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# Impact of the Ileal Microbiota on Surgical Site Infections in Crohn's Disease: A Nationwide Prospective Cohort

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## Abstract

**Background and Aims:** Surgery is performed in 50–70% of Crohn's disease [CD] patients, and its main risk is surgical site infection [SSI]. The microbiota has been extensively assessed in CD but not as a potential risk factor for septic morbidity. The objective of this study was to assess the impact of the gut microbiota on SSI in CD.

**Methods:** We used the multicentric REMIND prospective cohort to identify all patients who experienced SSI after ileocolonic resection for CD, defined as any postoperative local septic complication within 90 days after surgery: wound abscess, intra-abdominal collection, anastomotic leakage or enterocutaneous fistula. The mucosa-associated microbiota of the ileal resection specimen was analysed by 16S gene sequencing in 149 patients. The variable selection and prediction were performed with random forests [R package VSURF] on clinical and microbiotal data. The criterion of performance that we considered was the area under the Receiver Operating Characteristic [ROC] curve [AUC].

**Results:** SSI occurred in 24 patients [16.1%], including 15 patients [10.1%] with major morbidity. There were no significant differences between patients with or without SSI regarding alpha and beta diversity. The top selected variables for the prediction of SSI were all microbiota-related. The maximum AUC [0.796] was obtained with a model including 14 genera, but an AUC of 0.78 had already been obtained with a model including only six genera [*Hungatella*, *Epulopiscium*, *Fusobacterium*, *Ruminococcaceae\_ucg\_009*, *Actinomyces* and *Ralstonia*].

**Conclusion:** The gut microbiota has the potential to predict SSI after ileocolonic resection for CD. It might play a role in this frequent postoperative complication.

**Key Words:** Gut microbiota; Crohn's disease; prediction

## Background and context:

The main risk associated with surgery in Crohn's disease [CD] is surgical site infection [SSI]. Several clinical parameters have been already described. The gut microbiota is involved in the pathogenesis of CD and in postoperative recurrence.

## New findings:

In this nationwide, French, population-based cohort study of patients with CD-related intestinal surgery [ileocolonic resection], we identified a microbiota-based model able to accurately predict surgical site infection by a random forest-based approach.

## Limitations:

This analysis involved patients with ileocolonic resection for which clinical parameters were known, and patient management took them into account. The importance of clinical parameters should not be underestimated.

## Impact:

Our study shows promising results to better define patients at risk of SSI using gut microbiota and represents a therapeutic target to prevent surgical site infection in CD.

## 1. Introduction

Despite significant progress in the medical treatment of Crohn's disease [CD], at least 50–70% of patients eventually undergo surgery.<sup>1</sup> Indeed, in medical treatment failure, complications [haemorrhage, perforation, obstruction] and neoplasia, surgery is indicated.<sup>2</sup> Serious postoperative complications such as anastomotic leak and intra-abdominal sepsis occur more frequently in CD than in other indications for intestinal resection.<sup>3</sup> The short-term outcomes after surgery for ileocolonic CD have been markedly improved by better perioperative management,<sup>4</sup> and the introduction of the laparoscopic approach.<sup>5</sup> However, despite better knowledge of the risk factors for surgical complication in CD,<sup>6–9</sup> the occurrence of serious complications remains high. Therefore, some of these risk factors probably still remain unknown.

The gut microbiome has emerged as a major actor in several diseases<sup>10</sup> and was shown to play a role in postoperative recurrence in CD.<sup>11,12</sup> It might also play a role in postoperative complications such as anastomotic leakage, which is associated with a low microbial diversity and a higher abundance of mucin-degrading members of the families Bacteroidaceae and Lachnospiraceae.<sup>13</sup> Despite a huge number of studies exploring the alterations of the microbiome in inflammatory bowel disease [IBD],<sup>14</sup> there is a lack of information about its potential impact in postoperative septic complications.

In this context, we analysed a large prospective multicentric cohort of patients with CD who underwent ileocolonic resection<sup>11,15</sup> with the aim of identifying predictors of postoperative surgical site infection [SSI].

## 2. Patients and Methods

### 2.1. Study design and patient selection

The present study was undertaken in parallel with a prospective multicentre study performed by the REMIND study

group and aimed to identify predictors of early postoperative endoscopic recurrence.<sup>16</sup> Inclusion criteria were age over than 18 years, ileal or ileocolonic CD, and an indication of CD-related intestinal surgery [ileocolonic resection] in the absence of intestinal dysplasia or cancer. Mucosal samples were collected from the surgical specimen in the inflamed portion of the ileum.

All patients presenting with SSI were identified in the prospective database. All centres involved were expert in IBD, with surgeons taking into account clinical and biological risk factors for septic complications that were known until 2015, to choose between stoma creation and primary anastomosis, namely: pre-operative steroids within 3 months before surgery, malnutrition, smoking habit, intra-operative abscess or fistula, and iterative surgery.<sup>9,17</sup> Stoma creation was chosen when there were at least three of these risk factors. Pre-operative anti-tumour necrosis factor  $\alpha$  [anti-TNF $\alpha$ ] therapy and anaemia <10 g/dL were not included as risk factors by the surgeons as they were identified in 2016.<sup>8</sup>

The collected clinical data included the following: demographic data, such as gender, age at the time of surgery, body mass index [BMI], malnutrition and smoking status; pre-operative data, such as CD phenotype according to the Montreal classification,<sup>17,18</sup> associated perianal disease, CD activity according to the Harvey Bradshaw index,<sup>19</sup> CD medical treatment within the 3 months before surgery [i.e. steroids, immunomodulators and biotherapies, such as anti-TNF $\alpha$ ], systemic antibiotics within 1 month before surgery, pre-operative blood count and parenteral nutritional support; intra-operative data, such as the type of surgical approach [i.e. open or laparoscopic], operative time, and the presence of a primary anastomosis; and postoperative data, such as SSI, haemorrhage, major morbidity, unplanned surgery with or without stoma creation, and mortality.

### 2.2. Outcome definitions

SSI was defined as any postoperative local septic complication, with or without systemic sepsis<sup>20</sup> and occurring until Day 90 after surgery, namely: wound abscess, enterocutaneous fistula, intra-abdominal collection treated by antibiotics and/or drainage, and anastomotic leakage. The diagnosis of anastomotic leakage was confirmed by computed tomography [CT] with iodine injection and CT enterography; intra-abdominal collections located around the anastomosis were considered as anastomotic leakage. Major morbidity was defined as any complication requiring surgical, radiological or endoscopic intervention [Clavien-Dindo III], life-threatening complications requiring intensive care management [Clavien-Dindo IV] or death [Clavien-Dindo V].<sup>21</sup> Malnutrition was defined as a BMI  $\leq 18.5$  kg/m<sup>2</sup>, weight loss >10% or albuminaemia <30 g/L. All patients had a clinical review 1 month after discharge, and complications occurring after hospitalization were collected.

### 2.3. Ileal biopsies collection, DNA extraction and 16S DNA sequencing and analysis

Biopsies collected on ileal resection specimens were used for global microbiota analysis (samples stored at Nice Hospital Biobank [BB-0033-00025], University Côte d'Azur, France). DNA was extracted and 16S gene sequencing data were generated as previously described.<sup>11</sup> Briefly, a 16S rDNA fragment comprising the V3 and V4 hypervariable regions was amplified using the following

primers: 16S sense 5'-TACGGRAGGCAGCAG-3' and anti-sense 5'-CTACCNGGGTATCTAAT-3', as previously described.<sup>11</sup> Sequencing was performed using a 300-bp paired-end sequencing protocol on an Illumina MiSeq platform [Illumina] at GenoScreen. Raw sequence data [already published]<sup>11</sup> are accessible in the European Nucleotide Archive [Accession number PRJEB31684]. Following sequencing, raw paired-end reads were processed in a data curation pipeline that includes a step of removal of low-quality reads [Qiime2 2020.6].<sup>22</sup> Remaining sequences were assigned to samples based on barcode matches, and barcode and primer sequences were then trimmed. The sequences were denoised using the DADA2 method, and reads were classified using the Silva reference database [version 138].<sup>23</sup> Alpha and beta diversity were computed using the phyloseq package [v1.24.2].<sup>24</sup> The number of observed features and Shannon indexes were calculated using rarefied data [depth = 6000 sequences per sample] and used to characterize alpha diversity.

## 2.4. Statistical analyses

The quantitative data are reported as means and standard deviations, and compared using the two-tailed non-parametric Mann–Whitney test. The qualitative data are reported as the number of patients [percentage of patients] and were compared using Pearson's  $\chi^2$  test or Fisher's exact test, as appropriate. GraphPad Prism v6.0 and R software v3.5.2 [R Foundation for Statistical Computing] were used for analyses and graph preparation.

The MaAsLin2 R package was used to find associations between SSI and microbial community abundance.<sup>25</sup> The following potential confounding factors were taken into account in the analysis: corticosteroid, thiopurine or methotrexate treatments in the 3 months before surgery, antibiotics and anti-TNF $\alpha$  in the last month before surgery, preoperative nutritional support, primary anastomosis [or stoma], planned or emergency surgery, laparoscopic [vs laparotomy] surgery, the type of anastomosis, gender, presence of inflammation or of a stenosis seen during the surgery. Default statistical parameters were used [ $p < 0.05$  with  $q < 0.25$  were considered significant].

The R package VSURF [Variable Selection Using Random Forests] was used to perform variable selection and prediction by minimizing the classification error. This method assigned a score to each variable, which allowed us to rank the variables by decreasing order of importance. It also computed a threshold such that all variables with a score lower than this threshold were considered not to be useful for explaining the outcomes and could therefore be discarded from the model. However, to avoid overfitting of the data, 80% of the initial population was randomly sampled several times and VSURF was trained on each subsample. The final score of each variable was the average value obtained over all subsamples. Moreover, the final model only contained variables that had been selected at least 50% of the times, in order to exclude variables contributions for which might have been overestimated in a few subsamples. All variables were then ranked by decreasing order of importance so that it only remained to decide how many variables would be kept in the final model. This number was chosen according to two goals: first, determining which subset of variables provided the best results in terms of prediction score, and second, what was the minimum number of variables that were necessary to reach a reasonable predictive performance.

The criterion of performance that we considered was the area under the Receiver Operating Characteristic [ROC] curve, referred to as AUC. For a subsample of size 80% of the initial population, we performed the previously described algorithm and then predicted the outcomes on the remaining 20% of the population, which we compared to the actual outcomes. This procedure also allowed us to compute empirical confidence intervals, bounds of which were the values that contained 95% of the AUC obtained over all subsamples. We used the R package pROC to create the ROC curves.

## 3. Results

### 3.1. Characteristics of the study population

In total, 149 patients from the REMIND cohort enrolled between September 1, 2010 and September 30, 2017 were included. Their characteristics are presented in Table 1. Mean age was  $35 \pm 12$  years, and 74 patients [49.7%] were men, presenting with predominantly ileal or ileo-colonic involvement [55% and 43.6%, respectively]. CD phenotype was divided into stricturing [49.7%], penetrating [34.9%] and inflammatory [15.4%]. Twenty-four patients presented with SSI [16.1%], including intra-abdominal sepsis in 18 patients [12.1%], anastomotic leakage in eight [5.4%], intra-abdominal collection in 15 [10.1%] and wound-abscesses in six [4%].

Malnutrition was more frequent and albumin level was lower in SSI patients [Table 1]. The other pre-operative items were comparable between groups: overall, 69 patients [46.3%] received anti-TNF $\alpha$ , and 55 patients [36.9%] received steroids within 3 months before surgery. Thirty patients [20.1%] had a history of previous intestinal resection. Forty-eight patients [32.2%] required pre-operative nutritional support, which was parenteral in 26 cases [17.4%].

Regarding intra- and postoperative factors, 39 patients [26.2%] had no primary anastomosis because of septic risk factors, which was not different between groups [ $p = 0.72$ ]. Major morbidity occurred in 16 cases [10.7%] and was significantly more frequent with SSI [58.3% vs. 1.6%,  $p < 0.001$ ], along with unplanned surgery [41.7% vs. 0.8%,  $p < 0.001$ ], stoma creation [20.8% vs. 0%,  $p < 0.001$ ] and radiological drainage [20.8% vs. 0%,  $p < 0.001$ ]. The only unplanned surgery which was not performed for SSI was indicated by postoperative haemorrhage. There were no postoperative deaths.

### 3.2. Ileal mucosa-associated microbiota features associated with surgical site infection

The gut microbiota analysis did not show any difference according to the occurrence of SSI in term of alpha or beta diversity [Figure 1A–C; Supplementary Figure 1].

To identify the microbial features associated with SSI occurrence and control for potential confounding factors, we used the second version of Microbiome Multivariable Associations with Linear Models [Maaslin2, see 'Patients and methods' for details], which relies on general linear models to determine multivariable associations between phenotypes, covariates and gut microbiota data.<sup>25</sup> The strongest signals was observed with the genera *Fusobacterium*, *Epulopiscium* and *Hungatella* for which an increased abundance was significantly associated with SSI occurrence [Figure 1D].

Intestinal inflammation [defined by the presence of patent inflammation during surgery or fistula] was associated with

**Table 1.** Perioperative characteristics of 149 patients undergoing intestinal resection for Crohn's disease

	Total, <i>n</i> = 149		SSI, <i>n</i> = 24		No SSI, <i>n</i> = 125		Univariate <i>P</i>
Men	74	[49.7]	14	[58.3]	60	[48.0]	0.35
Mean age at surgery [years]	35	[±12]	32	[±13]	35	[±12]	0.21
BMI <sup>a</sup>	21.6	[±4]	21.2	[±4.5]	21.7	[±4.1]	0.61
Active smoking at surgery	16	[10.7]	5	[20.8]	11	[8.8]	0.14
Montreal A							0.93
A1	18	[12.1]	2	[8.3]	16	[12.8]	
A2	112	[75.2]	19	[79.2]	93	[74.4]	
A3	19	[12.7]	3	[12.5]	16	[12.8]	
Montreal L							0.30
L1	82	[55.0]	12	[50.0]	69	[55.2]	
L2	1	[0.7]	1	[4.2]	0	[0]	
L3	65	[43.6]	11	[45.8]	54	[43.2]	
L4	1	[0.7]	0	[0]	1	[0.8]	
Montreal B							0.57
B1	23	[15.4]	2	[8.3]	21	[16.8]	
B2	74	[49.7]	12	[50.0]	61	[48.8]	
B3	52	[34.9]	10	[41.7]	42	[33.6]	
Surgical indication:							0.827
Symptomatic stenosis resistant to medical treatment	79	[53.0]	11	[45.8]	68	[54.4]	
Inflammatory form resistant to medical treatment	8	[5.4]	1	[4.2]	7	[5.6]	
Fistulizing presentation	43	[28.9]	8	[33.3]	35	[28.0]	
Combination of several criteria	19	[12.8]	4	[16.7]	15	[12.0]	
Anoperineal lesion	34	[22.8]	5	[20.8]	29	[23.2]	1
<b>Malnutrition</b>	<b>43</b>	<b>[28.9]</b>	<b>15</b>	<b>[62.5]</b>	<b>28</b>	<b>[22.4]</b>	<b>&lt;0.001</b>
<b>Albumin [g/L]<sup>b</sup></b>	<b>34.7</b>	<b>[±6.0]</b>	<b>30.6</b>	<b>[±6.5]</b>	<b>35.5</b>	<b>[±5.7]</b>	<b>0.001</b>
Previous intestinal resection	30	[20.1]	3	[12.5]	27	[21.6]	0.41
Preoperative steroids	23	[15.4]	5	[20.8]	18	[14.4]	0.54
Preoperative IS	11	[7.4]	0	[0]	11	[8.8]	0.21
Preoperative anti-TNFα	31	[20.8]	4	[16.7]	27	[21.6]	0.79
Parenteral nutrition	26	[17.4]	5	[20.8]	21	[16.8]	0.57
HB index	5.1	[±3.9]	5.8	[±3.1]	5.0	[±4.1]	0.36
Preoperative antibiotics	86	[57.7]	14	[58.3]	72	[57.6]	0.95
Laparoscopic surgery	82	[55.0]	14	[58.3]	68	[54.4]	0.72
Primary anastomosis	110	[73.8]	17	[70.8]	93	[74.4]	0.72
<b>SSI</b>	<b>24</b>	<b>[16.1]</b>	<b>24</b>	<b>[100]</b>	<b>0</b>	<b>[0]</b>	<b>&lt;0.001</b>
<b>Only wall abscess</b>	<b>6</b>	<b>[4.0]</b>	<b>6</b>	<b>[25.0]</b>	<b>0</b>	<b>[0]</b>	<b>&lt;0.001</b>
<b>Clavien-Dindo ≥ 3</b>	<b>16</b>	<b>[10.7]</b>	<b>14</b>	<b>[58.3]</b>	<b>2</b>	<b>[1.6]</b>	<b>&lt;0.001</b>
Haemorrhage	5	[3.4]	2	[8.3]	3	[2.4]	0.18
<b>Unplanned surgery</b>	<b>11</b>	<b>[7.4]</b>	<b>10</b>	<b>[41.7]</b>	<b>1</b>	<b>[0.8]</b>	<b>&lt;0.001</b>
with stoma	5	[3.4]	5	[20.8]	0	[0]	<0.001

Data are reported as *n* [%] or means and standard deviation [±]. SSI = surgical site infection; BMI = body mass index; IS = immuno-suppressive drugs; anti-TNFα = anti-tumor necrosis factor alpha; HB index = Harvey-Bradshaw index.

Statistically significant differences are indicated in bold.

<sup>a</sup>Missing data for 17 patients.

<sup>b</sup>Missing data for 24 patients.

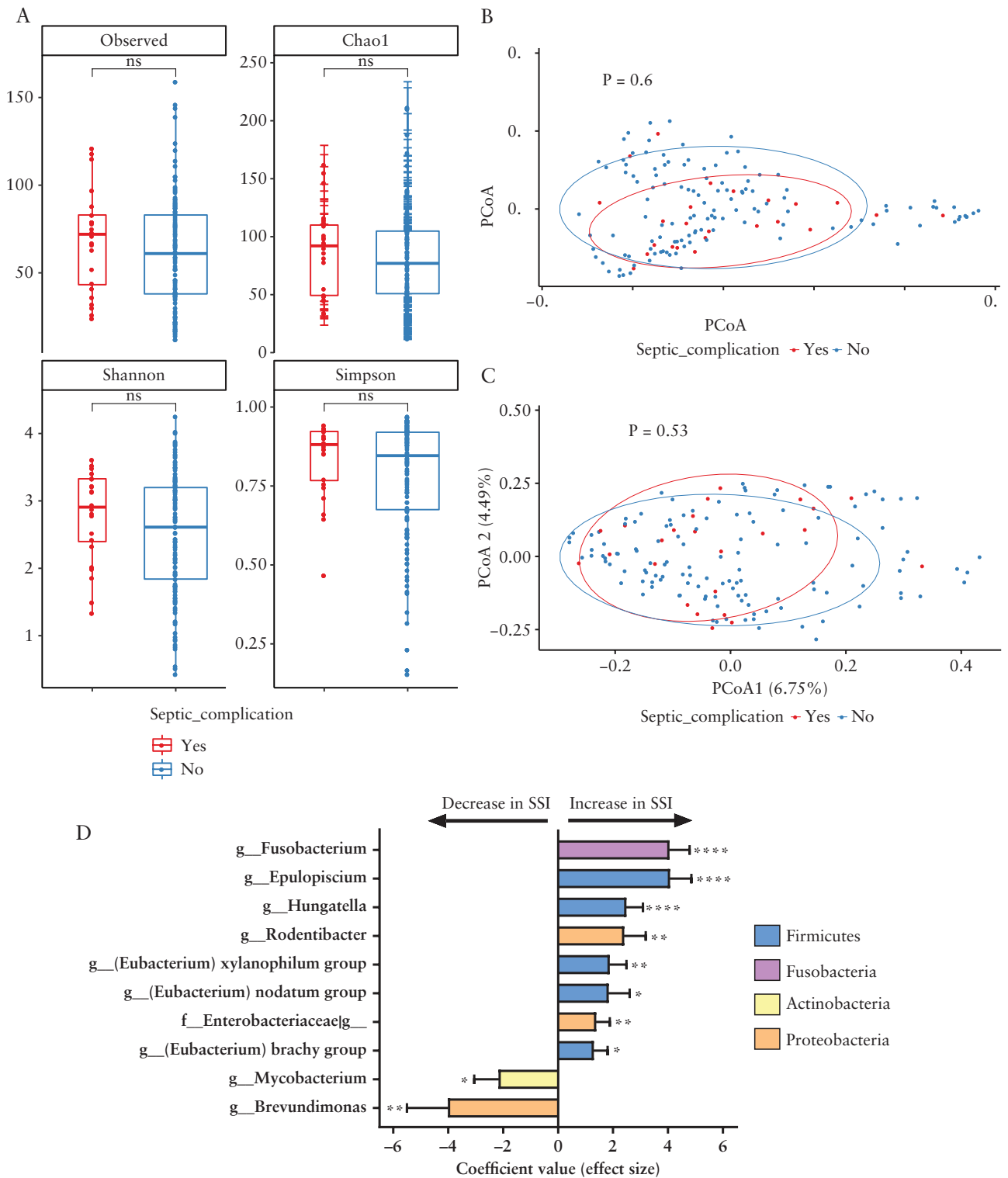
alterations in alpha and beta diversity [Supplementary Figure 2A and B]. Maaslin2 identified several taxa significantly associated with intestinal inflammation [Supplementary Figure 2C].

### 3.3. Ileal mucosa-associated microbiota at surgery as a predictor of SSI

To identify factors predicting SSI, we used a random forest-based approach taking into account both clinical param-

eters [gender, age, CD phenotype, malnutrition, smoking status, previous surgery, occurrence of abscess or fistula during the surgery, pre-operative treatment including steroids, anti-TNFα and antibiotics, laparoscopic approach, and anastomosis characteristics] and microbiota features at the genus level.

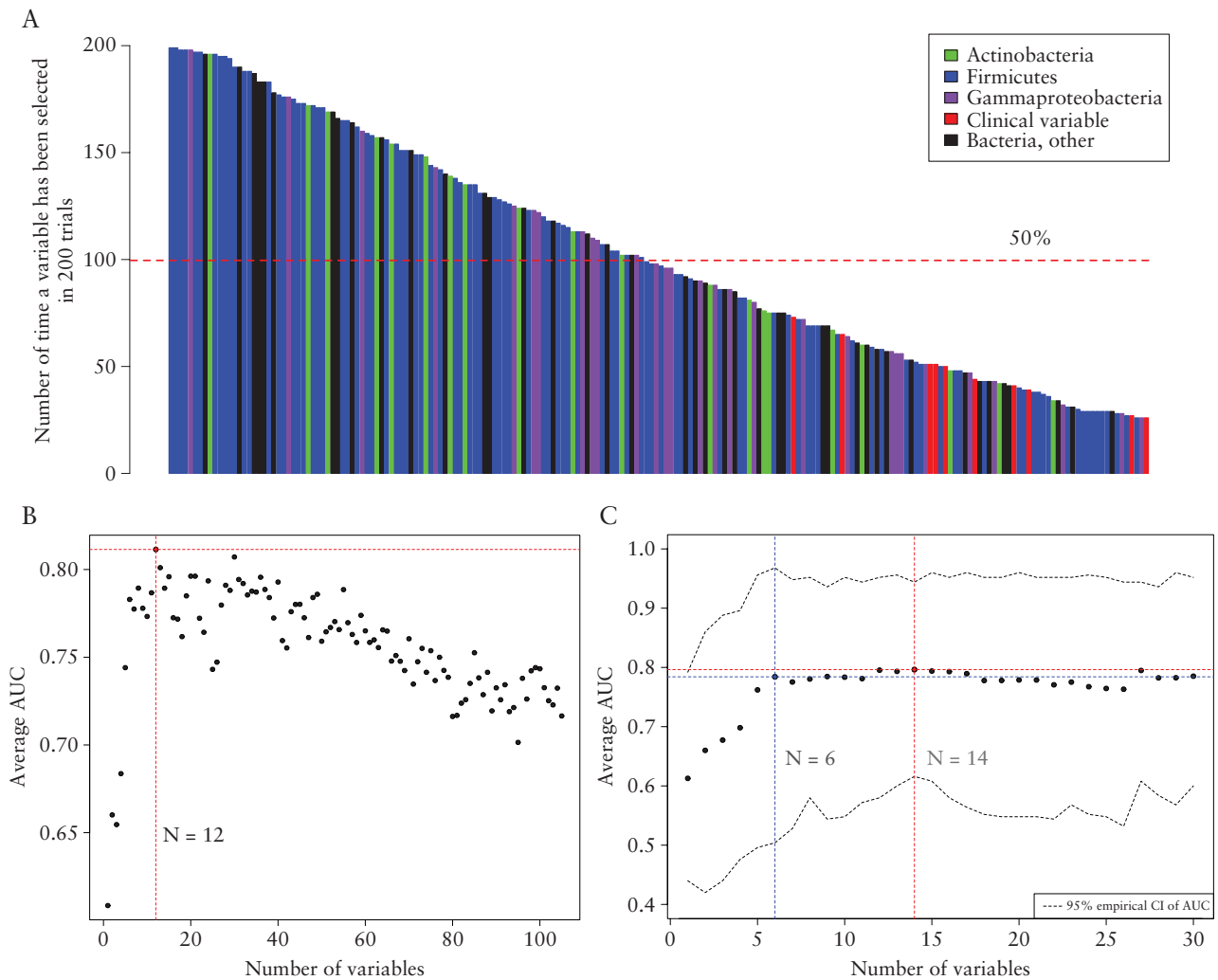
For this, 80% of the initial population was randomly sampled 200 times and VSURF was applied on each subsample, allowing us to rank the variables according to their respective



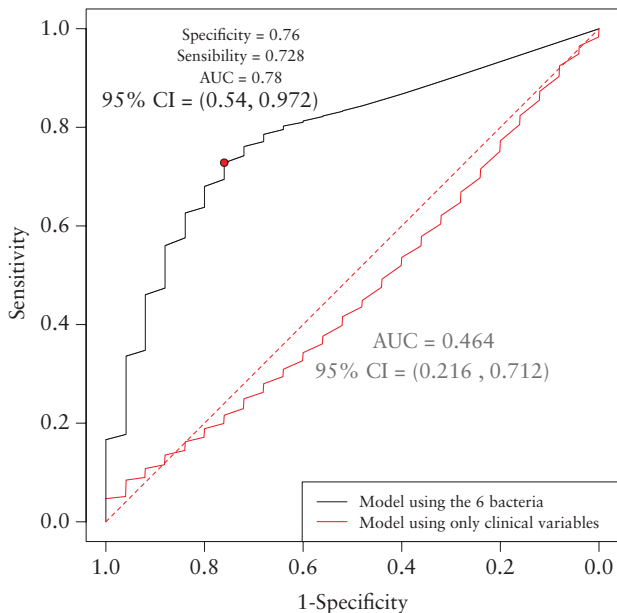
**Figure 1.** Ileal mucosa-associated microbiota features associated with surgical site infection. [A] Alpha diversity; [B] beta diversity [Bray–Curtis]; [C] beta diversity [Jaccard]; [D] bacterial taxa-associated SSI detected using Maaslin2. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$ .

importance in predicting models. Strikingly, no single clinical variable was among the most relevant, and only gut microbiota features were selected [Figure 2A]. We then determined the optimal number of variables [N] to include in the predictive model. In a first analysis, we tested one to more than 100 variables and evaluated the AUC in 100 trials, showing that the optimal number of variables to include in the model was

around 12 [Figure 2B]. To refine this result, we performed the same analysis testing one to 30 variables in 1000 trials. Although the maximum AUC [0.79] was obtained for  $N = 14$ , only six variables was sufficient to get an AUC of 0.78 (95% confidence interval [CI] 0.55–0.96) [Figures 2C and 3]. As expected, a model including only the clinical variables was not relevant [Figure 3]. The variables ranked according to their



**Figure 2.** Construction of the predictive model of SSI. [A] Number of times a variable was selected by VSURF in 200 trials; [B] average AUC on 100 trials using random forests according to the number of variables in the model; [C] average AUC on 1000 trials using random forests according to the number of variables in the model.



**Figure 3.** Performance of the predictive model: average ROC curves generated by random forests on 1000 random trials.

importance in the model are shown in Table 2. Interestingly, the first three genera [*Hungatella*, *Epulopiscium* and *Fusobacterium*] were also the best ones identified in Maaslin2 analysis [Figure 1D].

### 3.4. Low albuminaemia is a predictor of SSI but ileal mucosa-associated microbiota features improve the prediction accuracy

As pre-operative malnutrition [defined as a BMI  $\leq 18.5$  kg/m<sup>2</sup>, weight loss >10% or albuminaemia <30 g/L] was significantly more frequent in SSI patients than in non-SSI patients, we explored this parameter further. Malnutrition was not associated with changes in alpha or beta diversity [Supplementary Figure 3A and B], but Maaslin2 analysis identified several taxa associated with it [Supplementary Figure 3C]. We then focused on albuminaemia. We re-ran our analysis excluding the 24 patients [16.1% of the global population] in which albuminaemia was not available. Among the most relevant variables, the only clinical one selected by VSURF was albuminaemia [Figure 4A]. When albuminaemia was included in the predictive model, it was necessary to add six more microbiota variables to reach the accuracy we obtained in the whole population with six microbiota variables alone [Figure 4B].

**Table 2.** AUC according to the number of variables in the predictive model

Number of variables	Genus added to the model	Average AUC on 1000 trials	Number of times the variable was selected by VSURF
1	g_Hungatella	0.612	197
2	g_Epulisium	0.659	190
3	g_Fusobacterium	0.677	190
4	g_Ruminococcaceae_ucg_009	0.698	118
5	g_Actinomyces	0.762	196
6 <sup>a</sup>	g_Ralstonia	0.784	160
7	g_Anaerostipes	0.775	102
8	g_Eubacterium_brachy_group	0.78	172
9	g_Ruminococcaceae_ucg_004	0.784	126
10	g_Pseudoxanthomonas	0.783	125
11	g_Roseburia	0.781	199
12	g_Eubacterium_eligens_group	0.795	135
13	g_Lachnoanaerobaculum	0.793	120
14	g_Peptoniphilus	0.796	183
15	g_Bradyrhizobium	0.793	169
16	g_Gemella	0.792	151
17	g_Blautia	0.789	151
18	g_Campylobacter	0.777	129
19	g_Uba1819	0.778	144
20	g_Erysipelatoclostridium	0.778	127
21	g_Roseomonas	0.778	131
22	g_Eubacterium_nodatun_group	0.77	171
23	g_Terrisporobacter	0.775	129
24	g_Anaerotruncus	0.767	156
25	g_Olsenella	0.764	148
26	g_Rothia	0.763	124
27	g_Cellulomonas	0.794	172
28	f_Ruminococcaceae g_uncultured_9	0.782	199
29	g_Acidovorax	0.782	198
30	g_Butyricoccus	0.785	175

<sup>a</sup>The 6th row provides the AUC for the model that contains the top six variables.

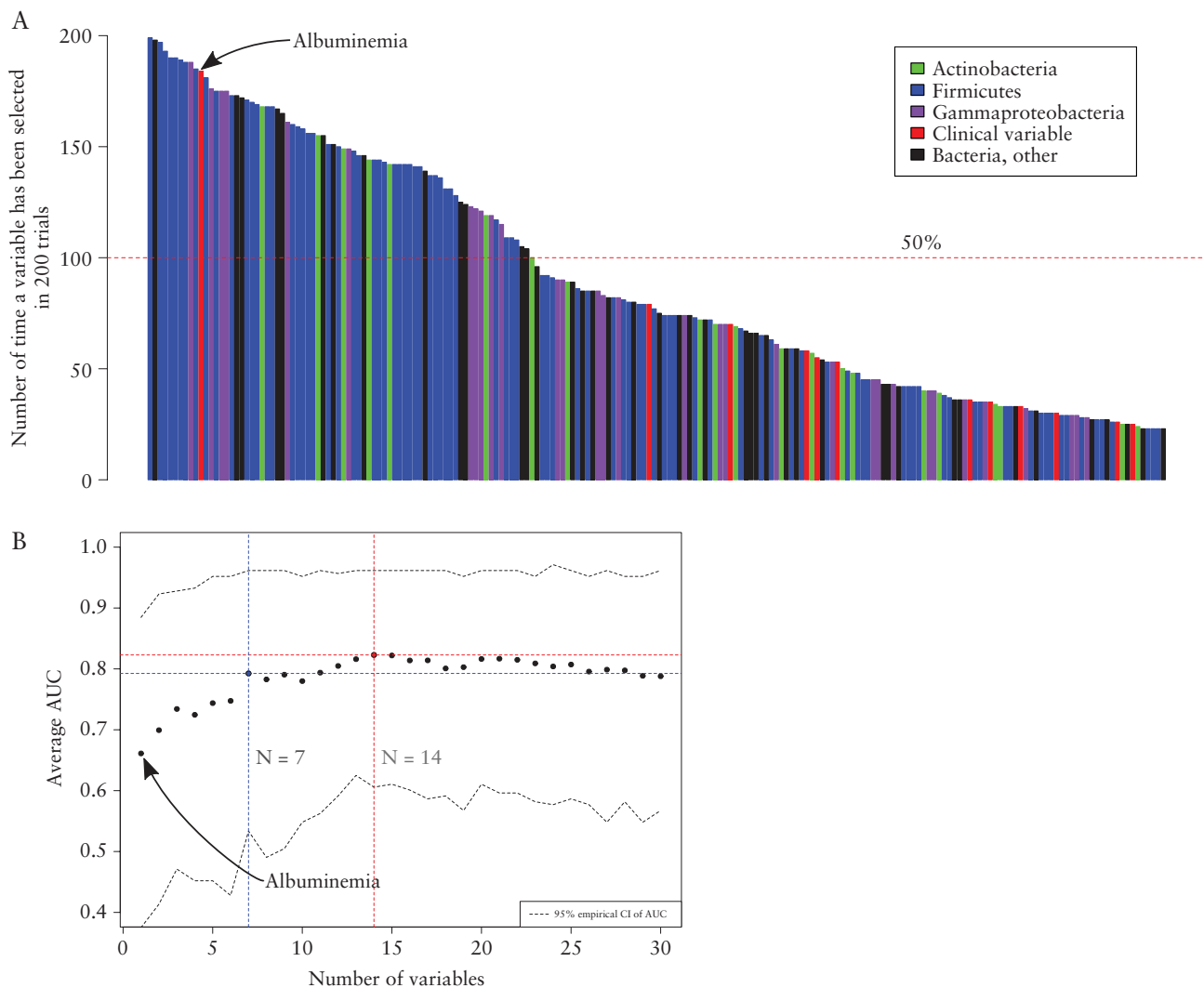
#### 4. Discussion

In this study, we showed that the ileal mucosa-associated microbiota might serve as a predictive factor for SSI in patients with CD. SSI represents a challenge in the surgical management of CD patients.<sup>3</sup> Several risk factors are known and have already been well studied, such as smoking, malnutrition, corticosteroid therapy within 3 months before surgery, and abscess or fistula during surgery.<sup>9,17</sup> Other risk factors remain of debate, such as previous surgical resection,<sup>7,9,17</sup> anti-TNF $\alpha$  therapy, operating time and pre-operative anaemia.<sup>6-8</sup> However, these factors alone may not explain every septic complication. In this study, we investigated the potential role of the ileal mucosa-associated microbiota at the time of surgery as a predictive factor for SSI in CD, a condition particularly involving gut microbiota alterations.<sup>26,27</sup> Using a random forest-based approach, we identified a microbiota-based model that is able to accurately predict SSI. Interestingly, just six genera [i.e. *Hungatella*, *Epulisium*, *Fusobacterium*, *Ruminococcaceae\_ucg\_009*, *Actinomyces*, *Ralstonia*] was enough to achieve an AUC of 0.78 [95% CI 0.55–0.96]. Among these genera, the three most important in the predictive model were also identified by Maaslin2.

None of the clinical parameters studied [including malnutrition but not albuminaemia per se] were selected in the predictive model. This result may be explained by the fact that these factors were already integrated in the surgical strategy.<sup>4,6-9,17</sup> Such findings have already been observed in the recent prospective study by Brouquet *et al.*<sup>8</sup> In their study, ileo-colostomy was more frequent than in the previous retrospective cohorts that have defined the clinical risk factors for postoperative sepsis.<sup>9,17</sup> Indeed, in Brouquet *et al.* as well as in the present study, primary anastomosis was contraindicated when three or more of the following risk factors were present: pre-operative steroids within 3 months before surgery, malnutrition, smoking habit, intraoperative abscess or fistula, and iterative surgery. Such changes in the modern surgical management of CD patients explain why previous clinical risk factors have been ‘erased’ from statistical analysis in recent studies, but these risk factors should still be taken into account.

We then focused on the potential role of pre-operative nutritional status as it was different in patients with or without SSI. Although no alteration in alpha or beta diversity was associated with malnutrition, changes in several taxa were noted, in accordance with previous studies showing an al-





**Figure 4.** Predictive model of SSI taking albuminaemia into account. [A] Number of times a variable was selected by VSURF in 200 trials; [B] average AUC on 1000 trials using random forests according to the number of variables in the model.

teration of the gut microbiota in malnourished children.<sup>28–30</sup> When we studied the subgroup of patients in which data for albuminaemia level at surgery were available, it was selected in the SSI predictive model. Nevertheless, the predictive efficiency of albuminaemia alone was low and adding microbiota features improved the model. Indeed, a model including albuminaemia and six gut microbiota features was roughly as good as one with six microbiota features alone.

In the present series, SSI occurred in 16.1% of patients, including 12.1% with intra-abdominal sepsis and 6% with wound abscess. These results were consistent with the literature, in which intra-abdominal septic morbidity is the most common complication in this high-risk population.<sup>17,31</sup> In their case-matched study of 2007, Alves *et al.* reported 9.3% with intra-abdominal sepsis and 7.3% with wound abscess.<sup>17</sup> Ten years later, Brouquet *et al.* found 8% with intra-abdominal sepsis and 4% with wound abscess.<sup>31</sup> This shows that despite improvements in both medical therapies and knowledge of clinical risk factors for septic morbidity, the incidence of such morbidity remains quite stable, implying that some other risk factors remain unknown. Improving our knowledge of these risk factors is also especially relevant in this setting, as

the consequences of SSI are serious in this young CD population, including 58.3% with major morbidity, 41.7% with unplanned surgery and 20.8% with unplanned stoma creation in the present series.

The predictive ability of the microbiota regarding septic morbidity is relatively high in our study. However, this does not preclude a causality link between the gut microbiota alteration and SSI, as microbiota alterations and SSI might be under the influence of similar factors such as inflammation and oxygen exposure. Indeed, Winter *et al.* explained that the host inflammatory response generates by-products [namely, electron acceptors] that modify the microbiota as these electron acceptors selectively feed facultative anaerobic bacteria, thereby increasing their prevalence.<sup>32</sup> The bacterial variations between SSI and non-SSI patients could therefore be explained as a consequence of a more aggressive disease and not as the potential cause of SSI. This hypothesis is supported by a recent publication showing a correlation between CD severity, faecal amino acid concentrations and/or bacterial nitrogen flux.<sup>33</sup> Regarding the use of antibiotic therapy, which could have theoretically explained these variations, the criterion of preoperative antibiotic therapy was collected and taken into

account in the analysis of clinical co-factors. Moreover, peri-operative antibiotic prophylaxis was homogeneous in all patients, and in accordance with the French recommendations.<sup>34</sup>

Interestingly, the bacterial genera identified in the predictive model have already been associated with IBD features. *Fusobacterium* abundance is increased in both faecal and mucosal tissues of patients with CD.<sup>14,15,35</sup> This pathogen is considered to be pro-inflammatory and pro-oncogenic.<sup>35</sup> Moreover, *Fusobacterium* has been previously associated with postoperative and chronic inflammation in both CD and ulcerative colitis [UC].<sup>36</sup>

*Epulopiscium* abundance was shown to be increased in patients with active IBD, and particularly CD, compared to inactive IBD or healthy controls.<sup>37</sup> Compared to healthy subjects, patients with CD requiring an ileocecal resection exhibited an increased abundance of *Epulopiscium* in their mucosal microbiota.<sup>12</sup> Interestingly, *Epulopiscium* has been associated with newly diagnosed CD in paediatric populations.<sup>38</sup>

Recently, in a paediatric study, *Hungatella* was found to be decreased in patients with CD not responding to exclusive enteral nutrition in comparison to healthy subjects or responders.<sup>39</sup> Similarly, in an adult CD cohort, exclusive enteral nutrition was associated with an increase in *Hungatella*.<sup>40</sup> Thereby, preoperative management of malnutrition and exclusive enteral nutrition could alter the incidence of *Hungatella*. In TNF<sup>delta</sup>ARE mice, Schaubek and colleagues observed that different strains of *Hungatella* were found in mice developing and not developing a spontaneous ileitis, suggesting that different strains from the genus *Hungatella* may have different proinflammatory behaviour.<sup>41</sup>

Understanding the microbiota variations only as a consequence and not a root-cause of intestinal inflammation is discordant with the results of faecal microbiota transplantation. Faecal microbiota transplantation in CD is still emerging,<sup>42</sup> but promising results are already available in UC.<sup>43</sup> Moreover, it is reasonable to think that the microbiota not only has an impact on the severity of the disease but also on the risk of SSI, as an indirect reflection of the healing capacity of the tissues. Indeed, Kumar *et al.* suggested that commensal bacteria modulate the protein degradation machinery of several essential signalling components, which in turn influence diverse physiological processes of the gut cells.<sup>44</sup>

Beyond the field of IBD, Van Praagh *et al.* reported a possible impact of the microbiota in colorectal anastomotic fistulas.<sup>13</sup> In their study, a low microbiota diversity as well as a high abundance of bacteria from the families Bacteroidaceae and Lachnospiraceae were associated with an increased risk of anastomotic fistula. Although the disease and the type of anastomosis are different from our study, they still support the concept of a predictive role of the gut microbiota in SSI.

Finally, although suggest that the microbiota can predict SSI in patients operated on for CD, validation in an external cohort is needed, and we should remain aware that the quality of prediction can vary substantially from one sample to another [the confidence intervals of the AUC values are quite large]. This is an additional motivation to develop new mathematical methods more adapted to the microbiota data, which could for instance take into account their hierarchical structure.

In conclusion, our study shows that the mucosal microbiota has the potential to predict the occurrence of SSI after surgery for CD. Forthcoming studies should confirm these findings and assess if preoperative analysis of the microbiota may impact postoperative morbidity in CD patients. Beside

its potential role as a biomarker, if the gut microbiota plays an active role in SSI pathogenesis, therapeutic or preventive intervention targeting the gut microbiota might be considered to reduce the risk of postoperative complications and increase the rate of primary anastomosis in patients with CD.

## Supplementary Data

Supplementary data are available online at *ECCO-JCC* online.

## Conflict of Interest

H.S. received unrestricted study grants from Biocodex; board membership, consultancy or lecture fees from Carenity, Abbvie, Astellas, Danone, Ferring, Mayoly Spindler, MSD, Novartis, Roche, Tillots, Enterome, Maat, BiomX, Biose, Novartis and Takeda; and is a co-founder of Exeliom bioscience. M.N. received board membership, consultancy or lecture fees from Abbvie, Adacyte, Amgen, Arena, Biogen, CTMA, Celltrion, Ferring, Fresenius-Kabi, Janssen, Mayoli-Spindler, MSD, Pfizer and Takeda. J.H.L. reports lecture fees from Ethicon, Takeda, Intuitive and B-Braun, invitation to a medical congress by Biomup and MD start. J.H.L. is a consultant for Safeheal and Coloplast and a personal investor in digital companies, medical device companies or biotech companies. S.N. declares counselling, board, transport or fees from Abbvie, Biogen, HAC-pharma, Janssen, MSD, Novartis, Pfizer, Takeda, Tillots, BMS, Amgen and Fresenius. The other authors declare no conflict of interest. The data underlying this article will be shared on reasonable request to the corresponding author.

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C.J.: acquisition of data, drafting of the manuscript; E.A., S.L.C., A.B.: mathematical and statistical analysis; L.B.B.: drafting of the manuscript; H.S.: analysis and interpretation of the data, drafting of the manuscript; all authors: acquisition of data, critical revision of the manuscript. All the authors revised and approved the manuscript.

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