (2.7)	4 (2.0)	4 (3.7)	
Enteropathogenic <i>E. coli</i>	9 (2.9)	5 (2.5)	4 (3.7)	
Enterotoxigenic <i>E. coli</i>	0 (0)	0 (0)	0 (0)	
<i>Giardia</i>	1 (0.3)	1 (0.5)	0 (0)	
Norovirus	4 (1.3)	1 (0.5)	3 (2.8)	
<i>Plesiomonas shigelloides</i>	0 (0)	0 (0)	0 (0)	
Rotavirus	4 (1.3)	1 (0.5)	3 (2.8)	
<i>Salmonella</i> species	2 (0.6)	2 (1.0)	0 (0)	
Sapovirus	2 (0.6)	0 (0)	2 (1.9)	
Shigalike toxin-producing <i>E. coli</i> STX/ST2	2 (0.6)	1 (0.5)	1 (0.9)	
<i>Shigella/enteroinvasive E. coli</i>	2 (0.6)	0 (0)	2 (1.9)	
<i>Vibrio cholerae</i>	0 (0)	0 (0)	0 (0)	
<i>Vibrio</i> species	0 (0)	0 (0)	0 (0)	
<i>Yersinia enterocolitica</i>	0 (0)	0 (0)	0 (0)	

Discussion

In this retrospective study of hospitalized patients tested for COVID-19, we found that patients with COVID-19 were less likely to test positive for co-infection with typical gastrointestinal pathogens compared to those without COVID-19. This difference was driven by non-*C. difficile* pathogens. *C. difficile* infection rates were similar among COVID-19 positive and negative patients, although patients with COVID-19 had longer median hospital stays and were diagnosed with *C. difficile* later during the course of hospitalization.

Despite the high prevalence of gastrointestinal symptoms described in COVID-19 patients [3–8], a relatively small percentage of patients overall were tested during the study period. Overall, rates of *C. difficile* testing were relatively unchanged by the COVID-19 pandemic whereas rates of testing for other GI pathogens were less than half normal.

Our study suggests that *C. difficile* should remain in the differential for patients presenting with diarrhea regardless of COVID-19 status, especially later in their admission, given that patients with COVID-19 had *C. difficile* infections at the same rates as those without COVID-19. Other gastrointestinal pathogens may be less likely in patients with COVID-19, though this may have been affected by decreased rates of testing.

To our knowledge, this is the first study to report the prevalence of co-infection with enteric pathogens using PCR-based stool assays in patients under evaluation for COVID-19. Disruption of normal mucosal defenses by one enteric infection is recognized as a risk factor for other infections, and prior studies have found that up to 26% of patients undergoing stool pathogen testing have multiple pathogens detected [16]. Only 0.9% of the overall study population were positive for an enteric infection,

Table 3 Univariable predictors of GI pathogen detection in patients with stool studies

Variables	All tested <i>n</i> = 311	GI pathogen detected <i>n</i> = 45	No GI pathogen detected <i>n</i> = 266	<i>p</i> Value
COVID-19 infection	204 (66)	21 (47)	183 (69)	< 0.01
Age, years mean (SD)	62.0 (16.5)	65.5 (14.8)	62.0 (16.8)	0.91
Male sex <i>n</i> (%)	187 (60)	29 (64)	158 (59)	0.52
<i>Race/ethnicity n (%)</i>				
Non-Hispanic white	66 (21)	13 (20)	53 (20)	0.25
Hispanic	103 (33)	17 (37)	86 (32)	
Black	80 (26)	10 (22)	70 (26)	
Other	62 (20)	5 (11)	57 (21)	
<i>BMI n (%)</i>				
< 25 kg/m2	95 (30)	17 (38)	78 (29)	0.08
25–30 kg/m2	90 (29)	14 (31)	76 (29)	
> 30 kg/m2	102 (33)	8 (18)	94 (35)	
Unknown	24 (8)	6 (13)	18 (7)	
<i>Modified Charlson comorbidity score</i>				
0 points	90 (28.9)	8 (17.8)	82 (30.8)	0.09
1 point	59 (19.0)	7 (15.6)	52 (19.6)	
2 + points	162 (52.1)	30 (66.6)	132 (49.6)	

Table 4 Multivariable model of predictors of GI pathogen detection in tested patients (*n* = 311)

Variables	Odds ratio	Confidence interval	<i>p</i> Value
COVID-19 infection	0.49	0.24–0.97	0.04
Age (per additional year)	1.01	0.99–1.03	0.38
Male sex	1.31	0.66–2.62	0.44
<i>Race/ethnicity</i>			
Non-Hispanic white	Reference	–	
Hispanic	0.98	0.43–2.27	0.97
Black	0.7	0.28–1.77	0.45
Other	0.42	0.13–1.31	0.14
<i>BMI</i>			
< 25 kg/m ²	Reference	–	
25–30 kg/m ²	1.1	0.48–2.51	0.81
> 30 kg/m ²	0.5	0.20–1.34	0.18
Unknown	1.44	0.48–4.38	0.51
<i>Modified Charlson comorbidity score</i>			
0 points	Reference	–	
1 point	1.65	0.54–5.00	0.38
2+ points	2.04	0.85–4.89	0.11

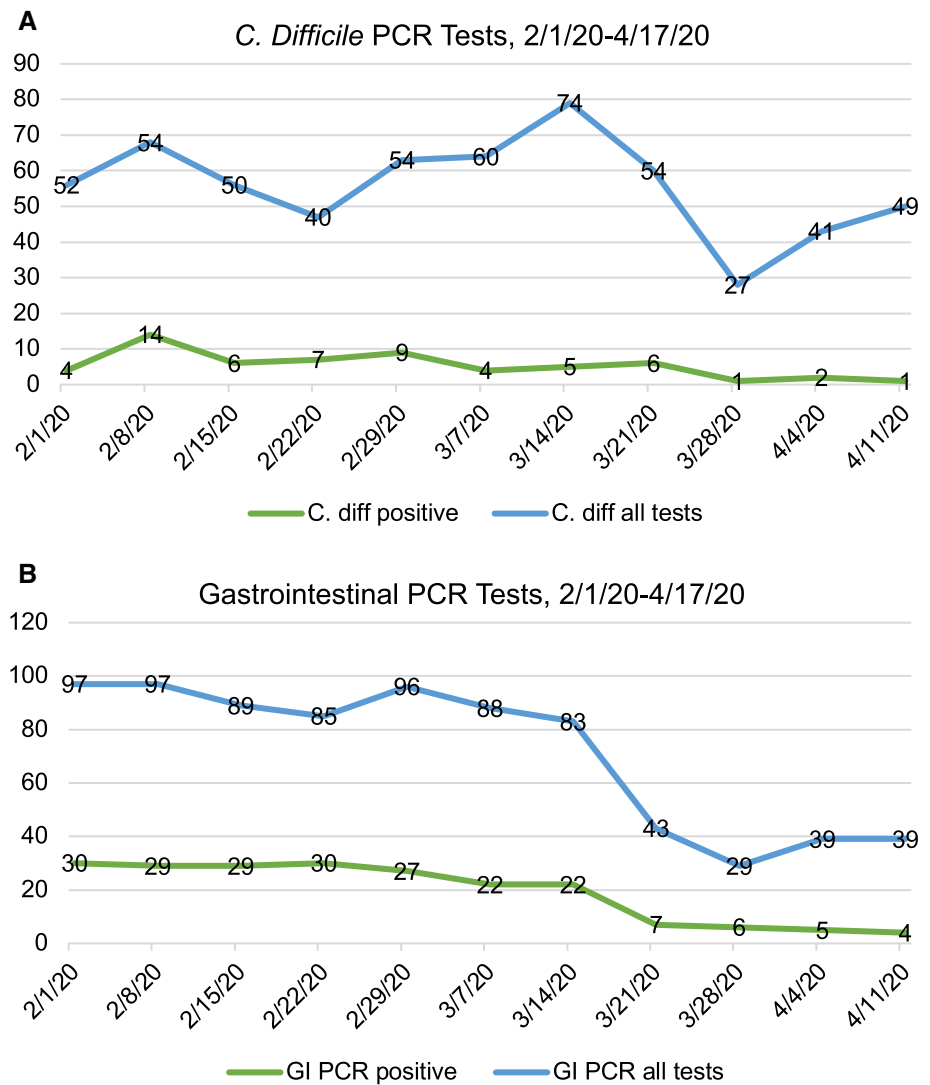
although the prevalence of infections among those who were actually tested for enteric pathogens was much higher, at 14.5%. The majority of gastrointestinal symptoms in COVID-19 patients may be attributable to the virus itself. This is in line with the findings of the study by Jin, et al. where 0 of 74 COVID-19 patients in China

with gastrointestinal symptoms had positive stool cultures [8]. Aside from *C. difficile*, the most commonly identified enteric infections were *E. coli* species, which would be expected given these are relatively common pathogens. Although a similar number of COVID-19 positive and COVID-19 negative patients had multiple pathogens detected on GI PCR, this was observed in a small number of patients making it difficult to draw definitive conclusions regarding risks of co-infection in these populations.

Patients with COVID-19 likely have low rates of non-*C. difficile* enteric infections because SARS-CoV-2 itself is the cause of diarrhea on presentation; however, over time these patients become more similar to other hospitalized patients and have a similar rate of *C. difficile* infections compared to patients without COVID-19. This is particularly notable given that the standard of care for COVID-19 positive patients includes heightened contact precautions and hand hygiene. Furthermore, while COVID-19 positive patients had fewer comorbidities than COVID-19 negative patients, the similar rates of *C. difficile* infections in these groups speak to the power of prolonged hospitalization as a risk factor for this infection.

We also found that testing for enteric infections decreased at our institution since the onset of the COVID-19 pandemic. There are several possible explanations for this. First, during the COVID-19 pandemic, patients may be less likely to seek care for symptoms that may otherwise prompt presentation to a hospital, such as diarrhea. Second, some providers evaluating patients with diarrhea may attribute these symptoms to COVID-19 and therefore defer testing for enteric

Fig. 1 Trends in stool testing and results over time during COVID-19 pandemic (2/1/2020–4/11/2020) for **a** *C. difficile* testing and **b** gastrointestinal PCR testing



pathogens (although testing rates were significantly higher in COVID-19 positive than negative patients). It is also possible that, due to concerns over fecal shedding [17], providers may have been discouraged from ordering stool tests to limit nursing contact with stool specimens during collection. Not surprisingly, the rate of *C. difficile* testing, which nadired on March 28, seemed to subsequently be increasing. This is expected based on the number of critically ill patients and number of patients requiring broad spectrum antibiotics and longer hospital length of stay as the COVID-19 pandemic continued.

There are limitations to this study inherent to its retrospective and observational nature. These associations do not establish causality, and there may be an inherent bias in the types of patients that providers chose to test with stool studies in the setting of the pandemic. While this study was performed in high-volume centers, the testing

practice may be subject to institutional biases limiting generalizability. The ordering of the tests was done at the discretion of the physician caring for the patient. For some patients, only one of the two stool tests were available. There may have been differences in the approach to the workup of diarrhea given physicians were redeployed from different specialties and different institutions. Some may not have been aware of the availability of GI PCR testing options. Since the tests are collected in distinct containers, it is possible some may not have been run due to improper collection. Furthermore, both tests may not have been appropriate depending on the clinical scenario. We included all available results to maximize the available data. This study highlights the need for ongoing awareness among clinicians regarding the correct diagnosis of GI infections, particularly *C. difficile*, in these populations.

Despite the large study size, a relatively small absolute number of patients (311, 6.3%) underwent stool tests.

Prior reports from our institution demonstrated that 18% of individuals tested for COVID-19 presented with diarrhea, and 35% presented with gastrointestinal symptoms including nausea and vomiting [3]. Given that stool testing decreased institutionally as diarrhea become known as a COVID-19-related symptom, the actual prevalence of enteric infection among patients may have been underestimated in our study. In addition, a positive result on a gastrointestinal PCR test could represent chronic shedding or potential false positive, in addition to actual infectious gastroenteritis.

Conclusions

In conclusion, we identified that COVID-19 positive patients were less likely to test positive for enteric infections than COVID-19 negative patients overall; however, the frequency of *C. difficile*, in particular, was not significantly different between the two groups. Given the high prevalence of GI symptoms in COVID-19 patients, there may be decreased utility in using extensive gastrointestinal pathogen panels, as pathogens aside from *C. difficile* rarely contribute to diarrhea in COVID-19 positive patients. Our results suggest the need for ongoing vigilance regarding *C. difficile* infection in these populations. Furthermore, the possible long-term impact of COVID-19 on gastrointestinal problems as well as risk for future infections requires further study.

Acknowledgment None.

Author's contribution ML, JK, ASF, AMJ, CH, and DEF contributed to study concept and design. ML, JK, ASF, MI, HT, ERS, and BM contributed to acquisition of data. ML, JK, ASF, MI, HT, ERS, BM, WGG, JZ, BL, CH, and DEF contributed to analysis and interpretation of data. ML, JK, and AMJ contributed to drafting of the manuscript. ML, JK, ASF, AMJ, HT, ERS, MI, BM, WGG, JZ, BL, DEF, and CH contributed to critical revision of the manuscript for important intellectual content. All authors approved the final version of the manuscript.

Funding ASF: NIH T32DK083256, BL: The Louis and Gloria Flanzer Philanthropic Trust.

Compliance with Ethical Standards

Conflict of interest They authors declare that they have no conflict of interest.

References

- Huang C, Wang Y, Li X et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan. *China. Lancet*. 2020;395:497–506
- Guan WJ, Ni ZY, Hu Y et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. 2020;382:1708–1720
- Nobel YR, Phipps M, Zucker J et al. Gastrointestinal symptoms and COVID-19: case-control study from the United States. *Gastroenterology*. 2020;159:e2
- Redd WD, Zhou JC, Hathorn KE, McCarty TR, Bazarbashi AN, Thompson CC, Shen L, Chan WW. Prevalence and characteristics of gastrointestinal symptoms in patients with SARS-CoV-2 infection in the United States: A multicenter cohort study. *Gastroenterology*. 2020
- Hajifathalian K, Krisko T, Mehta A, et al. Gastrointestinal and hepatic manifestations of 2019 novel coronavirus disease in a large cohort of infected patients from New York: clinical implications [published online ahead of print, 2020 May 8]. *Gastroenterology*. 2020; <https://doi.org/10.1053/j.gastro.2020.05.010>
- Cholankeril G, Podboy A, Aivaliotis VI, Tarlow B, Pham EA, Spencer S, Kim D, Hsing A, Ahmed A. High prevalence of concurrent gastrointestinal manifestations in patients with SARS-CoV-2: early experience from California. *Gastroenterology*. 2020
- Pan L, Mu M, Yang P et al. Clinical characteristics of COVID-19 patients with digestive symptoms in Hubei, China: a descriptive, cross-sectional, multicenter study. *Am J Gastroenterol*. 2020;115:766–773
- Jin X, Lian JS, Hu JH et al. Epidemiological, clinical and virological characteristics of 74 cases of coronavirus-infected disease 2019 (COVID-19) with gastrointestinal symptoms. *Gut*. 2020;69:1002–1009
- Zhou P, Yang XL, Wang XG et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020;579:270–273
- Zhang H, Kang Z, Gong H, et al. Digestive system is a potential route of COVID-19: an analysis of single-cell coexpression pattern of key proteins in viral entry process. *Gut*. 2020;69:1010–1018
- Buss SN, Leber A, Chapin K et al. Multicenter evaluation of the BioFire FilmArray gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis. *J Clin Microbiol*. 2015;53:915–925
- Bélanger SD, Boissinot M, Clairoux N et al. Rapid detection of clostridium difficile in feces by real-time PCR. *J Clin Microbiol*. 2003;41:730–734
- Surawicz CM, Brandt LJ, Binion DG et al. Guidelines for diagnosis, treatment, and prevention of clostridium difficile infections. *Am J Gastroenterol*. 2013;108:478–498
- Feuerstadt P, Das R, Brandt LJ. The evolution of urban *C. difficile* infection (CDI) in 2009–2011 is less severe and has better outcomes than CDI in 2006–2008. *Am J Gastroenterol*. 2014;109:1265–1276
- Han C, Duan C, Zhang S, et al. Digestive symptoms in COVID-19 patients with mild disease severity: clinical presentation, stool viral RNA testing, and outcomes. *Am J Gastroenterol*. 2020; <https://doi.org/10.14309/ajg.0000000000000664>.
- Axelrad JE, Joelson A, Green PHR, Lawlor G, Lichtiger S, Cadwell K, Lebowitz B. Enteric infections are common in patients with flares of inflammatory bowel disease. *Am J Gastroenterol*. 2018;113:1530–1539
- Chen Y, Chen L, Deng Q et al. The presence of SARS-CoV-2 RNA in the feces of COVID-19 patients. *J Med Virol*. 2020;92:833–840

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Patients with More Severe IBD Get *Clostridioides difficile* Rather than *Clostridioides difficile* Increasing the Severity of IBD

Sanskriti Varma¹ · Adam S. Faye² · Adithya Kannan³ · Garrett Lawlor² · Abhishek Verma⁴ · Jordan Axelrad⁵ · Daniel E. Freedberg²

Received: 19 May 2020 / Accepted: 18 July 2020 / Published online: 29 July 2020
© Springer Science+Business Media, LLC, part of Springer Nature 2020

Abstract

Background Inflammatory bowel disease (IBD) patients who have *Clostridioides difficile* infection (CDI) have worse outcomes.

Aims We aimed to determine whether such outcomes are the result of CDI or whether CDI occurs in patients who have more severe IBD.

Methods This was a retrospective study of patients hospitalized for ≥ 2 IBD flares from 2010 to 2019. The primary outcome was time to IBD flare between hospitalizations. First, time to flare was compared between patients who were hospitalized for a flare complicated by CDI and subsequently for a CDI-negative flare (cohort A, denoted +/–) versus patients who were hospitalized for two CDI-negative flares (cohort B, –/–). Second, time between flares was compared within the subset of cohort A patients who had three flares (cohort C, –/+–) before and after CDI.

Results Time between flares was a median of 4 months (IQR 1–9) among 51 cohort A patients versus 12 months (IQR 6–38) among 51 cohort B patients (log-rank $P < 0.01$). In contrast, the median time between flares was similar within cohort C before and after CDI (log-rank $P = 0.54$). At time of the second IBD flare, patients in cohort A (+/–) were more likely to have moderate or severe disease compared to patients in cohort B (–/–).

Conclusions Patients with prior CDI had shorter time to subsequent IBD flare relative to their CDI-negative counterparts. This is not likely due to CDI itself because there was no difference in time between flares before versus after acquiring CDI. Rather, patients who acquire CDI may have more severe IBD.

Keywords Inflammatory bowel disease · *Clostridioides difficile* infection · IBD severity · IBD relapse

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10620-020-06504-y>) contains supplementary material, which is available to authorized users.

✉ Sanskriti Varma
sav9046@nyp.org
Adam S. Faye
asf2160@cumc.columbia.edu
Adithya Kannan
ak3676@cumc.columbia.edu
Garrett Lawlor
gl2501@cumc.columbia.edu
Abhishek Verma
abhishek.verma@nyulangone.org
Jordan Axelrad
Jordan.axelrad@nyulangone.org
Daniel E. Freedberg
def2004@cumc.columbia.edu

¹ Department of Medicine, New York Presbyterian Columbia University Medical Center, 630 West 168th Street, New York, NY 10032, USA

² Division of Digestive and Liver Diseases, Department of Medicine, College of Physicians and Surgeons, Columbia University, 622 West 168th Street, New York, NY 10032, USA

³ Vagelos College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York, NY 10032, USA

⁴ Department of Medicine, NYU Langone Health, 550 First Avenue, New York, NY 10016, USA

⁵ Division of Gastroenterology and Hepatology, NYU School of Medicine, 550 First Avenue, New York, NY 10016, USA

Introduction

A variety of environmental triggers have been implicated in the development and relapse of inflammatory bowel disease (IBD). Of these potential triggers, gastrointestinal pathogenic bacteria are a leading candidate [1–5]. *Clostridioides difficile* infection (CDI) is up to tenfold more prevalent in those with IBD compared to the general population [6]. IBD may predispose to CDI due to a reduction in microbiome diversity and an increase in the proportion of pro-inflammatory gut microbial species [4]. Mucosal inflammation, host immune alterations, and/or genetic susceptibility may also play a role in the acquisition and severity of CDI among patients with IBD [1].

While IBD may predispose patients to CDI, it is also possible that CDI may change the course of IBD. CDI encompasses a wide spectrum of disease ranging from mild diarrhea to fulminant colitis [7]. CDI may worsen IBD through direct effects on the mucosa or because it is inevitably accompanied by a state of ultra-low luminal microbial diversity. Whether CDI worsens IBD or whether higher IBD disease severity predisposes to CDI has often been mooted as a problem of origin and first cause (i.e., a “chicken-and-egg” problem).

Understanding which event occurs first—IBD or CDI—may shed light on how patients with concurrent CDI and IBD should be optimally managed. To better understand the causal chain of events, we tested (1) whether there was a shorter time to the next IBD flare within a given individual with IBD and CDI after successful clearance of *C. difficile*, and (2) whether individuals with IBD who successfully cleared CDI have a shorter time between IBD flares compared to individuals with IBD and no history of CDI.

Methods

Study Population

This was a retrospective study using the electronic medical records of IBD patients hospitalized at NewYork-Presbyterian Columbia University Irving Medical Center, a quaternary care institution in New York City that serves patients from the surrounding urban area, the tristate region (New York, New Jersey, and Connecticut), and those seeking quaternary care from more distant regions. We identified IBD patients (International Classification of Disease, Clinical Modification code for UC [556.x, K51.x] or CD [555.x, K50.x]) of any age admitted to the institution from January 2010 to September 2019. Patients were included

in the study if they were hospitalized at least 48 h for an IBD flare on two or more separate occasions, tested for CDI during both flares, and met additional criteria for inclusion (see below). IBD flare was manually confirmed by searching the electronic medical records for the following data: clinical (diarrhea, bloody stools, abdominal pain/cramping, fevers, fatigue, reduced appetite), laboratory (abnormal inflammatory markers), endoscopic (evidence of active disease), and/or axial imaging (evidence of active disease). This study was approved by the Institutional Review Board at Columbia University Medical Center.

Clostridioides difficile Infection

CDI was defined by a clinical picture compatible with CDI (diarrhea with three or more loose bowel movements in 24 h), microbiological evidence of toxin-producing *C. difficile* with a positive result from a polymerase chain reaction (PCR) test for *C. difficile* toxin B gene using the Cepheid Xpert *C. difficile* Assay (Cepheid, Sunnyvale, California, USA) from an unformed stool, followed by receipt of appropriate anti-CDI antibiotics (i.e., intravenous metronidazole and/or oral vancomycin [8]). Patients were stratified based on whether they had CDI that was successfully cleared. We selected 2010 for the start of the study because it represents the earliest time that our institution began exclusively using the toxin-based stool PCR for the diagnosis of *C. difficile*.

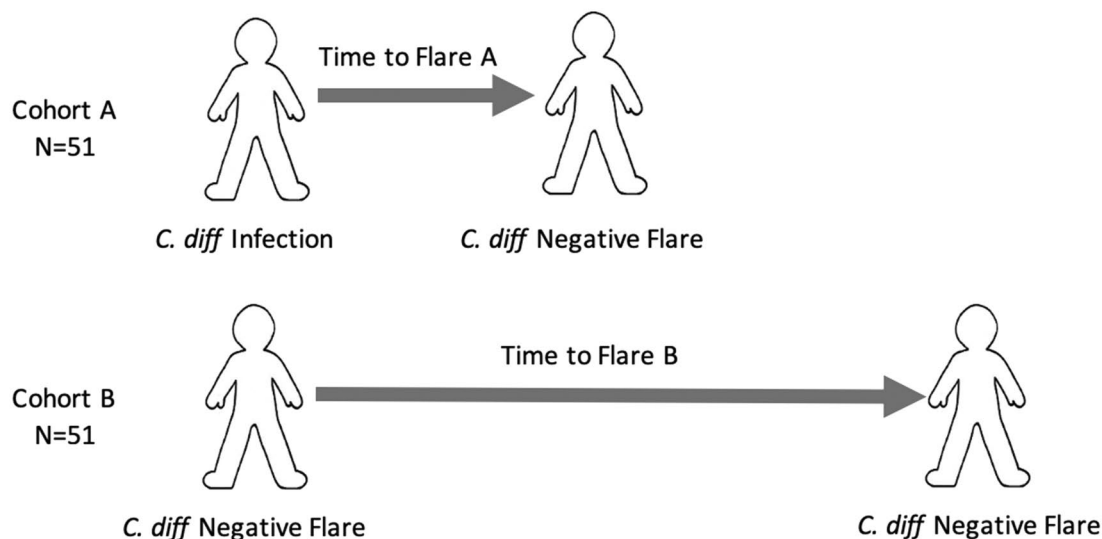
Study Cohorts

Our analysis included three cohorts of patients (Fig. 1). Patients in cohort A (Fig. 1, top panel, CDI+/-) had an index hospitalization for IBD flare, during which they tested positive for CDI. These patients were only included if they subsequently had another hospitalization for an IBD flare during which they tested negative for CDI and, as such, were considered to have cleared *C. difficile*. Cohort B patients (Fig. 1, top panel, CDI-/-) were chosen at random from a pool of patients meeting eligibility. Patients in cohort B also had two hospitalizations for IBD flares during the study period but, unlike the cohort A patients, tested negative for *C. difficile* on both occasions and also had no known prior history of CDI. Last, a subset of patients in cohort A, who had three hospitalizations for flares, were included in cohort C (-/+/-): CDI testing was negative, then positive, then negative at each respective flare hospitalization. No patients in cohort C had CDI prior to the episode included in this study.

Primary Outcome

The primary outcome was the time to flare between hospitalizations (Fig. 1), a metric that has often been used

Primary Hypothesis: Time to Flare A < Time to Flare B



Secondary Hypothesis: Time to Flare C > Time to Flare D

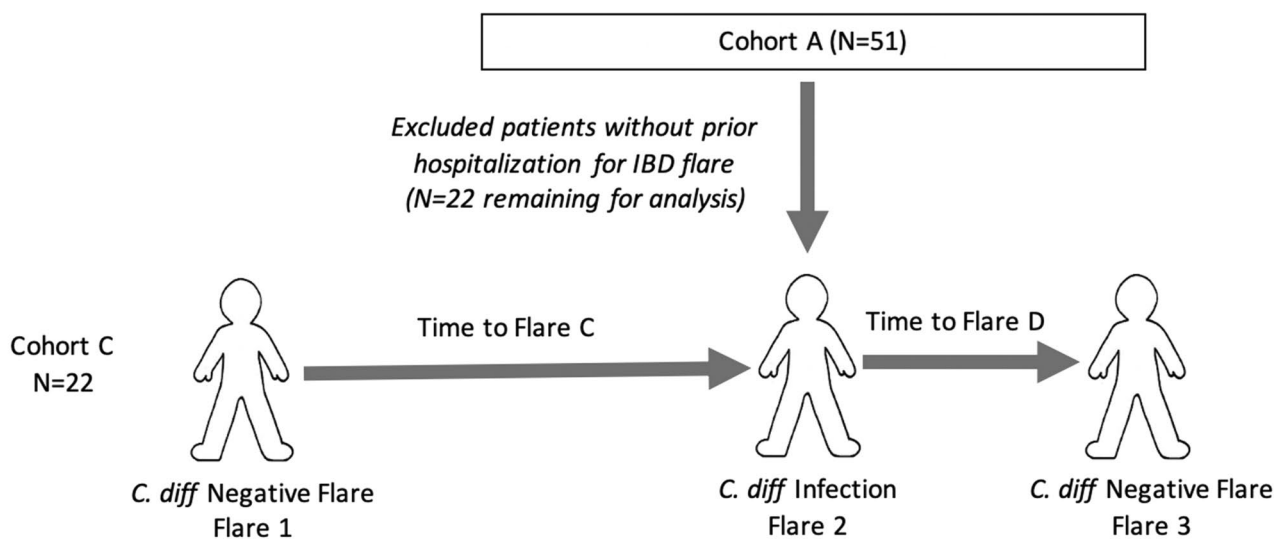


Fig. 1 Illustration of study cohorts. The primary outcome was the time to flare between hospitalizations for IBD flare. Patients in cohort A (top panel, CDI+/-) had an index hospitalization for diarrheal illness, during which they tested positive for CDI; these patients had a subsequent hospitalization for IBD flare during which they tested negative for CDI. Patients in cohort B (top panel, CDI-/-) tested

negative for CDI during both flare hospitalizations. A subset of patients in cohort A, who had three hospitalizations for flares, were included in cohort C (bottom panel, -/+/-): CDI testing was negative, then positive, and then negative at each respective flare hospitalization

to gauge severity of IBD [9–12]. For the comparison of cohort A versus cohort B, time to flare was calculated using the time between the discharge date of the index hospitalization and the admission date of the second hospitalization. For the comparison made within cohort C, time to

flare was calculated using the following: (1) time between the discharge date of the first hospitalization (CDI-) and the admission date of the second hospitalization (CDI+); (2) time between the discharge date of the second

hospitalization (CDI+) and the admission date of the third hospitalization (CDI−).

Baseline Demographic and Clinical Characteristics

We recorded the following values from the medical record: dates of admissions, dates of discharges, date of birth, date of IBD diagnosis, IBD subtype (UC, CD) and location/behavior (using the Montreal Classification of UC or CD), race/ethnicity (white, Hispanic, black, other), body mass index (BMI, categorized as $< 18.5 \text{ kg/m}^2$ and $\geq 18.5 \text{ kg/m}^2$), Charlson comorbidity index (CCI, stratified as < 3 and ≥ 3), smoking status (never, former, current), IBD medications taken immediately prior to index hospitalization [steroids (systemic vs. budesonide), aminosalicylates, biologics, immunomodulators, none], and escalation of IBD medication during or after index hospitalization (considered to be an increase in frequency/dosage of a medication or transition to another class of IBD medications). Duration of disease was calculated using time between the date of IBD diagnosis and the date of the analysis (February 2020).

Other IBD-Related Outcomes

Several IBD-related outcomes were collected for patients in each cohort at the time of the second flare hospitalization. These included duration of hospitalization, severity of the second flare (defined as: mild, Crohn's disease activity index (CDAI) score 150–220 or Mayo score 3–5; moderate, CDAI 220–450 or Mayo 6–10; or severe, CDAI > 450 or Mayo 11–12) [13], highest temperature, highest heart rate, lowest systolic blood pressure, highest white blood cell count, lowest hemoglobin, highest creatinine, lowest albumin, highest c-reactive protein, highest erythrocyte sedimentation rate, use of steroids, use of antibiotics, intensive care unit admission during hospitalization, death during admission, and IBD-related surgery during admission. We also collected data on long-term outcomes after the second flare hospitalization (occurring within 1 year of discharge), including resolution of c-reactive protein level, resolution of active disease on endoscopy/axial imaging (computed tomography and magnetic resonance imaging), escalation of IBD therapy, number of recurrent flares, death, and IBD-related surgery.

Statistical Approach

For continuous variables, means and standard deviations (SD) were computed if data were normally distributed, or medians and interquartile ranges (IQR) if data were skewed. Differences in means were calculated using Student's *T* tests, and differences in medians were compared using Wilcoxon rank-sum tests. Categorical variables were compared

using χ^2 or Fisher's exact test when 5 or fewer events were expected in any category. With regard to within-individual comparisons evaluated in cohort C, McNemar's test was used for binary outcomes, paired *T* tests for normally distributed continuous variables, and rank-sum tests for skewed continuous variables. The comparisons of time to flare between cohorts were made using the log-rank test. Cox proportional hazard modeling was used to test the association between parameters and time to disease relapse. The reduced model retained predictor variables associated with relapse ($P < 0.10$). All analyses were performed using STATA version 13.1 (College Station, TX, USA). Associations were considered statistically significant at a *P* value < 0.05 .

Results

Population

We identified 51 IBD patients who met criteria for inclusion in cohort A (+/−) and 51 IBD patients who met criteria for inclusion in cohort B (−/−) (Table 1). Most participants had UC (57%) and were between 18 and 65 years of age, with a median age of 32 (IQR 20–47). Of all participants, 54% were female, and the majority were white (36%) or Hispanic (34%). The median duration of IBD was 9 (IQR 6–13) years. Patients without CDI (−/−) had significantly higher BMI ($P = 0.04$) and fewer comorbidities ($P < 0.01$) compared to those with CDI (+/−). There was no difference between groups in the use of antibiotics within 90 days prior to index hospitalization ($P = 0.81$). Those without CDI were more likely to have received budesonide ($P = 0.03$) and less likely to have received biologics ($P = 0.01$) prior to their first IBD-related hospitalization. After hospitalization, those without CDI were more likely to have an escalation in IBD therapy ($P < 0.01$). Six patients in cohort A had CDI prior to the index hospitalization with CDI, and no patients in cohort B had a prior history of CDI. In all cases of prior CDI, more than a year had elapsed without any interval flares or testing for CDI.

Characteristics of Cohort A (+/−) Versus B (−/−)

The median time to flare for cohort A (+/−) was 4 months (IQR 1–9) and for cohort B was 12 months (IQR 6–38, $P < 0.01$, Fig. 2). There was no difference in time between flares when results were stratified by CD vs. UC. Among 58 patients with UC, time to relapse for cohort A was a median of 4 months (IQR 1–9) and for cohort B was 14 months (IQR 6–38, $P < 0.01$). Among 44 patients with CD, time to relapse for cohort A was a median of 2 months (IQR 1–9) and for cohort B was 10 months (IQR 7–26, $P < 0.01$). Table 2 compares the characteristics between cohorts A and

Table 1 Baseline characteristics of 102 patients hospitalized for an index flare of inflammatory bowel disease, stratified based on whether the flare was associated with *C. difficile* infection (+/–) or not (–/–)

Characteristics	All (N=102)	CDI (+/–) (cohort A, N=51)	No CDI (–/–) (cohort B, N=51)	P value
Age at diagnosis, median years (IQR)	32 (20–47)	34 (21–48)	27 (15–46)	0.25
Age at diagnosis, years				0.26
< 18	21 (20%)	14 (8%)	7 (0%)	
≥ 18 and < 65	74 (72%)	34 (73%)	40 (84%)	
≥ 65	8 (8%)	3 (19%)	4 (16%)	
Sex				0.16
Female	55 (54%)	31 (61%)	24 (47%)	
Male	47 (46%)	20 (40%)	27 (53%)	
Race/ethnicity				0.72
White	37 (36%)	19 (37%)	18 (35%)	
Hispanic	35 (34%)	18 (35%)	17 (33%)	
Black	24 (24%)	10 (20%)	14 (27%)	
Other	6 (6%)	4 (8%)	2 (4%)	
BMI, kg/m ²				0.04
< 18.5	25 (25%)	17 (33%)	8 (17%)	
≥ 18.5	77 (75%)	34 (67%)	43 (84%)	
Charlson comorbidity index				< 0.01
Score < 3	69 (68%)	28 (55%)	41 (80%)	
Score ≥ 3	33 (32%)	23 (45%)	10 (20%)	
Inflammatory bowel disease subtype				0.69
Ulcerative colitis	58 (57%)	28 (55%)	30 (59%)	
E1 Proctitis	14 (24%)	8 (29%)	6 (20%)	
E2 Left-sided colitis	20 (34%)	6 (21%)	14 (46%)	
E3 Extensive colitis	24 (41%)	14 (50%)	10 (33%)	
Crohn's disease	44 (43%)	23 (45%)	21 (41%)	
L1 Ileal	5 (11%)	0 (0%)	5 (24%)	
L2 Colonic	14 (32%)	7 (30%)	7 (33%)	
L3 Ileocolonic	25 (57%)	16 (70%)	9 (43%)	
L4 Upper gastrointestinal	2 (5%)	1 (4%)	1 (5%)	
B1 Non-stricturing, non-penetrating	32 (73%)	15 (65%)	17 (81%)	
B2/B3 Stricturing, penetrating	12 (27%)	8 (35%)	4 (19%)	
Duration of disease, median years (IQR)	9 (6–13)	9 (6–13)	9 (6–12)	0.73
Smoking status				0.05
Never	81 (79%)	37 (73%)	44 (86%)	
Former	16 (16%)	9 (18%)	7 (14%)	
Current	5 (5%)	5 (10%)	0 (0%)	
Duration of index hospitalization, median days (IQR)	6.5 (3–10)	7 (4–12)	5 (3–9)	0.26
Severity of index flare				0.35
Mild	35 (34%)	14 (27%)	21 (41%)	
Moderate	62 (61%)	34 (67%)	28 (55%)	
Severe	5 (5%)	3 (6%)	2 (4%)	
IBD medications immediately prior to hospitalization				
Steroids	33 (32%)	11 (22%)	22 (43%)	0.02
Systemic	25 (25%)	10 (20%)	15 (29%)	0.36
Budesonide	8 (8%)	1 (2%)	7 (14%)	0.03
5-ASAs	38 (37%)	16 (31%)	22 (43%)	0.22
Biologics	25 (25%)	18 (35%)	7 (14%)	0.01
Immunomodulators	19 (19%)	11 (22%)	8 (16%)	0.45

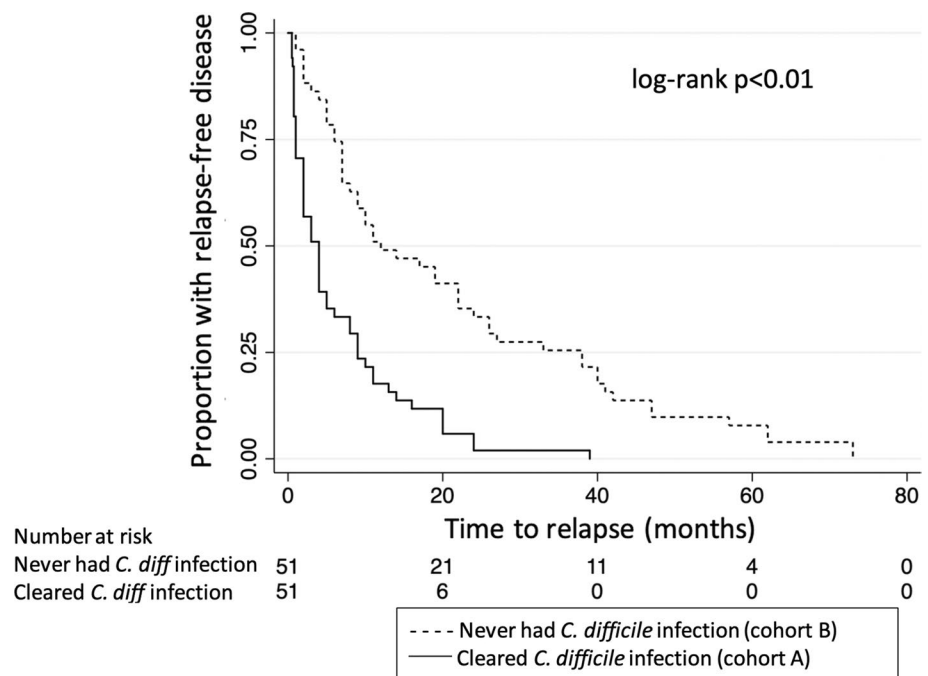
Table 1 (continued)

Characteristics	All (N=102)	CDI (+/–) (cohort A, N=51)	No CDI (–/–) (cohort B, N=51)	P value
None	15 (15%)	6 (12%)	9 (18%)	0.40
Antibiotics within the last 90 days	23 (23%)	12 (24%)	11 (22%)	0.81
Escalation of IBD therapy during/after index hospitalization	51 (50%)	14 (27%)	37 (73%)	< 0.01

P values < 0.05 are given in bold

This table compares patient characteristics at the time of the index hospitalization for IBD flare in cohort A (+/–) versus cohort B (–/–) (see Fig. 1, top panel). *CDI* *Clostridioides difficile* infection, *SD* standard deviation, *IQR* interquartile range, 5-ASA aminosalicylate. Severity of flare was defined as the following: mild—Crohn's disease activity index (CDAI) score 150–220 or Mayo score 3–5; moderate—CDAI 220–450 or Mayo 6–10; severe—CDAI > 450 or Mayo 11–12

Fig. 2 Cumulative incidence of relapse-free disease in 102 patients with inflammatory bowel disease, stratified based on whether they had a prior episode of *C. difficile* infection. The comparison made by this table is graphically depicted in Fig. 1 (top panel)



B at the time of the second hospitalization, during which all participants experienced an IBD flare that was CDI-negative. Those who never had CDI (–/–) were more likely to have received budesonide between flares ($P=0.02$) and had milder flares based on defined severity scores ($P<0.01$). During the 1-year period after the second IBD flare requiring hospitalization, the cohort B patients (–/–) were more likely to have endoscopic resolution of disease ($P=0.01$) and had fewer flares ($P=0.02$) compared to the cohort A patients (+/–).

Multivariable Model, Cohort A Versus Cohort B

In a multivariable model constructed to examine the independent relationship between prior history of CDI and time to IBD relapse among all 102 patients (i.e., the risk associated with being in cohort A versus cohort B, Table 3), prior history of CDI was strongly associated with shorter time to

IBD relapse (aHR 3.99, 95% CI 2.48–6.43). Age of diagnosis between 18 and 65 years (aHR 1.90, 95% CI 1.11–3.23) and former smoking status (aHR 1.89, 95% CI 1.06–3.37) were also associated with shorter time to IBD relapse.

Within-Individual Comparison in Cohort C (–/+–)

Within cohort C, the time between the first and second flares was a median of 5 months (IQR 2–12) and the time between the second and third flares was a median of 2 months (IQR 1–8) ($P=0.54$, Fig. 3). Table 4 shows the inpatient characteristics of participants in cohort C at each of three flare hospitalizations (–/+–) and compares the within-individual characteristics at the time of the first versus the third flare (i.e., a within-individual comparison of the two flares when subjects did not have CDI). More participants had moderate or severe disease at the time of the third flare compared to the first flare ($P=0.03$).

Table 2 Characteristics of the second admission for 102 patients, at which time all were negative for *C. difficile*

Characteristics	All (N = 102)	Cleared CDI (+/−) (cohort A, N = 51)	Never had CDI (−/−) (cohort B, N = 51)	P value
Duration of hospitalization for subsequent flare, median days (IQR)	6 (3–10)	6 (4–12)	4 (3–8)	0.09
IBD medications immediately prior to subsequent flare hospitalization				
Steroids	31 (30%)	10 (20%)	21 (41%)	0.02
Systemic	22 (22%)	9 (18%)	13 (25%)	0.34
Budesonide	9 (9%)	1 (2%)	8 (16%)	0.02
5-ASAs	35 (34%)	14 (27%)	21 (41%)	0.14
Biologics	38 (37%)	20 (39%)	17 (33%)	0.41
Immunomodulators	17 (17%)	9 (18%)	9 (18%)	1.00
None	11 (11%)	7 (14%)	4 (8%)	0.53
IBD medications escalated prior to discharge	65 (64%)	30 (59%)	35 (69%)	0.30
Severity of second flare				< 0.01
Mild	27 (26%)	4 (8%)	23 (45%)	
Moderate	55 (54%)	34 (67%)	21 (41%)	
Severe	20 (20%)	13 (25%)	7 (14%)	
Characteristics at second flare				
Highest temperature, mean (SD)	39 (9)	39 (9)	39 (9)	0.89
Highest heart rate, mean (SD)	121 (90)	115 (25)	125 (27)	0.58
Lowest systolic blood pressure, mean (SD)	94 (19)	93 (20)	94 (18)	0.91
Highest white blood cell count, median (IQR)	11 (8–16)	11 (8–19)	11 (8–15)	0.81
Lowest hemoglobin, mean (SD)	9.2 (2.7)	9.0 (2.4)	9.4 (2.9)	0.56
Highest creatinine, median (IQR)	0.9 (0.7–1.2)	0.8 (0.6–1.1)	0.9 (0.7–1.3)	0.28
Lowest albumin, mean (SD)	3.0 (0.9)	2.9 (0.8)	3.2 (0.9)	0.03
Highest c-reactive protein, median (IQR) (N = 79)	39 (5–109)	37 (8–75)	40 (4–124)	0.92
Highest erythrocyte sedimentation rate, median (IQR) (N = 74)	30 (15–60)	33 (16–60)	29 (12–55)	0.58
Use of steroids	50 (49%)	26 (51%)	24 (47%)	0.69
Intensive care unit admission	3 (3%)	1 (2%)	2 (4%)	1.00
Death	4 (4%)	2 (4%)	2 (4%)	1.00
Abdominal surgery	7 (7%)	5 (10%)	2 (4%)	0.24
Long-term outcomes after second flare (within 1 year)				
Resolution of c-reactive protein (N = 93)	40 (39%)	18 (35%)	22 (43%)	0.57
Resolution of active disease on endoscopy/imaging (N = 91)	32 (35%)	9 (21%)	23 (47%)	0.01
Escalation of IBD therapy	50 (49%)	24 (47%)	26 (51%)	0.69
Number of subsequent flares				0.02
No flares	48 (47%)	18 (35%)	30 (59%)	
One or more flares	54 (53%)	33 (64%)	21 (41%)	
Death	3 (3%)	2 (4%)	1 (2%)	0.56
Abdominal surgery	19 (19%)	12 (24%)	7 (14%)	0.20

P values < 0.05 are given in bold

Results have been stratified based on whether patients had a prior episode of *C. difficile* infection (+/−) or not (−/−)

This table compares patient characteristics at the time of the second hospitalization for IBD flare in cohort A versus cohort B (see Fig. 1, top panel). Missing values: 28 ESR; 23 CRP; 9 resolution of CRP; 11 resolution on endoscopy/imaging. *CDI* *Clostridioides difficile* infection, *SD* standard deviation, *IQR* interquartile range, 5-ASA aminosalicylate. Severity of flare was defined as the following: mild—Crohn's disease activity index (CDAI) score 150–220 or Mayo score 3–5; moderate—CDAI 220–450 or Mayo 6–10; severe—CDAI > 450 or Mayo 11–12

Otherwise, characteristics were similar between the two CDI-negative flares. Of the participants who had surgery, 2 had colectomies, 1 had a proctectomy, and 2 had fistula repairs. Supplemental Table 1 shows the baseline characteristics of cohort C participants.

Discussion

In this retrospective study evaluating patients hospitalized for IBD flares, we found that those with prior CDI had significantly shorter time to IBD relapse when compared to

Table 3 Cox proportional hazards model for time to relapse among 102 patients with inflammatory bowel disease

Risk factors	Full model (HR, 95% CI)	Reduced model (HR, 95% CI)
History of <i>C. difficile</i> infection	5.5 (3.05–10.14)	3.99 (2.48–6.43)
Age at diagnosis, years		
< 18	Reference	Reference
≥ 18 and < 65	2.37 (1.29–4.32)	1.90 (1.11–3.23)
≥ 65	3.01 (0.10–9.06)	2.22 (0.87–5.63)
BMI, kg/m ²		
< 18.5	Reference	–
≥ 18.5	1.19 (0.67–2.13)	–
Inflammatory bowel disease subtype		
Ulcerative colitis	Reference	–
Crohn's disease	1.22 (0.77–1.92)	–
Charlson comorbidity index		
Score < 3	Reference	–
Score ≥ 3	0.70 (0.41–1.17)	–
Smoking status		
Never	Reference	Reference
Former	1.72 (0.93–3.17)	1.89 (1.06–3.37)
Current	0.59 (0.21–1.70)	0.67 (0.26–1.74)
Duration of index hospitalization (per additional day)	1.00 (0.96–1.03)	–
Severity of index flare		
Mild	Reference	–
Moderate	1.26 (0.79–2.03)	–
Severe	0.77 (0.24–2.51)	–
Medications immediately prior to index hospitalization		
Steroids	1.24 (0.75–2.06)	–
5-ASAs	0.78 (0.46–1.33)	–
Biologics	1.07 (0.56–2.07)	–
Immunomodulators	0.77 (0.40–1.49)	–
None	1.15 (0.54–2.45)	–

P values < 0.05 are given in bold

BMI body mass index, CI confidence interval, IQR interquartile range, 5-ASA aminosalicylate

those without history of CDI. Conversely, among those who had CDI, the time between flares was similar before CDI compared to afterward. These results suggest that the acquisition of CDI may not necessarily change the course of IBD, but rather, the shorter time to disease relapse among those with CDI may be related to intrinsic disease and/or patient characteristics that predispose patients to CDI and ultimately impact time to flare. Our study's findings shed light on the “chicken and egg” relationship between CDI and IBD relapse and progression.

IBD patients are more likely to acquire CDI than the general population and often do so without the classic risk factors. CDI more commonly affects IBD patients in the community and at younger ages [14]. Up to 40% of patients do not have documented antibiotic exposure prior to presentation, and the use of corticosteroids has been associated with an increased risk of CDI, relative to biologics and

other IBD maintenance drugs [15–18]. IBD itself has been shown to be a risk factor for CDI, particularly when there is colonic disease involvement [16, 19]. In our patient population, all patients contracted CDI in the community and were more likely to have been on steroids and biologic maintenance therapy as opposed to 5-ASA agents, which may be evidence of more severe disease. Those who acquired CDI had a significantly lower BMI at baseline, which may reflect this group's overall illness severity and overall state of inflammation.

Few prior studies have addressed the cause-and-effect sequence related to CDI and IBD disease progression and outcomes. The combination of CDI and IBD is associated with an established risk for poor outcomes when compared with either condition alone [19–23]. Similarly, we found that CDI was associated with shorter time to disease relapse; further, at the time of the second IBD flare

Fig. 3 Cumulative incidence of relapse-free disease in 22 patients with inflammatory bowel disease, before and after *C. difficile* infection. The comparison made by this table is graphically depicted in Fig. 1 (bottom panel)

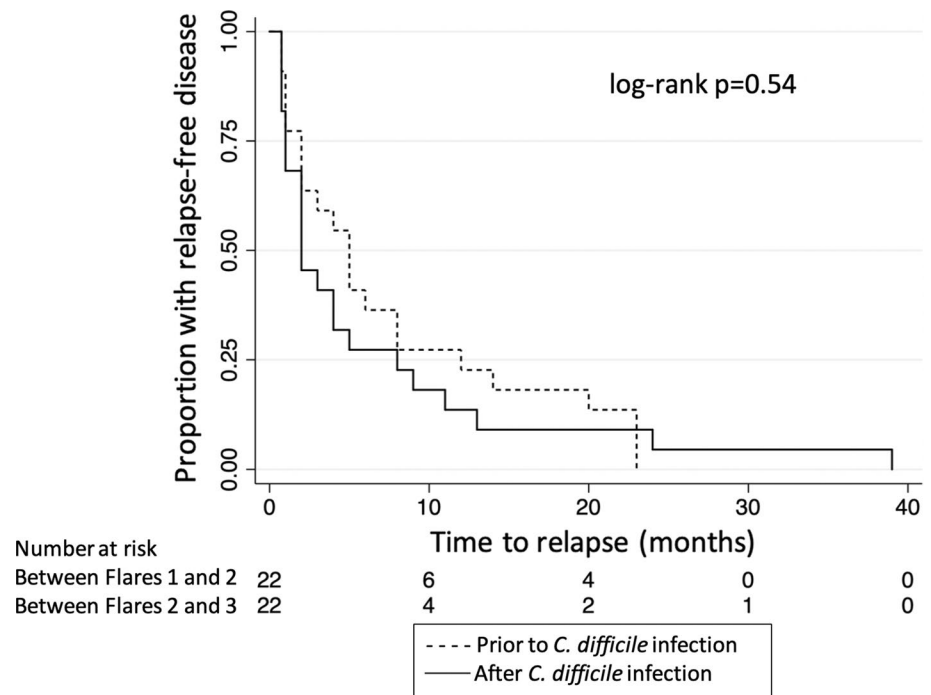


Table 4 Characteristics of 22 patients hospitalized for inflammatory bowel disease flares that occurred prior to *C. difficile* infection, with *C. difficile* infection, and after *C. difficile* infection (–/+/–)

Characteristics at the time of flares	Flare 1: prior to CDI (N=22)	Flare 2: with CDI (N=22)	Flare 3: after clearing CDI (N=22)	P value, comparing Flare 1 versus Flare 3
Duration of hospitalization, median days (IQR)	6 (4–8)	5 (3–10)	5 (3–9)	0.38
Severity of flare				0.03
Mild	8 (36%)	3 (14%)	1 (5%)	
Moderate	12 (55%)	18 (82%)	17 (77%)	
Severe	2 (9%)	1 (5%)	4 (18%)	
Inpatient outcomes at flare				
Highest temperature, mean (SD)	37.4 (0.95)	37.9 (1.3)	37.9 (0.97)	0.10
Highest heart rate, median (IQR)	102 (95–118)	106 (92–133)	107 (98–123)	0.43
Lowest systolic blood pressure, mean (SD)	93 (23)	97 (15)	97 (12)	0.99
Highest white blood cell count, mean (SD)	10 (7)	11 (4)	12 (7)	0.37
Lowest hemoglobin, mean (SD)	8.6 (2.9)	9.3 (3.0)	9.4 (2.0)	0.20
Highest creatinine, median (IQR)	0.8 (0.7–1.0)	0.9 (0.7–1.0)	0.8 (0.7–1.0)	0.44
Lowest albumin, mean (SD)	3.1 (0.7)	2.9 (1.0)	2.9 (0.9)	0.23
Highest c-reactive protein, median (IQR) (N=40)	9 (8–41)	38 (6–77)	58 (5–95)	0.56
Highest erythrocyte sedimentation rate, median (IQR) (N=52)	26.5 (10–42)	22 (9–73)	24 (12–60)	0.69
Use of steroids	15 (68%)	6 (27%)	10 (45%)	0.13
Use of antibiotics	4 (18%)	22 (100%)	7 (32%)	0.29
Intensive care unit admission	0 (0%)	2 (9%)	0 (0%)	1.0
Abdominal surgery	1 (5%)	2 (9%)	2 (9%)	0.55

P values < 0.05 are given in bold

This table compares patient characteristics before and after *C. difficile* infection (see Fig. 1, bottom panel). Missing values: 26 CRP; 14 ESR. CDI *Clostridioides difficile* infection, SD standard deviation, IQR interquartile range. Severity of flare was defined as the following: mild—Crohn's disease activity index (CDAI) score 150–220 or Mayo score 3–5; moderate—CDAI 220–450 or Mayo 6–10; severe—CDAI > 450 or Mayo 11–12

requiring hospitalization, those with prior CDI had lower albumin, greater number of subsequent flares, and fewer had resolution of disease on endoscopy/imaging relative to their CDI-negative counterparts. While it is possible that these differences are the result of CDI, we believe that they are more likely to mark those with more severe IBD because all patients in this comparison were negative for *C. difficile* at the time of the second flare. Interestingly, these differences cannot be accounted for by typical markers of disease severity. Even after adjusting for IBD disease severity and other clinical factors, CDI remained associated with shorter time to disease relapse. An alternative hypothesis is that CDI changes the course of IBD (i.e., frequency of flares) before and after infection. However, our exploratory analysis of cohort C competes against this hypothesis, as there was no difference in the time to flare before compared to after CDI. This supports the notion that severity of IBD is likely the primary determinant of outcomes, rather than CDI itself [17, 18]. Certainly, whether CDI worsens IBD or whether worse IBD predisposes to CDI is a complex and underdeveloped topic, which warrants further study.

There is disagreement among gastroenterologists regarding the optimal management approach to CDI in IBD flare [24]. Treatments for IBD may be held out of concern for worsening infection when the flare is complicated by CDI. In the past, co-administration of immunotherapy for aggressive treatment of IBD with antibiotics for CDI has been associated with worse outcomes [25]. However, this paradigm may be shifting. In more recent data, aggressive management of IBD after recent CDI was associated with clinical improvement [26]. We found that when IBD flares were associated with CDI, providers were less likely to intensify or initiate IBD therapy relative to when flares were CDI-negative. Although we did not study the optimal treatment strategy for patients with IBD flares who test positive for CDI, our results suggest that aggressive treatment of IBD is warranted even when CDI is present.

There are strengths to this study. While it was relatively small, the study utilized granular patient data. The primary outcome—time to flare—was rigorously adjudicated. A novel paradigm was used to address the study question and to test competing hypotheses. This study also has limitations. It was observational and retrospective and was not powered to detect small differences in time to IBD flare, especially for the within-patient comparison. Stool *C. difficile* toxin PCR testing fails to discriminate between active infection and asymptomatic colonization, and there is uncertainty regarding the clinical interpretation of such assays in IBD flares. Data regarding antibiotic use within the 90 days prior to index hospitalization may be incompletely captured given the limitations of chart review. We did not collect patient samples and therefore cannot report on genetic,

microbiome-related, or immunologic data, which warrants future investigation.

In summary, patients with prior CDI had shorter time to IBD relapse compared to their counterparts without history of CDI. Among those hospitalized for three IBD flares, there was no difference in time to relapse before or after acquiring CDI. To our knowledge, this is one of few studies to suggest that acquiring CDI may not necessarily affect the course of IBD, but rather that patients with severe IBD are more susceptible to CDI and therefore have worse clinical outcomes, including earlier time to IBD relapse. A comprehensive understanding of the factors associated with more severe IBD subtype, especially those related to the microbiome and immunologic responses, may provide further insight into the interaction between CDI and IBD pathophysiology, adequate treatment of the underlying disease process, and outcomes.

Funding None.

Compliance with Ethical Standards

Conflict of interest The authors declare that there is no conflict of interests.

References

1. Malik T, Mannon P. Inflammatory bowel diseases: emerging therapies and promising molecular targets. *Front Biosci (Schol Ed)*. 2012;4:1172–1189.
2. Ananthakrishnan AN. Epidemiology and risk factors for IBD. *Nat Rev Gastroenterol Hepatol*. 2015;12:205–217.
3. Azimi T, Nasiri MJ, Chirani AS, Pouriran R, Dabiri H. The role of bacteria in the inflammatory bowel disease development: a narrative review. *APMIS*. 2018;126:275–283.
4. Kostic AD, Xavier RJ, Gevers D. The microbiome in inflammatory bowel disease: current status and the future ahead. *Gastroenterology*. 2014;146:1489–1499.
5. Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature*. 2012;491:119–124.
6. Nguyen GC, Kaplan GG, Harris ML, Brant SR. A national survey of the prevalence and impact of *Clostridium difficile* infection among hospitalized inflammatory bowel disease patients. *Am J Gastroenterol*. 2008;103:1443–1450.
7. Surawicz CM, Brandt LJ, Binion DG, et al. Guidelines for diagnosis, treatment, and prevention of *Clostridium difficile* infections. *Am J Gastroenterol*. 2013;108:478–498. **quiz 99**.
8. Larson AM, Fung AM, Fang FC. Evaluation of tcdB real-time PCR in a three-step diagnostic algorithm for detection of toxigenic *Clostridium difficile*. *J Clin Microbiol*. 2010;48:124–130.
9. Brignola C, Campieri M, Bazzocchi G, Farruggia P, Tragnone A, Lanfranchi GA. A laboratory index for predicting relapse in asymptomatic patients with Crohn's disease. *Gastroenterology*. 1986;91:1490–1494.
10. D'Haens G, Baert F, van Assche G, et al. Early combined immunosuppression or conventional management in patients with newly diagnosed Crohn's disease: an open randomised trial. *Lancet*. 2008;371:660–667.

11. Sahmoud T, Hochtin-Boes G, Modigliani R, et al. Identifying patients with a high risk of relapse in quiescent Crohn's disease. The GETAID Group. The Groupe d'Etudes Therapeutiques des Affections Inflammatoires Digestives. *Gut*. 1995;37:811–818.
12. Henriksen M, Jahnsen J, Lygren I, et al. Clinical course in Crohn's disease: results of a five-year population-based follow-up study (the IBSEN study). *Scand J Gastroenterol*. 2007;42:602–610.
13. Peyrin-Biroulet L, Panes J, Sandborn WJ, et al. Defining disease severity in inflammatory bowel diseases: current and future directions. *Clin Gastroenterol Hepatol*. 2016;14:348–54.e17.
14. Kutty PK, Woods CW, Sena AC, et al. Risk factors for and estimated incidence of community-associated *Clostridium difficile* infection, North Carolina, USA. *Emerg Infect Dis*. 2010;16:197–204.
15. Bossuyt P, Verhaegen J, Van Assche G, Rutgeerts P, Vermeire S. Increasing incidence of *Clostridium difficile*-associated diarrhea in inflammatory bowel disease. *J Crohns Colitis*. 2009;3:4–7.
16. Rodemann JF, Dubberke ER, Reske KA, Seo DH, Stone CD. Incidence of *Clostridium difficile* infection in inflammatory bowel disease. *Clin Gastroenterol Hepatol*. 2007;5:339–344.
17. Schneeweiss S, Korzenik J, Solomon DH, Canning C, Lee J, Bressler B. Infliximab and other immunomodulating drugs in patients with inflammatory bowel disease and the risk of serious bacterial infections. *Aliment Pharmacol Ther*. 2009;30:253–264.
18. Kariv R, Navaneethan U, Venkatesh PG, Lopez R, Shen B. Impact of *Clostridium difficile* infection in patients with ulcerative colitis. *J Crohns Colitis*. 2011;5:34–40.
19. Ananthakrishnan AN, McGinley EL, Binion DG. Excess hospitalisation burden associated with *Clostridium difficile* in patients with inflammatory bowel disease. *Gut*. 2008;57:205–210.
20. Issa M, Vijayapal A, Graham MB, et al. Impact of *Clostridium difficile* on inflammatory bowel disease. *Clin Gastroenterol Hepatol*. 2007;5:345–351.
21. Berg AM, Kelly CP, Farraye FA. *Clostridium difficile* infection in the inflammatory bowel disease patient. *Inflamm Bowel Dis*. 2013;19:194–204.
22. Khanna S, Pardi DS. IBD: poor outcomes after *Clostridium difficile* infection in IBD. *Nat Rev Gastroenterol Hepatol*. 2012;9:307–308.
23. Jodorkovsky D, Young Y, Abreu MT. Clinical outcomes of patients with ulcerative colitis and co-existing *Clostridium difficile* infection. *Dig Dis Sci*. 2010;55:415–420. <https://doi.org/10.1007/s10620-009-0749-9>.
24. Yanai H, Nguyen GC, Yun L, et al. Practice of gastroenterologists in treating flaring inflammatory bowel disease patients with *Clostridium difficile*: antibiotics alone or combined antibiotics/immunomodulators? *Inflamm Bowel Dis*. 2011;17:1540–1546.
25. Ben-Horin S, Margalit M, Bossuyt P, et al. Combination immunomodulator and antibiotic treatment in patients with inflammatory bowel disease and *Clostridium difficile* infection. *Clin Gastroenterol Hepatol*. 2009;7:981–987.
26. Lukin DJ, Lawlor G, Hudesman DP, et al. Escalation of immunosuppressive therapy for inflammatory bowel disease is not associated with adverse outcomes after infection with *Clostridium difficile*. *Inflamm Bowel Dis*. 2019;25:775–781.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Prevalence and Risk Factors for Inappropriate Continuation of Proton Pump Inhibitors After Discharge From the Intensive Care Unit

John W. Blackett, MD; Adam S. Faye, MD; Meaghan Phipps, MD; Jianhua Li, MD; Benjamin Lebwohl, MD, MS; and Daniel E. Freedberg, MD, MS

Abstract

Objective: To determine the prevalence and risk factors for inappropriate discharge on proton pump inhibitor (PPI) therapy started in the intensive care unit (ICU) for stress ulcer prophylaxis.

Patients and Methods: This was a retrospective cohort study of adults initiated on treatment with a PPI in any of 9 affiliated ICUs from January 1, 2014, to December 31, 2018. Patients were excluded if they had an appropriate long-term PPI indication. Logistic regression modeling was used to identify characteristics associated with discharge on treatment with an inappropriate PPI.

Results: Of 24,751 patients admitted to an ICU, 4127 were initiated on treatment with a new PPI, with 2467 (60%) lacking a long-term PPI indication. Of these 2467, a total of 1122 (45%) were continued on PPI therapy after transfer to the floor and 668 (27%) were discharged on PPI therapy. On multivariable analysis, risk factors for inappropriate discharge on PPI therapy included having an upper endoscopy (adjusted odds ratio [aOR], 1.70; 95% CI, 1.08-2.66), admission to the surgical compared with medical ICU (aOR, 2.03; 95% CI, 1.32-3.10), and discharge to a nursing home or rehabilitation facility (aOR, 1.43; 95% CI, 1.04-1.96; and aOR, 2.29; 95% CI, 1.62-3.24, respectively).

Conclusion: Among patients started on treatment with a PPI in the ICU without an indication for outpatient PPI use, 27% (668 of 2467) were nonetheless discharged on PPI therapy. Medically complex and surgical ICU patients are at increased risk for receiving PPIs without appropriate documented indications, and careful review of medication lists at discharge should occur in these high-risk groups.

© 2020 Mayo Foundation for Medical Education and Research ■ Mayo Clin Proc. 2021;96(10):2550-2560



For editorial
comment, see
page 2511

From the Division of Digestive and Liver Diseases, Department of Medicine (J.W.B., A.S.F., M.P., B.L., D.E.F.), and Department of Biomedical Informatics (J.L.), New York Presbyterian Columbia University Medical Center, New York.

Proton pump inhibitors (PPIs) are one of the most commonly prescribed medications in the world, and their use has increased substantially in recent years.^{1,2} Although they are highly effective and safe when used for appropriate indications, they are also one of the most frequently overused medications.³⁻⁵ Prior studies have suggested that 60% to 80% of PPI therapy started in the hospital lacks an appropriate long-term indication.⁶⁻⁸ Despite earlier concerns about long-term adverse consequences of PPI use, the overall safety profile of PPIs is probably favorable.⁹⁻¹⁶ Still, it is wasteful to continue them in the outpatient setting unless they have some reasonable probability of benefit.¹⁷⁻²¹ For this reason, PPIs have been a

common target of initiatives aimed at reducing low-value care.²²⁻²⁵

Stress ulcer prophylaxis with short-term PPI therapy is common in the intensive care unit (ICU). Clinically important gastrointestinal (GI) bleeding occurs in approximately 2% to 3% of ICU patients, with as many as 70% of ICU patients receiving some form of acid suppression in prior studies.^{26,27} Guidelines vary but typically recommend acid suppression with PPIs or histamine 2 receptor antagonists for ICU patients with coagulopathy, prolonged mechanical ventilation, history of upper GI bleeding, traumatic brain injury or burn injury, severe sepsis, or use of steroids or antiplatelet medications.^{28,29}

Prior studies have suggested that PPIs prescribed during hospitalization are frequently continued after hospitalization without indication,^{17,30,31} including 3 small studies of ICU patients in which most patients were continued on PPI therapy at discharge after transfer from the ICU.^{32,33} However, there are no studies identifying which ICU patients are most at risk for being inappropriately continued on PPI therapy at discharge. We sought to estimate the prevalence of this problem among ICU patients at our institution and also to identify predictors of inappropriate PPI therapy continuation.

PATIENTS AND METHODS

Population

This was a retrospective cohort study of patients 18 years or older who were admitted to 1 of the 9 ICUs at Columbia University Medical Center between January 1, 2014, and December 31, 2018, and survived to discharge. For patients who were admitted multiple times during the period, only the first hospitalization was included. During each patient's hospitalization, medication reconciliation notes documenting the medications at admission, transfer, and discharge were electronically parsed for use of PPIs. Patients were included in the study if they received a PPI while in the ICU and had not been on PPI therapy earlier in the hospitalization or before admission, as determined by the medication reconciliation notes. Patients were then excluded from the primary analysis if they had a documented indication for long-term PPI use based on clinical notes from the same time points. Indications for PPI use included ulcers, esophagitis, or erosions on upper endoscopy; diagnostic codes for gastroesophageal reflux disease, Barrett esophagus, peptic ulcer disease, or *Helicobacter pylori* infection; having received a lung transplant; or discharge on treatment with nonsteroidal anti-inflammatory drugs, dual antiplatelet therapy, or an antiplatelet medication with an anticoagulant (Supplemental Table 1, available online at <http://www.mayoclinicproceedings.org>). This left 2467

patients (60% of the 4127 initiated on PPI therapy in the ICU) who lacked a documented long-term indication and made up the primary analysis population.

Exposures

Demographic, hospitalization, and medical covariates were extracted from the electronic medical record using automated queries. These covariates included age, sex, race, ICU type, admission service, discharge destination, length of stay, number of discharge medications, ventilation requirement during admission, Charlson Comorbidity Index score calculated from *International Classification of Diseases* (versions 9 and 10) codes, and upper endoscopy findings. Continuous variables such as length of stay and number of discharge medications were converted to categorical variables by dividing the data into equal tertiles.

Primary Outcome

The primary outcome was inappropriate continuation of PPI therapy at the time of hospital discharge. This was classified based on the discharge medication reconciliation note, which must be completed before discharge in all patients (ie, there is a computerized "hard stop" preventing finalization of the electronic discharge order unless the medication reconciliation is performed and documented). A secondary outcome was inappropriate continuation of PPI therapy at the first primary care follow-up visit among patients who had primary care visits available to review in our health care system. Again, PPI assertion was through the electronic medication reconciliation that must be performed before finalizing this outpatient visit.

Statistical Approach

Categorical variables were compared using χ^2 tests, and continuous variables were compared using Student *t* test or Wilcoxon rank sum test for non-normally distributed data. A multivariable logistic regression model was developed to determine covariates significantly associated with discharge on PPI therapy among patients with no

long-term PPI indication who were initiated on PPI therapy in the ICU. Potential predictors were identified based on clinical expertise as those most likely to be associated with inappropriate PPI therapy continuation post-ICU. The initial model was developed by including the predictors that were significant at alpha level of .20 on univariable analysis. To determine variables that were retained in the final reduced model, we manually removed nonsignificant variables 1 by 1 beginning with those believed to be the least important clinically. Variables in the final model all had a significant independent relationship with the outcome at the .05 level.

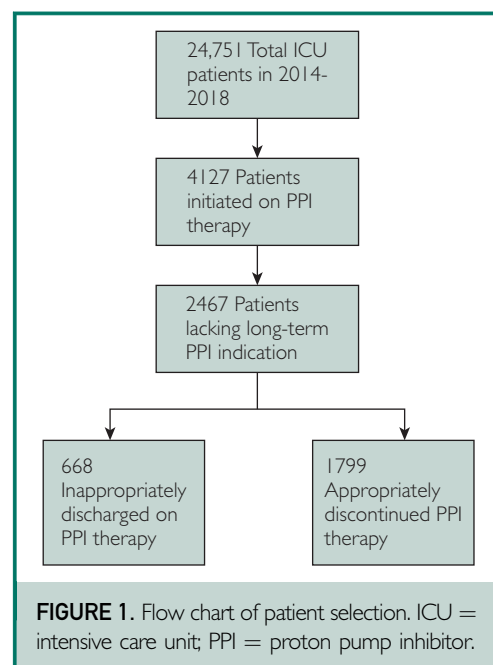
Statistical analysis was performed using Stata (StataCorp, version 16), with alpha level of .05 unless otherwise stated. The Columbia University Institutional Review Board approved this study.

RESULTS

Population

There were 24,751 patients admitted to one of the 9 adult ICUs at 2 affiliated hospitals in 2014 to 2018 who survived to discharge (Figure 1). These included 3 medical ICUs (n=5300; 21%), 2 cardiac ICUs (n=4885; 20%), 2 cardiothoracic ICUs (n=6585; 27%), 1 surgical ICU (n=3266; 13%), and 1 neurological ICU (n=4715; 19%). Of these 24,751 patients, 4127 (17%) were initiated on PPI therapy while in the ICU (had not received a PPI earlier in the hospitalization and no PPI was listed on the admission medication reconciliation).

Of the 4127 patients initiated on PPI therapy in the ICU, 2467 (60%) lacked documentation of a long-term indication and were therefore presumed to be for short-term use, such as stress ulcer prophylaxis. Further, 1122 of these patients (45% of those lacking an indication, 27% of all PPI therapy initiated in ICU) were continued on the PPI therapy at transfer to the floor. Finally, 668 (27% of those lacking an indication, 16% of all PPIs initiated in ICU) were ultimately discharged on PPI therapy without a long-term indication (Figure 2). Of the patients discharged on unnecessary PPI therapy



who had primary care records available, 18 of 28 (64%) were continued on the PPI therapy at their follow-up appointment without documentation of an indication for its prescription.

Patient Characteristics

On univariable analysis, compared with patients whose PPI therapy was appropriately discontinued, patients inappropriately continued on PPI therapy were more likely to be women, be discharged from the neurosurgery service, have a higher number of providers during their hospitalization, have undergone upper endoscopy, have a longer length of stay and more discharge medications, and be discharged to a skilled nursing or rehabilitation facility (Table 1). Patients discharged on inappropriate PPI therapy were less likely to have been intubated during admission. On univariable analysis, patients inappropriately discharged on PPI therapy had a higher mean Charlson Comorbidity Index score at 4.03 compared with 3.66 in patients whose PPI was appropriately discontinued ($P=.001$).

Upper Endoscopy

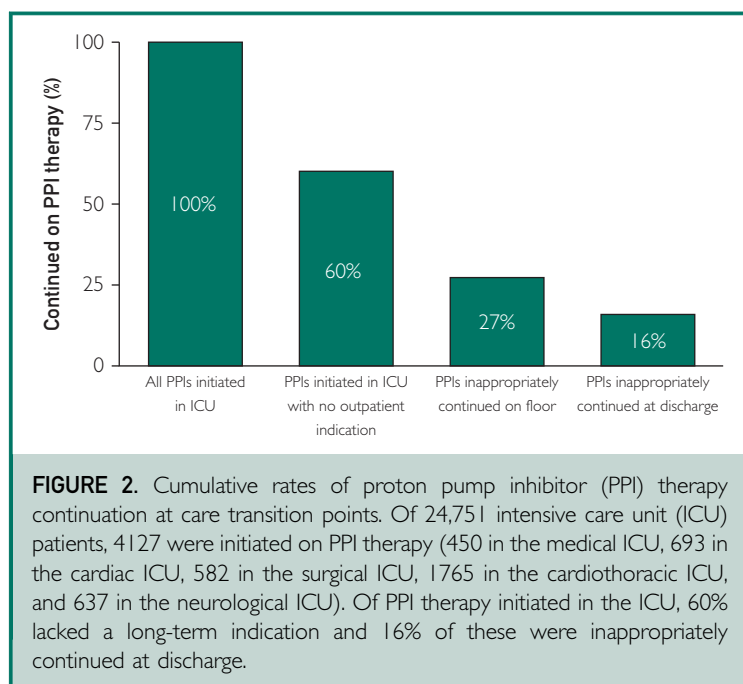
There was a significant association between undergoing upper endoscopy during

hospitalization and discharge on PPI therapy, even after excluding patients with findings that would warrant acid suppression. There were 288 patients initiated on PPI therapy in the ICU who underwent upper endoscopy during admission (28% [n=82] for melena, 15% [n=42] for hematemesis, 4% [n=12] for coffee ground emesis, 7% [n=19] for hematochezia, 8% [n=24] for anemia or heme-positive stool, 9% [n=25] for endoscopic retrograde cholangiopancreatography, 11% [n=31] for percutaneous endoscopic gastrostomy tube placement, 6% [n=16] for dysphagia, and the remaining 13% [n=37] for other reasons, including abnormal imaging results, varices screening, vomiting, or abdominal pain).

Of these 288 patients, 115 had normal endoscopy findings. Of these, 59 (51%) were still discharged on PPI therapy compared with 137 of 173 (79%) patients with abnormal findings ($P<.001$). Among the 137 patients with abnormal findings were 8 patients who had blood or clot seen during endoscopy without an observed underlying lesion. These were considered potentially obscuring lesions that would warrant acid suppression, and continuation on PPI therapy for these patients was considered appropriate. Of the 4 of those who did not have an additional reason for acid suppression, none were discharged on PPI therapy. After excluding patients with PPI indications (such as gastroesophageal reflux disease, ulcers, esophagitis, *H pylori* infection, or erosions on endoscopy), there were 98 patients who underwent upper endoscopy, with 83 of these having normal findings. Of these, 39 of 83 (47%) were still discharged on PPI therapy compared with 8 of 15 (53%) of those who had abnormal findings on endoscopy that would not necessarily warrant PPI prescription, such as arteriovenous malformations, masses, and varices ($P=.65$).

Mechanical Ventilation

Of the 2467 patients without a long-term PPI indication, 1715 (70%) were mechanically ventilated during admission, and 781 (32%) were ventilated for more than 48



hours. Mechanical ventilation overall was associated with a reduced likelihood of discharge on PPI therapy (in 23% [386 of 1715] of ventilated patients vs 38% [282 of 752] of never ventilated patients; $P<.001$), but there was no significant difference in rates of PPI therapy continuation at discharge in those ventilated for more than 48 hours vs those who were not (29% [224 of 781] vs 26% [444 of 1686], respectively; $P=.22$).

ICU Type

There were substantial differences in the rates at which ICUs of different types started and managed PPI therapy (Table 2). The cardiothoracic ICUs (CTICUs) had the highest proportion of patients initiated on new PPI therapy at 27% (1765 of 6588), while the medical ICUs had the lowest proportion at 8.5% (450 of 5300). However, most PPI therapy started in the CTICU (1465 of 1765; 83%) was only given in the ICU and not continued on the floor or at discharge. The surgical ICU and neurological ICU had the highest percentages of patients discharged on PPI therapy without an indication, at 6% (191 of 3266) and 4% (196 of 4715), respectively, among all patients

TABLE 1. Characteristics of Patients Started on PPI Therapy in the Intensive Care Unit Without Documented Long-term Indication

Table 1: Characteristics of Patients on Started on Proton Pump Inhibitors in the ion			
Characteristic	PPI Appropriately Discontinued (n=1799)	PPI Inappropriately Continued at Hospital Discharge (n=668)	P
Age tertile (y), no. (%)			
18-56	707 (39)	287 (43)	.23
56-70	609 (34)	207 (31)	
>70	483 (27)	174 (26)	
Sex, no. (%)			
Male	1081 (60)	363 (54)	.01
Female	718 (40)	305 (46)	
Race, no. (%)			
White	808 (45)	302 (45)	.77
Black	183 (10)	72 (11)	
Asian	73 (4)	32 (5)	
Other/unknown	735 (41)	262 (39)	
Admit service, no. (%)			
Medicine	540 (30)	216 (32)	<.001
Neurology	193 (11)	74 (11)	
Surgery	840 (47)	255 (38)	
Neurosurgery	153 (9)	108 (16)	
Other	73 (4)	15 (2)	
Charlson Comorbidity Index score, no. (%)			
0-2	652 (36)	198 (30)	.003
3-5	759 (42)	294 (44)	
>5	388 (22)	176 (26)	
Intubated during admission, no. (%)			
No	470 (26)	282 (42)	<.001
Yes	1329 (74)	386 (58)	
No. of providers, no. (%)			
1-11	718 (40)	211 (32)	<.001
12-18	638 (35)	225 (34)	
>18	443 (25)	232 (35)	
No. of hospital locations, no. (%)			
1-3	858 (48)	352 (53)	.06
4	434 (24)	155 (23)	
>4	507 (28)	161 (24)	
Length of hospital stay (d), no. (%)			
1-7	733 (41)	198 (30)	<.001
8-15	587 (33)	201 (30)	
>15	479 (27)	269 (40)	
Underwent upper endoscopy, no. (%)			
No	1748 (97)	621 (93)	<.001
Yes	51 (3)	47 (7)	
Discharge destination, no. (%)			
Home	732 (41)	186 (28)	<.001
Home with services	607 (34)	223 (33)	
Rehabilitation facility	147 (8)	115 (17)	
Skilled nursing facility	273 (15)	133 (20)	
Other/against medical advice	18 (1)	6 (1)	

Continued on next page

TABLE 1. Continued

Table 1: Characteristics of Patients on Started on Proton Pump Inhibitors in the ion			
Characteristic	PPI Appropriately Discontinued (n=1799)	PPI Inappropriately Continued at Hospital Discharge (n=668)	P
Discharge destination, no. (%), continued			
Hospice	22 (1)	5 (1)	
No. of discharge medications, no. (%)			
0-7	992 (55)	265 (40)	<.001
8-10	424 (24)	155 (23)	
>10	383 (21)	248 (37)	

PPI = proton pump inhibitor.

admitted to an ICU during the period ($P<.001$).

Multivariable Logistic Regression Model

A multivariable logistic regression model was developed among all patients initiated on PPI therapy in the ICU who lacked a long-term indication for acid suppression (Table 3). The outcome was inappropriate continuation of PPI therapy compared with appropriate discontinuation of PPI therapy at hospital discharge. The predictors significantly associated with discharge on inappropriate PPI therapy in this model were admission to the surgical ICU relative to the medical ICU (adjusted odds ratio [aOR], 2.03; 95% CI, 1.32 to 3.10), discharge to a rehabilitation facility (aOR, 2.29; 95% CI, 1.62 to 3.24) or skilled nursing facility (aOR, 1.43; 95% CI, 1.04 to 1.96) relative to home, undergoing upper endoscopy (aOR, 1.70; 95% CI, 1.08 to 2.66), and increasing number of medications at discharge (aOR, 2.40 for >10 medications relative to <8 medications; 95% CI, 1.88 to 3.06). Predictors associated with decreased odds of discharge on inappropriate PPI therapy were intubation during hospitalization (aOR, 0.57; 95% CI, 0.44 to 0.73), an increasing number of physical locations (ie, more transfers) during hospitalization (aOR, 0.70 for >4 compared with <4 locations; 95% CI, 0.54 to 0.91), and admission to the CTICU relative to the medical ICU (aOR, 0.33, 95% CI, 0.21 to 0.51). Although increasing Charlson Comorbidity Index

score was significantly associated with discharge on an inappropriate PPI on univariable analysis ($P=.001$), it did not remain significantly associated with inappropriate discharge on PPI therapy on multivariable analysis.

Modified Poisson Regression Model

The primary analysis in this study was a multivariable logistic regression model, which provides an estimate of the odds ratio (OR) for inappropriate discharge on PPI therapy for the various predictors. Because the OR may overestimate the relative risk as the outcome of interest becomes more frequent, a sensitivity analysis was performed using a modified Poisson regression model with robust error variance to determine the relative risks of the exposures of interest.³⁴ These results did not substantively differ from the original logistic regression model. Though as expected, the adjusted relative risks for each predictor were somewhat lower than the aORs in the primary logistic regression model, all of the significant predictors remained significant in the sensitivity analysis (Supplemental Table 2, available online at <http://www.mayoclinicproceedings.org>).

DISCUSSION

Low-value medications are a common cause of waste in the modern health care system, with several campaigns such as the Choosing Wisely Initiation of the American Board of Internal Medicine aimed at reducing unnecessary drug prescriptions.^{35,36} Proton pump

TABLE 2. Percent of Patients From Each ICU Discharged on PPI Therapy

ICU type	Started on PPI in ICU, no. (%)	PPI Continued Inappropriately on Transfer to Hospital Floor, no. (%)	PPI Continued Inappropriately at Hospital Discharge, no. (%)	PPI Appropriately Discontinued at Hospital Discharge, no. (%)
Medical ICU (n=5300)	450 (9)	164 (3)	84 (2)	80 (2)
Cardiac ICU (n=4885)	693 (14)	166 (3)	99 (2)	67 (1)
Surgical ICU (n=3266)	582 (18)	268 (8)	191 (6)	77 (2)
Cardiothoracic ICU (n=6585)	1765 (27)	155 (2)	98 (2)	57 (1)
Neurological ICU (n=4715)	637 (14)	369 (8)	196 (4)	173 (4)

ICU = intensive care unit; PPI = proton pump inhibitor.

inhibitors are one of the most commonly overused medications in the United States, with most estimates suggesting that half the PPI prescriptions are medically unnecessary.^{6-8,37} New medications are frequently started during acute hospitalizations and then continued at discharge despite absence of a clear long-term indication.³⁸ Because PPIs are used in the acute setting for stress ulcer prophylaxis in the ICU, they often fall into this category. This study sought to measure the prevalence of discharge on an inappropriate PPI therapy among patients who first received one in the ICU and determine risk factors for inappropriate discharge on long-term PPI therapy after hospitalization in the ICU. We found that among ICU patients started on PPI therapy without a long-term indication, 45% (1122 of 2467) were continued on the PPI therapy on the floor and 27% (668 of 2467) were ultimately discharged on treatment with the PPI.

Three prior small studies were identified that sought to estimate the prevalence of inappropriate continuation of acid suppression started during an ICU admission.^{32,33,39} These 3 studies included a total of 849 patients in 4 medical and surgical ICUs and found rates of inappropriate PPI therapy continuation at the time of hospital discharge from 24% to 39%. In a study of

248 patients who were started on acid suppression in the surgical ICU, Murphy et al³⁹ found that patients with longer hospitalizations and more risk factors for GI bleeding were more often continued on acid suppression therapy, but that only 5% of patients continued on acid suppression therapy had a compelling reason for it at discharge. Unlike these prior studies, this study focused on predictors of inappropriate PPI therapy continuation, including differences in PPI prescribing patterns based on ICU type. A total of 9 ICUs were involved in this study, including medical, surgical, neurological, and cardiac ICUs. We found that the medical ICU started the lowest proportion of its patients on PPI therapy at 9% (450 of 5300) compared with 28% (1765 of 6585) of patients in the CTICU and 18% (582 of 3266) of patients in the surgical ICU (Table 2). However, most PPI therapy initiated in the CTICU was stopped before transfer to the floor. Only 2% (155 of 6585) of CTICU patients were transferred to the floor with an inappropriate PPI therapy compared with 8% (268 of 3266) of surgical ICU patients and 3% (164 of 5300) of medical ICU patients. Patients in the CTICU were commonly admitted after cardiac surgery, often still intubated and receiving systemic anticoagulation, and therefore

TABLE 3. Multivariable Model of Predictors of Discharge on PPI Therapy Among Patients Initiated on PPI in the ICU Without Long-term Indication

Characteristic	Adjusted Odds Ratio (95% CI)	P
ICU where PPI started		
Medical ICU	I (reference)	
Cardiac ICU	1.31 (0.90-1.92)	.16
Surgical ICU	2.03 (1.32-3.10)	.001
Cardiothoracic ICU	0.33 (0.21-0.51)	<.001
Neurological ICU	1.49 (0.79-2.83)	.22
Discharge destination		
Home	I (reference)	
Home with services	1.27 (0.98-1.63)	.07
Rehabilitation facility	2.29 (1.62-3.24)	<.001
Skilled nursing facility	1.43 (1.04-1.96)	.028
Other/against medical advice	0.97 (0.37-2.59)	.96
Hospice	0.60 (0.22-1.68)	.33
Admit service		
Medicine	I (reference)	
Neurology	0.51 (0.27-0.96)	.037
Surgery	1.22 (0.87-1.71)	.26
Neurosurgery	1.03 (0.54-1.98)	.93
Other	0.32 (0.17-0.60)	<.001
Underwent upper endoscopy		
No	I (reference)	
Yes	1.70 (1.08-2.66)	.021
No. of discharge medications		
0-7	I (reference)	
8-10	1.66 (1.29-2.14)	<.001
>10	2.40 (1.88-3.06)	<.001
No. of locations		
1-3	I (reference)	
4	0.83 (0.64-1.07)	.15
>4	0.70 (0.54-0.91)	.008
Length of stay (d)		
1-7	I (reference)	
8-15	1.07 (0.82-1.39)	.64
>15	1.56 (1.15-2.12)	.004
Intubated during admission		
No	I (reference)	
Yes	0.57 (0.44-0.73)	<.001

ICU = intensive care unit; PPI = proton pump inhibitor.

frequently had clear indications for stress ulcer prophylaxis. A total of 98% (1725 of 1765) of CTICU patients were intubated during hospitalization compared with 47% (328 of 693) of cardiac ICU patients, 71% (320 of 450) of medical ICU patients, and 70% (405 of 582) of surgical ICU patients. Providers may be more likely to discontinue

PPI therapy in patients who are started on prophylaxis for an indication with a more defined end point, such as mechanical ventilation, as opposed to coagulopathy or severe sepsis. This is supported by our finding that a history of mechanical ventilation during admission was a negative risk factor for discharge on inappropriate PPI therapy.

Interestingly, patients inappropriately continued on PPI therapy had a higher mean Charlson Comorbidity Index score on univariable analysis, indicating higher level of illness. However, this association was modest in strength and did not remain significant on multivariable analysis after adjusting for ICU type, length of stay, number of discharge medications, and other significant predictors and was therefore not retained in the final multivariable model. It appears that after adjusting for other variables that also capture the concept of comorbid conditions (eg, length of stay, ICU type, and number of medications), the number of baseline comorbid conditions as measured by the Charlson score may not have a significant impact on whether PPI therapy is inappropriately continued.

Although few outpatient follow-up appointments were available to review, 64% (18/28) of patients discharged on inappropriate PPI therapy were continued on the PPI treatment at follow-up. The ongoing propagation of unnecessary medications may be due to a reluctance by providers to discontinue medications that they did not start. Although PPIs are often considered benign with few side effects, the financial cost of unnecessary PPIs has been well documented.¹⁷⁻²¹ Additionally, although not evaluated in this study, it is also possible that adding an extra unnecessary medication in a medically complex and (likely) overburdened patient can be harmful.^{40,41}

The risk factors for discharge on unnecessary PPI therapy included admission to the surgical ICU compared with the medical ICU, discharge to a rehabilitation or skilled nursing facility, greater number of discharge medications, and greater length of stay. This suggests that provider type and patient medical complexity significantly influence whether unnecessary PPI treatment is continued after an ICU stay. The more floors on which a patient was located during the hospitalization, the less likely they were to be discharged on PPI therapy. This result

may be because a medication reconciliation is required during every patient transfer, and at these times, each medication must be reordered. This provides an additional step (similar to transfer from ICU to floor or floor to discharge) in which providers are prompted to reorder or discontinue medications. Our findings suggest that medication reconciliation, in which providers must review all medications at times of admission, transfer, or discharge, may therefore be a useful tool in eliminating certain unnecessary medications.

We also noted that an upper endoscopy (even in patients without ulcers, *H pylori* infection, esophagitis, Barrett esophagus, or any other clear reasons for acid suppression) is associated with unnecessary PPI use. This may reflect that PPI therapy is often started preemptively in patients with occult GI bleeding or anemia but not stopped after a negative endoscopy result. Gastroenterologists may need to take a more proactive role in recommending the discontinuation of PPI therapy after a negative upper endoscopy result.

The strengths of this study included its large size and the inclusion of multiple ICU types. This study also evaluated risk factors associated with discharge on inappropriate PPI therapy rather than estimating the prevalence of the problem.

A limitation is that it was conducted at a single academic medical center composed of 2 affiliated hospitals. Only a small number of patients had outpatient follow-up records available, so only a subset of charts could be reviewed for ongoing refilling of the PPI after discharge (ie, we did not know how long the PPI treatments were continued in the outpatient setting). It is also important to note that our primary results for predictors of inappropriate PPI therapy continuation were ORs, which can overestimate the risk as the outcome of interest becomes more common. Finally, the classification of PPIs as indicated (or not) relied on the documentation of relevant *International Classification of Diseases* codes.

CONCLUSION

In summary, nearly half the patients who start PPI therapy in the ICU without a long-term indication have the PPI therapy continued on the hospital floor, and more than a quarter are ultimately discharged home on the PPI therapy. Put another way, 16%, or approximately 1 in every 6 patients initiated on PPI therapy in the ICU, are inappropriately continued at hospital discharge. More medically complex patients and those in the surgical ICU were more likely to be discharged on PPI therapy without a documented indication. Encouragingly, some ICU types, for example, cardiac specialty ICUs, started more PPI therapy but were also better at PPI deprescribing. Future quality improvement initiatives can be designed based on these data to reduce the rate of inappropriate PPI prescriptions after ICU stay.

SUPPLEMENTAL ONLINE MATERIAL

Supplemental material can be found online at <http://www.mayoclinicproceedings.org>. Supplemental material attached to journal articles has not been edited, and the authors take responsibility for the accuracy of all data.

Abbreviations and Acronyms: aOR, adjusted odds ratio; CTICU, cardiothoracic intensive care unit; GI, gastrointestinal; ICU, intensive care unit; OR, odds ratio; PPI, proton pump inhibitor

Grant Support: The work was supported by The Louis and Gloria Flanzer Philanthropic Trust (B.L.); National Institutes of Health T32DK083256 (A.S.F.); National Institutes of Health K23 DK111847 (D.E.F.); and Department of Defense PR181960 (D.E.F.).

Potential Competing Interests: The authors declare no competing interests.

Correspondence: Address to John W. Blackett, MD, Department of Medicine, New York Presbyterian Columbia University Medical Center, 622 W 168th St, New York, NY. (jwb2158@cumc.columbia.edu) or Daniel E. Freedberg, MD, MS, Department of Medicine, New York Presbyterian Columbia, University Medical Center, 622 W 168th St, New York, NY (def2004@cumc.columbia.edu).

ORCID

John W. Blackett:  <https://orcid.org/0000-0002-8680-9489>

REFERENCES

1. Kantor ED, Rehm CD, Haas JS, Chan AT, Giovannucci EL. Trends in prescription drug use among adults in the United States from 1999-2012. *JAMA*. 2015;314(17):1818-1831.
2. Schumock GT, Li EC, Suda KJ, et al. National trends in prescription drug expenditures and projections for 2016. *Am J Health Syst Pharm*. 2016;73(14):1058-1075.
3. Mafi JN, May FP, Kahn KL, et al. Low-value proton pump inhibitor prescriptions among older adults at a large academic health system. *J Am Geriatr Soc*. 2019;67(12):2600-2604.
4. Leontiadis GI, Sharma VK, Howden CW. Proton pump inhibitor therapy for peptic ulcer bleeding: Cochrane collaboration meta-analysis of randomized controlled trials. *Mayo Clin Proc*. 2007;82(3):286-296.
5. Dean BB, Gano AD Jr, Knight K, Ofman JJ, Fass R. Effectiveness of proton pump inhibitors in nonerosive reflux disease. *Clin Gastroenterol Hepatol*. 2004;2(8):656-664.
6. Leri F, Ayzenberg M, Voyce SJ, Klein A, Hartz L, Smego RA Jr. Four-year trends of inappropriate proton pump inhibitor use after hospital discharge. *South Med J*. 2013;106(4):270-273.
7. Reid M, Keniston A, Heller JC, Miller M, Medvedev S, Albert RK. Inappropriate prescribing of proton pump inhibitors in hospitalized patients. *J Hosp Med*. 2012;7(5):421-425.
8. Ladd AM, Panagopoulos G, Cohen J, Mar N, Graham R. Potential costs of inappropriate use of proton pump inhibitors. *Am J Med Sci*. 2014;347(6):446-451.
9. McDonald EG, Milligan J, Frenette C, Lee TC. Continuous proton pump inhibitor therapy and the associated risk of recurrent *Clostridium difficile* infection. *JAMA Intern Med*. 2015;175(5):784-791.
10. Lazarus B, Chen Y, Wilson FP, et al. Proton pump inhibitor use and the risk of chronic kidney disease. *JAMA Intern Med*. 2016;176(2):238-246.
11. Vaezi MF, Yang YX, Howden CW. Complications of proton pump inhibitor therapy. *Gastroenterology*. 2017;153(1):35-48.
12. Lambert AA, Lam JO, Paik JJ, Ugarte-Gil C, Drummond MB, Crowell TA. Risk of community-acquired pneumonia with outpatient proton-pump inhibitor therapy: a systematic review and meta-analysis. *PLoS One*. 2015;10(6):e0128004.
13. Freedberg DE, Kim LS, Yang YX. The risks and benefits of long-term use of proton pump inhibitors: expert review and best practice advice from the American Gastroenterological Association. *Gastroenterology*. 2017;152(4):706-715.
14. Moayyedi P, Eikelboom JW, Bosch J, et al. COMPASS Investigators. Safety of proton pump inhibitors based on a large, multi-year, randomized trial of patients receiving rivaroxaban or aspirin. *Gastroenterology*. 2019;157(3):682-691.e2.
15. Corley DA. Safety and complications of long-term proton pump inhibitor therapy: getting closer to the truth. *Gastroenterology*. 2019;157(3):604-607.
16. Lee JK, Merchant SA, Schneider JL, et al. Proton pump inhibitor use and risk of gastric, colorectal, liver, and pancreatic cancers in a community-based population. *Am J Gastroenterol*. 2020;115(5):706-715.
17. Thomas L, Culley EJ, Gladowski P, Goff V, Fong J, Marche SM. Longitudinal analysis of the costs associated with inpatient initiation and subsequent outpatient continuation of proton pump inhibitor therapy for stress ulcer prophylaxis in a large managed care organization. *J Manag Care Pharm*. 2010;16(2):122-129.
18. Venkataraman R, Rashid M, Shrestha H. Inappropriate medication use and cost comparison analysis of proton pump inhibitors: evidence from an Indian tertiary care facility. *Curr Drug Saf*. 2020;15(2):147-155.
19. Heidelbaugh JJ, Goldberg KL, Inadomi JM. Overutilization of proton pump inhibitors: a review of cost-effectiveness and risk [corrected; erratum in: *Am J Gastroenterol*. 2009;104(4):1072; erratum in: *Am J Gastroenterol*. 2009;104(2)(suppl):S39]. *Am J Gastroenterol*. 2009;104(suppl 2):S27-S32.

20. Heidelbaugh JJ, Inadomi JM. Magnitude and economic impact of inappropriate use of stress ulcer prophylaxis in non-ICU hospitalized patients. *Am J Gastroenterol*. 2006;101(10):2200-2205.
21. Shin S. Evaluation of costs accrued through inadvertent continuation of hospital-initiated proton pump inhibitor therapy for stress ulcer prophylaxis beyond hospital discharge: a retrospective chart review. *Ther Clin Risk Manag*. 2015;11:649-657.
22. Yachinski PS, Farrell EA, Hunt DP, Reid AE. Proton pump inhibitors for prophylaxis of nosocomial upper gastrointestinal tract bleeding: effect of standardized guidelines on prescribing practice. *Arch Intern Med*. 2010;170(9):779-783.
23. Buckley MS, Park AS, Anderson CS, et al. Impact of a clinical pharmacist stress ulcer prophylaxis management program on inappropriate use in hospitalized patients. *Am J Med*. 2015;128(8):905-913.
24. Odenthal DR, Philbrick AM, Harris IM. Successful deprescribing of unnecessary proton pump inhibitors in a primary care clinic. *J Am Pharm Assoc* (2003). 2020;60(1):100-104.
25. Lin D, Eke C, Cai C, Thrift AP, Shukla R. Decreasing overall and inappropriate proton pump inhibitor use: perspective from a large safety-net healthcare system. *Clin Gastroenterol Hepatol*. 2019;18(4):763-766.e2.
26. Krag M, Perner A, Wetterslev J, et al; SUP-ICU co-authors. Prevalence and outcome of gastrointestinal bleeding and use of acid suppressants in acutely ill adult intensive care patients. *Intensive Care Med*. 2015;41(5):833-845.
27. Krag M, Marker S, Perner A, et al; SUP-ICU trial group. Pantoprazole in patients at risk for gastrointestinal bleeding in the ICU. *N Engl J Med*. 2018;379(23):2199-2208.
28. Barletta JF, Bruno JJ, Buckley MS, Cook DJ. Stress ulcer prophylaxis. *Crit Care Med*. 2016;44(7):1395-1405.
29. The PEPTIC Investigators for the Australian and New Zealand Intensive Care Society Clinical Trials Group, Alberta Health Services Critical Care Strategic Clinical Network, and the Irish Critical Care Trials Group, Young PJ, Bagshaw SM, Forbes AB, et al. Effect of stress ulcer prophylaxis with proton pump inhibitors vs histamine-2 receptor blockers on in-hospital mortality among ICU patients receiving invasive mechanical ventilation: the PEPTIC Randomized Clinical Trial. *JAMA*. 2020;323(7):616-626.
30. Gardner TB, Robertson DJ. Stress ulcer prophylaxis in non-critically ill patients: less may be more. *Am J Gastroenterol*. 2006;101(10):2206-2208.
31. Zink DA, Pohlman M, Barnes M, Cannon ME. Long-term use of acid suppression started inappropriately during hospitalization. *Aliment Pharmacol Ther*. 2005;21(10):1203-1209.
32. Wohlt PD, Hansen LA, Fish JT. Inappropriate continuation of stress ulcer prophylactic therapy after discharge. *Ann Pharmacother*. 2007;41(10):1611-1616.
33. Farley KJ, Bamed KL, Crozier TM. Inappropriate continuation of stress ulcer prophylaxis beyond the intensive care setting. *Crit Care Resusc*. 2013;15(2):147-151.
34. Zou G. A modified Poisson regression approach to prospective studies with binary data. *Am J Epidemiol*. 2004;159(7):702-706.
35. Cassel CK, Guest JA. Choosing wisely: helping physicians and patients make smart decisions about their care. *JAMA*. 2012;307(17):1801-1802.
36. Levinson W, Born K, Wolfson D. Choosing wisely campaigns: a work in progress. *JAMA*. 2018;319(19):1975-1976.
37. Hamzat H, Sun H, Ford JC, Macleod J, Soiza RL, Mangoni AA. Inappropriate prescribing of proton pump inhibitors in older patients: effects of an educational strategy. *Drugs Aging*. 2012;29(8):681-690.
38. Betteridge TM, Frampton CM, Jardine DL. Polypharmacy—we make it worse! A cross-sectional study from an acute admissions unit. *Intern Med J*. 2012;42(2):208-211.
39. Murphy CE, Stevens AM, Ferrentino N, et al. Frequency of inappropriate continuation of acid suppressive therapy after discharge in patients who began therapy in the surgical intensive care unit. *Pharmacotherapy*. 2008;28(8):968-976.
40. Scott IA, Hilmer SN, Reeve E, et al. Reducing inappropriate polypharmacy: the process of deprescribing. *JAMA Intern Med*. 2015;175(5):827-834.
41. Atkin PA, Veitch PC, Veitch EM, Ogle SJ. The epidemiology of serious adverse drug reactions among the elderly. *Drugs Aging*. 1999;14(2):141-152.

Proton Pump Inhibitor Prescribing and Monitoring Patterns Among Gastroenterology Practitioners

David A. Leiman, MD, MSHP,*† Karthik Ravi, MD,‡
Daniel E. Freedberg, MD, MS,§ and C. Prakash Gyawali, MD||

Goals: The aim was to quantify proton pump inhibitor (PPI) practice habits among gastroenterology (GI) practitioners.

Background: Reports of side effects have prompted patients and practitioners alike to discontinue PPI use. Emerging evidence-based literature on PPI risks and safety seek to guide practitioners, but the impact of this literature on PPI prescribing patterns has not been evaluated.

Study: We performed an anonymous online survey of US GI practitioners across 6 academic and community affiliated medical centers. Demographic data including practice type and number of weekly gastroesophageal reflux disease patients seen were obtained. Survey questions evaluated practitioners' monitoring for PPI side effects, dose adjustments, and sources of information about PPI risks.

Results: The survey response rate was 60% (256/429). The majority of respondents were male (169, 66%) attending physicians (178, 70%) practicing general GI (63, 25%). There were 92 (36%) respondents who reported testing for PPI side effects at least once a year. Most respondents (143, 56%) reported discontinuing PPIs at least 50% of the time because of patients' concerns about PPI side effects. The majority of respondents reported getting their information regarding PPI safety from published journals (239, 98%) as well as colleagues (222, 91%).

Conclusions: Despite best available evidence suggesting safety of long-term PPI use without routine monitoring, stopping PPIs and monitoring for potential side effects occurs frequently, even within a cohort of mostly academic GI practitioners. Alternative strategies are needed to improve adherence to best practices, especially since gastroenterologists often serve as PPI experts.

Key Words: proton pump inhibitors, gastroesophageal reflux disease, patient safety

(*J Clin Gastroenterol* 2021;00:000–000)

Received for publication April 23, 2021; accepted September 4, 2021.
From the *Division of Gastroenterology, Duke University; †Duke Clinical Research Institute, Durham, NC; ‡Division of Gastroenterology, Mayo Clinic, Rochester, MN; §Division of Gastroenterology, Columbia University, New York, NY; and ||Division of Gastroenterology, Washington University School of Medicine, St. Louis, MO.

This work was performed under the auspices of the American Gastroenterological Association's Future Leaders Program, with sponsorship from the program.

The authors declare that they have nothing to disclose.

Address correspondence to: David A. Leiman, MD, MSHP, Division of Gastroenterology, Duke University School of Medicine, 200 Morris Street, Suite 6524, Durham, NC 27701
(e-mail: david.leiman@duke.edu).

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website, www.jcge.com.

Copyright © 2021 Wolters Kluwer Health, Inc. All rights reserved.
DOI: 10.1097/MCG.0000000000001623

Proton pump inhibitors (PPIs) are effective for treating gastroesophageal reflux disease (GERD),¹ peptic ulcer disease,² and eosinophilic esophagitis,³ and their use has risen dramatically since initial introduction. These medications account for nearly \$10 billion in direct health care costs,⁴ in large part because of the profound prevalence of GERD symptoms, affecting as many as 30% to 40% of the US adult population.⁵

While helpful in the majority of patients with GERD symptoms, recent scientific literature has described an increasing litany of potential PPI-associated side effects.⁶ Prominent coverage in the lay media, concerns regarding drug-drug interactions,⁷ fear of dementia,⁸ and even worries of increased risk of the novel coronavirus⁹ among others, have prompted patients and practitioners to discontinue PPI use even in situations in which PPIs have clear benefits.¹⁰

As a result, several studies have carefully re-examined these putative risks, and gastroenterology (GI) societies have endorsed evidence-based recommendations reflective of a comprehensive assessment of PPI risks and benefits.¹¹ The majority of the studies reporting increased side effect risks with PPI use are retrospective, observational and often limited by methodological flaws, including selection biases, and residual confounding that results in relatively small risk ratios. Together, these limitations should deter practitioners from adjusting their PPI prescribing patterns.¹² Despite this, there remains evidence that practitioners overestimate PPI risk, resulting in inappropriate discontinuation.¹³ Patients, too, have reported self-discontinuation based on perceived long-term risks.¹⁴ Consequently, even though the best available evidence indicates appropriate PPI use should continue without monitoring, patient attitudes may influence prescriber patterns.

Prior studies evaluating PPI prescribing have focused mainly on primary care providers rather than gastroenterologists, who are in the best position to be experts on PPI therapy. Thus, the impact of PPI safety evidence and GI society recommendations on PPI prescribing patterns among specialists has not been thoroughly evaluated. We therefore aimed to quantify PPI practice habits among GI practitioners.

MATERIALS AND METHODS

Study Population

Participants were recruited from 6 geographically diverse academic medical centers and their affiliated community hospitals, including Arizona, Florida, Minnesota, Missouri, New York, and North Carolina. Inclusion criteria included current GI clinical practice, though no restrictions were made on frequency of clinical care. Physicians, trainees (fellows), and advanced practice providers were recruited. A total of 429 practitioners were invited to participate through

e-mail. Participants who completed $\geq 50\%$ of the questions were considered respondents. All authors had access to the study data and reviewed and approved the final manuscript. This study was reviewed and deemed exempt by the institutional review boards at Duke University, Columbia University, Washington University and the Mayo Clinic.

Survey Development and Distribution

We developed a 16-question survey instrument. Survey domains included demographic information concerning practice history and frequency of GERD patient management, comfort level regarding PPI safety and monitoring practices, and sources of PPI risk and safety information. The specific questions included a description of practice location, experience level of practitioner, primary practice area, years in practice, and percentage of time directed to clinical care. We also queried the frequency of GERD patients seen, as well as comfort in prescribing PPIs, and adjusting PPI dosage, and discussing potential PPI side effects in GERD patients. Additional questions evaluated circumstances in which PPIs are discontinued and the motivation for dose adjustments (eg, patient or practitioner concerns). Professional society affiliations and sources of information for PPI risks and safety were also queried.

Comfort level was assessed using a sliding visual analog scale from 0 to 100.¹⁵ The survey was iteratively developed and underwent face validity testing. The final survey is provided as Appendix 1 (Supplemental Digital Content 1, <http://links.lww.com/JCG/A779>).

The first e-mail invitation was sent in November 2019. Three additional reminder emails were sent to nonresponders before the survey was closed in March 2020. There was no compensation provided for survey completion.

Analysis

For categorical variables, we calculated descriptive statistics, including frequencies and percentages. The primary outcome of testing for PPI side effects was dichotomized as any testing versus no testing performed. We performed χ^2 , the Fisher exact, and Wilcoxon rank sum tests as appropriate for comparisons among survey questions. We then performed a multivariable regression analysis to evaluate for an association between identified factors and testing for PPI side effects. In order to include all potentially relevant variables, our final model included all exposure variables with a prespecified P -value of ≤ 0.15 on univariate analysis. The association between discontinuing PPIs because of patient and practitioner concerns was evaluated using Spearman rank correlation coefficient.

All analysis was performed using STATA 13 (StataCorp, College Station, TX).

RESULTS

A total of 429 practitioners across 6 academic medical centers were invited to participate in the survey. Among the 262 invited participants who responded, 6 (2.3%) were excluded because they completed fewer than 50% of the questions, including the primary question of interest regarding frequency for discontinuing PPIs. Therefore, the overall response rate was 60% (256/429).

The majority of respondents were male (169, 66%), attending physicians (178, 70%) practicing general GI (63, 25%) in an academic environment (233, 91%). Among nontrainees, the highest proportion of respondents have been practicing for > 20 years (63/193, 33%).

PPI Testing Patterns

There were 92 (36%) respondents who reported testing for PPI side effects at least once a year. While practice patterns did not vary by level of training, years in practice or practice location (eg, academic vs. community-based practice), using the dichotomized outcome of performing any testing related to PPIs versus no testing, there was a significant association between testing and several other domains (Table 1). Specifically, practice area ($P < 0.001$) was significantly associated with variable testing patterns with hepatology and inflammatory bowel disease (IBD) focused practitioners more likely to perform testing (Fig. 1). In addition, practitioners who discontinued PPIs because of their own concerns ($P < 0.001$) or patient concerns ($P = 0.01$) were significantly more likely to test for side effects. Practitioners who tested for side effects were significantly more likely to also reduce PPI dose because of safety concerns ($P = 0.009$). Similarly, practitioners with higher comfort discussing PPI side effects ($P = 0.007$) and increased frequency of attempting to reduce PPIs in patients in whom GERD symptoms were controlled ($P = 0.026$), were also more likely to test for side effects.

In our multivariable model, only comfort discussing PPI safety (odds ratio: 1.03, confidence interval: 1.01-1.06) and discontinuing PPI because of practitioner concern about side effects (odds ratio: 1.02, confidence interval: 1.01-1.03) were independently associated with performing any testing. No evaluated sociodemographic factors were significantly associated with performing testing when assessed in logistic regression modeling.

PPI Practice Patterns

A majority of respondents (143, 56%) reported discontinuing PPIs at least 50% of the time because of patients' concerns about PPI side effects, and 89 (35%) practitioners reported discontinuing PPIs at least 50% of the time because of their own (practitioner) concerns. When assessing independently by whether or not testing was ever performed, there was fair to moderate correlation between discontinuing PPIs because of patient concerns and practitioner concerns ($\rho = 0.55$, $P < 0.001$) (Fig. 2).

Source of PPI Safety Information

The majority of respondents reported getting their information regarding PPI risks and safety from published journals (239, 98%), GI periodicals (157, 63%), colleagues (222, 91%), and GI society websites (154, 63%). There were no significant differences identified between practitioners who did and did not test for PPI side effects based on the sources of PPI safety information (Table 2). However, a substantial minority among those who received information from academic journals (88, 37%), GI periodicals (58, 37%), society websites (61, 40%), and colleagues (83, 37%), reported testing for PPI side effects.

DISCUSSION

Although broadly effective in the management of a number of common gastrointestinal conditions, the use and safety of PPIs remains an area of frequent scientific and lay interest.¹⁶ Despite numerous reported associations for long-term health-related consequences, studies have failed to show direct harm.¹⁷ Recent reviews of the evidence regarding PPI safety have stated that these medications can be safely used without drug monitoring in the appropriate clinical condition at the lowest effective dose.¹¹ In this multicenter survey of GI practitioners, despite the majority

TABLE 1. Sociodemographic Factors and Univariate Analysis of Practice Patterns Among Those Practitioners Who Do and Do Not Test for Proton Pump Inhibitor (PPI) Side Effects

Survey Variables	Do Not Test for PPI Side Effects	Test for PPI Side Effects	P
Sociodemographic and practice characteristics			
Male, n (%)	110 (44)	55 (22)	0.13
Practice type			
Academic, n (%)	149 (59)	79 (31)	0.12
Practice level			
Attending, n (%)	109 (43)	67 (27)	0.15
Practice area			
General, n (%)	34 (14)	28 (11)	<0.001
Practice years			
> 20, n (%)	31 (12)	30 (12)	0.20
Percentage of weekly time spent seeing patients (median, IQR)*	80 (50-91)	80 (50-90)	0.65
Weekly frequency of GERD patients			
0-5/week, n (%)	77 (31)	43 (17)	0.82
PPI prescribing patterns, reported on a visual analog scale from 0 to 100			
Comfort prescribing PPIs for GERD (median, IQR)*	100 (92-100)	100 (94-100)	0.84
Comfort discussing PPI side effects (median, IQR)*	90 (75-100)	96 (90-100)	0.01
Frequency of attempting to reduce PPI in controlled GERD (median, IQR)*	75 (50-90)	80 (55-97.5)	0.03
Discontinue PPI because of practitioner concerns (median, IQR)*†	20 (5-50)	46 (12-73)	<0.001
Discontinue PPI because of patient concerns (median, IQR)*‡	50 (20-70)	50 (25-79)	0.01
Reduce PPI dose because of safety concerns (median, IQR)*§	50 (20-75)	70 (25-88.5)	0.01
Change PPI formulation because of safety concerns (median, IQR)*§	15 (0-40)	20 (0-38.5)	0.76

*Wilcoxon rank sum.

Total responses was 251 except: †249, ‡230, §221.

IQR indicates interquartile range.

of respondents working in an academic environment with high self-reported use of academic literature to obtain information about PPI safety, over half reported discontinuing PPIs because of patient concerns regarding side effects at least 50% of the time, and over a third reported at least annual testing for side effects.

PPIs continue to receive substantial online and literature attention regarding long-term safety, spanning a wide diversity of purported side effects, including when the mechanism is not firmly established.¹⁸ The reason for this focus is uncertain, but may be related to their widespread use.¹⁹ There are also substantial data that these medications are often prescribed

inappropriately or continued when no longer indicated.²⁰ In recent years, the Choosing Wisely campaign has highlighted reducing PPI overuse as a central priority in the management of GERD.²¹ One unintended consequence is that many patients with appropriate indications for PPIs are taken off their therapy or PPIs are not prescribed when indicated.²²

It is increasingly clear from recent national surveys that patients are aware of putative long-term risks of PPI, with nearly half of all respondent patients indicating awareness of at least one potential risk.¹⁴ One survey reported 46% of

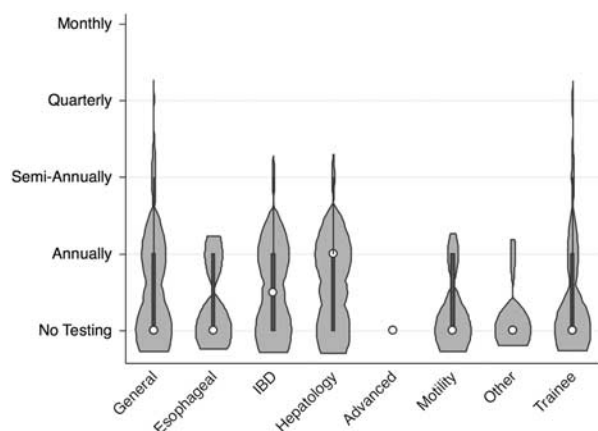
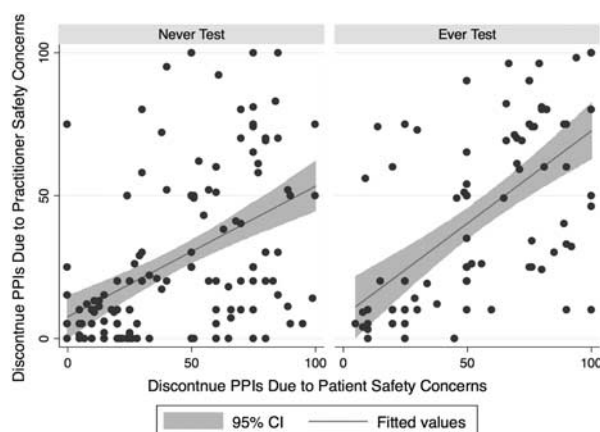
**FIGURE 1.** There was a significantly ($P < 0.001$) increased frequency of testing for proton pump inhibitor side effects when comparing by provider type, with hepatology and inflammatory bowel disease (IBD) focused practitioners more likely to perform testing compared with others.**FIGURE 2.** Among practitioners who ever test for proton pump inhibitor (PPI) side effects, those practitioners who are more likely to discontinue PPIs because of patient concerns about side effects are also more likely to discontinue because of their own concerns about side effects. Overall, there was fair to moderate correlation between discontinuing PPIs because of patient concerns and practitioner concerns ($\rho = 0.55$, $P < 0.001$). CI indicates confidence interval.

TABLE 2. Although There Were No Significant Differences Identified Among Those Who Did and Did Not Test for PPI Side Effects Based on the Sources of PPI Safety Information, a Substantial Minority Among Those Who Received Information From Academic Journals, GI Periodicals, Society Websites, and Colleagues Still Reported Testing for PPI Side Effects

Source	Source Used and Ever Test, n (%)	Source Not Used and Ever Test, n (%)	P
Published media	13 (44.8)	76 (35.5)	0.33
Journal*	88 (36.8)	1 (25.0)	0.53
Internet	7 (31.8)	82 (37.1)	0.62
Society websites	61 (39.6)	28 (31.5)	0.20
News	17 (39.5)	72 (36.0)	0.66
GI periodicals	58 (39.5)	31 (32.3)	0.26
Colleagues	83 (37.4)	6 (28.6)	0.42
Online education	57 (40.7)	32 (31.1)	0.12

Total number of respondents for each category was 243. Percentages are proportions by source use.

*Fisher's exact test.

GI indicates gastroenterology; PPI, proton pump inhibitor.

outpatients, and nearly 100% of their physicians indicated awareness of PPI-related side effects.²³ This nearly ubiquitous awareness was similar to a national sample of physicians.¹⁰ Although these studies reveal 60% to 70% of physicians have concerns about PPI side effects, there was inconsistency regarding whether physicians change their practice as a result. Among the national sample, 76% indicated either somewhat or very much changing their PPI prescribing habits because of perceptions regarding PPI adverse events, whereas others have found a minority of practitioners have changed their management because of concerns about PPIs.²³ Although these studies provide important insights into practitioner concerns and habits regarding PPIs, only 1% of the national sample and <20% of the outpatient provider sample queried gastroenterologists. As experts in the management of patients with GERD and other conditions for which PPIs are frequently prescribed, these data may therefore not be representative of specialty providers' opinions.

Our current survey, which included GI trainees, physicians, and advanced practice providers, offers important new insights. It is striking that over a third of respondents reported testing for side effects despite the lack of evidence to do so. This finding further conflicts with the 37% of practitioners checking for side effects even when obtaining information regarding PPI safety from academic journals, and was a persistent finding regardless of clinical years of experience and practice environment. In our multivariable model, while the effect size was small, only comfort discussing PPI safety and discontinuing PPIs because of practitioner concern about side effects were independently associated with performing any testing. These findings were independent of practice area or frequency of seeing patients with GERD, though no socio-demographic factors included in the model were significantly associated with testing for PPI side effects. These findings might be considered concordant with our other results.

Most practitioners in our study were academically affiliated and reported high use of academic literature and society-related materials. As such, increased knowledge of presumed PPI-related risks may be prompting practitioners to exercise higher caution. Further, in the face of conflicting data and

opinion, practitioners employ a "better safe than sorry" approach. Nonetheless, this is contrary to the best available evidence, and indicates that current educational efforts are insufficient to encourage best practices. Lack of adherence to guidelines is observed in other realms of gastrointestinal care²⁴ and not unique to PPI prescriptions.²⁵ But while perfect adherence to best practices may be desirable, this is rarely achieved in clinical practice. Going forwards, the creation of quality indicators and/or fully specified quality measures could track performance across patient subgroup populations when the strength of recommendation and quality of evidence supports changing practice patterns.²⁶ Such efforts have been shown to improve gastroenterologist quality in other contexts²⁷ and could part of a more robust effort to affect practice patterns. In the case of monitoring for side effects, this may include caveats for practitioners caring for populations with alternate risks for adverse events resembling those reported with PPIs, such as those with IBD or cirrhosis, even when the effect size may be small.

There was a notable correlation that practitioners who test for PPI side effects because of patient concerns are also more likely to discontinue PPIs because of their own concerns about side effects. This may reflect self-reinforcing behavior, even though not consistent with best practices. Also, the frequent reported use of scientific literature and other society resources may in fact produce a paradoxical effect whereby conflicting studies increase confusion rather than produce a consistent message. In this situation, such information could lead to the observed findings. Furthermore, although testing for side effects is discordant with best practices, an acknowledged limitation of our study is that we cannot account for how patients' comorbid conditions may influence the tendency of GI subspecialists with varying primary expertise for side effect monitoring. For example, given the increased risk of enteric infection in cirrhotic patients, it is possible hepatologists may be more vigilant to check for PPI side effects in this population. While it is also possible that performing some diagnostic tests might be consistent with age-appropriate or guideline-based disease monitoring, such as evaluating bone mineral density in patients with IBD, this less likely explains the current data given prior findings that many gastroenterologists do not consider such chronic management part of the focus of GI visits.²⁸ While unclear, motivations for the observed practice patterns could include ongoing concerns regarding adverse events, fear of litigation, or other yet undefined concerns. Although we cannot conclude as much from our data, attributes associated with those who test for side effects are potentially modifiable. This should be an area of further study, as a greater understanding of the motivation to practice out of step with evidence-based medicine has broad implications.

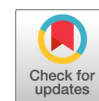
Our study, though provocative and providing important insights into practice patterns, is not without limitations. A self-reported survey, although generally representative of individual practitioners' attitudes, may not entirely reflect true practice patterns because of respondent bias. No study centers were located on the West Coast and, together with the academic focus of most respondents, our results may therefore not represent all practicing gastroenterologists. However, our survey had a high (60%) response rate that did include a broad and diverse national sample. We did not assess duration of PPI therapy as a factor in practice patterns, which has been evaluated with respect to adverse outcomes in the past.¹⁷ Also, we neither inquired about particular side effects that motivate PPI prescribing behavior, since this has previously been investigated,²³ nor did we query respondents about which

specific tests were ordered to monitor for side effects. As a result, we cannot conclude which side effects are most motivating to check for or how increased cost of testing compared with benefit may impact practitioners' decision making; while current best practices do not consider incremental costs in the decision making regarding testing,¹¹ a deeper understanding of practice patterns should be the focus of future study. Further, the number of GERD patients managed across all respondents was relatively small (0 to 5 GERD patients per week: n=120, 48%), which could influence prescribing or testing patterns. Nonetheless, the overall comfort in using PPIs to treat GERD was very high and not different between those who do or do not test for side effects. Although some provider types surveyed may have particular expertise in prescribing PPIs, such as esophageal specialists, with broad indications for their use it is expected all GI practitioners would have baseline proficiency in using PPIs. Indeed, among the strengths of our study was not focusing exclusively on those with esophageal expertise since our primary aim was to fill a knowledge gap related to evaluating a representative assessment of general GI practices. Whereas prior reports have focused on practice habits of primary care providers, the current study includes all practice areas within GI.

In conclusion, we found that despite practicing in an academic environment with high self-reported use of GI literature to obtain information about PPI risks and safety, a substantial number of practitioners report monitoring for side effects and discontinuing PPIs when there is conformity between their patients' and their own concerns about side effects. Therefore, current education efforts are not sufficient to promote best practices and alternative strategies are needed to encourage guideline and evidence concordant practice patterns regarding PPI safety.

REFERENCES

1. Gyawali CP, Kahrilas PJ, Savarino E, et al. Modern diagnosis of GERD: the Lyon Consensus. *Gut*. 2018;67:1351–1362.
2. Barkun AN, Almadi M, Kuipers EJ, et al. Management of nonvariceal upper gastrointestinal bleeding: guideline recommendations from the International Consensus Group. *Ann Intern Med*. 2019;171:805–822.
3. Dellon ES, Liacouras CA, Molina-Infante J, et al. Updated International Consensus Diagnostic Criteria for Eosinophilic Esophagitis: Proceedings of the AGREE Conference. *Gastroenterology*. 2018;155:1022–1033.e10.
4. Gawron AJ, French DD, Pandolfino JE, et al. Economic evaluations of gastroesophageal reflux disease medical management. *Pharmacoeconomics*. 2014;32:745–758.
5. El-Serag HB, Sweet S, Winchester CC, et al. Update on the epidemiology of gastro-oesophageal reflux disease: a systematic review. *Gut*. 2014;63:871–880.
6. Vaezi MF, Yang YX, Howden CW. Complications of proton pump inhibitor therapy. *Gastroenterology*. 2017;153:35–48.
7. Gilard M, Arnaud B, Cornily JC, et al. Influence of omeprazole on the antiplatelet action of clopidogrel associated with aspirin: the randomized, double-blind OCLA (Omeprazole CLopidogrel Aspirin) study. *J Am Coll Cardiol*. 2008;51:256–260.
8. Gomm W, von Holt K, Thome F, et al. Association of proton pump inhibitors with risk of dementia: a pharmacoepidemiological claims data analysis. *JAMA Neurol*. 2016;73:410–416.
9. Almario CV, Chey WD, Spiegel BMR. Increased risk of COVID-19 among users of proton pump inhibitors. *Am J Gastroenterol*. 2020;115:1707–1715.
10. Kurlander JE, Rubenstein JH, Richardson CR, et al. Physicians' perceptions of proton pump inhibitor risks and recommendations to discontinue: a national survey. *Am J Gastroenterol*. 2020;115:689–696.
11. Freedberg DE, Kim LS, Yang YX. The risks and benefits of long-term use of proton pump inhibitors: expert review and best practice advice from the American Gastroenterological Association. *Gastroenterology*. 2017;152:706–715.
12. Savarino V, Dulbecco P, Savarino E. Are proton pump inhibitors really so dangerous? *Dig Liver Dis*. 2016;48:851–859.
13. Kurlander JE, Kolbe M, Scheiman JM, et al. The right idea for the wrong patient: results of a national survey on stopping PPIs. *Clin Gastroenterol Hepatol*. 2017;15:1475–1476.
14. Kurlander JE, Kennedy JK, Rubenstein JH, et al. Patients' perceptions of proton pump inhibitor risks and attempts at discontinuation: a national survey. *Am J Gastroenterol*. 2019;114:244–249.
15. Kushnir VM, Prakash Gyawali C. High resolution manometry patterns distinguish acid sensitivity in non-cardiac chest pain. *Neurogastroenterol Motil*. 2011;23:1066–1072.
16. Lin D, Eke C, Cai C, et al. Decreasing overall and inappropriate proton pump inhibitor use: perspective from a large safety-net healthcare system. *Clin Gastroenterol Hepatol*. 2020;18:763–766.e2.
17. Moayyedi P, Eikelboom JW, Bosch J, et al. Safety of proton pump inhibitors based on a large, multi-year, randomized trial of patients receiving rivaroxaban or aspirin. *Gastroenterology*. 2019;157:682–691.e2.
18. Yuan J, He Q, Nguyen LH, et al. Regular use of proton pump inhibitors and risk of type 2 diabetes: results from three prospective cohort studies. *Gut*. 2021;70:1070–1077.
19. Wallerstedt SM, Fastbom J, Linke J, et al. Long-term use of proton pump inhibitors and prevalence of disease- and drug-related reasons for gastroprotection—a cross-sectional population-based study. *Pharmacoepidemiol Drug Saf*. 2017;26:9–16.
20. Gawron AJ, Rothe AJ, Fought AJ, et al. Many patients continue using proton pump inhibitors after negative results from tests for reflux disease. *Clin Gastroenterol Hepatol*. 2012;10:620–625; quiz e57.
21. American Gastroenterological Association. Five things physicians and patients should question. [ABIM Web site]. 2016. Available at: <https://www.choosingwisely.org/societies/american-gastroenterological-association/>. Accessed October 8, 2020.
22. Platt KD, Saini SD, Kurlander JE. Selecting the appropriate patients for proton pump inhibitor discontinuation: a teachable moment. *JAMA Intern Med*. 2019;179:1276–1277.
23. Ghosh G, Schnoll-Sussman F, Mathews S, et al. Reported proton pump inhibitor side effects: what are physician and patient perspectives and behaviour patterns? *Aliment Pharmacol Ther*. 2020;51:121–128.
24. Spiegel BM, Farid M, van Oijen MG, et al. Adherence to best practice guidelines in dyspepsia: a survey comparing dyspepsia experts, community gastroenterologists and primary-care providers. *Aliment Pharmacol Ther*. 2009;29:871–881.
25. Djinbachian R, Dube AJ, Durand M, et al. Adherence to post-polypectomy surveillance guidelines: a systematic review and meta-analysis. *Endoscopy*. 2019;51:673–683.
26. Adams MA, Allen JI, Saini SD. Translating best practices to meaningful quality measures: from measure conceptualization to implementation. *Clin Gastroenterol Hepatol*. 2019;17:805–808.
27. Kaminski MF, Wieszczy P, Rupinski M, et al. Increased rate of adenoma detection associates with reduced risk of colorectal cancer and death. *Gastroenterology*. 2017;153:98–105.
28. Wagnon JH, Leiman DA, Ayers GD, et al. Survey of gastroenterologists' awareness and implementation of AGA guidelines on osteoporosis in inflammatory bowel disease patients: are the guidelines being used and what are the barriers to their use? *Inflamm Bowel Dis*. 2009;15:1082–1089.



Oral Microbiome Alterations and SARS-CoV-2 Saliva Viral Load in Patients with COVID-19

Emily Happy Miller,^a Medini K. Annavajhala,^a Alexander M. Chong,^{a,b} Heekuk Park,^{a,b} Yael R. Nobel,^a Ali Soroush,^a John W. Blackett,^a Anna Krigel,^a Meaghan M. Phipps,^a Daniel E. Freedberg,^a Jason Zucker,^a Ellen D. Sano,^c Anne-Catrin Uhlemann,^{a,b}  Julian A. Abrams^a

^aDepartment of Medicine, Columbia University Irving Medical Center, New York, New York, USA

^bMicrobiome and Pathogen Genomics Collaborative Center, Department of Medicine, Columbia University Irving Medical Center, New York, New York, USA

^cDepartment of Emergency Medicine, Columbia University Irving Medical Center, New York, New York, USA

Emily Happy Miller and Medini K. Annavajhala contributed equally to this article. Author order was determined by alphabetical order of first names.

ABSTRACT Bacterial-viral interactions in saliva have been associated with morbidity and mortality for respiratory viruses such as influenza and SARS-CoV. However, such transkingdom relationships during SARS-CoV-2 infection are currently unknown. Here, we aimed to elucidate the relationship between saliva microbiota and SARS-CoV-2 in a cohort of newly hospitalized COVID-19 patients and controls. We used 16S rRNA sequencing to compare microbiome diversity and taxonomic composition between COVID-19 patients ($n = 53$) and controls ($n = 59$) and based on saliva SARS-CoV-2 viral load as measured using reverse transcription PCR (RT-PCR). The saliva microbiome did not differ markedly between COVID-19 patients and controls. However, we identified significant differential abundance of numerous taxa based on saliva SARS-CoV-2 viral load, including multiple species within *Streptococcus* and *Prevotella*.

IMPORTANCE Alterations to the saliva microbiome based on SARS-CoV-2 viral load indicate potential biologically relevant bacterial-viral relationships which may affect clinical outcomes in COVID-19 disease.

KEYWORDS COVID-19, SARS-CoV-2, saliva microbiome, viral load

Biological factors that influence SARS-CoV-2 acquisition and subsequent disease severity are not well understood, particularly with respect to potential interactions between the virus and the human microbiome. Given the extensively reported detection of SARS-CoV-2 in saliva and the possibility of transmission via saliva (1, 2), the oral microbiome may represent one such key constituent and viral reservoir. Oral dysbiosis has been linked to many local and systemic diseases, including periodontitis, and could potentially influence COVID-19 disease severity (3–5). Defining the oral microbiome in COVID-19 disease is a necessary step in determining if factors such as oral hygiene could be a modifiable risk factor for severe disease (6).

It has been suggested that oral and respiratory tract microbiota could similarly express enzymes such as the transmembrane serine protease 2 (TMPRSS2), which may enhance viral entry into host cells and further viral infection (7). Results of a recent study of bronchoalveolar lavage fluid from patients with and without COVID-19 suggest that components of the host microbiome can modify heparan sulfate, a key cofactor to SARS-CoV-2 infectivity (8). Direct interaction between influenza and components of the oral microbiome, such as neuraminidase-producing streptococci, is thought to result in increased viral load (9). These studies support the hypothesis that the host microbiome is an important mediator of disease severity in respiratory viral infections. However, the relationship between oral microbiota and SARS-CoV-2 is currently poorly defined.

Citation Miller EH, Annavajhala MK, Chong AM, Park H, Nobel YR, Soroush A, Blackett JW, Krigel A, Phipps MM, Freedberg DE, Zucker J, Sano ED, Uhlemann A-C, Abrams JA. 2021. Oral microbiome alterations and SARS-CoV-2 saliva viral load in patients with COVID-19. *Microbiol Spectr* 9:e00055-21. <https://doi.org/10.1128/Spectrum.00055-21>.

Editor Paul A. Jensen, University of Illinois at Urbana Champaign

Copyright © 2021 Miller et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Anne-Catrin Uhlemann, au2110@cumc.columbia.edu, or Julian A. Abrams, ja660@cumc.columbia.edu.

Received 19 April 2021

Accepted 7 September 2021

Published 13 October 2021

TABLE 1 Demographic and clinical characteristics of newly hospitalized COVID-19 and non-COVID-19 patients^a

Characteristic	Value for non-COVID-19 ^b (n = 59)	Value for COVID-19 ^b (n = 53)	P value ^c
Age (mean [SD])	56.2 (16.8)	56.5 (16.1)	0.93
Male (%)	36 (61.0)	24 (45.3)	0.14
Race (%)			0.49
Black	16 (27.1)	20 (37.7)	
White	14 (23.7)	11 (20.8)	
Other/unreported	29 (49.2)	22 (41.5)	
Ethnicity (%)			0.88
Hispanic/Latinx	26 (44.1)	24 (45.3)	
Not Hispanic/Latinx	22 (37.3)	21 (39.6)	
Not specified	11 (18.6)	8 (15.1)	
BMI (median [IQR])	26.4 (23.2, 30.3)	29.5 (24.7, 35.8)	0.04 ^c
HTN (%)	35 (59.3)	29 (54.7)	0.76
DM (%)	25 (42.4)	19 (35.8)	0.61
CKD (%)	16 (27.1)	6 (11.3)	0.06
Underlying kidney disease (%)	17 (28.8)	8 (15.1)	0.13
Any pulmonary disease (%)	14 (23.7)	12 (22.6)	1.00
Any liver disease (%)	5 (8.5)	3 (5.7)	0.72 ^e
CAD (%)	14 (23.7)	3 (5.7)	0.02
Charlson comorbidity index (median [IQR])	3 (2, 5.5)	2 (1, 4)	0.01 ^d
Oxygen rank severity (%)			0.03 ^e
No supplemental oxygen	49 (83.1)	32 (60.4)	
Nasal cannula	8 (13.6)	16 (30.2)	
Noninvasive ventilation	2 (3.4)	5 (9.4)	
WBC (median [IQR]) ^f	8.99 (7.08, 11.89)	7.01 (5.37, 10.28)	<0.01
NLR (median [IQR]) ^f	4.20 (2.35, 8.29)	3.84 (2.57, 7.52)	0.65
Antibiotics ≤48 h prior to saliva collection (%) ^g	24 (40.7)	25 (47.2)	0.62
Composite outcome (%)			0.68
Deceased	3 (5.1)	4 (7.5)	
Decompensated (no death)	1 (1.7)	2 (3.8)	
Discharged (no death)	55 (93.2)	47 (88.7)	

^aBMI, body mass index; HTN, hypertension; DM, diabetes mellitus; CKD, chronic kidney disease; CAD, coronary artery disease; WBC, white blood cell count; NLR, neutrophil-lymphocyte ratio.

^bAs determined via clinical testing of nasopharyngeal swabs using RT-PCR.

^cCategorical variables were compared using the chi-squared test, unless indicated as below. Continuous variables were assessed for normal or normal-like distribution and compared using *t*-tests, unless indicated as below.

^dKruskal-Wallis test was used due to nonnormal distribution.

^eFisher's exact test was used due to at least one expected value of ≤5.

^fLab values as measured upon admission; not available for all patients (WBC *n* = 58/59 of controls, 53/53 of cases; NLR *n* = 56/59 of controls, 47/53 of cases).

^gInpatient antibiotic use, as determined through chart review.

Here, we characterize the saliva microbiome and saliva SARS-CoV-2 viral load in COVID-19 and control patients hospitalized during the peak of the COVID-19 outbreak in New York City. Bacterial composition did not differ markedly between COVID-19 patients and controls. However, we identified numerous differentially abundant bacterial taxa associated with SARS-CoV-2 saliva viral load, providing evidence for bacterial-viral interactions in the saliva and suggesting that the host microbiome may represent a cofactor affecting disease course.

RESULTS

Clinical characteristics of patient cohort. We collected saliva within 24 h of hospitalization from 53 COVID-19 patients who tested positive for SARS-CoV-2 based on nasopharyngeal swabs and who did not require intensive care at admission. During the study period, SARS-CoV-2 testing was performed on all patients admitted to the hospital, and we also enrolled 59 control patients who were newly hospitalized and confirmed to be SARS-CoV-2 negative (Table S2). Demographics of case and control cohorts were largely comparable, with no significant differences in age, sex, race, or ethnicity (Table 1). However, SARS-CoV-2 positive patients did have significantly higher median body mass index (BMI; 29.5 versus 26.4, *P* = 0.04). Notably, in our cohort,

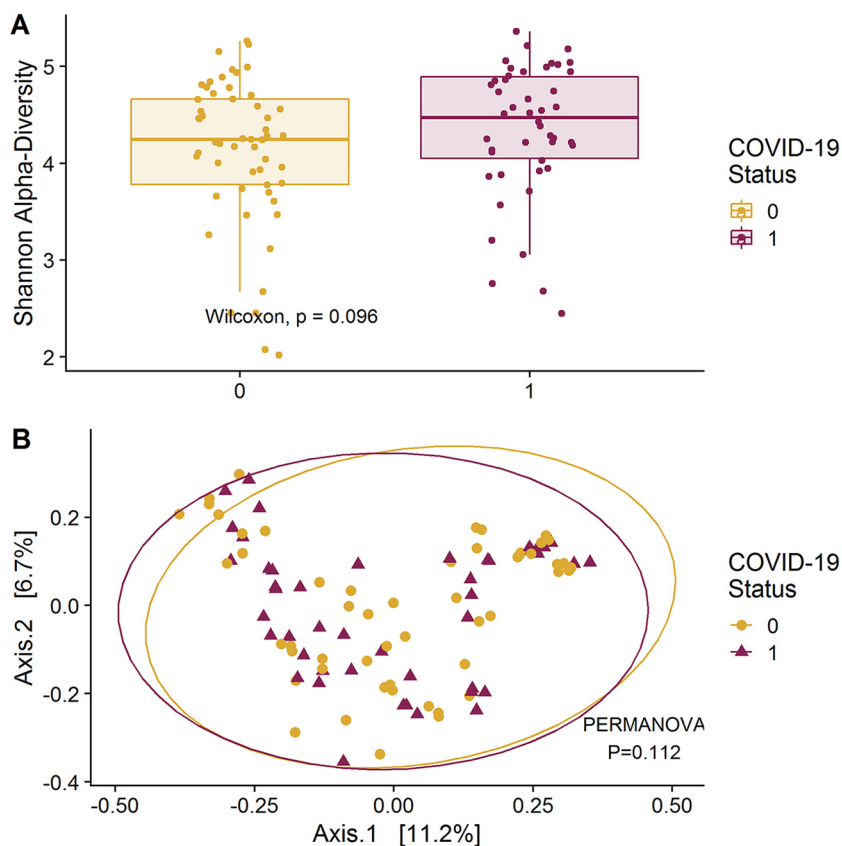


FIG 1 Saliva microbiome diversity in patients with versus without COVID-19. (A) Shannon α -diversity and (B) UniFrac β -diversity in saliva collected from patients with SARS-CoV-2-positive versus -negative nasopharyngeal swabs via clinical testing upon admission. Kruskal-Wallis test was used to compare Shannon α -diversity, and permutational ANOVA (PERMANOVA) was used to compare UniFrac β -diversity. Normal data ellipses for each group are shown over the β -diversity plot in panel B.

control patients had a significantly higher median Charlson comorbidity index (3 versus 2, $P = 0.02$), driven by higher rates of coronary artery disease (CAD; 23.7% versus 5.7%, $P = 0.02$) and chronic kidney disease (CKD; 27.1% versus 11.3%, $P = 0.06$). Compared to controls, COVID-19 patients had similar rates of receipt of antibiotics within 48 h of admission ($P = 0.62$) but were more likely to receive supplemental oxygen ($P = 0.03$), primarily via nasal cannula (30.2% of COVID-19 patients). One patient received remdesivir prior to saliva sample collection. With regard to hospital course and outcomes, relatively few COVID-19 patients had clinical decompensation ($n = 6$, 11.3%) or died in the hospital ($n = 4$, 7.5%).

Saliva microbiome in COVID-19 patients and controls. Of the enrolled patients, we were able to perform saliva microbiome analyses by 16S rRNA gene sequencing on 46 COVID-19 patients (86.8%) and 54 controls (91.5%). There were no significant differences in α -diversity comparing COVID-19 patients and controls (Shannon $P = 0.10$; Chao $P = 0.21$), and there was no evidence of clustering by COVID-19 status in β -diversity analyses (permutational multivariate analysis of variance [PERMANOVA] $P = 0.11$) (Fig. 1; Fig. S1). However, differential abundance analyses did show significant alterations in three amplicon sequence variants (ASVs), including enrichment of *Prevotella pallens* in COVID-19 patients and enrichment of *Rothia mucilaginosa* and a *Streptococcus* spp. in control patients (DESeq2, adjusted P value [P_{adj}] of <0.05).

Saliva SARS-CoV-2 viral load in COVID-19 patients. Cycle threshold (C_T) values from SARS-CoV-2 reverse transcription (RT-PCR) testing can be used as a surrogate for viral load and have been associated with patient outcomes (10). We performed RT-PCR on saliva from both COVID-19 and control patients. Among COVID-19 patients, 28

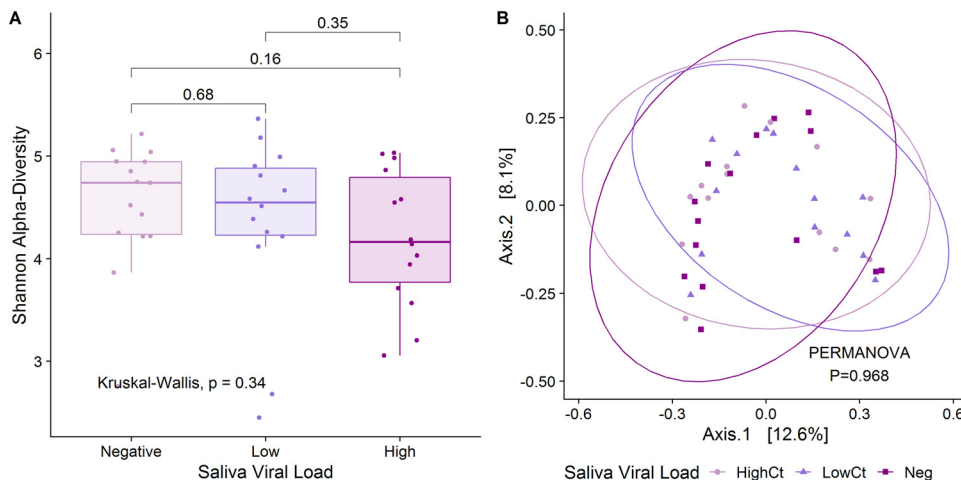


FIG 2 Saliva microbiome diversity compared across SARS-CoV-2 saliva viral load. (A) Shannon α -diversity and (B) UniFrac β -diversity in saliva collected from patients with SARS-CoV-2-positive nasopharyngeal swabs via clinical testing upon admission. Saliva samples were stratified by SARS-CoV-2 RT-PCR cycle threshold (C_T) values into the following categories: negative ($C_T > 40$), low viral load ($C_T > 30$), and high viral load ($C_T < 30$). Kruskal-Wallis test was used to compare Shannon α -diversity, and permutational ANOVA (PERMANOVA) was used to compare UniFrac β -diversity. Normal data ellipses for each group are shown over the β -diversity plot in panel B.

(62%) had detectable SARS-CoV-2 in the saliva, and one patient who tested negative for SARS-CoV-2 on nasopharyngeal swab had detectable viral load in their saliva. Thus, RT-PCR testing of saliva in this cohort had 65.1% (95% confidence interval [CI] 49.1% to 79.0%) sensitivity and 98.1% (95% CI 90.1% to 99.9%) specificity to identify COVID-19 patients (with nasopharyngeal swab results as the gold standard). Detection of SARS-CoV-2 in saliva was not significantly associated with clinical characteristics among COVID-19 patients.

We then categorized COVID-19 patients based on saliva viral load as follows: negative, low viral load (C_T of ≥ 30.4 [the median value among COVID-19 patients with detectable SARS-CoV-2 in saliva] and < 40), and high viral load ($C_T < 30.4$). Across these groups, we did not observe any significant differences in demographic or clinical characteristics (Table S3). There was no association between saliva viral load and symptom duration prior to hospital admission. Saliva viral load was not associated with clinical decompensation or death, although these outcomes occurred in relatively few patients.

Differences in saliva microbiome composition based on saliva SARS-CoV-2 viral load in COVID-19 patients. We next tested whether SARS-CoV-2 viral load in saliva was associated with saliva microbiome diversity or composition. There were no differences in Shannon ($P = 0.34$) or Chao ($P = 0.66$) α -diversity based on saliva viral load, and there was no evidence of clustering by viral load on β -diversity analyses (PERMANOVA $P = 0.97$) (Fig. 2; Fig. S1). However, we identified multiple taxa that were differentially abundant between saliva-negative COVID-19 patients and those with detectable saliva viral load, with alterations in numerous ASVs in genera *Streptococcus*, *Prevotella*, and *Actinomyces*, among others (Fig. 3; Table S4). COVID-19 patients with detectable SARS-CoV-2 in saliva had increased *P. pallens*, *Streptococcus infantis*, *Streptococcus parasanguinis* clade 411, *Streptococcus sanguinis*, *Actinomyces* sp. HMT180, and *Treponema* spp. and decreased *Prevotella denticola*, *Prevotella oris*, *Saccharibacteria* strain HMT356, *Streptococcus peroris*, and an additional *Streptococcus* spp. Comparisons of COVID-19 patients with low viral load versus undetectable virus and high viral load versus undetectable virus produced highly similar results (Fig. 3; Table S4).

While there was no difference in the proportion of COVID-19 patients requiring supplemental oxygen based on saliva viral load (Table S3), we speculated that receipt of supplemental oxygen could affect the relationship between saliva viral load and microbiome composition. However, after adjusting the DESeq2 generalized linear model for

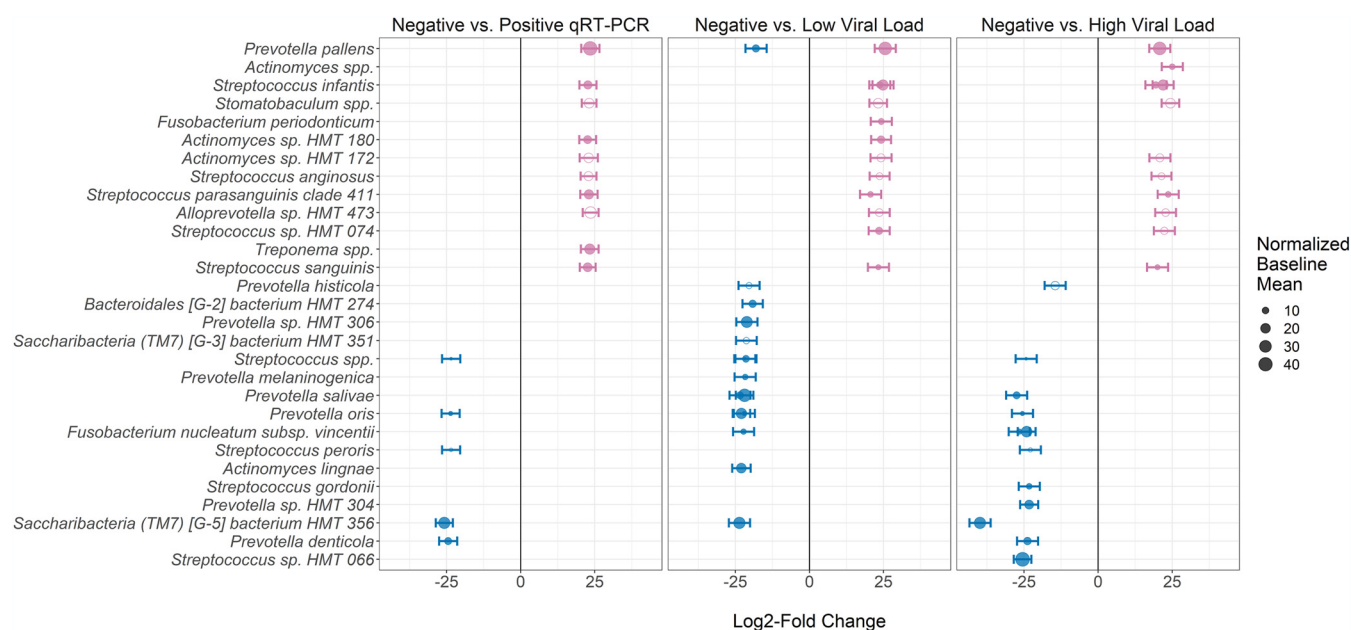


FIG 3 Differentially abundant taxa based on SARS-CoV-2 viral load in saliva from SARS-CoV-2-positive patients. Panels show taxa classified at the genus, species, and/or strain level, which are differentially enriched (*DESeq2*, Benjamini-Hochberg $P_{adj} < 0.05$) across the following comparisons (left to right): (i) SARS-CoV-2-positive (pink) versus -negative (blue) saliva, (ii) SARS-CoV-2-positive with low viral load (pink) versus negative (blue) saliva, and (iii) SARS-CoV-2-positive with high viral load (pink) versus negative (blue) saliva. Median cycle threshold (C_T) across all positive samples was used to determine the threshold between low ($C_T > 30$) versus high ($C_T < 30$) viral load. Each point represents one amplicon sequence variant (ASV), scaled in size by the average normalized read counts in baseline (saliva SARS-CoV-2 negative) samples (i.e., larger circles represent ASVs with higher read counts in baseline samples). Filled circles represent ASVs which remained differentially abundant after adjusting for whether the patient received supplemental oxygen to account for effects of oxygen on the microbiome. Standard error bars for the $\log_2$ fold change calculation across groups are shown for each point. Note: for low-viral-load versus negative saliva samples, different *P. pallens* ASVs were enriched in either group (middle panel, first row). For all other species and strains, all ASVs assigned to a specific taxon were differentially enriched in the same group.

receipt of supplemental oxygen, the large majority of originally identified taxa that were altered based on saliva viral load remained significantly differentially abundant (Fig. 3; Table S4).

DISCUSSION

In our cohort of hospitalized patients, we did not find marked differences in the saliva microbiome between those with and without COVID-19 disease. However, among COVID-19 patients, we did find significant differences in saliva microbiome communities based on saliva SARS-CoV-2 viral load. Further, differential abundance of these taxa was also independent of administration of supplemental oxygen, which suggests that the addition of oxygen to the local oral environment by either nasal cannula or face mask does not markedly affect the relationship between the microbiome and viral load.

Prior studies of other respiratory viruses, such as influenza, have established that the saliva microbiome can be a mediator of disease severity. Bacterial-viral interactions between neuraminidase-producing streptococci and influenza may directly affect viral load (9). The nasal and posterior pharyngeal microbiome is highly distinct in household contacts who do or do not develop influenza after close contact exposure to an index patient (11), which suggests that host microbiome influences susceptibility to influenza infection after exposure. Recently, evidence has been emerging that similar transkingdom relationships may exist with SARS-CoV-2. Analyses of bronchoalveolar lavage fluid from COVID-19 patients and controls identified alterations in bacterial communities capable of modifying heparan sulfate, which is required for SARS-CoV-2 binding to angiotensin converting enzyme 2 (ACE2) (8). Bacterial components of both the respiratory and gut microbiomes influence expression of and cofactors needed for viral binding to ACE2, the host-derived receptor for SARS-CoV-2 (8, 12, 13). Our findings are also consistent with bacterial-viral associations which may have potential clinical significance

and warrant further study. Additionally, the role of inflammation in the oral microbiome should also be considered. One hallmark of COVID-19 disease is a robust inflammatory response in patients, which can lead to a hyperinflammatory state and death (8, 14–16). Specific components of the oral microbiome, such as *Prevotella nigrescens* and *Streptococcus mutans*, are known to be associated with inflammation and oral dysbiosis (3). It is also possible that COVID-19-induced inflammation could directly alter oral bacterial composition. Further investigation into the effect of inflammation on the oral microbiome during SARS-CoV-2 infection is necessary.

Interestingly, we observed only minimal differences in the saliva microbiome of COVID-19 patients compared to that of controls. This suggests that the presence of SARS-CoV-2 may not in and of itself markedly alter the oral microbiome. This is consistent with previous work demonstrating that influenza infection does not appreciably affect oropharyngeal microbiome composition (17). However, possible mediation by the microbiome of susceptibility to SARS-CoV-2 infection cannot be excluded, as we did not assess SARS-CoV-2 exposure in controls. However, two other studies did recently observe an oral microbiome diversity in COVID-19 patients significantly lower than that in healthy controls (18, 19), along with a decrease in butyrate-producing bacteria (18). A metagenomic analysis identified enrichment in opportunistic oral pathogens *Veillonella* and *Megasphaera* in COVID-19 patients, but, similarly to this study, did not find significant changes in alpha-diversity when comparing noncritically ill COVID-19 patients to healthy controls (20). These findings taken along with the findings in this study underscore the need for larger studies of the oral microbiome in COVID-19 patients.

Assessing the diagnostic performance of saliva testing for COVID-19 was not a focus of the current study, and the study was underpowered to identify clinical features associated with SARS-CoV-2 viral load in saliva. However, the results do add to growing data on saliva testing to identify COVID-19 patients. In this study, RT-PCR for SARS-CoV-2 in the saliva had lower sensitivity compared to that seen in prior reports on saliva-based testing in patients hospitalized with COVID-19 disease (1, 21, 22). These studies included patients presenting with more severe disease, and saliva testing may have decreased sensitivity in less symptomatic and asymptomatic patients. Another consideration is the quality of saliva sample the patients produce. COVID-19 patients can have difficulty producing saliva (23, 24), and hyposalivation in COVID-19 patients can be exacerbated by delivery of supplementary oxygen, especially via facemask. Both hyposalivation and supplemental oxygen delivery could affect the production of high-quality saliva samples and adversely affect the performance of RT-PCR SARS-CoV-2 testing on these samples in real-life clinical situations.

This study represents one of the first comprehensive analyses of the saliva microbiome in patients with active, symptomatic SARS-CoV-2 infection. Strengths include the rapid saliva collection in newly admitted patients, the availability of relevant clinical and laboratory data for both cases and controls, and the ability to integrate RT-PCR and 16S rRNA sequencing data on the saliva samples collected. The study included a large proportion of black and Hispanic patients, populations disproportionately affected by COVID-19 yet historically underrepresented in clinical studies. Limitations include a relatively small sample size, which may have affected our ability to detect biologically meaningful differences in microbiome composition between COVID-19 patients and controls. Given the extraordinary circumstances the medical center faced at the height of the COVID-19 surge in New York City, recruiting and consenting patients for research studies was challenging. Clinical C_T values for corresponding nasopharyngeal specimens from this cohort were also largely unavailable due to these constraints. Assessment of the corresponding nasopharyngeal microbiome may have been informative but was beyond the scope of this study. Additionally, data on oral health such as periodontal disease were not available, so any relationship between oral health and the oral microbiome could not be evaluated in this study. Future

studies are needed to address whether oral health and preexisting periodontal disease affect the saliva microbiome during SARS-CoV-2 infection.

As the COVID-19 pandemic continues to pose a global public health threat, it is essential to understand how the virus interacts with the host and how these interactions affect severity of disease. The saliva microbiome represents an array of commensal and potentially pathogenic bacteria which can act as cofactors in the disease process. With high rates of hospitalized patients with COVID-19, common complications, such as bacterial pneumonia, underscore the importance of understanding viral-bacterial community dynamics. Further studies are needed to better elucidate the mechanisms of interactions between the saliva microbiome and SARS-CoV-2 and what effect these interactions have on the course of COVID-19 disease. Additionally, an improved understanding of saliva microbiome dynamics in the setting of SARS-CoV-2 infection may allow for the development of strategies to decrease transmission and complications.

MATERIALS AND METHODS

Sample processing and nucleic acid extraction. Consented patients provided approximately 1 to 2 ml saliva via self-collection in Zymo DNA/RNA Shield saliva collection kits containing 2 ml Zymo DNA/RNA Shield for viral inactivation and stabilization. Inactivated samples were separated into two 1-ml aliquots and stored at -80°C . DNA and RNA were extracted in parallel from 300 μl sample using the ZymoBIOMICS DNA/RNA miniprep kit (Zymo).

SARS-CoV-2 RT-PCR. We used the 2019-nCoV research use only (RUO) primers and probes kit to amplify the SARS-CoV-2 N1 gene and human RNase P (RP) gene (IDT). Reverse transcription PCR (RT-PCR) was performed using the TaqMan Fast Virus 1-step master mix (ThermoFisher), with a total reaction volume of 20 μl , including 5 μl of RNA template. Single RT-PCRs were performed for each sample on 96-well plates, with each plate including a no template control (NTC) with 5 μl of molecular-grade water. Cycle threshold (C_T) values were obtained from RT-PCR amplification curves, and samples with C_T of <40 were considered SARS-CoV-2 positive.

16S rRNA sequencing and data processing. We amplified the 16S rRNA V1-V2 region using Illumina adapter-ligated primers (25), with 2.5 μl (5 ng) DNA template in a total reaction volume of 25 μl (12.5 μl KAPA HiFi HotStart ReadyMix, 5 μl each of forward and reverse primers) with the following cycling protocol: 95°C for 3 min, 25 cycles of 95°C for 30s, 55°C for 30s, and 72°C for 30s, and 72°C for 5 min. The Illumina Nextera XT v2 index sets A to D were used to barcode sequencing libraries. Libraries were sequenced on an Illumina MiSeq using the v3 reagent kit (600 cycles) and a loading concentration of 12 pM with 10% phiX spike-in.

Raw sequencing reads were adapter-trimmed and demultiplexed after FASTQ conversion in BaseSpace (Illumina). Quantitative Insights Into Microbial Ecology (QIIME2) and DADA2 were used to trim, dereplicate, and filter chimeric sequences before generating amplicon sequence variant (ASV) tables (26). Based on the quality score profiles of sequencing reads, forward reads were truncated at 280 bp and reverse reads were truncated at 260 bp prior to merging, ambiguities in the overlap region were not allowed, and default parameters were otherwise applied in the R *dada2* package `filterAndTrim()` function [`truncLen=c(280,260)`; `trimLeft=c(5,5)`, `maxN = 0`, `maxEE=c(2,2)`, `truncQ = 2`]. After dereplication and merging of reads, chimeric reads were identified by consensus across samples using the *dada2* function `removeBimeraDenovo()`. All samples passed the imposed minimum of 10,000 reads after quality filtering for inclusion in this analysis. The MAFFT and FastTree modules in QIIME2 were used to generate a phylogenetic tree of all ASV sequences. ASV taxonomy was assigned using a naive Bayesian classifier trained on a custom database consisting of Greengenes 99% clustered operational taxonomic units (OTUs) and the eHOMD 16S rRNA RefSeq version 15.2 database for increased resolution of oral taxa assignments (27, 28). Unique sequences across the Greengenes and eHOMD databases were clustered at 99% identity and used as the training data set for the QIIME2 naive Bayesian taxonomic classifier. Read counts before and after quality filtering and ASV assignment are provided in Table S1. The R *phyloseq* package was used to calculate microbiome α - and β -diversity metrics (Shannon, Chao indices, and UniFrac distance matrix) (29).

Quantification and statistical analysis. Categorical variables were compared using chi-square or Fisher's exact test. Continuous variables were assessed for normal or normal-like distribution using visualization of QQ-plots. Variables with normal or near-normal distribution were compared using two-sided *t*-tests, and nonnormally distributed variables were compared using Kruskal-Wallis or Mann-Whitney-U nonparametric tests. UniFrac β -diversity distance matrices were compared across groups using permutation ANOVA (PERMANOVA). For all comparisons, significance was defined as a *P* value of <0.05 . Differential abundance analyses were performed using DESeq2, with significance defined as Benjamini-Hochberg adjusted P_{adj} of <0.05 . The statistical tests used are clearly indicated for each comparison throughout the text and in table and figure legends. All statistical analysis and table and figure generation were performed using R version 3.6.1, and R Markdown files detailing all statistical analyses and visualizations are available in a public GitHub repository (https://github.com/mka2136/covid19_saliva_microbiome).

Experimental model and subject details. This was a prospective cohort study of patients newly admitted to the medical service at Columbia University Irving Medical Center between 7 April 2020 and 9 May 2020. The study was approved by the Columbia University Institutional Review Board. The

electronic medical record was screened to identify patients admitted within the previous 24 h to an inpatient, non-intensive care medical service. Inclusion criteria included age of ≥ 18 years, ability to produce a saliva sample, and ability to provide informed consent. Patients were excluded if they were intubated, otherwise medically unstable or unconscious, or admitted to an intensive care setting. Hospitalized patients with a nasopharyngeal swab negative for SARS-CoV-2 but with history of prior COVID-19 disease were excluded. Patients included in the control group had a negative nasopharyngeal swab for SARS-CoV-2 on admission, as per our institution's clinical practice at the time of the study, and otherwise met the same inclusion and exclusion criteria as those in the case group.

Per routine clinical practice during the study period, all patients were tested in the emergency room for SARS-CoV-2 by nasopharyngeal swab PCR. Written informed consent was obtained from SARS-CoV-2-negative patients. Verbal consent was obtained from patients with a positive or pending SARS-CoV-2 test to minimize patient contact.

The following patient-level data were extracted from the electronic medical record: age, sex, race, ethnicity, body mass index (BMI), comorbidities, and receipt of antibiotics prior to or at admission. Additional hospitalization data collected included oxygen requirement at the time of admission, COVID-19 treatment (including methylprednisolone and remdesivir) received during the hospitalization, and relevant laboratory results (white blood cells [WBC], C-reactive protein [CRP], erythrocyte sedimentation rate [ESR], neutrophil-to-lymphocyte ratio [NLR], interleukin-6 [IL-6], ferritin). Clinical outcomes were recorded, including hospital length of stay, clinical decompensation (defined as need for intubation and/or transfer to an intensive care setting), discharge to hospice, discharge to home, and death. All details regarding sample size and demographic characteristics of the full cohort are included in Table 1.

The study was approved by the Columbia University Institutional Review Board (protocol number IRB-AAAS9837).

Data availability. Raw sequencing reads generated in this study have been deposited to the NCBI Short Read Archive (SRA) under NCBI BioProject [PRJNA669421](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA669421). All R code used for statistical analyses and table and figure generation are available in a public GitHub repository (https://github.com/mka2136/saliva_covid19_microbiome). Further information and requests for resources or reagents should be directed to the lead contact, Julian A. Abrams. This study did not generate new unique reagents.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

ACKNOWLEDGMENTS

This work was supported in part by T32 AI100852-08 (E.H.M.), TL1 TR001875 (M.K.A.), R01 CA245894 (A.-C.U.), and R01 CA238433 (J.A.A.). Conceptualization, J.A.A., A.-C.U.; methodology, M.K.A.; formal analysis, M.K.A.; investigation, E.H.M., M.K.A., A.M.C., H.P., Y.R.N., A.S., J.W.B., A.K., M.M.P., J.Z., E.D.S.; resources, J.A.A., A.-C.U.; data curation, E.H.M., M.K.A., J.Z., A.M.C., H.P.; writing – original draft, E.H.M., M.K.A., J.A.A.; writing – review and editing, all authors; supervision, A.-C.U., J.A.A.; funding acquisition, A.-C.U., J.A.A.

We declare no conflicts of interest.

REFERENCES

- Azzi L, Carcano G, Gianfagna F, Grossi P, Gasperina DD, Genoni A, Fasano M, Sessa F, Tettamanti L, Carinci F, Maurino V, Rossi A, Tagliabue A, Baj A. 2020. Saliva is a reliable tool to detect SARS-CoV-2. *J Infect* 81:e45–e50. <https://doi.org/10.1016/j.jinf.2020.04.005>.
- Han P, Ivanovski S. 2020. Saliva—friend and foe in the COVID-19 outbreak. *Diagnostics* 10:290. <https://doi.org/10.3390/diagnostics10050290>.
- Kleinstei SE, Nelson KE, Freire M. 2020. Inflammatory networks linking oral microbiome with systemic health and disease. *J Dent Res* 99: 1131–1139. <https://doi.org/10.1177/0022034520926126>.
- Xiang Z, Koo H, Chen Q, Zhou X, Liu Y, Simon-Soro A. 2020. Potential implications of SARS-CoV-2 oral infection in the host microbiota. *J Oral Microbiol* 13:1853451. <https://doi.org/10.1080/20002297.2020.1853451>.
- Williams DW, Greenwell-Wild T, Brechley L, Dutzan N, Overmiller A, Sawaya AP, Webb S, Martin D, Hajishengallis G, Divaris K, Morasso M, Haniffa M, Moutopoulos NM, NIDCD/NIDCR Genomics and Computational Biology Core. 2021. Human oral mucosa cell atlas reveals a stromal-neutrophil axis regulating tissue immunity. *Cell* 184:4090–4104. <https://doi.org/10.1016/j.cell.2021.05.013>.
- Patel J, Sampson V. 2020. The role of oral bacteria in COVID-19. *Lancet Microbe* 1:e105. [https://doi.org/10.1016/S2666-5247\(20\)30057-4](https://doi.org/10.1016/S2666-5247(20)30057-4).
- Honarmand Ebrahimi K. 2020. SARS-CoV-2 spike glycoprotein-binding proteins expressed by upper respiratory tract bacteria may prevent severe viral infection. *FEBS Lett* 594:1651–1660. <https://doi.org/10.1002/1873-3468.13845>.
- Martino C, Kellman BP, Sandoval DR, Clausen TM, Marotz CA, Song SJ. 2020. Bacterial modification of the host glycosaminoglycan heparan sulfate modulates SARS-CoV-2 infectivity. *bioRxiv*. <https://doi.org/10.1101/2020.08.17.238444>.
- Kamio N, Imai K, Shimizu K, Cueno ME, Tamura M, Saito Y, Ochiai K. 2015. Neuraminidase-producing oral mitis group streptococci potentially contribute to influenza viral infection and reduction in antiviral efficacy of zanamivir. *Cell Mol Life Sci* 72:357–366. <https://doi.org/10.1007/s00018-014-1669-1>.
- Magleby R, Westblade LF, Trzebucki A, Simon MS, Rajan M, Park J, Goyal P, Safford MM, Satlin MJ. 2020. Impact of SARS-CoV-2 viral load on risk of intubation and mortality among hospitalized patients with coronavirus disease 2019. *Clin Infect Dis*. <https://doi.org/10.1093/cid/ciaa851>.
- Tsang TK, Lee KH, Foxman B, Balmaseda A, Gresh L, Sanchez N, Ojeda S, Lopez R, Yang Y, Kuan G, Gordon A. 2020. Association between the respiratory microbiome and susceptibility to influenza virus infection. *Clin Infect Dis* 71:1195–1203. <https://doi.org/10.1093/cid/ciz968>.
- Hashimoto T, Perlot T, Rehman A, Trichereau J, Ishiguro H, Paolino M, Sigl V, Hanada T, Hanada R, Lipinski S, Wild B, Camargo SMR, Singer D, Richter A, Kuba K, Fukamizu A, Schreiber S, Clevers H, Verrey F, Rosenstiel P, Penninger JM. 2012. ACE2 links amino acid malnutrition to microbial

- ecology and intestinal inflammation. *Nature* 487:477–481. <https://doi.org/10.1038/nature11228>.
13. Yang T, Chakraborty S, Saha P, Mell B, Cheng X, Yeo J-Y, Mei X, Zhou G, Mandal J, Golonka R, Yeoh BS, Putluri V, Piyaarthna DWB, Putluri N, McCarthy CG, Wenceslau CF, Sreekumar A, Gewirtz AT, Vijay-Kumar M, Joe B. 2020. Gnotobiotic rats reveal that gut microbiota regulates colonic mRNA of Ace2, the receptor for SARS-CoV-2 infectivity. *Hypertension* 76: e1–e3. <https://doi.org/10.1161/HYPERTENSIONAHA.120.15360>.
 14. Cummings MJ, Baldwin MR, Abrams D, Jacobson SD, Meyer BJ, Balough EM, Aaron JG, Claassen J, Rabbani LE, Hastie J, Hochman BR, Salazar-Schicchi J, Yip NH, Brodie D, O'Donnell MR. 2020. Epidemiology, clinical course, and outcomes of critically ill adults with COVID-19 in New York City: a prospective cohort study. *Lancet* 395:1763–1770. [https://doi.org/10.1016/S0140-6736\(20\)31189-2](https://doi.org/10.1016/S0140-6736(20)31189-2).
 15. Wu Z, McGoogan JM. 2020. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China. *JAMA* 323: 1239–1242. <https://doi.org/10.1001/jama.2020.2648>.
 16. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395:497–506. [https://doi.org/10.1016/S0140-6736\(20\)30183-5](https://doi.org/10.1016/S0140-6736(20)30183-5).
 17. Ramos-Sevillano E, Wade WG, Mann A, Gilbert A, Lambkin-Williams R, Killingley B, Nguyen-Van-Tam JS, Tang CM. 2019. The effect of influenza virus on the human oropharyngeal microbiome. *Clin Infect Dis* 68: 1993–2002. <https://doi.org/10.1093/cid/ciy821>.
 18. Ren Z, Wang H, Cui G, Lu H, Wang L, Luo H, Chen X, Ren H, Sun R, Liu W, Liu X, Liu C, Li A, Wang X, Rao B, Yuan C, Zhang H, Sun J, Chen X, Li B, Hu C, Wu Z, Yu Z, Kan Q, Li L. 2021. Alterations in the human oral and gut microbiomes and lipidomics in COVID-19. *Gut* 70:1253–1265. <https://doi.org/10.1136/gutjnl-2020-323826>.
 19. Iebba V, Zanutta N, Campisciano G, Zerbato V, Di Bella S, Cason C, Luzzati R, Confalonieri M, Plamara AT, Comar M. 2020. Profiling of oral microbiota and cytokines in COVID-19 patients. *Front Microbiol* 12:671813. <https://doi.org/10.1101/2020.12.13.422589>.
 20. Ma S, Zhang F, Zhou F, Li H, Ge W, Gan R, Nie H, Li B, Wang Y, Wu M, Li D, Wang D, Wang Z, You Y, Huang Z. 2021. Metagenomic analysis reveals oropharyngeal microbiota alterations in patients with COVID-19. *Signal Transduct Target Ther* 6:191. <https://doi.org/10.1038/s41392-021-00614-3>.
 21. To KK-W, Tsang OT-Y, Yip CC-Y, Chan K-H, Wu T-C, Chan JM-C, Leung W-S, Chik TS-H, Choi CY-C, Kandamby DH, Lung DC, Tam AR, Poon RW-S, Fung AY-F, Hung IF-N, Cheng VC-C, Chan JF-W, Yuen K-Y. 2020. Consistent detection of 2019 novel coronavirus in saliva. *Clin Infect Dis* 71:841–843. <https://doi.org/10.1093/cid/ciaa149>.
 22. Wylie AL, Fournier J, Casanovas-Massana A, Campbell M, Tokuyama M, Vijayakumar P, Warren JL, Geng B, Muenker MC, Moore AJ, Vogels CBF, Petrone ME, Ott IM, Lu P, Venkataraman A, Lu-Culligan A, Klein J, Earnest R, Simonov M, Datta R, Handoko R, Naushad N, Sewanan LR, Valdez J, White EB, Lapidus S, Kalinich CC, Jiang X, Kim DJ, Kudo E, Linehan M, Mao T, Moriyama M, Oh JE, Park A, Silva J, Song E, Takahashi T, Taura M, Weizman O-E, Wong P, Yang Y, Bermejo S, Odio CD, Omer SB, Dela Cruz CS, Farhadian S, Martinello RA, Iwasaki A, Grubaugh ND, et al. 2020. Saliva or nasopharyngeal swab specimens for detection of SARS-CoV-2. *N Engl J Med* 383:1283–1286. <https://doi.org/10.1056/NEJMc2016359>.
 23. Baghizadeh Fini M. 2020. Oral saliva and COVID-19. *Oral Oncol* 108: 104821. <https://doi.org/10.1016/j.oraloncology.2020.104821>.
 24. Freni F, Meduri A, Gazia F, Nicastro V, Galletti C, Aragona P, Galletti C, Galletti B, Galletti F. 2020. Symptomatology in head and neck district in coronavirus disease (COVID-19): a possible neuroinvasive action of SARS-CoV-2. *Am J Otolaryngol* 41:102612. <https://doi.org/10.1016/j.amjoto.2020.102612>.
 25. Schirmer M, Ijaz UZ, D'Amore R, Hall N, Sloan WT, Quince C. 2015. Insight into biases and sequencing errors for amplicon sequencing with the Illumina MiSeq platform. *Nucleic Acids Res* 43:e37. <https://doi.org/10.1093/nar/gku1341>.
 26. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* 37:852–857. <https://doi.org/10.1038/s41587-019-0209-9>.
 27. Escapa IF, Chen T, Huang Y, Gajare P, Dewhirst FE, Lemon KP. 2018. New insights into human nostril microbiome from the expanded human oral microbiome database (eHOMD): a resource for the microbiome of the human aerodigestive tract. *mSystems* 3:e00187-18. <https://doi.org/10.1128/mSystems.00187-18>.
 28. Escapa I, Huang Y, Chen T, Lin M, Kokaras A, Dewhirst FE, Lemon KP. 2020. Construction of habitat-specific training sets to achieve species-level assignment in 16S rRNA gene datasets. *Microbiome* 8:65. <https://doi.org/10.1186/s40168-020-00841-w>.
 29. McMurdie PJ, Holmes S. 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 8: e61217. <https://doi.org/10.1371/journal.pone.0061217>.

Prevalence and risk factors for gastrointestinal symptoms after recovery from COVID-19

John W. Blackett¹  | Jianhua Li² | Daniela Jodorkovsky¹ | Daniel E. Freedberg¹

¹Department of Medicine, Division of Digestive and Liver Diseases, New York Presbyterian Columbia University Medical Center, New York, NY, USA

²Department of Biomedical Informatics, New York Presbyterian Columbia University Medical Center, New York, NY, USA

Correspondence

John W. Blackett, Department of Medicine, New York Presbyterian Columbia University Medical Center, 622 West 168th Street, New York, NY, USA.
Email: jwb2158@cumc.columbia.edu

Abstract

Background: COVID-19 frequently presents with acute gastrointestinal (GI) symptoms, but it is unclear how common these symptoms are after recovery. The purpose of this study was to estimate the prevalence and characteristics of GI symptoms after COVID-19.

Methods: The medical records of patients hospitalized with COVID-19 between March 1 and June 30, 2020, were reviewed for the presence of GI symptoms at primary care follow-up 1 to 6 months later. The prevalence of new GI symptoms was estimated, and risk factors were assessed. Additionally, an anonymous survey was used to determine the prevalence of new GI symptoms among online support groups for COVID-19 survivors.

Key Results: Among 147 patients without pre-existing GI conditions, the most common GI symptoms at the time of hospitalization for COVID-19 were diarrhea (23%), nausea/vomiting (21%), and abdominal pain (6.1%), and at a median follow-up time of 106 days, the most common GI symptoms were abdominal pain (7.5%), constipation (6.8%), diarrhea (4.1%), and vomiting (4.1%), with 16% reporting at least one GI symptom at follow-up (95% confidence interval 11 to 23%). Among 285 respondents to an online survey for self-identified COVID-19 survivors without pre-existing GI symptoms, 113 (40%) reported new GI symptoms after COVID-19 (95% CI 33.9 to 45.6%).

Conclusion and inferences: At a median of 106 days after discharge following hospitalization for COVID-19, 16% of unselected patients reported new GI symptoms at follow-up. 40% of patients from COVID survivor groups reported new GI symptoms. The ongoing GI effects of COVID-19 after recovery require further study.

KEYWORDS

coronavirus, COVID-19, functional gastrointestinal disorders, irritable bowel syndrome

1 | INTRODUCTION

The SARS-CoV-2 virus has infected nearly 70 million people worldwide, including nearly 15 million in the United States.¹ While over 1.5 million patients have died, many more have recovered. The medical community continues to grapple with the long-term effects of coronavirus disease 2019 (COVID-19). It is clear that persistent

symptoms are common after recovery, in particular fatigue and dyspnea.²⁻⁵

COVID-19 often presents with gastrointestinal (GI) symptoms such as diarrhea, abdominal pain, and nausea or vomiting.⁶⁻⁹ Other infections that cause acute GI symptoms frequently lead to persistent symptoms such as post-infectious irritable bowel syndrome (IBS) and post-viral gastroparesis.^{10,11} It has been estimated that

Abbreviations: COVID-19, coronavirus disease 2019; GI, gastrointestinal; IBS, Irritable bowel syndrome; IBS-SSS, irritable bowel syndrome symptom severity score; IQR, interquartile range.

post-infectious IBS occurs in approximately 10% of patients after acute infectious gastroenteritis.^{12,13} Given the millions of people affected by COVID-19, even a small percentage with persistent GI symptoms would have important public health implications for patients and their providers.

In this study, we sought to identify the prevalence, severity, and risk factors for GI symptoms in the months following initial infection with COVID-19, through both a retrospective analysis of patients hospitalized for COVID-19 with primary care follow-up at our institution, and through an online survey of “long-haulers” drawn from two COVID-19 support groups.

2 | METHODS

This was a two-part study in which we determined the prevalence of new gastrointestinal symptoms during the months following diagnosis of COVID-19 using two fundamentally different cohorts. First, we performed a retrospective study of patients hospitalized with COVID-19 at our institution, using manual chart review to determine the prevalence of GI symptoms 1–6 months after hospital discharge. Second, we administered an online survey to users of two support groups for survivors of COVID-19 assessing the prevalence, type, and severity of GI symptoms, as well as risk factors and demographic information.

In the first study cohort, we included all adult patients who were hospitalized for COVID-19 at Columbia University Irving Medical Center between March 1 and June 30, 2020, and had at least one primary care follow-up appointment at our institution between 30 and 180 days post-discharge. Patients were excluded if they had a pre-existing history of irritable bowel syndrome, inflammatory bowel disease, or other chronic GI conditions, determined by both manual chart review and the presence of International Classification of Disease diagnostic codes identifying these conditions at the time of COVID-19 diagnosis. They were also excluded if they had a diagnosis of any other acute enteric infection (eg, *Clostridioides difficile* infection) during hospitalization for COVID-19. Patients who were not admitted for COVID-19 based on the discharge diagnosis (such as women admitted for labor and delivery found to screen positive for SARS-CoV-2) were also excluded. Thus, the cohort included all adults admitted for COVID-19, whether or not they had any gastrointestinal symptoms at the time of admission. The primary outcome was the documentation of prespecified GI symptoms (diarrhea, abdominal pain, nausea or vomiting, and constipation) based on manual chart review at follow-up. Other covariates including age, sex, hospital length of stay, symptoms at presentation, intensive care unit or ventilator requirement, and history of anxiety or depression were also extracted from the electronic medical record.

In the second study cohort, we administered an anonymous, online survey using Qualtrics to adult (≥ 18 years of age) users of two support groups for survivors of COVID-19 (www.reddit.com/r/covid19positive, and Survivor Corps). The survey consisted of questions

related to demographics, COVID-19 severity, and pre-COVID and post-COVID GI symptoms (complete survey available in the online Methods Supplement). Questions were designed to determine whether respondents met the Rome IV criteria for irritable bowel syndrome (IBS) with IBS questions pertaining to both pre- and post-COVID-19 symptoms so that we could clearly identify whether symptoms were new.^{14,15} Additional questions related to the current presence of gastrointestinal symptoms including abdominal pain, diarrhea, constipation, and nausea/vomiting were used to provide additional information about specific symptoms outside of the Rome criteria for IBS. Respondents also completed the IBS Symptom Severity Score (IBS-SSS), a validated survey used to determine the severity of IBS symptoms on a 0–500 point scale, with ≤ 175 indicating mild, 175–300 moderate, and ≥ 300 severe IBS.¹⁶ The survey was administered between November 12 and December 15, 2020. The primary outcome was again the presence of post-COVID GI symptoms, defined as the report of any GI symptoms (same symptoms as above) post-COVID-19 that were not reported pre-COVID-19. A secondary outcome was the presence of new symptoms meeting Rome IV criteria for IBS post-COVID-19, among those who did not meet criteria for IBS pre-COVID-19.

Participation in the survey was voluntary and anonymous with no financial or medical incentive. The survey was distributed by email and by posting on the relevant forum with the permission of the group administrators. The Columbia University Institutional Review Board approved this study.

2.1 | Statistical analysis

For both parts of the study, continuous variables were compared in patients with and without persistent GI symptoms using the Student's *t* test or Mann-Whitney *U* test for non-parametric data. Categorical variables were compared using chi-square tests. A multi-variable logistic regression model was also used to identify independent risk factors for ongoing GI symptoms, adjusting for predictors that were significant at alpha level 0.20. Among the unselected patients coming for routine follow-up visits, we sought to test whether the prevalence of GI symptoms diminished with increasing time from hospital discharge. To do this, the cohort was divided *a priori* into those presenting for follow-up from 30 to 89 days after hospitalization for COVID-19, 90–119 days after hospitalization, 120–149 days after hospitalization, and 150–180 days after hospitalization. The trend across the proportions of those with GI symptoms within each time interval was then assessed using the Cochran-Armitage test. Among the second study cohort of online survey respondents, the prevalence of GI symptoms meeting criteria for irritable bowel syndrome was compared before and after COVID-19 diagnosis using McNemar's test for paired categorical data. Confidence interval for proportions was calculated using the exact binomial (Clopper-Pearson) method. All statistical analyses were performed with Stata version 16 (College Station, Tx). Alpha.05 was considered statistically significant for all analyses.

3 | RESULTS

3.1 | Unselected patients hospitalized for COVID-19

There were 187 adult patients hospitalized for COVID-19 at Columbia University Irving Medical Center between March 1 and June 30, 2020, with at least one primary care outpatient clinic appointment at least 30 days after hospital discharge. Of these, 37 were excluded for pre-existing GI symptoms (documentation of chronic diarrhea, constipation, abdominal pain, celiac disease, irritable bowel syndrome, inflammatory bowel disease, or gastroparesis). An additional 3 patients were excluded for *C. difficile* infection during the COVID-19 hospitalization, leaving 147 patients in the cohort. The most common GI symptoms at initial presentation with COVID-19 among this group were diarrhea (23%), nausea/vomiting (21%), and abdominal pain (6.1%), with 51 patients (35%) having at least one GI symptom at the time of hospital admission.

3.2 | Unselected patients: persistent new GI symptoms at routine follow-up

After a median follow-up time of 106 days (IQR 78–141), 24/147 (16%) of patients had at least one ongoing GI symptom (95% confidence interval 11%–23%) at the time of their most recent follow-up appointment after hospitalization for COVID-19, which had not been present prior to their diagnosis of COVID-19. These included 10 patients with constipation (6.8%), 6 with diarrhea (4.1%), 6 with nausea/vomiting (4.1%), and 11 with abdominal pain (7.5%), with 14 patients having more than one symptom. An additional 5% of patients experienced transient GI symptoms at follow-up, which had resolved by the time of their latest clinic appointment. Among the 113 patients with at least 3 months of follow-up, the prevalence of ongoing abdominal pain in association with bowel disturbance (diarrhea or constipation) was 6.2%. As a point of comparison, the prevalence of post-infectious IBS after infectious gastroenteritis has been estimated at 10%,^{12,13} though it should be noted that this estimate varies widely depending on pathogen and definition used. Furthermore, the diagnosis of IBS requires at least 6 months of symptoms, which patients in this cohort did not have.

3.3 | Unselected patients: GI symptom characteristics and baseline risk factors

Among patients hospitalized for COVID-19, the prevalence of constipation increased from 0% at hospital admission to 6.8% at most recent outpatient follow-up ($p < 0.01$). The prevalence of diarrhea decreased from 23% at admission to 4.1% at follow-up ($p < 0.01$). The prevalence of abdominal pain was similar at 6.1% at admission and 7.5% at follow-up ($p = 0.64$). The prevalence of nausea/vomiting decreased from 21% at admission to 4.1% at follow-up

($p < 0.01$). The prevalence of each GI symptom present at the time of the most recent follow-up is shown in Table 1. Women were significantly more likely to report new abdominal pain compared to men (14% vs. 1.3%, $p < 0.01$), though there was no significant difference in the prevalence of overall GI symptoms by sex (21% in women vs. 12% in men, $p = 0.15$). There were no significant differences in GI symptoms by age, race, or ethnicity. Prior history of depression was also a risk factor for persistent GI symptoms (28% vs. 13%, $p = 0.04$).

3.4 | Unselected patients: hospital risk factors for persistent new GI symptoms

The prevalence of persistent GI symptoms stratified by hospitalization characteristics is shown in Table 2. While patients who had GI symptoms at initial presentation had a higher prevalence of persistent GI symptoms at follow-up, this difference was not statistically significant (22% vs. 14%, $p = 0.21$). Length of stay, intensive care unit admission, and use of antibiotics, steroids, or hydroxychloroquine were not associated with a higher prevalence of GI symptoms at follow-up. The peak laboratory values of inflammatory markers during hospitalization were also identified for each patient, and there were no significant differences in the median erythrocyte sedimentation rate, C-reactive protein, white blood cell count, ferritin, or interleukin-6 levels in patients with and without persistent GI symptoms at follow-up (Table S1).

3.5 | Unselected patients: multivariable analysis of predictors of ongoing GI symptoms at follow-up

A multivariable logistic regression model was also used to evaluate for independent risk factors of ongoing GI symptoms at the time of follow-up (Table 3). After adjusting for age, sex, and admission to intensive care unit (predictors that were significant at alpha level 0.20), only a history of depression remained significantly associated with the presence of GI symptoms at follow-up, with adjusted odds ratio 3.07, 95% confidence interval 1.04–9.04, $p = 0.042$.

3.6 | Unselected patients: gastrointestinal symptoms over time

The prevalence of GI symptoms was 24% among patients whose most recent follow-up was 30–89 days after discharge, 20% for those 90–119 days post-discharge, 17% for those 120–149 days post-discharge, and 11% for those 150–180 days post-discharge (Figure 1, Cochran-Armitage test for trend $p = 0.10$). Of the 24 patients with ongoing GI symptoms at follow-up, 5 had undergone evaluation with upper endoscopy and 3 with colonoscopy at the time of chart review, with only minor findings noted (one patient with grade A esophagitis and 1 with esophageal candidiasis).

TABLE 1 Prevalence of gastrointestinal symptoms among 147 patients hospitalized for COVID-19 after a median 106 days of follow-up

	Gastrointestinal symptoms at most recent follow-up appointment				
	Constipation	Diarrhea	Abdominal pain	Nausea or vomiting	Any symptoms
Sex					
Female (n = 72)	4	4	10*	3	15 (21%)
Male (n = 75)	6	2	1*	3	9 (12%)
Age					
18–44 (n = 24)	3	0	4	0	5 (21%)
45–59 (n = 36)	3	3	4	2	9 (25%)
60–69 (n = 34)	0	1	1	1	3 (8.8%)
≥70 (n = 53)	4	2	2	3	7 (13%)
Race					
White (n = 37)	2	2	2	2	5 (14%)
Black (n = 27)	0	2	0	0	2 (7.4%)
Asian (n = 3)	1	0	1	0	2 (67%)
Other/decline (n = 80)	7	2	8	4	15 (19%)
Ethnicity					
Hispanic (n = 83)	7	4	8	3	15 (18%)
Non-Hispanic (n = 38)	2	2	1	1	5 (13%)
Unknown/decline (n = 26)	1	0	2	2	4 (15%)
Depression					
No (n = 115)	7	3	7	4	15 (13%)*
Yes (n = 32)	3	3	4	2	9 (28%)*
Anxiety					
No (n = 120)	7	5	7	5	19 (16%)
Yes (n = 27)	3	1	4	1	5 (19%)
Fibromyalgia					
No (n = 128)	9	5	7*	6	19 (15%)
Yes (n = 19)	1	1	4*	0	5 (26%)

* $p < 0.05$.

3.7 | Respondents to an online survey

There were 355 respondents to the online survey (229 from Survivor Corps and 126 from the COVID19positive Reddit group) who were ≥18 years of age and answered “yes” to the question “Have you been diagnosed with COVID-19.” There were 70 respondents (20%) who reported a history of IBS or symptoms consistent with IBS prior to their COVID-19 diagnosis leaving 285 in the primary analysis. Among these, 91 (32%) reported diagnosis less than 1 month prior to the survey, 38 (13%) 1–3 months prior, 44 (15%) 3–6 months prior, and 112 (39%) more than 6 months prior.

3.8 | Survey respondents: post-COVID-19 abdominal pain with bowel disturbance

After excluding survey respondents with pre-existing IBS, there were 113/285 (40%) respondents with post-COVID-19 abdominal pain with bowel disturbance at the time of the survey (ie, with

weekly abdominal pain associated with at least two of the following: related to defecation, change in bowel frequency, or change in stool form). In the most recent 10 days prior to survey completion, 165/285 (58%) respondents reported diarrhea, 173 (61%) reported abdominal pain, 130 (46%) reported constipation, and 134 (47%) reported nausea or vomiting. Among all survey respondents, 123/355 (35%) reported taking a new medication for a new gastrointestinal symptom after diagnosis with COVID-19.

3.9 | Survey respondents: risk factors for abdominal pain with bowel disturbance

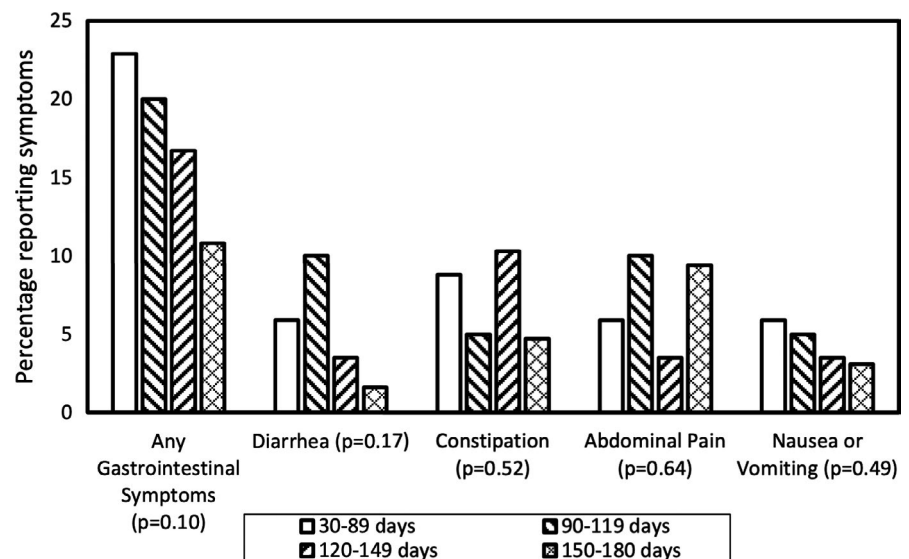
The characteristics of the survey respondents, excluding those with pre-existing IBS, is shown in Table 4, comparing those with and without weekly abdominal pain with bowel disturbance after recovery from COVID-19. Women had a higher prevalence of GI symptoms, but the difference was not statistically significant (42% vs. 35%, $p = 0.25$). There was no association between post-COVID GI symptoms and

TABLE 2 Hospitalization characteristics of patients with GI symptoms at follow-up

	GI Symptoms at most recent follow-up appointment				
	Constipation	Diarrhea	Abdominal pain	Nausea or vomiting	Any symptoms
GI symptoms at diagnosis					
No (n = 96)	5	3	8	3	13 (14%)
Yes (n = 51)	5	3	3	3	11 (22%)
Length of Stay					
0 to 3 days (n = 33)	4	3	4	1	8 (24%)
4–7 days (n = 34)	0	0	1	2	3 (8.8%)
8–14 days (n = 43)	3	0	3	1	5 (12%)
>14 days (n = 37)	3	3	3	2	8 (22%)
ICU admission					
No (n = 128)	8	4	9	5	19 (15%)
Yes (n = 19)	2	2	2	1	5 (26%)
Hydroxychloroquine					
No (n = 46)	5	3	2	3	9 (20%)
Yes (n = 101)	5	3	9	3	15 (15%)
Steroids					
No (n = 111)	5	3	7	4	15 (14%)
Yes (n = 36)	5	3	4	2	9 (25%)
Antibiotics					
No (n = 43)	4	3	1	3	9 (21%)
Yes (n = 104)	6	3	10	3	15 (14%)

* $p < 0.05$.

FIGURE 1 Prevalence of gastrointestinal symptoms by time period of most recent follow-up among patients hospitalized with COVID-19: p -value refers to trend of symptom prevalence over time within each symptom category



race/ethnicity, or history of depression, anxiety, or fibromyalgia. There was no significant association with the method of COVID-19 diagnosis. Those who had GI symptoms at the time of diagnosis were significantly more likely to report GI symptoms at follow-up (45% vs. 27%, $p = 0.01$), as were those who received antibiotics (51% vs. 35%, $p = 0.02$) or hydroxychloroquine (69% vs. 38%, $p = 0.03$).

3.10 | Survey respondents: new symptoms consistent with IBS lasting ≥ 6 months

There were 134 survey respondents who were diagnosed with COVID-19 at least 6 months prior to the survey (including 22 with and 112 without pre-COVID symptoms of IBS). These respondents

TABLE 3 Multivariable logistic regression of predictors of gastrointestinal symptom at follow-up

Variable	Any GI Symptom at most recent follow-up appointment		
	Adjusted odds ratio	95% confidence interval	p-value
Age			
18–44	1 (reference)		
45–59	1.52	0.40–5.81	0.54
60–69	0.37	0.07–1.97	0.24
≥70	0.64	0.15–2.67	0.54
Sex			
Male	1 (reference)		
Female	2.13	0.74–6.13	0.16
ICU admission			
No	1 (reference)		
Yes	3.25	0.81–13.00	0.096
Depression			
No	1 (reference)		
Yes	3.07	1.04–9.04	0.042

thus could be evaluated for chronic symptoms of post-infectious IBS by the Rome IV criteria. Among them, the prevalence of IBS symptoms (abdominal pain with altered bowel habits) increased from 22/134 (16%) pre-COVID-19 to 55/134 (41%) post-COVID-19 ($p < 0.01$). Among these 134 respondents, there were 92 who answered all questions from the IBS-SSS pertaining to their current symptoms, as well as to their symptoms prior to developing COVID-19. The mean IBS-SSS score pre-COVID was 61 and increased to 248 post-COVID ($p < 0.01$). Among the 112 respondents with at least 6 months of follow-up, excluding 22 with pre-existing history or symptoms of IBS, the prevalence of post-COVID IBS symptoms was 44/112 or 39%.

4 | DISCUSSION

This study evaluated the prevalence of new gastrointestinal symptoms after diagnosis of COVID-19 using two methods: (1) during a routine primary care visit a median 3 months after hospitalization for COVID-19 and (2) using an anonymous survey of COVID-19 survivors drawn from two online support groups. In the unselected cohort of hospitalized patients, among those without previous chronic GI symptoms, the prevalence of at least one new GI symptom at follow-up was 16%. In contrast, the prevalence of new GI symptoms among self-identified COVID-19 survivors (after excluding those with pre-existing GI symptoms) was 40%. Routine primary care visits may under-estimate the true prevalence of GI symptoms due to under-documentation whereas selection bias among COVID long-haulers is likely to over-estimate the true prevalence of GI symptoms. We believe the true prevalence of persistent GI symptoms (meaning any

symptoms for one month or more) after COVID-19 is likely between 16 and 40%, but much closer to the former than the latter.

The prevalence of post-infectious IBS after acute gastroenteritis has been estimated at 10%.^{12,13} There are important caveats to recognize that prevent the direct comparison of this study's 16% prevalence of GI symptoms at a median of 3 months after COVID-19 to the 10% estimate of post-infectious IBS after acute gastroenteritis. First, patients in this study did not all have the length of follow-up time to meet the Rome IV criteria for IBS. When the definition for "persistent GI symptoms" within the unselected cohort was restricted to patients with both persistent abdominal pain and altered bowel habits for ≥ 3 months, the prevalence of abdominal pain with bowel disturbance fell to 6.2%. Second, the prevalence of GI symptoms appeared to decrease with increasing time since discharge, although this trend was not statistically significant. A subset of patients may have had lingering symptoms from COVID-19 rather than a true post-infectious IBS, and it is likely that estimates for symptom prevalence would decline if the duration of follow-up was extended.

In the second study cohort, we evaluated self-identified COVID-19 survivors via online survey of COVID-19 support groups and found a higher prevalence of ongoing GI symptoms. In this survey, we directly asked questions to determine whether patients met the Rome IV criteria for IBS, as well as questions regarding the severity of their GI symptoms using the IBS-SSS, which was not possible through a retrospective chart review (as clinicians may document diarrhea without specifically asking about the presence of abdominal pain, for example). Among 134 patients diagnosed with COVID-19 at least 6 months prior to the survey, the prevalence of symptoms consistent with IBS increased from 16% pre-COVID-19 to 41% post-COVID-19. The severity of GI symptoms as measured by the IBS Symptom Severity Score also increased significantly from a mean of 61 pre-COVID-19 to 248 post-COVID-19 (corresponding to moderate IBS symptoms). A recent study found that 21% of patients with gastroenteritis due to *Campylobacter* developed post-infectious IBS, in most cases diarrhea predominant or mixed.¹⁷ Among patients with pre-existing IBS, 38% experienced an increase in frequency of abdominal pain after *Campylobacter* infection, similar to our finding that IBS symptom severity score increased after COVID-19.

These data are intriguing, but it is unknown how well these self-identified COVID-19 survivors represent the general population. "Long hauler" patients with persistent symptoms are more likely to complete surveys evaluating such symptoms and have the potential to create a powerful selection bias. Nonetheless, given the high numbers of patients with so-called "long COVID," it remains valuable to determine the prevalence and severity of gastrointestinal symptoms in this population. Our results suggest that in addition to dyspnea, fatigue, and "brain fog" symptoms, abdominal pain and altered bowel habits are common and can be severe.^{4,18}

Our study did not uncover any surprising risk factors for new GI symptoms after COVID-19. Markers of more serious illness such as length of stay or intensive care unit admission were not associated with new GI symptoms. Among the hospitalized group, women were more likely to have abdominal pain at follow-up, and patients with a

TABLE 4 Prevalence of Post-COVID GI symptoms in an anonymous survey of survivors.

	All respondents (n = 285)	No post-COVID GI symptoms (n = 172)	Post-COVID GI symptoms* (n = 113)	p-value
Age				
18–29	48 (17%)	35 (73%)	13 (27%)	0.31
30–39	67 (24%)	41 (61%)	26 (39%)	
40–49	49 (17%)	26 (53%)	23 (47%)	
50–59	58 (21%)	31 (53%)	27 (47%)	
60–69	47 (17%)	29 (62%)	18 (38%)	
≥70	12 (4.3%)	6 (50%)	6 (50%)	
Sex				
Female	198 (71%)	114 (58%)	84 (42%)	0.25
Male	80 (29%)	52 (65%)	28 (35%)	
Race				
White	242 (86%)	141 (58%)	101 (42%)	0.36
Black	9 (3.2%)	7 (78%)	2 (22%)	
Asian	10 (3.6%)	8 (80%)	2 (20%)	
Other	21 (7.5%)	13 (62%)	8 (38%)	
Ethnicity				
Hispanic	25 (9.4%)	14 (56%)	11 (44%)	0.73
Non-Hispanic	240 (91%)	143 (60%)	97 (40%)	
Depression				
No	202 (71%)	125 (62%)	77 (38%)	0.41
Yes	83 (29%)	47 (57%)	36 (43%)	
Anxiety				
No	192 (67%)	118 (61%)	74 (39%)	0.58
Yes	93 (33%)	54 (58%)	39 (42%)	
Fibromyalgia				
No	272 (95%)	166 (61%)	106 (39%)	0.28
Yes	13 (4.6%)	6 (46%)	7 (54%)	
Diagnosis Type				
PCR	192 (67%)	111 (58%)	81 (42%)	0.57
Antibody	21 (7.4%)	15 (71%)	6 (29%)	
Symptoms	63 (22%)	40 (63%)	23 (37%)	
Unsure	9 (3.2%)	6 (67%)	3 (33%)	
GI symptoms at diagnosis				
No	85 (30%)	62 (73%)	23 (27%)	0.01
Yes	200 (70%)	110 (55%)	90 (45%)	
Hospitalized				
No	249 (88%)	150 (60%)	99 (40%)	0.87
Yes	34 (12%)	20 (59%)	14 (41%)	
Intubated				
No	279 (98%)	169 (61%)	110 (39%)	0.60
Yes	6 (2.1%)	3 (50%)	3 (50%)	
Received Antibiotics				
No	206 (72%)	133 (65%)	73 (35%)	0.02
Yes	79 (28%)	39 (49%)	40 (51%)	

Received Hydroxychloroquine

(Continues)

TABLE 4 (Continued)

	All respondents (n = 285)	No post-COVID GI symptoms (n = 172)	Post-COVID GI symptoms* (n = 113)	p-value
No	272 (95%)	168 (62%)	104 (38%)	0.03
Yes	13 (4.6%)	4 (31%)	9 (69%)	
Received Steroids				
No	233 (82%)	141 (61%)	92 (39%)	0.91
Yes	52 (18%)	31 (60%)	21 (40%)	
Time from diagnosis				
< 1 month	91 (32%)	64 (70%)	27 (30%)	0.01
1–3 months	38 (13%)	15 (39%)	23 (61%)	
3–6 months	44 (15%)	25 (57%)	19 (43%)	
>6 months	112 (39%)	68 (61%)	44 (39%)	

*Presence of at least weekly abdominal pain, associated with altered bowel habits, among all respondents without pre-existing IBS. Patients with any follow-up length of time were included in this analysis.

pre-existing history of depression were more likely to have at least one GI symptom, consistent with previous literature demonstrating an association of functional GI disorders with these groups.^{19–22} Post-COVID GI symptoms may be related to ongoing psychological factors such as post-traumatic stress disorder, disturbance of the gut microbiome, or persistent intestinal inflammation,²³ and future studies should aim to elucidate the mechanism underlying this association.

There are few studies reporting on the frequency and severity of new GI symptoms post-COVID-19 in detail. A study of 100 patients in Italy reported that 3% had “new bowel control problems” a mean of 48 days post-discharge for COVID-19.²⁴ Another study of 150 non-critically ill COVID-19 patients in France found that 30 days post-discharge, 17% reported ongoing diarrhea or vomiting, which decreased to 12% at 60 days.²⁵ A recent study of hospitalized patients in China found that 80/1,655 (5%) reported diarrhea or vomiting six months later, which was similar to our finding of 4.1% for both diarrhea and vomiting as individual symptoms among hospitalized patients.⁵ However, they did not survey patients for abdominal pain or constipation. These studies were relatively limited in their assessment of persistent GI symptoms, so it is challenging to compare their results to ours.

Some limitations should be mentioned. The retrospective cohort included only patients who were hospitalized during the early phase of the pandemic, when testing for SARS-CoV-2 was limited and often reserved for those with more severe symptoms. Therefore, it may not be generalizable to the broader population of all COVID-19 patients who typically have a milder course. As mentioned, due to the retrospective nature of the study, it was not possible to assess for the Rome IV criteria for post-infectious IBS. On the contrary, the online survey allowed for more precise questioning on symptom type, chronicity, and severity, in keeping with Rome IV. But this part of the study was limited by selection bias, as patients suffering from ongoing symptoms are more likely to be users of online support groups.

In summary, new GI symptoms were common after COVID-19. This was true in unselected patients coming for routine primary care visits and more so among self-identified COVID-19 survivors in an

online survey. Outpatient providers caring for COVID-19 survivors should be aware that GI symptoms may persist or develop after COVID-19 and the long-term GI impact of COVID-19 merits additional study.

CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

JWB, DJ, and DEF contributed to study concept and design. JWB and JL involved in acquisition of data. JWB, DJ, and DEF involved in analysis and interpretation of data. JWB and DEF involved in drafting of the manuscript. JWB, JL, DJ, and DEF involved in critical revision of the manuscript for important intellectual content.

ETHICS APPROVAL

The Columbia University Institutional Review Board approved this study.

DATA AVAILABILITY STATEMENT

Anonymized data are available from the corresponding author upon reasonable request.

ORCID

John W. Blackett  <https://orcid.org/0000-0002-8680-9489>

REFERENCES

1. COVID-19 Dashboard by the Center for Systems Science and Engineering. Johns Hopkins University. <https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6>. Published 2020. Accessed December 8.
2. Carfi A, Bernabei R, Landi F. Persistent symptoms in patients after acute COVID-19. *JAMA*. 2020;324(6):603–605.
3. Davido B, Seang S, Tubiana R, de Truchis P. Post-COVID-19 chronic symptoms: a postinfectious entity? *Clin Microbiol Infect*. 2020;26(11):1448–1449.

4. Garrigues E, Janvier P, Kherabi Y, et al. Post-discharge persistent symptoms and health-related quality of life after hospitalization for COVID-19. *J Infect*. 2020;81(6):e4-e6.
5. Huang C, Huang L, Wang Y, et al. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. *Lancet*. 2021;397(10270):220-232.
6. Redd WD, Zhou JC, Hathorn KE, et al. Prevalence and characteristics of gastrointestinal symptoms in patients with severe acute respiratory syndrome coronavirus 2 infection in the United States: a multicenter cohort study. *Gastroenterology*. 2020;159(2):765-767 e762.
7. Zhou Z, Zhao N, Shu Y, Han S, Chen B, Shu X. Effect of gastrointestinal symptoms in patients with COVID-19. *Gastroenterology*. 2020;158(8):2294-2297.
8. Nobel YR, Phipps M, Zucker J, et al. Gastrointestinal symptoms and coronavirus disease 2019: a case-control study from the United States. *Gastroenterology*. 2020;159(1):373-375 e372.
9. Pan L, Mu M, Yang P, et al. Clinical characteristics of COVID-19 patients with digestive symptoms in Hubei, China: A Descriptive, Cross-Sectional, Multicenter Study. *Am J Gastroenterol*. 2020;115(5):766-773.
10. Camilleri M, Chedid V, Ford AC, et al. Gastroparesis. *Nat Rev Dis Primers*. 2018;4(1):41.
11. Card T, Enck P, Barbara G, et al. Post-infectious IBS: defining its clinical features and prognosis using an internet-based survey. *United European Gastroenterol J*. 2018;6(8):1245-1253.
12. Klem F, Wadhwa A, Prokop LJ, et al. Prevalence, risk factors, and outcomes of irritable bowel syndrome after infectious enteritis: a systematic review and meta-analysis. *Gastroenterology*. 2017;152(5):1042-1054 e1041.
13. Halvorson HA, Schlett CD, Riddle MS. Postinfectious irritable bowel syndrome—a meta-analysis. *Am J Gastroenterol*. 2006;101(8):1894-1899. quiz 1942.
14. Palsson OS, Whitehead WE, van Tilburg MA, et al. Rome IV diagnostic questionnaires and tables for investigators and clinicians. *Gastroenterology*. 2016;150(6):1481-1491.
15. Palsson OS, Whitehead W, Tornblom H, Sperber AD, Simren M. Prevalence of Rome IV functional bowel disorders among adults in the United States, Canada, and the United Kingdom. *Gastroenterology*. 2020;158(5):1262-1273 e1263.
16. Francis CY, Morris J, Whorwell PJ. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. *Aliment Pharmacol Ther*. 1997;11(2):395-402.
17. Berumen A, Lennon R, Breen-Lyles M, et al. Characteristics and risk factors of post-infection irritable bowel syndrome after *Campylobacter* Enteritis. *Clin Gastroenterol Hepatol*. 2020;19(9):1855-1863.
18. Rubin R. As their numbers grow, COVID-19 "Long Haulers" Stump Experts. *JAMA*. 2020;324(14):1381.
19. Ballou S, Katon J, Singh P, et al. Chronic diarrhea and constipation are more common in depressed individuals. *Clin Gastroenterol Hepatol*. 2019;17(13):2696-2703.
20. Lee SP, Sung IK, Kim JH, Lee SY, Park HS, Shim CS. The effect of emotional stress and depression on the prevalence of digestive diseases. *J Neurogastroenterol Motil*. 2015;21(2):273-282.
21. Chang L, Heitkemper MM. Gender differences in irritable bowel syndrome. *Gastroenterology*. 2002;123(5):1686-1701.
22. Heitkemper MM, Jarrett ME. Update on irritable bowel syndrome and gender differences. *Nutr Clin Pract*. 2008;23(3):275-283.
23. Schmulson M, Ghoshal UC, Barbara G. Managing the inevitable surge of post-COVID-19 functional gastrointestinal disorders. *Am J Gastroenterol*. 2021;116(1):4-7.
24. Halpin SJ, Mclvor C, Whyatt G, et al. Postdischarge symptoms and rehabilitation needs in survivors of COVID-19 infection: a cross-sectional evaluation. *J Med Virol*. 2020;93(2):1013-1022.
25. Carvalho-Schneider C, Laurent E, Lemaignan A, et al. Follow-up of adults with noncritical COVID-19 two months after symptom onset. *Clin Microbiol Infect*. 2020;27(2):258-263.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Blackett JW, Li J, Jodorkovsky D, Freedberg DE. Prevalence and risk factors for gastrointestinal symptoms after recovery from COVID-19. *Neurogastroenterology & Motility*. 2021;00:e14251. <https://doi.org/10.1111/nmo.14251>



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Potential Long Coronavirus Disease 2019 Gastrointestinal Symptoms 6 Months After Coronavirus Infection Are Associated With Mental Health Symptoms

Over 200 million people globally have been infected with the severe acute respiratory syndrome coronavirus 2, also known as coronavirus disease 2019 (COVID-19).¹ Some COVID-19 survivors recover from acute infection yet suffer from persistent symptoms including fatigue, anxiety, and depression.² New, chronic gastrointestinal (GI) symptoms are also commonly reported after COVID-19.^{3–7} There is an established association between mental health symptoms and chronic GI symptoms, especially irritable bowel syndrome (IBS).^{8,9} However, the association between mental health symptoms and chronic GI symptoms after COVID-19 has not been fully explored.

The Columbia University Institutional Review Board approved this study. This survey study, nested within a prospective cohort of COVID-19 survivors with a minimum of 6 months of follow-up, assessed the hypothesis that mental health symptoms both before and after COVID-19 infection would be associated with post-COVID-19 chronic GI symptoms (see [Supplementary Methods](#) for full study details). Of 1783 patients surveyed, 749 (42%) responded (67% women; median age, 43 years). The cohort comprised 61% white, 9.6% black, and 20% other races (with 9.6% choosing not to answer) and 28% Hispanic and 70% non-Hispanic (with 2% choosing not to answer). The survey was completed in English by 93% of respondents and in Spanish by 6.8%. Fifteen percent of patients had been hospitalized for COVID-19, with 1.7% requiring mechanical ventilation.

Six months after COVID-19 diagnosis, 220 patients (29%) reported GI symptoms self-perceived to be related to COVID-19. These included 72 (9.6%) with diarrhea, 83 (11%) with constipation, 70 (9.4%) with abdominal pain, 53 (7.1%) with nausea/vomiting, and 122 (16%) with heartburn ([Table 1](#)). A GI symptom was the most bothersome current symptom in 83 patients (11%).

Thirty-nine patients (5.2%) reported pre-COVID-19 mental health symptoms (which were not further classified in the survey), and 280 (37%) reported post-COVID-19 mental health symptoms (27% with sadness, 34% with anxiety). Patients with pre-COVID-19 mental health symptoms were more likely than those without to report GI symptoms post-COVID-19 (49% vs 28%, respectively; $P = .01$) ([Table 1](#) and [Supplementary Figure 1A](#)). Similarly, patients who reported sadness or anxiety post-COVID-19 were more likely to also have GI symptoms post-COVID-19 (55% with sadness/anxiety vs 14% without, $P < .01$) ([Table 1](#) and [Supplementary Figure 1B](#)). Of the 39 patients reporting pre-COVID-19 mental health symptoms, 29 (74%) reported current anxiety or sadness and 19 (49%) reported current GI symptoms, all of whom also reported current sadness or anxiety. The likelihood of sadness or anxiety

increased with more severe GI symptoms (Cochran-Armitage test for trend $P = .02$).

On multivariable logistic regression, the presence of both pre- and post-COVID-19 mental health symptoms was significantly associated with post-COVID-19 GI symptoms (adjusted odds ratio [aOR], 16.5; 95% confidence interval [CI], 6.97–38.9), as was post-COVID-19 mental health symptoms alone (aOR, 6.16; 95% CI, 4.21–9.01) ([Supplementary Table 1](#)).

Neither black race nor Hispanic ethnicity was associated with GI symptoms after COVID-19, although preference for the Spanish language was (aOR, 2.55; 95% CI, 1.14–5.73). This discrepancy may represent subtle differences in translation or unmeasured factors—such as access to care, socioeconomic status, or strength of social networks—that fundamentally differ between Hispanics who do or do not prefer Spanish.

Of 33 patients with abdominal pain that started after COVID-19 who answered additional questions pertaining to IBS, 13 (39%) reported having pain at least weekly that was associated with a change in stool form or frequency, with onset at least 6 months prior, and thus met Rome IV criteria for IBS. Women were no more likely than men to report post-COVID-19 GI symptoms ($P = .63$) or to meet criteria for IBS ($P = .56$).

Of 110 patients hospitalized with COVID-19, 51% reported post-COVID-19 GI symptoms compared with 26% of nonhospitalized patients ($P < .01$). This association remained significant on multivariable analysis (aOR, 2.30; 95% CI, 1.38–3.84), suggesting that more severe infection may be an important risk factor for post-COVID-19 GI symptoms.

Overall, these results highlight the potential bidirectionality of mental health and chronic GI symptoms after COVID-19, as has previously been observed in non-COVID-19 studies. Anxiety or sadness may exacerbate functional GI symptoms, and functional GI symptoms may lead to anxiety or sadness.¹⁰ Alternatively, a common underlying mechanism could explain both GI and mental health symptoms. This study, which involved an assessment made at a single time point, cannot fully determine the temporality between mental health and GI symptoms after COVID-19. This may be a fruitful area for future

Abbreviations used in this article: aOR, adjusted odds ratio; COVID-19, coronavirus disease 2019; GI, gastrointestinal; IBS, irritable bowel syndrome.

Most current article

© 2022 by the AGA Institute
0016-5085/\$36.00

<https://doi.org/10.1053/j.gastro.2021.10.040>

Table 1. Prevalence, Severity, and Frequency of Specific GI Symptoms 6 Months After COVID-19 Diagnosis (N = 749)

	Diarrhea	Constipation	Abdominal pain	Nausea/vomiting	Reflux or heartburn	Any GI symptom
Mental health symptoms ^a						
None	20 (27.8)	18 (21.7)	13 (18.6)	8 (15.1)	33 (27.1)	67 (30.5)
Pre-COVID-19 only	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Post-COVID-19 only	42 (58.3)	55 (66.3)	48 (68.6)	38 (71.7)	76 (62.3)	134 (60.9)
Pre- and post-COVID-19	10 (13.9)	10 (12.1)	9 (12.9)	7 (13.2)	13 (10.7)	19 (8.6)
Overall reporting symptom	72 (9.6)	83 (11.1)	70 (9.4)	53 (7.1)	122 (16.3)	220 (29.4)
Most bothersome symptom	10 (1.3)	25 (3.3)	18 (2.4)	11 (1.5)	46 (6.1)	83 (11.1)
Severity (% reporting each severity among all patients with the symptom)						
Very mild	24 (33.3)	29 (34.9)	23 (32.9)	23 (43.4)	45 (36.9)	85 (38.6)
Mild	25 (34.7)	25 (30.1)	18 (25.7)	16 (30.2)	33 (27.1)	61 (27.7)
Moderate	13 (18.1)	8 (9.6)	15 (21.4)	4 (7.6)	22 (18.0)	32 (14.6)
Severe	8 (11.1)	12 (14.5)	7 (10.0)	7 (13.2)	12 (9.8)	25 (11.4)
Very severe	2 (2.8)	9 (10.8)	7 (10.0)	3 (5.7)	10 (8.2)	17 (7.7)
Frequency (% reporting each frequency among all patients with the symptom) ^b						
	(n = 67)	(n = 82)	(n = 67)	(n = 47)	(n = 121)	(n = 214)
Occasionally	28 (41.8)	20 (24.4)	24 (35.8)	23 (48.9)	30 (24.8)	63 (29.4)
Sometimes	23 (34.3)	30 (36.5)	27 (40.3)	16 (34.0)	40 (33.1)	72 (33.6)
Often	13 (19.4)	18 (22.0)	11 (16.4)	7 (14.9)	36 (29.8)	52 (24.3)
Always	3 (4.5)	14 (17.1)	5 (7.5)	1 (2.1)	15 (12.4)	27 (12.6)

NOTE. Values are n (%).

^aRow percentages stratified by mental health symptoms are as follows: None, 67/459 (14.6%) with GI symptoms; Pre-COVID-19 only, 0/10 (0%) with GI symptoms; Post-COVID-19 only, 134/251 (53.4%) with GI symptoms; Pre- and post-COVID-19, 19/29 (65.5%) with GI symptoms.

^bValues of n are lower because some respondents who reported a symptom did not report frequency.

studies that can use longitudinal assessment strategies to tease apart the causal chain of events and evaluate strategies to improve long-term GI outcomes after COVID-19. Although all patients with chronic GI symptoms should receive screening for comorbid mental health symptoms, this study provides specific evidence that screening COVID-19 survivors will be high yield.

The study has some limitations. The response rate was 42%, raising the possibility of response bias, but, reassuringly, the estimate of prevalence for new GI symptoms after COVID-19 (29%) fell squarely in the middle of the range of 16%–40% from a retrospective study.⁷ Although post-COVID-19 symptoms were assessed prospectively, reporting of mental health symptoms before COVID-19 was assessed retrospectively and may have been under-reported because of recall bias related to the stigma of such symptoms. Because the survey instrument from which these data were generated was focused primarily on new physical symptoms after COVID-19, there was limited detail on the characteristics of current mental health symptoms. Importantly, the association found between mental health symptoms and GI symptoms after COVID-19 is not necessarily causal. It is possible that post-COVID-19 GI symptoms may be partially due to heightened stress and anxiety arising from pandemic-related lifestyle changes rather than the direct consequence of severe

acute respiratory syndrome coronavirus 2 infection. In future studies, a control group assessing the incidence of new GI symptoms in patients who tested negative for COVID would help to clarify this question.

In summary, we observed very strong associations between mental health symptoms and post-COVID-19 GI symptoms. Nearly one-third of patients (29%) reported at least 1 new GI symptom after COVID-19. These were not trivial, because over 10% of the cohort reported a GI symptom as their most bothersome COVID-19-related condition.

Supplementary Material

Note: To access the [supplementary material](#) accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org and at <https://doi.org/10.1053/j.gastro.2021.10.040>.

JOHN W. BLACKETT

Department of Gastroenterology and Hepatology
Mayo Clinic
Rochester, Minnesota

MILTON WAINBERG

Department of Psychiatry
New York Presbyterian Columbia University Medical Center
New York, New York

MITCHELL S.V. ELKIND

Department of Neurology
New York Presbyterian Columbia University Medical Center
New York, New York

DANIEL E. FREEDBERG

Department of Medicine
Division of Digestive and Liver Diseases
New York Presbyterian Columbia University Medical Center
New York, New York

References

1. Johns Hopkins COVID-19 Dashboard 2021. <https://coronavirus.jhu.edu/map.html>.
2. Huang C, et al. *Lancet* 2021;397:220–232.
3. Nobel YR, et al. *Gastroenterology* 2020;159:373–375.
4. Halpin SJ, et al. *J Med Virol* 2021;93:1013–1022.
5. Carvalho-Schneider C, et al. *Clin Microbiol Infect* 2021;27:258–263.
6. Dong ZY, et al. *J Clin Gastroenterol* 2021;55:67–76.
7. Blackett JW, et al. *Neurogastroenterol Motil* 2021 Sep 1:e14251.

8. Zamani M, et al. *Aliment Pharmacol Ther* 2019;50:132–143.
9. Vu J, et al. *Neurogastroenterol Motil* 2014;26:1323–1332.
10. Jones MP, et al. *Clin Gastroenterol Hepatol* 2017;15:1014–1020.

Correspondence

Address correspondence to: John W. Blackett, MD, Department of Gastroenterology and Hepatology, Mayo Clinic, 200 1st Street NW, Rochester, Minnesota 10032. e-mail: blackett.john@mayo.edu.

Acknowledgment

The authors thank Sheila O'Byrne for her assistance with the Columbia University COVID-19 Biobank (CUB) data.

CRediT Authorship Contributions

John William Blackett, MD (Conceptualization: Equal; Data curation: Equal; Formal analysis: Lead; Investigation: Lead; Writing – original draft: Lead; Writing – review & editing: Equal). Milton Wainberg, MD (Conceptualization: Supporting; Investigation: Supporting; Writing – review & editing: Equal). Mitchell S. V. Elkind, MD, MS, MPhil (Conceptualization: Equal; Investigation: Supporting; Supervision: Supporting; Writing – review & editing: Equal). Daniel E. Freedberg, MD, MS (Conceptualization: Equal; Data curation: Supporting; Investigation: Supporting; Supervision: Lead; Writing – original draft: Equal; Writing – review & editing: Equal).

Conflicts of interest

The authors disclose no conflicts.

Funding

None

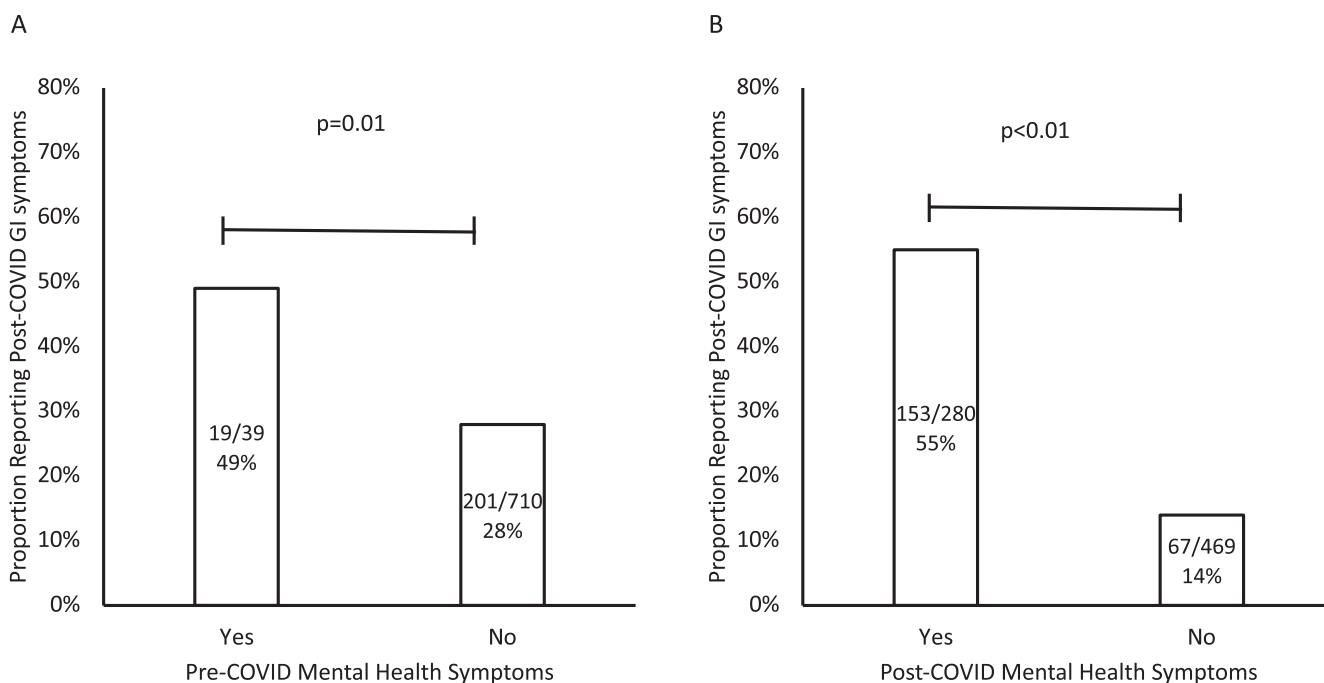
Supplementary Methods

This was a prospective, nested case-control study among 1783 patients diagnosed with COVID-19 at a single center in New York City. Patients with a positive severe acute respiratory syndrome coronavirus 2 polymerase chain reaction test at Columbia University Irving Medical Center who survived and consented to follow-up were prospectively enrolled in a COVID-19 cohort from April to November 2020.

Six months after their diagnosis, they received an electronic survey regarding ongoing self-perceived COVID-19-related symptoms and pre-COVID-19 mental health symptoms. Specifically, they were asked whether they experienced emotional, mental health, or psychosocial symptoms before they were diagnosed with COVID-19. For all current symptoms, they were asked about the presence, severity (very mild, mild, moderate, severe, or very severe), and frequency

(occasionally, sometimes, often, always) of a wide range of symptoms. Current GI symptoms queried in the survey were abdominal pain, nausea/vomiting, heartburn/reflux, diarrhea, or constipation. Current mental health symptoms were sadness and anxiety. Patients who experienced abdominal pain were also asked whether the pain occurred at least weekly, whether it was associated with a change in stool form or stool frequency, whether it started after COVID-19, and whether it had been present at least 6 months.

Demographic factors of age, sex, race, and ethnicity and clinical information related to the initial COVID-19 infection including hospitalization or mechanical ventilation requirement were also collected. The proportion of patients experiencing GI symptoms was compared among patients with and without pre- and post-COVID-19 mental health symptoms using χ^2 testing. A multivariable logistic regression model was used to identify independent predictors of GI symptoms 6 months after COVID diagnosis.



Supplementary Figure 1. Prevalence of post-COVID-19 GI symptoms (A) in patients with and without pre-COVID-19 mental health symptoms and (B) in patients with and without post-COVID-19 mental health symptoms.

Supplemental Table 1. Multivariable Logistic Regression Model of Predictors Associated With New GI Symptoms After COVID-19

Predictor	No. of cases with GI symptoms/no. at risk (%)	Adjusted odds ratio	95% confidence interval
Mental health symptoms			
Neither pre-COVID-19 nor post-COVID-19	67/459 (14.6)	1 (reference)	...
Pre-COVID-19 only ^a	0/10 (0)	Unable	NA
Post-COVID-19 only	134/251 (53.4)	6.16	4.21–9.01
Both pre-COVID-19 and post-COVID-19	19/29 (65.5)	16.5	6.97–38.9
Sex			
Male	70/248 (28.2)	1 (reference)	
Female	150/501 (29.9)	1.03	0.69–1.53
Age			
Under 30 y	26/110 (23.6)	1 (reference)	
30–39 y	48/204 (23.5)	1.36	0.73–2.55
40–59 y	101/272 (37.1)	2.29	1.27–4.12
≥60	45/163 (27.6)	1.79	0.92–3.48
Race			
White	113/454 (24.9)	1 (reference)	
Black	23/72 (31.9)	0.97	0.51–1.84
Asian/Pacific Islander	25/73 (34.3)	2.40	1.30–4.44
Native American	4/8 (50.0)	5.81	1.01–33.59
Other	29/70 (41.4)	1.23	0.59–2.56
Did not answer	26/72 (36.1)	1.45	0.68–3.09
Ethnicity			
Non-Hispanic	138/524 (26.3)	1 (reference)	
Hispanic	80/208 (38.5)	1.15	0.68–1.94
Did not answer	2/17 (11.8)	0.15	0.03–0.85
Language			
English	189/698 (27.1)	1 (reference)	
Spanish	31/51 (60.8)	2.55	1.14–5.73
Hospitalized			
No	164/639 (25.7)	1 (reference)	
Yes	56/110 (50.9)	2.30	1.38–3.84

^aThe independent association between pre-COVID-19 mental health symptoms and post-COVID-19 GI symptoms cannot be computed because 19 patients had pre-COVID-19 mental health symptoms and post-COVID-19 GI symptoms and all 19 (100%) also had post-COVID-19 mental health symptoms.



Relationship Between Body Composition and Death in Patients with COVID-19 Differs Based on the Presence of Gastrointestinal Symptoms

Yael R. Nobel¹ · Steven H. Su² · Michaela R. Anderson³ · Lyndon Luk⁴ · Jennifer L. Small-Saunders⁵ · Gissette Reyes-Soffer⁶ · Dympna Gallagher⁷ · Daniel E. Freedberg¹

Received: 10 August 2021 / Accepted: 8 November 2021

© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2021

Abstract

Background Patients with SARS-CoV-2 who present with gastrointestinal symptoms have a milder clinical course than those who do not. Risk factors for severe COVID-19 disease include increased adiposity and sarcopenia.

Aims To determine whether body composition risk factors are associated with worse outcomes among patients with gastrointestinal symptoms.

Methods This was a retrospective study of hospitalized patients with COVID-19 who underwent abdominal CT scan for clinical indications. Abdominal body composition measures including skeletal muscle index (SMI), intramuscular adipose tissue index (IMATI), visceral adipose tissue index (VATI), subcutaneous adipose tissue index (SATI), visceral-to-subcutaneous adipose tissue ratio (VAT/SAT ratio), and liver and spleen attenuation were collected. The association between body composition measurements and 30-day mortality was evaluated in patients with and without gastrointestinal symptoms at the time of positive SARS-CoV-2 test.

Results Abdominal CT scans of 190 patients with COVID-19 were evaluated. Gastrointestinal symptoms including nausea, vomiting, diarrhea, or abdominal pain were present in 117 (62%). Among patients without gastrointestinal symptoms, those who died had greater IMATI ($p=0.049$), less SMI ($p=0.010$), and a trend toward a greater VAT/SAT ratio. Among patients with gastrointestinal symptoms, those who died had significantly greater IMATI ($p=0.025$) but no differences in other measures.

Conclusions Among patients with COVID-19, those without gastrointestinal symptoms showed the expected associations between mortality and low SMI, high IMATI, and trend toward higher VAT/SAT ratio, but those with gastrointestinal symptoms did not. Future studies should explore the mechanisms for the altered disease course in patients with COVID-19 who present with gastrointestinal symptoms.

Keywords COVID-19 · Obesity · Adiposity · Gastrointestinal symptoms · Diarrhea

✉ Yael R. Nobel
yrn2102@cumc.columbia.edu

¹ Division of Digestive and Liver Diseases, Columbia University Irving Medical Center, 630 West 168th Street, 3rd Floor, New York, NY 10032, USA

² College of Physicians and Surgeons, Columbia University, New York, NY, USA

³ Division of Pulmonary and Critical Care, Columbia University Irving Medical Center, New York, NY, USA

⁴ Department of Radiology, Columbia University Irving Medical Center, New York, NY, USA

⁵ Division of Infectious Diseases, Columbia University Irving Medical Center, New York, NY, USA

⁶ Division of Endocrinology, Columbia University Irving Medical Center, New York, NY, USA

⁷ Institute of Human Nutrition, Columbia University Irving Medical Center, New York, NY, USA

Introduction

In coronavirus disease 2019 (COVID-19), obesity has emerged as a leading patient risk factor for severe clinical disease and mortality [1–4]. Among hospitalized patients with COVID-19, elevated body mass index (BMI) has been independently associated with increased risk for death or intubation [5].

BMI, which does not incorporate measures related to body fat and muscle stores, does not fully capture the increased health risks associated with adiposity. Adipose tissue distribution—specifically, increased visceral adiposity—is more closely associated with adverse clinical outcomes than BMI across multiple categories of disease, including stroke, heart disease, and cancer [6–8]. This relationship also appears to hold for visceral adiposity and outcomes related to sepsis, with a limited number of studies, suggesting that increased visceral adiposity is associated with a more severe COVID-19 clinical course [9, 10].

COVID-19 is primarily a respiratory illness, but gastrointestinal symptoms—such as nausea, vomiting, or change in bowel habits—appear in half of patients [11–13]. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which causes COVID-19, enters host cells via the ACE-2 receptor, which is densely expressed on enteric epithelial cells and in the hepatobiliary system. The presence of gastrointestinal symptoms at disease onset has been associated with different disease course and disease severity than when such symptoms are absent [14–17]. Because patients with gastrointestinal symptoms seem to have a different phenotype of COVID-19, it is possible that risk factors for disease severity may vary between patients with or without gastrointestinal symptoms.

The primary aim of this study was to evaluate the relationship between body composition measures of obesity using abdominal computed tomography (CT) scans—including skeletal muscle, intramuscular adipose tissue, visceral adipose tissue, subcutaneous adipose tissue, and hepatic steatosis—and clinical outcomes related to COVID-19, and to determine whether this relationship differed based on the presence or absence of gastrointestinal symptoms.

Methods

Patient Population

We performed a retrospective cohort study using data from the electronic medical record at New York Presbyterian-Columbia University Irving Medical Center.

Adults ≥ 18 years of age were considered for the study if they were hospitalized for COVID-19 from March 6, 2020 (the start of the COVID-19 pandemic at our institution), to May 1, 2020. Patients were included if they had a positive SARS-CoV-2 test during or within 72 h before an inpatient admission and had an abdominal CT scan performed within 30 days before or after the SARS-CoV-2 test. If patients were tested more than once, the first positive SARS-CoV-2 test was used. This study was approved by the Columbia University Medical Center Institutional Review Board.

Gastrointestinal Symptoms

The presence or absence of gastrointestinal symptoms was evaluated at the time of positive SARS-CoV-2 test based on manual review of provider documentation at the time the respective test was performed. Gastrointestinal symptoms could include nausea or vomiting, abdominal pain, or new onset diarrhea or constipation. Respiratory signs/symptoms included cough, shortness of breath, or hypoxia.

Clinical Outcomes

Pertinent clinical outcomes, including admission to an intensive care unit, death, discharge to a hospice facility, and final hospital disposition, were ascertained from the electronic medical record, which interfaces with the national social security death index. The primary outcome of interest was death or discharge to hospice within 30 days after positive SARS-CoV-2 test. Secondary outcomes were admission to ICU within 30 days and a pooled outcome of admission to ICU or death/hospice within 30 days.

Abdominal CT Scans

Patients were included in the study only if an analyzable CT scan was available within 30 days of their first positive SARS-CoV-2 test. CT scans could be performed for any indication and could be performed before or after COVID-19 was diagnosed, provided they fell within the 30-day time frame. For example, a CT scan performed when a patient presented to the emergency department for diarrhea and was diagnosed with COVID-19 and an outpatient CT scan performed to stage malignancy weeks prior to presentation for COVID-19 would both meet criteria for the study, provided both scans were analyzable and fell within the 30-day window of COVID-19 diagnosis. To evaluate indications for CT scans, provider documentation immediately prior to the scan was reviewed. CT scan indications were classified as: suspected abdominal, intestinal, or hepatobiliary pathology; as part of multi-organ evaluation of fever or sepsis, often performed in combination with CT

scan of the chest; suspected retroperitoneal hemorrhage; cancer diagnosis, staging, or surveillance; suspected genitourinary pathology such as for hematuria or suspected kidney stone; gastrointestinal bleeding; or other. If more than one abdominal CT scan was available, the scan closest to the date of SARS-CoV-2 test was used.

Body Composition Measurements

Body composition measurements for skeletal muscle (SM) tissue area, intramuscular adipose tissue (IMAT) area, visceral adipose tissue (VAT) area, and subcutaneous adipose tissue (SAT) area were taken on a single axial CT slice at the L3 vertebral level, using TomoVision sliceOmatic version 5.0 software (Quebec, Canada). All included CT scans were protocolled with standard slice thickness of 2.5–3.0 mm. The L3 vertebral level was selected due to prior evidence that this location provides the most accurate single-slice estimates of visceral adiposity [18–21]. Segmentation of muscle and adipose tissue compartments was performed according to the software specifications and as previously described, using tissue demarcation thresholds of 29 to 150 Hounsfield units (HU) for SM, –190 to –30 HU for IMAT, –150 to –50 HU for VAT, and –190 to –30 HU for SAT [22, 23]. All measures were normalized to height, to generate SM Index, IMAT Index, VAT Index, and SAT Index, respectively. VAT/SAT ratio was calculated by dividing non-normalized VAT area by SAT area.

The presence of hepatic steatosis was determined based on the measurement of liver and spleen attenuation from non-contrast CT scans as previously described [24, 25]. Liver and spleen attenuation in HU was calculated on a single axial CT slice using regions of interest (ROIs) between 80 and 100 mm² in area, with two ROIs placed in the right lobe of the liver anteriorly and one ROI placed in the spleen. Absolute liver attenuation was defined as average attenuation of both right liver lobe ROIs; lower absolute liver attenuation is consistent with increased hepatic steatosis. Liver-to-spleen attenuation ratio was calculated by dividing the absolute liver attenuation by the spleen attenuation; hepatic steatosis was defined as liver/spleen attenuation ratio less than 1.

All scans were analyzed by a single reader (SS). A random selection of 19 scans (10% of the study population) were analyzed by a second reader (YRN) and demonstrated high inter-reader reliability, with intraclass correlations above 90% for all measures (Supplementary Table 1). SAT was excluded if any of the subcutaneous tissue was outside the field of the scan. Index measures were excluded if patient height was not available in the electronic medical record.

Co-variables

Patient clinical characteristics were extracted from the electronic medical record, including age, sex, race/ethnicity, body mass index (BMI), height, and the presence of specific medical comorbidities (hypertension, diabetes mellitus, or chronic liver disease) based on ICD codes. Laboratory values—including white blood cell (WBC) count, platelet count, aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), ferritin, international normalized ratio (INR), D-dimer, lactate dehydrogenase (LDH), creatine phosphokinase (CPK), high-sensitivity troponin-T, lactate, and interleukin-6 (IL-6)—were extracted, and peak value for each laboratory measure in the time frame between 3 days prior to 14 days after SARS-CoV-2 test was included in the study.

Statistical Analyses

Continuous variables were summarized using medians, and categorical variables were summarized using proportions. Continuous variables were compared using rank-sum tests, and categorical variables were compared using Fisher's exact tests. For validation of CT scan measurements, intraclass correlations were calculated using a two-way random effects model. Statistical calculations were performed using STATA version 16 (College Station, TX).

Results

Study Population

A total of 190 inpatients with COVID-19 were included in the study. Of these, 117 (62%) presented with gastrointestinal symptoms at the time of positive SARS-CoV-2 test (Supplementary Table 2). The median time between abdominal CT scan and SARS-CoV-2 test was zero days among patients with gastrointestinal symptoms (i.e., scan was performed on the same day as the test) and 7 days in patients without gastrointestinal symptoms. The most common indications for CT scan were suspicion for intestinal, hepatobiliary, or abdominal compartment pathology, as part of a multi-organ evaluation of fever/sepsis, or evaluation for retroperitoneal hemorrhage (Supplementary Table 3).

Gastrointestinal Symptoms

Clinical characteristics of study participants based on the presence or absence of gastrointestinal symptoms at presentation are shown in Table 1. There were no differences in age, sex, race/ethnicity, body mass index (BMI), or presence

Table 1 Clinical characteristics among 190 patients hospitalized for COVID-19 based on the presence of gastrointestinal (GI) symptoms

	GI symptoms (n = 117)	No GI symptoms (n = 73)	P value
Age, median (IQR)	66 (52–76)	64 (51–74)	0.599
Sex, n (%)			0.918
Female	52 (44.4)	33 (45.2)	
Male	65 (55.6)	40 (54.8)	
Race/ethnicity, n (%)			0.281
White	13 (11.1)	15 (20.5)	
Black non-Hispanic	22 (18.8)	10 (13.7)	
Hispanic	60 (51.3)	33 (45.2)	
Other	22 (18.8)	15 (20.5)	
BMI, median (IQR)	26.1 (22.7–30.1)	26.4 (22.6–30.4)	0.930
BMI category, n (%)			0.517
Underweight (BMI < 18.5)	4 (3.4)	2 (2.7)	
Normal weight (BMI 18.5–24.9)	43 (36.8)	26 (35.6)	
Overweight (BMI 25–29.9)	36 (30.8)	20 (27.4)	
Class I obesity (BMI 30–34.9)	18 (15.4)	13 (17.8)	
Class II obesity (BMI 35–39.9)	5 (4.3)	8 (11.0)	
Class III obesity (BMI ≥ 40.0)	11 (9.4)	4 (5.5)	
Medical comorbidities, n (%)			
Hypertension	78 (66.7)	43 (58.9)	0.279
Diabetes mellitus	55 (47.0)	24 (32.9)	0.055
Chronic liver disease	9 (7.7)	10 (13.7)	0.398
Respiratory symptoms, n (%)	71 (60.7)	52 (71.2)	0.139

of respiratory symptoms between the two groups. While there were no statistically significant differences in the presence of major medical comorbidities in the two groups, there was a trend toward higher prevalence of diabetes mellitus among patients with gastrointestinal symptoms (47% versus 33% of patients without gastrointestinal symptoms; $p = 0.06$).

Clinical Outcomes

Of the 190 study patients, 46 (24%) were admitted to the ICU and 31 (16%) died or were discharged to hospice within 30 days after first positive SARS-CoV-2 test. Patients with gastrointestinal symptoms were significantly less likely to be admitted to the ICU, but rate of death or discharge to hospice within 30 days did not differ between those with versus without gastrointestinal symptoms (Fig. 1).

Body Composition Measures

Body composition parameters and metrics of hepatic steatosis were measured from abdominal CT scans (Fig. 2). SAT was excluded in 30 patients because the tissue area exceeded the boundaries of the scan, and SM and IMAT were excluded for one patient in whom segmentation of these compartments was not possible due to poor image quality (Supplementary Fig. 1). There were no significant

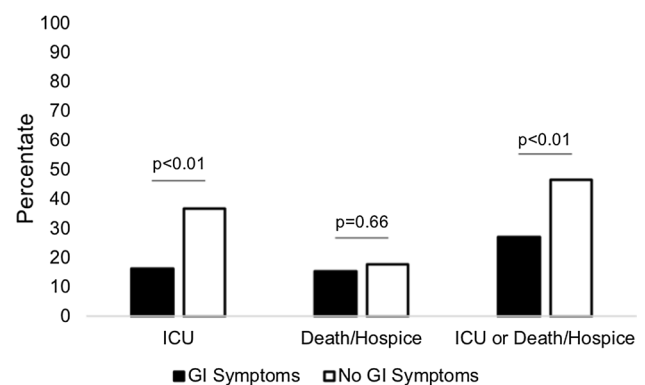


Fig. 1 Clinical outcomes based on the presence or absence of gastrointestinal symptoms

differences in skeletal muscle index (SMI), intramuscular adipose tissue index (IMATI), visceral adipose tissue index (VATI), subcutaneous adipose tissue index (SATI), or VAT/SAT ratio between those with and without gastrointestinal symptoms. Although absolute liver attenuation did not differ significantly between those with and without gastrointestinal symptoms, hepatic steatosis based on liver-to-spleen attenuation ratio was present in significantly fewer patients with gastrointestinal symptoms compared to patients without (23% versus 38% of patients, respectively; $p = 0.04$).

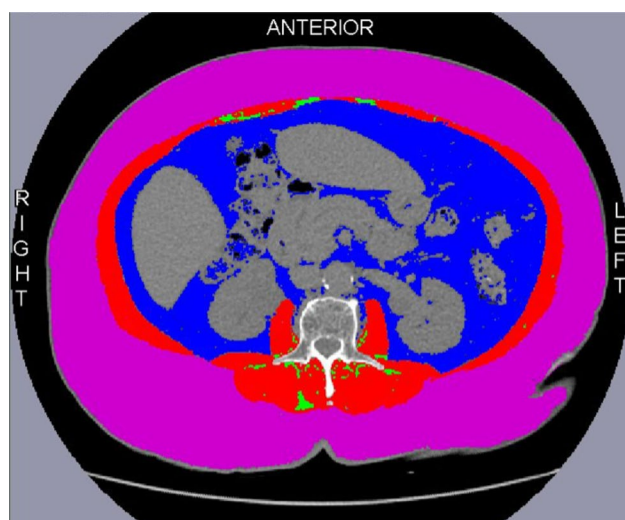


Fig. 2 Representative abdominal CT scan with segmentation of skeletal muscle, intramuscular adipose, visceral adipose, and subcutaneous adipose tissue compartments. Red: skeletal muscle. Green: intramuscular adipose tissue. Blue: visceral adipose tissue. Pink: subcutaneous adipose tissue

Relationship Between Body Composition and Clinical Outcomes Based on the Presence of Gastrointestinal Symptoms

Body composition measures in patients who did or did not meet the primary clinical outcome of death or discharge to hospice within 30 days are shown in Table 2. Among all patients either with or without gastrointestinal symptoms, those who died had a significantly higher IMATI compared to those who did not die. Only among patients without gastrointestinal symptoms was SMI associated with mortality: SMI was 42.7 (IQR 35.0–50.4) among those who died compared to 32.3 (IQR 30.1–41.5) among those who did not die (Table 2). Among patients without gastrointestinal symptoms, there was a nonsignificant trend toward elevated VAT/SAT ratio in those who died, which was not seen in patients with gastrointestinal symptoms. No other measures differed with respect to clinical outcomes comparing those with versus those without gastrointestinal symptoms.

Table 2 Body composition measurements among 117 patients with GI symptoms and 73 patients with no GI symptoms based on death/hospice at 30 days

	Overall (n = 190)	Death/hospice at 30 days (n = 31)	No death/hospice at 30 days (n = 159)	P value
<i>Body composition, median (IQR)</i>				
SMI				
GI symptoms	44.9 (35.8)	42.7 (35.0–50.4)	45.5 (35.9–57.7)	0.394
No GI symptoms	43.8 (31.5–57.0)	32.3 (30.1–41.5)	46.0 (34.5–59.4)	0.010
IMATI				
GI symptoms	2.26 (1.28–4.47)	3.55 (2.16–5.78)	2.08 (1.20–4.13)	0.025
No GI symptoms	2.65 (1.72–4.28)	3.72 (2.65–4.77)	2.43 (1.61–4.28)	0.049
VATI				
GI symptoms	53.9 (35.1–79.1)	50.7 (26.2–83.3)	54.7 (35.4–75.8)	0.754
No GI symptoms	50.1 (27.4–73.5)	39.8 (22.0–54.9)	50.8 (31.0–78.2)	0.221
SATI				
GI symptoms	69.5 (43.2–88.7)	58.1 (44.7–82.1)	71.6 (41.6–90.3)	0.529
No GI symptoms	68.0 (49.9–89.8)	68.6 (28.6–81.9)	68.0 (51.7–92.4)	0.276
VAT/SAT ratio				
GI symptoms	0.73 (0.43–1.20)	0.72 (0.48–1.77)	0.75 (0.43–1.14)	0.498
No GI symptoms	0.65 (0.41–0.94)	0.80 (0.41–1.06)	0.64 (0.41–0.83)	0.419
<i>Hepatic steatosis</i>				
Liver/spleen attenuation ratio < 1, no. (%)				
GI symptoms	27 (23.1)	5 (45.5)	22 (64.7)	0.257
No GI symptoms	17 (37.8)	3 (37.5)	14 (37.8)	0.986
Liver attenuation (HU), median (IQR)				
GI symptoms	56.4 (47.6–64.0)	60.5 (54.2–62.9)	54.6 (45.9–68.8)	0.649
No GI symptoms	57.2 (53.2–64.3)	56.5 (48.8–62.8)	60.1 (53.2–64.3)	0.694

Statistically significant values are given in bold

Index measures (SMI, IMATI, VATI, and SATI) were normalized to patient height

Inflammatory Markers

Associations between body composition and serologic measurements, which provide additional information about disease severity, were evaluated in patients with and without gastrointestinal symptoms. Patients with gastrointestinal symptoms had significantly lower peak levels of WBC, ESR, transaminases (AST and ALT), D-dimer, LDH, and high-sensitivity troponin-T compared to patients without gastrointestinal symptoms (Table 3).

Discussion

Obesity—typically measured by BMI—is a risk factor for severe disease and poor clinical outcomes in patients with COVID-19 [1, 2, 4, 26]. Prior studies suggest that patients with prominent gastrointestinal symptoms at the time of COVID-19 diagnosis have better outcomes compared to patients who present with isolated respiratory symptoms [14–17]. The intersection of obesity and gastrointestinal symptoms in COVID-19 was the focus of this retrospective cohort study of 190 patients with COVID-19 who underwent abdominal CT scan for clinical indications. The study findings support the conclusion that gastrointestinal symptoms at the time of COVID-19 diagnosis mark a fundamentally different and milder clinical course, with lower rates of ICU admission and less aberrant laboratory values seen in patients with gastrointestinal symptoms. Less clear is the relationship between standard measures of body composition and adverse outcomes in COVID-19. High intramuscular adipose tissue index (IMATI, a measure of the fat content

within muscle) was associated with death among all patients, with and without gastrointestinal symptoms. On the other hand, low skeletal muscle index (SMI, a measure of skeletal muscle mass) was only associated with death among those without gastrointestinal symptoms. Surprisingly, other common measures of body composition—including the visceral adipose tissue index (VATI, representing the amount of visceral fat), which has been linked with systemic inflammation—were not associated with death. This remained true even when the VATI was corrected for subcutaneous adipose tissue (VAT/SAT ratio) and was true regardless of the presence or absence of gastrointestinal symptoms.

A limited number of prior studies have evaluated cross-sectional adiposity in patients with COVID-19, and several have identified an association between increased VAT or VAT/SAT ratio and ICU admission or need for mechanical ventilation [27–33]. Only one of these investigated IMATI and found a relationship between IMATI and COVID-19 outcomes. Low muscle mass and intramuscular adiposity are associated with poor clinical outcomes in other diseases. For example, high IMAT content, low SM mass, and high VAT/SAT ratio are associated with mortality following liver transplantation and with risk of death or delisting prior to lung transplantation [34, 35]. Among patients with liver disease, reduced SM mass is associated with increased severity of liver injury, increased rate of complications such as infections or encephalopathy, and increased mortality [36–38]. In the context of sepsis and critical illness, higher IMAT content and lower SM mass are associated with increased ICU mortality and overall 30-day mortality [39]. Our results are consistent with these findings related to muscle mass/adiposity, but differ with respect to visceral fat.

Table 3 Two-week peak laboratory values based on the presence or absence of gastrointestinal symptoms

14-day peak value, median (IQR)*	GI symptoms (n = 117)	No GI symptoms (n = 73)	P value
WBC (n = 186)	10.1 (7.1–15.8)	13.8 (8.9–24.8)	< 0.01
Platelets (n = 186)	277 (209–418)	325 (228–453)	0.31
AST (n = 184)	55 (28–85)	83.5 (39–166)	< 0.01
ALT (n = 184)	35 (24–77)	53 (31–108)	0.01
Total bilirubin (n = 184)	0.6 (0.4–1.0)	0.7 (0.5–1.3)	0.26
ESR (n = 166)	77 (48–112)	95 (60–130)	0.04
CRP (n = 173)	127.1 (62.9–245.5)	160.8 (47.2–297.3)	0.19
Ferritin (n = 169)	812 (329–1677)	998 (383–2068)	0.42
INR (n = 172)	1.2 (1.1–1.4)	1.3 (1.2–1.6)	0.05
D-Dimer (n = 150)	2.7 (1.2–5.6)	3.0 (2.1–13.1)	0.04
LDH (n = 169)	378 (267–540)	468 (353–816)	< 0.01
CPK (n = 149)	142 (65–387)	191 (74–578)	0.23
High-sensitivity troponin-T (n = 165)	24 (12–70)	53 (20–156)	< 0.01
Lactate (n = 127)	2.4 (1.8–3.5)	2.9 (2.2–3.9)	0.08
IL-6 (n = 136)	38 (8–90)	58 (12–158)	0.30

Statistically significant values are given in bold

*Where values are missing, corrected sample size shown within each cell

The finding that VATI, a more accurate measure of obesity than BMI, was not associated with death in patients with COVID-19 is surprising. First, it is possible that the association between BMI and poor clinical outcomes in COVID-19 is unrelated to adipose tissue distribution; while an association between VAT and disease severity has been identified in prior studies, these studies used small sample sizes and varying metrics of adiposity quantification. Second, the gut microbiome is altered among patients with COVID-19, and the presence of gastrointestinal symptoms may reflect intestinal differences in SARS-CoV-2 viral load within the bowel [40–44]. If this is the case, the relationship between sarcopenia (muscle mass and intramuscular adiposity) and clinical outcomes in COVID-19 may be related to a microbiome-mediated “gut-muscle axis” [45]. Skeletal muscle wasting is mediated in part by inflammatory cytokines, including tumor necrosis factor (TNF)- α and nuclear factor (NF)- κ B [46]. In our study, subjects with gastrointestinal symptoms had lower serologic inflammatory markers and did not show the expected association between SM index and mortality. It is possible that the physiology represented by the presence of gastrointestinal symptoms involves a reduced inflammatory response to COVID-19. Finally, it must be recognized that patients who underwent abdominal CT scans during the height of the COVID-19 pandemic—when access to imaging, even in the hospital, was highly limited—reflect a unique patient population.

Our study has limitations. While the majority of abdominal CT scans in our study were performed at the time of initial COVID-19 diagnosis, some scans were performed earlier or later within the included timeframe, and it is possible that body composition measures differed based on timing of CT scan during the disease course. SAT measurements were excluded if the patient’s SAT extended past the scan window, so we were unable to evaluate SATI and VAT/SAT ratio in patients with the highest SAT. Finally, it is possible that patients who underwent abdominal CT scans were dissimilar from patients in the general population of hospitalized patients with COVID-19, limiting generalizability of our findings. There are also study strengths. To our knowledge, ours is the largest study to date of body composition measures in patients with COVID-19. Body composition measurements were taken according to established, validated methods, and inter-reader consistency was confirmed, demonstrating reliability. These measures provide more accurate, specific information about body composition than BMI and may be less variable over the course of illness than measured weights.

Our findings are consistent with prior reports that patients with COVID-19 and gastrointestinal symptoms have improved outcomes compared to patients without gastrointestinal symptoms; whether the effect of adiposity differs based on gastrointestinal symptoms, and how this may

impact outcomes, was less clear. Specifically, patients without gastrointestinal symptoms showed the expected associations between mortality and low SMI, high IMATI, and trend toward high VAT/SAT ratio, but this pattern was not observed in patients with gastrointestinal symptoms. Future studies are needed to elucidate the mechanisms underlying the altered disease course in patients with COVID-19 who present with gastrointestinal symptoms.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10620-021-07324-4>.

Funding YRN: NIH T32 DK083256-12. MRA: NIH K23 HL150280. JLS: Doris Duke Physician Scientist Fellowship and Columbia Integrated Program in Infectious Diseases Research (NIH T32 AI100852).

Declarations

Conflict of interest All authors declare that they have no conflict of interest.

References

1. Simonnet A, Chetboun M, Poissy J et al. High prevalence of obesity in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) requiring invasive mechanical ventilation. *Obesity (Silver Spring)*. 2020;28:1195–1199.
2. Lighter J, Phillips M, Hochman S et al. Obesity in patients younger than 60 years is a risk factor for Covid-19 hospital admission. *Clin Infect Dis*. 2020;71:896–897.
3. Goyal P, Choi JJ, Pinheiro LC et al. Clinical characteristics of Covid-19 in New York City. *N Engl J Med*. 2020;382:2372–2374.
4. Cai Q, Chen F, Wang T et al. Obesity and COVID-19 severity in a designated hospital in Shenzhen, China. *Diabetes Care*. 2020;43:1392–1398.
5. Anderson MR, Geleris J, Anderson DR et al. Body mass index and risk of intubation or death in SARS-CoV-2 infection: a retrospective cohort study. *Ann Intern Med*. 2020;173:782–790.
6. Kaess BM, Pedley A, Massaro JM, Murabito J, Hoffmann U, Fox CS. The ratio of visceral to subcutaneous fat, a metric of body fat distribution, is a unique correlate of cardiometabolic risk. *Diabetologia* 2012;55:2622–2630.
7. Fox CS, Massaro JM, Hoffmann U et al. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. *Circulation*. 2007;116:39–48.
8. Kim JH, Choi KH, Kang KW et al. Impact of visceral adipose tissue on clinical outcomes after acute ischemic stroke. *Stroke*. 2019;50:448–454.
9. Lee JGH, Genga KR, Pisitsak C et al. Survival benefit of a low ratio of visceral to subcutaneous adipose tissue depends on LDL clearance versus production in sepsis. *Crit Care*. 2018;22:58.
10. Foldi M, Farkas N, Kiss S et al. Visceral adiposity elevates the risk of critical condition in COVID-19: a systematic review and meta-analysis. *Obesity (Silver Spring)*. 2021;29:521–528.
11. Nobel YR, Phipps M, Zucker J et al. Gastrointestinal symptoms and coronavirus disease 2019: a case-control study from the United States. *Gastroenterology*. 2020;159:373–375.

12. Sultan S, Altayar O, Siddique SM et al. AGA institute rapid review of the gastrointestinal and liver manifestations of COVID-19, meta-analysis of international data, and recommendations for the consultative management of patients with COVID-19. *Gastroenterology*. 2020;159:320–334.
13. Cholanteril G, Podboy A, Aivaliotis VI et al. High prevalence of concurrent gastrointestinal manifestations in patients with severe acute respiratory syndrome coronavirus 2: early experience from California. *Gastroenterology*. 2020;159:775–777.
14. Dong ZY, Xiang BJ, Jiang M, Sun MJ, Dai C. The prevalence of gastrointestinal symptoms, abnormal liver function, digestive system disease and liver disease in COVID-19 infection: a systematic review and meta-analysis. *J Clin Gastroenterol*. 2021;55:67–76.
15. Redd WD, Zhou JC, Hathorn KE et al. Prevalence and characteristics of gastrointestinal symptoms in patients with severe acute respiratory syndrome coronavirus 2 infection in the United States: a multicenter cohort study. *Gastroenterology*. 2020;159:765–767.
16. Aziz M, Haghbin H, Lee-Smith W, Goyal H, Nawras A, Adler DG. Gastrointestinal predictors of severe COVID-19: systematic review and meta-analysis. *Ann Gastroenterol*. 2020;33:615–630.
17. Livanos AE, Jha D, Cossarini F et al. Intestinal host response to SARS-CoV-2 infection and COVID-19 outcomes in patients with gastrointestinal symptoms. *Gastroenterology*. 2021;160:2435–2450.
18. Irlbeck T, Massaro JM, Bamberg F, O'Donnell CJ, Hoffmann U, Fox CS. Association between single-slice measurements of visceral and abdominal subcutaneous adipose tissue with volumetric measurements: the Framingham Heart Study. *Int J Obes (Lond)*. 2010;34:781–787.
19. Zopfs D, Theurich S, Grosse Hokamp N et al. Single-slice CT measurements allow for accurate assessment of sarcopenia and body composition. *Eur Radiol*. 2020;30:1701–1708.
20. Demerath EW, Shen W, Lee M et al. Approximation of total visceral adipose tissue with a single magnetic resonance image. *Am J Clin Nutr*. 2007;85:362–368.
21. Shen W, Punyanitya M, Chen J et al. Visceral adipose tissue: relationships between single slice areas at different locations and obesity-related health risks. *Int J Obes (Lond)*. 2007;31:763–769.
22. Aubrey J, Esfandiari N, Baracos VE et al. Measurement of skeletal muscle radiation attenuation and basis of its biological variation. *Acta Physiol (Oxf)*. 2014;210:489–497.
23. Brown JC, Caan BJ, Prado CM et al. Body composition and cardiovascular events in patients with colorectal cancer: a population-based retrospective cohort study. *JAMA Oncol*. 2019;5:967–972.
24. Zeb I, Li D, Nasir K, Katz R, Larijani VN, Budoff MJ. Computed tomography scans in the evaluation of fatty liver disease in a population based study: the multi-ethnic study of atherosclerosis. *Acad Radiol*. 2012;19:811–818.
25. Torgersen J, So-Armah K, Freiberg MS et al. Comparison of the prevalence, severity, and risk factors for hepatic steatosis in HIV-infected and uninfected people. *BMC Gastroenterol*. 2019;19:52.
26. Anderson MR, Geleris J, Anderson DR et al. Body mass index and risk for intubation or death in SARS-CoV-2 infection: a retrospective cohort study. *Ann Intern Med*. 2020;173:782–790.
27. Battisti S, Pedone C, Napoli N et al. Computed tomography highlights increased visceral adiposity associated with critical illness in COVID-19. *Diabetes Care*. 2020;43:e129–e130.
28. Deng M, Qi Y, Deng L et al. Obesity as a potential predictor of disease severity in young COVID-19 patients: a retrospective study. *Obesity (Silver Spring)*. 2020;28:1815–1825.
29. Petersen A, Bressen K, Albrecht J et al. The role of visceral adiposity in the severity of COVID-19: Highlights from a uni-center cross-sectional pilot study in Germany. *Metabolism*. 2020;110:154317.
30. Watanabe M, Caruso D, Tuccinardi D et al. Visceral fat shows the strongest association with the need of intensive care in patients with COVID-19. *Metabolism*. 2020;111:154319.
31. Yang Y, Ding L, Zou X et al. Visceral adiposity and high intramuscular fat deposition independently predict critical illness in patients with SARS-CoV-2. *Obesity (Silver Spring)*. 2020;28:2040–2048.
32. Pediconi F, Rizzo V, Schiaffino S et al. Visceral adipose tissue area predicts intensive care unit admission in COVID-19 patients. *Obes Res Clin Pract*. 2021;15:89–92.
33. Favre G, Legueult K, Pradier C et al. Visceral fat is associated to the severity of COVID-19. *Metabolism*. 2021;115:154440.
34. Hamaguchi Y, Kaido T, Okumura S et al. Impact of skeletal muscle mass index, intramuscular adipose tissue content, and visceral to subcutaneous adipose tissue area ratio on early mortality of living donor liver transplantation. *Transplantation*. 2017;101:565–574.
35. Anderson MR, Easthausen I, Gallagher G et al. Skeletal muscle adiposity and outcomes in candidates for lung transplantation: a lung transplant body composition cohort study. *Thorax*. 2020;75:801–804.
36. Al-Azzawi Y, Albo B, Fasullo M et al. Sarcopenia is associated with longer hospital stay and multiorgan dysfunction in alcoholic hepatitis. *Eur J Gastroenterol Hepatol*. 2020;32:733–738.
37. Hara N, Iwasa M, Sugimoto R et al. Sarcopenia and sarcopenic obesity are prognostic factors for overall survival in patients with cirrhosis. *Intern Med*. 2016;55:863–870.
38. Hsu CS, Kao JH. Sarcopenia and chronic liver diseases. *Expert Rev Gastroenterol Hepatol*. 2018;12:1229–1244.
39. Loosen SH, Schulze-Hagen M, Pungel T et al. Skeletal muscle composition predicts outcome in critically ill patients. *Crit Care Explor*. 2020;2:e0171.
40. Cheung KS, Hung IFN, Chan PPY et al. Gastrointestinal manifestations of SARS-CoV-2 infection and virus load in fecal samples from a Hong Kong cohort: systematic review and meta-analysis. *Gastroenterology*. 2020;159:81–95.
41. Chen Y, Chen L, Deng Q et al. The presence of SARS-CoV-2 RNA in the feces of COVID-19 patients. *J Med Virol*. 2020;92:833–840.
42. Guo M, Tao W, Flavell RA, Zhu S. Potential intestinal infection and faecal-oral transmission of SARS-CoV-2. *Nat Rev Gastroenterol Hepatol*. 2021;18:269–283.
43. Zuo T, Zhang F, Lui GCY et al. Alterations in gut microbiota of patients with COVID-19 during time of hospitalization. *Gastroenterology*. 2020;159:944–955.
44. Zuo T, Liu Q, Zhang F et al. Depicting SARS-CoV-2 faecal viral activity in association with gut microbiota composition in patients with COVID-19. *Gut*. 2021;70:276–284.
45. Ticinesi A, Mancabelli L, Tagliaferri S et al. The gut-muscle axis in older subjects with low muscle mass and performance: a proof of concept study exploring fecal microbiota composition and function with shotgun metagenomics sequencing. *Int J Mol Sci*. 2020;21:8946.
46. Thoma A, Lightfoot AP. NF- κ B and inflammatory cytokine signalling: role in skeletal muscle atrophy. *Adv Exp Med Biol*. 2018;1088:267–279.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Lack of Effect of Gluten Challenge on Fecal Microbiome in Patients With Celiac Disease and Non-Celiac Gluten Sensitivity

Yael R. Nobel, MD¹, Felix Rozenberg, BA², Heekuk Park, PhD², Daniel E. Freedberg, MD, MS¹, Martin J. Blaser, MD³, Peter H.R. Green, MD^{1,4}, Anne-Catrin Uhlemann, MD, PhD^{2,5} and Benjamin Lebwohl, MD, MS^{1,4}

INTRODUCTION: Celiac disease (CD) may be associated with gut microbial dysbiosis. Whether discrete gluten exposure in subjects with well-controlled disease on a gluten-free diet impacts the gut microbiome is unknown and may have implications for understanding disease activity and symptoms. We conducted a prospective study to evaluate the impact of gluten exposure on the gut microbiome in patients with CD and nonceliac gluten sensitivity (NCGS).

METHODS: Subjects with CD (n = 9) and NCGS (n = 8) previously on a gluten-free diet were administered a 14-day gluten challenge (5 g of gluten per day) and compared with controls (n = 8) on a usual gluten-containing diet. Stool was collected for fecal microbiome analysis using 16S rRNA gene and metagenomic sequencing before, during, and after the gluten challenge. Symptoms were assessed using 2 validated clinical scales.

RESULTS: Among subjects with CD and NCGS, there were no significant fecal microbial changes in response to gluten challenge. Gut microbiome composition differed among controls, subjects with CD, and subjects with NCGS at baseline, and these differences persisted despite gluten exposure. Gastrointestinal and general health symptoms reported by subjects with CD and NCGS were worst in the middle of gluten challenge and lessened by its end, with no consistent associations with gut microbiome composition.

DISCUSSION: Pre-existing fecal microbiome diversity was unaffected by gluten challenge in adult subjects with CD and NCGS. These findings suggest that current microbiome status is unrelated to current disease activity and disease severity.

SUPPLEMENTARY MATERIAL accompanies this paper at <http://links.lww.com/CTG/A734>.

Clinical and Translational Gastroenterology 2021;12:e00441. <https://doi.org/10.14309/ctg.0000000000000441>

INTRODUCTION

Celiac disease (CD) is a multisystem autoimmune disease triggered by ingestion of gluten in genetically susceptible individuals and is treated by avoidance of dietary gluten (1). Another condition, nonceliac gluten sensitivity (NCGS), describes patients who develop symptoms—such as diarrhea, abdominal pain, or nausea—in response to gluten intake, but do not carry serologic or histologic markers of CD (2). In patients with CD and NCGS observing a gluten-free diet, there is wide variation in symptoms and, in CD, degree of biomarker recurrence that develops in response to gluten exposure, ranging from negligible to severe.

Investigations of CD have posited a role of the gut microbiome in multiple aspects of disease development and progression (3–5). Certain taxa, including *Bifidobacterium* and Clostridial species such as *Faecalibacterium prausnitzii*, have decreased abundance in patients with active CD or treated CD compared with controls (6,7). However, not all studies have shown consistent trends, and some of the dysbiosis observed in patients with CD may be attributable to gluten-free diet rather than to the underlying disease (8,9).

In this study, we investigated the impact of gluten exposure on gut microbiome composition among a clinic-based cohort of patients with CD and NCGS compared with controls. We

¹Celiac Disease Center, Columbia University Irving Medical Center, New York, New York, USA; ²Microbiome and Pathogen Genomics Collaborative Center, Columbia University Irving Medical Center, New York, New York, USA; ³Center for Advanced Biotechnology and Medicine, Rutgers University, New Brunswick, New Jersey, USA; ⁴Department of Epidemiology, Mailman School of Public Health, Columbia University Irving Medical Center, New York, New York, USA; ⁵Division of Infectious Diseases, Department of Medicine, Columbia University Irving Medical Center, New York, New York, USA.

Correspondence: Benjamin Lebwohl, MD, MS. E-mail: BL114@columbia.edu.

Received October 11, 2021; accepted October 24, 2021; published online December 20, 2021

© 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of The American College of Gastroenterology

hypothesized that a 14-day gluten challenge would alter gut microbiome composition in subjects with CD and NCGS and that symptom severity in response to gluten challenge would be associated with microbiome composition.

METHODS

We performed a prospective study in which patients with CD and NCGS maintaining a long-term gluten-free diet were given a 14-day gluten challenge in parallel with controls, who remained on a nonrestrictive, gluten-containing diet (Figure 1). Stool sample collection for gut microbiome analysis and symptom assessments were performed before, during, and after the gluten challenge or corresponding period for the controls. We characterized baseline differences, stability over time, and response to gluten challenge of the gut microbiome and correlation of the gut microbiome with clinical symptoms in patients with CD and NCGS.

Study design and subject recruitment

Adult subjects 18 years and older were included in the study. All subjects with CD ($n = 9$) and subjects with NCGS ($n = 8$) were recruited by their physicians at the Celiac Disease Center at Columbia University, New York, New York, and control subjects ($n = 8$) were recruited through fliers posted at Columbia University Irving Medical Center. Four other study participants with CD were enrolled but did not complete the study because of severe symptoms at the outset of gluten challenge ($n = 2$) or loss to follow-up ($n = 2$). CD diagnosis was based on intestinal biopsy performed as standard care before study screening. NCGS was defined by the presence of gastrointestinal and/or extraintestinal symptoms that improved on cessation of gluten ingestion, with negative genetic, serologic, or duodenal histologic testing for celiac disease while on a gluten-containing diet. Controls had no clinical history of gluten intolerance or intestinal disease. Exclusion criteria included a personal history of inflammatory bowel disease, hospitalization in the 2 months before study enrollment, history of severe symptomatic response to gluten exposure, or use of antibiotics, corticosteroids, or probiotics (at least once per week) in the 3 months before study enrollment. Baseline clinical characteristics were compared among the 3 study groups using ANOVA for continuous variables and the χ^2 test or Fisher exact test for categorical variables.

The study duration was 6 weeks. This was composed of 3 phases for subjects in the CD and NCGS groups: a 2-week lead-in period of usual (gluten-free) diet; a 2-week period of gluten challenge, during which subjects consumed 5 g of gluten (2 slices of whole wheat bread, with gluten content confirmed through R5 ELISA by Bia Diagnostics [Colchester, VT], an accredited food and nutraceutical testing laboratory) per day; and a 2-week follow-up period of usual (gluten-free) diet. Controls continued their usual (gluten-containing) diets for the duration of the study. Clinical symptom questionnaires and stool samples were collected at 5 timepoints before, during, and after the gluten challenge.

This study was approved by the Columbia University Irving Medical Center Institutional Review Board.

Symptom assessments and symptom group assignments

The Gastrointestinal Symptom Rating Scale (GSRS; AstraZeneca, East Hartford, CT) is a validated clinical rating scale for describing patient-reported gastrointestinal symptoms across 5 symptom dimensions, with higher score indicating more severe

symptoms. The RAND 36-Item Health Survey 1.0 short-form survey (SF-36; RAND Corporation, Santa Monica, CA) is a validated questionnaire assessing patient-reported outcomes in 8 health domains, with lower score indicating more severe symptoms (10). At timepoints 2, 3, and 4, the total number of symptom dimensions (GSRS) or health domains (SF-36) indicating worsening, improving, or unchanged severity compared with timepoint 1 was assessed, with each of the symptom complexes weighted equally. Subjects were considered to have high (more severe) overall symptoms for a given timepoint if the number of worsened symptom complexes exceeded the number of symptom complexes that improved and/or were unchanged compared with baseline timepoint 1 and considered to have low overall symptoms otherwise.

Because diarrhea is a prominent symptom in clinically active CD and NCGS, the diarrhea component of the GSRS was also independently examined. For each subject, the diarrhea score at timepoint 2, 3, or 4 was compared with timepoint 1. Subjects were considered to have high (more severe) diarrhea symptoms for a given timepoint if the diarrhea score increased compared with baseline timepoint 1 and to have low diarrhea symptoms if the diarrhea score decreased or stayed the same.

Symptom severity within each study group at timepoints 2, 3, and 4 was compared with timepoint 1 using t tests with P value adjusted for multiple comparisons.

Stool sample collection

Study subjects collected stool specimens at each of the pre-specified timepoints. Stool was submitted within 24 hours of collection and stored as unprocessed aliquots at -80°C .

16S rRNA sequencing and analysis

DNA was extracted from thawed stool samples using the Qiagen MagAttract PowerSoil Kit. The V3-V4 region of the 16S rRNA gene was polymerase chain reaction amplified using standard primers with overhang for Nextera XT index adapters (Illumina, San Diego CA) (11). Final libraries were quantitated using Quant-iT Broad Range dsDNA Assay Kit (Thermo Fisher Scientific, Waltham, MA) and were sequenced on the MiSeq platform to achieve 300-bp paired-ended reads, yielding an average of 37,890 reads per sample. Analysis was performed using Divisive Amplicon Denoising Algorithm version 2 (DADA2) v1.10.1 and R version 3.6.1 (12). DADA2 was used for quality filtering, trimming, error correction, chimeric sequence removal, and generation of the amplicon sequence variant (ASV) table. Each ASV was classified using the Greengenes 97% reference database. ASVs composing on average less than 0.05% of relative abundance were removed. Alpha diversity (Shannon index) and beta diversity (unweighted UniFrac, weighted UniFrac, and Bray-Curtis) were determined using the phyloseq v1.30.0 package in R (13). Differences in alpha diversity were compared across disease group and across timepoints using a pairwise Kruskal-Wallis test. Beta diversity was visualized using principal coordinate analysis (PCoA), with differences tested using permutational analysis of variance (PERMANOVA) using the R package, vegan v2.5.6 (14). Differentially abundant taxa were identified using DESeq2 after false discovery rate adjustment for multiple comparisons of the P values (15). Bacterial DNA sequences are publicly available in the NCBI Sequencing Read Archive (SRA) (Accession number PRJNA778253).

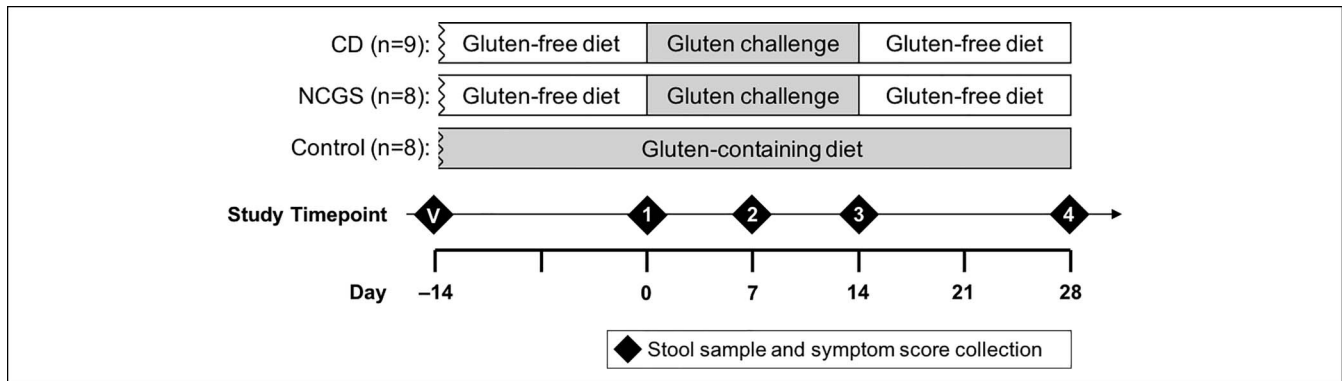


Figure 1. Study design. CD, celiac disease; NCGS, nonceliac gluten sensitivity; V, validation timepoint.

Shallow shotgun metagenomic sequencing and analysis

The same extracted DNA was used to create shotgun metagenomics libraries on select samples using the Nextera DNA Flex Library Prep kit (Illumina) according to the standard protocol while using the minimum number of cycles for polymerase chain reaction amplification. Samples from subjects with CD and NCGS at timepoints 1 and 3, immediately before and after gluten challenge, were selected because these were expected to best demonstrate changes to the gut microbiome in response to gluten exposure. Libraries were quantitated using Quant-iT Broad Range dsDNA Assay Kit (Thermo Fisher Scientific), and fragment length was validated using the 2,100 Bioanalyzer system (Agilent, Santa Clara, CA) with a High-Sensitivity DNA Chip. Sequencing was performed on the NextSeq platform to generate 150-bp single-ended reads. Raw reads were filtered to remove low-quality reads and adapters using Trim Galore version 0.6.4. After quality control filtering, the average read count per sample was approximately 4.4 million reads with no samples reaching below 2.5 million reads. Use of shotgun metagenomics sequenced at a shallow read depth, beginning with 0.5 million reads, has recently been validated for beginning to investigate species-level taxonomic and functional microbiome data (16). Kraken2 version 2.0.8 was used for taxonomic classification using the available bacterial genome information available on the National Center for Biotechnology Information (17). The taxonomic read count table was

normalized using reads per million raw reads (RPKM) to control for bias introduced by variable genome size. RPKM was then used to determine the relative abundance of each taxon. Functional analysis of gene and pathway abundance was performed using HUMAnN2 version 2.8.1 with default settings using the UniRef 50 database (18). Identified functional pathway differences and the associated taxonomic drivers were presented using Functional Shifts Taxonomic Contributors (FishTaco) analysis version 1.1.1 (19).

RESULTS

Baseline clinical characteristics and microbiome composition of study subjects

Baseline clinical characteristics of the study population are shown in Table 1. There were no significant differences in demographics among the 3 groups or in previous dietary exposures between the CD and NCGS groups. Study design is shown in Figure 1.

To assess baseline consistency of the gut microbiome composition within each group, samples were collected at a validation timepoint, 14 days before timepoint 1. Between the validation timepoint and timepoint 1, microbiome composition was stable within each study group, with few differences in relative abundance of specific taxa and no differences in alpha or beta diversity (Figure 2).

Table 1. Characteristics of study population (N = 25 subjects)

	Control (n = 8)	NCGS (n = 8)	CD (n = 9)	P value
Age at start of study (y), median (IQR)	40.4 (34.5–51.4)	51.9 (40.7–76.5)	57.9 (48.8–70.6)	0.07
Sex, no. (%)				
Female	4 (50.0)	8 (100.0)	5 (55.6)	1.0 ^b
Male	4 (50.0)	0 (0.0)	4 (44.4)	
Duration of GFD before study start (y), median (IQR)	N/A	3.2 (1.2–9.1)	8.4 (3.6–13.0)	0.15
Time since last suspected inadvertent gluten exposure at study start (d), median (IQR) ^a	N/A	37 (6–84)	9 (1–48)	1.0

CD, celiac disease; GFD, gluten-free diet; IQR, interquartile range; NCGS, nonceliac gluten sensitivity.

^aData missing for 1 patient with CD.

^bComparing only CD with NCGS: *P* = 0.08.

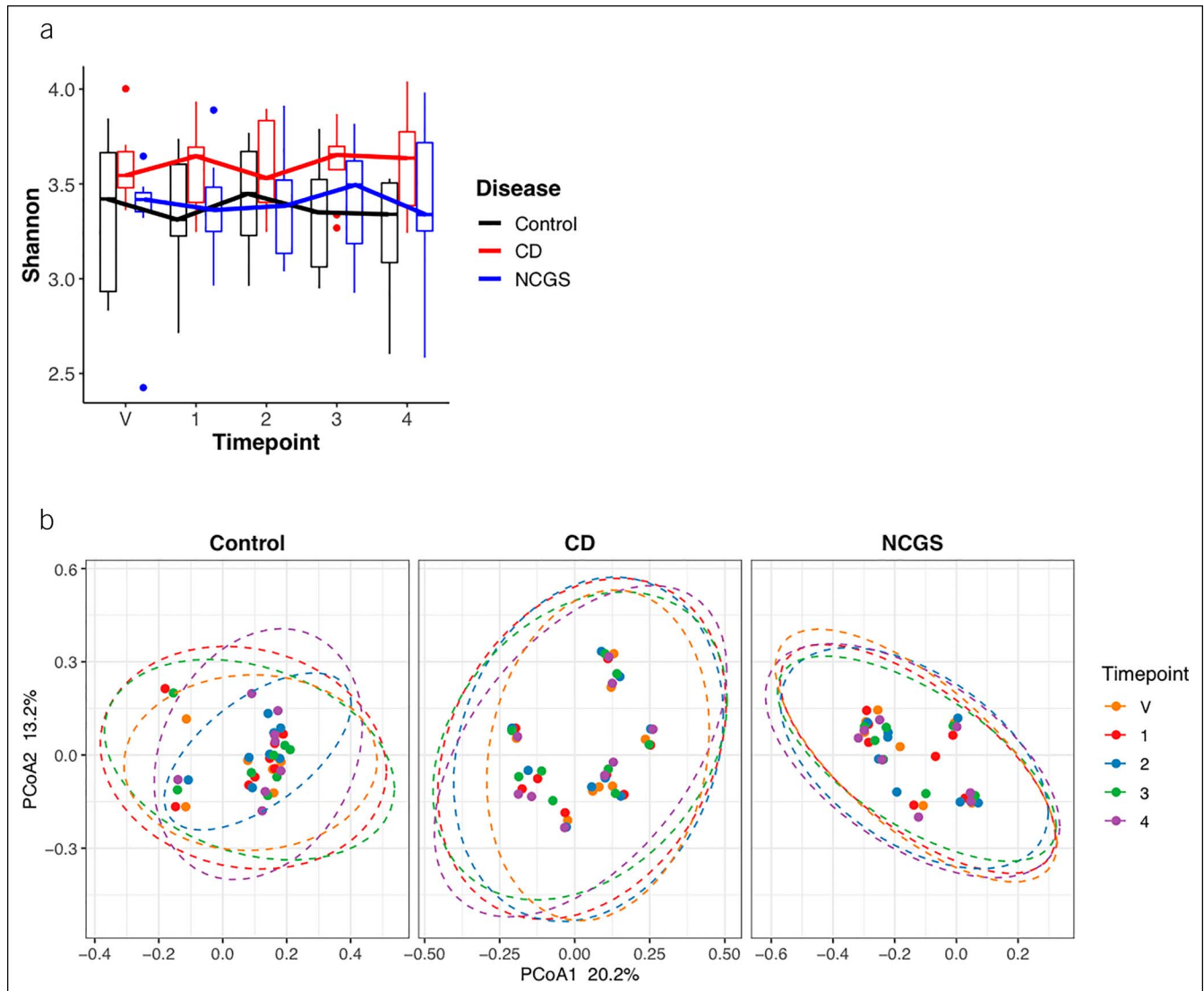


Figure 2. (a) Alpha diversity in control, CD, and NCGS study groups over time. (b) Beta diversity based on unweighted UniFrac within groups over time. All pairwise comparisons of timepoints within each study group: not significant at $\alpha = 5\%$. CD, celiac disease; NCGS, nonceliac gluten sensitivity.

Gut microbiome composition in response to gluten challenge

There were no significant changes in gut microbiome composition during or after gluten challenge in either the CD or NCGS group. Between start and end of gluten challenge (timepoint 1 vs timepoint 3), there were no differences in alpha diversity, beta diversity, or differential abundance of specific taxa within either group (Figure 2; see supplementary Figure 1A, Supplementary Digital Content 1A, <http://links.lww.com/CTG/A734>, see supplementary Figure 2A, Supplementary Digital Content 2A, <http://links.lww.com/CTG/A734>).

Baseline gut microbiome composition differed among the 3 study groups, and these differences persisted after gluten challenge (Figure 3A). Although alpha diversity did not differ among the 3 study groups over time (Figure 2A), beta diversity was significantly different among the 3 study groups at all timepoints (Figure 2B; see supplementary Figure 1B, Supplementary Digital Content 1B, <http://links.lww.com/CTG/A734>, see supplementary Figure 2B, Supplementary Digital Content 2B, <http://links.lww.com/CTG/A734>). Based on 16S rRNA analyses, multiple specific taxa were

differentially abundant in CD and NCGS groups compared with controls at timepoints 1, 2, 3, and 4 (see supplementary Figures 4-6, Supplementary Digital Content 4-6, <http://links.lww.com/CTG/A734>). *Akkermansia muciniphila* was enriched in subjects with CD compared with the control group at all timepoints and in NCGS compared with the control group at timepoint 2 only.

Shotgun metagenomics and functional analyses

Shotgun metagenomic sequencing was performed on CD and NCGS groups at baseline (timepoint 1) and end of gluten challenge (timepoint 3). Using this method, there remained no significant differences in alpha diversity, beta diversity, or differential abundance of any specific taxon between the 2 timepoints within either group. The metagenome-based and taxabased functional capacity of the microbiome was evaluated using Functional Shifts Taxonomic Contributors (FishTaco) analysis (19); comparing timepoints 1 and 3, there were no significant differences in functional pathways within either group.

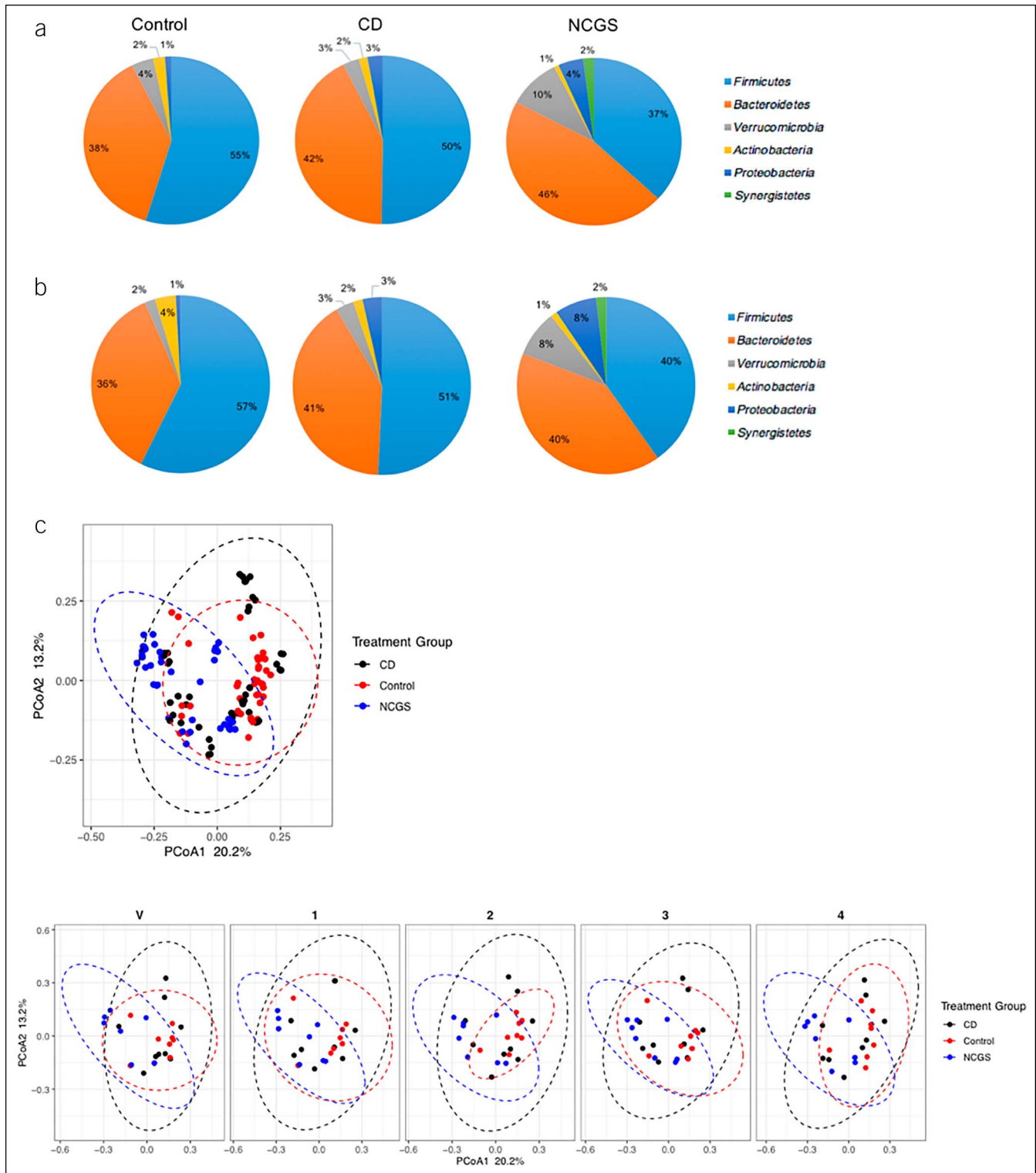


Figure 3. Microbiome composition in control, CD, and NCGS study groups over time. Relative abundance of phyla at (a) timepoint 1 and (b) timepoint 3. All other phyla had relative abundance <0.5%. (c) Unweighted UniFrac within each group and in the 3 treatment groups over time. Pairwise comparisons between each group, $P < 0.01$. CD, celiac disease; NCGS, nonceliac gluten sensitivity.

Association of gut microbiome composition with clinical outcomes after gluten challenge

The CD and NCGS groups had more severe symptoms than controls at all timepoints (see supplementary Figures 7 and 8,

Supplementary Digital Content 7 and 8, <http://links.lww.com/CTG/A734>), with nonsignificant trend toward worsening of some symptoms over the course of the study. One question in the GSRS specifically evaluated nausea and vomiting; this symptom was

most severe in the NCGS group throughout the study, but rose in subjects with CD after gluten exposure. The number of patients with high (more severe) or low (less severe) composite overall symptoms during and after gluten challenge relative to baseline is shown in supplementary Figure 9, Supplementary Digital Content 9, <http://links.lww.com/CTG/A734>.

Gut microbiome composition was evaluated within CD and NCGS study groups comparing subjects with high vs low overall symptoms at the end of gluten challenge, timepoints 3 and 4. In both groups, there were no significant differences in alpha or beta diversity at either timepoint between those with high and low overall symptoms. Differentially abundant taxa at each timepoint in the CD and NCGS groups are shown in supplementary Figure 10, Supplementary Digital Content 10, <http://links.lww.com/CTG/A734>. In both subjects with CD and NCGS, *Akkermansia muciniphila* was enriched in subjects with low overall symptoms at the end of gluten challenge (timepoint 3), but in subjects with high overall symptoms 2 weeks later (timepoint 4). In the CD group, *Faecalibacterium prausnitzii* was enriched at both timepoints after gluten challenge.

When comparing subjects with high vs low diarrhea symptoms after gluten challenge, there were no differences in alpha or beta diversity within either the CD or NCGS group. Differentially abundant specific taxa based on high or low diarrhea symptom in the CD and NCGS groups are shown in supplementary Figure 11, Supplementary Digital Content 11, <http://links.lww.com/CTG/A734>.

DISCUSSION

In this prospective study of subjects with CD and NCGS undergoing a 14-day gluten challenge, we found that baseline fecal microbiome composition differed among subjects with CD, subjects with NCGS, and controls, but that within the CD and NCGS groups, gluten challenge led to no significant changes in gut microbiome composition or functional capacity over time. In addition, we found no differences in alpha and beta diversity between subjects with more vs less severe symptoms after gluten challenge and inconsistent differences in differential abundance of specific taxa. These findings provide strong evidence that periodic gluten exposure does not meaningfully disrupt the gut microbiome in people with CD and NCGS observing a gluten-free diet.

Previous studies have posited a role of the gut microbiome in the development and clinical manifestations of CD. In infancy, events that perturb the gut microbiome—including enteric infections and systemic antibiotic exposure—are associated with CD later in life (20-23). Relative abundance of *Bifidobacterium* and *F. prausnitzii*, a Clostridial species believed to serve an anti-inflammatory role in the intestine, has been found to be lower in children with CD than controls (6,7,24). Although it is likely that gut microbiome composition in childhood contributes to the pathogenesis of CD through its role in gut mucosal and systemic immune system development, the impact of gut dysbiosis later in life is less clear. Studies have suggested that adults with endoscopically, serologically, or symptomatically active CD may have higher relative abundance of *Proteobacteria* and lower relative abundance of *Firmicutes* in duodenal samples; however, consistent patterns of dysbiosis and the impact of gluten exposure or avoidance remain incompletely understood (5,24,25).

Long-term diet is a potent modulator of the gut microbiome and may contribute to a variety of pathology, ranging from

metabolic dysregulation to colon cancer (26-30). In healthy controls, gluten-free diet is associated with reductions in *Bifidobacterium* abundance, changes in abundance of Clostridial species, and changes in metabolic pathways related to carbohydrate metabolism, mirroring changes observed in patients with CD (31-33). We found, however, that gluten challenge in patients with established CD or NCGS produced few changes in fecal microbial diversity or relative abundance of specific taxa. Subjects with CD and NCGS had increased relative abundance of *Bacteroidetes* and *Proteobacteria* and decreased relative abundance of *Firmicutes*; these differences were present at the start of the study and persisted despite gluten intake. Although composition of the microbiome does not always reflect its metabolic function, we also found no changes in functional capacity of the microbiome after gluten challenge.

We investigated the possibility that changes in gut microbiome composition mediate variable symptomatic response to gluten ingestion among patients with CD and NCGS. Both within our study over time and in our study compared with previous studies, enrichment of specific taxa correlating to specific symptoms was variable. In our study, *Bifidobacterium* was enriched in both subjects with CD and NCGS with more severe diarrhea, and *F. prausnitzii* was enriched in subjects with CD with more severe overall symptoms. *Akkermansia muciniphila* was enriched in both subjects with CD and NCGS with less severe overall symptoms at the end of gluten challenge, but in subjects with more severe symptoms by 2 weeks later. Some of our findings (e.g., the association of diarrhea with reduced abundance of *A. muciniphila*) have been identified previously (34), whereas others contradict some previous findings (e.g., that endoscopically active CD was associated with reduced abundance of *Bifidobacterium* and *F. prausnitzii*) (6). Based on the lack of change in microbial diversity during gluten challenge observed in our study, it seems that microbiome composition in adults with established CD is unlikely to contribute to symptom control, and interventions targeting the gut microbiome at this point in the disease process may have limited utility.

Our study has limitations. There was a small sample size in each study group, so it is possible that there was inadequate power to detect more subtle differences in gut microbiome composition. Symptom variability was limited, and patients with CD and NCGS with more severe baseline symptoms may be less likely to participate in a gluten challenge study. Because this study did not include children or adolescents, our null findings regarding microbial diversity may not necessarily apply across the lifespan. Longitudinal microbiome analysis was not possible for the 4 subjects with CD who left the study, including 2 who left because of symptoms; we were therefore unable to investigate associations between gut microbiome composition and CD in those with the most severe response to gluten. We studied the fecal microbiome, which is removed from the primary site of disease in CD; it is possible that duodenal dysbiosis would develop more quickly in response to gluten exposure. Future studies should consider endoscopic evaluation for duodenal microbial sampling after gluten challenge.

There were also several strengths. The patients were prospectively recruited and well-characterized, including biopsy-proven disease in subjects with CD. Because of the potential for significant symptoms including nausea, pain, and diarrhea, patients with CD and NCGS are often reluctant to participate in research requiring intentional repeated gluten exposure; our

study is valuable in that it provides longitudinal analysis of the gut microbiome in these patients during and after gluten exposure. This important information is often difficult to obtain, and the analysis of serially collected fecal microbiome samples during a gluten challenge as it correlates with symptoms has not been evaluated by other studies. Subjects were sampled serially over time, allowing for prospective evaluation of changes to the gut microbiome before, during, and after gluten challenge. The dose of gluten (5 g per day) administered was standardized, and the duration of exposure used in our study has been demonstrated to be adequate for induction of histologic and serologic changes in patients with CD (35) and approach that of average daily gluten intake among US adults (36).

In conclusion, in this study of subjects with CD and NCGS previously on a long-term gluten-free diet, we found that a 14-day gluten challenge resulted in no significant changes to fecal microbiome composition in either group. Controls, subjects with CD, and subjects with NCGS had distinct microbiome composition at baseline, and differences persisted regardless of gluten exposure. When patients were stratified based on more severe or less severe symptoms in response to gluten challenge, there were no consistent patterns in gut microbial dysbiosis. Our findings indicate that in people with CD and NCGS on a long-term gluten-free diet, short-term gluten consumption does not alter gut microbiome composition. Gut microbial dysbiosis in patients with established CD or NCGS is unlikely to meaningfully impact disease activity and symptom severity in patients with these conditions.

CONFLICTS OF INTEREST

Guarantor of the article: Benjamin Lebwohl, MD, MS.

Specific author contributions: Study concept and design: B.L., Y.R.N., M.J.B., P.H.R.G., A.C.U., and D.E.F. Data acquisition and analysis: Y.R.N., F.R., H.P., A.C.U., and B.L. Manuscript preparation: Y.R.N. and B.L. Manuscript review: Y.R.N., F.R., H.P., D.E.F., M.J.B., P.H.R.G., A.C.U., and B.L.

Financial support: Y.R.N.: NIH 5T32DK083256-12. D.E.F.: Columbia University Irving Institute for Clinical and Translational Research, and Department of Defense Peer-Reviewed Medical Research Program (PR181960). M.J.B.: Sergei S. Zlinkoff Fund for Medical Research & Education, Inc.; Canadian Institute of Advanced Research. ACU: R01 AI116939. B.L.: The Louis V. Gerstner, Jr. Scholars Program, the Celiac Disease Foundation Young Investigator Award, and the Louis and Gloria Flanzer Philanthropic Trust.

Potential competing interests: None to report.

Study Highlights

WHAT IS KNOWN

- ✓ Gut dysbiosis early in life may contribute to celiac disease (CD) pathogenesis.
- ✓ Whether gluten exposure in adults with established CD or nonceliac gluten sensitivity (NCGS) perturbs the gut microbiome is unknown.

WHAT IS NEW HERE

- ✓ In this gluten challenge study, fecal microbiome composition differed across study groups, but was not significantly altered by gluten exposure in the CD and NCGS groups.

REFERENCES

1. Lebwohl B, Sanders DS, Green PH. Coeliac disease and dermatitis herpetiformis—Authors' reply. *Lancet* 2018;392(10151):917.
2. Verdu EF, Armstrong D, Murray JA. Between celiac disease and irritable bowel syndrome: The "no man's land" of gluten sensitivity. *Am J Gastroenterol* 2009;104(6):1587–94.
3. Nylund L, Kaukinen K, Lindfors K. The microbiota as a component of the celiac disease and non-celiac gluten sensitivity. *Clin Nutr Exp* 2016;6: 17–24.
4. Francavilla R, Cristofori F, Vacca M, et al. Advances in understanding the potential therapeutic applications of gut microbiota and probiotic mediated therapies in celiac disease. *Expert Rev Gastroenterol Hepatol* 2020;14(5):323–33.
5. Wacklin P, Laurikka P, Lindfors K, et al. Altered duodenal microbiota composition in celiac disease patients suffering from persistent symptoms on a long-term gluten-free diet. *Am J Gastroenterol* 2014;109(12): 1933–41.
6. De Palma G, Nadal I, Medina M, et al. Intestinal dysbiosis and reduced immunoglobulin-coated bacteria associated with coeliac disease in children. *BMC Microbiol* 2010;10:63.
7. Collado MC, Donat E, Ribes-Koninckx C, et al. Imbalances in faecal and duodenal Bifidobacterium species composition in active and non-active coeliac disease. *BMC Microbiol* 2008;8:232.
8. Krishnareddy S. The microbiome in celiac disease. *Gastroenterol Clin North Am* 2019;48(1):115–26.
9. Olshan KL, Leonard MM, Serena G, et al. Gut microbiota in celiac disease: Microbes, metabolites, pathways and therapeutics. *Expert Rev Clin Immunol* 2020;16(11):1075–92.
10. Hays RD, Sherbourne CD, Mazel RM. The RAND 36-item health survey 1.0. *Health Econ* 1993;2(3):217–27.
11. 16S Metagenomic Sequencing Library Preparation Illumina, Inc.: San Diego, CA, 2013. (https://emea.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf).
12. Callahan BJ, McMurdie PJ, Rosen MJ, et al. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016;13(7): 581–3.
13. McMurdie PJ, Holmes S. Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 2013;8(4):e61217.
14. Oksanen J, Blanchet FG, Friendly M, et al. Community Ecology Package; Package "vegan.", 2019 (<https://cran.r-project.org/web/packages/vegan/vegan.pdf>).
15. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014;15(12): 550.
16. Hillmann B, Al-Ghalith GA, Shields-Cutler RR, et al. Evaluating the information content of shallow shotgun metagenomics. *mSystems* 2018; 3(6):e00069–18.
17. Wood DE, Lu J, Langmead B. Improved metagenomic analysis with Kraken 2. *Genome Biol* 2019;20(1):257.
18. Abubucker S, Segata N, Goll J, et al. Metabolic reconstruction for metagenomic data and its application to the human microbiome. *PLoS Comput Biol* 2012;8(6):e1002358.
19. Manor O, Borenstein E. Systematic characterization and analysis of the taxonomic drivers of functional shifts in the human microbiome. *Cell Host Microbe* 2017;21(2):254–67.
20. Dydenborg Sander S, Nybo Andersen AM, Murray JA, et al. Association between antibiotics in the first year of life and celiac disease. *Gastroenterology* 2019;156(8):2217–29.
21. Stene LC, Honeyman MC, Hoffenberg EJ, et al. Rotavirus infection frequency and risk of celiac disease autoimmunity in early childhood: A longitudinal study. *Am J Gastroenterol* 2006;101(10):2333–40.
22. Bouziat R, Hinterleitner R, Brown JJ, et al. Reovirus infection triggers inflammatory responses to dietary antigens and development of celiac disease. *Science* 2017;356(6333):44–50.
23. Kempainen KM, Lynch KF, Liu E, et al. Factors that increase risk of celiac disease autoimmunity after a gastrointestinal infection in early life. *Clin Gastroenterol Hepatol* 2017;15(5):694–702.e5.
24. Zafeiropoulou K, Nichols B, Mackinder M, et al. Alterations in intestinal microbiota of children with celiac disease at time of diagnosis and on a gluten-free diet. *Gastroenterology* 2020;159(6):2039–51.e20.

25. D'Argenio V, Casaburi G, Precone V, et al. Metagenomics reveals dysbiosis and a potentially pathogenic *N. Flavescens* strain in duodenum of adult celiac patients. *Am J Gastroenterol* 2016;111(6):879–90.
26. Wu GD, Chen J, Hoffmann C, et al. Linking long-term dietary patterns with gut microbial enterotypes. *Science* 2011;334(6052):105–8.
27. Mehta RS, Nishihara R, Cao Y, et al. Association of dietary patterns with risk of colorectal cancer subtypes classified by *Fusobacterium nucleatum* in tumor tissue. *JAMA Oncol* 2017;3(7):921–7.
28. Desai MS, Seekatz AM, Koropatkin NM, et al. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell* 2016;167(5):1339–53.e21.
29. Turnbaugh PJ, Bäckhed F, Fulton L, et al. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe* 2008;3(4):213–23.
30. Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. *Nature* 2009;457(7228):480–4.
31. Hansen LBS, Roager HM, Søndertoft NB, et al. A low-gluten diet induces changes in the intestinal microbiome of healthy Danish adults. *Nat Commun* 2018;9(1):4630.
32. De Palma G, Nadal I, Collado MC, et al. Effects of a gluten-free diet on gut microbiota and immune function in healthy adult human subjects. *Br J Nutr* 2009;102(8):1154–60.
33. Bonder MJ, Tigchelaar EF, Cai X, et al. The influence of a short-term gluten-free diet on the human gut microbiome. *Genome Med* 2016;8(1):45.
34. Di Biase AR, Marasco G, Ravaoli F, et al. Gut microbiota signatures and clinical manifestations in celiac disease children at onset: A pilot study. *J Gastroenterol Hepatol* 2021;36(2):446–54.
35. Leffler D, Schuppan D, Pallav K, et al. Kinetics of the histological, serological and symptomatic responses to gluten challenge in adults with coeliac disease. *Gut* 2013;62(7):996–1004.
36. Lebowitz B, Cao Y, Zong G, et al. Long term gluten consumption in adults without celiac disease and risk of coronary heart disease: Prospective cohort study. *BMJ* 2017;357:j1892.

Open Access This is an open access article distributed under the Creative Commons Attribution License 4.0 (CCBY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



A Simple Emergency Department-Based Score Predicts Complex Hospitalization in Patients with Inflammatory Bowel Disease

Abhishek Verma¹ · Sanskriti Varma² · Daniel E. Freedberg³ · Jordan E. Axelrad⁴ 

Received: 9 November 2020 / Accepted: 24 January 2021 / Published online: 19 February 2021
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC part of Springer Nature 2021

Abstract

Background and Aims Thirty percent of inflammatory bowel disease (IBD) patients hospitalized with flare require salvage therapy or surgery. Additionally, 40% experience length of stay (LOS) > 7 days. No emergency department (ED)-based indices exist to predict these adverse outcomes at admission for IBD flare. We examined whether clinical, laboratory, and endoscopic markers at presentation predicted prolonged LOS, inpatient colectomy, or salvage therapy in IBD patients admitted with flare.

Methods Patients with ulcerative colitis (UC) or colonic involvement of Crohn's disease (CD) hospitalized with flare and tested for *Clostridioides difficile* infection (CDI) between 2010 and 2020 at two urban academic centers were studied. The primary outcome was complex hospitalization, defined as: LOS > 7 days, inpatient colectomy, or inpatient infliximab or cyclosporine. A nested k-fold cross-validation identified predictive factors of complex hospitalization.

Results Of 164 IBD admissions, 34% (56) were complex. Predictive factors included: tachycardia in ED triage (odds ratio [OR] 3.35; confidence interval [CI] 1.79–4.91), hypotension in ED triage (3.45; 1.79–5.11), hypoalbuminemia at presentation (2.54; 1.15–3.93), CDI (2.62; 1.02–4.22), and endoscopic colitis (4.75; 1.75–5.15). An ED presentation score utilizing tachycardia and hypoalbuminemia predicted complex hospitalization (area under curve 0.744; CI 0.671–0.816). Forty-four of 48 (91.7%) patients with a presentation score of 0 (heart rate < 99 and albumin ≥ 3.4 g/dL) had noncomplex hospitalization.

Conclusions Over 90% of IBD patients hospitalized with flare with an ED presentation score of 0 did not require salvage therapy, inpatient colectomy, or experience prolonged LOS. A simple ED-based score may provide prognosis at a juncture of uncertainty in patient care.

Keywords Inflammatory bowel disease · Infliximab · Predictive score · Colectomy · Length of stay

Introduction

Hospitalization for flare of inflammatory bowel disease (IBD) is associated with significant morbidity and high resource utilization [1, 2]. Though corticosteroids are the mainstay of acute treatment, nearly 40% of patients will experience a worsening clinical course and require surgery

or salvage therapy with either infliximab or cyclosporine [3–6]. Not only are hospitalization rates for IBD flare rising at a rate of 4.3% annually [7–9], rates of in-hospitalization surgery are rising in those with severe disease [9]. Though biologics help avoid urgent surgery, they may not mitigate long-term surgical risk [10], indicating the higher morbidity surrounding patients who require inpatient second-line

✉ Jordan E. Axelrad
Jordan.Axelrad@nyulangone.org

Abhishek Verma
Abhishek.Verma@nyulangone.org

Sanskriti Varma
sav9046@nyp.org

Daniel E. Freedberg
def2004@cumc.columbia.edu

¹ Department of Medicine, NYU Langone Health, 550 First Avenue, NBV 16 North 30, New York, NY 10016, USA

² Department of Medicine, New York Presbyterian Columbia University Medical Center, 622 West 168th Street, New York, NY 10032, USA

³ Division of Digestive and Liver Diseases, Department of Medicine, College of Physicians and Surgeons, Columbia University, 630 West 168th Street, 3rd Floor, New York, NY 10032, USA

⁴ Division of Gastroenterology, NYU Langone Health, Inflammatory Bowel Disease Center at NYU Langone Health, 240 East 38th Street, 23rd Floor, New York, NY 10016, USA

therapy. Therefore, early identification of patients who will require rescue therapy or surgery during admission is critical to directly improving IBD care.

Several scores utilize a combination of clinical, biochemical, and/or endoscopic data to predict those patients whose clinical course will worsen [11–19]. Truelove and Witts in the 1950s employed frequency of bloody stools, elevated inflammatory markers, fever, anemia, and tachycardia to define severe ulcerative colitis (UC) [11]. Recently, the ACE (albumin, C-reactive protein [CRP], and Endoscopy) score developed by Grant et al. used albumin, CRP, and the endoscopic Mayo score [20] to predict which UC patients would require second-line therapy or colectomy [19]. Existing scores, however, are limited to patients with UC, do not account for *Clostridioides difficile* infection (CDI), and do not predict prolonged length of stay (LOS).

Objective

The objective of this study was to create a simple, emergency department-based index to predict complex hospitalization, defined as: (a) prolonged length of stay, (b) inpatient tumor necrosis factor (TNF) inhibitor or cyclosporine, or (c) inpatient colectomy in all IBD patients admitted with flare symptoms.

Methods

Selection Criteria

All patients admitted through the ED for IBD flare and tested for CDI were considered for inclusion in this retrospective dual-center study. Only those tested for CDI were considered in order to examine CDI as a risk factor for complex hospitalization. IBD diagnosis (UC or Crohn's disease [CD]) was confirmed by International Statistical Classification of Diseases and Related Health Problems 10th revision and by manual review of gastroenterology consultant documentation during admission. Admissions between January 2010 and September 2019 at New York-Presbyterian Columbia University Irving Medical Center, and between January 2013 and June 2020 at NYU Langone Health were considered. Inclusion criteria were limited to the following: age over 18 at admission, index hospitalization for IBD flare, hospitalization for at least 48 h, and presentation with clinical symptoms, laboratory, imaging, or endoscopy findings consistent with IBD flare. Only hospitalizations of at least 48 h in duration were considered in order to exclude observation visits. Patients with ileocolonic and colonic Crohn's disease were included, but Crohn's patients with isolated ileal, small bowel, or perianal disease were excluded. Additionally, Crohn's patients admitted with small bowel obstructions,

fistulizing disease, or intra-abdominal abscesses were excluded. Other exclusion criteria were incomplete documentation and admission for another primary diagnosis or for elective surgical procedures. Patient selection occurred prior to the extraction of exposure variables from all considered charts.

Data Collection

Demographic data and laboratory values were collected by manual chart review; all laboratory values were recorded from the first blood draw performed within the ED. Hemodynamics at presentation were recorded from vital signs in ED triage. It was noted whether colitis was read by a radiologist on in-hospital axial imaging. It was also noted whether endoscopy performed during admission demonstrated colitis, defined as a Mayo endoscopic subscore of 1 or greater for patients with UC [20] and the presence of colitis for patients with CD.

Data on IBD-related outcomes that were collected included: LOS, new inpatient TNF inhibitor or cyclosporine, and inpatient colectomy for refractory colitis. Any new IBD medication initiated while inpatient was also recorded: steroids, 5-aminosalicylates, immunomodulators (6-mercaptopurine, azathioprine, methotrexate), TNF inhibitors, anti-interleukin (IL)-12/IL-23, anti-integrin, cyclosporine, and Janus kinase inhibitor.

Outcomes and Definitions

The primary outcome of this study was complex hospitalization, defined as a composite of the following outcomes: (1) prolonged LOS (7 days or longer), (2) inpatient colectomy, or (3) requiring either inpatient TNF inhibitor or cyclosporine. Each component of the primary outcome was analyzed separately as a secondary outcome. ACE score at ED presentation (1 point each for albumin ≤ 3.0 g/dL and CRP ≥ 50 mg/L) and during hospitalization (an additional 1 point for colitis on endoscopy) was also calculated for all patients [19].

Statistical Analysis

Continuous variables are presented as medians with interquartile ranges (IQR). Categorical variables are presented as frequency using percentages. Regression-derived statistics are presented using odds ratio (OR) and confidence intervals (CI).

A nested k-fold cross-validation approach was used for the final data analysis to allow for simultaneous validation of the study findings. In the internal cross-validation, lasso regression was performed on a subset of data to identify variables predictive of complex hospitalization.

Significant variables were then included in a reduced multivariate logistic regression using all available data.

Multiple indices were created using combinations of variables predictive of complex hospitalization. Cutoff analyses were performed for all linear variables; each variable was allocated a score of 0 for a value below cutoff and a score of 1 for a value above cutoff. ‘Presentation’ indices were created using significant variables only immediately available at ED presentation. For each presentation index, a companion ‘early admission’ index was created which also included significant variables whose results would be available shortly after admission, including results of *C. difficile* testing and endoscopy. Receiver operator characteristic (ROC) curves were generated using the Youden index for all indices. Area under the curve (AUC) is presented with 95% confidence intervals (CI).

Study data were collected and managed using REDCap electronic data capture tools hosted at NYU [21, 22]. Statistical analyses and graphic generation were performed using R version 3.3.3 [23]. A *p* value of < 0.05 was considered statistically significant. This study was approved by the Institutional Review Board of New York University Langone Health.

Results

Patient Characteristics and Outcomes

Of the 258 patients hospitalized for IBD flare and tested for CDI in the study period, 164 patients met inclusion criteria (Fig. 1). Of these 164 patients, 41.5% (68) had CD and 57.9% (95) had UC. The median age at hospitalization was 34.4 years (IQR 25.8–49.8). Complex hospitalization was present in 34% (56 of 164) of patients studied. While the median LOS was 3.74 days (IQR 2.43–6.61), 21.3% of patients (35 of 164) had a prolonged LOS of > 7 days. Inpatient TNF inhibitor therapy was initiated in 11.6% (19 of 164) of patients and inpatient cyclosporine was initiated in 3.0% of patients (5 of 164). Ten (6.1%) patients underwent inpatient surgery during hospitalization. Demographics, medications prior to admission, and laboratory results on admission are outlined in Table 1.

Variable Selection

In the internal k-fold cross-validation on 134 patients, lasso regression was performed on the following variables: age, body mass index, race, sex, Charlson comorbidity

Fig. 1 Patient selection for the study. Supplementary Table 4 details the non-flare primary hospitalization diagnoses. Abbreviations: IBD (inflammatory bowel disease); CDI (*Clostridioides difficile* infection); CD (Crohn’s disease)

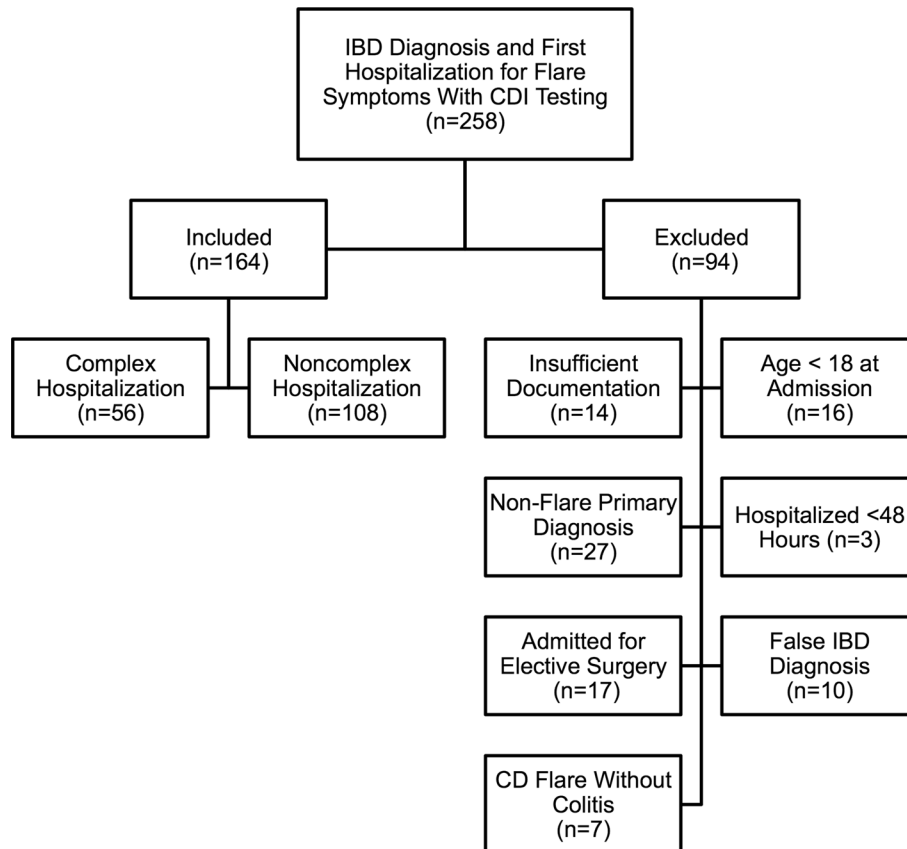


Table 1 Baseline demographics of the study population

Characteristic	Total (n = 164)
Median age, years (IQR)	34.4 (25.8–49.8)
Female, % (n)	54.3 (89)
Race, % (n)	
White	50.6 (83)
Other	25.6 (40)
Black/African-American	18.3 (30)
Asian	3.7 (6)
Not reported	2.4 (4)
More than one race	0.61 (1)
Body mass index, median (IQR)	23.4 (20.2–26.8)
Charlson comorbidity Index, median (IQR)	0 (0–2)
Tobacco use, % (n)	13.4 (22)
IBD subtype, % (n)	
Crohn's disease	41.5 (68)
Ulcerative colitis	57.9 (95)
Indeterminate colitis	0.61 (1)
Disease duration at admission (years), median (IQR)	7.56 (2.65–14.7)
IBD medications prior to admission	
Oral 5-aminosalicylates, % (n)	39.6 (65)
Oral steroids, % (n)	27.4 (45)
Immunomodulators (6-MP, AZA, MTX), % (n)	13.4 (22)
TNF inhibitor, % (n)	19.5 (32)
Cyclosporine, % (n)	0 (0)
JAK inhibitor, % (n)	0.61 (1)
New IBD medications initiated during admission	
Oral 5-aminosalicylates, % (n)	12.8 (21)
IV steroids, % (n)	65.2 (107)
Immunomodulators (6-MP, AZA, MTX), % (n)	0 (0)
TNF inhibitor, % (n)	11.6 (19)
Cyclosporine, % (n)	3.0 (5)
JAK inhibitor, % (n)	1.3 (2)
Temperature (C), median (IQR)	37.3 (37.1–37.7)
Heart rate (/min), median (IQR)	100 (90–113)
Systolic blood pressure (mmHg), median (IQR)	96 (91–105)
Diastolic blood pressure (mmHg), median (IQR)	53 (47–57)
Admission laboratory values	
White blood count (10 ⁹ /L), median (IQR)	9.64 (7.50–13.5)
Hematocrit (%), median (IQR)	36.5 (31.7–40.8)
Platelet Count (10 ⁹ /L), median (IQR)	330 (254–451)
Albumin (g/dL), median (IQR)	3.6 (3.1–4.0)
Creatinine (mg/dL), median (IQR)	0.80 (0.70–0.99)
CRP (mg/L), median (IQR)	33.0 (9.10–82.0)
Erythrocyte sedimentation rate (mm/h), median (IQR)	38.0 (22.8–63.3)
Lactate (mmol/L), median (IQR)	1.14 (0.86–1.46)
Cross-axial imaging during admission	
Cross-axial imaging with colitis, % (n)	51.4 (75)
Cross-axial imaging not performed, % (n)	40.2 (66)

Table 1 (continued)

Characteristic	Total (n = 164)
Endoscopy during admission	
Endoscopy with colitis, % (n)	56.7 (93)
Endoscopy not performed, % (n)	37.2 (61)
<i>C. difficile</i> positive, % (n)	26.8 (44)
Complex hospitalization, % (n)	34.1 (56)
Length of stay (days), median (IQR)	3.74 (2.43–6.61)
Prolonged length of stay (> 7 days), % (n)	21.3 (35)
Inpatient surgery, % (n)	6.1 (10)
New TNF inhibitor or cyclosporine, % (n)	14.6 (24)

CRP C-reactive protein, IBD inflammatory bowel disease, IQR inter-quartile range, JAK Janus kinase, TNF tumor necrosis factor

index, temperature, heart rate, systolic blood pressure, diastolic blood pressure, white blood count, hematocrit, platelet, albumin, creatinine, C-reactive protein, CDI, cross-axial imaging demonstrating colitis, and endoscopy demonstrating colitis. Prior biologic use did not correlate with the primary outcome. In the multivariate logistic regression on all 164 patients, the following variables predicted complex hospitalization: tachycardia (OR 3.35; CI 1.79–4.91), hypotension (OR 3.45; CI 1.79–5.11), hypoalbuminemia (OR 2.54; CI 1.15–3.93), CDI (OR 2.62; CI 1.02–4.22), and colitis seen on endoscopy (OR 4.75; CI 1.75–5.15) (Table 2).

These variables remained significant in analyses of a subset of 141 patients who had *C. difficile* testing performed within 48 h of ED presentation to confirm that delays in *C. difficile* testing were not a confounding factor for the primary outcome.

The optimal cutoff for heart rate was 99 BPM (AUC 0.716; CI 0.631–0.801; sensitivity 78.6%, specificity 58.8%), and 100 mm Hg for systolic blood pressure (AUC 0.689; CI 0.605–0.774; sensitivity 85.7%, specificity 43.3%). The optimal cutoff for albumin was 3.4 g/dL (AUC 0.696; CI 0.611–0.780; sensitivity 67.3%, specificity 63.1%).

Table 2 Significant variables from the multivariable logistic regression

Variable	Odds ratio	Confidence interval
Tachycardia (HR ≥ 99 BPM)	3.35	1.79–4.91
Hypoalbuminemia (dL/g)	2.54	1.15–3.93
<i>C. difficile</i> positive	2.62	1.02–4.22
Endoscopy showing colitis	4.75	1.75–5.15
Hypotension (SBP < 100 mmHg)	3.45	1.79–5.11

All variables demonstrated statistical significance ($p < 0.05$)

BPM beats per minute, HR heart rate, SBP systolic blood pressure

Index Development and Selection

Two ‘presentation’ indices were generated using the significant variables that would reasonably be available at ED presentation: heart rate, systolic blood pressure, and albumin. Systolic blood pressure was omitted in the second presentation index; this was done to augment index simplicity given that the covariate tachycardia is an earlier sign of clinical dehydration. Companion ‘early admission’ indices were created by sequentially adding endoscopy and the presence of CDI. Sensitivity, specificity, and area under the curve for each index are shown in Table 3. There were no significant differences in the area under the curve among the indices created (Table 3). Consequently, the simplest ‘presentation’ index of heart rate and albumin (AUC 0.744; CI 0.671–0.816; sensitivity 54.5%; specificity 80.2%) and its companion ‘early admission’ index adding CDI and

endoscopy results (AUC 0.795; CI 0.727–0.864; sensitivity 58.2%; specificity 85.1%) were chosen for further analysis. The ideal cutoff for a positive presentation score was a score of 2. The ideal cutoff for a positive early admission score was a score of 3 or greater. Figure 2 demonstrates ROC curves for the two indices. Supplementary Table 1 outlines the performances at each cutoff for the presentation and early admission indices. There were no differences in score performance by AUC between study centers (Supplementary Table 2).

Index Performance

Of all patients with a presentation score of 0—heart rate below 99 and albumin of at least 3.4 g/dL—91.7% (44 of 48) had a noncomplex hospitalization. Similarly, 91.5% (54 of 59) of all patients with an early admission score of 1 or

Table 3 Comparing multiple severity indices

	HR + albumin	HR + SBP + albumin	HR + albumin + endoscopy	HR + SBP + albumin + endoscopy	HR + albumin + CDI + endoscopy	HR + SBP + albumin + CDI + endoscopy
Sensitivity	54.5%	85.5%	85.5%	76.3%	58.2%	83.6%
Specificity	80.2%	59.4%	61.4%	72.2%	85.1%	66.3%
PPV	60%	53.4%	54.7%	60%	68.1%	57.5%
NPV	76.5%	88.2%	88.6%	84.9%	78.9%	88.2%
AUC	0.744 (0.671–0.816)	0.775 (0.704, 0.846)	0.773 (0.701–0.845)	0.793 (0.723–0.862)	0.795 (0.727–0.864)	0.817 (0.75–0.883)

A comparison of the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC) of multiple severity indices

CDI *Clostridioides difficile* infection, HR heart rate, SBP systolic blood pressure

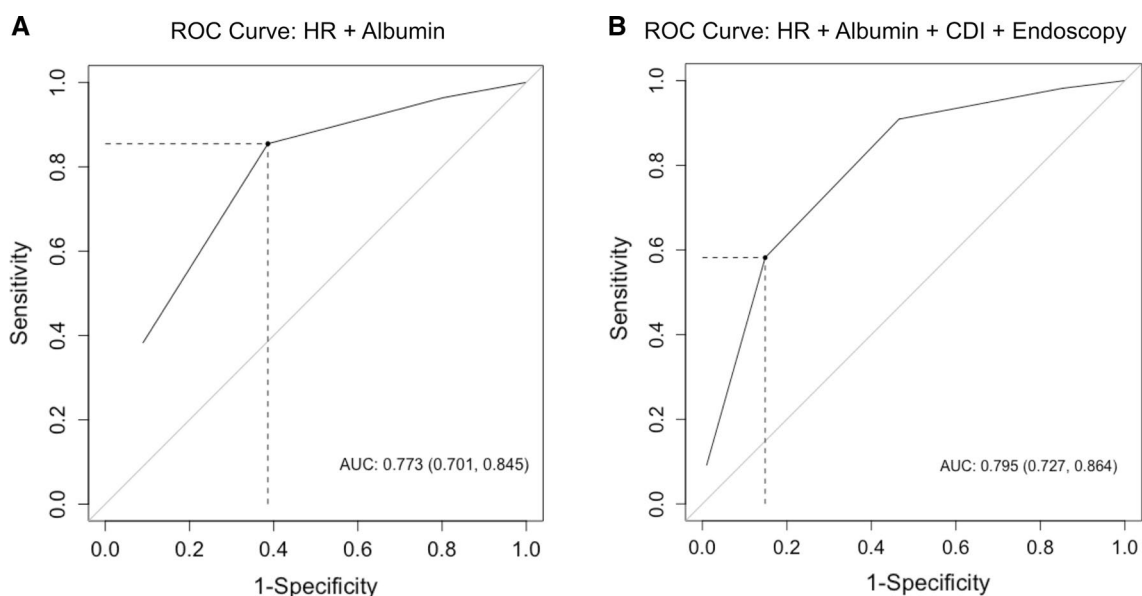


Fig. 2 Receiver operator characteristic (ROC) curves for (a) presentation and (b) early admission indices. Dotted lines demonstrate the optimal cutoff. Area under curve (AUC) ± confidence interval included

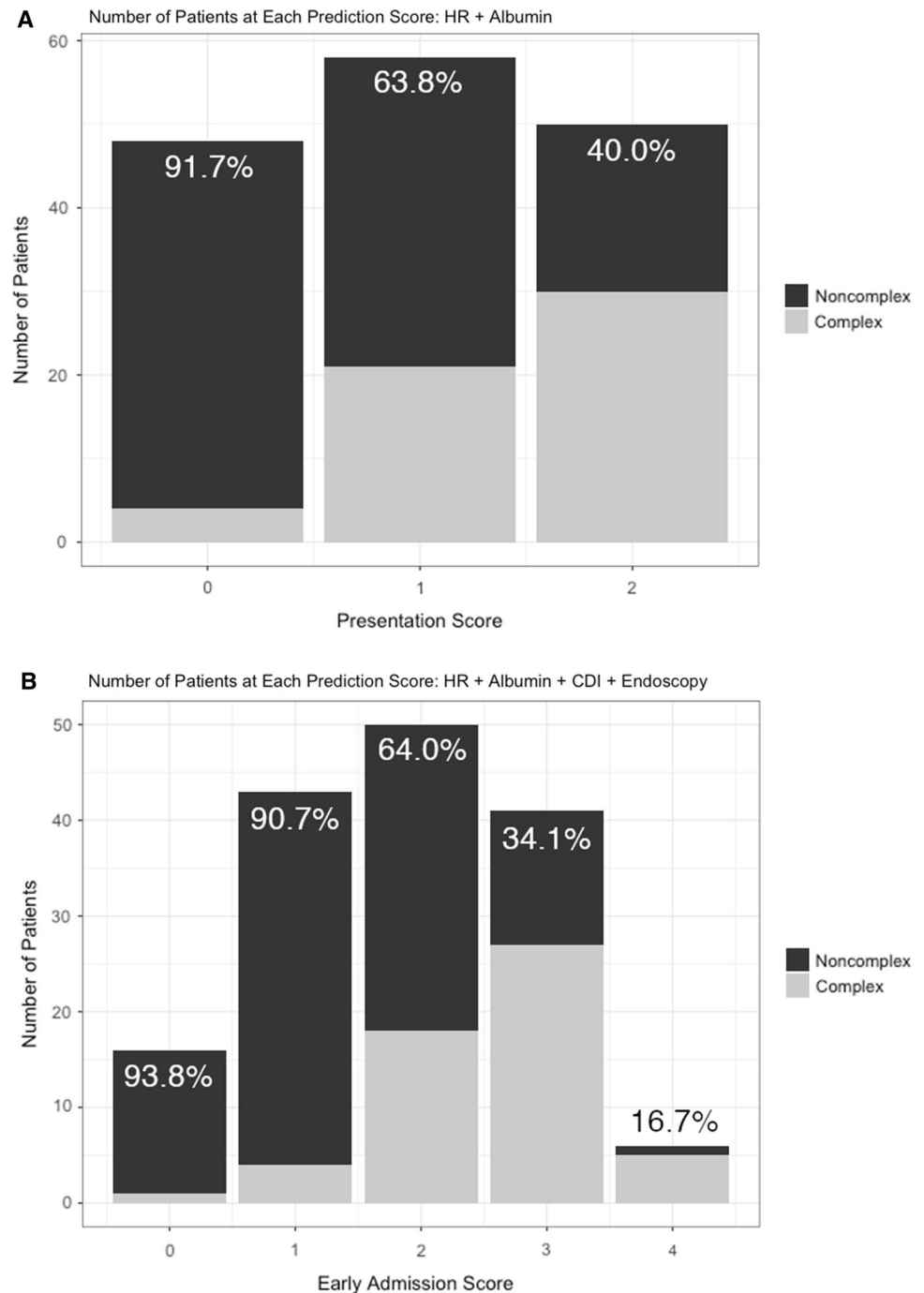
below had a noncomplex hospitalization. Conversely, 30 of 50 (60%) patients with a maximum presentation score of 2 and 5 of 6 (83.3%) patients with a maximum early admission score of 4 had a complex hospitalization (Fig. 3).

Secondary Outcomes

Of all patients with a presentation score of 0, 97.9% (47/48) had a LOS less than 7 days, 100% (48 of 48) did not have surgery, and 93.8% (45 of 48) did not receive a

TNF inhibitor or cyclosporine. Of all patients with an early admission score of 0, 100% (16 of 16) had a LOS less than 7 days, 100% (16 of 16) did not receive inpatient surgery, and 93.8% (15 of 16) did not receive a TNF inhibitor or cyclosporine. Complete sensitivities, specificities, positive predictive values, and negative predictive values are demonstrated in Supplementary Table 3.

Fig. 3 The number of patients at each prediction score for the presentation (a) and early admission (b) indices. Percentage of patients with each score who have a noncomplex stay are shown



Comparison to ACE Score

Optimal cutoffs were a score of 1 for ACE at presentation (AUC 0.683; CI 0.599–0.767; sensitivity 72.0%; specificity 62.6%), and a score of 2 for the full ACE score (AUC 0.710; CI 0.627–0.794; sensitivity 60%; specificity 73.3%). Of all patients with an ACE at presentation score of 0, 80.3% (57 of 71) had a noncomplex stay, while 76.7% (66 of 86) patients with an ACE at hospitalization score of 1 or below had a noncomplex stay (Supplementary Fig. 1). The early admission score trended toward a higher AUC than the ACE score; however, this difference did not cross the statistical significance threshold (0.795 [early admission score; 95% CI 0.727–0.864] vs. 0.710 [ACE Score; 95% CI 0.627–0.794], $p=0.065$; Supplementary Fig. 2).

Restriction to Patients Treated with Intravenous Steroids

A sub-analysis was performed solely on patients who received intravenous (IV) steroids at admission ($n=104$) to study score performances in this subgroup of patients. Of all patients initially treated with IV steroids with a presentation score of 0, 92.9% (26 of 28) had a noncomplex stay. Of all patients initially treated with IV steroids with an early admission score of 1 or less, 91.9% (34 of 37) had a noncomplex stay (Supplementary Fig. 3). Complete performance metrics can be seen in Supplementary Table 5.

Sub-analysis by Disease Subtype

In a separate sub-analysis, the performances of the scores were evaluated separately for ulcerative colitis patients ($n=90$) and for patients with Crohn's disease with colonic involvement ($n=65$). Of UC patients with a presentation score of 0, 91% (21 of 23) had a noncomplex hospitalization. Of those with an early admission score of 1 or less, 93% (26 of 28) had a noncomplex hospitalization (Supplementary Fig. 4). The performances of the presentation and early admission scores in the subgroup of CD patients were similar (Supplementary Fig. 4). Complete metrics can be seen in Supplementary Table 6.

Discussion

In this multicenter study, we present an internally validated simple score that can be employed at ED presentation to determine complex hospitalization in all IBD patients admitted with flare of UC and Crohn's with colonic involvement. At a time point of undifferentiation, this score demonstrates value in prognosticating the hospitalization course, including duration of stay, requirement of salvage therapy, or

inpatient surgery. Specifically, those with a presentation score of 0 (heart rate <99 , albumin ≥ 3.4 g/dL) have a 92% likelihood of having a noncomplex hospitalization. Additionally, we created an early admission score, factoring in the presence of CDI and colitis on endoscopy, demonstrating that 92% patients with a score of 1 or below have a noncomplex hospitalization.

Distinguishing Factors of this Study

This study addresses multiple limitations of existing scores. First, the broad inclusion criteria of this study translate to a higher real-world applicability at a critical time point in patient care. Prior scores have solely focused on acute severe UC patients as defined by the modified Truelove and Witts score (TWS) [13, 14, 16, 24]. Many non-severe patients, however, experience adverse in-hospital outcomes: in a 2010 study, 16.4% of UC patients hospitalized with non-severe flare required infliximab, cyclosporine, or surgical therapy [9, 15]. This subset limitation has also led to a paucity of guidance for patients with flare of colonic CD. In this study, not only are Crohn's colitis patients included, but also all illness levels are considered. By not limiting the severity level of our studied population at the outset, we are able to identify relevant predictive components of severity (i.e., tachycardia, a component of the modified TWS) while maintaining broad applicability.

Another important factor that has either been excluded or controlled for in prior predictive scores [19, 24] is CDI, despite the known interplay between CDI and flare. IBD patients are 5–8 times as likely as non-IBD patients to develop CDI [25, 26]. Patients with concurrent CDI and flare have worse outcomes, including higher mortality, LOS, and resource utilization [27, 28]. Of the 44 *C. difficile* positive patients in this study, 19 (38.6%) received concurrent steroids, one received infliximab, one started cyclosporine, one underwent colectomy, and 18 (40.9%) had a prolonged LOS. These findings highlight the importance of including CDI testing when evaluating for complex hospitalization, and provide early prognosis when it is unclear whether symptoms are due to flare or CDI.

Although predictive factors of prolonged LOS in hospitalized IBD patients have been evaluated separately [29, 30], this study is the first to incorporate prolonged LOS in a composite of adverse outcomes. Predicting extended hospitalization duration likely helps prognosticate the known associated risks of venous thromboembolism [31] and hospital-acquired infections [32], although these associated outcomes were not directly evaluated in this study. It is unlikely that CDI was acquired during hospitalization in this study; 141 of 164 (86%) of CDI tests were sent within 48 h of presentation. Even in this subset of patients, CDI remained a significant predictor of the primary outcome in

the multivariate logistic regression. This is consistent with prior findings that over 75% of CDI in hospitalized IBD patients are community acquired [33].

Additionally, with the exception of the ACE score, the majority of indices can only be calculated at day 3 of hospitalization. The presentation score developed in this study can be calculated at ED presentation, averting the loss of valuable time for early intervention and potential escalation of therapy.

Comparison of Outcomes

Our study had a similar median LOS [30] and surgery rate [10] compared to prior studies, though rates of TNF inhibitor and cyclosporine administration varied. In our study, 11.6% of patients received TNF inhibitors and 3.0% received cyclosporine. In prior studies, the TNF inhibitor rate has ranged from 5.5 to 21.1% [10, 19], while cyclosporine administration rates have been as high as 22% [19]. These differences in administration rates are likely due to differing institutional practices and different patient populations.

Comparison of Predictive Factors

The variables predictive of complex hospitalization in this study were tachycardia at presentation, albumin at presentation, endoscopic inflammation, and CDI. This is largely consistent with prior findings; multiple studies have found day 3 albumin to be predictive of inpatient colectomy in UC patients [14, 15, 19]. Tachycardia approached significance for predicting prolonged LOS in a study by Kelso et al [30]. The majority of studies examining surgery and second-line therapy outcomes had likely already selected for tachycardic patients by limiting their evaluation to severe patients by TWS.

Endoscopic inflammation is a strong predictor in multiple other studies: the Mayo score had an OR of 3.17 in one study [19]. Additionally, the Ulcerative Colitis Endoscopic Index of Severity was an independent predictor of needing rescue therapy or colectomy [17, 18], even outperforming the endoscopic Mayo score [24].

Temperature [12] and CRP [12, 13, 19] were significant factors in other studies; although included in our study, these were not significant in the multivariate analysis. Though Jain et al. found fecal calprotectin of over 1000 as an independent predictor of complex hospitalization [18], the clinical utilization of fecal calprotectin during admission in our study was not high enough to perform meaningful analyses.

Validation of ACE Score

Our analyses provide external validation to the ACE score (Supplementary Fig. 1), albeit not for its original purpose

[19] of predicting non-response to IV steroids in UC patients hospitalized with flare. Here we show that the ACE score is also able to predict those patients who will have complex hospitalization with an AUC of 0.710 (CI 0.627–0.794). As previously discussed, our indices have overlapping components of albumin and inflammation on endoscopy. CRP, however, was not a significant predictor in our study. Although our equivalent early admission score had a higher specificity (85.1%) compared to the ACE score (73.3%), the higher AUC compared to that of the ACE score did not cross the statistical significance threshold ($p=0.065$).

Limitations

This study has certain limitations. First, it is a retrospective evaluation of electronic medical record data. As such, the IBD diagnoses in the medical record may not always be accurate. However, diagnosis was manually confirmed for all patients using gastroenterology consultant documentation to mitigate this possibility. Another limitation is that patients who were not tested for CDI were excluded; though it is rare for IBD patients admitted with flare symptoms to not be tested for CDI, it is possible we may be missing a portion of hospitalized IBD patients. Although we did not collect data on symptoms—such as frequency and characteristic of bowel movements—these are known to be difficult to characterize with high subjectivity in interpretation.

It may be argued that including both UC and CD patients with colonic disease creates excess heterogeneity within our study population. However, even when patients were separated by subtype, the scores performed well in both the UC and CD subgroups. We believe that including Crohn's colitis patients lends insight into a previously unstudied population, and is a strength of our study.

Although this study was not limited solely to patients who were initially treated with IV steroids, the sub-analysis focusing on such patients demonstrates the persistent ability of the proposed scores to predict noncomplex hospitalization well. A limitation of the early admission score is that patients who have endoscopy will inevitably have longer hospitalization than those who do not, thus potentially introducing bias in predicting prolonged LOS. Finally, we have a relatively small sample size; though k-fold cross-validation decreases the effect of small sample size, our findings will need external validation.

Conclusion

Here we present a simple, ED-based score to recognize those IBD patients presenting with flare who are at risk for prolonged length of stay, requiring salvage therapy, or inpatient surgery. Additionally, we report an early admission score

that includes the factors of CDI and endoscopy. These presentation and early admission scores may provide prognostic information and guide subsequent management in IBD patients who require hospitalization.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10620-021-06877-8>.

Acknowledgments None.

Funding JEA reports receiving research grants from BioFire Diagnostics; consultancy fees or honorarium from BioFire Diagnostics and Janssen; and holds U.S. patent 2012/0052124A1. JEA receives research support from the Crohn's and Colitis Foundation, the Judith & Stewart Colton Center for Autoimmunity, Lyanne and Michael Saperstein, and the NIH NIDDK Diseases K23DK124570.

Conflict of interest The authors declare that they have no conflict of interest.

References

- Kappelman MD, Rifas-Shiman SL, Porter C et al. Direct health care costs of Crohn's disease and ulcerative colitis in United States Children and Adults. *Gastroenterology* 2008;135:1907–1913
- Gibson TB, Ng E, Ozminowski RJ et al. The direct and indirect cost burden of Crohn's disease and ulcerative colitis. *J Occup Environ Med* 2008;50:1261–1272
- Sedano R, Quera R, Simian D et al. An approach to acute severe ulcerative colitis. *Expert Rev Gastroenterol Hepatol* 2019;13:943–955
- Assche GV, Vermeire S, Rutgeerts P. Treatment of severe steroid refractory ulcerative colitis. *World J Gastroenterol* 2008;14:5508–5511
- Turner D, Walsh CM, Steinhart AH et al. Response to corticosteroids in severe ulcerative colitis: a systematic review of the literature and a meta-regression. *Clin Gastroenterol Hepatol* 2007;5:103–110
- Narula N, Marshall J, Colombel J-F et al. Systematic review and meta-analysis: infliximab or cyclosporine as rescue therapy in patients with severe ulcerative colitis refractory to steroids. *Am J Gastroenterol* 2016;111:477–491
- Nguyen GC, Tuskey A, Dassopoulos T et al. Rising hospitalization rates for inflammatory bowel disease in the United States between 1998 and 2004. *Inflamm Bowel Diseases* 2007;13:1529–1535
- Bewtra M, Su C, Lewis JD. Trends in hospitalization rates for inflammatory bowel disease in the United States. *Clin Gastroenterol Hepatol* 2007;5:e1
- Ananthakrishnan AN, McGinley EL, Binion DG et al. A Nationwide analysis of changes in severity and outcomes of inflammatory bowel disease hospitalizations. *J Gastrointest Surg* 2011;15:267–276
- Aratari A, Papi C, Clemente V et al. Colectomy rate in acute severe ulcerative colitis in the infliximab era. *Digest Liver Disease* 2008;40:821–826
- Truelove SC, Witts LJ. Cortisone in ulcerative colitis. *Br Med J* 1955;2:1041–1048
- Lindgren SC, Flood LM, Kilander AF et al. Early predictors of glucocorticosteroid treatment failure in severe and moderately severe attacks of ulcerative colitis. *Eur J Gastroenterol Hepatol* 1998;10:831–836
- Travis SP, Farrant JM, Ricketts C et al. Predicting outcome in severe ulcerative colitis. *Gut* 1996;38:905–910
- Ho GT, Mowat C, Goddard CJR et al. Predicting the outcome of severe ulcerative colitis: development of a novel risk score to aid early selection of patients for second-line medical therapy or surgery. *Aliment Pharmacol Ther* 2004;19:1079–1087
- Lynch RW, Churchhouse AMD, Protheroe A et al. Predicting outcome in acute severe ulcerative colitis: comparison of the Travis and Ho scores using UK IBD audit data. *Aliment Pharmacol Ther* 2016;43:1132–1141
- Gibson DJ, Hartery K, Doherty J et al. CRP/albumin ratio: an early predictor of steroid responsiveness in acute severe ulcerative colitis. *J Clin Gastroenterol* 2018;52:e48
- Corte C, Fernandopulle N, Catuneanu AM et al. Association between the ulcerative colitis endoscopic index of severity (UCEIS) and outcomes in acute severe ulcerative colitis. *J Crohns Colitis* 2015;9:376–381
- Jain S, Kedia S, Bopanna S et al. Faecal calprotectin and UCEIS predict short-term outcomes in acute severe colitis: prospective cohort study. *J Crohns Colitis* 2017;11:1309–1316
- Grant RK, Jones G-R, Plevris N, et al. The ACE (Albumin, CRP and Endoscopy) Index in acute colitis: a simple clinical index on admission that predicts outcome in patients with acute ulcerative colitis. *Inflamm Bowel Dis*.
- Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. *New England Journal of Medicine* 1987;317:1625–1629
- Harris PA, Taylor R, Thielke R et al. Research Electronic Data Capture (REDCap)—A metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009;42:377–381
- Harris PA, Taylor R, Minor BL et al. The REDCap consortium: building an international community of software platform partners. *J Biomed Inform* 2019;95:103208
- R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing, 2013. <http://www.R-project.org/>.
- Xie T, Zhang T, Ding C et al. Ulcerative Colitis Endoscopic Index of Severity (UCEIS) versus Mayo Endoscopic Score (MES) in guiding the need for colectomy in patients with acute severe colitis. *Gastroenterol Rep (Oxf)* 2018;6:38–44
- Singh H, Nugent Z, Yu BN et al. Higher incidence of *Clostridium difficile* infection among individuals with inflammatory bowel disease. *Gastroenterology* 2017;153:e2
- Nguyen G, Kaplan G, Harris M et al. A National Survey of the Prevalence and Impact of *Clostridium difficile* infection among hospitalized inflammatory bowel disease patients. *Am J Gastroenterol* 2008;103:1443–1450
- Khanna S, Shin A, Kelly CP. Management of *Clostridium difficile* infection in inflammatory bowel disease: expert review from the clinical practice updates committee of the AGA institute. *Clin. Gastroenterol. Hepatol.* 2017;15:166–174
- Liu K, Bellaguarda E, Gregory D et al. The role of intravenous corticosteroids in patients with inflammatory bowel disease and *Clostridium difficile* infection: 1905. *Off J Am College Gastroenterol* 2015;110:S810
- Erlach J, Rubin DT. Predictors of IBD-related length of hospital stay and 30 day readmission in a tertiary center: 602. *Am J Gastroenterol* 2016;111:S277
- Kelso M, Weideman RA, Cipher DJ et al. Factors associated with length of stay in veterans with inflammatory bowel disease hospitalized for an acute flare. *Inflamm Bowel Dis* 2018;24:5–11
- Nguyen G, Sam J. Rising prevalence of venous thromboembolism and its impact on mortality among hospitalized inflammatory bowel disease patients. *Am J Gastroenterol* 2008;103:2272–2280

32. Karagozian R, Johannes RS, Sun X et al. Increased mortality and length of stay among patients with inflammatory bowel disease and hospital-acquired infections. *Clin Gastroenterol Hepatol* 2010;8:961–965
33. Issa M, Vijayapal A, Graham MB et al. Impact of *Clostridium difficile* on inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2007;5:345–351

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Appendix 9.10

March 8, 2022

Daniel Freedberg
751800X - MED Medicine

Protocol Number: IRB-AAAS2576

Title: Inulin the ICU

Protocol Version #: 1

Grant #: PR181960

Approval Date: 02/23/2022 Expiration Date: 02/22/2023

Event Identifier: Renewal (Y04M00)

The above-referenced event was reviewed by Columbia University IRB 5.

Level of review and outcome: Convened IRB review IRB Meeting Date: 02/23/2022

To view a list of documents that were included in this approval (if applicable) and all other currently approved documents for this study, please refer to the Print Menu for this Event in Rascal. It is important to confirm the status of each document, e.g., active, stamped, etc. Only stamped, active documents can be used with research participants.

Study Status: Open to enrollment or ongoing review of records/specimens

Consent Requirements:

Informed consent with written documentation will be obtained from the research participant or appropriate representative

HIPAA Authorization:

Authorization will be obtained

Please access the following link for a list of tasks that should be addressed prior to your next submission:

<https://www.rascal.columbia.edu/irb/protocol/AAAS2576/353206/researcherTasks>

Reminders:

1. A request for continuation or completion of a research protocol is due at least 60 days before this research protocol's expiration date, unless otherwise requested by the Board. This renewal was submitted on 2/14/2022 with an expiration of 3/16/2022.

2. As you plan on enrolling Spanish speaking subjects, administrative IRB approval of the Spanish-translated patient-facing documents (e.g., consent, recruitment materials, questionnaires, etc.) is required prior to their enrollment. Please see the IRB's policy on the Enrollment of Non-English Speaking Subjects in Research for further details

<https://research.columbia.edu/sites/default/files/content/HRPO/Nonenglishspeakingsubjects.Revised.FINAL%20111909.pdf>

Modifications to the protocol included with this renewal:

- Department of Defense Annual Report (for renewal process)
- DSMB_deaths
- DSMB_June_2021

Electronically signed by: Collazo, Yaritza

Researcher Responsibilities:

Any proposed changes in the protocol must be immediately submitted to the IRB for review and approval prior to implementation, unless such a change is necessary to avoid immediate harm to the participants.

Any unanticipated problems that involve risks to subjects must be reported to the IRB in accordance with the Unanticipated Problems: Reporting to the IRB of Unanticipated Problems Involving Risks policy. All submissions for modifications and unanticipated problems must be submitted through Rascal.

Renewal applications should be submitted 60 days before the expiration date of this study through Rascal. Failure to obtain renewal of your study prior to the expiration date will require discontinuance of all research activities for this study, including enrollment of new subjects.

You must file a Closure Report in Rascal when your study has been completed.