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Identification of cancer hallmarks in patients with non-metastatic colon cancer after surgical resection.

Facoltà di Medicina e Odontoiatria

Dipartimento di Chirurgia "Pietro Valdoni"

Dottorando

Dott.ssa Mariarita Tarallo

Tutor

Prof. Enrico Fiori

Coordinatore

Prof. Andrea Mingoli

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INTRODUCTION

In recent years the diagnostic imaging field has found new strategies to exceed its limitations related to uncertainties in the response to therapy and prognostic evaluation looking for new tools able to detect tissue characterization with a more precise and deeper approach than just visual assessment through a quantitative evaluation of images. With the genomic revolution in the early 1990s, medicine and biology fields has started to conceive that diseases has a genetic specific fingerprint, medical research has been driven to deepen the basis of human disease on a genomic level with the aim of provide precise therapies' strategies tailored to the specific genetic makeup of a pathology [1; 2]. The "Omic" revolution has also involved the field of medical imaging, through radiomics [3; 4]. Such technology aims to extract quantitative features from imaging studies to improve disease characterization.

Radiomics, is a new emerging Imaging field consisting of an automated high-throughput extraction of huge number of quantitative features and has the capability to analyze intratumoral heterogeneity in a non-invasive manner in order to extract a quantitative imaging biomarkers potentially, aiming at exploiting personalized medicine [2; 5]. Clearly, Radiomics is a method of research that extracts quantitative radiologic data from medical images (radiomic data) and explores the correlation with clinical outcomes [6; 7]. In addition, radiomics profiling has shown to be superior to conventional approaches in predicting a patient's response to treatment. The process used in radiomics involves the identification of vast amount of quantitative features within digital images, storage of such data in federated databases (that is, a system in which several independent databases function as a single entity) and the subsequent mining of the data for knowledge extraction and application. Innumerable quantitative features already be extracted using high-throughput computing from medical images such as CT, MR, PET and US [8; 9].

To date, Radiomics has becoming central in emerging precision personalized-medicine especially for oncologic patients; traditional Imaging has been shifting from diagnostic and staging to be a quantitative tool in detecting cancer, prognosis prediction and assessment of response to therapy [10]. Today, Radiomics has the expectancy to be an additional skill to provide a quantitative evaluation of tumor in a complementary manner to the observational approach. It should be considered as a helpful tool for the physicians to manage oncologic patients by building a structured workflow by adding some objective data to traditional clinical evaluation.

Radiomics parameters are extracted from a specific regions of interest (ROIs) [11], selected on encrypted medical images, and the mineable quantitative data reflect neoplasm phenotypes and heterogeneity, usually correlated with tumor aggressiveness [11-13]. Then, radiologists in a single image evaluation can obtain an estimation of volumetric tumor heterogeneity in terms of radiomic parameters (i.e. tumor shape and textural parameters). In that scenario, Oncologists may have an additional non-invasive biomarker to complement the biopsy that often can result too reductive or not diagnostic especially in case of small lesion [14].

This recent landscape of Imaging, which is showing fundamental for improving clinical practice of oncologic patients, has been attracting the attention of many researchers, driven by promising results achieved through integration of Radiomics data with clinical biomarkers, in fact between 2013 and 2018 were published 553 original articles concerning Radiomics [15]. Radiomics-approach resulted to be a promising tool for oncologists to set, modify or assess treatment protocol according to evidence-based medicine [16]. In this way, Radiomics could provide a quantitative measurable difference of radiomic parameters between baseline and post-treatment images that may reflect intralesional radiomics changes that could be induced by therapy, such as necrosis or vessel tortuosity, or by tumor progression [17; 18].

The aim of this thesis is to investigate the pivotal role of Radiomics in diagnosis, prognosis and evaluation of response to therapy in cancer patients, through an accurate description of promising results and main limitations of quantitative approach.

SURGERY IN COLORECTAL CANCER

Surgery remains the mainstay for the treatment of colon and rectal cancer.

Localization of the tumor and its histopathology are important in selecting an operative plan and the optimal resection margins. The presence of a lesion at watershed areas of vascular supply may require a more extensive resection of the colon. Colonoscopy is widely used today and represents the optimal means of detecting a cancer, identifying its location, providing histopathologic material, and tattooing for intraoperative localization when required. Precise localization of the lesion with ink tattooing is paramount in the era of laparoscopy since manual palpation is not possible. The lesion should be inked in three separate areas around the circumference of the colon wall distal to the lesion. Computer tomography (CT) allows the localization of larger lesions, identification of local organ invasion, and provides important staging information regarding the presence of extracolonic disease, particularly liver involvement. For rectal cancer patients, the tumor-related factors of prognostic significance which may be evaluated prior to the treatment of rectal cancer include the depth of penetration of the tumor through the rectal wall, the presence or absence of metastases to the regional and pelvic lymph nodes, and the presence of distant metastases. So preoperative staging includes assessment of local staging by ERUS, MRI, and CT.

The preoperative detection of distant metastasis can influence the initial management of a colorectal cancer patient. Computed tomography is the most widely used imaging modality to screen for liver metastasis because of its availability and relative low cost compared to positron emission tomography (PET).

Effective preparation of the patient requiring a colonic resection for colon cancer or an anterior resection for rectal cancer requires knowledge of the tumor location, clinical stage and patient's physiologic status. A variety of scoring systems are available for grading operative risk of surgical patients. The most widely applied scoring system is the American Society of Anesthesia (ASA) score; however, this tool only provides information regarding the risk of an anesthesia complication given a certain physiologic status.

Bowel preparation has historically been considered an essential component of the preoperative preparation of the patient. Mechanical cleansing combined with oral antibiotics reduces the concentration of aerobic and anaerobic bacteria within the colon and decreases the incidence of wound infection from 35 to 9%. However, more recent prospective randomized studies have questioned the additional benefit of luminal preparation, compared to the use of appropriate intravenous antibiotics administered in a timely manner.

For colon cancer, the principles of an oncologic resection were a wide mesenteric resection achieved by ligating the feeding artery at its origin with adequate distal and proximal margins. It is recommended that a minimum of 12 lymph nodes should be examined. There are several studies that support a survival benefit for patients who have 12 or more lymph nodes examined after surgical resection. This benefit most likely occurs for two reasons. First, the greater number of lymph nodes examined increases the accuracy of the final pathologic staging, a phenomenon known as stage migration. Second, there is clearly an oncologic benefit to a radical mesenteric resection, where all involved lymph nodes are resected. For rectal cancer, total mesorectal excision (TME) should typically be performed.

Right colectomy

The patient is placed supine on the operating table. If laparotomy rather than a minimally invasive technique is chosen, a vertical midline incision is made sufficiently long to allow complete visualization of the operative field. After the incision is fashioned, a thorough examination of the abdominal and pelvic contents should be performed. Particular attention should be paid to potential metastatic sites, especially the liver. The resectability of the tumor should be assessed with minimal manipulation of the lesion. It is important to determine if disease is adherent to adjacent viscera which should then be included as an en bloc resection. It is rare that a right-sided tumor is unresectable, however, extensive involvement of the vena cava, superior mesenteric artery, or the pancreas may dictate a palliative resection or bypass procedure. The key to an oncologically safe and effective resection of a colon cancer requires clear lateral margins, resection of the locoregional lymph node bearing mesentery for both cure and staging, and performance of an accurate and well-vascularized anastomosis. The right colon mesentery is elevated off the retroperitoneum, and the duodenum is identified. The lateral attachments are incised and the hepatic flexure is fully mobilized. For cancer operations, it is best to resect the omentum with the specimen, so when entering the lesser sac, the lesser omentum or gastrocolic attachments are divided. The ileocolic and right or hepatic branch of the middle colic vessels are ligated at their origins. The terminal ileum should be divided 10–15 cm proximal to the ileocecal valve to allow for good vascular supply. The transverse colon is divided just to the right of the main trunk of the middle colic artery. The ileocolic anastomosis can be fashioned according to the desire of the operating surgeon. We usually perform an end-to-lateral ileo-colic anastomosis by anastomosing the bowel segments with a circular stapler and closing the remaining colostomy with a linear stapler or sutures.

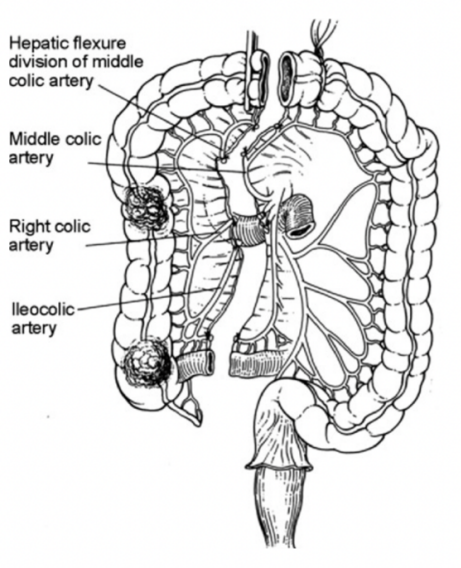


Figure 1. Levels for vascular ligation and colonic transition for a right hemicolectomy

Extended Right Colectomy

An extended right colectomy should usually be performed for any lesion involving the transverse colon. This procedure once again should achieve complete resection, lymph node clearance, and most importantly two optimally vascularized bowel segments for anastomosis. The operation proceeds in similar fashion as the right colectomy described above. However, rather than proceeding through the transverse colon mesentery to ligate and divide the right branch of the middle colic artery, dissection continues in the retroperitoneal plane to identify the main middle colic arterial trunk anterior to the pancreas. This vessel is ligated and divided. The right colon is then mobilized medially as before, and the lesser omentum is divided along the entire transverse colon. The splenic flexure is released and the bowel with its mesentery is divided just proximal to the left colic artery which is preserved for right-sided lesions. The left colic may be sacrificed for left transverse colon lesions, where a more distal colonic anastomosis is desired. The ileocolic anastomosis is then constructed based upon surgeon preference.

Left Colectomy

The left colon can be mobilized in either a lateral to medial or medial to lateral approach. For the lateral to medial approach, the small bowel is packed to the right upper quadrant. The lateral peritoneum from the sigmoid colon to the splenic flexure is incised. The left colon mesentery is elevated off the retroperitoneum, so the left ureter is exposed and the colon and its mesentery are brought to the midline. This allows the inferior mesenteric artery (IMA) to be ligated at its origin at the aorta and the inferior mesenteric vein (IMV) to be ligated near the ligament of Treitz and the inferior border of the pancreas. For the medial to lateral approach, the small bowel mesentery is mobilized to the right upper quadrant to expose the origin of the inferior mesenteric artery located just caudal to the third portion of the duodenum. The superior rectal artery is grasped at the level of the sacral promontory, the peritoneum is incised, and the retroperitoneum is entered. The left ureter is reflected into the retroperitoneum and the IMA is traced up to its origin. A window is then created on the cephalad side of the artery, medial to the IMV, and the artery is then ligated. The inferior mesenteric vein is ligated at the base of the pancreas. The mesentery is elevated off the retroperitoneum toward the abdominal wall and the lateral attachments are then incised. For either approach, the splenic flexure is mobilized by separating the omentum from the transverse colon. This completely opens the lesser sac and allows the posterior attachments to the inferior border of the pancreas to be divided. The bowel is transected with at least a 5 cm proximal margin and the distal site of resection on the top of the rectum. We perform an end-to-end circular stapled anastomosis, dividing the rectosigmoid junction with a linear stapler or purse-string suture. A leak test with air insufflation of a submerged anastomotic segment should be performed in all cases. Resection of proximal left colon lesions may require division of the middle colic artery to allow the right transverse colon to reach the rectal stump for an anastomosis. However, an

extended right colectomy and ileosigmoid or ileorectal anastomosis may be preferable if there is any concern related to the blood supply. Another alternative is to perform a retroileal right colon to rectum anastomosis if maintenance of the right colon is desired. The type of anastomosis is left to the discretion of the surgeon.

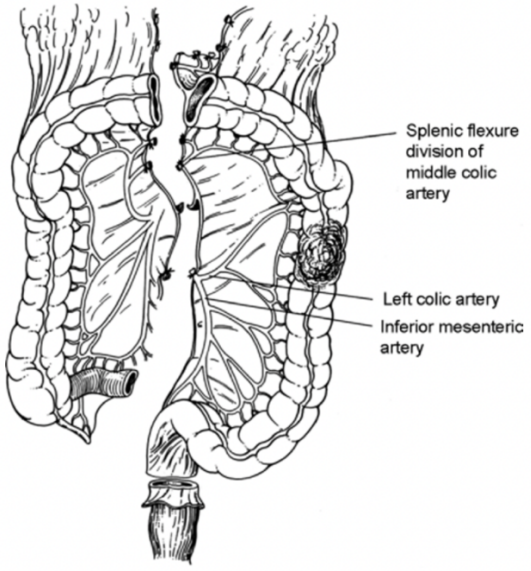


Figure 2. Left colon cancer

The small bowel mesentery is mobilized to the right upper quadrant to expose the origin of the IMA located just caudal to the third portion of the duodenum. An incision running along the base of the left colic and sigmoid mesentery from the sacral promontory to the ligament of Treitz, exposes the aorta, bifurcation of the common iliac arteries, and IMA vein. The IMA is ligated and divided proximal to the take-off of the left colic artery. The left branch of the middle colic vessels requires ligation and division for a formal left colectomy.

Laparoscopic Colon Resection for Cancer

The application of laparoscopic techniques has been used in colorectal surgery for more than 15 years. The short-term benefits of laparoscopy have been demonstrated, such as faster return of bowel function, shorter length of stay, and less narcotic use, but there was minimal short-term quality of life benefit with laparoscopy. Further studies stated that overall survival was equivalent between the laparoscopic and open groups. Additionally, there was no difference in survival or recurrence for any stage of cancer. Furthermore, it was demonstrated that conversion did not have a negative impact on the oncologic outcome of these patients. Equivalent survival and recurrence rates stage for stage have been reported for laparoscopic and open colectomy for colon cancer. A significant learning curve is associated with laparoscopic colectomy. Nevertheless, with adequate experience, laparoscopic colectomy for right- or left-sided colon cancers is safe and provides similar outcomes to open colectomy.

Rectal Cancer

The type of surgery used for rectal cancer depends on the stage of the cancer, where it is, and the goal of the surgery. Radiation and chemotherapy are often given before and/or after surgery.

Local excision is an appropriate treatment modality for carefully selected patients with cT1N0 rectal cancer without high-risk features. Transanal excision may also be appropriate for patients with more advanced cT disease but who are considered medically unfit for radical cancer surgery. Whereas local excision offers advantages of minimizing operative risk and functional sequelae, it does not adequately remove or pathologically stage the mesorectal lymph nodes.

Distinguishing early depth of invasion (ie, Tis, T1, T2) may be difficult with MRI, and EUS may be utilized as a complementary staging tool in certain situations. Clinical criteria for local excision typically include small (<3 cm) adenocarcinomas limited to <30% of the rectal circumference, that are well or moderately differentiated, without lymphovascular invasion, perineural invasion, tumor budding on tissue biopsy, and no clinical nodal involvement, and that are accessible transanally for full-thickness excision. In general, local excision is considered an oncologically inadequate treatment for cT2 lesions because the local recurrence rate ranges from 26% to 47%, and these tumors have an elevated risk for harboring occult nodal disease. Radical resection should typically be recommended under these circumstances.

For curative resection of tumors of the upper third of the rectum, a tumor-specific mesorectal excision should typically be performed as part of a low anterior resection (LAR) with the mesorectum divided, ideally, at least 5 cm below the distal margin of the tumor.

Total mesorectal excision (TME) in conjunction with an LAR or an abdominal perineal resection involves precise sharp dissection and removal of the entire rectal mesentery, including that distal to the tumor, as an intact unit. For tumors of the middle and lower thirds of the rectum, TME should typically be performed. Appropriate surgical technique is integral to optimizing oncological outcomes and minimizing morbidity and should follow the principles and anatomic planes of a TME. Dissection between the visceral and parietal layers of the endopelvic fascia facilitates en bloc removal of the rectal cancer and associated mesentery, lymphatics, and tumor deposits. Mesorectal excision can preserve the autonomic nerves and reduce intraoperative bleeding and the rate of local recurrence.

Conventional rectal surgery is associated with a significant incidence of sexual and urinary dysfunction. The extent of resection margins in rectal cancer remains controversial. Although the first line of rectal cancer spread is upward along the lymphatic course, tumors

below the peritoneal reflection also spread distally by intramural or extramural lymphatic and vascular routes. When distal intramural spread occurs, it is usually within 2.0 cm of the tumor, unless the lesion is poorly differentiated or widely metastatic.

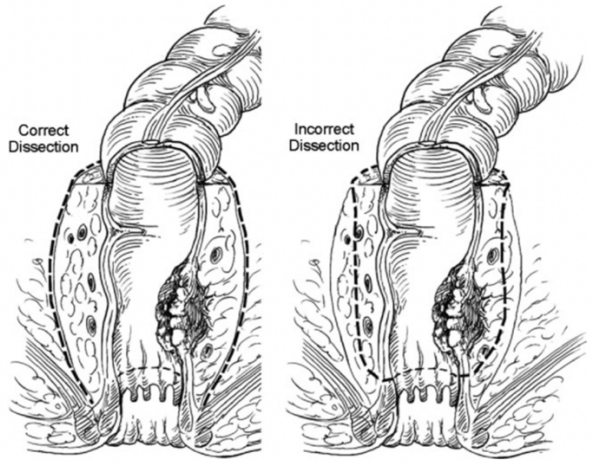


Figure 3. Correct TME dissection versus an incorrect dissection. The dissection should proceed between the mesorectal fascia and the pelvic wall fascia to ensure a “complete” TME.

Sphincter-sparing procedures for resection of mid and some distal rectal cancers have become increasingly prevalent as their safety and efficacy have been established. The advent of circular stapling devices is largely responsible for their increasing popularity and utilization. An LAR involves dissection and anastomosis below the peritoneal reflection with ligation of the superior and middle hemorrhoidal arteries. An extended LAR indicates complete mobilization of the rectum down to the pelvic floor with division of the lateral ligaments and posterior mobilization through Waldeyer’s fascia to the tip of the coccyx. Additionally, there is dissection of the plane between the anterior rectal wall and the vagina in a female patient and dissection of the plane between the rectum and the prostate in a male patient to a level distal to the inferior margin of the prostate gland. As long as the surgeon can obtain a distal margin of at least 2 cm, an anastomosis can be considered appropriate if technically feasible. Body habitus, adequacy of the anal

sphincter, encroachment of the tumor on the anal sphincters, and adequacy of the distal margin are all factors in determining the applicability of a sphincter-sparing operation.

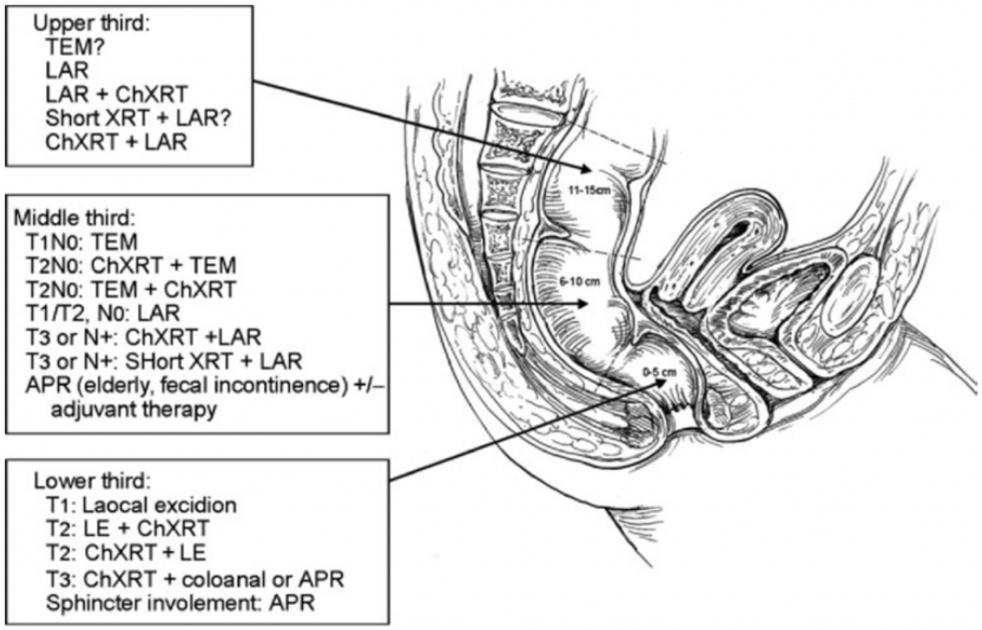


Figure 4. Treatment options for rectal cancer depending on stage and location.

Stage I (T1N0, T2N0 – the cancer is confined to the rectal wall, and no nodes are involved). Distal rectal cancers: T1 (invasion into the submucosa only): Local excision; Radical resection, often an APR; Adjuvant therapy is usually not recommended. Distal rectal cancers: T2 (invasion into the muscularis propria): Local excision with preoperative or postoperative adjuvant therapy; Radical resection without adjuvant therapy, often an APR. Mid rectal cancer: T1: TEM (transanal endoscopic microsurgery); Radical resection, usually an LAR with low anastomosis. A temporary proximal diverting ostomy is often required; Adjuvant therapy is usually not recommended. Mid rectal cancer: T2: TEM with either preoperative or postoperative adjuvant therapy; Radical resection similar to a T1 cancer; Adjuvant therapy is not recommended if a radical resection is performed but is recommended before or after a TEM resection. Upper rectal cancers: T1 and T2: LAR; TEM?

Stage II and Stage III cancers [Stage II cancers have invasion into the mesorectal fat (T3) but no involved mesorectal lymph nodes. Stage III cancers are any rectal cancer (T1, T2, or T3) but with involved lymph nodes.] Distal rectal cancers: Preoperative adjuvant therapy is most often recommended followed by a radical resection, usually an APR; If preoperative imaging does not clearly define the stage of the cancer, resection can be done first followed by postoperative adjuvant therapy. Mid rectal cancers: Same as above for distal rectal cancers except an LAR is usually performed instead of an APR. Upper rectal cancers: LAR, with either preoperative or postoperative adjuvant therapy.

Stage IV cancers: Treatment for any cancer is dependent on the extent of metastasis. With better surgical and medical treatments for metastatic disease, locoregional control of the primary should be aggressive and similar to the above recommendations except in the most advanced cases. Key: LE local excision, short XRT short-course radiation therapy given two times a day for 5 days in larger fractions, ChXRT long-course therapy given in 30 smaller fractions over weeks in combination with chemotherapy

Laparoscopically Assisted Resections for Rectal Cancer

The application of laparoscopy for the treatment of intraabdominal malignancies including proctectomy for rectal cancer is now being performed. In these operations, part of the procedure is done using the laparoscope, and completion of the procedure is in the traditional manner. In particular, exploration and mobilization of the colon and rectum can be done with the laparoscope and laparoscopic instruments. Ligation of the vascular pedicle is performed with laparoscopic clips, vascular stapling devices, or radiofrequency coagulation devices. The improved optics of laparoscopy can provide a much better view in the pelvis, thus facilitating rectal dissection. In the recent years, there has been an increased use of robotic more than laparoscopic resection for rectal cancer. Most often, however, the actual resection of the bowel and an anastomosis are still more easily performed in an extracorporeal manner. The main questions about laparoscopic- and robotic-assisted proctectomy for colorectal cancer are whether they provides the same TME specimen as traditional open techniques, and whether there is any other unique biologic alteration in the laparoscopic procedure that leads to a change in survival or in recurrence patterns. It is of paramount importance that laparoscopic and robotic resection follows the same oncologic principle as open surgery including precise or better TME. The blood loss is usually less. Most of the studies report earlier return of bowel function, decreased hospital stay, and reduction in pain. The rate of anastomotic leak in sphincter sparing rectal surgery is comparable between two approaches and is approximately 10% and can be as high as 17%. Also, there have been two reports of an increase in erectile dysfunction with the minimally invasive rectal resection versus open surgery.

Robotic assisted surgery

The advantages of a robotic platform over laparoscopy include stability of visualization, tireless retraction, improved exposure and precision, better instruments, and suturing movements. For rectal cancer specifically, the robot allows for a finer dissection of the rectum out of the tight space where it is located. The advantages of robotic surgery are especially applicable in cases like these, due to the difficulty of laparoscopic pelvic surgery. The improved ergonomics that robotics offer are therefore hugely beneficial in the treatment of colorectal conditions. The advantages of robotic colorectal surgery are expressed in a better postoperative recovery, lower anastomotic leak rate, lower conversion rate and better functional outcomes. There was no difference in survival or recurrence for any stage of cancer. It was demonstrated that conversion rate to open surgery was lower than laparoscopy. Equivalent survival and recurrence rates stage for stage have been reported for laparoscopic and robotic colorectal resections. A significant learning curve is associated with robotic surgery.

Technical Background

Imaging has an important role in healthcare and is considered as a complementary knowledge to lab tests, patient demographic, and family information. The major topics in imaging informatics, include image resolution, image enhancement, denoising, fusion, and knowledge extraction. Commonly, after the images are acquired from the imaging modalities, they are processed by these methods in a processing pipeline with the end goal of producing image features or actionable knowledge to improve healthcare.

Figure 5 illustrates the radiomics workflow for any imaging dataset, which may be 2D, 3D, or of higher dimension. Component parts are: (1) identification of the location of the VOI to be analyzed, (2) annotation of the tissue with semantic features, (3) VOI segmentation, i.e., identification of the entire imaged volume of tissue to be analyzed, and (4) feature computation via human-engineered image features. In some cases, “delta” features [19; 20] may be computed by comparing individual feature values derived from different images acquired at different times. Collections of imaging features may be also created that combine features computed from multiple imaging methods. These imaging features may be summarized in a feature vector.

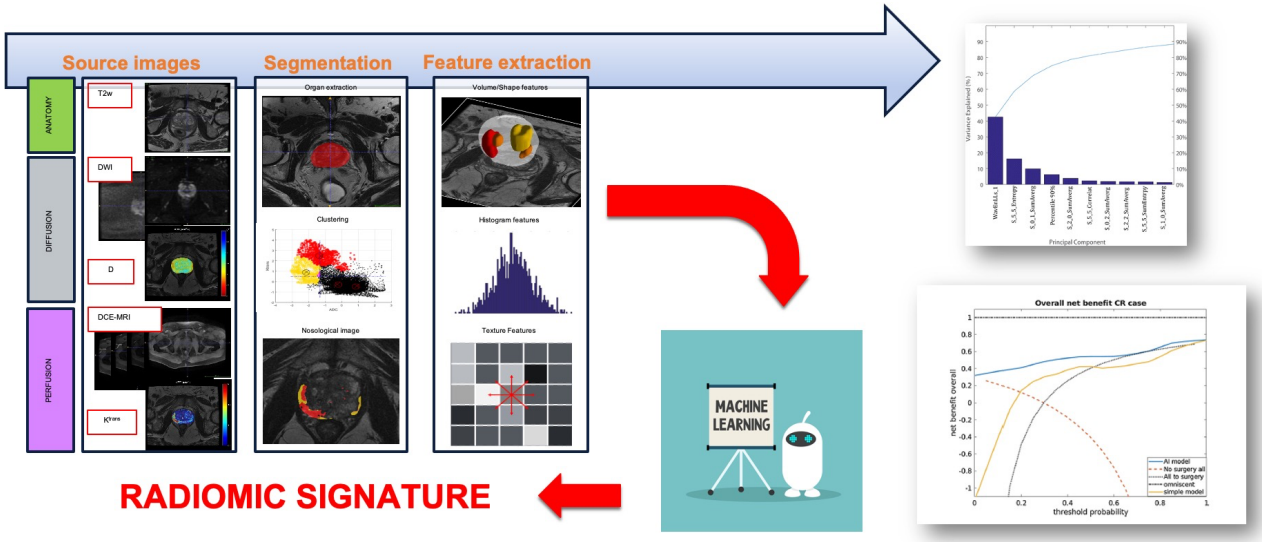


Figure 5. Radiomic workflow

- *VOI identification*: Each and every VOI to be processed must first be identified, either semi-automatically or manually by a radiologist or automatically using computer aided detection approaches. In cancer imaging, e.g., when multiple tumors are present in a single imaging study, human effort is generally required to identify those that are clinically relevant, as in the case of index lesions scored with RECIST ("response evaluation criteria in solid tumors"). When computing delta radiomics features, matching of tumors across observations will also be required.
- *Annotation with semantic features:* Semantic features are descriptive observations of image content. For example, semantic features of a lung tumor might include "left lower lobe," "pleural attachment," "spiculated," "ground-glass opacity," etc. However, extraction of semantic content from unstructured radiology reports may not be appropriate due to inconsistent and/or ambiguous vocabulary across observations and observers. Further, structured reports may not support the kinds of detailed observations required for making fine distinctions among tumor characteristics that may prove useful in classification or assessing response. Some semantic features, such as location, other morbidities, etc., are meant to be complementary to computational features; others, such as "spherical," "heterogeneous," etc., are correlated with computational features. One advantage of semantic annotations is that they are immediately translatable, i.e., they can be elicited in clinical environments without specialized algorithms (e.g., segmentation) or workstations. As such, they have shown interesting results in several radiogenomic studies [21; 22]
- *VOI segmentation*: Radiomics features can be extracted from arbitrary regions within the image volume: In cancer imaging, e.g., a given region may contain an entire tumor, a subset of the tumor and/or a peritumoral region thought to be involved with or affected by the tumor. In all cases, these regions must be unambiguously identified (segmented) and input to the radiomics feature computation algorithms. This

segmentation step is the single most problematic aspect of conventional radiomics workflows, as the features computed from tumor volumes may be extremely sensitive to the specification of the volume to be analyzed. Each combination of tumor type and image modality presents its own challenges (including volume averaging of tissues within each voxel, tumor contrast with surrounding/adjacent structures, image contrast-to-noise characteristics, and variations of image quality across vendor implementations and time). In addition, many algorithms require operator inputs, such as bounding boxes and/or seed points, and the segmentation outlines and radiomics features computed from them may be sensitive to these inputs. Thus, the state-of-the art today is such that each segmentation must be reviewed and possibly edited by a human observer in order for the radiomics features computed from it to be trusted. One potential mitigation is to ignore segmentation altogether and to compute only features that do not require complete edge-to-edge coverage of the tumor, i.e., histogram and texture features, which may be less sensitive to the exact tumor definition, and to ignore shape and margin sharpness features, which require accurate and consistent edge delineations.

- *Image feature computation:* Conventional or human-engineered computational image features can be divided into four classes: those that describe (1) shape, (2) margin sharpness, (3) histogram features (e.g., mean, variance, kurtosis, maximum, minimum), and (4) texture features, which describe the spatial variation of gray values within the tumor. Within each class there are hundreds to thousands of individual features, for example, some texture features quantify the spatial variation of gray values across multiple scales and orientations, and shape features can similarly quantify edge irregularity at multiple scales. It is important to recognize that many image features are inter-correlated and, as a result, not all features may add independent predictive power to radiomics models. A standard approach in many

studies is to generate an autocorrelation matrix and combine correlated features into a single descriptor. Several groups have made available computer code and processing pipelines for the calculation of image features from volumetric image data and segmentations (or at least volumes of interest): see, e.g., the imaging biomarker explorer, the Quantitative Image Feature Engine, and pyRadiomics.

An additional challenge is the need to standardize the methods for calculation of radiomics features so that identically intended features computed from the same data by different algorithms have the same name and values. Indeed, the same study referenced above that compared features computed from multiple segmentations also revealed that implementations from separate institutions of purportedly the same feature sometimes produced different values [23]. Much effort is underway to standardize feature naming and computation conventions, predominately led by the image biomarker standardization initiative and the Quantitative Imaging Network [24].

In addition to the challenges raised by segmentation, a final challenge is sensitivity of radiomics features to image acquisition and reconstruction, i.e., the heterogeneity of image acquisitions. Each clinical study uses their own combination of acquisition parameters, such as slice thickness, reconstruction kernel, MR pulse sequences, etc. In addition, many acquisition parameters are optimized for the particular patient under study (e.g., kilovolts [kV], milliamperes [mA] field of view). While radiologist interpretations are somewhat immune to these differences, computational radiomics features are by design sensitive to these choices.

IMAGE ANALYSIS

Imaging modalities can be divided into anatomic, functional, and molecular screening. Each of them can contribute to disease interpretation, and the combination of their extracted information can supply a significant added value. Anatomic imaging modalities such as computerized tomography (CT), mammography, and MRI need to have high spatial resolution to accurately identify the structure of objects of interest (e.g., organs, lesions). Other imaging techniques focus on functional imaging and tend to have a lower spatial resolution, comparing with anatomic imaging. Relying on these techniques for an accurate structural identification will not be accurate enough, though they will able to help in under- standing the functional significance of the specific tissue. Example functional modalities include ultrasound Doppler that can help to analyze the blood flow and positron emission tomography that can help in recognizing metabolic processes. Therefore, functional imaging can help in finding ischemia, inflammation, necrotic regions, and cancer tissue. Another emerging technique is molecular imaging, a sub-field of functional imaging, in which we measure the expression of particular genes. This provides a potential platform for linking specific imaging analysis with a specific molecular gene expression pattern.

Lastly, the importance of integrating the image information from two imaging types (e.g., molecular, functional, anatomical) to detect tissue changes was recognized and became very popular over the recent years. Such a cross-modality analysis can help in understanding the role of specific genes on the tissue structure/functionality. Two approaches were introduced: (1) a single machine such as functional magnetic resonance (fMRI) that can supply both anatomical and functional analyses, and (2) fusion of the information that is obtained from two separate modalities and will be detailed below in the “Image Registration” section.

Image fusion

Image fusion is applied to construct a more detailed and representative output image by using image registration, feature extraction, and semantic information conclusion [25]. Image registration is the process of mapping input images with the help of reference image. Image registration is considered as an optimization problem whose goal is to maximize the similarity between the images. Applications in the medical field usually include registration of anatomical modalities (e.g., CT, MRI) with functional modalities such as PET, single-photon emission computed tomography (SPECT), or f-MRI. This kind of registration supplies complementary information that can help a lot for intervention and treatment planning, computer-aided diagnosis and disease following-up, surgery simulation, radiation therapy, assisted/guided surgery, anatomy segmentation, computational model building, and image subtraction for contrast-enhanced images [26; 27]. Image registration can be roughly done by three different approaches. First, by measuring the intensity similarity between different pixels/voxels in the images. It can be done by applying rigid or non-rigid techniques. The main difference between them is that in case of rigid approach, we assume that the whole object is moving together and in non-rigid—different local distortions can occur in different locations within the object. Second, by detecting key points within the images and then matching those points. In this group of techniques, one can find SIFT and SURF. These key points must be characterized by a distinction to the spatial neighbors, invariance to the original image variations, robustness against noise, and with high computational efficiency. Image descriptors are then used to represent the extracted key points. After defining the coordinates of the key points, the transformation function estimates the geometric relation between the images. The transformation functions can be selected based on the images that are needed to be registered, although, it is hard to find a single transformation function that is better for all types of images due to the strengths and weaknesses associated with each function. After

registering the images, multiple image features can be extracted, and the information that was extracted from the different imaging modalities can be fused and lead to the overall clinical decision. Third, an image registration can be done by atlas-based approaches [28].

Image Resolution

Image quality depends, among all, on image resolution. Image resolution is divided into spatial, temporal, and contrast resolutions. Spatial resolution refers to the ability of differentiating between two points in the space. High spatial resolution means that we can separate well between two points that are very close to each other in the space. Temporal resolution represents the number of images that can be acquired per second. Real-time application must have a high frame rate, means high number of images per second. An optimal imaging modality would produce images with high spatial and temporal resolution, however, usually there is a trade-off between these two. There are many super-resolution methods that were developed to improve the image resolution. Most of these techniques can be divided into four different groups—prediction models, edge- based models, image statistics, and patch-based models.

Contrast resolution refers to differences in spatially adjacent pixels or their local surrounding and is the basis for recognizing anatomic structures and abnormalities, which differ from adjacent regions through local differences in pixel values. Imaging contrast agents can help to increase the contrast resolution. Contrast agents have different imaging characteristics than the body tissues, and as a result, they can enhance the contrast differences between themselves and the surrounding regions. The composition of contrast agents varies according to the modality characteristics and to optimally be visible based on the physical basis of image formation. Over the recent years, advances in molecular biology have led to the ability to design contrast agents that are highly specific for

individual molecules, and as a result, only the specific region of interest is highlighted in the image.

Image Enhancement

The goal of image enhancement is to improve the visual appearance of the image by converting a low- quality image to a high-quality image and to enable better automated image analysis such as detection, segmentation, and recognition. An optimal image enhancement technique should supply: (1) time and computational efficiency, (2) simple implementation, (3) robustness to noise to avoid noise enhancement and to different kinds of images, (4) structure preservation to keep image texture, and (5) continuity, which means that a small change in the input should cause a only a small change in the output. Enhancement procedures can be mainly divided into two classes—spatial domain methods and transform domain methods. Spatial domain methods are very popular for image enhancement, and they incorporate different histogram manipulations such as histogram equalization to automatically determine a transformation function producing an output image with a uniform histogram. Another approach is histogram matching, wherein we generate an image that has the same intensity distribution as a predefined desired histogram. Gamma correction is another popular technique to stretch the histogram of a region of interest, separately and independently enhancing each local region. Transform enhancement frameworks incorporate techniques such as Fourier transforms, and as a result, the image is enhanced by modifying the frequency substance of the image.

Image Denoising

Similar to image enhancement, image denoising approaches can be categorized as spatial domain, transform domain, and dictionary learning-based approaches. Spatial domain methods include local and non-local filters, which exploit the similarities between the

statistics of different regions in the image. The main difference between local and non-local frameworks is the size of the surrounding region that an examined region is compared with. A large number of local filtering algorithms have been designed for noise reduction such as wavelet filter Wiener filter, least mean squares filter, bilateral filter, anisotropic filtering, blind source separation, and co-occurrence filter. Local methods are effective in terms of time complexity and also for considering the more relevant information within closer regions only. However, local frameworks are more sensitive than non-local ones to high amounts of image noise. Even though they are better than local filters for dealing with high noise levels, their major drawback is that they still create artifacts such as over-smoothing. The second category is transforming domain methods, wherein the image patches are represented by their frequency content. These methods usually achieve better performance compared to spatial domain methods, because they have higher level properties such as sparsity and multiresolution.

Image Segmentation

Segmentation of images involves an automated annotation of a relevant ROI within an image. Traditional segmentation approaches mostly include thresholding, region- growing, watershed, clustering, active contours and level sets, atlas- based, and graph-based models. Popular segmentation techniques can be divided into edge-based, regions-based, model-based or knowledge-based, and machine learning-based approaches. Edge-based segmentation relies on detecting and analyzing the boundaries of an object. However, in cases of noisy images, low contrast images, or incomplete broken boundaries, edge-based approaches will not perform well. Region-based techniques can handle better with these challenges because they con- sider the statistics inside a ROI, thus an object will be accurately segmented as long as the background- foreground statistics are different from each other. Both region-based and edge-based segmentation are essentially low-level

techniques that only focus on local regions in the raw image data. A popular alternative method for medical image segmentation is a model-based deformable models (e.g., active contour, level set, active appearance models). These segmentation approaches have been established as one of the highly successful methods for image analysis. By developing a model that contains information about the expected shape and appearance of the structure of interest to new images, the segmentation is conducted in a top-down fashion. Due to the significant a priori information, this approach is more stable against local image artifacts and perturbations than conventional low-level algorithms that consider the image data only. Information about common variations has to be included in the model. A straight-forward approach to gather this information is to examine a number of training shapes by statistical means, leading to statistical shape models (SSMs). Well-known methods in that area are the active shape models and active appearance models.

Feature Extraction

After segmenting the desired ROI, image features can be extracted. These images can be first-order features such as pixels' intensity or higher-order features such as more complex texture features. These features can be considered as a sparse representation of the whole image data. In order to reduce the feature space dimensionality, the feature extraction procedure is usually followed by a feature selection step. Feature selection is the technique of selecting a subset of dominant features for building robust learning models by keeping the most dominant features only. Feature selection also helps people acquire better understanding about their data by telling them what are the important features and how they are related with each other and with the image itself. There are several common-used methods for features selection, including principal components analysis (PCA), linear discriminant analysis (LDA), least absolute shrinkage and selection operator (LASSO), and generalized linear models with elastic-net penalties (GLMNET) that

are usually used in case that we have many more features than patients. It combines L1 and L2 losses by integrating LASSO and ridge regression.

Deep Learning

Machine learning approaches also have been popular for medical image analysis as the strong computational resources became available. Machine learning and specifically deep learning architectures can be used in different types of tasks. However, their main limitation is the need of a lot of training labeled data. Many recent methods were developed to tackle those limitations. Generative adversarial networks (GANs), w-GANs, and stacked-GANs can be used for data augmentation and for the improvement of the image quality. Convolutional neural networks such as U-Net and V-Net were designed specifically to deal with medical domain challenges such as small amount of labeled data. Autoencoders, variational autoencoders, and stacked-autoencoders can be used for image denoising and as an unsupervised features extractor. Other methods were designed to handle with various of classifications tasks such as lesion detection, segmentation, and disease classification.

EXPERIMENTAL PART

From

Radiomic Cancer Hallmarks to Identify High-Risk Patients in Non-Metastatic Colon Cancer.

Caruso D, Polici M, Zerunian M, Del Gaudio A, Parri E, Giallorenzi MA, De Santis D, Tarantino G, Tarallo M, Dentice di Accadia FM, Iannicelli E, Garbarino GM, Canali G, Mercantini P, Fiori E, Laghi A. Cancers (Basel). 2022 Jul 15;14(14):3438

Introduction

Colon cancer is the fifth most common cancer for incidence and mortality with 1.480.000 new cases in 2020 worldwide [29]. The main therapeutic options are the surgical resection and adjuvant chemotherapy in non-metastatic colon cancer; however, the evaluation of overall adjuvant chemotherapy benefit, in patients with high risk of recurrency, is a clinical challenge [30]. The decision is based on TNM staging system [31], which represents the most important parameter, colon cancer at stage III are globally recognized as patients who can benefit from chemotherapy, while regarding stage II with other clinical risk factors the advantages of chemotherapy are still debated [30; 32]. In presence of clinical risk factors the final strategy is often arbitrary decided by the oncologist. Nevertheless, several evidence reveal that not all clinical risk features are equal, not all affect the overall survival, and the decision to treat colon cancer with adjuvant chemotherapy should be assessed in a multidisciplinary approach [33].

In that context, Radiomics could have a pivotal role in the colon cancer workup with the expectancy to help the clinicians in identifying patients with high-risk disease. Radiomics might be used as a non-invasive imaging biomarker, able to provide a

quantitative evaluation of medical images, with the chance to shift the imaging approach from conventional, which is qualitative and subjective, to quantitative. This new field of imaging has the ability to extract a large amount of data from specific regions of interest (ROIs), including differences in image texture, spatial resolution and pixel interrelations, that are rather imperceptible to the human eye, in order to quantitatively outline image phenotypic characteristics at an ultrastructural level [11; 34]. Up to date, radiomic approach has been extensively investigated in the cancer patients with a specific focus on tumor diagnosis, staging, prediction prognosis and long-term monitoring [34].

Concerning colon cancer, several managerial aspects were explored with the aim to test the performance of radiomics as an adding tool in clinical setting. In particular, the main fields examined were the preoperative assessment of mutational panel, the differentiation between low- and high-grade colon cancer, and the prediction of nodal metastases [35-37]. Almost studies were performed on baseline CT scans by outlining the primary tumor; overall, results achieved good and consistent efficiency especially in mutational paneling and in identifying hisk-risk clinical factors, reinforcing the idea that radiomics could have a central role in colon cancer patient's workup. Nevertheless, radiomics knows numerous shortcomings, which make the daily use extremely difficult. Among these, lack of standardization and validation, poor reproducibility, and missing prospective multicentric studies represent the main drawbacks which must be overcome to introduce the radiomic approach in clinical routine [34].

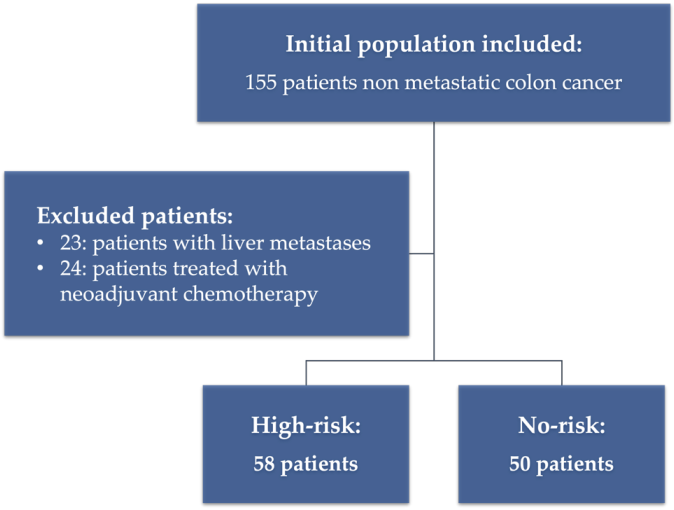
On the best of our knowledge, no studies assessed the performance of radiomics to stratify patients with high-risk disease in patients with non-metastatic colon cancer. We built and validated a radiomic model with the purpose to preoperatively identify patients with high-risk colon cancer, who could benefit of adjuvant chemotherapy.

Methods

Patient selection

This retrospective observational study was in accordance with the Declaration of Helsinki, and it was approved by ethical committee of Sant'Andrea University Hospital (ref. nr. CE 6597/2021). All patients enrolled were new diagnoses of non-metastatic colon cancer from January 2015 to June 2020, all patients provided the informed consensus. For each patient were collected epidemiological and clinical data, including age, sex, perineural invasion (PNI), lymphovascular invasion (LVI), budding, staging. Population was selected in accordance with the following inclusion criteria: I) radical surgery, II) availability of clinical and histological data, III) availability of portal phase on baseline CT scan, IV) stage I, II and III. Exclusion criteria: I) stage IV, II) patients previously treated with neoadjuvant chemotherapy. The internal cohort (n=108) was divided into High-risk (n=58) and No-risk (n=50) according to the presence of least one of the following risk factors: staging T4, LVI, PNI, budding, and nodal metastases [30] (Fig. 6). An external validation cohort of 40 non metastatic colon cancer (27 male and 13 female), selected following the same inclusion and exclusion criteria described for the internal cohort, was used to test the predictive models.

Figure 6. Patients recruitment flow-chart



CT Acquisition Protocol

All patients were studied with contrast enhanced CT scans, by using 128-slices CT (GE Revolution EVO Slice CT Scanner, GE Healthcare, Milwaukee, WI, USA), before the surgery. CT scans were acquired with the patients in supine position, performing the scans at end-inspiration in cranio-caudal direction, the Z-axis was set covering the entire abdomen.

The contrast medium (CM) volume was tailored for each patients, following the lean body weight [38; 39]:

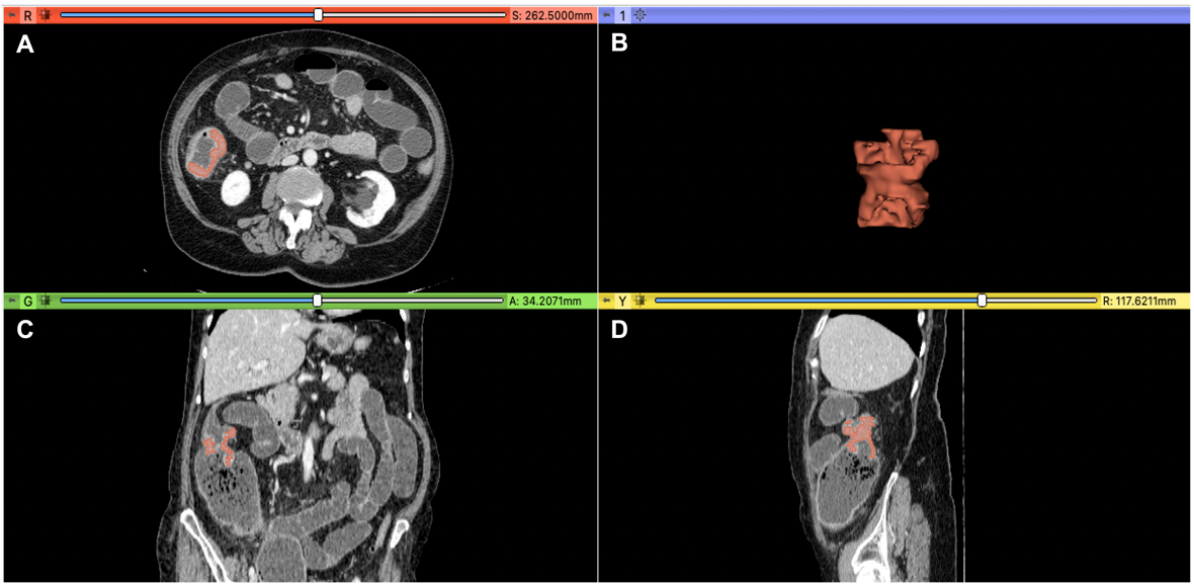
$$\text{CM volume (mL)} = \frac{0.7\text{gI x LBW (kg)}}{\text{CM concentration } (\frac{\text{mgI}}{\text{mL}})}$$

The bolus of contrast medium (Iodixanolo 320 mg I/mL, Visipaque 320; GE Healthcare, USA) and the subsequent saline solution (50mL) were injected by the contrast media injection system (MEDRAD® Centargo CT Injection System) fixing a flow rate of 3.5 mL/s through an antecubital venous access (18-20 gauge). The bolus-tracking method (Smart Prep, GE, Milwaukee, WI) was used for the multiphases CT scans acquisition by setting a 100 HU-threshold region of interest, at tripod celiac level, within the abdominal aorta. For each patient were performed the unenhanced, late arterial (18s from threshold) and portal venous (70s from threshold achieved) phases. The following CT technical specification were set: tube voltage 100kV; spiral pitch factor 0.98; tube current modulation 130-300mAs by using SMART mA (GE Healthcare, Milwaukee, USA); time of rotation 0.6s; collimation 64x0.625mm.

CT scans segmentation analysis

All colon cancers were segmented by two expert abdominal radiologists (E.I. and D.C. of 25 and 10 years of experience), who independently performed a volumetric segmentation of colon cancer on preoperative CT scans at portal phase. The open-source 3D Slicer software (version 4.10.2, http://www.slicer.org) was used for the segmentation. The volumetric region of interest was manually outlined slice-by-slice, in order to cover the entire colon cancer volume and avoiding to include the surrounding pericolic fat and healthy large bowel wall in the segmentation (Fig. 7).

Figure 7. Colon cancer 3D segmentation of in portal phase, performed by using Slicer software (version 4.10.2, http://www.slicer.org). Panel (A) displays axial, (B) 3D volumetric segmentation, (C) coronal, (D) sagittal.



Radiomics extraction

To extract 107 radiomic features from CT portal venous phase was used the 3D Slicer Radiomics extension (pyradiomics library [40]). The 107 features extracted including: First Order statistics, 19 features, 2D and 3D Shape, 13 features, Neighbouring Gray Tone Difference Matrix (NGTDM), 5 features, Grey Level Size Zone Matrix (GLSZM), 16

features, Gray Level Co-Occurrence Matrix (GLCM), 24 features, Gray Level Dependence Matrix (GLDM), 14 features, Gray Level Run Length Matrix (GLRLM), 16 features.

Statistical Analysis

All continuous data were evaluated as mean ± standard deviation. Interobserver variability, evaluating the inter-class correlation (ICC), was used to select the stable radiomic features and radiomic features achieving ICC>0.8 were maintained for the next statistical steps of analysis [41]. Student T-test and Mann-Whitney U test were used in the comparison of continuous variables of High-risk and No-risk patients, according to Gaussian normality or non-normality, respectively. Univariate enter logistic regression was used to test stable radiomic features (ICC>0.8) as predictors of high risk cancer. All parameters resulted significant (P<0.05), were selected for the multivariable enter logistic regression analysis with the goal to build a radiomic model to predict High-risk colon cancer. This predictive radiomic model was validated through the external cohort. Statistically significance was considered with a P<0.05. Statistical analysis was achieved with MedCalc (MedCalc Software, version15, Ostend, Belgium).

Results

Study population

Internal population included 108 patients (Median age 72, Male 56/108), 58 patients were stratified as High-risk and 50 as No-risk. In the sub analysis of the High-risk patients, concerning T staging, 1 (1,7%) was T1, 3 (5.2%) were T2, 33 (56.9%) were T3, 17(29.3%) were T4a, and 4 (6.9%) were T4b. About the presence of risk factors 36 (62%) were LVI positive, 4 (6.9%) were PNI positive, 34 (58.6%) were budding positive, and 16 (27.6%)

were N positive. In the sub analysis of the No-risk patients, concerning T staging, 1 (2%) was T1, 8 (16%) was T2, 41 (82%) were T3 (Table 1).

Table 1. Patient clinical data

High-risk (58/108)	N patients	%	No risk (50/108)	N patients	%
T			**T**		
○ **T1**	1	1.7	○ **T1**	1	2
○ **T2**	3	5.2	○ **T2**	8	16
○ **T3**	33	56.9	○ **T3**	41/50	82
○ **T4a**	17	29.3	○ **T4a**	0/50	0
○ **T4b**	4	6.9	○ **T4b**	0/50	0
LVI			**LVI**		
○ **LVI+**	36/58	62	○ **LVI+**	0/50	-
○ **LVI-**	22/58	38	○ **LVI-**	50/50	100
PNI			**PNI**		
○ **PNI+**	4/58	6.9	○ **PNI+**	0/50	-
○ **PNI-**	54/58	93.1	○ **PNI-**	50/50	100
BUDDING			**BUDDING**		
○ **Budding +**	34/58	58.6	○ **Budding +**	0/50	-
○ **Budding-**	24/58	41.4	○ **Budding-**	50/50	100
Nodes			○ **Nodes**		
○ **N0**	42/58	72.5	○ **N0**	50/50	100
○ **N1a**	4/58	6.9	○ **N1a**	-	-
○ **N1b**	5/58	8.6	○ **N1b**	-	-
○ **N2a**	5/58	8.6	○ **N2a**	-	-
○ **N2b**	2/58	3.4	○ **N2b**	-	-

*T: T staging; LVI: lymphovascular invasion; PNI: perineural invasion

Feature selection and radiomic analysis

107 radiomic features were extracted from the 3D segmentations of colon cancer on portal phase of baseline CT scans. The analysis of ICC revealed that only 35 radiomic features (8 Shape, 5 First order, 3 GLCM, 5 GLDM, 6 GLRLM, 7 GLSZM, and 1 NGTDM features) resulted to be stable ($0.81 \leq ICC < 0.92$). Among the stable features, 28 features (7 Shape, 3 First order, 1 GLCM, 4 GLDM, 6 GLRLM, 6 GLSZM, and 1 NGTDM features) resulted to be significantly different in the comparison between High-risk and No risk patients ($0.004 \leq P < 0.05$) (Table 2).

Univariate and Multivariate analyses

All significant stable radiomic features were tested by using univariable logistic regression analysis to evaluate the correlation with High-risk colon cancer. Univariate analysis showed that 9 radiomic features (1 First Order, 1GLCM, 5GLRLM, and 2 GLSZM features) were significantly associated with high-risk cancer, P values ranging from 0.01 to 0.05 with OR <1. Among these features, 1 Shape (SurfaceVolumeRatio), 3 GLRLM (RunLengthNonUniformityNormalized, RunPercentage, and ShortRunEmphasis), and 1 GLSZM (ZonePercentage) resulted to be predictors of High-risk cancer, with P values ranging from 0.01 to 0.05 and OR between 13.6 and 157x104. While 1 GLCM (Idmn), 2 GLRLM (LongRunEmphasis and RunVariance), and 1 GLSZM (SmallAreaEmphasis) showed inverse correlation with High-risk cancer, with P value from 0.01 to 0.02 and OR between 0.84 and 4.2004e-17. The remanent stable radiomic features showed no significant correlation with high-risk cancer or indifferent value of OR. Multivariate analysis was tested to build the radiomic model by including the radiomic features showing significant correlated with high-risk cancer.

Table 2. Stable radiomic features resulted in comparison between High-risk and No risk patients.

Radiomic features	High-risk	No risk	ICC	P
	Mean ± SD	Mean ± SD		
›e_LeastAxisLength	23.34 ± 10.43	28.38 ± 12.24	0.82	**0.02**
›e_Maximum2DDiameterColumn	43.20 ± 18.48	56.74 ± 23.58	0.87	**0.003**
›e_Maximum2DDiameterSlice	49.30 ± 19.09	58.63 ± 22.76	0.90	**0.02**
›e_MeshVolume	21047.06 ± 26389.25	39659.83 ± 43204.46	0.81	**0.02**
›e_MinorAxisLength	31.52 ± 11.29	38.45 ± 13.76	0.91	**0.004**
›e_SurfaceArea	6507.93 ± 4960.29	10070.17 ± 7988.04	0.87	**0.02**
›e_SurfaceVolumeRatio	0.46 ± 0.19	0.38 ± 0.16	0.85	**0.02**
›e_Maximum3DDiameter	56.72 ± 22.63	65.57 ± 24.46	0.89	0.07
Order_VoxelVolume	21532.50 ± 26508.12	40253.22 ± 43386.76	0.91	**0.009**
Order_Energy	5272857.19 ± 6465846.03	9614816.02 ± 10922495.83	0.90	**0.03**
Order_TotalEnergy	142367144.12 ± 174577842.8	259600032.54 ± 294907387.3	0.86	**0.03**
Order_Maximum	149.91 ± 30.88	147.02 ± 27.59	0.82	0.61
Order_Mean	74.72 ± 15.85	72.08 ± 18.64	0.88	0.81
‘M_Idmn	0.98 ± 0.01	0.98 ± 0.01	0.89	**0.03**
‘M_Icm2	0.29 ± 0.10	0.26 ± 0.09	0.85	0.08
‘M_SumAverage	9.55 ± 6.30	11.28 ± 7.01	0.85	0.16
)M_DependenceNonUniformity	39.95 ± 42.32	70.22 ± 72.70	0.87	**0.02**
)M_GrayLevelNonUniformity	439.23 ± 537.16	877.22 ± 985.89	0.86	**0.01**
)M_LargeDependenceEmphasis	152.75 ± 71.20	185.66 ± 83.91	0.88	**0.02**
)M_SmallDependenceEmphasis	0.06 ± 0.03	0.05 ± 0.02	0.90	**0.03**
)M_SmallDependenceLowGrayLevelEm ‹is	0.02 ± 0.01	0.01 ± 0.01	0.89	0.06
.LM_GrayLevelNonUniformity	199.18 ± 202.85	342.55 ± 321.67	0.88	**0.02**
.LM_LongRunEmphasis	4.34 ± 2.62	5.61 ± 3.63	0.85	**0.03**
.LM_RunLengthNonUniformityNormali	0.47 ± 0.09	0.43 ± 0.10	0.81	**0.02**
.LM_RunPercentage	0.62 ± 0.09	0.58 ± 0.11	0.82	**0.04**
.LM_RunVariance	1.37 ± 1.10	2.04 ± 1.92	0.87	**0.04**
.LM_ShortRunEmphasis	0.70 ± 0.07	0.67 ± 0.08	0.87	**0.03**
ZM_ LargeAreaEmphasis	12801.55 ± 22785.03	32877.79 ± 45848.79	0.90	**0.006**
ZM_ ‹eAreaHighGrayLevelEmphasis	609276.79 ± 1704878.107	1908734.67 ± 4536097.03	0.90	**0.01**
ZM_ LargeAreaLowGrayLevelEmphasis	693.99 ± 1234.32	1714.36 ± 3566.59	0.82	**0.03**
ZM_SmallAreaEmphasis	0.58 ± 0.17	0.64 ± 0.11	0.87	**0.04**
ZM_ZonePercentage	0.07 ± 0.04	0.05 ± 0.04	0.89	**0.01**
ZM_ZoneVariance	12224.58 ± 22231.55	31666.31 ± 44755.72	0.91	**0.008**
ZM_ llAreaHighGrayLevelEmphasis	16.35 ± 29.18	23.66 ± 31.59	0.90	0.06
‘DM_ Coarseness	0.04 ± .06	0.02 ± 0.04	0.88	**0.01**

: Standard Deviation; ICC: inter-class correlation; P: P value; GLCM: Grey Level Co-occurrence Matrix; GLDM: Gray Level Dependence ‹ix; GLRLM: Gray Level Run Length Matrix; GLSZM: Grey Level Size Zone Matrix; NGTDM: Neighbouring Gray Tone Difference ‹ix

The radiomic model showed a good performance with AUC of 0.73 (95% CI, 0.63-0.82; P<0.001), with positive predictive power of 71.43% and negative predictive power of 69.7%. Results were validated through the external cohort, in which the radiomic model yielded an AUC of 0.75 (95% CI, 0.55-0.94; P=0.02), with positive predictive power of 70% and negative predictive power of 77.3% (Figure 8 and Table 3).

Figure 8. Performance of radiomic model to identify the High-risk colon cancer in the internal (dotted black line) and external cohort (solid grey line).

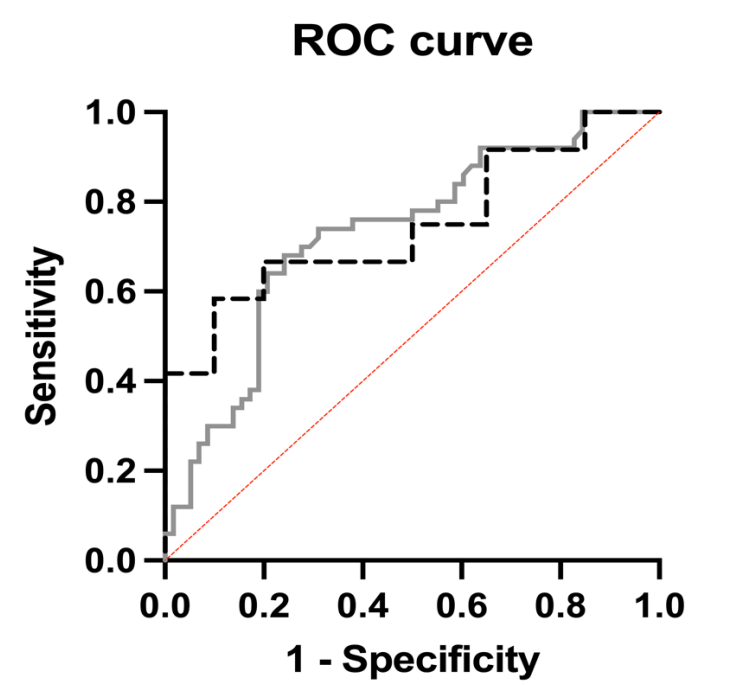


Table 3. Multivariate logistic regression to test the performance of radiomic model in predicting High-risk colon cancer in internal and external cohorts.

Radiomic variable	Internal Cohort Radiomic model		External Cohort	
	OR(95%CI)	Coefficient	OR(95%CI)	Coefficient
Shape_SurfaceVolumeRatio	0.79 (7.82e-022 to 5.42e+030)	-0.24	227.1 (6.65e-005 to 1771984111)	5.42
GLCM_Idmn	3647282668 (2.973e-010 to 1.16e+0.30)	22.02	1.21e+020 (2.07e-028 to 1.05e+074)	46.25
GLRLM_LongRunEmphasis	0.02 (0.0003 to 1.42	-3.63	58.36 (0.0004 to 183464701)	4.067
GLRLM_RunLengthNonUniformityNormalized	5.99e+014 (3.38e-015 to 1.37e+044)	34.03	8.20e+0.38 (9.29e-060 to 5.55e+145)	89.60
GLRLM_RunPercentage	4.7e+018 (0.005 to 1.66e+042)	42.99	1.54e-054 (1.33e-131 to 126781)	-123.9
GLRLM_RunVariance	1537 (1.24 to 4121443)	7.34	1.89e-005 (1e-017 to 36401)	-10.87
GLRLM_ShortRunEmphasis	3.54e-045 (2.35e-088 to 0.0006)	102.4	735727550 (1.49e-077 to 1.94e+100)	20.42
GLSZM_SmallAreaEmphasis	38.22 (0.49 to 3684)	3.64	0.89 (9.58e-006 to 64647)	-0.11
GLSZM_ZonePercentage	6.87e-008 (6.04e-019 to 1659)	-16.49	42583803 (1.39e-022 to 1.75e+040)	17.57
P value	**<0.0001**		**0.02**	
AUC	**0.73**		**0.75**	
Positive Predictive Power	**71.4%**		**70%**	
Negative Predictive Power	**69.7%**		**77.3%**	

*OR: Odds Ratio; AUC: Area under curve; GLCM: Grey Level Co-occurrence Matrix; GLRM: Gray Level Run Length Matrix; GLSZM: Grey Level Size Zone Matrix.

Discussion

In this study, we developed a radiomic model to predict High-risk disease in non-metastatic colon cancer by performing a volumetric segmentation of primary tumors on baseline CT scans. All patients were treated with surgical resection and we considered clinicopathological data as reference standard to divide the starting population in High-risk and No-risk patients, according to the presence of at least one of clinical risk factors between staging T4, LVI, PNI, budding, and nodal metastases [33; 42]. We analyzed all pre-operative CT scans on portal phase, extracting from each volumetric tumor segmentation multiple radiomic features which were reduced according to the value of ICC, to maintain only the stable features. Then, the stable radiomic features were compared by testing the differences between high-risk and no-risk patients, and the significant radiomic features were used to build a radiomic predictive model. This model reached a good performance in predicting high-risk disease with an AUC of 0.73, highlighting the promising role of radiomics in patient risk stratification. Finally, we also validated the radiomic model through an external cohort, in which the AUC was confirmed good, yielding the value of 0.75.

To date, Radiomics has been widely described as the new field of quantitative imaging having the ability to outline the micro-architecture and heterogeneity of the tissues through the large volume of numeric data extracted from medical images [34]. These high-dimensional data could be expression of tumor aggressiveness, with the possible opportunity to overcome the limitations of conventional imaging, that is subjective and qualitative [12]. Focusing on colon cancer, conventional imaging knows consistent limitations in identifying the main high-risk clinical factors, such as nodal metastases, LVI, and PNI. Among these, nodal involvement was the factor mostly investigated by using

conventional imaging and no consistent results were reached. In fact, almost all qualitative evaluation to predict the risk of nodal metastases were found to be non-performing [43].

In that context, radiomics could be seen as a novel tool to stratify the patients affected by colon-cancer providing some additional quantitative data, with the goal to outline tumor phenotype and to predict patient prognosis before starting therapeutic workflow. Recently, the group of Yao X. [44] demonstrated the chance to use a radiomic approach to predict the disease free survival in colon cancer patients. They compared the predictive value of TNM staging system, clinical model, and radiomics. Radiomic signature resulted to be more efficient than TNM and clinical model in predicting the patient's prognosis. Similar results were showed by Dai W. et al., who tested radiomics as imaging biomarker to identify patients with poor prognosis. They evaluated the potentiality of a quantitative approach to assess overall survival and relapse free survival by analyzing preoperative CT scans. Authors obtained good performance for both the endpoints, reaching an AUC of 0.77 and 0.74 in predicting overall survival and relapse free survival, respectively. These studies enhanced the potential value of radiomics as imaging biomarker in non-metastatic colon cancer, that will help the clinicians in choosing the best treatment option according to patient risk-stratification.

Nowadays, all colon cancers at stage III and II with high-risk clinical features are recommended to be treated with adjuvant chemotherapy. However, the benefit of adjuvant chemotherapy in stage II with high-risk clinical features is debated, mainly due to the conflicting results of some clinical studies [45; 46]. Then, the option of adjuvant chemotherapy in high-risk colon cancer at stage II is still arbitrary, and often guided from subjective evaluation of the oncologists. In such scenario, we decided to use the clinicopathological data only to stratify the patients in High-risk and no-risk groups and to test only the performance of radiomic model. The study design was weighted on the basis

of the controversial results present in literature about the combined model, clinical-radiomic, to preoperatively identify colon cancer at stage III. In fact, on the one hand, a recent study stated that a clinical-radiomic nomogram was superior in preoperative prediction of nodal metastases [47]. Conversely, in a different study it was reported that radiomic signature achieved the best performance in N staging in comparison with combined model [37]. These opposite results guided our decision to consider the histological data only to stratify the patients and not to build a combined model, even considering that our main investigation was to look at radiomic approach as a supporting tool for the clinicians without any possibilities to replace clinical approach. Nevertheless, we did not include to stratify patients several novel biomarkers concerning the mutational panel (e.g. BRAF, KRAS, and microsatellite instability) [30; 32]. The paneling of mutational status was not widely used as routine in colon cancer, especially in the previous years, and these information were not available at the moment of analysis also considering the retrospective nature of the study.

In the new era of personalized medicine, quantitative imaging could be central in management of colon cancer, by providing to the clinicians a non-invasive imaging biomarker to properly tailor the therapy especially in doubtful cases. The arbitrary decisions should be reduced, and a structured workflow is required to ensure a therapeutic program fitted per patient. Despite the high potentialities of radiomic analysis in pre-operative clinical setting, the real strengths in predicting patient outcome have been verified, however the leading limitations are dependent on a poor standardization, low reproducibility of results, and different acquisition parameters among different centers [34]. In fact, between the various cancer-research centers there is a disparity concerning several factors inherent to CT acquisition workflow, such as contrast enhanced CT

phases, iterative reconstructions, and the total volume of contrast medium, which could affect the consistency of radiomics[48].

The study has several limitations, firstly, the retrospective nature of the study; secondly, the small sample of internal and external cohorts; thirdly, data of patient outcome are missing and a survival analysis was not perfumed.

Conclusion

To sum up, we can conclude that radiomic model might have a pivotal role in the future colon-cancer workup, focusing on patient risk stratification in a pre-operative clinical setting. This approach might serve as a supporting tool for the clinicians, with the expectancy to enter in the structured treatment management, allowing to get a personalized therapeutic strategy.

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