3D IMAGE PROCESSING AND RECONSTRUCTION FOR THE ASSESSMENT OF TUMOR INFILTRATION IN COLON CANCER: PROSPECTIVE OBSERVATIONAL NON-RANDOMIZED TRIAL

Background and objectives

Colon cancer is one of the most prevalent oncologic pathologies in the abdomen, so specific surgical strategies needed to treat this pathology must be known. Currently, there is evidence on the standardization of the surgical technique in colon resections by using a complete mesocolic excision (CME). However, in certain cases such as suspected infiltration of the retroperitoneum (pre-renal fat, duodenum, etc.) or neighboring anatomical structures, the technique should be modified. Thus, performing wider resections to obtain better oncologic results, leaving neither macro nor microscopic tumor (R0) as well as free surgical margins. Likewise, there is published evidence that the rates of disease-free patients increase after R0 surgeries, as well as a decrease in the rates of local tumor recurrence.

On the other hand, the gold standard technique for colon cancer staging is computed tomography (CT). However, in more advanced cases in which a possible or suspicious infiltration of the retroperitoneal margin or neighboring structures is observed, sensitivity and specificity decrease. For this reason, we propose the use of mathematical 3D reconstruction models applied to assess cases in which infiltration of neighboring structures and/or the retroperitoneal margin is suspected. This means that artificial intelligence algorithms are used from the CT images to create a three-dimensional model showing the different anatomical structures. In addition, this software (CELLA®) has the ability to "look" more closely and in more detail at possible areas of infiltration known as 3D image processing and reconstruction (3D-IPR). Subsequently, we will analyze the surgical specimens together with the anatomic pathology department to confirm whether CT and 3D-IPR are sensitive for the detection of infiltration in cases of colon cancer. In this way, it constitutes an important tool for general and digestive surgeons during surgical strategy planification and to be able to assess whether it is necessary to extend the resection to obtain optimal oncologic results.

Obtaining surgical specimens with surgical margins free of infiltration or R0 type surgery is the key concept to reduce the risk of local recurrence and carcinomatosis in colorectal oncologic surgery.

Current diagnostic techniques show percentages of diagnostic accuracy of infiltration of neighboring structures that could be improved. In rectal cancer, the technique of choice is magnetic resonance imaging (MRI) with a diagnostic accuracy of 65%, while in colon cancer CT is the gold standard imaging technique with a diagnostic accuracy of 60% for possible tumor infiltration.

Colon oncological surgery has already been standardized in different works, being described as complete excision of the right and left mesocolon (CME), just as the complete excision of the mesorectum was standardized in rectal cancer. The concept of CME refers to dissection following the embryological planes, high ligation of the corresponding blood vessels to keep the mesocolon intact.

However, when there is suspicion of a surgical margin threatened by infiltration, the concept of CME changes and a diversion of the surgical strategy must be performed to achieve a free resection margin (R0). This means that when there are organs or extra-colonic structures attached to the tumor, the dissection plane should be extended towards the invaded structure. This is done *in-bloc* with the rest of the surgical specimen, to avoid peritoneal dissemination with the consequent increase of loco-regional recurrence and/or peritoneal carcinomatosis that would worsen the patient's prognosis. Thus, infiltration of the retroperitoneal margin or

neighboring structures in right colon cancer, for example, is associated with increased risk of loco-regional recurrence and consequent decreased survival rates.
Furthermore, infiltration of the mesocolon and retroperitoneum in colon cancer is an independent prognostic factor, which together with peritoneal dissemination, tumor perforation and venous invasion would fall within the group of high-risk tumors. This is why surgeon's knowledge of embryological planes is very important to be able to perform R0 resections in colon cancer with a threatened retroperitoneal margin.
Colon cancer staging is performed based on the anatomopathological study of the surgical specimen: T1, T2, T3, T4; T4a and T4b according to the depth of invasion in each layer of the colon. CT has the capacity to detect tumor invasion beyond the intestinal wall (T1/T2 vs. T3/T4), but current studies have great variability with sensitivity of 55 to 98% and specificity of 33 to 100%. Furthermore, when comparing the diagnostic accuracy of CT with pathological anatomy in cases of T3-T4 tumors, a correct diagnosis by CT is only observed in 66%, 76% and 54% in T3, T4a and T4b, respectively. This translates into a high percentage of patients with unknown invasion of neighboring structures.
For this reason, new methods have been proposed to improve the specificity when assessing the retroperitoneal margin or the infiltration of neighboring structures in colon cancer. These are the use of three-dimensional image processing and mathematical reconstruction models (3D-IPR) to assess possible infiltrations that require a change in surgical approach. Previous studies have already demonstrated the use of 3D-IPR to assess possible infiltrations in rectal tumors and the consequent change of surgical approach to achieve an R0. Despite this, there is still no evidence regarding colon tumors and the threatened retroperitoneal margin.
In addition, there is increasing evidence for the need to perform an extended lymphadenectomy (D3 lymphadenectomy) in right colon tumors. This requires surgeons to have a good knowledge of the venous anatomical variations of the trunk of Henle to perform a correct oncological surgery. For this reason, 3D-IPR allows the surgeon a more visually representative model of these anatomical variations to improve the quality of D3 lymphadenectomy.
For this reason, this study aims to demonstrate the usefulness of 3D-IPR to obtain surgeries with R0 resection in patients with suspected infiltration of neighboring structures in right, transverse, and left colon cancer. This is why we propose the following hypothesis and objectives:

- **HYPOTHESIS**: A 3D-IPR model based on mathematical algorithms from CT could improve the diagnostic accuracy of suspected tumor infiltration of the retroperitoneal margin and neighboring structures in advanced tumors of the right, transverse, and left colon. This is a novel tool to establish a correct surgical strategy with the aim of increasing the percentage of R0 type resection in this type of tumors.
- **OBJECTIVES**: The main objective of this study is to assess the usefulness of 3D-IPR to obtain surgeries with R0 resection in patients with Threatened Surgical Margin (TSM); either by threatened retroperitoneal margin or suspected infiltration of neighboring structures in cancer of the right, transverse, and left colon.
    - Secondary objectives are:
        - To assess the usefulness of 3D-IPR as a preoperative surgical strategy tool.
        - To compare the diagnostic accuracy of 3D-IPR with the radiological report of CT regarding the infiltration of neighboring structures and retroperitoneal margin in right and left colon tumors with TSM.
        - To evaluate the usefulness of 3D-IPR to detect pathological or suspicious adenopathy.

- To evaluate the use of 3D-IPR to point out the main vascularization, as well as the possible anatomical variants of the right and left colon.

Research methodology

We propose a ***Prospective observational comparative non-randomized not-for-profit study***

Inclusion criteria:

- Patients diagnosed of primary colon cancer located in right, transverse and/or left colon by colonoscopy. Locally advanced tumors with suspected infiltration of neighboring structures and/or retroperitoneal margin or those considered as T3 or T4 (TNM staging classification) according to radiologist's report from the extension CT.
- Over 18 years of age
- Patient who agree and sign informed consent for surgical intervention.

Exclusion criteria:

- Preoperative chemotherapy or radiotherapy (neoadjuvant treatment).
- Suspicion of carcinomatosis in preoperative CT scan.
- Suspected distant metastasis on preoperative CT scan.
- Patients with infiltrating tumors considered unresectable (preoperatively or intraoperatively), since anatomo-pathological analysis will not be available.

Mathematical method of 3D reconstruction (3D-IPR) and evaluation of retroperitoneal infiltration:

- Mathematical 3D reconstruction is extracted from the pre-operative CT, which is performed in all patients with colon neoplasms, to assess the location of the primary colon tumor and possible infiltration of neighboring/retroperitoneal structures.
- Evaluation of the same parameters exposed for the CT of the abdomen.
- 3D-IPR will be based on two concepts:
    - Preprocessing of the CT of extension using "Bias Field Correction" algorithms and anisotropic diffusion filters of the image.
    - Medical image segmentation in the different sequences provided using sequence of algorithms based on active contour methods, modified dynamic search and based on atlases. Finally, the 3D surface will be reconstructed using modified "*marching cubes*" algorithms.

All this analysis will be performed in conjunction with the 3D Reconstruction and 3D Printing Unit of the Hospital Universitario Son Espases.

Surgical Technique/Ethical Review:
The intervention will always be performed according to the radiological report from the preoperative CT scan. The assessment of tumor infiltration by the 3D reconstruction technique will not modify the surgical technique decided preoperatively based on the radiological report of the preoperative CT.

Anatomo-pathological study:

- Surgical specimens are processed and fixed in 10% formalin and the quality of the complete excision of the mesocolon will be evaluated as well as the presence of a complete D3 lymphadenectomy in the case of having performed it in a right colectomy.

- Transverse sections 4mm thick will be made on the specimen (each section will be digitally photographed) from the proximal portion including the tumor area to visualize the relationship of the cancer with the circumferential margin of resection. Thus, the location of the tumor is evaluated in relation to the retroperitoneal margin: above, at the level or below it; and with the rest of the adjacent resected structures.
- For the microscopic study, quadrant sections are included in blocks, choosing the sections with the greatest infiltrative component in the intestinal wall, closest to the circumferential margin painted with Chinese or orange ink.
- The samples are included and stained with hematoxylin-eosin following the standard methodology for histological evaluation.
- The parameters analyzed are those of the protocol already established in the reference unit.

Variables:

- Tumor infiltration zone will be considered after the anatomopathological study: Presence of infiltrating tumor cells in neighboring anatomical structure.
- Evaluation of diagnostic accuracy of 3D-IPR with respect to tumor infiltration:
    - Microscopic analysis of each of the structures marked as infiltrated by 3D-IPR.
- Comparison with CT report regarding:
    - Tumor infiltration of neighboring structures: comparison by microscopic study of each of the anatomical structures suspected or not of being infiltrated according to CT and 3D-IPR.
- Comparison of oncologic results and morbidity & mortality rates with the control group:
    - Historical control group of patients operated at the centers the last 5 years for right, transverse and/or left colon cancer with T4 pathological anatomy.
    - The results of perioperative morbimortality and oncologic results objectifiable by analysis of the surgical specimen and survival will be compared.
    - Survival variables at three and five years will be: local recurrence, distant recurrence and survival.

Sample size:

As this is an innovative study in which a preoperative technique utility is going to be used and has not been analyzed yet, there is no previous bibliography.

It will be carried out in all cases that meet the inclusion and exclusion criteria established for three years in the participating centers.

It is considered that the number of patients operated on in the five centers will be 180 according to current publications. Each center will intervene each year around 12-14 patients with the characteristics described in the methodology. This adds up to 84 patients per each year of study.

Study Variables:

	VARIABLE	VALUES
DEMOGRAPHIC VARIABLES	HOSPITAL	Text
	ID – 3D	Numeric text
	Gender	0: Female 1: Male

	Date of birth	DD/MM/YYYY
	Age	Years
	Comorbidities	0: Arterial hypertension 1: Diabetes Mellitus 2: Ischemic cardiopathy 3: Anticoagulant therapy 4: Immunosuppression/Immunodepression 5: Malnutrition 6: Severe COPD 7: Hepatopathy/Cirrhosis 8: Chronic kidney disease
	American Society of Anesthesiologists (ASA) physical status classification system	0: ASA I 1: ASA II 2: ASA III 3: ASA IV 4: ASA V 5: ASA VI
	Diagnosis	0: Right colon neoplasm 1: Transverse colon neoplasm 2: Left/sigmoid colo neoplasm
	Affirmation or suspicion of infiltration of neighboring structures in CT?	0: No 1: Yes
	Neighboring structures infiltrated in CT scan	Text
	Distance Metastases	0: No 1: Yes
CT SCAN VARIABLES	Primary Tumor (TNM Staging)	0: cT0 1: cT1 2: cT2 3: cT3 4: cT4a 5: cT4b
	Regional lymph nodes in CT scan (TNM Staging)	0: N0 1: N1a 2: N1b 3: N1c 4: N2a 5: N2b
3D-IPR VARIABLES	Affirmation or suspicion of infiltration of neighboring structures in 3D-IPR?	0: No 1: Yes
	Neighboring structures infiltrated in 3D-IPR.	Text
	Volume of infiltration in 3D-IPR	Continuous numeric (mm^3)

	Multidisciplinary committee decision	0: non-resectable 1: Resectable
	Complete Match between 3D-IPR and CT scan?	0: No 1: Yes
	Surgical strategy modification after 3D-IPR?	0: No 1: Yes
	Grade of satisfaction of 3D-IPR for surgical planning	1: Unsatisfied 2: Little satisfied 3: Satisfied 4: Very Satisfied
INTRAOPERATIVE VARIABLES	Surgical approach	0: Laparotomy 1: Laparoscopy 2: Robot-assisted
	Type of surgical intervention	0: Complete Right Mesocolic Excision (R-CME) 1: Complete Right Mesocolic Excision (R-CME) + D3 Lymphadenectomy 2: Complete Left Mesocolic Excision (L-CME) 3: Extended right colectomy 4: Extended left colectomy 5: Transverse colon segment resection
	Visceral resections performed (other than colon)	Descriptive text
	Intraoperative subjective resection margins	0: R0 1: R1 2: R2
POSTOPERATIVE VARIABLES	Surgical intervention date	DD/MM/YYYY
	Discharge date	DD/MM/YYYY
	Postoperative stay	Days
	Clavien-Dindo (Complications)	0: 1 1: 2 2: 3a 3: 3b 4: 4a 5: 4b 6: 5
	Cause of complication	Text
	Tumor size in pathology report	Quantitative continous (mm)
	Affected margins	0: No 1: Yes
	Histology	Text
	T in Pathology Report (TNM Staging)	0: pT0 1: pT1 2: pT2 3: pT3 4: pT4a

		5: pT4b
	N: Lymph nodes (TNM Staging)	0: N0 1: N1a 2: N1b 3: N1c 4: N2a 5: N2b
	Infiltrated neighbor structures	Text
	Pathology report matches CT scan?	0: No 1: Yes
	Pathology report matches 3D-IPR?	0: No 1: Yes

Oncologic variables analyzed at 3 and 5 years:

- Loco-regional recurrence: intraluminal tumor growth near the suture or within the cavity near the previously operated location.
- Distant recurrence: distant metastasis outside the abdominal cavity.
- Peritoneal carcinomatosis: It will be considered carcinomatosis when there are tumor implants in at least two different areas of the abdominal cavity with anatomopathological diagnosis of the presence of tumor cellularity. This makes it possible to differentiate it from loco regional recurrence.
- Disease-free survival: Time from surgery to the date on which recurrence is documented.
- Overall survival: time from admission to death from any cause (includes 90-day post-operative mortality).
- Mortality due to oncologic progression.

All patients with recurrent disease should be confirmed, if possible, histologically or by radiological imaging or new surgery.

Statistical method

Qualitative variables will be expressed by sample size and percentage. Quantitative variables will be expressed by median and range.

In non-parametric univariate analysis, continuous variables will be compared by Kruskal-Wallis test, while categorical variables will be compared by Fisher's Exact test. A p-value < 0.05 will be considered statistically significant.

Data protection database:

Database (DB) data access and storage policy report: The information is stored in cloud servers owned by Oracle. The information is encrypted in DB so any direct access to the DB is useless unless the private key is known. No data that could be used to locate the patient such as name, surname, ID number, etc., is requested. The only data that could be used is the Clinical history ID, which is trimmed, so that the user who reports data can locate the correspondence in his/her hospital, i.e. he must have the real data written down in some place controlled only by him (such as the hospital management program itself) to be able to locate that patient.

In the event of access to the application by someone unauthorized but who has obtained a username and password with access to read patients from their own and other hospitals, the information available does not serve to "de-anonymize" the data. The accesses to the application, writings, readings, etc. are registered by the application so that any access without express authorization can be located.

Work plan, calendar, and percentage breakdown of expenditure between the participating centers

Work plan

For three years, the surgical center will carry out the study:

- Identification of patients who are candidates for the study according to material and methods.
- Referral of the pre-operative CT scan to Cella Medical Innovation and Technology (software owner) for the elaboration of the 3D reconstruction and assessment of tumor infiltration.
- Surgical intervention.
- Detailed microscopic study of the surgical specimen and definitive anatomopathological report.
    - It important to state at this point that these results could be used to create 3D reconstructions based on artificial intelligence. In other words, a convolutional neural network is created to which two elements must be sent: the medical images and the already validated result (*ground truth*) of a significant number of cases (estimated at over 80 cases for an acceptable network). This leads to an artificial intelligence training phase; the network is given 80 cases with two types of information: The medical image + segmentation validated by pathological anatomy (*ground truth*). Then the network learns to segment. Subsequently, a test is performed in which the network is given 20 cases with only the medical image to see what segmentation it performs. This segmentation is compared with *the ground truth* for those 20 cases and its hit rate is observed giving a correlation level. This research will have sufficient number of cases to initiate artificial intelligence phase.
- Incorporation of data to the prospective database.
- Remission of all microscopic images to the main investigator for storage.

During the following 6 months, the analysis of results will be carried out. Assessment of strategies and communication of results.

Coordination program of the project:

- *Phase 1*. Familiarization of the center with the study protocol. The center must present a perfect coordination with four different specialties: colorectal surgery, pathological anatomy, radiology, and oncology.
- *Phase 2.* Selection of the cases. The selection of cases will follow the guidelines set out in the protocol. If there is any doubt, the main coordinator of the study will provide a contact telephone number and e-mail.
- *Phase 3.* Surgical intervention of the patients.
- *Phase 4.* Analysis of the surgical specimen by pathologists. For the anatomopathological study of the cases, the guidelines set out in the protocol will be followed. If there is any doubt, the main coordinator of the study will provide a contact telephone number and email of the pathologist assigned as a reference.
- *Phase 5.* Incorporation of results into the database. Database created especially online for this study. A specific workshop will be held to explain how the database works. If

there are any doubts, the main coordinator of the study will provide a contact telephone number and email of the database creator as a reference.
- *Phase 6*. Analysis of results by the main coordinator of the study.
- *Phase 7*. Analysis of survival results at 3 years.
- *Phase 8.* Analysis of survival results at 5 years.

If greater diagnostic accuracy is observed for this mathematical method of 3D-IPR with respect to infiltration of neighboring structures in locally advanced colon tumors:
- Radiologists would have an objective tool to delimit tumor extension.
- Surgeons would have an objective tool to program the type of surgical intervention with less probability of modifying it during the operative time. In addition, this 3D tool facilitates the preoperative visualization of the tumor location in a compartment where numerous anatomical structures coincide.
- This type of intervention may require the collaboration of other specialties such as gynecology, urology, vascular surgery, traumatology, and plastic surgery. Correct programming of the type of extended resection to be performed is essential. To this end, this tool would provide the necessary data for this purpose.
- The discussion of this type of pathology in a Multidisciplinary Committee within the Colorectal Units is frequent and knowing exactly the degree of infiltration of anatomical structures facilitates decision making.
- The surgery of locally advanced colon tumors presents a high rate of post-surgical complications as well as of affected surgical resection margin. This tool could improve these results considerably.
- The fact of not subjecting the patient to a new diagnostic test to obtain this reconstruction is an important advantage since it is performed using a technique used by protocol to stage this oncologic pathology.

The discussion of this type of pathology in a multidisciplinary committee within a referral colorectal unit is frequent and knowing the exact degree of infiltration of anatomical structures facilitates decision making. At the present time, there are different therapeutic lines for locally advanced colon tumors. Among them we find direct surgery and subsequent adjuvant chemotherapy, there is also evidence of performing neoadjuvant chemotherapy and subsequent resection and finally recent studies propose surgery with intraoperative hyperthermic peritoneal chemotherapy (HIPEC). For this reason, correct staging is essential to avoid overtreatment.
An R0 surgery decreases the risk of oncologic recurrence. This fact reduces the number of patients requiring chemotherapy treatment for this reason, long-term follow-up, the number of face-to-face consultations and complementary tests.
In this way, 3D reconstruction is a tool that could provide digital solutions for healthcare, promoting the development and use of these innovative techniques to improve the quality of life of patients. This explains the need to promote the development of these technologies, considering the security, confidentiality, and standardization, to improve the quality of life of patients.
The surgery of locally advanced colon tumors presents a rate of post-surgical complications that can be high, as well as of affected surgical resection margin. This tool could improve these results considerably. For this reason, if the results manage to change the surgical attitude, the radiology and pathological anatomy services will be informed about this observational study in order to carry out new clinical trials.
The fact of not subjecting the patient to a new diagnostic test to obtain this reconstruction is an important advantage, since it is based on a technique used by protocol to stage this oncologic pathology.

Calendar

Before starting the study, a training day will be required at the Hospital Universitario Son Espases for the collaborating researchers and the pathologists of each center.

In this day will be demonstrated by means of real cases already performed, how to:

- Use the 3D reconstruction software.
- Perform the anatomopathological study of the surgical specimen.

The proposed schedule to be followed is:

	Months					
Activity	**0-6**	**7-12**	**13-18**	**19-24**	**25-30**	**26-36**
Ethics Committee report						
Patient identification						
3D-IPR preparation						
Surgical Intervention						
Microscopic study						
Incorporation of data into the prospective database						
Historical group retrospective data collection						
Results Analysis						
Reporting of results						

Resources available for the implementation of the project

Available resources: material (provided by the center itself):

- Reference center in the Autonomous Community of the Balearic Islands for locally advanced colon surgery.
- 3D reconstruction performed by CELLA Medical Solutions.
- Oncological multidisciplinary committee.

Available resources: research team (provided by the center itself):

- Specialized Colorectal Surgeons with national and European accreditations.
- Anatomopathologist specialized in colorectal surgery.
- Radiologists specialized in colorectal surgery .
- Technological innovations necessary for this type of surgery.
- Different surgical and intensive care units for a multidisciplinary preoperative, intraoperative and postoperative approach.

Results dissemination and utilization plan

Dissemination of the results in the National Congress of Surgery (Asociación Española de Cirujanos), National Congress of Coloproctology (Asociación Española de Coloproctología – AECP), International Congress of European (ESCP) and American Coloproctology and European Congress of Endoscopic Surgery (EAES). Articles resulting from this research are aimed to be published in Q1 Journal Citation Reports (JCR) journals.