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Facoltà di Medicina e Chirurgia

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Prof.ssa Fabiola Paiar

PATIENTS WITH LOCALLY ADVANCED RECTAL CANCER: PREDICTIVE FACTORS OF PATHOLOGICAL RESPONSE AND PROGNOSTIC FACTORS

Relatore

Dott. Aldo Sainato

Candidato

Dott.ssa Sabrina Montrone

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Introduction

Colorectal cancer is the most common gastrointestinal malignancy and the third leading cause of cancer-related death in Western countries (1); in this countries it represents the second leading cause of death in both sexes (2,3). More than half of rectal cancer patients (pts) have been diagnosed with locally advanced tumour (LARC: T3/T4 tumour and/or positive limphonodes) (4). In this cases, preoperative radiochemotherapy (RTCT) followed by total mesorectal excision (TME) is the standard of cure (5,6).

The aim of neoadjuvant RTCT is to reduce the local recurrence (from 25-40% to less than 10%) and to increase the rate of sphincter preservation in pts with low-lying tumour, thanks to downstaging and downsizing. Many studies have also shown a benefit in overall survival (OS) derived from the use of preoperative RTCT (6,7).

Neoadjuvant radiotherapy is generally administered in 28 fractions (Long Course Radiotherapy); in recent years neoadjuvant short course radiotherapy (SRT: 25 Gy in 5 fractions) has proven effectiveness as long as the long course radiotherapy in terms of local recurrence and distant relapses. Although, SRT seems to be less effective on the rate of sphincter preservation in pts with low-lying tumour, due to the little waiting time between radiotherapy and surgery (usually around 7-10 days) that does not allow a T downsizing (8,9). A delayed surgery could increase that rate (10).

At present, TME, that involves resection of both the tumor and the surrounding mesorectal fat, is the surgical treatment of choice for pts with T2-T4 rectal cancer; when associate with preoperative treatment, the recurrence rate is less than 2,4% at 2 years (11,12).

After neoadjuvant treatment the rate of histopathologic tumour downstaging and the rate of complete pathologic response (pCR) range between 30%-60% and 4%-30%, respectively (13,14). The pathological response has been reported in several studies to be closely related to oncologic outcomes (15-18) and many studies (19-21) are trying to find predictive factors of complete response to neoadjuvant treatment in order to select pts who could benefit from sphincter-preserving procedures or organ-preserving options such as local excision of residual tumor or the omission of surgery altogether.

This could be important to avoid late toxicities related to surgery (either after abdominal-perineal resection or after rectal anterior resection) (19-21).

Recognize in advance whether the patient would respond to neoadjuvant treatment may also be useful to decide whether intensify the schedule of preoperative radiotherapy in order to improve the downstaging/dowsizing and the rate of pCR.

Some studies have shown a correlation between the rate of pCR and many factors: the tumor dimension at diagnosis (22,23), the interval between neoadjuvant treatment and surgery (24), the carcinoembryonic antigen (CEA) level at diagnosis (25), the distance of the tumor from the anal verge before neoadjuvant treatment (26) and the nodal stage at diagnosis (23).

To date, however, there is not a real consensus regarding the independent predictive factors for achieve a pathological response after neoadjuvant RTCT. Instead, there is a greater consensus regarding the prognostic factors and the evaluation of these parametres could be useful for deciding whether intensify the schedule of radiotherapy and chemotherapy in order to improve the outcomes.

Many studies regarding prognostic factors in pts with LARC have reported both clinical and pathological parameters. The pCR is the main factor that could influenced the local and distant rate of relapses (27). Another important parameter is the nodal status: the number of pathological positive nodes (28,29), the total number of nodes removed (30-32) and the nodes ratio (30-32), defined as the number of metastatic nodes divided by the total number harvested (30-32).

Associated with a poor prognosis it is also the circumferential resection margin of surgical specimens ≤1mm (33-35). This is related to an higher rate of local recurrence and to a worst quality of TME that has been shown to be an independent factor of local and overall relapses in some studies (36,37).

The differentiation grade and/or the mucinous aspects seem to be important prognostic factors in the staudy carried out by Qui HZ on ninty-six patints (38) and in the study of Oberholzer published in 2012 (39). Both these studies have shown a poor prognosis in pts with mucinous tumor and low grade of differentiation.

The prognostic clinical factors (strumental and haematological) individuated in some studies are: the T clinical stage, the tumor size before RTCT (38,40) and the pretreatment CEA level (30,40,41).

The primary endpoint of this retrospective analysis was to identify predictive factors of response to neoadjuvant RTCT, which could be used in the next future for treatment decision making. The second endpoint was to identify in this subset of pts the prognostic factors related to OS and disease free survival (DFS).

Materials and methods

In this retrospective study we analyzed the data of 119 pts affected by LARC, without evidence of distant metastases, treated with neoadjuvant chemoradiotherapy, between January 2008 and April 2014, in Pisa Universitary Hospital. All pts were initially submitted to a multidisciplinary discussion (surgeon, medical oncologist, radiation-oncologist and radiologist).

Pts characteristics are listed in Table 1.

All pts were staged by clinical examination (a digital rectal examination, a physical examination and a complete history was obtained), total-body tomography (CT), rectum-colonscopy and pelvic magnetic resonance (MRI); many of them were also submitted to endorectal ultrasound (EUS).

For our analysis we restaged all pts according to AJCC cancer staging manual ed.7th 2010.

Histological diagnosis of rectal cancer was obtained in all pts before the start of treatments.

The planned treatment was neoadjuvant long course RTCT followed by TME-surgery +/- adjuvant chemotherapy.

Clinical T and nodal stage was established by radiologists based on MRI images and/or EUS.

Restaging with pelvic MRI +/- abdominal CT was performed 4 weeks after the end of RTCT.

This analysis was conducted in accordance with: 1) recommendations for physicians involved in research on human subjects adopted by the Helsinki declaration of 1964 and later revisions and 2) Directive 2001/20/EC April 4, 2001 of the European Parliament. The study was also approved by local Medical Ethics Committee. Informed consent was obteined in all pts.

Treatment (RTCT and surgery)

Radiotherapy

In all pts, before treatment, axial CT images were obtained using a Light Speed RT 16-slice simulator (GE HealthCare) with a 5mm steps; they were posizioned in prone position and in many cases was utilized the belly-board device to displace the small bowel out of the treatment field. All pts were treated by 3DCRT (three or four fields) or

VMAT technique based on the percentage of small bowel eventually included in high level of dose.

The clinical target volume (CTV) included the rectal tumour with a safety margin of 4-5 cm, the mesorectum, the presacral and internal iliac nodes. The external iliac nodes were included only in T4 tumours for infiltration of anterior pelvic structures; obturatory nodes were included if primary tumor was located under the peritoneal reflection. For tumors located in the distal rectum, the anal canal was included but avoiding the perineal skin. Planning target volume (PTV) consisted of an isotropic 4-5mm expansion of the CTV. The planned total dose was 5040 cGy (energy level of 6-15 MV) prescribed at the ICRU point, in 28 fractions of 180 cGy/day, 5 fractions/week.

Concurrent Chemotherapy

Before the start of chemotherapy, all pts were submitted to a cardiological and haemathological evaluation. Blood cells counts was repeated weekly.

Chemotherapy consisted in continuous infusion of 5-FU at the dose of 225 mg/m2/day or capecitabine at dose of 825 mg/m2/BID administered everyday for the entire radiotherapy course.

Surgery

Surgery was planned between 6 and 8 weeks after the end RTCT. All pts were treated by the same surgical equipe. The surgical procedure, AR (anterior resection) or APR (abdominoperineal resection) with protective ileostomy, was decided by the surgeon and in most cases was based on the initial tumour extension and location. TME was specifically recommended.

Pathologic evaluation

Surgical specimens were evaluated according to a standardized procedure. Their macroscopic and microscopic characteristics were registered in a specific patient's form, reporting the pathologic stage (UICC classification), the number of examined and involved lymph nodes, the status of the margins, the differentiation grade, the presence of mucine, the Quirke's grade (3: intact mesorectum with only minor irregularities of a smooth mesorectal surface; 2: moderate bulk to the mesorectum but irregularity of the mesorectal suface; 1: little bulk to mesorectum with defects down onto m.propria and/or very irregular cirumferential resection margin.) and the Dworak's tumor regression grade (TRG 0 tumor without regression; TRG 1 dominant tumor mass with obvious

fibrosis and/or vasculopathy; TRG 2 dominantly fibrotic changes with few tumor cells or groups; TRG 3 very few tumor cells in the fibrotic tissue with/without mucous substance; TRG 4 fibrotic mass without tumor cells exist).

Postoperative chemotherapy

The administration of adjuvant chemotherapy was prescribed by the physician responsible for the patient based on personal experience.

When adjuvant chemotherapy was prescribed, it was administered 3-5 weeks after surgery, using 5FU /capecitabine as single agent or 5FU /capecitabine plus oxaliplatin. Four-six cycles were generally planned.

Follow-up

After surgery, all pts were monitored every 4 months for the first year, every 6 months until the fifth year and then once a year. The follow-up included clinical examination, abdominal ultrasound and chest X-Ray alternated to total body CT scan; rectoscopies were prescribed every 6 months for the first 2 years and than yearly; pan-colonoscopies were performed at 1, 3 and 5 years from surgery. Local recurrence was defined as any tumour reappearance, clinically or histologically proven, occurring in the pelvis or in the perineum.

Statistical analysis

Before testing of inferential statistics, an exploration phase was performed.

All variables were described by statistical characteristics: categorical data were described by frequency and percentage, whereas continuous data by mean, median and range. Differences were considered significant at $p<0,05$.

The primary endpoint of this retrospective analysis was to identify predictive factors of pathological response to neoadjuvant RTCT. The second endpoint was to identify prognostic factors related to OS and DFS.

- *Predictive factors of T and N response*:

Based on RM-images, we analyzed T and N characteristics at diagnosis and at restaging (before surgery) and their variations.

For T parameter we considered: clinical stage, T site respect to anal verge (low: ≤ 7cm, medium: 7-11 cm, high: >11 cm), cranio-caudal extantion, number of involved quadrants, T volume and the distance from mesorectal fascia. For the last 2 parameters we also analyzed the differences between diagnosis and restaging data (ΔT).

For N parameter we considered: clinical stage (established by radiologists based on first MR and/or EUS with inclusion of all parameters of malingnancy), the number of nodes ≥ 5mm (short axis diameter) and their distance from mesorectal fascia.

The time between surgery and the end of RTCT (> or ≤ 8 weeks) was analyzed for both T and N predictive factors.

The univariate analysis was performed by a binary logistic regression. All variables significantly influenzing the T and N response in the univariate analysis were analyzed together in a multivariate binary logistic regression to assess the independent contribution of each predictive factor. The results of the regression model were calculated by Wald test and expressed using p-value and both the regression coefficients and odds ratio with its related confidence interval.

- *Prognostic factors:*

We analyzed all factors previously described for predictive factors plus age, sex and pathological caracteristics (type of surgery, ypT, ypN, total nodes removed, nodes ratio, mucinous aspect, grading, state of margins, Quirke grade and Dworak's tumor regression).

Follow-up length and survival were expressed as median and range. The interval time was calculated from the diagnosis.

The variables were assessed in the DFS and OS univariate survival analysis. Univariate survival analysis was performed including each variable in a Cox regression model and calculating related p- value by Wald test. All variables significantly influencing survival in the univariate analysis were analyzed together in a Cox regression model as multivariate analysis, with the aim of studying the independent contribution of each variable in explaining survivorship. Furthermore, the proportional hazard was always verified by the use of log(-log) curves. The results of the Cox regression were expressed using hazard ratios with related confidence intervals and related p-value.

Differences were considered significant at $p<0.05$. Analyses were performed using SPSS technology v.23.

Results

All pts completed the planned radiotherapy. In 88 pts (73,9%) was used 3DCRT planning (three or four fields based on dose distribution), in 13 pts (10,9%) VMAT (most of all in the last 3 years) and in 18 pts (15,1%) this data was unknow (they underwent radiotherapy in other structures). In 38/119 pts (31,9 %) radiotherapy was delivered in more than 45 days (median 43 days; range 36-79) but in only 3 pts because of toxicities (2 diarrhea G3 and 1 perianal skin toxicity G3). The concurrent chemotherapy was not administered in 1 patient bacause of kidney failure; in 17 pts (14,3 %) it was interrupped for $\geq$ 7 days (range 7-30) due to toxicities $\leq$ G3 (35,3 % haematological and 17,6 % gastrointestinal toxicity).

In most cases was administered concomitant capecitabine (103 pts; 86,5%) and only 16 pts (13,5%) received i.c. of 5FU.

The mean time between the end of RTCT and surgery was 8,6 weeks (range: 4,7-15,1).

Fifteen pts (13,5%), with primary tumor located in the lower part of the rectum, were submitted to abdominalperineal resection (APR); 103 pts (86,5%) to anterior resection (AR).

In all pts was confirmed a diagnosis of Adenocarcinoma.

Downstaging was evaluated by comparing clinical staging and pathological staging. At the pathological findings 30 pts had a complete pathological response (25,2%), 60 pts (50,4%) a partial T response and 29 pts (24,4%) had a stable disease of the primary tumor. No progression disease was observed.

The TRG was measured using the Dworak scale: TRG4, TRG3, TRG2 and TRG1 was obtained in 30 (25,2%), 35 (29,5%), 41 (34,4%) and 13 (10,9%) pts, respectively.

Seventeen pts (14,3%) had mucinous aspects on the tumor; the grade of differentiation was G1, G2 and G3 in 2 (1,7%), 52 (43,7%) and 14 (11,8%) pts, respectively (52 unknown).

Four pts (4,4%) had a positive radial margin ($\leq$1mm) and 1 patient (0,8%) had a close radial margin (2mm).

Twenty-five pts (21%) had metastatic nodes at the pathological examination; twenty of them (80%) were clinically staged as clinical N+ both by radiologists and by our calculation (nodes >5mm at pelvic MR before RTCT). The mean number of removed nodes was 21 (range 5-55). (Tab.2)

Adjuvant chemotherapy was administered in 71 pts (59,7%), three-five weeks after the surgery; fortynine pts received a monochemotherapy (5FU or capecitabine) and 22 a

doublet of drugs (5Fu/capecitabine + oxaliplatin).

After a median follow-up of 50,7 months (range 17,0 - 96,8), one hundred and six pts (89,1%) are still alive (90 without evidence of disease, 5 with local recurrence and 11 with distant metastases). Ten of 13 pts who die had distant relapse without local recurrence; the other 3 pts died without evidence of disease (Tab.3).

The percentage of ypN+ on the pathological findings was 61,9%, 60% and 12,2% in pts with distant relapse, local relapse and without recurrences, respectively.

The probability of overall survival at 2 and 5 years was 97,3% and 88,5%, respectively (Fig.1).

Two and five years DFS was 91,5% and 77,5%, respectively (Fig.2).

The results in terms of predictive and prognostic factors were analyzed separately.

Predictive Factors

Based on RM-images, we analyzed T and N characteristics at diagnosis, at restaging (before surgery) and their variations.

We analyzed separately the predictive factors of TRG3-4 (the main and-point) and the predictive factors of pathological N0.

The statistical analysis showed a correlation between TRG3-4 and two of the parameters analyzed: the number of involved quadrants at diagnosis (p=0,002) and the cranio-caudal extension of the tumour at diagnosis (p=0,043). The analysis showed also a trend for the volume of the tumor at diagnosis (p=0,122). At the multivariate analysis, the number of quadrants resulted as the only parameter statistically significant (p=0,012) and the T extension lost is significance (p=0,418).

These results are shown in Tab.4.

Regarding to the predictive factors of N0, the analysis showed the correlation with just one parameter: the number of nodes ≥ 5mm (smaller diameter). It resulted statistically significant both as a continuous variable (p= 0,004) and as a dichotomous variable (number of nodes <3 vs ≥4; p<0,0001). (Fig. 3).

These results are shown in Tab.5.

Prognostic Factors

We analyzed the role of clinical and pathological characteristics in terms of DFS and OS.

Clinical parameters releted to DFS were: the volume of the tumor at diagnosis, both as a

continous variable (p=0,046) and as a dichotomous variable (median value of 40cc; p=0,015), the number of involved quadrants (p=0,011) and the distance between the tumor and the mesorectal fascia, both as dichotomous (cut-off: 1mm; p=0,015) (Fig.4) and as continuous variable (p=0,016).

Pathological parameters related to DFS were: the pathological T stage as continuous (p= 0,001) and dichotomous variable (T0 vs T1-4; p=0,041), the pathological N stage as dichotomous parameter (N0 vs N+; p<0,0001) (Fig. 5) and continuous parameter (p<0,0001) and the nodal ratio as dichotomous parameter (≤0,2 vs >0,2; p<0,0001). We also analyzed the grade of differentiation of the tumor (p=0,045) but we decided to avoid it in the multivariate analysis due to the high percentage of unknown date (43,7%). Even the TRG parameter resulted statistically correlated to DFS, both as dichotomous variable (TRG 1-2 vs TRG 3-4; p=0,041) (Fig. 6) and as continuous variable (p= 0,001), but we decided to not analized it in multivariate analysis because of its correlation with pathological T stage.

Our analysis also showed a correlation between DFS and adjuvant chemotherapy in favour of those who did it (P= 0,028).

At the multivariate analysis, the pathological N stage resulted as the only parameter statistically significant (p= 0,006); the nodal ratio and the T volume lost their significance and the other four parameters analyzed showed a trend of significance. These results are shown in Tab.6.

In our analysis, the only clinical parameter releted to OS was the number of involved quadrants (p=0,011). Instead, the pathological parameters related with OS were: the pathological N stage as dichotomous parameter (N0 vs N+; p=0,009), the number of resected nodes both as dichotomous variable (median value: ≤21 vs >21; p=0,048) and as continuous variable (p= 0,042) and the nodes ratio as dichotomous parameter (≤0,2 vs >0,2; p=0,002).

Our analysis also showed a correlation between OS and adjuvant chemotherapy (P= 0,005).

At the multivariate analysis, the pathological N stage (P=0,037), the number of nodes resected as dichotomous variable (P=0,049) and the adjuvant chemotherapy (P=0,023) resulted as the only parameters statistically significant; the N ratio lost is significance (P=0,832) and number of involved quadrants showed a trend toward statistical significance (P=0,097).

These results are shown in Tab.7.

Discussion

The primary endpoint of this analysis was to identify predictive clinical parameters of pathological response. To know these parameters could be important because pCR seems to be related to a better 5-year disease-free survival (86% for TRG4 vs 75% for TRG 2-3 and 63% for TRG 0-1) and to a very low rate of local recurrence (near to zero) (17). These findings have been corroborated in many studies and a meta-analysis including 3105 pts with LARC reported that a pCR after preoperative CTRT was associated with a 5-year crude DFS of 83% vs 66% for pts without a complete response (42).

This improvement in systemic and local control in pts with a pCR suggested a biological basis for treatment response and that pCR could be a prognostic marker for better survival.

After preoperative RTCT, TME represents the standard treatment with excellent oncologic outcomes but this approach (radical surgery after pelvic radiotherapy) could be associated with significant toxicity. The rate of perioperative mortality is as high as 2.4% and postoperative complications occur in over one-third of pts (43,44). Late complications related to both surgery and radiotherapy can include bowel obstructions, urinary incontinences and bowel and sexual dysfunctions; pts with distal rectal tumors may require a permanent colostomy, often associated with poor body image (45).

Based on the significant rate of pCR after neoadjuvant RTCT (13,14) and the potential toxicity of radical surgery, many studies are exploring a conservative menagement ("watch-and-wait" approach or local excision) in pts with clinical complete response (cCR) or near complete response to RTCT.

The "watch-and-wait" approach for pts with rectal cancer who achieved a cCR to neoadjuvant RTCT was initially described by Brazilian investigators in 2004 (46).

In an updated series published in 2006, Habr-Gama et al. reported the resultes of 361 pts with cT2-T4N0 rectal cancer treated with RTCT (50.4 Gy with concurrent leucovorin and 5-FU). At 8 weeks, all pts underwent repeated evaluations, including endoscopies with biopsies. The presence of any significant residual ulcer or positive biopsies was considered incomplete clinical response and the pts were submitted to radical resection. Pts deemed to have a cCR were closely monitored with a monthly serum CEA, digital rectal examination (DRE), proctoscopy and biopsy of any suspicious lesion for 1 year, then continued under surveillance every 3 months for an additional year and every 6 months thereafter (47). A total of 99 pts had a sustained cCR for ≥ 12 months and were

managed nonoperatively. At a mean follow-up of 60 months, this cohort experienced 13 (13%) recurrences. Of these, 5 (5%) recurrences were endorectal, 7 (7%) systemic, and 1 (1%) combined. The 5 isolated endorectal recurrences were effectively salvaged. The 5-year OS and DFS rate was 93% and 85%, respectively (48).

In a recent prospective study, the same authors reported that 47/70 pts (68%) analyzed demonstrated an initial cCR; eight of them developed a local recurrence in the first 12 months of follow-up and other four pts had a local relapse > 12 months of follow-up. Overall, 35 (50%) pts never underwent surgery due to sustained cCR.(49)

Recent evidences show that the tumor response to RTCT is time-dependent and that tumor regression could take more than 6 weeks (46); the longer interval from the end of RTCT was found to be associated with a significantly improved pCR rate in many studies (24, 50). Also Petrelli et al (51) showed an improvement of pCR (19.5% vs 13.7%) in pts who waited more than 6-8 weeks.

The omission of surgery is based on the ability to identify pts who have achieved a cCR before surgery. Unfortunately, clinical evaluations like DRE, endoscopic assessment and imaging modalities, such as CT, MRI or proton emission tomography (PET) are limited in their ability to distinguish post-radiation changes from residual disease; none of them can identify a cCR with sufficient reliability as a single modality (52,53).

The question of how to accurately identify pts who have achieved a true pCR is still being evaluated and the “watch-and-wait” approach remains investigational.

The clinical parameters commonly analyzed to predict the pathological complete response are: age, gender, clinical T and N stage, T size, circumferential involvment, distance from anal verge and interval between RTCT and surgery.

The initial T size was one of the most common factors identified as predictive for pCR (22,23,25,54,55). Some studies indicated 4-5 cm (cranio-caudal extension) as a limit value to obtain or not a pCR. Also in our data, the T dimension was indipendentely correlated with a pathological response (TRG3-4) as continous variable (inversely proportional to TRG3-4, without a significant value of cut-off) and only in the univariate analysis (P= 0,043).

Another variable analyzed in many studies was the number of involved quadrants at diagnosis (26,50,54) but it resulted statistically correlated with the rate of pCR only in one study wich analyzed 562 pts affected by LARC (54). The results of this study indicated that a tumor circumferential extent >60% was associated with a lower pCR rate (P=0,033). Also in our analysis the number of involved quadrants seemed to be a very significant predictive factor of pCR in the univariate (P=0,002) and in the

multivariate analysis (P=0,012). The high significant value of this parameter probably mask the significance of the T extension in the multivariate analysis also because they are both dimensional measurements.
In the same study of Das et al (54), the tumor distance from the anal verge resulted as a predictive factor of response (P=0,035) and this data was confirmed in the article published by Santos in 2016 (P<0,005) (26). In our study this distance was not statistically correlated with pathological response (P=0,567); similar data were reported in the majority of the studies (23,24,50).
Regard to the interval between RTCT and surgery, recently considered as important predictive factor of response (24,36,50), our data did not confir the direct correlation between the time and the rate of pCR (P=0,717), probably because of most pts were submitted to surgery in the planned time or a bit longer. Maybe, if we had waited longer we would have observed an higher rate of T regression.

Many studies analyzed the role of T and N clinical stage to predict the pathological response (23,26,50,54,55) but none showed a statistical correlation. In the study of Park CH et al (55) it was examined the data of 249 pts affected by LARC and treated with neoadjuvant RTCT and they did not find a correlation between pCR and the clinical T and N stage at diagnosis but only with the ycT (p<0,001) and ycN (p<0,001) stage; this correlation lost its significance in the multivariate analysis. In our study, we analyzed the prognostic value of clinical T and N stage just before neoadjuvant treatment and we found a correlation between the initial number of nodes >5mm (lower diameter) and the rate of pathological negative nodes; our results showed that pts with a lower number of nodes at diagnostic MRI (cut-off: ≥4) had an increased probability of pN0 stage (p<0,0001). In our analysis, as well as in the studies cited above (23,26,50,54,55), we did not find a correlation between the T clinical stage and the rate of pathological response (p=0,200).

The secondary endpoint of our retrospective analysis was to identify the prognostic factors related to DFS and OS.
In pts with LARC, after neoadjuvant RTCT and TME-surgery, the rate of local failure is less than 2,4% at 2 years (11,12), moreover the number of distant metastases is still high, around 24-30% at 5 years (56); should be therefore necessary to find prognostic factors related to DFS and OS in order to improve the therapeutic strategies.
Pts with high risk of metastases at diagnosis may undergo intensified chemotherapy

regimens or may be subjected to earliest systemic treatment (57). Instead, pts with a low risk of metastases may avoid chemotherapy and could be treated by intensified radiotherapy in order to decrease the percentage of local recurrences.

Many studies have tried to find clinical and pathological factors related to the rate of local and distant relapses in order to classify the pts in “at high” or “at low risk” of relapse.

One of the most important parameters related to the long-term outcome is the pCR; many studies showed a close correlation between pCR and the rate of local and distant recurrences. Martin S.T. and his colleagues reviewed 16 studies involving 3363 pts and showed that pts with pCR were 4 times less likely to develop local failure, 4.3 times more likely to be disease free at 5 years and 3.3 times more likely to be survival at 5 years (16). Also Maas et her colleagues reviewed 484 articles and concluded that pCR was related to better DFS and to a lower rate of local recurrences and distant metastases (42).

In our study to evaluate the response to neoadjuvant RTCT, we used Dworak’s TRG and we decided to divide the pts in two groups (TRG1-2 and TRG3-4) because TRG3 and TRG4 could benefit from conservative menagement or “watch-and-wait” approach. Pts with TRG3 and TRG4 had a significant better DFS than pts with TRG1 and TRG2 (p=0.041). This difference was not evident for OS (p=0.696). Rodel et al, in 2005, analyzing Dworak’s TRG separately, had similar results in terms of DFS: pts with TRG4, TRG2-3 and TRG0-1 had 86%, 75% and 63% 5 years DFS, respectively (p=0.006) (17).

Regard to T parameter (size, involved quadrants, volume and ypT stage) Luna-Perez on 61 pts affected by LARC, showed a correlation between tumor size and local relapses (35); pts with tumor < 3cm had a lower rate of local recurrences (p=0,039). The same author did not report a correlation between T size and distant relapses (p=0,08). In our analysis, the initial tumor size was not related to DFS (p=0,235) or to OS (p=0,272), while the number of involved quadrants at diagnosis, resulted correlated with DFS (p=0,011).

In our work we analyzed also the prognostic impact of the tumor volume at diagnosis, after RTCT and its variation, based on MRI images. Our results showed a correlation between the T volume at diagnosis and the DFS (p=0,046) but we decided to not include it in the multivariate due to its close correlation with the T size and the number of involved quadrants. Inversely, we did not find a correlation between the volume-parameters and OS; Finally, also the ΔT volume did not seem to correlate with the OS

(P=0,960) and DFS (p=0,533). In our study, a bigger initial T volume seemed to be related more to local than to distant relapses: all pts (100%) who had local failure had a T volume ≥40cc instead, only 3/21 pts (12,3%) who had distant relapses had a T volume ≥40cc.

In our analysis we showed a strong correlation between the number of involved quadrants at diagnosis and both DFS (p=0,011) and OS (p=0,011) and we noticed that pts who developed a local or distant relapse had more likely ≥3 involved quadrants (80% and 80,9%) than those who did not have e relapse (52%).

As regard to the pathological T stage, in our analysis it was not related to OS (p=0,270) but only to DFS (p=0,001). We noticed that none of the pts with local recurrence had a pCR vs the 9,5% of them with distant relapses. The absence of correlation between the T stage and the OS could be related, in our opinion, to the low rate of events caused by the relatively short follow-up. In the study published by Rodel in 2005 (17) the pathological T stage was independently correlated to DFS (p=0,016).

Regard to the prognostic impact of N parameter (ypN stage, nodes ratio and number of resected nodes) many studies showed a correlation between the pathological N stage and the rate of local and distant recurrences (17,42,58). In the study published by Rodel and his colleagues (17) the ypN was one of the most independent parameter at the multivariate analysis (<0,0001).

In our analysis, we showed a strong correlation of the nodal involvement (ypN+) with DFS (p<0,0001) and OS (p=0,009) and this parameter maintained its significance in the multivariate analysis, for DFS (p=0,006) as well as for OS (p=0,037). In fact, our analysis showed that pts who developed distant or local recurrence had an higher percentage of ypN+ (61,9% and 60%) than pts who did not developed a recurrence.

Some studies analyzed the role of the lymph nodes ratio on the outcome (58,59). Leonard and his colleagues, recently, published a work where was shown that pts with node ratio > 0,2 had recurrences 4-5 times more likely than pts with node ratio <0,2 (59). After a reviewed of the literature, we decided to use this cut-off also in our study and we found similar results; the node ratio >0,2 was associated with a lower DFS (p<0,0001) and OS (p=0,002). In both cases, in the multivariate analysis, this variable lost its significance.

In the study of Pedro Luna-Perez was also analyzed the impact of the total number of resected nodes on DFS and OS: when this number was <11 it seemed to be related to distant relapses and not to local failures (35). In a study that Zuo conducted on 264 pts affected by LARC, the number of resected nodes (cut-off:12) was not correlated with 5

years DFS (p=0,87) and OS (p=0,62). In our work we analyzed the same factor and we found a correlation only with OS. The reference values analyzed were 12 (p=0,264) and 21 (the median; p=0,048).

In Leonard's study, he showed that the type of surgery and the quality of TME resulted connected with the survival (59). Maas and her colleagues confirmed these results in their pooled analysis published in 2010; the prognosis of pts submitted to APR was poorer than for those who underwent AR (42).

Another parameter partially dependent to the quality of surgery is the state of the circumferential margins that was analyzed by Pedro Luna-Perez in 2005 (35); in this analysis it was shown to be related to distant relapses (p=0,02) but not to overall local recurrence (p=0,33).

In our analysis we also looked for a correlation between the type of surgery and DFS/OS and we found that pts who underwent Miles had not a lower rate of DFS and OS but just a trend of it (p=0,080 and p=0,098). We also analyzed the quality of surgery in term of Quirke's scale and we did not find a correlation with DFS as well as with OS, maybe due to the very low number of pts with a bad Quirke's score (21/119 pts with Quirke 2 and 1/119 patient with Quirke 1). This could be also the reason of the absence of correlation between the positive radial margins and the local and distant failures. In fact, in our group of pts, there were only 5 positive radial margins and they were not correlated with DFS (p=0,204) and OS (p=0,611).

The involvement of the radial margin seems to be related to the distance between the tumor and the mesorectal fascia; pts with a distance ≤1mm should undergo to long course radiochemotherapy, instead of SCRT, in order to obtain a T downsizing and to reduce the rate of positive margins (guidelines AIRO 2012). In our analysis, for this reason, we studied the correlation between the T distance from the mesorectal fascia and the outcomes; this parameter, continuous as well dichotomous (cut-off:1mm) resulted correlated with DFS (p=0,016 and p=0,015) but not with OS (p=0,673). In the multivariate analysis it lost its significance and maintained only a trend (p=0,097).

In the pooled analysis published by Maas in 2010 (42), was analyzed the influence of adjuvant chemotherapy on DFS and OS; the administration of chemotherapy did not have an effect on DFS, although it was associated with an improved OS. In the metanalysis published by Breugom in 2015 (60), which included also the Italian CNR-study (61) was shown that adjuvant chemotherapy after neoadjuvant RTCT did not improve DFS and OS. In our analysis we also analyzed the role of adjuvant treatments and we found a better OS (p=0,005) and DFS (p=0,028) in pts who received it. This

discrepancy could be due to the casual higher incidence of ypN+ in the group of pts that did not undergo to adjuvant chemotherapy (26% vs 17%) and to the rate of pCR that was higher in pts submitted to postoperative chemotherapy (28% vs 17%). Finally, we had not full data of adjuvant chemotherapy in terms of number of cycles so it could be interesting to better evaluate these data in the future.

Conclusions

Our study is retrospective and, thus, has certain inherent limitations.

know predictive factors of complete or near complete pathological response in pts with LARC, after neoadjuvant RTCT, could influence the surgical approach. Many authors have analyzed different parameters predictive of response but their conclusions are inhomogeneous; the T size and its distance from the anal verge seem to be two common predictive factors of pCR. Based on our results, our opinion is that the number of nodes (≥5mm) at diagnosis and the number of involved quadrants could be additional predictive parameters. In our study was not possible to analyzed the predictive value of the interval between RTCT and surgery but it seems to be an important parameter to investigate.

For these reasons, the question of how to accurately identify pts who have achieved a pCR needs further studies, including the analysis of biologic aspects of the tumor; "watch-and-wait" approach as well as the local excision remains investigational.

As regard to the prognostic factors related to DFS and OS, our study is aligned with the conclusion of other authors. Analyzing our data, we could conclude that pathological N parameters (stage, number of resected nodes and limph nodes ratio) and the number of involved quadrants are strongly related to an higher incidence of local and distant relapses. Instead, T parameters (the volume and the ypT stage) seem to be related to the risk of local recurrence.

A better knowledge of prognostic factors related to local and distant relapses will be necessary to decide whether intensify local or systemic treatments.

Figures

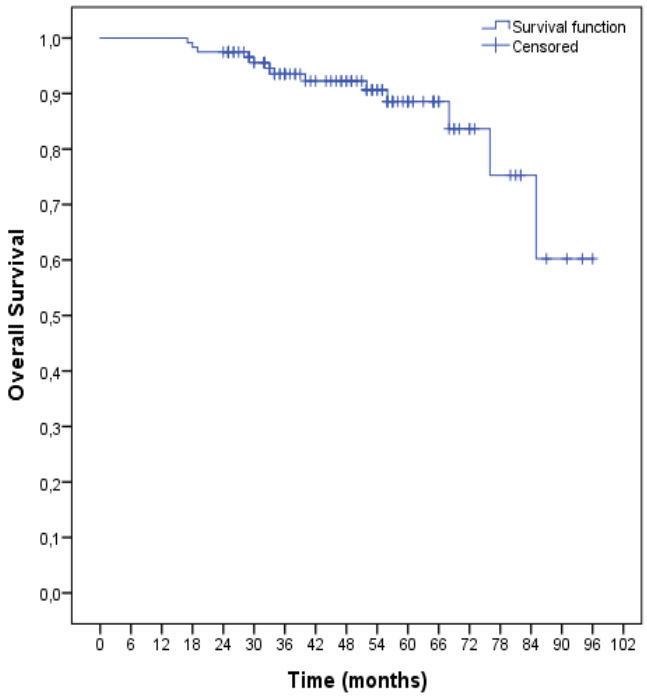

Fig.1: Overall Survival Curve

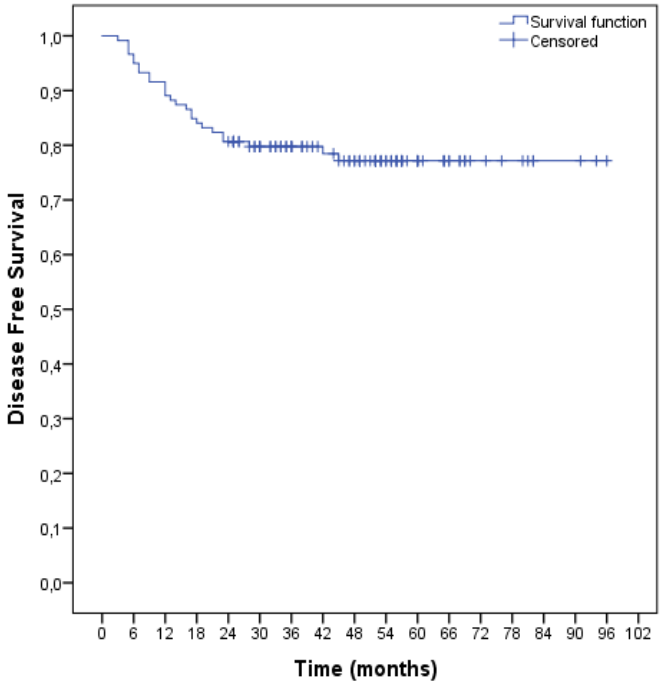

Fig.2: Disease Free Survival Curve

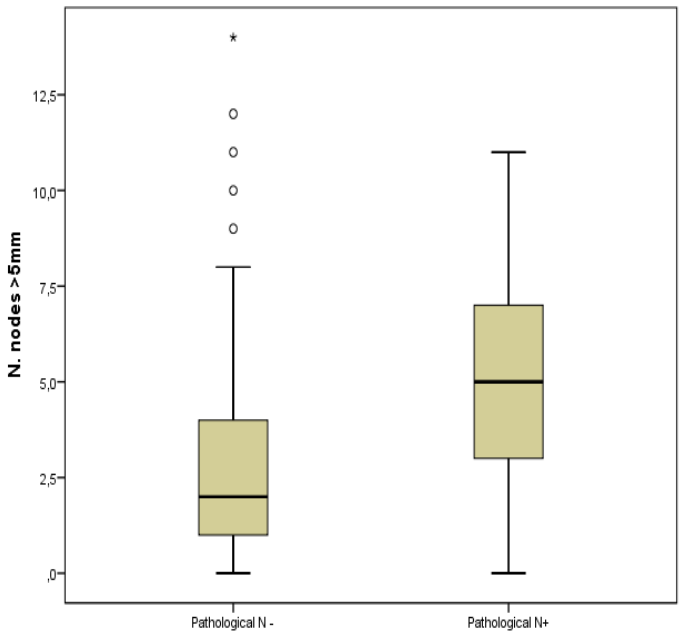

Fig.3: Clinical N stage as predictive factor of ypN-

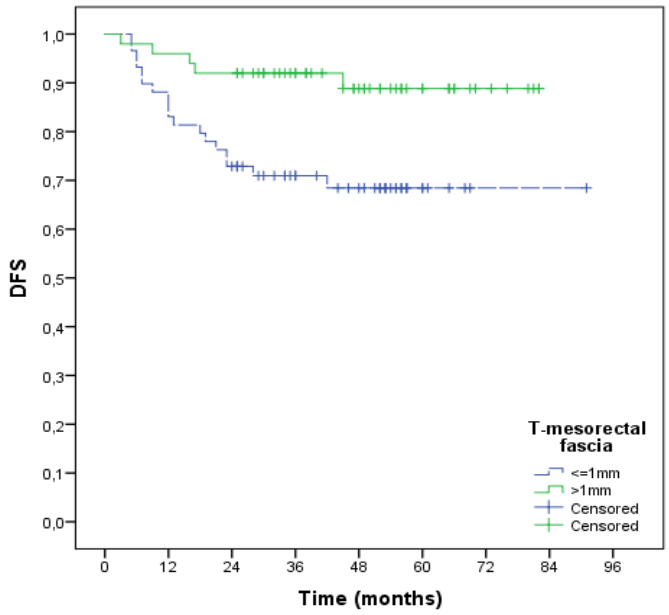

Fig.4: Distance T-mesorectal fascia as prognostic factor of DFS

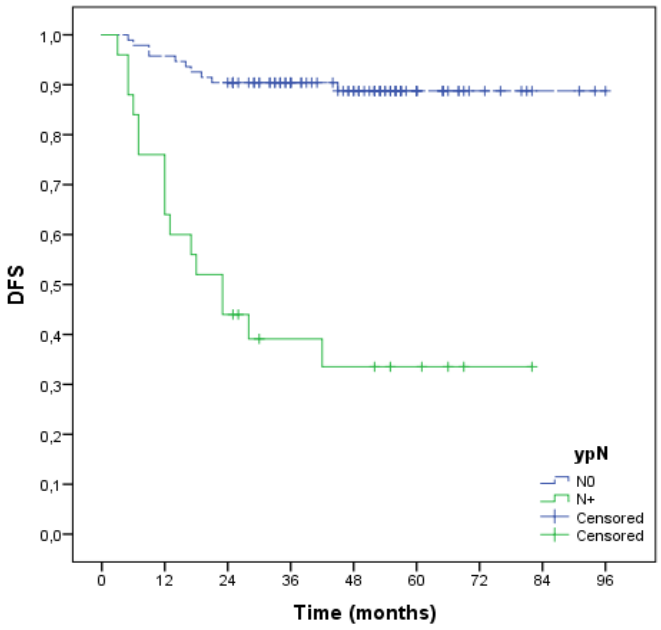

Fig.5: ypN as prognostic factor of DFS

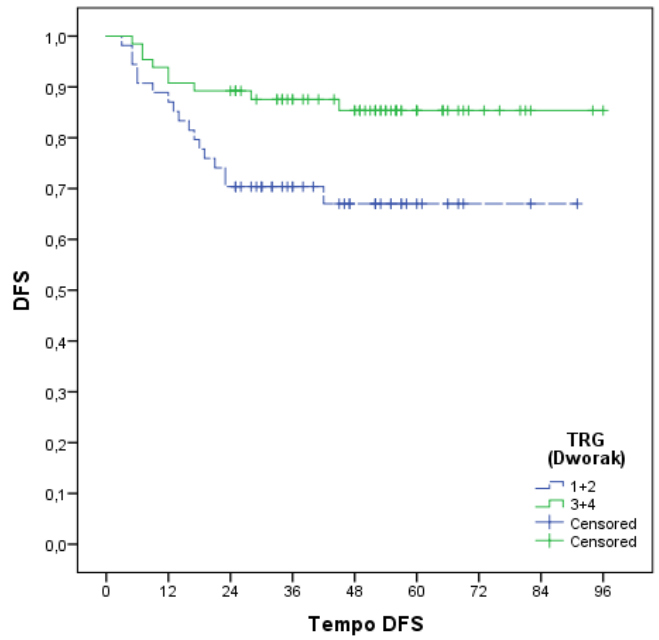

Fig.6: Dworak TRG as prognostic factor of DFS

Tables

Tab 1. Patients characteristics

	N°	%
Patients	**119**	**100**
Age (median 65 years)		
≤ 65	60	50,4
> 65	59	49,6
Sex		
Male	77	64,7
Female	42	35,3
Clinical T stage		
2	2	1,7
3	95	79,8
4	22	18,5
Clinical N stage		
N+	108	90,7
N-	11	9,3
N° of involved quadrants		
1	9	7,5
2	44	37,0
3	17	14,3
4	49	41,2
T-anal verge distance (cm)		
≤ 7	61	51,3
7-11	50	42,0
> 11	8	6,7
T-mesorectal fascia distance (mm)		
>1	52	43,7
≤ 1	59	49,6
Not evaluable	8	6,7
T volume (median 30 cc)		
≤ 30	70	58,8
> 30	49	41,2
T extention (median 50 mm)		
≤ 50	75	63,0
> 50	44	37,0
N ≥ 5mm at diagnosis		
≥ 4	45	37,8
< 4	68	57,1
Not evaluable	6	5,1

Tab 2. Pathological characteristics

	N°	%
Patients	**119**	**100**
Type of surgery		
AR	104	87,4
APR (Miles)	15	12,6
ypT		
0	30	25,2
1	13	10,9
2	41	34,4
3	34	28,6
4	1	0,9
ypN		
N+	25	21
N-	94	79
N° resected nodes (mean: 21)		
≤ 21	64	53,8
> 21	55	46,2
Nodes ratio (cut-off 0,2)		
≤ 0,2	111	93,3
> 0,2	8	6,7
Mucinous aspect		
yes	17	14,3
No	102	85,7
Grading		
1	2	1,7
2	52	43,7
3	14	11,8
Unknow	51	42,8
Positive radial margin		
Yes	5	4,2
No	114	95,8
Quirke grade		
1	1	0,9
2	21	17,6
3	97	81,5
TRG (Dworak)		
1	13	11
2	41	34,4
3	35	29,4
4	30	25,2

Tab 3. Local and distant relapses

	N°	%
Patients	**119**	**100**
Local relapses only	5	4,2
Distant relapses only	21	17,7
Local and distant relapses	0	0
Non evidence disease	93	78,1

Tab 4. Predictive factors of TRG 3-4

	Univariate			Multivariate			
	OR	**IC 95%**	**P-**	**RC**	**OR**	**IC 95%**	**p-**
Clinical T stage (T2-T3-T4)	0,562	0,233-1,357	0,200				
T site (low-medium-high)	0,839	0,478-1,471	0,540				
N°of involved quadrants	0,551	0,378-0,802	**0,002**	-0,527	0,591	0,392-0,891	0,012
Distance T-anal verge	1,010	0,976-1,046	0,567				
Distance T-mesorectal fascia	1,554	0,951-1,168	0,314				
Cranio-caudal T extension	0,979	0,959-0,999	**0,043**	-0,009	0,991	0,969-1,013	0,418
Initial T volume	0,987	0,970-1,004	0,122				
ΔT volume	0,118	0,992-1,045	0,170				
Weeks RTCT-surgery	0,150	0,541-2,445	0,717				
Costant				2,199	9,020	2,317-35,11	0,418

Tab 5. Predictive factors of pN0

	Univariate			Multivariate			
	OR	**IC 95%**	**P-value**	**RC**	**OR**	**IC 95%**	**p-value**
Clinical N stage (N+/N-)	0,933	0,725-1,201	0,592				
Nodes ≥5mm (dic:<4/≥4)	6,033	2,230-16,18	**<0,0001**				
Nodes ≥5mm (continous)	0,803	0,693-0,932	**0,004**				
Distance N-mesorectal fascia	1,110	0,917-1,343	0,983				
Weeks RTCT-surgery (≤8/>8)	1,006	0,840-1,195	0,950				

Tab 6. Prognostic factors of DFS

	Univariate			Multivariate			
	HR	**IC 95%**	**p-value**	**RC**	**HR**	**IC 95%**	**p-value**
Age (cut-off:62 years)	1,422	0,490-4,128	0,517				
Sex (M/F)	1,199	0,544-2,642	0,653				
Clinical N stage (N+/N-)	0,734	0,220-2,452	0,615				
Clinical T stage (T2-T3-T4)	1,482	0,628-3,495	0,369				
T site (low-medium-high)	0,673	0,351-1,288	0,232				
Involved quadrants	1,727	1,131-2,638	**0,011**	0,399	1,491	0,871-2,550	0,145
T-anal verge distance(cm)	0,994	0,980-1,009	0,448				
T-mesorectal fascia(mm)	0,706	0,533-0,936	**0,016**	-0,126	0,881	0,759-1,023	0,097
T extension	1,010	0,993-1,028	0,235				
T volume (cut-off : 40cc)	2,710	1,216-6,042	**0,015**	0,346	1,413	0,533-3,743	0,487
ΔT volume	0,992	0,969-1,016	0,533				
ypN (N+/N-)	8,650	3,903-19,17	**<0,0001**	1,539	4,659	1,561-13,911	0,006
ypT (continuous)	2,360	1,450-3,843	**0,001**	0,485	1,624	0,834-3,164	0,154
Type of surgery (APR/AR)	0,442	0,167-1,102	0,080				
Mucinous aspect	0,802	0,241-2,671	0,719				
Grading	2,7	1,021-7,142	**0,045**				
Quirke	1,342	0,476-3,785	0,578				
TRG (TRG3-4/TRG1-2)	4,485	1,060-18,98	**0,041**				
TRG (continuous)	0,494	0,319-0,763	**0,001**				
Radial margin	2,550	0,602-10,81	0,204				
Resected nodes	0,979	0,935-1,024	0,353				
Nodes ratio (cut-off:0,2)	10,162	4,005-25,78	**<0,0001**	0,498	1,645	0,407-6,653	0,485
Adjuvant chemotherapy	0,404	0,179-0,909	**0,028**	-0,791	0,453	0,174-1,183	0,106

Tab 7. Prognostic factors of OS

	Univariate			Multivariate			
	HR	**IC 95%**	**p-value**	**RC**	**HR**	**IC 95%**	**p-value**
Age (cut-off:62years)	1,079	1,012-1,150	0,099				
Sex (M/F)	2,090	0,667-6,545	0,206				
Clinical N stage (N+/N-)	4,572	1,463-14,28	0,009				
Clinical T stage (T2-T3-T4)	1,352	0,628-3,495	0,369				
T site (low-med-high)	0,673	0,792-2,309	0,270				
Involved quadrants	2,738	1,260-5,948	**0,011**	0,667	1,948	0,887-4,279	0,097
T-anal verge distance(cm)	0,997	0,978-1,017	0,794				
T-mesorectal fascia(mm)	0,964	0,814-1,142	0,673				
T extension	1,013	0,990-1,037	0,272				
T volume (diagnosis)	1,001	0,961-1,043	0,960				
ΔT volume	0,992	0,969-1,016	0,533				
ypN (N+/N-)	4,572	1,463-14,18	**0,009**	1,633	5,120	1,100-23,83	0,037
ypT (continuous)	1,352	0,792-2,309	0,270				
Type of surgery (APR/AR)	0,364	0,110-1,205	0,098				
Mucinous aspect	0,647	0,083-5,061	0,678				
Grading	0,518	0,032-8,387	0,643				
Quirke	0,785	0,230-2,679	0,699				
TRG (TRG3-4/TRG1-2)	1,305	0,343-4,973	0,696				
TRG (continuous)	0,858	0,469-1,570	0,619				
Radial margin	1,705	0,218-13,33	0,611				
Resected nodes (cut-off:21)	0,127	0,016-0,983	**0,048**	-2,093	0,123	0,015-0,992	0,049
Nodes ratio (cut-off:0,2)	8,146	2,134-31,10	**0,002**	-0,209	0,811	0,118-5,571	0,832
Adjuvant chemotherapy	0,102	0,020-0,511	**0,005**	-1,966	0,140	0,026-0,758	0,140

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