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TESI DI DOTTORATO

Dose intensification of Radiotherapy in neoadjuvant rectal cancer treatment
Personalized Therapy with the use of Magnetic Resonance Imaging
Preliminary Results

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1. Abstract

Colorectal cancer is one of the most common types of cancer. Staging of rectal cancer differs from colon cancer in that it is important to assess for locally advanced disease, which is an indication for treatment with chemotherapy and/or radiation prior to surgical intervention. Magnetic resonance imaging (MRI) of the pelvis with specific rectal cancer protocol is the current standard of care to assess for local tumor advancement and lymph node involvement . Surgery is the primary treatment modality for patients with Rectal Cancer . Preoperative chemoradiation is now considered standards of care for local advance rectal cancer patients.

The usual dose in chemoradiation protocol given to initial pelvic fields is 45 Gy in 25 fractions of 1.8 Gy each , an additional tumor boost may be administered, should include the tumor with a 2- to 5-cm margin, to an additional 5.4 Gy , the concomitant usual chemotherapy is capecitabine 825 mg/mq/bid per os . In this study was decided to use the Diffusion weighted (DW) magnetic resonance (MR) imaging and the ADC maps as a tool for evaluating patients and dividing them into two categories good e bad responder. After obtaining for each patient images of MR pre (baseline) and MR during (treatment) , variation in ADC in percentage (**Δ** ADC%) for each patient have been calculated , and then the data obtained were compared and matched with the histological exams . At this point was calculated the receiver operating characteristic (ROC) curves and the area under the curve (AUC) to investigate the discriminatory capacity of the **Δ** ADC . With this analysis it was possible , to divide the patients between bad and good responder . This study has two steps . Starting from the data analysis of step 1 , hence the first group of 39 patients , we observed that pathologic examination of the entire surgical specimen showed 14 patients considered as good responder (TRG 0-1) 36% of the patients , and 25 patients considered as bad responder (TRG 2-3) 64% . Using the Δ ADC formula , we found a significantly higher value in good responder patients than in bad responder patients. In step 2, the decision to perform an additional radiotherapy dose

boost (5.4 Gy in 3 fractions) was introduced with respect to step 1 for patients who were considered bad responders at MRI during treatment . In the second phase of the project , 15 patients have been enrolled , 10 of which received an additional radiotherapy dose boost (5.4 Gy in 3 fractions) , but to date it has not been possible to recover the data of the histological examination for all patients undergoing the boost.

This study is one of the first studies that aims to change the therapeutic approach of rectal cancer based on MR results during radiotherapy.

What is expected for the future in the treatment of rectal cancer is to perform increasingly personalized treatments aimed at improving the complete response to neoadjuvant treatment, without increasing the treatments related toxicity. For this reason studies such as this may play a role in defining which patients may be eligible for a dose intensification protocol .

The trend in oncology today is to perform therapies more and more personalized taking into account the multiple characteristics of each case.

2. Backgraund

2.1 Epidemiology of colorectal Cancer

Colorectal cancer is one of the most common types of cancer. Worldwide it is estimated that 1.7 million cases of colorectal cancer were diagnosed in 2015 [1]. Around 6.3 million people in the world live with colorectal cancer which is the second most diagnosed cancer estimated to have caused around 860,000 deaths in 2018 [1, 2]. It is also a disease associated with a high rate of morbidity and loss of healthy life years [3].

The incidence of rectal cancer in the European Union is 125 000 per year, i.e. 35% of the total colorectal cancer incidence, reflecting 15–25 cases/100 000 population per year and is predicted to increase further in both genders. The mortality is 4–10/100 000 population per year. Median age at diagnosis is 70 years, but predictions suggest that this figure will rise in the future [4].

2.2 Risk Factor of colorectal Cancer

Many factors have been postulated as either determinants of colorectal cancer or increasing its risk. The possible analysis of risk factors suffers from the same shortcomings of analytical epidemiological studies investigating the complex issues of diet and lifestyle. High body mass index, body or abdominal fatness and diabetes type II are seen as risk factors. Longstanding ulcerative colitis and Crohn's disease affecting the rectum, excessive consumption of red or processed meat and tobacco as well as moderate/heavy alcohol use increase the risk. A healthy lifestyle and exercise can reduce the risk of developing rectal cancer [5 , 6]. Consumption of garlic, milk, calcium and high dietary fibre are regarded as protective [7]. Approximately 20% of cases of colorectal cancer are associated with familial clustering , and first-degree relatives of patients with colorectal adenomas or invasive colorectal cancer [8-9] are at increased risk for colorectal cancer . Genetic susceptibility to colorectal cancer includes well defined inherited syndromes , such as

Lynch Syndrome (also known as Hereditary non polyposis colorectal cancer (HNPCC) [10-11] and familial adenomatous polyposis (FAP) [12].

Colorectal cancer is a heterogeneous disease. An international consortium has recently reported a molecular classification, defining four different subtypes: CMS1 (MSI Immune), hypermutated, microsatellite unstable (see *Lynch Syndrome*, above), with strong immune activation; CMS2 (Canonical), epithelial, chromosomally unstable, with marked WNT and MYC signalling activation; CMS3 (Metabolic), epithelial, with evident metabolic dysregulation; and CMS4 (Mesenchymal), prominent transforming growth factor β activation, stromal invasion, and ngiogenesis. [13]. However, this classification is not yet recommended in clinical practice.

2.3 Staging of Colorectal Cancer

The initial clinical workup of patients with rectal cancer provides important preoperative information on the clinical stage of disease. Since the clinical stage is used to direct decisions regarding choice of primary treatment, including surgical intent and whether to recommend preoperative chemoradiotherapy (CRT), the implications of either clinically understaging or overstaging rectal cancer can be substantial. A specialized and dedicated multidisciplinary team (MDT) of named radiologists, surgeons, radiation oncologists, medical oncologists and pathologists should discuss all patients . Patients who present with rectal cancer require a complete staging evaluation, which includes total colonoscopy to evaluate for synchronous lesions or other pathologic conditions of the colon and rectum. Rigid proctoscopy can also be considered. Tumours with distal extension to <15 cm from the anal margin (as measured by rigid sigmoidoscopy) are classified as rectal and more proximal tumors as colonic. Cancers are categorized as low (up to 5cm), middle (from> 5 to 10cm) or high (from> 10 up to 15 cm). Patients with rectal cancer also require a complete physical examination, including full blood count, liver and renal function tests , carcinoembryonic antigen (CEA) determination and assessment of performance status to determine operative risk. Imaging also plays a critical role in preoperative evaluation, both for evaluation of the primary tumor and to assess for the presence of distant metastases. Preoperative imaging for rectal cancer includes chest/abdominal computed tomography (CT) and pelvic Magnetic Resonance Imaging (MRI) or chest CT and abdominal/pelvic MRI. Positron emission tomography (PET) may provide additional information in terms of disease outside the pelvis. However, current evidence is not considered strong enough to recommend the use of PET in all patients. Chest imaging should be by CT scan, whereas imaging of the abdomen can be performed with CT or MRI. Lung metastases occur in approximately 4% to 9% of patients with colon and rectal cancer,[14-15] and studies have shown that 20% to 34% of patients with colorectal cancer present with synchronous liver metastases [16-17]. Staging of rectal cancer differs from colon cancer in

that it is important to assess for locally advanced disease, which is an indication for treatment with chemotherapy and/or radiation prior to surgical intervention. Magnetic resonance imaging (MRI) of the pelvis with specific rectal cancer protocol is the current standard of care to assess for local tumor advancement and lymph node involvement [18-19]. The circumferential margin or circumferential resection margin (CRM) is an important pathologic staging parameter in rectal cancer [20]. Pelvic MRI has the ability to provide accurate images of soft tissue structures in the mesorectum, including the mesorectal fascia so as to provide information useful in the prediction of the CRM prior to radical surgery [21-22]. The CRM by MRI is measured at the closest distance of the tumor to the mesorectal fascia. The clear CRM is defined as > 1 mm from mesorectal fascia and elevator muscles and not invading into the intersphincteric plane. An involved CRM, in contrast, is within 1 mm of mesorectal fascia; or, for lower third rectal tumors, within 1 mm from elevator muscle. The current Guidelines used to define TNM (tumor, node, metastases) for Rectal Cancer are the staging system of the AJCC Cancer Staging Manual (Table 1).The TNM categories reflect very similar survival outcomes for rectal and colon cancer; these diseases therefore share the same staging system.

American Joint Committee on Cancer (AJCC) TNM Staging Classification for Rectal Cancer 8th ed., 2017

Table 1. **Definitions for T, N, M**

T	**Primary Tumor**
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma *in situ*: intramucosal carcinoma (involvement of lamina propria with no extension through muscularis mucosae)
T1	Tumor invades the submucosa (through the muscularis mucosa but not into the muscularis propria)
T2	Tumor invades the muscularis propria
T3	Tumor invades through the muscularis propria into pericolorectal tissues
T4	Tumor invades* the visceral peritoneum or invades or adheres** to adjacent organ or structure
T4a	Tumor invades* through the visceral peritoneum (including gross perforation of the bowel through tumor and continuous invasion of tumor through areas of inflammation to the surface of the visceral peritoneum)
T4b	Tumor directly invades* or adheres** to adjacent organs or structures

N	**Regional Lymph Nodes**
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	One to three regional lymph nodes are positive (tumor in lymph nodes measuring ≥0.2 mm), or any number of tumor deposits are present and all identifiable lymph nodes are negative
N1a	One regional lymph node is positive
N1b	Two or three regional lymph nodes are positive
N1c	No regional lymph nodes are positive, but there are tumor deposits in the subserosa, mesentery, or nonperitonealized pericolic, or perirectal/mesorectal tissues
N2	Four or more regional lymph nodes are positive
N2a	Four to six regional lymph nodes are positive
N2b	Seven or more regional lymph nodes are positive
M	**Distant Metastasis**
M0	No distant metastasis by imaging, etc.; no evidence of tumor in distant sites or organs. (This category is not assigned by pathologists)
M1	Metastasis to one or more distant sites or organs or peritoneal metastasis is identified
M1a	Metastasis to one site or organ is identified without peritoneal metastasis
M1b	Metastasis to two or more sites or organs is identified without peritoneal metastasis
M1c	Metastasis to the peritoneal surface is identified alone or with other site or organ metastases

2.4 Therapeutic approach of colorectal Cancer

2.4.1 Surgery

Surgery is the primary treatment modality for patients with Rectal Cancer . A variety of surgical approaches, depending on the location and extent of disease, are used to treat primary rectal cancer lesions.These methods include local procedures, such as polypectomy, transanal local excision, and transanal endoscopic microsurgery (TEM), and more invasive procedures involving a transabdominal resection , low anterior resection [LAR], proctectomy with total mesorectal excision (TME) and coloanal anastomosis, abdominoperineal resection [APR]) [23-24]. Even when total mesorectal excision (TME) is performed from stage I rectal cancer, perioperative morbidity remains between 20 and 25%, which does not reflect the surgical and psychological impact of stoma creation [25]. Long-term complications related to ileostomy and colostomy creation include parastomal hernia and stomal prolapse, which are associated with significant morbidity and often require surgical correction [26]. the high morbidity and mortality rates associated with TME have driven the quest for less invasive local surgical approaches . Transanal local excision (TAE) is only appropriate for selected T1, N0 early stage cancers. Small (<3 cm), well to moderately differentiated tumors that are within 8 cm of the anal verge and limited to less than 30% of the rectal circumference and for which there is no evidence of nodal involvement can be approached with transanal local excision with negative margins [27]. Transanal endoscopic microsurgery (TEM) was developed by Gerald Buess in 1982 as an endoscopic approach for local excision of low and mid-rectal lesions [28]. This approach represented a significant technical advancement relative to conventional TAE and endoscopic piecemeal polypectomy, with improved visualization and exposure of lesions, particularly those in the proximal rectum. Until recently, adoption of transanal endoscopic surgery was confined to a few high volume and centers of expertise. Wider adoption was limited by the prohibitively high costs of the rigid TEM and TEO platforms, scarcity of training centers, and long learning curve required to achieve technical expertise in these procedures.

In 2009, at the height of popularity of single incision laparoscopy, an alternate transanal endoscopic setup using single-incision laparoscopic disposable transanal ports was reported, which was called transanal minimally invasive surgery (TAMIS) [29 -30]. Although data are limited, a 2015 meta-analysis found that TEM may achieve superior oncologic outcomes compared with transanal local excision [31]. Both transanal local excision and TEM involve a full-thickness excision performed perpendicularly through the bowel wall into the perirectal fat. Negative (>3 mm) deep and mucosal margins are required, and tumor fragmentation should be avoided. Patients with rectal cancer who do not meet requirements for local surgery should be treated with a trans abdominal resection Organ-preserving procedures that maintain sphincter function are preferable, but not possible in all cases. Total mesorectal excision (TME) appears to have been described by Abel in 1931 [32] well before Heald brought it to worldwide attention. Rectal cancer surgery was considered morbid and difficult surgery with high rates of abdominoperineal excision of rectum (APER), high rates of local recurrence and poor long-term survival [33-34]. Heald first published the Basingstoke experience in 1979 describing proctectomy with an emphasis on sharp dissection and complete removal of the mesorectum and called it TME [35]. Heald went on to publish the first series of TME for rectal cancer with an extremely low local recurrence rate of 2.7% and an overall 5-year survival of 87.5% [36]. The low recurrence rates have since been verified, and similar results have been reported in other series [37–38]. A TME involves an en bloc removal of the mesorectum, including associated vascular and lymphatic structures, fatty tissue, and mesorectal fascia as a “tumor package” through sharp dissection and is designed to spare the autonomic nerves [39-40-41]. The lymphatic drainage regions of rectal tumors are influenced by their position in the rectum. More distal tumors are more likely to be characterized by both upward and lateral lymphatic drainage, whereas the likelihood of only upward mesorectal drainage is much higher for more proximal tumors [42]. TME can lead to increase in bowel frequency, urgency and associated faecal incontinence. This results from the removal of rectal reservoir function and is exacerbated by possible sphincter and neural injury resulting from rectal and pelvic dissection. Anorectal function is compromised after TME with or

without the use of preoperative radiotherapy though functional symptoms are worse after neoadjuvant radiotherapy. Anorectal function appears to improve after 12 months especially in the absence of neoadjuvant radiotherapy [43]. Laparoscopic rectal surgery is oncologically equivalent to open TME. In addition laparoscopic TME has significant short-term benefits with lower pain, quicker recovery and lower incidence of some complications [44].

2.4.2 Concurrent Radiotherapy with Chemotherapy

Radiation therapy plays an important role in the management of patients with rectal cancer. Radiation therapy can improve local control and increase sphincter preservation rates. Preoperative chemoradiation and preoperative short course radiotherapy are now considered standards of care for appropriate rectal cancer patients. Several years before the widespread adoption of preoperative chemoradiation, multiple clinical trials established the role of postoperative chemo- radiation for rectal cancer, in all series patients treated with a combination of postoperative radiotherapy and chemotherapy had significantly improved overall survival . Several studies have compared the administration of radiotherapy preoperatively versus postoperatively for stage II/III rectal cancer [45-46]. A large prospective, randomized trial from the German Rectal Cancer Study Group (the CAO/ARO/AIO-94 trial) compared preoperative versus postoperative CRT in the treatment of clinical stage II/III rectal cancer [45]. Results of this study indicated that preoperative therapy was associated with a significant reduction in local recurrence (6% vs. 13%; P = .006) and treatment associated toxicity (27% vs. 40%; P = .001), although OS was similar in the 2 groups. Although both preoperative and postoperative adjuvant therapy can be effective, neoadjuvant treatment has emerged as the standard of care. Neoadjuvant therapy is associated with tumor downstaging , improved resectability and tolerance (both acute and chronic), and potential for expanded sphincter preservation options in the distal rectum. In parts of Europe where a hypofractionated preoperative radiotherapy regimen is preferred, a study to determine whether a short-course approach (5 Gy for five fractions) to neoadjuvant therapy is better than a protracted approach (50.4 Gy using 1.8- to 2-Gy fractions with concomitant bolus 5-FU/LV given during weeks 1 and 5) was undertaken by the Polish Rectal Cancer Group [47]. With respect to the type of chemotherapy administered concurrently with radiotherapy , recent studies have shown that capecitabine is equivalent to 5-FU in perioperative chemoRT therapy [48-49]. External-beam treatment fields for rectal carcinoma

should encompass potential sites at greatest risk for harboring disease, including the presacral space, primary tumor site, and (for post-APR cases) the perineum. The external iliac nodes should also be included for T4 tumors involving anterior structures; inclusion of the inguinal nodes for tumors invading into the distal anal canal can also be considered. Generally, the risk of disease involvement of the para-aortic region is sufficiently low, and the morbidity from treatment is sufficiently high, to exclude this region from radiation fields. The usual dose given to initial pelvic fields is 45 Gy in 25 fractions of 1.8 Gy each. An additional tumor boost may be administered, should include the tumor or tumor bed with a 2- to 5-cm margin, to an additional 5.4 to 9 Gy. Small bowel should be excluded from the boost volume after about 50 Gy in an effort to minimize acute and late toxicity. Intensity-modulated radiotherapy (IMRT) has improved the tolerance of the preoperative RT for rectal cancer, especially with regard to radiation enteritis. The above-discussed DVH parameters were partially obtained using 3D radiotherapy conformal (3D-RT), without volumetric imaging, and are therefore not strictly applicable to modern IMRT treatments. However in rectal cancer, despite a number of published phase II trials, and single and multicentre series, including the RTOG 0822 trial; IMRT has failed to show any significant benefit in terms of reduced toxicity or improved cancer outcomes [50-51]. Unfortunately, the RTOG 0822 trial did not use simultaneous integrated boost (SIB) , instead using IMRT to deliver 45 Gy in 25 fractions followed by a conformal 5.4 Gy in a three-fraction boost. This failed to meet its primary end point of a reduction in gastrointestinal toxicity, but it should be recognised that a two-phase technique with a longer overall treatment time is not the optimal method of minimising toxicity to the gastrointestinal system. The failure of this trial should not deter research and appropriate use of IMRT, as the question of reduced toxicity with the use of SIB has not been answered. [52]. Image-guided Radiation Therapy (IGRT) allows to correct set-up errors and to evaluate target motion and shape variation. It also reduces radiation-induced toxicity by enabling adaptive radiotherapy and contributes to a better tumor response by facilitating dose-painting. Decisions made on the basis of IGRT have drastically improved the quality of rectal cancer radiotherapy.

Highly-conformal radiotherapy techniques and IGRT are synergetic and should be simultaneously applied in the clinic.

2.4.3 Chemotherapy

In attempts to improve on the outcomes achieved with neoadjuvant 5-FU/RT or capecitabine/RT, several large randomized phase III trials (ACCORD 12, STAR-01, R-04, CAO/ARO/AIO-04, and FOWARC) addressed the addition of oxaliplatin to the regimens. In a planned interim report of primary tumor response in the STAR-01 trial, grade 3 and 4 adverse events occurred more frequently in patients receiving infusional 5-FU/oxaliplatin/RT than in those receiving infusional 5-FU/RT (24% vs. 8%, $P < .001$), while there was no difference in pathologic response between the arms of the study (16% pathologic complete response in both arms) [54]. Recently reported results of the NSABPR-04 trial also showed that the addition of oxaliplatin did not improve clinical outcomes including the endpoints of locoregional events, DFS, OS, pathologic complete response, sphincter-saving surgery, and surgical downstaging, while it increased toxicity [49-53]. Some authors have hypothesized the use of cetuximab in addition to chemotherapy (the randomized phase II EXPERT-C trial). Patients in the control arm received CAPEOX followed by capecitabine/RT, then surgery followed by CAPEOX. Patients randomized to the cetuximab arm received the same therapy with weekly cetuximab throughout all phases. A significant improvement in OS was seen in patients with *KRAS* exon 2/3 wild-type tumors treated with cetuximab (HR, 0.27; 95% CI, 0.07–0.99; $P = .034$). However, the primary endpoint of complete response rate was not met [55]. Other Authors have tried to add bevacizumab in the neoadjuvant setting with capecitabine and oxaliplatin (FOLFOX/bevacizumab) The 5-year OS rate was 80%, and the 5-year RFS rate was 81%. However, the primary endpoint of pathologic complete response was not met, significant toxicities

were observed [56]. Adjuvant chemotherapy is recommended for all patients with stage II/III rectal cancer following neoadjuvant chemoRT and surgery if they did not receive neoadjuvant chemotherapy regardless of the surgical pathology results; however, few studies have evaluated the effect of adjuvant chemotherapy in patients with rectal cancer, and its role is not well-defined [57-58].

2.4.4 Total Neoadjuvant Therapy (TNT)

The standard of care for rectal cancer is neoadjuvant chemoradiotherapy (nCRT) followed by surgery and then postoperative multiagent chemotherapy based on several randomized trials [59-60]. A novel approach called total neoadjuvant therapy (TNT) has recently gained favor whereby patients are given both nCRT as well as multiagent chemotherapy prior to surgery. TNT use has been associated with better compliance, decrease in toxicities, and higher rates of anal sphincter preservation[61-63]. This change in approach has been based on increasing evidence that there is better quality of life with improved functionality, decreased toxicity, and lower rates of recurrence with preoperative treatment[62-63]. In the Spanish GCR-3 randomized phase II trial, patients were randomized to receive CAPEOX either before chemoRT or after surgery [64-65]. Possible benefits of using chemotherapy first include the early prevention or eradication of micrometastases , higher rates of pathologic complete response, minimizing the length of time patients need an ileostomy, facilitating resection, and improving the tolerance and completion rates of chemotherapy.

2.4.5 Watch-and Wait Nonoperative Approach (WW)

A watch-and-wait (WW) approach for patients with rectal cancer following a clinical complete response (cCR) to NAT is a nonstandard approach, but it has become more widely practiced with the advent of total neoadjuvant therapy [66]. and with increasing demand by patients in the context of a cCR [67]. As preoperative treatment and imaging modalities have improved, some have suggested that patients with a clinical complete response to chemoRT may be able to be spared the morbidities of surgery. In 2004, Habr-Gama et al [68] retrospectively compared the outcomes of 71 patients who were observed without surgery following complete clinical response (27% of patients) to the outcome of 22 patients (8%) who had incomplete clinical responses but complete pathologic responses post-TME. The OS and DFS rates at 5 years were 100% and 92%, respectively, in the nonoperative group compared to 88% and 83%, respectively, in the resected group. However, other studies did not achieve as impressive results, and many clinicians were skeptical of the approach.

3. Treatment Intensification

Rectal cancer treatment has continued to improve in recent years as a result of optimized surgical technique, advances in staging, pathological quality control and multi- disciplinary management. Neoadjuvant chemoradiotherapy (CRT) is considered the standard of care for locally advanced rectal cancer (LARC). It is well recognized that the response to neoadjuvant CRT is both variable and unpredictable for the individual patient, and techniques to risk-stratify patients and predict response are an expanding area of research. Radiosensitizers are employed routinely to improve the radiosensitivity of rectal cancer to radiotherapy ; the standard of care is a concurrent single-agent fluoropyrimidine. A num- ber of studies have analysed novel agents or combination therapies that aim to improve radiosensitivity and cCR and/or pCR rates. The critical target for RT is DNA and

the accumulation of DNA damage, particularly DNA double-strand breaks, and the ability of tumour cells to repair this damage, contributes significantly to the therapeutic effect. Some agents and combination therapies (such as oxaliplatin, irinotecan and poly(ADP-ribose) polymerase (PARP) inhibitors) might typically take advantage of this by creating additional DNA damage or inhibiting DNA damage repair, exacerbating the effects of RT. Neoadjuvant chemoradiotherapy with 5-FU or capecitabine is standard of care for rectal cancer . Oxaliplatin is a third-generation platinum-based drug that enhances radiation-induced cytotoxicity via irreparable DNA damage through formation of interstrand and intrastrand crosslinks, induction of G2/M cell-cycle arrest, blockage of DNA replication and inhibition of transcription [69-70]. Preclinical data indicated potent radiosensitizing properties of the drug, with synergism between oxaliplatin and RT [71-72] ; these findings have been applied to several clinical trials for patients with LARC. The evidence at present, including subsequent meta-analyses [73-74] , still supports the use of a single-agent fluoropyrimidine as the standard of care because of a lack of consistent improvement in pCR and 3-year DFS rates [75] with the combined regimen, and the greater toxicity due to oxaliplatin [76] . Irinotecan, a topoisomerase (TOPO) 1 inhibitor, inhibits religation of single-strand DNA breaks through the formation of camptothecin 11–TOPO-1–DNA complexes [77] . A preclinical study [78] has demonstrated irinotecan to be not only a feasible addition to 5-FU chemotherapy, but also a potent radiosensitizing agent in colorectal cancer, even under hypoxic conditions. Varius phase II studies have been published with the aim of evaluating the possibility of adding this agent to standard therapy , but the result to date demostrade that there was no significant difference between the treatment arms in terms of pCR or downstaging, but an increased rate of acute toxicity was reported in the irinotecan group. Epidermal growth factor receptor (EGFR), a member of the ErbB family of receptors, is relevant in colorectal cancer because overexpression or up-regulation of the *EGFR* gene occurs in 60 – 80 per cent of cases [79-80] . Expression of the gene is also associated with poor survival [81-82]. The anti-EGFR monoclonal antibodies cetuximab and panitumumab are already approved for the treatment of RAS wild-type metastatic colorectal cancer [83], but their role

in LARC remains unclear. There have been several clinical trials of EGFR-targeting monoclonal antibodies as radiosensitizers in neoadjuvant therapy for LARC. However, these studies did not investigate tumour RAS status, which is used as a predictive biomarker for anti-EGFR monoclonal antibody response in metastatic colorectal cancer [84-85]. Potentially, optimal ordering of chemotherapy, RT and the EGFR inhibitor might unlock the full radiosensitizing potential of anti-EGFR monoclonal antibodies [86]. Cetuximab did not improve the primary outcome (pCR), so it was not felt to have contributed significantly to increased radiation-induced cytotoxicity. Bevacizumab is a monoclonal antibody that targets vascular epithelial growth factor (VEGF). In combination with cytotoxic chemotherapy, it has shown potential for rectal cancer treatment; the evidence is, however, currently limited to phase I – II trials [87] which however did not demonstrate the expected improvment [88-89] . Sorafenib is a multikinase inhibitor that blocks the receptor tyrosine kinase of VEGF, platelet-derived growth factor and the RAF serine–threonine kinases along the RAF – mitogen-activated protein kinase kinase–extracellular signal-related kinase pathway. Jeong and colleagues [90] assessed its potential as a radiosensitizer using three colorectal cell lines, and a xenograft animal model. Van Moos *et al.* [91] evaluated its effect in a cohort of 54 patients with KRAS-mutated rectal tumours in combination with capecitabine-based CRT. The pCR rate was 60 per cent, with downstaging in 82 per cent. A second phase I study [92] also produced encouraging results, with a pCR of 36 per cent. PARPs, particularly PARP-1, play a critical role in the recognition and repair of DNA single- and double-strand breaks. Higher PARP activity has been noted in cancer cells with increased proliferation and chemoradio- resistance, and this has led to the development of PARP inhibitors, which reduce the cancer cell's ability to repair single and double-strand breaks generated by RT and lead to cell death. Preclinical trials have demonstrated radiosensitizing effects in multiple colorectal cell lines [93]. Veliparib (ABT-888), a potent orally bioavailable PARP-1/2 inhibitor, has been shown to enhance the antitumor activity of chemotherapy and RT in preclinical colorectal cancer models [94] . As with the EGFR monoclonal antibodies, this class of potential radiosensitizer remains an area of interest and future studies are

needed to elucidate its role in rectal cancer. The immune system plays an intricate and complex role in all aspects of cancer from carcinogenesis to treatment [95]. Over the past 10years, a great deal of work has been done to better understand that role, with the development of therapies such as programmed cell death protein 1 (PD-1)/programmed death ligand 1 (PD-L1) inhibitors, cancer vaccines and adoptive cell therapy. Specifically, the PD-1 immune check- point inhibitors pemrolizumab and nivolumab have shown promising activity in DNA mismatch repair-deficient (dMMR)/microsatellite instability – high colorectal cancers, which carry a high mutation load and an active immune microenvironment[96-97]. The R-IMMUNE phase II study [98] is currently recruiting to compare the use of atezolizumab as a radiosensitizer with 5-FU-based neo- adjuvant CRT. An alternative potential method of enhancing the effectiveness of CRT is by increasing the radiation dose. Since response to radiotherapy is dose dependent in rectal cancer, dose escalation may lead to higher complete response rates [99-100]. A recent mathematical prediction model on pCR-rate indicated that 50% of patients could reach pCR after 92 Gy and that response exponentially increased after 60 Gy [102]. This was in line with a prediction-curve based on a large systematic review on dose response in patients with LARC [101-102]. A prospective single- center study [102] from Denmark in patients with T2 – 3 cancers within 6 cm of the anal verge used radiation dose intensification to the primary tumour delivered with intensity-modulated external-beam RT to 60 Gy in 30 fractions over 6 weeks, with 50 Gy to the pelvic nodes, combined with an endorectal brachytherapy tumour boost to 5 Gy and tegafur/uracil on treatment days. Of the 51 patients treated, 78 per cent achieved a cCR and organ preservation; the local recurrence rate was 26 per cent at 2years. Grade 3 diarrhoea occurred in 8 per cent, and long-term rectal bleeding was of concern during follow-up. An alternative strategy to dose escalation is the development of novel delivery methods that reduce toxicity, particularly to the small bowel. Intensity-modulated RT is one such technique that has been proposed owing to its highly conformal dose distribution. There are currently few published prospective data to support its routine use; however, a recent meta-analysis [103] of retrospective studies has suggested that it has a significantly lower toxicity profile

than routine three-dimensional CRT. Finally, accelerated treatment (higher dose per fraction, i.e. simultaneous integrated boost) increases the biological effective dose which may benefit response [104-105] , especially when tumor-regrowth time is short [106-107]. Nevertheless, some of these accelerated schedules remain challenging because of considerable toxicity [108-109] and peri/post-operative complications [109-110].

4. Evaluation of the response to treatments

The standard of care for rectal cancer is neoadjuvant chemoradiotherapy (nCRT) followed by surgery histopathologic parameters of response to neoadjuvant therapy are major determinant factors to predict tumor biology and long-term disease-specific outcome for patients with rectal adenocarcinoma and a valid endpoint to assess response to investigational therapeutic approach applied in neoadjuvant setting [111]. More than one study have shown that downstaging of tumor stage (ypT < cT) is an independent prognostic factor of disease-free survival in node- positive and node-negative rectal adenocarcinoma [112]. Pathologic T stage is decided based on the depth of invasion of tumor in rectal wall and/or adherent organs. Only difference as compared to the specimens without neoadjuvant therapy is that presence of tumor cells is required in a layer of rectal wall or adjacent organ for appropriate ypT stage designation. Presence of acellular mucin, necrosis, or fibrosis without tumor cells is not considered to be a histologic evidence of tumor for ypT. Radiation-induced injury also includes thinning of the colon wall with partial destruction of submucosa and/or muscularis pro-pria which can lead to an understaging of ypT3 to ypT1 or ypT2. Grading system of response to neoadjuvant therapy based on macroscopic examination is not well characterized and unlikely to be reproducible. Microscopic findings indicative of changes secondary to neoadjuvant chemoradiation are reduction of tumor cells and replacement by the granulation

tissue, fibrosis, mononuclear inflammation, necrosis, calcification, and radiation- induced vascular changes like intimal thickening and medial muscular hypertrophy. Various grading systems have been proposed for TRG, however, resulting in interobserver variability in grading (Table 2) this systems have shown clinical relevance in predicting disease-specific survival in patients with advanced locoregional rectal adenocarcinoma [113–114]. The Mandard and Dworak TRG systems are classified according to five-point grades based on residual tumor and fibrosis. Complete pathologic response characterized by absence of residual tumor cells in rectal wall and perirectal soft tissue has been observed in ~20% of patients in majority of the prior studies. These groups of patients with complete pathologic response are likely to have more than 95% or higher 5-year disease-free survival [115- 116].

Table 2 Histopathologic regression grading systems

Tumor regression grade (TRG), Ryan et al.	AJCC 7th[TR] edition (TRG) system	Mandard five-tier system	Modified Mandard three-tier system	Dowrak/Rodel five-tier system	Dowrak/Rodel three-tier system	MSKCC system	UTMDACC system
No tumor cells (1)	No residual tumor cells (0)	Absence of histologically identifiable tumor cells in rectal wall	Absence of histologically identifiable tumor cells in rectal wall	No regression	Complete regression	100% tumor response	Complete pathologic response
Single tumor cells or small groups of tumor cells (2)	Single tumor cell or small groups of tumor cells (1)	Rare residual tumor cells scattered through the fibrosis	Rare residual tumor cells scattered through the fibrosis	Dominant tumor mass with obvious fibrosis and/or vasculopathy	Fibrosis occupying 25–99% of tumor mass	86–99% tumor response	Near-complete pathologic response
Residual cancer outgrown by fibrosis (3)	Residual tumor outgrown by fibrosis (2)	Increased number of tumor cells but fibrosis predominant	Increased number of tumor cells but fibrosis predominant to absence of regressive changes	Dominant fibrosis with few tumor cells (easy to find)	Fibrosis occupying <25% of tumor mass	<85% tumor response	Major response
Significant fibrosis outgrown by tumor (4)	Minimal or no tumor cell kill (3)	Residual cancer outgrowing fibrosis		Very few (difficult to find) tumor cells in fibrotic tissue			Minor response
No fibrosis with extensive residual cancer (5)		Absence of regressive changes		No tumor cells, only fibrotic mass (total regression or response)			
Kappa = 0.64 for 5-grade system Kappa = 0.84 for 3-grade system (TRG2 + 3 = TRG2, TRG 4 + 5 = TRG3)		Kappa = 0.84	Kappa = 0.89 m $p < 0.001$				$r^2 = 0.77$ Kappa = 0.72

The gold standard for assessing the tumoral response to preoperative RCT is conventional histopathological analysis. This method, however, is only applicable in the postoperative setting and consequently cannot be used for the preoperative selection for an individualized treatment. Nowadays, the diagnostic challenge for an organ preservation approach is to find an adequate surrogate of histology able to discriminate responders from non-responders. Computed tomography (CT), endorectal ultrasound (EUS) and conventional magnetic resonance imaging (MRI) have shown to lack accuracy for restaging after RCT [117-118]. In recent years, there has been growing interest in functional imaging techniques to improve clinical response assessment. These imaging modalities depict the microstructural and metabolic characteristics of the tumor, allowing assessment of treatment-induced changes before morphological changes become apparent. In this respect, diffusion-weighted imaging (DWI) and ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F- FDG PET/CT) have emerged as powerful tools in the response prediction before, during and after neoadjuvant RCT for rectal cancer. DWI is a non-invasive imaging modality, providing functional information on the microstructure of tissues through the assessment of differences in water proton mobility [119]. Water diffusion characteristics depend on several factors such as cell density, vascularity, viscosity of the extracellular fluid and cell membrane integrity. By quantifying these properties as the apparent diffusion coefficient (ADC), DWI can be used as an imaging biomarker to monitor and predict tumoral response to RCT [120-121]. ^{18}F-FDG PET semi-quantitatively assesses tumor glucose metabolic activity through changes in FDG-uptake. A decrease in FDG-uptake after radiotherapy and/or chemotherapy has been correlated with pathological response in several tumor types [122-123]. CEA is the most widely used tumor marker in patients with rectal cancer. Compared with other potential predictive markers, measurement of serum CEA level is inexpensive, standardized, widely used, and easily performed. In recent years, many studies have focused on the predictive value of CEA level in patients with rectal cancer receiving preoperative CRT. Most studies have

shown low pre-CRT CEA (CEA-pre) levels with different cut-off values to be associated with good tumor response or pCR, although the results of the CEA-pre predictive values are not consistent [124-125-126-127-128-129].CD133, CD44, and CD24 have been widely known as colorectal cancer stem cell markers. Cancer stem cell has the characteristics of resistance to chemotherapy and radiotherapy. Therefore, there have been some efforts to investigate the correlation of cancer stem cell markers with the treatment response to CRT. CD133 expression means the existence of cancer stem cell and high level is correlated with resistance to CRT in LARC [130]. Until now, there is no confirmative result that cancer stem cell marker is predictive of CRT response and useful in clinical field. Many molecular markers have been assessed for evaluation and prediction of tumor response to preoperative CRT in patients with rectal cancer according to IHC or direct gene sequencing analysis. More than 40 different biomarkers have been explored in the literature, with conflicting results in predicting the outcomes of nCRT.

5. Time interval between neoadjuvant chemoradiotherapy and surgery

Apart from sporadic observations and reports between the 1960s and 1990s, not much attention has been placed on the interval between CRT and surgery [131]. The reasons for this are probably multifaceted, but the arbitrary 6–8-week interval between the end of CRT and surgery has spread widely andis still maintained today. Nevertheless, in the 1990s, Brierley and colleagues from the Princess Margaret Hospital used radiation alone for 229 patients whose tumors were deemed unresectable or who were medically unfit for surgery or declined to have surgery [132]. They made a key observation that out of 66 patients who had a clinical complete response (cCR) to radiation, approximately 60% had tumor involution at 4 months and the remainder at 8 months, far longer than the standard 6–8 weeks. This suggested that the extent of radiation-induced tumor necrosis would have continued beyond the time of surgery. The reluctance to extend the duration is partly due to the well-recognized increase in fibrosis following completion of RT, which increases the difficulty of the operation . At least one retrospective study reported increased morbidity and worse outcomes associated with a delay in surgery [133]. However, this has yet to be verified in any prospective cohort. In fact, many retrospective studies since then have found that a prolonged interval between CRT and surgery is associated with a greater tumor response [134-135]. In recent years some authors have tried to delay surgery using chemotherapy adjuvant to chemoradiotherapy obtaining an improvement in results with the increase in time between radiochemotherapy and surgery [136]. Despite the encouraging data to date there is no recommendation to exceed 10 weeks between chemoradiotherapy and surgery.

6. Aim of the study

This project has as its object the neoadjuvant treatment of rectal cancer in particular in the locally advanced stages , therefore from cT2 cN1 to cT4 cN2 , which today represents the standard treatment for these stages of disease.

The project aims to identify a diagnostic algorithm capable of identifying the subgroup of patients who respond to neoadjuvant treatment and the subgroup that does not respond.

Literature data suggest that approximately 20% of patients undergoing neoadjuvant treatment have a complete response, approximately 20% of patients do not have a response to treatment and 60% of patients undergoing neoadjuvant treatment have a variable response.

On the basis of these data in recent years more teams have tried to intensify neoadjuvant treatments trying to increase or change the doses of chemotherapy drugs or alternatively increasing the dose of radiotherapy ; obtaining different results with improvement in some cases of percentage of complete responses, but in other cases an increase in treatment-related toxicity was observed.

Observing the literature data it can be argued that there are two groups of patients : those who respond to treatment and those who do not respond to neoadjuvant treatment ; to identify these two groups , the effect of neoadjuvant therapy is , obviously, evaluated by evaluating the histological report and then compare it with the data available before the start of the treatments (MR-ECO TR-TC- PET/TC).

If we base the classification of patients who respond to treatment from patients who do not respond to treatment on histological report only, it would be impossible to use this differentiation to improve the results of neoadjuvant therapy. For this reason, during the last decade, it has been progressively hypothesized to find , through the diagnostic imaging , a system to differentiate the patients in two groups (good / bad responder) . In regard of the method of trans rectal ultrasound is concerned, it is an invasive method , it is poorly tolerated by patients during or after neoadjuvant

radiochemotherapy treatment , moreover, this method is in a certain part dependent operator and therefore has been discarded as gold standard in the restaging after neoadjuvant treatments .

Another method is CT scan with contrast , this method is often used to restaging patients after neoadjuvant treatment , this method is not able to correctly evaluate the downstaging , in particular is unable to assess the degree of infiltration of the disease with respect to the viscera.

In recent years there has been increasing interest in the study of metabolic methods such as MR with DW and TC/PET with fdg.

The role of TC/PET with fdg has been underlined by several studies that have demonstrated its validity , but not being included in the list of tests required for the staging , and due to the high cost, for these reasons, this method is not commonly used in clinical practice.

In order to identify a metabolic method without adding other instrumental exams probably to date Diffusion weighted (DW) magnetic resonance (MR) imaging and subsequent creation of the apparent diffusion coefficient (ADC) maps is one of the best tools to evaluate the response to neoadjuvant treatment before performing surgery.

On the basis of these premises, it was decided in this study to use the Diffusion Weighted (DW) magnetic resonance (MR) imaging and the ADC maps as a tool for evaluating patients and dividing the patients into two categories good e bad responder.

This study consists of two phases , at first (step 1) the MR data are compared with histological results and a cut-of value is sought to divide patients into the two categories good and bad responder , in a second phase (step2) the data obtained in the first phase are used to intensify the radiotherapy dose in patients who are identified as bad responders , and maintain a standard dosage of radiotherapy those patients identified as good responders.

With regard to concomitantly used chemotherapy, all patients performed in a standard protocol involving the use of oral capecitabine with a dosage of 825 mg/mq/bid.

The aim of this project is to find a diagnostic and therapeutic algorithm that allows us to increase the rate of complete pathological responses at the time of the surgery , without increasing treatment-

related toxicity , with the intention to not subject all patients to an overtreattment and to intensify treatment only to patients who could benefit from this procedure.

With the ultimate goal of increasing the percentage of patients with complete pathological response to the surgery and thus improving the clinical outcome of these patients.

With regard to the data in the literature there are several interesting experiences confirming the possibility to use the Diffusion weighted (DW) magnetic resonance (MR) imaging and the ADC maps as a tool to evaluate the response to cancer treatments , one of the most concerned factors of this work is first to reproduce the data obtained from other working groups with respect to step 1 and secondly to apply the data observed in order to try to improve the results of a neoadjuvant treatment , which has been the standard of treatment for many years , but to date there are no major improvements in response rates without increasing toxicity also varying drugs , doses of radiotherapy and surgical timing.

7. Materials and Methods

This study was carried out with the collaboration of several radiotherapy centers with the creation of a single data base to monitor patients.

From March 2017 to March 2019 , 39 patients were enrolled in this study, and then from March 2019 to December 2019 another 15 patients were enrolled , in all cases, the type of treatment has been evaluated with the assistance of a surgeon, oncologist and radiotherapist and the decision on the treatment procedure has been shared between the various specialists.

Patients have been treated with no different treatment than in national and international guidelines, all patients have signed an informed consent to treatment , as is the practice in each treatment centers .

For the staging of the disease, all eligible patients were required to have a biopsy colonoscopy and histological examination confirming the presence of an infiltrating adenocarcinoma , CT with and without total body mdc for evaluation of local and especially for distance disease stage , MR with and without mdc for evaluation of local disease extension , and during this MR exam was asked to perform a DWI sequence with the following parameters using three different b values (b=50-500-1000) for all patients .

The criteria for inclusion in the protocol were , over 18 years of age , Karnofsky performance status *(*KPS) >70 , adenocarcinoma histology , and only patients from stage cT2 cN1 to stage cT4 cN2 were considered ; exclusion criteria for this study were patients with KPS < 70 , and patients with even single distance metastases , histology different from adenocarcinoma (e.g., mucinosis) chemotherapy regimens different than capecitabine.

Before to radiotherapy as usual all patients performed a CT simulation for the definition of the target, at CTV was given a margin of 2 cm as indicated by AIRO guidelines , with regard to the choice of radiotherapy technique, no limits were given to the various centers, therefore patients were treated in according with the internal procedures of each center , there were no limits in the

use of photon energies , in the use of complex techniques (3D , VMAT , Tomotherapy) and in choosing the treatment position.

Standard neoadjuvant rectal cancer treatment by AIRO guidelines provides for radiotherapy treatment up to a dose of 45Gy in 25 fractions, followed by a radiotherapy boost of dose of 5.4gy in 3 fractions using 1.8Gy in each fractions; with combination of capecitabine for oral dosing of 825 mg/mq/bid.

In the first step of the study, once started neoadjuvant treatment , to all patients who have met the inclusion criteria , an MR with sequences in DWI using three different b values (b=50-500-1000) was programmed between the twentieth and the 22nd fraction of radiotherapy (39.6 – 43.2 Gy).

Subsequently, patients performed a re-staging of the disease between approximately 6-8 weeks and again the MR sequence of DWI was required , and finally patients were initiated into surgery as originally planned.

The timing with surgery is dependent on each individual center where the patient decided to perform such treatment, but in any case the time passed was between 6 and 12 weeks.

Once the surgery was performed, each individual center was responsible for recovering the histological data and then the histological data were compared with the MRI performed during radiotherapy , in order to evaluate the diagnostic accuracy.

After obtaining for each patient images of MR pre (baseline), MR during (treatment) and MR post-treatment (pre surgical re-staging) ; an ADC map was created for each MR based on the DWI sequences using three different b values (b=50-500-1000) and on that map the ADC value of the disease was calculated using a special ROI (region of interest) of about 0.6 cm^2 diameter to try to homogenize the data (ADC value) , has been calculated the difference in ADC values between the MR pre and MR During , using the same dedicate work station .

Variation in ADC in percentage (**Δ** ADC%) for each patient have been calculated with the following formula $ADC = \left[\left(ADC_{\ d} - ADC_{\ p}\right)/ADC_{\ p}\right] \times 100$, where $ADC_{\ d}$ is the value of the

coefficient during radiotherapy (39.6 – 43.2 Gy) ; while where ADC_p is the value of the coefficient before the start of radiotherapy.

And then the data obtained were compared and matched with the histological reports from the various centers where patients were undergoing surgery.

With regard to the TRG classification, we chose to use the 7th edition AJCC classification (TRG) system , classifying good responders in classes 0-1 and bad responders in classes 2 – 3.

For statistical analysis matlab tools were used assuming a p.value < 0.05 considered as statistically significant .

At this point was calculated the receiver operating characteristic (ROC) curves and the area under the curve (AUC) to investigate the discriminatory capacity of the **Δ** ADC , so as to be able to distinguish for what value of difference between ADC pre and During is to be considered a patient in response or not .

With this analysis it was possible in step 2 , to divide the patients between bad and good responder.

With the analysis of this pattern STEP 1 of the work has been concluded.

In the second part of the work patients have the same characteristics as in the first step , and the criteria for inclusion and exclusion remained the same , also with regard to the processing modalities and the use of MRI with sequences in DWI and the subsequent creation of ADC map for the staging and re-evaluation during the treatment .

Once we have obtained the difference (DELTA Δ) between ADC values observed in MRI pre and ADC values observed in MR during , data from the first phase ROC curve were used to distinguish patients in two groups , good and bad responder.

In step 2, the decision to perform an additional radiotherapy dose boost (5.4 Gy in 3 fractions) was introduced with respect to step 1 for patients who were considered bad responders at MRI during treatment with the aim of improving the response rate to neoadjuvant treatment , trying to perform this intensification of treatment only to patients who had not shown a good response at the date of re-evaluation (MR during) , and in addition, the benefit of not intensifying treatment, with the

following increase in the rate of side effects , to the group of patients who were considered responded to treatment (good responder) .

In the second phase of the project from March 2019 to today 15 patients have been enrolled , 10 of which received an additional radiotherapy dose boost (5.4 Gy in 3 fractions) , but to date it has not been possible to recover the data of the histological examination for all patients undergoing the boost.

Obviously due to the low number of cases , available today ,we are not allowed to evaluate how the additional dose boost (5.4 Gy in 3 fractions) has produced a positive result in terms of improved treatment response.

8. *Results*

Starting from the data analysis of step 1 , hence the first group of 39 patients , we observed that pathologic examination of the entire surgical specimen showed 14 patients considered as good responder (TRG 0-1) 36% of the patients , and 25 patients considered as bad responder (TRG 2-3) 64% . In order to verify whether the mean value of the distribution between good responders and bad responders differs significantly , the T test was performed . After dividing the patients in the two categories good and bad responder , we analyzed the respective ADC values pretreatment , we found ADC mean 794 +/-45 mm^2/s for the good responder and we found ADC mean 929 +/- 137 mm^2/s for the bad responder, with p value of 10^{-3} . Therefore this result shows that , in this case study , the pre treatment value of ADC was correlated to the response of histological examination , indicating a significantly lower ADC value in good responder than in bad responder . Receiver operating characteristics curve analysis (ROC), AUC 0.89 , reveled that the maximum efficiency cut-off point for pre treatment ADC is 820 mm^2/s ,this point can identify patients who will achieve a good response to the treatment with a sensitivity of 71% , specificity of 92% , positive predictive value (PPV) of 83% and negative predictive value (NPV) of 85% (Fig.1). This cut-off point indicates that patients having an ADC lower than 820 mm^2/s have a good probability to be considered as good responders.

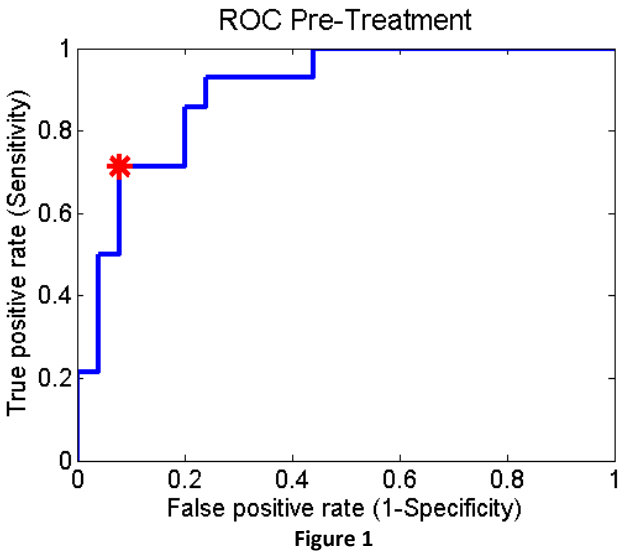

Figure 1

Using the Δ ADC formula , we found a significantly higher value of Δ ADC in good responder patients than in bad responder patients , in %: Δ ADC: 85% +/-10 VS. 53% +/28 p value= 2 x10^{-4} (Fig 2).

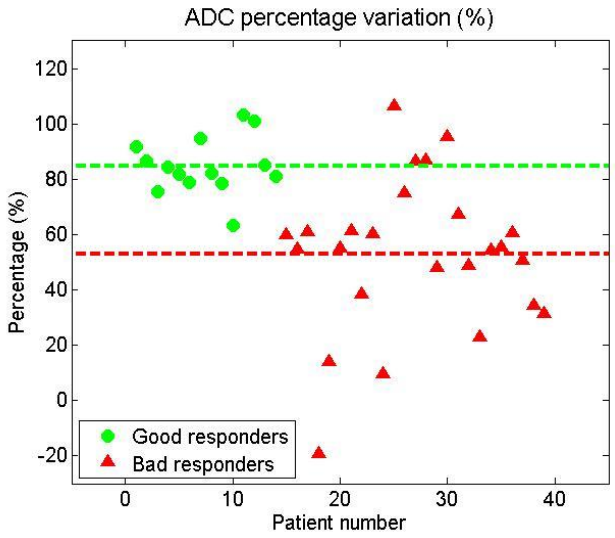

Figure 2

ROC analysis showed an AUC of 0.87 (Fig. 3) , at an optimal cut-off value of 75% . This allowed for prediction of good response with sensitivity of 93% and specificity of 84% , PPV of 76% and NPV 95% . In this second analysis if the variation of ADC between pre treatment and during treatment is higher than 75% , the patients could be considered as good responder , compared with histological analysis.

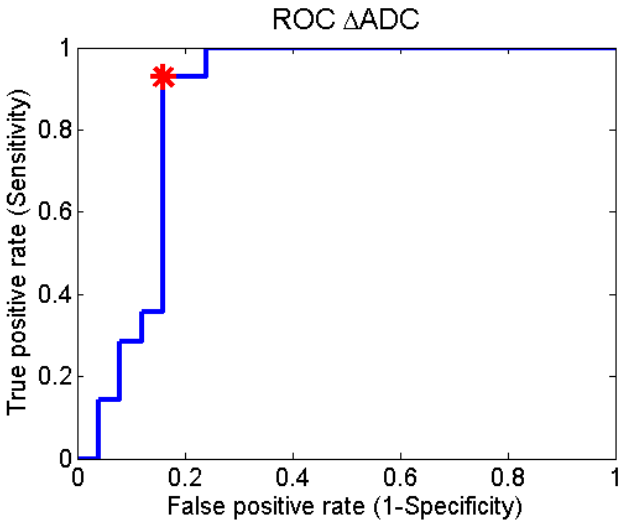

Figure 3

9. Discussion

The aims of project is to identify a diagnostic algorithm , using the diffusion weighted magnetic resonance (DW MR) imaging and apparent diffusion coefficient (ADC) maps , capable of identifying the subgroup of patients who respond to neoadjuvant treatment and the subgroup that does not respond.

This is a multi-center study with all the potential benefits and potential bias in data analysis.

As far as MR data is concerned, it should first be borne in mind that not all MRs device are the same and that not all centers use the same type of sequences , a particular DWI sequence with pre-set values was requested in our study and to reduce the differences relative to the different work stations of elaboration all the maps have been re-evaluated with the same dedicated work station .

As regards the calculation of the value of apparent diffusion coefficient (ADC) , are available software that allow to contour the area of disease and extract the average value of ADC , but in our work this software is not available , we used the positioning of a ROI (region of interes) of the predetermined and equal diameter for all patients (0.6cm2) , placed in an area representative of the disease in the ADC maps , lymph node disease values have not been calculated even when present.

The available literature data on functional imaging technique, during or post radiotherapy , such as PET/TC with fdg are numerous ; whereas for PET/TC with fdg there are more data on MR during radiotherapy and in particular on response evaluation using the ADC maps there are few data available on literature and there is no unanimous agreement on when perform this procedure .

The decision to perform the magnetic resonance between the twentieth and the 22nd fraction of radiotherapy (39.6– 43.2 Gy) has a practical motivation because it allows , especially in the second phase , to have time to study the images and evaluate the response to treatment and therefore time to decide when whether or not to overdose when to perform or when not to perform an overdose of radiotherapy .

As for the dosage decided as overdose (5.4Gy in additional 3 fractions) is probably a low dosage to observe large changes in results in terms of complete response, even in some literature cases the dosages were more generous , but as a multi-center study it was decided not to exceed the recommended doses and fractions of Italian Association of Radiotherapy (AIRO).

In this work, data have been collected from different radiotherapy centers and especially different surgical centers , it is known in the literature how the time factor can influence histological results after neoadjuvant therapy of rectal tumors . It was stressed to all patients the importance of performing surgery over a period of 8-11 weeks, but due to different logistical problems of the individual centers, a timing with surgery between 6 and 13 weeks was observed.

As regards the number of patients observed, this is in line with the data reported in other cases of neo-adjuvant radiochemotherapy , and is not far from the expected . It is certain that in order to obtain results from the data of the second phase it will be necessary to continue recruiting other patients and perhaps involve other centers .

What is expected for the future in the treatment of rectal cancer is to perform increasingly personalized treatments aimed at improving the complete response to neoadjuvant treatment , without increasing the treatments related toxicity. For this reason studies such as this may play a role in defining which patients may be eligible for a dose intensification protocol . It will also be very important to assess whether in our case patients undergoing overdose will show an increase in acute or post-surgical toxicity.

10 . Conclusion

The trend in oncology today is to perform therapies more and more personalized taking into account the multiple characteristics of each case.

This study is one of the first studies that try to change the therapeutic approach of rectal cancer based on MR results during radiotherapy.

The case studies analyzed in this work, although similar to other literature work, do not allow definitive conclusions to be drawn on this argument.

The hope is to involve other radiotherapy centers , then recruit more patients to improve and reproduce the data obtained.

It would also be useful in the future to tend to homogenize treatments both from the point of view of radiotherapy technique and patient positioning during the treatment .

Furthermore, if the data obtained in the second phase confirmed an increase in the complete response at the histological examination, as already observed in other papers ; it would be interesting to increase or modify dose and fractionation of radiotherapy boost dose.

In the final analysis, it may be interesting to input or associate data from other metabolic response methods, e.g. PET/TC with fdg , with the aim of strengthening data and improving the ability to distinguish groups of responder or non-responder patients , creating maybe even more than two classes. Or alternatively associate the data obtained using MR with the evaluation of the expression-based molecular subtypes gene.

The future also for radiotherapy is to identify factors that can guide treatment, and in this regard the manufacturers of LINAC are also pushing, In fact to date are already on the market two different companies that produce MR/LINAC that is machines that allow to perform MR imaging on board , hoping that such machines provide data that allow to individualize the treatments for each case.

Therefore we believe that in the future more consideration will be given to the molecular subtypes of the rectal cancer to evaluate the type of treatment most suitable , and maybe there will be a development of different drugs to be associated , or not , with capecitabine for each patient group .

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