De Nardi, Paola and Testoni, Sabrina Gloria Giulia and Corsetti, Maura and Andreoletti, Hulda and Giollo, Patrizia and Passarett, Sandro and Testoni, Pier Alberto (2017) Manometric evaluation of anorectal function in patients treated with neoadjuvant chemoradiotherapy and total mesorectal excision for rectal cancer. Digestive Diseases and Sciences, 49 (1). pp. 91-97. ISSN 1573-2568

Manometric evaluation of anorectal function in patients treated with neoadjuvant chemoradiotherapy and total mesorectal excision for rectal cancer

Paola De Nardi°MD, Sabrina Gloria Giulia Testoni*MD, Maura Corsetti£MD PhD, Hulda Andreoletti^MD, Patrizia Giollo*MD, Sandro Passaretti*MD, Pier Alberto Testoni MD*.

°Gastrointestinal Surgery, San Raffaele Scientific Institute, Milan, Italy

£Translational Research Center for Gastrointestinal Disorders. Department of Clinical and Experimental Medicine, University of Leuven,Leuven, Belgium

^ Service de Anesthésiologie,Etablissements Hospitaliers du Nord Vaudois, Yverdon-les-Bain, Switzerland

*Gastroenterology Division, San Raffaele Scientific Institute, Milan, Italy.

Address for correspondence

Paola De Nardi M.D.

Gastrointestinal Surgery

San Raffaele Scientific Institute

Via Olgettina 60

20132 Milan, Italy

Tel +390226435574

FAX +390226432159

denardi.paola@hsr.it

Electronic word count: 2832

Abstract

Background: An altered ano-rectal function is reported after chemoradiotherapy and surgery for rectal cancer.

Aim of this study was to clarify the relative contribution of neoadjuvant chemoradiation and surgical resection on the impairment of anorectal function as evaluated by anorectal manometry.

Methods Thirty-nine patients with rectal cancer, who underwent neoadjuvant chemoradiotherapy and laparoscopic rectal resection, were evaluated with the Pescatori Fecal Incontinence score, and with anorectal manometry: before neoadjuvant therapy (T0), after neoadjuvant therapy and before surgery (T1), 12 months after stoma closure (T2).

Results Resting and/or maximum squeeze pressure and/or volume thresholds for urgency were below the normal values in 12 (30%) patients at baseline. After chemoradiotherapy the mean resting pressure significantly decreased (p= 0.007). Surgery determined a significantly decrease of the resting pressure (p=0.001), of the maximum squeeze pressure (p=0.001) and of the volume threshold for urgency (p=0.001). Impairment of continence was reported by 5, 11 and 18 patients at T0, T1 and T2, with a mean incontinence score of 3, 3.8 and 3.9 respectively.

Conclusions Chemoradiotherapy is detrimental to the function of the internal anal sphincter. Rectal resection significantly affects both internal and external anal sphincter function and the maximum tolerated volume of the neo-rectum, particularly in patients with low rectal cancer, significantly impairing anal continence.

Key words: anorectal function, manometry, chemoradiotherapy, rectal cancer, fecal incontinence

Introduction

Low anterior resection (LAR) with total mesorectal excision (TME) is the surgical standard procedure in sphincter-preserving therapy for cancer of the medium and low rectum [1-3]. Neoadjuvant chemoradiotherapy (CRT) is being used in patients with T3/T4 rectal cancer, or with lymph node involvement, with the purpose of reducing tumor extension and increasing the likelihood to perform a radical resection [4]. Literature data suggest that the preoperative approach is associated with less long-term toxicity and better oncological outcome than postoperative therapy [5].

Sphincter-saving operations may give rise to anterior resection syndrome (ARS) [6] which includes high bowel frequency, urgency, and fecal incontinence (FI); ARS occurrence increases as the level of anastomosis approaches the sphincters, with the highest incidence reported in patients with ultralow anterior resection and colo-anal anastomosis [7,8]. These symptoms are a consequence of direct damage to the sphincter complex [9], altered function of the anal sphincters, reduced capacity and distensibility of the neo-rectum, colonic dismotility and denervation [10,11]. Both radiotherapy and surgery may adversely affect ano-rectal function and contribute to the development of ARS symptoms. Anorectal manometry is the preferred technique to provide objective information about the function of continence mechanism since it can identify functional sphincter weakness, poor rectal compliance, and rectal sensation impairment [12]. Therefore we performed this study in order to evaluate the different role of neoadjuvant CRT and surgical resection in determining the onset of different anorectal dysfunctions. Although clinical data were also considered, the study focused on ano-rectal manometry alterations.

Methods

Patients with histologically proven medium or low rectal adenocarcinoma, referring to the Surgical Department of the San Raffaele Scientific Institute, between April 2011 and April 2012, were studied. Medium and low rectal cancer were defined as tumor 7 to 11 cm and 0 to 7 cm from the anal verge respectively, as measured by rigid proctoscopy [13]. The patients were staged with total body computed tomography scan, and magnetic resonance and echo-endoscopy of the pelvis. Patients with clinical stage T3/4 or N+ underwent neoadjuvant CRT. Only patients who performed both CRT and surgery at our Institution were included in the analysis. Patients with very low lying tumor, requiring an intersphincteric resection and patients whose stoma was not closed were also excluded (Fig 1). All the patients signed an informed consent for the procedures and for the use of their data for the study.

The preoperative chemotherapy scheme consisted of oxaliplatin 100 mg/mq iv plus a continuous infusion of 5-fluorouracil 200 mg/mq iv for fourteen days, to be repeated every fourteen days for a total of 3 cycles. Radiotherapy was added from the fourteenth day of treatment (i.e. from the second cycle) for a total of 41.4 Gy in 18 daily doses (2.3 Gy for each dose). Clinical target volume for RT included tumour visible at MRI, mesorectum, lymph-nodes of obturator, internal iliac, common iliac chains as well as the whole anterior surface of sacrum, coccyx and piriformis muscle, and ischiatic fossa; the volume was expanded by 0.5 cm in all directions.

All the patients were evaluated clinically and with anorectal manometry three times during their clinical course: T0: before neoadjuvant therapy, T1: after neoadjuvant therapy and before surgery, T2: 12 months after stoma closure.

Anorectal manometry was performed using a custom-designed, open-tip, 14-Fr diameter, PVC probe with seven lumens and a 4-cm latex balloon tied at the end of the probe

(Bioengineering Laboratories SpA, Milan, Italy). Six lumens were connected to side ports for pressure. A personal computer using version 6.4 of the Lower GI Edition Polygram for Windows program recorded the pressure of the side ports in a digital format. The protocol was applied as previously reported [14]. The following parameters were investigated and recorded: resting pressure, squeeze pressure, rectal sensation (first sensation, desire to defecate, urgency or discomfort during intermittent distension), anal responses to rectal distension.

At the same time the patients scored their anal continence by using the Pescatori Fecal Incontinence score [15].

The patients underwent surgery 8 to 10 weeks after completion of the neoadjuvant treatment. Preoperatively the patients underwent mechanical bowel preparation with 2 litres enema, and antimicrobial prophylaxis with cefazolin 2g ev plus metronidazole 500 mg ev, 30 min before the induction of the anaesthesia. After surgery antithrombotic prophylaxis with Nadroparin calcium according to patient’s weight was administered

Statistical analysis

Paired and unpaired data were compared with Student's t-test. Chi-square test was applied to comparison of percentages. Differences were considered significant with $p < 0.01$ (according to Bonferroni's correction). All data are given as mean±SD.

Results

Thirty-nine patients, 26 men (66.67%), median age 65 years (range 41-79), were studied. Twenty-five patients had cancer of the medium and 14 of the lower rectum. The mean distance of the neoplasm from the anal verge was 7.02 cm (range 3-11 cm). Clinical staging at diagnosis was as follow: 26 patients T3N+, 4 patients T3N0, 4 T2N+ and 5 T4N+. Demographic and clinical data of the patients are reported in Table 1. The two groups of patients with cancer of medium and low rectum did not differ for any of the evaluated parameter (Tab 2). All the patients underwent laparoscopic low anterior resection with total mesorectal excision. Attention was paid to preserve pelvic autonomic innervation. Depending on the level of the tumor, a Knight-Griffen end-to-end anastomosis with a circular stapler or a hand-sewn colo-anal anastomosis was performed. A transverse colostomy or an ileostomy was performed at surgeon's choice. All the patients underwent stoma closure 1 to 8 months after surgery

Manometric results before neoadjuvant chemoradiotherapy

Twelve out of 39 (30%) patients had an altered anorectal function before any treatment. The clinical characteristic and the FI score of the patients are reported in Table 1. The mean values of the manometric parameters are reported in Table 2. Mean incontinence score in the 5 patients was 3 (range 2-4)

Patients with medium or low rectal cancer as a group, did not differ for any of the clinical and manometric parameters, except for a lower volume threshold for urgency in the patients with medium compared with those with low rectal cancer (p= 0.01).

Manometric results after neoadjuvant chemoradiotherapy

FI was reported by 11 patients with a mean score of 3.8 (range 3-5). An anorectal dysfunction emerged for the first time after CRT in 9 (23%) patients. In the overall group CRT significantly reduced the resting pressure of the anal sphincter in comparison to the evaluation before any treatment (p=0.007) (Tab. 3).

The 2 groups of patients with cancer of the medium and low rectum did not differ for any of the manometric parameters.

Manometric results after surgery

All the 25 patients with medium rectal cancer had a mechanical anastomosis; among the 14 patients with low rectal cancer 2 had a mechanical and 12 a hand sewn colo-anal anastomosis. Mean distance of the tumor from the anal verge was 8.1±2.9 cm in patients with mechanical anastomosis and 4.4 ±1.5 cm in patients with hand sewn anastomosis (p=0.0005) respectively.

In the 2 groups globally an anorectal dysfunction arise for the first time in 14 (35%) patients, in 5 (28%) and 9 (64%) patients with tumor of the medium and low rectum respectively. Eighteen patients globally and 7 for the first time showed FI with a mean score of 3.9 (range 3-5).

Surgery significantly reduced the resting pressure, the maximum squeeze pressure and volume threshold for urgency in comparison with the evaluation after CRT (all p< 0.001) and before any treatment (all p< 0.001) (Tab. 3) these manometric alterations, as well as FI, occurred with a greater frequency in patients with low than medium rectal cancer (64% vs 28%, p< 0.03) (Tab. 4). Moreover incontinence symptoms were present in 9out of 12 patients (75%) with hand sewn and in 9 out of 27 (33%) with mechanical anastomosis (p=0.03).

Other predictive factors for anorectal function impairment were female sex, age >60, and hand sewn anastomosis (Tab …).

Discussion

Neoadjuvant chemo radiotherapy has become the standard treatment for patients with locally advanced rectal cancer. When compared to surgery alone, local tumor control, anal sphincter preservation and disease free survival improvement have been demonstrated [16,17]. Potential adverse effects of radiation therapy on the gastrointestinal tract, however, should not be neglected. After neoadjuvant therapy and surgery, patients frequently complain of altered continence which is associated with a corresponding alteration in the function of the anal sphincter and with an impaired capacity and distensibility of the neo-rectum [18]. Moreover irradiated patients, compared with patients who underwent TME alone, more likely reported fecal incontinence and less satisfaction with bowel function [19-21]. While pelvic irradiation may cause vascular toxicity and damage to the anal sphincter muscles and pudendal nerve [22], surgery may possibly lead to damage of the innervation of the lower gastrointestinal tract, and a reduced capacity and distensibility of the neo-rectum.

Many authors underlined the adverse effects of radiotherapy on maximal squeeze pressure [23], on incontinence score [24], or pudendal nerve terminal motor latency [25]. Histological changes have been demonstrated in the irradiated internal anal sphincter with increase in collagen fibers and alteration of myenteric plexus [26]. Recently Lorenzi at Al studied “in vitro” strips of irradiated internal anal sphincter and demonstrated a reduced spontaneous activity compared with controls [27].

Only few studies have addressed the role of radiotherapy as a contributing factor for the development of anorectal dysfunction or incontinence, and there are only limited data, and no randomized studies, concerning postoperative functional outcome of rectal cancer patients who underwent long course neoadjuvant CRT.

Ammann et Al. studied with anorectal manometry 2 groups of patients whit cancer of the medium or low rectum, who underwent TME surgery with or without nCRT, and found that chemoradiation adversely affect sphincter function, being the mean resting pressure, the resting vector volume and the maximum tolerated volume significatively impaired postoperatively in the chemoradiated patients, (28). In a similar study Canda et Al. observed a significant reduction in anal canal resting pressures and squeeze pressures, as well as Wexner Incontinence score, and Fecal Incontinence Quality of Life score immediately after the completion of preoperative chemoradiotherapy (23). On the basis of their results the Authors suggested to better identify patients at risk to develop functional problems and to select the patients who will benefit from neoadjuvant therapy. An impact on internal sphincter function and an higher incidence of incontinence in irradiated patients was also reported by Gervaz et Al. who suggested that individual characteristics may determine different susceptibility to radiation-induced damage (29).

Since the majority of the studies examined patients only after surgery, it is difficult to evaluate to which extent the post surgical impairment is caused by CRT or surgery. Our study is designed to try to clarify the weight of neoadjuvant therapy in determining anorectal dysfunction by evaluating an homogeneous group of patients, clinically and with anorectal manometry, who underwent to the same chemo-radiation protocol and surgical procedure, not only after surgery but also before surgery and after neoadjuvant therapy.

The present study demonstrates that at baseline, before any treatment, 8% of patients have a dysfunction of resting and squeeze pressure of anal sphincters and 23% have a lower maximum tolerated rectal volume. These dysfunctions have never been reported in the literature in patients with rectal cancer. Considering the range of age of the population of the patients in the study (median age 60), they confirm the decrease of anal resting tone and squeeze with advancing age [30-32]. However, since the sphincter integrity has not been evaluated preoperatively, unknown sphincter injuries could have also determined low

resting or squeezing pressure before CRT. On the contrary, no relationship has been demonstrated between age and rectal sensory thresholds, therefore in this setting, the abnormal values are probably the result of the presence of the tumor. Also the result concerning the presence of faecal incontinence in this population of patients has never been reported before but is in line with the prevalence of this dysfunction in population of patients in the same range of age [33].

After neoadjuvant treatment, another 23% of patients, showed the new onset of anorectal dysfunctions, mostly represented by a lower resting anal pressure. As the resting pressure mainly reflects the function of the internal anal sphincter, this data confirms the reported tropism of radiation damages for smooth muscle [34]. This finding was previously noted by Bakx that studied a group of patients after short term preoperative radiotherapy and found a significant difference in the resting pressure compared to healthy volunteers [35]. It is likely that radiotherapy may act as a detrimental factor causing fibrosis of internal anal sphincter, as found by Pollack with endoanal ultrasound [21]. In contrast, other studies reported either no influence of CRT on resting pressure [36] or a reduction of the squeeze pressure [25] or of both resting and squeeze pressure [23, 22]. These different results could be explained by several factors, such as the different dose and volume of irradiation among studies or the type of chemotherapy administered concurrently with RT. The National Comprehensive Cancer Network NCCN guidelines indicate either infusional FU or capecitabine combined with long course RT as suggested neoadjuvant treatment for patients with locally advanced rectal cancer [1]. Starting from 2002, in an attempt to reduce the risk of systemic metastases, in our Institute oxaliplatin was added to fluorouracil-based/RT regimens, which was reported to increase toxicity. Several studies addressed the addition of oxaliplatin to neoadjuvant treatment, with controversial results: while no improvement in pathological complete response has been found in some studies [37], in the recent CAO/ARO/AIO-04 trial, a significant improvement in 3 years disease-

free survival was noted. Moreover, with a schedule similar to our protocol, a good compliance, acceptable toxicity, and low surgical morbidity was reported [38]

To be noted that after chemoradiation faecal incontinence occurred also in patients without any anorectal dysfunction measured by anorectal manometry. This could be related to the altered bowel habit induced by the CRT that was not recorded in the present study.

After low anterior resection and TME, another 35% of patients showed an anorectal dysfunction represented by a lower resting and/or squeeze pressure with or without a lower maximum tolerated volume. The impairment of resting and squeeze pressures, which are considered to be the expression of the internal and external anal sphincter function respectively [39], has already been observed after rectal surgery [40,41]. Several factors may be implicated such as direct lesions due to stapling devices [9,42,43] or injury to the nervous pathway [44]. A significant difference can be observed between patients with medium or low rectal cancer in resting pressure, with the latter having worse resting pressure values both after CRT and after surgery. These data suggest that the localization of the rectal cancer might be predictive of the occurrence of anorectal dysfunction as measured by manometry and that this alteration mainly concerns the internal anal sphincter. Previous studies have suggested that a low level of anastomosis is a negative predictive index of anal continence after surgical treatment for rectal cancer however in these studies only surgical treatment and not neoadjuvant therapies were considered [45-48].

As expected we found a lower pressure threshold inducing urgency to defecate for the first time in 33% of the patients after surgery and in 6 of them it was the only manometric alteration. As previously reported, this sensory alteration most likely reflects the reduced capacity and distensibility of the rectum, [40]. It has been demonstrated that this alteration tends to recover after the sixth postoperative month [48], however in our study the manometric alteration persisted after 12 months of restored function.

A manometric alteration of the anorectal function does not necessarily correspond to symptoms, such as faecal incontinence. Indeed previous authors have demonstrated that faecal incontinence is determined by the simultaneous presence of different dysfunction of the anorectal structures [49].

A limit of our study is that a validated incontinence score was not employed and the relation between anorectal dysfunction revealed by anorectal manometry and symptoms has not been analysed, further studies should clarify this aspect.

In conclusion, the present study demonstrates that in a percentage of patients with rectal cancer an alteration of anal sphincter function as measured by anorectal manometry is already present before any treatment. CRT can be an aggravating factor, regardless of surgery, as it determines a decrease in anal sphincter resting pressure. Finally surgery represents a further detrimental factor significantly affecting both anal sphincter resting and squeeze pressure and reducing the sensory threshold, particularly in patients with low rectal cancer. The clinical implications of these results are that the maximal effort should be directed to develop therapeutical strategies to reduce anorectal dysfunction, and/or therapeutical options to manage the possible alterations.

Acknowledgements:

The Authors wish to thank Dr Matteo Alessandri who assisted in data collection.

Table 1. Clinical characteristics of patients' population and relative manometric findings before any treatment, (T0), after chemoradiotherapy (T1) and after surgery (T2). Changes in manometric findings between evaluations at the different time-points are highlighted.

ID	Sex	Age (ys)	Site	Cm from av	Comorbidity	Previous surgery	Clinical Stage	FI Score T0	Manometry T0	FI Score T1	Manometry T1	FI Score T2	Manometry T2	Pathologic Stage	Anastomosis
1	m	64	L	5			T3N+	0	N	0	N	3	RP+SP+UD	T3N0	HS
2	m	49	L	3	H	CH	T4N+	0	N	0	N	4	RP+SP	T0N0	HS
3	f	60	L	4	H		T3N0	0	UD	5	SP+UD	5	RP+SP+UD	T1N0	HS
4	m	52	L	4			T3N+	0	N	0	N	4	RP+SP+UD	T2N0	HS
5	m	70	L	6	H		T4N+	0	N	4	N	5	RP+SP+UD	T3N+	HS
6	m	41	L	5			T2N+	0	N	4	N	4	RP+UD	T1N0	HS
7	m	46	L	5			T3N+	0	N	0	N	5	RP+UD	T0N0	HS
8	m	43	L	5			T3N+	0	N	0	N	0	RP+SP+UD	T3N0	HS
9	f	72	L	4	HC		T3N+	0	N	0	N	3	RP+SP	T3N0	HS
10	m	48	L	4			T3N+	0	N	0	N	0	RP+SP+UD	T3N0	HS
11	m	75	L	4	H		T3N0	0	N	3	RP+UD	4	RP	T2N0	HS
12	f	79	L	4			T2N+	0	N	0	UD	0	UD	T3N+	HS
13	f	69	L	5			T3N+	0	N	0	RP+UD	0	RP+UD	T3N0	M
14	m	80	L	6	D2+H		T3N+	0	N	0	N	0	N	T3N+	M
15	f	67	M	8	D2	CH	T3N+	0	N	0	N	0	RP+SP+UD	T3N+	M
16	f	60	M	7	D1	CH+RC	T3N+	2	N	3	UD	4	UD	T1N0	M
17	f	74	M	7	H		T3N+	0	UD	0	UD	0	UD	T3N+	M
18	f	70	M	11	H	HY	T4N+	0	N	0	N	0	N	T3N+	M
19	m	65	M	8			T3N+	0	UD	0	RP+UD	4	RP	T2N0	M
20	m	66	M	7			T3N+	0	N	0	N	0	RP	T0N0	M
21	f	62	M	7	D2+HC		T3N+	0	UD	0	N	0	N	T2N+	M
22	m	49	M	8			T3N+	0	UD	0	UD	0	UD	T3N+	M
23	m	73	M	9	IHD	CH+PTCA	T3N+	0	N	0	RP+UD	0	RP+UD	T2N+	M
24	m	74	M	7	H+IHD	PTCA	T3N+	0	N	0	N	0	N	T0N0	M
25	f	77	M	10	H		T3N+	2	RP+SP	4	RP+SP+UD	5	SP+UD	T0N0	M
26	m	75	M	9			T3N+	3	RP+UD	3	RP	3	RP	T1N0	M
27	m	47	M	8			T4N+	0	UD	0	N	0	UD	T2N+	M
28	m	50	M	11	H		T3N+	0	N	3	SP+UD	4	UD	T2N0	M
29	m	72	M	8	BPH		T3N+	4	UD	5	UD	4	UD	T3N+	M
30	m	67	M	7	H+BPH		T3N0	0	N	0	N	0	N	T0N0	M
31	m	48	M	8			T2N+	0	N	0	RP	0	RP+SP+UD	T2N0	M
32	m	71	M	9			T3N+	0	SP	4	RP+SP+UD	4	N	T3N+	M
33	f	63	M	10	H		T4N+	4	UD	4	UD	4	UD	T3N0	M
34	m	63	M	10			T3N+	0	N	0	N	0	SP+UD	T1N0	M
35	m	74	M	9	D2+H		T3N+	0	N	0	RP	3	RP+SP+UD	T0N+	M
36	f	61	M	7	H	HY	T3N+	0	N	0	N	0	UD	T3N+	M
37	m	47	M	8			T3N0	0	N	0	UD	0	UD	T2N0	M
38	f	55	M	8			T3N+	0	UD	0	UD	0	UD	T0N0	M
39	m	58	M	9		HE	T2N+	0	N	0	N	0	RP+SP+UD	T2N0	M

L: low rectal cancer
M: medium rectal cancer
H: hypertension
HC: hypercholesterolemia
D2: type II Diabetes
D 1: type I Diabetes
IHD: ischemic heart disease
BPH: benign prostatic hypertrophy
CH: cholecistectomy
RC: right colectomy
HI: hysterectomy
PTCA: percutaneous transluminal coronary angioplasty
HE: hemorrhoidectomy
T0: before neoadjuvant therapy
T1: after neoadjuvant therapy and before surgery
T2: 12 months after stoma closure
FI Score: Pescatori incontinence score
RP: resting pressure below normal values
SP: squeeze pressure below normal values
UD: volume threshold for urgency below normal values
HS: hand sewn
M: mechanical

Table 2. Demographic, clinical and manometric parameters before any treatment in the whole patients' population and in the subgroups of patients with medium and low rectal cancer. Mean value and standard deviation for each parameter.

	Whole Patients Group	Medium Rectal Cancer	Low Rectal Cancer	P-value
Patient N°	39	25	14	NS
Females	13	9	4	NS
Males	26	16	10	NS
Age	62±11	63±10	62±10	NS
Comorbidities	20	18	7	NS
Previous pelvic surgery	3	3	0	NS
cT2N+	4	2	2	NS
cT3N0	4	2	2	NS
cT3N+	26	18	8	NS
cT4N+	5	3	2	NS
FI score	0.38 ± 1.06	0.6 ± 1.29	0 ± 0	NS
Resting	76±23	73±20	83±21	NS
Squeeze	239±99	234±90	249±89	NS
F	23±11	20±10	27±12	NS
D	42±22	40±21	48±22	NS
U	103±47	89±30	132±32	*0.01
RAIR	22±8	24±7	20±8	NS

Resting = resting pressure (mm Hg)
C= clinical stage at diagnosis
Squeeze = maximum squeeze pressure (mm Hg)
F = volume threshold inducing first sensation (ml)
D = volume threshold inducing desire to defecate (ml)
U = volume threshold inducing urgency to defecate (ml)
RAIR = recto-anal inhibitory reflex

* P= 0.01 Low vs Medium Rectal Cancer

Table 3. Manometric parameters in the whole patients' population before any treatment, after CRT and after surgery. Mean value and standard deviation for each parameter.

	T0	T1	P-value	T2	P-value
Resting	76±23	63±22	* 0.007	38±18	†‡<0.001
Squeeze	239±99	211±88	NS	142±60	†‡<0.001
F	23±11	20±3	NS	20±1	NS
D	42±22	39±21	NS	36±40	NS
U	103±47	93±43	NS	47±37	†‡<0.001
RAIR	22±8	22±8	NS	20±1	NS

T0 = before neoadjuvant therapy
T1 = after neoadjuvant therapy and before surgery
T2 = 12 months after stoma closure
Resting = resting pressure
Squeeze = maximum squeezing pressure
F = pressure threshold inducing first sensation
D = pressure threshold inducing desire to defecate
U = pressure threshold inducing urgency to defecate
RAIR = recto-anal inhibitory reflex

* P= 0.007 evaluation after chemoradiotherapy vs before any treatments
† P<0.001 evaluation after surgery vs after radiotherapy
‡ P<0.001 evaluation after surgery vs before any treatments

Table 4. Manometric parameters in the two subgroups of patients with medium and low rectal cancer before any treatment, after RCT and after surgery. Mean value and standard deviation for each parameter.

Medium rectal cancer	T0	T1	T2	P-value	Low rectal cancer	T0	T1	T2	P-value
Resting	73±20	61±22	§ 50±15	§ 0.001	**Resting**	83±23	66±22	*† 27±18	* <0.007 † <0.001
Squeeze	234±90	198±88	166±58	NS	**Squeeze**	249±99	226±88	*† 120±60	* <0.007 † <0.001
F	20±11	20±3	20±1	NS	**F**	27±11	21±3	20±1	NS
D	40±22	41±21	30±24	NS	**D**	48±22	37±21	43±40	NS
U	‡ 89±47	91±43	47±33	‡ 0.01	**U**	132±47	96±43	* 48±37	* <0.007
RAIR	24±8	22±8	30±16	NS	**RAIR**	20±8	21±8	20±1	NS

T0 = evaluation before neoadjuvant therapy
T1 = evaluation after neoadjuvant therapy and before surgery
T2 = evaluation 12 months after stoma closure
Resting = resting pressure
Squeeze = maximum squeezing pressure
F = pressure threshold inducing first sensation
D = pressure threshold inducing desire to defecate
U = pressure threshold inducing urgency to defecate
RAIR = recto-anal inhibitory reflex

*P< 0.007 evaluation after surgery vs after chemoradiotherapy in the subgroup of patients with low rectal cancer.
† P< 0.001 evaluation after surgery vs before any treatment in the subgroups of patients with low rectal cancer.
‡P= 0.01 evaluation before any treatments in the subgroup of patients with medium vs low rectal cancer.
§P=0.001 evaluation after surgery in the subgroup of patients with medium vs low rectal cancer.

Age > 60 years	T0	T1	T2	P-value	Age ≤ 60 years	T0	T1	T2	P-value
Resting	75.6± 23.8	59.4± 21.1	40±18.1	*0.02 †0.008 ‡<0.001	**Resting**	77.8± 22.8	71.4±23.4	36.1±19.9	†<0.001 ‡<0.001
Squeeze	223.4± 92.2	199±81	153±67.2	‡0.02	**Squeeze**	226.5± 109.6	232.1±98	130.1±51.5	†0.004 ‡0.001
F	21 ± 4.5	20±0	20±0	NS	**F**	26.7± 17.75	22.85±7.3	20±14.9	NS
D	38.9± 21.6	40.9± 26.4	48.6±54	NS	**D**	49.1± 24.3	36.9±11.1	23.3±8.2	†0.01 ‡0.02
U	98.9± 38.6	90.9± 41.7	56.9± 45.3	†0.03 ‡0.008	**U**	110±60	98.5±47.2	36±20.6	†<0.001 ‡0.001
RAIR	24.2± 10.7	22.7± 9.35	20±0	NS	**RAIR**	20±0	21.5±5.5	20±0	NS

D = pressure threshold inducing desire to defecate
F = pressure threshold inducing first sensation
RAIR = recto-anal inhibitory reflex
Resting = resting pressure
Squeeze = maximum squeezing pressure
T0 = evaluation before neoadjuvant therapy
T1 = evaluation after neoadjuvant therapy and before surgery
T2 = evaluation 12 months after stoma closure
U = pressure threshold inducing urgency to defecate
* P < 0.05 T0 vs T1
† P < 0.05 T1 vs T2
‡ P < 0.05 T0 vs T2
T0 > 60 vs T0 ≤ 60 NS
T1 > 60 vs T1 ≤ 60 NS
T2 > 60 vs T2 ≤ 60 NS

Male	T0	T1	T2	P-value	Female	T0	T1	T2	P-value
Resting	79.2± 20.5	68.1± 21.9	37.6± 19.7	†<0.001 ‡<0.001	**Resting**	70.3± 28.2	55.3±22	39.6± 16.9	‡0.02
Squeeze	269± 102.5	245.4± 81.7	158.1± 62.8	†<0.001 ‡<0.001	**Squeeze**	174.9± 53.6	141.1± 52.6	104.8± 33.4	‡0.008 § 0.01 ^<0.001 °0.04
F	24.5± 13.7	20.8±4	26.6±13	†<0.001	**F**	20±0	20±0	20±0	NS
D	42.7± 24.1	35.6± 13.4	30±21.4	NS	**D**	42.5± 19.8	47.3± 32.6	48± 62.6	NS
U	113.6± 47.6	102.6± 43.2	45±32.2	†<0.001 ‡<0.001	**U**	77.8± 38	76.7± 39.8	54.3± 49.9	NS
RAIR	21.9±6	22.5±9	20±0	NS	**RAIR**	25±14.1	21.8±6	20±0	NS

D = pressure threshold inducing desire to defecate
F = pressure threshold inducing first sensation
RAIR = recto-anal inhibitory reflex
Resting = resting pressure
Squeeze = maximum squeezing pressure
T0 = evaluation before neoadjuvant therapy
T1 = evaluation after neoadjuvant therapy and before surgery
T2 = evaluation 12 months after stoma closure
U = pressure threshold inducing urgency to defecate
† P < 0.05 T1 vs T2
‡ P < 0.05 T0 vs T2
§ P < 0.05 T0 Male vs T0 Female
^ P < 0.05 T1 Male vs T1 Female
° P < 0.05 T2 Male vs T2 Female

Hand-sewn anastomosis	T2	Mechanical anastomosis	T2	P-value
Resting	26.8±14.6	**Resting**	49.6±15.2	°0.001
Squeeze	119.6±54.7	**Squeeze**	165.6±58.9	NS
F	20±0	**F**	20±0	NS
D	42.8±52.2	**D**	30±24.5	NS
U	48.3±42.2	**U**	47.3±33.8	NS
RAIR	20±0	**RAIR**	20±0	NS

D = pressure threshold inducing desire to defecate
F = pressure threshold inducing first sensation
RAIR = recto-anal inhibitory reflex
Resting = resting pressure
Squeeze = maximum squeezing pressure
T2 = evaluation 12 months after stoma closure
U = pressure threshold inducing urgency to defecate
° P < 0.05 T2 Hand-sewn anastomosis vs T2 Mechanical anastomosis

Low rectal cancer < 5 cm from anal verge	T0	T1	T2	P-value	Low rectal cancer ≥ 5 cm from anal verge	T0	T1	T2	P-value
Resting	79± 25.5	68± 18.8	22.8± 11.9	†<0.001 ‡0.001	**Resting**	89.8± 22.5	74.4± 30.3	27.5± 18.1	†0.02 ‡0.002
Squeeze	228,5± 40.8	217.6± 99.9	115.7± 65.5	*0.01	**Squeeze**	277± 122.5	256.8± 86.1	138± 52.7	†0.03
F	25± 10	22.8± 7.5	20±0	NS	**F**	32± 26.8	20±0	20±0	NS
D	55± 34.1	45.7± 35.9	46.7± 56.1	NS	**D**	48± 22.8	31.4± 15.7	20±0	NS
U	115± 59.7	111.4± 68.2	73.3± 48.4	NS	**U**	156± 29.4	91.4± 27.9	20±0	*0.004 ‡<0.001 †<0.001
RAIR	20±0	22.8± 7.5	20±0	NS	**RAIR**	20±0	20±0	20±0	NS

D = pressure threshold inducing desire to defecate
F = pressure threshold inducing first sensation
RAIR = recto-anal inhibitory reflex
Resting = resting pressure
Squeeze = maximum squeezing pressure
T0 = evaluation before neoadjuvant therapy
T1 = evaluation after neoadjuvant therapy and before surgery
T2 = evaluation 12 months after stoma closure
U = pressure threshold inducing urgency to defecate
† P < 0.05 T1 vs T2
‡ P < 0.05 T0 vs T2
* P < o.o5 T0 vs T1
§ P < 0.05 T0 Low rectal cancer < 5 cm from anal verge vs T0 Low rectal cancer ≥ 5 cm from anal verge NS
^ P < 0.05 T1 Low rectal cancer < 5 cm from anal verge vs T1 T0 Low rectal cancer ≥ 5 cm from anal verge NS
° P < 0.05 T2 Low rectal cancer < 5 cm from anal verge vs T2 Low rectal cancer ≥ 5 cm from anal verge NS

Medium rectal cancer < 9 cm from anal verge	T0	T1	T2	P-value	Medium rectal cancer ≥ 9 cm from anal verge	T0	T1	T2	P-value
Resting	77.9± 18.9	64.7± 18.6	49.1± 14.6	‡0.001	**Resting**	66.8± 26.6	49.9± 21.4	46.2± 17.8	NS
Squeeze	266.5± 109.5	213.7± 73.9	177± 67.8	NS	**Squeeze**	190.3± 79.8	162.6± 95.7	126.7± 35.9	NS
F	20±0	20±0	20±0	NS	**F**	22± 6.3	20±0	20±0	NS
D	32.7± 13.5	42.9± 18.9	35±30	NS	**D**	46± 25	33.3± 10.3	20±0	NS
U	80± 41.8	98.6± 39.6	51.4± 41.4	†0.02	**U**	100± 36.5	68.6± 25.4	36.7± 15	‡0.001 †0.02
RAIR	20±0	24.3± 11.6	20±0	NS	**RAIR**	30± 15.1	20±0	20±0	NS §0.02

D = pressure threshold inducing desire to defecate
F = pressure threshold inducing first sensation
RAIR = recto-anal inhibitory reflex
Resting = resting pressure
Squeeze = maximum squeezing pressure
T0 = evaluation before neoadjuvant therapy
T1 = evaluation after neoadjuvant therapy and before surgery
T2 = evaluation 12 months after stoma closure
U = pressure threshold inducing urgency to defecate
† P < 0.05 T1 vs T2
‡ P < 0.05 T0 vs T2
* P < o.o5 T0 vs T1
§ P < 0.05 T0 Medium rectal cancer < 9 cm from anal verge vs T0 Medium rectal cancer ≥ 9 cm from anal verge
^ P < 0.05 T1 Medium rectal cancer < 9 cm from anal verge vs T1 T0 Medium rectal cancer ≥ 9 cm from anal verge NS
° P < 0.05 T2 Medium rectal cancer < 9 cm from anal verge vs T2 Medium rectal cancer ≥ 9 cm from anal verge NS

Fig. 1. Flow chart of included patients