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Prognostic value of a combination of innovative factors (gut microbiota, sarcopenia, obesity, metabolic syndrome) to predict surgical/oncologic outcomes following surgery for sporadic colorectal cancer: a prospective cohort study protocol (METABIOTE)

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ABSTRACT

Introduction Colorectal cancer (CRC) is still associated with poor prognosis, especially in patients with advanced disease. Development of new prognostic tools replacing or supplementing those routinely used is definitely needed, with the aim to optimise and personalise treatment strategies. Gut microbiota composition and body composition profile (obesity, sarcopenia and metabolic syndrome) have recently been reported separately as new relevant prognostic factors for postoperative surgical and oncologic outcomes following CRC surgery. However interactions that exist between these factors have been poorly studied. The purpose of this translational prospective cohort study (METABIOTE) is to investigate potential interactions between gut microbiota, body composition profile and postoperative outcomes and recurrence in patients undergoing surgery for non-metastatic sporadic CRC.

Methods and analysis This single-centre project aims to prospectively enrol 300 consecutive patients undergoing surgery for non-metastatic sporadic CRC at the University Hospital of Clermont-Ferrand, France for the identification of specific microbial signatures (from tumour, colonic mucosa and stools samples) associated with particular metabolic profiles that could impact postoperative morbidity and oncologic outcomes, using microbiological, molecular and imaging approaches. The primary outcome is the 5-year overall survival (OS). Other outcomes are 5-year CRC-related OS, 5-year disease-free survival, 30-day postoperative morbidity, 90-day postoperative mortality and length of hospital stay.

Ethics and dissemination This study protocol was reviewed and approved by an independent French regional review board (n°2018-A00352-53, 'Comité de Protection des Personnes Ile de France VII' on 4 July 2018, declared to the competent French authority ('Agence Nationale de Sécurité du Médicament et des produits de santé', France), and registered on the Clinical Trials web-based platform (NCT 03843905). Oral and written informed consent will be obtained from each included patient. Study results will be reported to the scientific community at conferences and in peer-reviewed scientific journals.

Trial registration number NCT03843905.

Strengths and limitations of this study

- This is one of the pioneering studies, investigating the combination of innovative prognostic markers such as gut microbiota and body composition profiles for the prediction of oncologic and surgical outcomes after colorectal cancer surgery.
- METABIOTE is a large prospective cohort study including 300 patients at a high-volume tertiary expert centre.
- The single-centre type of this study could be a limit, potentially exposing to recruitment biases.

INTRODUCTION

Colorectal cancer (CRC) is the third leading cancer worldwide, and is still associated with poor prognosis, especially in patients with advanced disease with less than 15% 5-year overall survival (OS).[1] Surgery remains the only chance of cure, but is associated with postoperative complications, particularly anastomotic leakage (AL), that could delay the administration of adjuvant treatments and then compromise oncologic outcomes.[2 3] Following CRC surgery, decision for adjuvant

chemotherapy is mainly made on pathology features and molecular characteristics, such as the tumor nodes metastasis (TNM) classification and *MSI/KRAS/BRAF* status.[4 5] However, these parameters remain imperfect for the assessment of CRC prognosis, especially in stage II CRC patients.[6–8] Therefore, there is still a need to develop new tools for CRC treatment optimisation and personalisation. Innovative factors have been recently linked to CRC prognosis and postoperative complications after CRC surgery, such as gut microbiota and body composition profile (eg, obesity, sarcopenia, metabolic syndrome (MS)).[9–14]

There are now strong evidences for the implication of the gut microbiota in colorectal carcinogenesis. Indeed, a dysbiosis has been reported in CRC patients,[1 2] with the emergence of some pathogenic bacterial species, including pathogenic *Escherichia coli*. Although most of the studies focused on the bacterial pro-carcinogenic effect in CRC, it appears increasingly obvious that dysbiosis could be a new prognostic biomarker and an innovative target for CRC therapies.[15 16] Recent evidences suggest a relationship between an abnormal colonic mucosa colonisation by pathogenic *E. coli* and advanced CRC.[17 18] Interestingly, some pathogenic *E. coli*, preferentially detected in CRC patients, are more prevalent in the mucosa of patients with stage III/IV advanced CRC than in those with stage I CRC.[17 18] This suggests that *E. coli* could be used as prognostic factor in CRC. Similar results have also been reported with other bacteria species (*Streptococcus bovis*, *Helicobacter pylori*, *Bacteroides fragilis*, *Enterococcus faecalis*, *Clostridium septicum*, *Fusobacterium* spp).[19] If the intestinal microbiota appears to be involved in carcinogenesis, it may also modulate the host response to anticancer therapies,[20] clinical trials targeting predictive value of the microbiota modulation being currently conducted on this topic. Intestinal microbiome has been shown to participate in the resistance to a wide range of anticancer treatments by direct interaction with the treatment or by indirectly stimulating host response through immunomodulation.[21] Some may have a direct action on anticancer drugs by metabolising them as β-glucuronidase-producing bacteria of the microbiota with irinotecan, a chemotherapy used in CRC treatment.[22] Moreover through its immunomodulation activity, intestinal microbiota can modulate the efficacy of chimiotherapy or immunotherapy. Indeed, Lida *et al*[23] described the oxaliplatin chemoresistance of tumours in germ-free or antibiotics-treated mice in comparison with specific pathogen-free mice. This resistance has been linked to the reactive oxygen species (ROS) producing myeloid antitumour cells activity. Similar results were observed with cyclophosphamide treatment by reducing Tregs and increasing Th1 and Th17 cells.[24] More recently, the impact of the gut microbiota on the immune checkpoint inhibitor therapy efficacy has also been largely explored and confirm the central role of remote lymphoid and myeloid cells modulation by the gut microbiota.[25] In the same way, a recent clinical study by Gopalakrishnan *et al* revealed that the composition of the gut microbiota could affect the antitumorous responses to anti-PD-1 immunotherapy in patients with metastatic melanomas.[26] Finally, gut microbiota has been recently linked to the occurrence of postoperative complications after colorectal surgery, especially AL.[10 27] Bacterial induction of an oxidative stress, notably through the production of ROS and the activation of specific pathways regulating intestinal scarring, is also suspected to be involved in the genesis of these postoperative complications.[12 28]

Both body composition parameters and metabolic profiles have been reported to be relevant prognostic factors in CRC.[10] Indeed, muscle exhaustion (sarcopenia), obesity, visceral obesity and MS may influence oncologic outcomes in patients undergoing CRC surgery, independently from TNM stage and other pathological risk factors.[9] Sarcopenia has been identified as an independent predictor of impaired survival, and has been associated with increased 30-day postoperative morbidity and mortality after CRC surgery as well.[13 29] It is also responsible for major toxicities of chemotherapy, thus resulting in dose reduction and delayed administration or even contraindication for adjuvant therapies. It is likely that early discontinuation of adjuvant treatment and vulnerability to postoperative infection contribute to impair survival in sarcopenic patients. Sarcopenia has also been associated with the systemic inflammatory response in CRC patients, and this relationship may explain why sarcopenic patients are subjected to poorer oncologic outcomes.[13] In addition, other studies reported higher postoperative morbidity and impaired survival in CRC patients with stage II/III obesity.[30 31] MS is a set of predictive risk factors predisposing to obesity. A study on non-metastatic CRC patients[30] showed (1) a 45% higher mortality risk in obese patients with MS, (2) a 9% higher risk in patients with MS only and (3) no increase in mortality in obese patients without MS. Finally, the combination of obesity and MS, especially in obese patients who also have sarcopenia (sarcopenic obesity), seems to be associated with an increase of severe complications within 30 days after colonic surgery.[32]

In this context, further studies are needed to assess impact and interactions of these innovative prognostic factors in CRC patients, with the goal to improve identification of patients at high risk for postoperative complications or recurrences, and to personalise perioperative strategies.[33] This large translational prospective research project aims to assess the impact of innovative prognostic tools combinations (MS, obesity, sarcopenia, gut microbiota composition) on surgical and oncologic outcomes following surgery for non-metastatic sporadic CRC. In addition to global microbiota composition, the pro-carcinogenic species described earlier could represent interesting prognostic factors. Among them, colibactin-producing *E. coli* could be a good candidate. Indeed, prevalence of this pathogenic *E. coli* seems to be correlated to aggressive CRC forms.[17] In the same way, Gopalakrishnan *et al* showed that resistance to anti-PD1

correlated with an enrichment of *E. coli* population in the intestinal microbiota of metastatic melanoma patients. In this context, the microbiota studies will be completed characterising the CRC-associated *E. coli* strains isolated from the samples of this cohort.

METHODS

Study design and setting

The present trial (METABIOTE) is an observational prospective cohort study conducted in a tertiary high-volume expert Digestive Surgery department at the University Hospital of Clermont-Ferrand, France. All consecutive patients with a non-metastatic sporadic CRC scheduled for surgery (corresponding to the ICD10 code: C18, C19) will be systematically proposed participation to the study, aiming inclusion of 300 patients from 2019 to 2021.

After oral and written informed consent has been obtained, patients will be enrolled and data will be prospectively collected using an electronic database (RedCap; Research Electronic Data Capture) hosted at the University Hospital of Clermont-Ferrand, France.[34] All data regarding patients/family medical and surgical history (mainly history of cancer and cardiovascular disease), usual treatments, co-morbidities (including tobacco and alcohol consumption), allergies and eating habits will be recorded at the initial visit. Then data on (1) metabolic profile, (2) postoperative outcomes and survivals following CRC surgery and (3) gut microbiota composition will be studied (figures 1 and 2).

Body composition profile assessment: obesity, MS and sarcopenia determination

To define these parameters, different measures and dosages will be performed

- Clinical parameters: weight (kg), height (cm), body mass index (BMI) calculation (kg/m^2) and waist circumference measurements will be collected preoperatively and at 3, 6 and 12 months postoperatively.
- Preoperative biological parameters: blood count, blood ionogram, urea, creatininemia, C-reactive protein, fasting plasma glucose, HbA1c, complete lipidic tests (high-density lipoprotein-c (HDL-c), low-density lipoprotein-c, total cholesterol and triglycerides), complete liver tests (aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, alkaline phosphatase), bilirubinemia, nutritional assessment: albuminemia, pre-albuminemia.
- Imaging features: a thoraco-abdomino-pelvic CT scan will be systematically performed as part of CRC preoperative assessment. In addition to the detection of metastases, it will be used to study metabolic profiles, analysing visceral fat, liver and splenic density

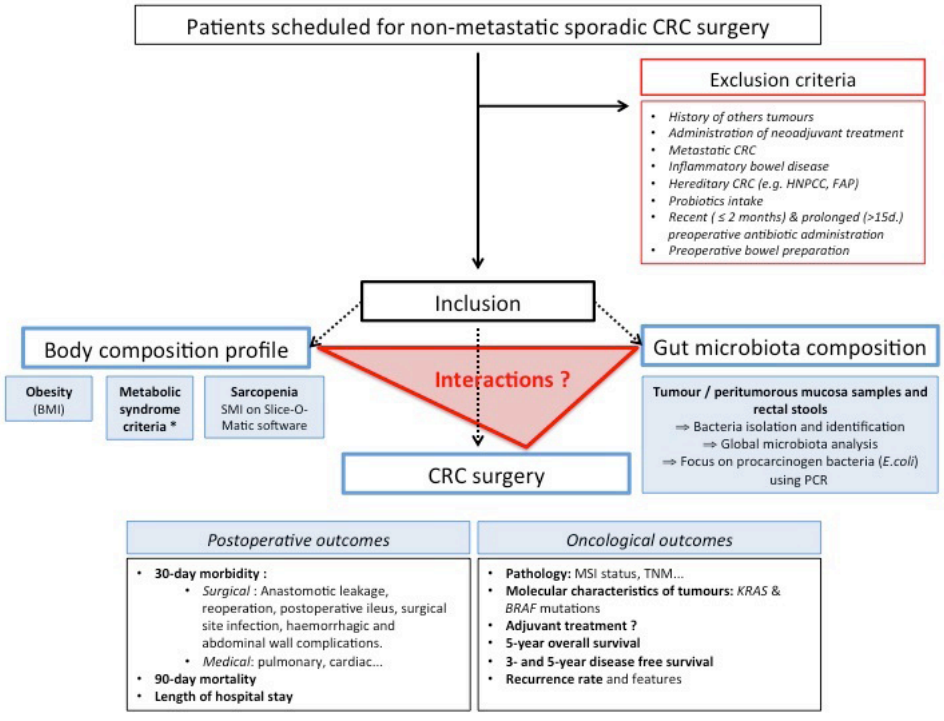

Figure 1 Investigated parameters on the METABIOTE project. *Metabolic syndrome according to new International Federation Diabete definition (IDF, 2006): central obesity (defined as waist circumference: ≥94 cm male; ≥80 cm female). Plus any two of the following four factors: raised triglycerides: >150 mg/dL (1.7 mmol/L) or specific treatment for this lipid abnormality; reduced HDL cholesterol: <40 mg/dL (1.03 mmol/L) in males, <50 mg/dL (1.29 mmol/L) in females or specific treatment for this lipid abnormality; raised blood pressure: systolic BP ≥130 or diastolic BP ≥85 mm Hg or treatment of previously diagnosed hypertension; raised fasting plasma glucose: ≥100 mg/dL (5.6 mmol/L) or previously diagnosed type 2 diabetes. BMI, body mass index; BP, blood pressure; CRC, colorectal cancer; FAP, familial adenomatous polyposis; HDL, high-density lipoprotein; HNPCC, hereditary non-polyposis colorectal cancer; PCR, polymerase chain reaction; SMI, skeletal muscle index.

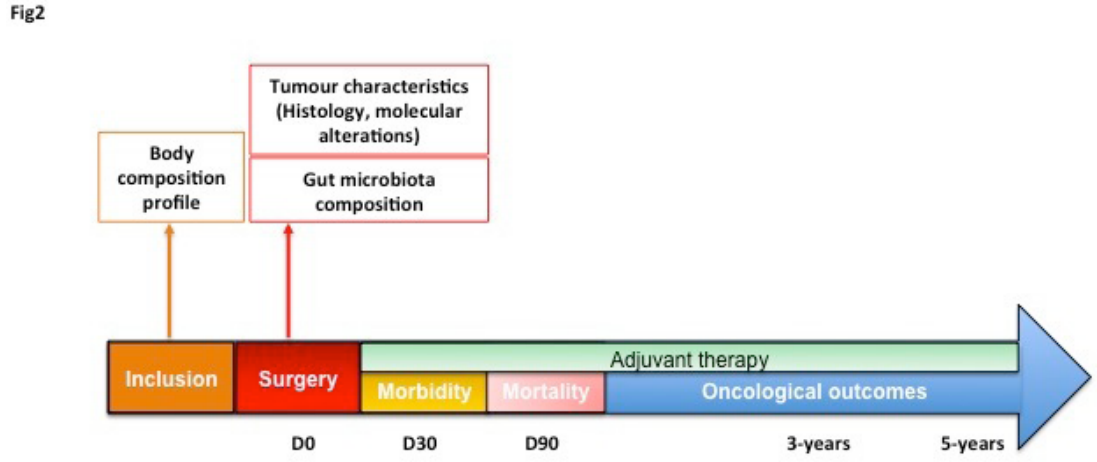

Figure 2 Design of the study for each patient.

and skeletal muscle index (SMI) on a L3 centred slice, using a dedicated software (Slice-O-Matic V.4.3 software, Tomovision, Montreal, Quebec, Canada) available at the Radiology Department, thus defining sarcopenia degree. CT scans will also be performed postoperatively as a part of the usual oncologic follow-up, every 3–6 months for up to 5 years.

According to the examinations carried out and described above

- *Obesity* is defined by the National Institutes of Health as a BMI≥30 kg/m^2.[35]
- *Metabolic syndrome* is defined according to the recommendations from the National Cholesterol Education Program Adult Treatment Panel[36] as a cluster of risk factors including abdominal obesity assessed by waist circumference, high blood pressure, elevated triglyceride level, hyperglycaemia and low HDL-cholesterol level.
- *Sarcopenia* is defined from CTs using Slice-O-Matic V.4.3 software. Skeletal muscle index (cm^2/m^2) is thus determined on a single slice at the third lumbar vertebra (L3). Sarcopenia is defined as reduced L3 SMI, identified using predefined sex-specific cut-off points defined by Prado *et al*.[32] Thereby, patients with a SMA <52·4 cm²/m² for men and <38.5 cm²/m² for women will be considered as sarcopenic. Sarcopenic obesity is defined as the combination of sarcopenia with a BMI>30 kg/m^2.

CRC features and postoperative outcomes

Data regarding CRC features and postoperative outcomes will be collected as following: pathology characteristics (TNM stage, *MSI*, *RAS* and *BRAF* status, presence of perineural or vascular invasion), 30-day postoperative morbidity, 90-day postoperative mortality, stay in intensive care unit, length of hospital stay, reintervention (radiological, endoscopic and/or surgical) and readmissions.

Postoperative morbidity and mortality will be defined as events occurring during hospital stay or within postoperative days 30 and 90, respectively. Postoperative complications will be assessed according to the Dindo-Clavien classification.[37]

Thromboembolic, cardiac, infectious, uro-nephrologic, psychiatric and pulmonary complications will be raised as postoperative medical morbidity.

Postoperative surgical morbidity includes:

- *AL*, according to the International Study Group of Rectal Cancer,[38] is defined as a communication between the intraluminal and extraluminal compartments-related defect at the anastomotic site. Thus any abscess adjacent to anastomosis, even in the absence of clearly identified communication on CT scan (opacified or not) or during a possible surgical revision, will be considered as an AL. A validated simple graduation system will be used to classify AL based on clinical management: grade A, no change in patient management; grade B, AL requires active therapeutic intervention (radiological and/or endoscopic drainage) and grade C, AL requires surgical reoperation (laparoscopy or laparotomy).
- *Postoperative ileus (POI)*: according to the Chapuis *et al* definition,[39] in the absence of mechanical bowel obstruction, POI is raised when the patient is bloated with a lack of bowel sounds and has experienced nausea or vomiting and no gas or stool for more than 3 days postoperatively. POI differs from mechanical occlusion in that it is resolved without surgery.
- *Surgical site infection (SSI)*[40] with a distinction between superficial incisional versus deep incisional or organ/space (including AL) SSI.
- *Haemorrhagic complication*: intra-abdominal or intraluminal haemorrhage, perioperative blood transfusion.
- *Abdominal wall complications*: incisional hernia and evisceration.

Collected postoperative oncologic outcomes are 3-year and 5-year OS, CRC-related OS, disease-free survival (DFS), recurrence rate and features. Time of recurrence is defined as the time of the first imaging that reported definitive or suspicious new tumours. For patients with biopsy-proven recurrence, the date of positive histological

results is defined as the time of recurrence. OS and DFS will be calculated from the time to CRC surgery to the time of death and first recurrence, respectively. For the purposes of this study, recurrence sites are recorded as liver, lung, peritoneum, retroperitoneal lymph nodes or other sites (eg, bones, brain), and biopsy-proven anastomotic recurrences will be recorded.

All these data will be collected during the hospital stay, and then at each postoperative outpatient visit starting at postoperative day 30 (cf. figure 2).

Gut microbiota composition

Tissues samples from adjacent peritumorous mucosa and tumour will be withdrawn in the operative room, as well as rectal stools, will be washed and immediately frozen (−80°C) and then moved to the M2iSH research unit for microbiota analysis. After thawing, samples will be next crushed in the presence of Triton 0.1X and incubated at room temperature. Lysate dilution will be plated on TBX agar and chromogenic agar chromID CPS3 (bioMérieux). Bacteria colonies of interest (around 48 per samples) will be then collected for molecular typing and identification will be confirmed with the automated Vitek II (bioMérieux) system. About 10 colonies of *E. coli* per sample will be typed with molecular methods to identify the *E. coli* genotypes colonising the samples. 'Enterobacterial Repetitive Intergenic Consensus' sequence PCR will be used as genotyping methods.[41] Identification of *E. coli* harbouring pks island was investigated using PCR methods on all CRC samples.[42]

Moreover, global microbiota modifications will be assessed using high-throughput sequencing of the bacterial 16S rRNA gene DNA extracted from tissues using the NucleoSpin Tissue XS kit (Macherey-Nagel, Hoerdt, France) according to the manufacturer's instructions. The V4 regions of the bacterial 16S rRNA gene will be amplified using 515F/806R primer pair and illumina high-throughput sequencing will be then performed on a MiSeq following the manufacturer's guidelines. To complete microbiota composition with functional data, metabolomics and shotgun metagenomics sequencing methods will be performed after selection of the more informative samples (eg, faeces). In addition, additional qPCR will be performed to quantify all the well-known pro-carcinogenic species such as *Fusobacterium nucleatum, E. faecalis, Bacteroides fragilis,* cyclomodulin-producing *E. coli* and to detect some virulence factors such as adhesins, collagenase and toxins (Bacteroides fragilis toxin or the colibactin pks island or VacA/Fad).

Objectives and endpoints

Primary and secondary objectives and endpoints

The main objective of this translational research project is to study the impact of the combination of innovative biomarkers (gut microbiota composition, body composition profile) on 3-year and 5-year OS in patients undergoing surgery for a non-metastatic sporadic CRC to ultimately propose a 5-year survival prognostic score.

The secondary objectives are to investigate the impact of such biomarkers combination on 3-year and 5-year CRC-related OS and DFS, recurrence rates and patterns, 30-day medical and surgical postoperative morbidity, reintervention (radiological, endoscopic and/or surgical) and unplanned readmissions, stay in reanimation/intensive care unit, length of hospital stay, 90-day postoperative mortality and kinetic of both postoperative sarcopenia and BMI.

Eligibility criteria

All the consecutive patients scheduled for surgery for a biopsy proven non-metastatic sporadic CRC at our institution will be systematically proposed participation to the study. Absence of metastases will be double-checked on a preoperative thoraco-abdomino-pelvic CT scan. All patients must have given both an oral and written informed consent. Patients cannot be included due to the presence of at least one of the followings:<18 years old, cognitive disorders or major disability, antibiotic intake within 2 months before CRC surgery, history of previous tumour except non-melanoma skin tumour, probiotics intake, inflammatory bowel disease, hereditary CRC (familial adenomatous polyposis or hereditary non-polyposis colorectal cancer syndrome), preoperative bowel preparation, administration of neoadjuvant treatment, metastatic disease on the preoperative assessment (figure 1).

Enrolment and follow-up

All patients fulfilling the inclusion and non-inclusion criteria will be proposed the study at the first preoperative outpatient visit (figure 1), and both oral and written information will be given. After oral and written informed consent has been obtained, patients will be enrolled. After discharge, follow-up will start at postoperative day 30 at the first postoperative outpatient visit consisting in a physical examination. Oncologic follow-up will be conducted according to the French guidelines,[43] every 3 months for 3 years, then every 6 months for 2 years, consisting in (1) a physical examination and carcinoembryonic antigen level assay and (2) an cross-sectional imaging. A thoraco-abdomino-pelvic CT scan will be performed annually for 5 years.

Patient and public involvement

Patients and public were not involved in the development of the research question, outcomes measures or design of the study. Patient care does not differ from the one usually carried out according to the recommendations, particularly for patient follow-up. Study participants will be able to find the results of the study in scientific publications or in conference presentations. They will not be directly informed.

Statistical considerations

Estimation of sample size

Sample size estimation is based on literature about statistical power and number of prognostic factors. According to recommendations reported by Harrell, Green and Hsieh *et al.*,[44–46] we plan to include 300 patients in order to identify prognostic factors for 5-year survival for a

two-sided type I error at 5%, a statistical power greater than 90% and 5-year survival equals 55% for rectal cancer patients and 60% for colon cancer patients.

A sequential analysis will be carried out every 100 inclusions in order to estimate the statistical power calculated from the work of Hsieh *et al*[45] and Demidenko[47] after estimating the number of factors actually tested and on the basis of preselected factors, determined according to univariate analysis and clinical relevance.

Statistical analysis

The statistical analysis will be performed with Stata software (V.13, StataCorp) for a two-sided type I error at 5%. The continuous data will be presented as mean±SD or median (interquartile interval) according to statistical distribution. The normality will be studied by the Shapiro-Wilk test. Since dichotomising implies loss of information and hence loss of statistical power,[48 49]continuous variables will be analysed without categorising them unless predefined clinical thresholds have been reported in the literature. The categorical variables will be presented with number of patients and percentages and the censored data will be estimated by Kaplan-Meier method.

To determine 5-year survival prognostic factors, we will start by univariate analyses using log-rank test for categorical variables and by Cox model for continuous parameters. Then, multivariable analysis will be performed using Cox proportional-hazards model. The covariates will be determined according to univariate results and to their clinical relevance. A particular attention will be paid to the study of multicollinearity and interactions between covariates (1) studying the relationships between the covariables: Student's t-test or Mann-Whitney test will be considered to compare continuous variables between groups whereas χ^2 or Fisher's exact will be applied for the study of relationships between categorical parameters and correlation coefficient (Pearson or Spearman according to statistical distribution) for the study of relationships between quantitative variables and (2) evaluating the impact to add or delete variables on multivariable model. The proportional-hazard hypothesis will be studied using Schoenfeld's test and plotting residuals. The results will be expressed as hazard ratios and 95% CIs and will be represented as a monogram. Tests of heterogeneity in risk associations, that is, in the relationships between tumour location and prognosis factors will be assessed using Wald test. All statistical analyses will be conducted according to TRIPOD recommendations,[50] in particular concerning the proposal of a prognostic survival score at 5 years, which will be based, in the absence of learning and validation samples, on a bootstrap approach.

For secondary objectives, univariate statistical analyses will consider the statistical tests aforementioned. For multivariable analyses, Cox model will be replaced (1) by a multiple linear regression model for the hospital duration: the normality of residuals will be studied and if appropriate a transformation, for example logarithmic, will be proposed to achieve the normality of dependent outcome and (2) by generalised linear logistic regression for dichotomous dependent variables (surgical and medical postoperative morbidity at 30 days, postoperative mortality at 90 days, anastomotic fistula rate, SSI rate, rehospitalisation rate). The results will be expressed as regression coefficients (for linear regression) and ORs (for logistic regression) and 95% CIs. Given the well-established differences between the microbiome between rectal and colon cancer, stratified analyses will be proposed. According to Consolidated Standards of Reporting Trials recommendations, subgroup analyses depending on rectal and colon cancer location will be proposed after the study of subgroup × randomisation group interaction in regression models aforementioned. Finally, a sensitivity analysis will be performed to study the statistical nature of missing data and then to use the most appropriate imputation data method.

ETHICS AND DISSEMINATION

Patients will be informed in a complete and fair fashion, in understandable terms, on objectives and constraints of the study, possible risks, necessary monitoring and safety measures, their rights to decline any participation in the study or the possibility of withdrawing at any time. Patient's oral and written informed consent will be collected by the investigator. An intermediate analysis will be performed at 100 enrolled patients. Study results will be reported to the scientific community at conferences and in peer-reviewed scientific journals.

DISCUSSION

The main expected point of this study is the identification of specific microbial signatures associated with particular metabolic profiles (obesity, sarcopenia, MS) in CRC patients who could predict high risk for postoperative complications and/or impaired oncologic outcomes. We focused this study on colon and upper rectum cancers. Indeed, patients who received neoadjuvant radiochemotherapy (mid and lower rectum cancer) were excluded from this study because this treatment is likely to significantly modify the intestinal microbiota before surgery and could thus bias our analysis.[51] It will especially assess whether the level of colonisation by some pathogenic bacterial species and/or the loss of protective bacteria could be associated with particular metabolic profiles and postoperative morbidity, effectiveness of anticancer therapies, development of recurrences and survival. The objective will be to validate microbial signatures first in tissues (tumour, colonic mucosa), and then on stool samples in order to obtain a non-invasive prognostic biomarker. In case of relevant results, this combination of innovative prognostic factors would therefore allow improved identification of high-risk CRC patients, leading to optimisation and personalisation of perioperative strategies, such as administration of adjuvant treatments and prevention of postoperative complications. To the best of our

knowledge, no translational studies have investigated the combined interactions between metabolic profiles, gut microbiota and the surgical and oncologic outcomes after surgery for CRC. Therefore the METABIOTE project will be the first study assessing this global approach in these settings, and could lead to innovative and clinically relevant proposals. In addition to the descriptive aspect of microbiota, bacterial virulence factors and microbiota functional data will be explored to better understanding host/pathogens interactions in CRC patients undergoing CRC surgery and in association to patients' metabolic profiles. If some bacteria and virulence factors will be associated with postoperative complications, preclinical mechanistic studies could be conducted to demonstrate effects of these factors on intestinal physiopathology. All these data could thus constitute a basis for the future development of microbiota-targeting therapies, such as perioperative administration of probiotics and/or colonic eradication of pathogenic bacterial species, and of prehabilitation and enhanced recovery programmes.[31 32]

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Competing interests None declared.

Patient consent for publication Obtained.

Ethics approval This study protocol was reviewed and approved by an independent French regional review board (n°2018-A00352-53, 'Comité de Protection des Personnes Ile de France VII' on 4 July 2018, declared to the competent French authority ('Agence Nationale de Sécurité du Médicament et des produits de santé', France), and registered on the Clinical Trials web-based platform (NCT 03843905).

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