



HAL
open science

YpN0 rectal cancer patients with sterilized lymph nodes after neoadjuvant chemoradiotherapy are of greater risk of recurrence

Gilles Manceau, Nicolas Margot, Jeremy Augustin, Armelle Bardier, Jean-Marc Simon, Jean-Baptiste Bachet, Jean-Philippe Spano, Philippe Maingon, Jean-Christophe Vaillant, Mehdi Karoui

► To cite this version:

Gilles Manceau, Nicolas Margot, Jeremy Augustin, Armelle Bardier, Jean-Marc Simon, et al.. YpN0 rectal cancer patients with sterilized lymph nodes after neoadjuvant chemoradiotherapy are of greater risk of recurrence. *Digestive and Liver Disease*, 2020, 52, pp.214 - 220. <10.1016/j.dld.2019.07.013>. <hal-03490001>

HAL Id: hal-03490001

<https://hal.science/hal-03490001v1>

Submitted on 21 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC 4.0 - Attribution - Non-commercial use - International License

ORIGINAL PAPER

YpN0 rectal cancer patients with sterilized lymph nodes after neoadjuvant chemoradiotherapy are of greater risk of recurrence

Gilles Manceau, MD-PhD (1), Nicolas Margot, MD (1)*, Jeremy Augustin, MD (2), Armelle Bardier, MD (2), Jean-Marc Simon, MD-PhD (3), Jean-Baptiste Bachet, MD-PhD (4), Jean-Philippe Spano, MD-PhD (5), Philippe Maingon, MD-PhD (3), Jean-Christophe Vaillant, MD (1), Mehdi Karoui, MD-PhD (1).

Sorbonne Université, Assistance Publique-Hôpitaux de Paris, Pitié-Salpêtrière University Hospital, Paris, France, Department of: (1) Digestive and Hepato-Pancreato-Biliary Surgery; (2) Pathology; (3) Radiotherapy; (4) Hepato-Gastroenterology and Digestive Oncology; (5) Medical Oncology

* The two first authors have equally contributed to the present work

Word count: 2 709 words

Corresponding author:

Mehdi Karoui, MD-PhD

Sorbonne Université, Assistance Publique de Hôpitaux de Paris, Pitié-Salpêtrière University Hospital, Department of Digestive and Hepato-Pancreato-Biliary Surgery
47-83 Boulevard de l'Hôpital, 75013 Paris, France

Tel : +33 1 42 17 56 51

Email: mehdi.karoui@aphp.fr

Source of Funding: none for all authors.

The present article is based on a previous communication to the ACHBT-SFCD meeting on November 2018.

Key words: rectal cancer, sterilized lymph node, neoadjuvant chemoradiotherapy, survival

ABSTRACT (198 words)

Background: Indication for adjuvant chemotherapy in ypN0 rectal cancer patients after chemoradiotherapy (CRT) is debated. The clinical significance of the presence of sterilized lymph nodes (LNS) in ypN0 patients remains to be determined.

Aims: To assess the prognostic value of LNS in ypN0 rectal cancers after neoadjuvant CRT.

Methods: From 2006-2016, 235 patients underwent TME surgery for non-metastatic mid-low rectal cancer after CRT. A lymph node was considered sterilized if there were signs of treatment response (fibrosis, necrosis or mucus) without residual tumor cells.

Results: 180 patients (77%) were classified ypN0 and 55 (23%) ypN+. LNS was present in 20 patients (9%). In ypN0 patients, 5-year OS was similar between patients with and without LNS. In contrast, 5-year DFS was significantly lower in ypN0/LNS+ patients (58% vs. 78%, $p=0.043$) and was similar to those staged ypN+. In multivariate analysis, two factors were independent predictors of DFS: mesorectal grading (OR=3.14; 95%CI: 1.10-8.34; $p=0.033$) and the presence of LNS (OR = 3.93, 95% CI: 1.06-11.81, $p = 0.042$)

Conclusion: The presence of LNS in ypN0 rectal cancer after neoadjuvant CRT is associated with an increased risk of recurrence and may be taken into account for the discussion of adjuvant chemotherapy.

INTRODUCTION

In patients with cT3/T4 and/or N+ mid or low rectal cancer, neoadjuvant chemoradiotherapy (CRT) followed by radical TME resection became the standard of care with a local recurrence rate of 4 to 8% [1-6]. However, 35 to 45% of these patients will develop metastatic recurrence at 5 years reflecting the relative failure of such a strategy to eradicate distant micrometastases [3, 4]. The initiation of systemic chemotherapy before CRT as induction treatment is currently under evaluation to improve the prognosis of these patients by upfront treatment of micrometastatic disease [7]. Better selection for adjuvant systemic chemotherapy may represent another option to improve oncological outcomes of rectal cancer patients after CRT. Although adjuvant chemotherapy is consensually accepted in stage III disease after CRT, its benefits for ypN0 patients remains unclear [8]. Adjuvant chemotherapy is usually proposed for ypN0 patients with high risk factors of recurrence including ypT4, tumor perforation, poorly differentiated tumor, neural and vascular invasion, abdominoperineal resection and positive circumferential margin [9-16]. Whether the presence of sterilized lymph nodes (LNS) within the mesorectum is a poor histological feature justifying adjuvant treatment is still to be determined [17]. Because the presence of LNS implies lymph node (LN) metastases before CRT and indicates a pretherapeutic cN+ status, it can be hypothesized that pN0/LNS+ patients had a more aggressive primary tumor at initial diagnosis compared to their pN0/LNS- counterparts and would therefore benefit from adjuvant chemotherapy. Conversely, pN0/LNS+ patients may be considered as very good responders to CRT with a prognosis similar to those initially staged cN0 and classified pN0/LNS-, implying that CRT is sufficient to prevent both local and systemic recurrence and that

the presence of LNS+ at pathological examination is not helpful in the decision making for adjuvant chemotherapy.

In the present study, we aimed to determine the prognostic value of LNS for patients with mid and low rectal cancer staged ypN0 after neoadjuvant CRT and radical TME resection. It is our hypothesis that the knowledge of the prognosis of ypN0 patients with LNS could help caregivers deciding the need for adjuvant chemotherapy or not.

MATERIALS AND METHODS

Patients

Between January 2006 and December 2016, all consecutive patients undergoing a radical TME resection for biopsy-proven, cT3-T4 and/or cN+ mid or low rectal adenocarcinoma after neoadjuvant CRT were included in a prospectively maintained database. Exclusion criteria were the following: metastatic rectal cancer, local excisions, induction chemotherapy before CRT, neoadjuvant CRT with additional chemotherapy during the resting period and patients who died within 30 days of surgery. Low rectal cancer was defined as a tumor located less than 6 cm from the anal verge. This observational non-therapeutic study was approved by the local research ethics committee.

Preoperative management

Pre-treatment clinical tumor-node-metastasis (TNM) stage was determined by physical examination with digital rectal examination, CT scan, endorectal ultrasonography, pelvic MRI or a combination of these exams. Indications for neoadjuvant CRT were discussed for all patients at multidisciplinary team meetings. Radiation therapy consisted of a total dose of 45-50.4 Gy delivered in daily fractions of 1.8-2 Gy by three beams in a 5- to 6-week period. The radiotherapy regimen remained the same for all patients; however, several chemotherapy regimens associated with radiotherapy were used.

Surgical management

Surgery was usually planned 6-8 weeks following the completion of neoadjuvant CRT and performed with curative intent according to the principles of total mesorectal excision. The surgical approach (open surgery or laparoscopy) was left to the surgeon's discretion. Abdominoperineal resection was performed for patients with external anal sphincter or levator ani muscle invasion [18]. A protective defunctioning ileostomy was usually performed in case of sphincter preservation.

Pathological examination

Pathologic examination was performed by pathologists specialized in gastrointestinal tract diseases. Macroscopic examination included an evaluation of the mesorectum aspect according to Quirke recommendations [19]. Tumors with a scar appearance were included totally and tumors with a vegetating appearance were sampled extensively. LN were carefully searched for and all LN were sampled for microscopic examination. The following criteria were systematically recorded: tumor size, tumor differentiation according to the World Health Organization, surgical resection margins and lymphovascular invasion. Tumor regression in primary tumor was assessed using the tumor regression grade (TRG) score mentioned in the College of American Pathologists and modified by Ryan [20] : no viable cancer cells (complete response) was scored 0 ; single cells or rare small groups of cancer cells (near complete response) was scored 1 ; residual cancer with evident tumor regression, but more than single cells or rare small groups of cancer cells (partial response) was scored 2 ; extensive residual cancer with no evident tumor regression (poor or no response) was scored 3.

All sampled LN were examined microscopically. LN with tumor invasion were considered positive. LN were considered sterilized (LNS) in case of lymphoid tissue

replacement by signs of sterilization: fibrosis, necrosis, foamy macrophages, giant cells, acellular mucin pool or calcifications (**Figure 1**). LN without any tumor or signs of sterilization was considered negative.

Adjuvant chemotherapy and oncological follow-up

Adjuvant chemotherapy was usually given in ypN+ patients, regardless of cN stage, and in patients with ypN0 tumor harboring histopathological criteria of poor prognosis. Decision for adjuvant chemotherapy was made during multidisciplinary team meetings. Oncological follow-up was standardized and performed according to international recommendations. Patients were seen every 3 months for the first 3 years after surgery, every 6 months for the next 2 years, and annually thereafter. During follow-up, patients were seen in clinic and underwent clinical examination, thoraco-abdominopelvic CT scan, and CEA determination. Colonoscopy was carried out at year 1 and every 3-5 years thereafter.

Study endpoints

The primary endpoint of this study was DFS, defined as the length of time between the date of rectal surgery and the date of first recurrence, or death. The secondary endpoint was OS. In order to limit interpretation biases, OS was analysed after exclusion of the patients who died during the first 30 postoperative days

Statistical analysis

Tumour staging was defined according to the 6th edition of the AJCC/UICC TNM staging system for colon and rectal cancer. Categorical variables were expressed as absolute numbers and percentages and compared with Pearson's chi-square test or

Fisher's exact test, as appropriate. Quantitative data are expressed as median and range and compared with the Mann-Whitney U-test. Survival curves were plotted according to the method of Kaplan and Meier and differences between survival distributions were assessed by the log-rank test. Prognostic factors that influence DFS were analysed in patients with ypN0 rectal cancer. Multivariate analysis was performed using Cox proportional stepwise procedure, including every non-redundant prognostic factor identified by the univariate analysis. A p-value <0.2 was defined for systematic entry into the model. A p-value <0.05 was considered as significant. Statistical analyses were performed using JMP (version 14.0.0; SAS Institute, Cary, North Carolina, USA) software.

RESULTS

Patient characteristics

During the study period, 235 patients with locally advanced mid or low rectal adenocarcinoma treated by neoadjuvant CRT were retrieved and fulfilled the inclusion criteria. Among these patients, 141 were male (60%). Ninety-three patients (40%) had a low rectal cancer. The median age at surgery was 63 (19.0-91.5) years. TME surgery included low anterior resection with colorectal anastomosis (n=69, 29%), coloanal anastomosis (n=100, 43%), intersphincteric resection (n=19, 8%), abdominoperineal resection (n=45, 19%) and low Hartmann's procedure (n=2, 1%). For patients with sphincter-preserving surgery (n=182, 77%), a temporary loop ileostomy was routinely performed, except in 3 cases (2%).

Pathological results

Among the 235 included patients, 180 patients (77%) were ypN0 and 55 (23%) ypN+. A total of 2,966 LN were analyzed, with a median of 11 (0-49) LN per patient. There were 151 positive LN (LN+), with a median of 2 (1-15) LN+ per patient, and 2,815 negative LN (LN-). Among the LN-, 37 (1.3%) were considered as sterilized (LNS) at pathological examination. At least one LNS was found in 20 of the 235 patients (9%) with a median number of 1 (1-9) LNS retrieved. All these LNS were located in the mesorectum. None was present along the superior rectal or inferior mesenteric arteries.

Overall, among ypN0 patients, 12 (7%) were staged ypN0/LNS+ and 168 (93%) ypN0/LNS- (**Table 1**). Eight out of the 12 (67%) patients ypN0/LNS+ were staged cN+ before neoadjuvant CRT. A better response to CRT was observed in the

primary tumour in the ypN0/LNS+ group than in the ypN0/LNS- group (TRG 0-1: 83% vs. 46%, $p=0.013$) (**Table 2**). Patients ypN0/LNS+ had a higher rate of T-downstaging (91%) and stage downstaging (100%) in comparison with those ypN0/LNS- (67% and 86%, respectively), although these differences were not statistically significant.

Oncological outcomes

The median duration of follow-up for the overall cohort was 31.8 months (range, 0.5-105.6 months) and was not different between ypN0/LNS+ and ypN0/LNS- patients (27.8 months vs. 32.5 months, $p=0.56$). Adjuvant chemotherapy was administered more often in the ypN0/LNS+ patients than in ypN0/LNS- patients, although the difference was not significant (45% vs. 20%, $p=0.066$). No significant difference was found between ypN0/LNS+ and ypN0/LNS- patients for OS (5-years OS: 100% vs. 91%, $p=0.47$, **Figure 2**). However, 5-years DFS was significantly lower in ypN0/LNS+ patients than in ypN0/LNS- patients (58% vs. 78%, $p=0.043$, **Figure 3**). Furthermore, 5-years DFS of ypN0/LNS patients + was similar to the one of those staged ypN+ (58% vs. 38%, $p=0.80$). Recurrence was diagnosed in 4 patients (33%) in the ypN0/LNS+ group: 2 were local recurrences and 2 were distant recurrences.

In univariate analysis, 3 of the 20 variables were statistically significant for reduced DFS: abdominoperineal resection, presence of LNS+, and lymphovascular emboli (**Table 3**). The multivariate analysis showed 2 variables as independent factors of poor DFS: the quality of the mesorectal excision (OR=3.14; 95%CI: 1.10-8.34; $p=0.033$), and the presence of LNS+ (OR=3.93; 95%CI: 1.06-11.81; $p=0.042$) (**Table 3**).

DISCUSSION

The prognostic value of LNS in ypN0 patients with mid and low rectal cancer after neoadjuvant CRT and radical TME resection remains unclear. We found that the presence of LNS at pathological examination in patients with ypN0 rectal cancer after CRT was associated with increased risk of recurrence and worse 5-years DFS. Patients staged ypN0/LNS+ had similar outcome to those staged ypN+, suggesting that the presence of LNS in ypN0 patients may be considered as a poor histological feature and taken into account for adjuvant chemotherapy administration.

To our knowledge, only one published study has evaluated the prognostic impact of LNS in ypN0 rectal cancer patients after preoperative CRT and radical surgery [17]. This retrospective study on 206 ypN0 patients operated on between 2007 and 2014 found no difference regarding oncological outcomes between ypN0/LNS+ and ypN0/LNS- patients, neither for 3-years OS (95.5% vs. 91.6%, $p=0.549$), nor for 3-years DFS (100% vs. 94.2%, $p=0.117$). The presence of LNS was not an independent prognostic factor associated with OS or DFS in multivariate analysis. Conversely, in the present study, LNS in ypN0 patients was associated with increased risk of recurrence and worse 5-years DFS to such a point that the prognosis of ypN0/LNS+ patients was close to the one observed in those staged ypN+. At multivariate analysis, the presence of LNS was a predictive factor for poor DFS in our series.

Several factors may explain our apparently contradictory results. First, in the Vychnevskaja et al. study [17], ypN0/LNS+ patients had significantly more primary tumor classified as ypT0 and were staged as ypT0N0 (ypCR) as compared to

ypN0/LNS- patients (38% vs. 20%, $p = 0.0009$). Because pathological complete response (ypT0N0) after neoadjuvant CRT has been demonstrated to be associated with excellent prognosis [21-24], it is our hypothesis that this difference could have corrected the poor spontaneous prognosis of patients with ypN0 tumor harboring LNS. Secondly, in the study reported by Vychnevskaja et al. [17], adjuvant chemotherapy was administered significantly more frequently to ypN0/LNS+ patients than to their ypN0/LNS- counterparts (53% vs. 33%, $p=0.012$) representing a confounding factor that could have led to interpretation biases. Third and importantly, there is no consensual definition for LNS, and the reported rates of LNS after CRT in rectal cancer vary from 24% to 51% in the literature [17, 25]. In the present work, the prevalence of LNS for the overall cohort was 9%. This low rate may be explained by the fact that there were not enough histological signs of sterilization to consider and classify a LN as sterilized. **In our opinion, coexistence of fibrosis, necrosis and foamy macrophages is highly specific of lymph node sterilization. Acellular pools of mucin is also a specific sign of sterilization, as mentioned in the College of American Pathologists guidelines [26]. However, fibrosis seems to be the less specific sign of sterilization, because it could be due to radiation. Indeed, it is unlikely to find one lymph node with fibrosis while adjacent lymph nodes are free from fibrosis, whereas they are all in the same irradiation field.** The techniques used for detection of LNS are furthermore not standardized. In our series, definition of LNS was based only on histopathological criteria. Conversely, in the study by Caricato et al. [25], LN regression grade was assessed by immunohistochemistry. We believe that immunohistochemistry may lead to “false positive” LNS, and that the use of another more precise method would have identified micrometastases in these LN classified

as sterilized.

The negative impact of LNS on oncological outcomes reported here may be surprising as downstaging of mesorectal lymph nodes to CRT has been reported to be a good prognostic factor in rectal cancer [25, 27-32]. None of these studies have however specifically assessed the prognostic value of LNS. In our series, ypN0/LNS+ patients underwent an increased primary tumor response as demonstrated by the TRG, the T- and/ or the stage-downstaging in comparison with ypN0/LNS- patients. Our results are in accordance with those reported by Nieman et al. [33] in a series of 90 patients with esophageal adenocarcinoma treated with neoadjuvant therapy. They found that ypN0 or ypN1 patients with histological signs of treatment-response within LN had a significantly poorer survival in comparison with those harboring no sign of treatment-response within LN [33].

In the present study, in lines with the literature [11, 34], mesorectal grading, reflecting the quality of TME surgery, was found to be an independent risk factor of recurrence in multivariate analysis. The presence of lymphovascular emboli and abdominoperineal resection, known to be independent negative prognostic factors in the literature [13-15, 35] were close to the significance for DFS in our series. It is to our knowledge that our study may be underpowered.

Two-thirds of ypN0/LNS+ patients were classified as cN+ before neoadjuvant CRT. This finding is in line with the literature regarding the accuracy of endorectal ultrasound and MRI to predict initial lymph node status. In a meta-analysis by Bipat et al. [36] including 90 studies, the performance of these two imaging methods was poor, with a sensitivity of 66% and a specificity of 77%. Furthermore, we could not find any predictive factor for the presence of LNS in our series. Such a predictor

could be interesting to discuss induction chemotherapy before neoadjuvant CRT, in order to enhance the poor spontaneous prognosis of these patients [7].

The benefit of adjuvant chemotherapy has long been proven in colon cancer after curative resection for stage III disease [37]. For patients with stage II colorectal cancer, based on the QUASAR trial, the absolute survival gain at 5 years following the use of adjuvant chemotherapy is weak, less than 4% [38]. For patients with stage II rectal cancer, adjuvant chemotherapy is still controversial and most practices are based on those for colon cancer [6, 8]. Adjuvant treatment may be administered only in fit ypN0 patients with high risk factors of recurrence including ypT4, tumor perforation, poorly differentiated tumor, neural and vascular invasion, abdominoperineal resection and positive circumferential margin [9]. We believe that the presence of LNS within the mesorectum in stage II disease after CRT may also be considered as an adverse feature justifying adjuvant treatment. Our results should be however confirmed in larger studies.

It is to our knowledge that our series suffers from several limitations including the relatively small number of patients with LNS, and the single-institution design. Furthermore, since this study is retrospective, some variables recently reported in the literature as being associated with an increased risk of recurrence could not be analyzed in uni- and multivariate analysis. This includes the presence of MRI-detected extramural vascular invasion [39, 40], or tumor budding [41]. However, in our series, the pretreatment characteristics of the tumors were comparable between the two groups, there was no difference between the two groups for adjuvant chemotherapy and all included patients had long-course CRT and curative surgery.

To conclude, the presence of LNS in stage II rectal cancer after neoadjuvant CRT

increases the risk of recurrence and negatively impacts DFS. Patients staged ypN0/LNS+ after CRT had similar outcome of those ypN+, suggesting that the presence of LNS in ypN0 patients may be considered for adjuvant chemotherapy administration.

Acknowledgements: None

REFERENCES

- [1] Sauer R, Becker H, Hohenberger W et al. Preoperative versus postoperative chemoradiotherapy for rectal cancer. *N Engl J Med* 2004;351:1731-40.
- [2] Peeters KC, Marijnen CA, Nagtegaal ID et al. The TME trial after a median follow-up of 6 years: increased local control but no survival benefit in irradiated patients with resectable rectal carcinoma. *Ann Surg* 2007;246:693-701.
- [3] Bosset JF, Collette L, Calais G et al. Chemotherapy with preoperative radiotherapy in rectal cancer. *N Engl J Med* 2006;355:1114-23.
- [4] Gerard JP, Conroy T, Bonnetain F et al. Preoperative radiotherapy with or without concurrent fluorouracil and leucovorin in T3-4 rectal cancers: results of FFCD 9203. *J Clin Oncol* 2006;24:4620-5.
- [5] Sebag-Montefiore D, Stephens RJ, Steele R et al. Preoperative radiotherapy versus selective postoperative chemoradiotherapy in patients with rectal cancer (MRC CR07 and NCIC-CTG C016): a multicentre, randomised trial. *Lancet* 2009;373:811-20.
- [6] Rodel C, Graeven U, Fietkau R et al. Oxaliplatin added to fluorouracil-based preoperative chemoradiotherapy and postoperative chemotherapy of locally advanced rectal cancer (the German CAO/ARO/AIO-04 study): final results of the multicentre, open-label, randomised, phase 3 trial. *Lancet Oncol* 2015;16:979-89.
- [7] Chua YJ, Barbachano Y, Cunningham D et al. Neoadjuvant capecitabine and oxaliplatin before chemoradiotherapy and total mesorectal excision in MRI-defined poor-risk rectal cancer: a phase 2 trial. *Lancet Oncol* 2010;11:241-8.
- [8] Hong YS, Nam BH, Kim KP et al. Oxaliplatin, fluorouracil, and leucovorin versus fluorouracil and leucovorin as adjuvant chemotherapy for locally advanced rectal cancer after preoperative chemoradiotherapy (ADORE): an open-label, multicentre, phase 2, randomised controlled trial. *Lancet Oncol* 2014;15:1245-53.
- [9] Lakkis Z, Manceau G, Bridoux V et al. Management of rectal cancer: the 2016 French guidelines. *Colorectal Dis* 2017;19:115-22.
- [10] Valentini V, van Stiphout RG, Lammering G et al. Nomograms for predicting local recurrence, distant metastases, and overall survival for patients with locally advanced rectal cancer on the basis of European randomized clinical trials. *J Clin Oncol* 2011;29:3163-72.
- [11] Nagtegaal ID, van de Velde CJ, van der Worp E et al. Macroscopic evaluation of rectal cancer resection specimen: clinical significance of the pathologist in quality control. *J Clin Oncol* 2002;20:1729-34.
- [12] Tilly C, Lefevre JH, Svrcek M et al. R1 rectal resection: look up and don't look down. *Ann Surg* 2014;260:794-9; discussion 9-800.

- [13] Chand M, Bhangu A, Wotherspoon A et al. EMVI-positive stage II rectal cancer has similar clinical outcomes as stage III disease following pre-operative chemoradiotherapy. *Ann Oncol* 2014;25:858-63.
- [14] Lee JH, Jang HS, Kim JG et al. Lymphovascular invasion is a significant prognosticator in rectal cancer patients who receive preoperative chemoradiotherapy followed by total mesorectal excision. *Ann Surg Oncol* 2012;19:1213-21.
- [15] Cienfuegos JA, Rotellar F, Baixauli J et al. Impact of perineural and lymphovascular invasion on oncological outcomes in rectal cancer treated with neoadjuvant chemoradiotherapy and surgery. *Ann Surg Oncol* 2015;22:916-23.
- [16] Netter J, Douard R, Durdux C et al. Advances in management of adjuvant chemotherapy in rectal cancer: Consequences for clinical practice. *Clin Res Hepatol Gastroenterol* 2016;40:546-52.
- [17] Vychnevskaja K, Dumont F, Agostini J et al. Prognostic Value of Sterilized Lymph Nodes After Preoperative Chemoradiotherapy for Patients with ypN0 Rectal Cancer. *Ann Surg Oncol* 2017;24:1304-11.
- [18] Rullier E, Denost Q, Vendrely V et al. Low rectal cancer: classification and standardization of surgery. *Dis Colon Rectum* 2013;56:560-7.
- [19] Campa-Thompson M, Weir R, Calcetera N et al. Pathologic processing of the total mesorectal excision. *Clin Colon Rectal Surg* 2015;28:43-52.
- [20] Ryan R, Gibbons D, Hyland JM et al. Pathological response following long-course neoadjuvant chemoradiotherapy for locally advanced rectal cancer. *Histopathology* 2005;47:141-6.
- [21] Maas M, Nelemans PJ, Valentini V et al. Long-term outcome in patients with a pathological complete response after chemoradiation for rectal cancer: a pooled analysis of individual patient data. *Lancet Oncol* 2010;11:835-44.
- [22] Yeo SG, Kim DY, Kim TH et al. Pathologic complete response of primary tumor following preoperative chemoradiotherapy for locally advanced rectal cancer: long-term outcomes and prognostic significance of pathologic nodal status (KROG 09-01). *Ann Surg* 2010;252:998-1004.
- [23] Zorcolo L, Rosman AS, Restivo A et al. Complete pathologic response after combined modality treatment for rectal cancer and long-term survival: a meta-analysis. *Ann Surg Oncol* 2012;19:2822-32.
- [24] Martin ST, Heneghan HM, Winter DC. Systematic review and meta-analysis of outcomes following pathological complete response to neoadjuvant chemoradiotherapy for rectal cancer. *Br J Surg* 2012;99:918-28.
- [25] Caricato M, Ausania F, De Dominicis E et al. Tumor regression in mesorectal lymphnodes after neoadjuvant chemoradiation for rectal cancer. *Eur J Surg Oncol* 2007;33:724-8.
- [26] <https://documents.cap.org/protocols/cp-colon-rectum-2017-v4000.pdf>.

- [27] Rodel C, Martus P, Papadopoulos T et al. Prognostic significance of tumor regression after preoperative chemoradiotherapy for rectal cancer. *J Clin Oncol* 2005;23:8688-96.
- [28] Kong JC, Guerra GR, Warrier SK et al. Prognostic value of tumour regression grade in locally advanced rectal cancer: a systematic review and meta-analysis. *Colorectal Dis* 2018;20:574-85.
- [29] Beppu N, Matsubara N, Noda M et al. Pathologic evaluation of the response of mesorectal positive nodes to preoperative chemoradiotherapy in patients with rectal cancer. *Surgery* 2015;157:743-51.
- [30] Li J, Yuan J, Liu H et al. Lymph nodes regression grade is a predictive marker for rectal cancer after neoadjuvant therapy and radical surgery. *Oncotarget* 2016;7:16975-84.
- [31] Choi JP, Kim SJ, Park IJ et al. Is the pathological regression level of metastatic lymph nodes associated with oncologic outcomes following preoperative chemoradiotherapy in rectal cancer? *Oncotarget* 2017;8:10375-84.
- [32] Kim TH, Chang HJ, Kim DY et al. Pathologic nodal classification is the most discriminating prognostic factor for disease-free survival in rectal cancer patients treated with preoperative chemoradiotherapy and curative resection. *Int J Radiat Oncol Biol Phys* 2010;77:1158-65.
- [33] Nieman DR, Peyre CG, Watson TJ et al. Neoadjuvant treatment response in negative nodes is an important prognosticator after esophagectomy. *Ann Thorac Surg* 2015;99:277-83.
- [34] Quirke P, Steele R, Monson J et al. Effect of the plane of surgery achieved on local recurrence in patients with operable rectal cancer: a prospective study using data from the MRC CR07 and NCIC-CTG CO16 randomised clinical trial. *Lancet* 2009;373:821-8.
- [35] How P, Shihab O, Tekkis P et al. A systematic review of cancer related patient outcomes after anterior resection and abdominoperineal excision for rectal cancer in the total mesorectal excision era. *Surg Oncol* 2011;20:e149-55.
- [36] Bipat S, Glas AS, Slors FJ et al. Rectal cancer: local staging and assessment of lymph node involvement with endoluminal US, CT, and MR imaging--a meta-analysis. *Radiology* 2004;232:773-83.
- [37] Andre T, Boni C, Mounedji-Boudiaf L et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. *N Engl J Med* 2004;350:2343-51.
- [38] Quasar Collaborative G, Gray R, Barnwell J et al. Adjuvant chemotherapy versus observation in patients with colorectal cancer: a randomised study. *Lancet* 2007;370:2020-9.
- [39] Chand M, Rasheed S, Heald R et al. Adjuvant chemotherapy may improve disease-free survival in patients with rectal cancer positive for MRI-detected

extramural venous invasion following chemoradiation. *Colorectal Dis* 2017;19:537-43.

[40] Schaap DP, Ogura A, Nederend J et al. Prognostic implications of MRI-detected lateral nodal disease and extramural vascular invasion in rectal cancer. *Br J Surg* 2018;105:1844-52.

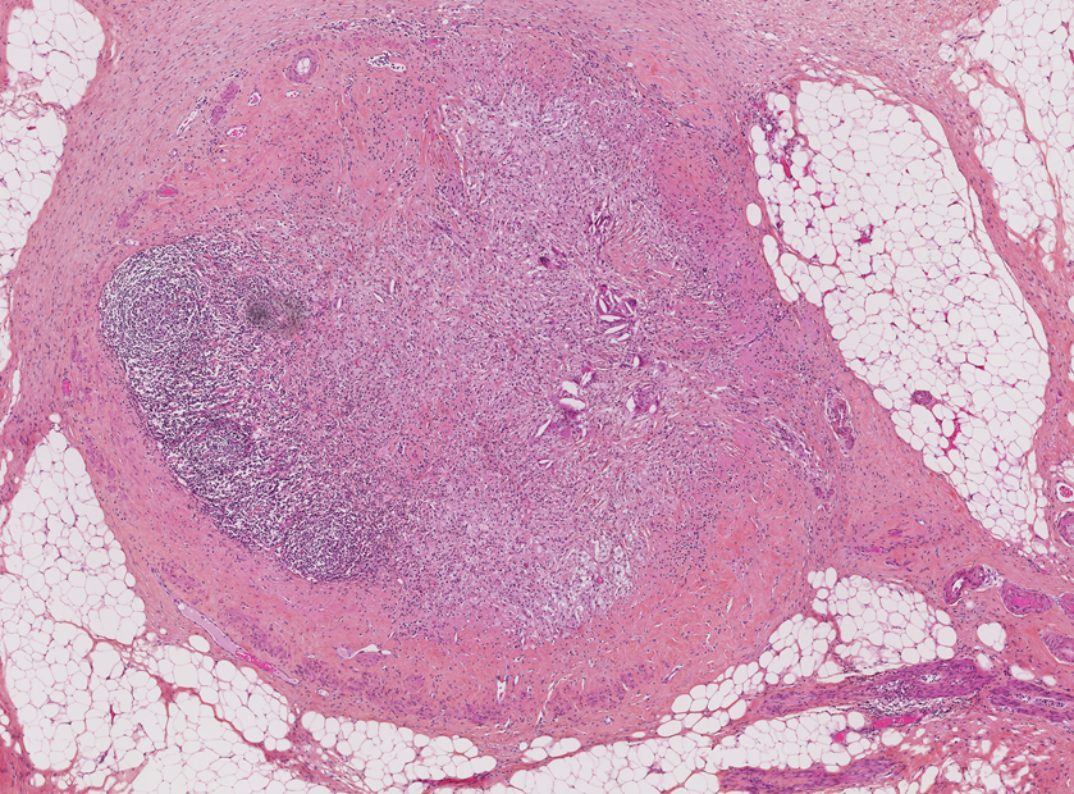
[41] Jager T, Neureiter D, Fallaha M et al. The potential predictive value of tumor budding for neoadjuvant chemoradiotherapy response in locally advanced rectal cancer. *Strahlenther Onkol* 2018;194:991-1006.

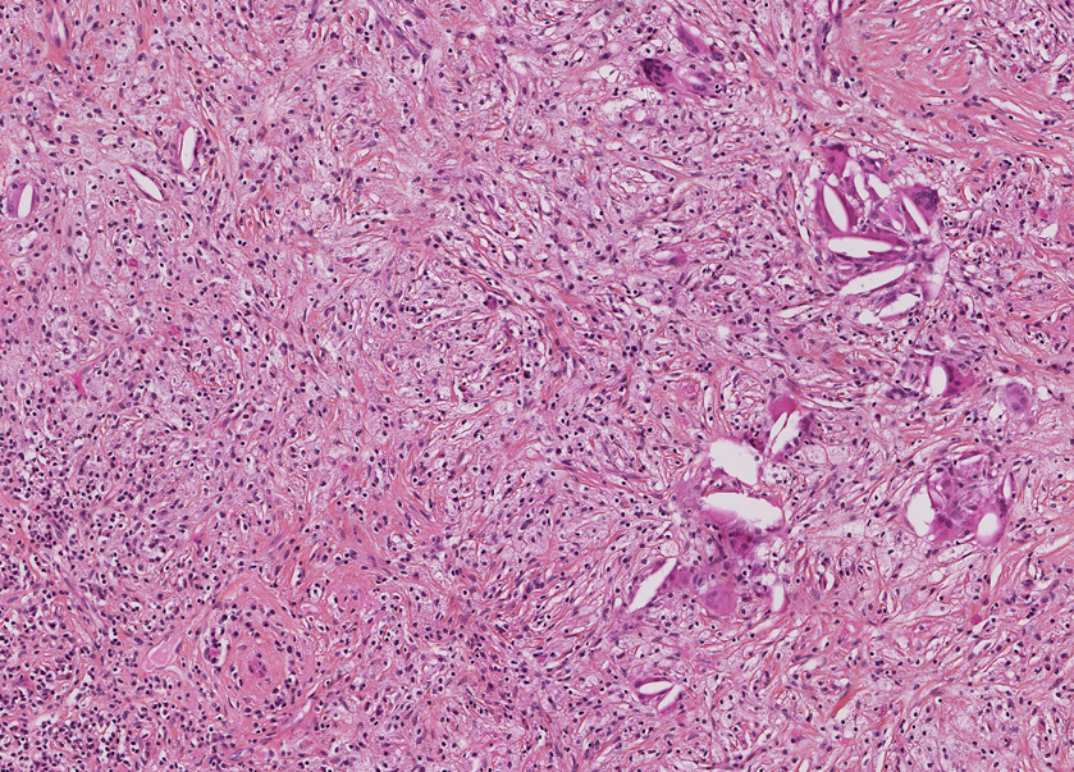
FIGURES LEGENDS

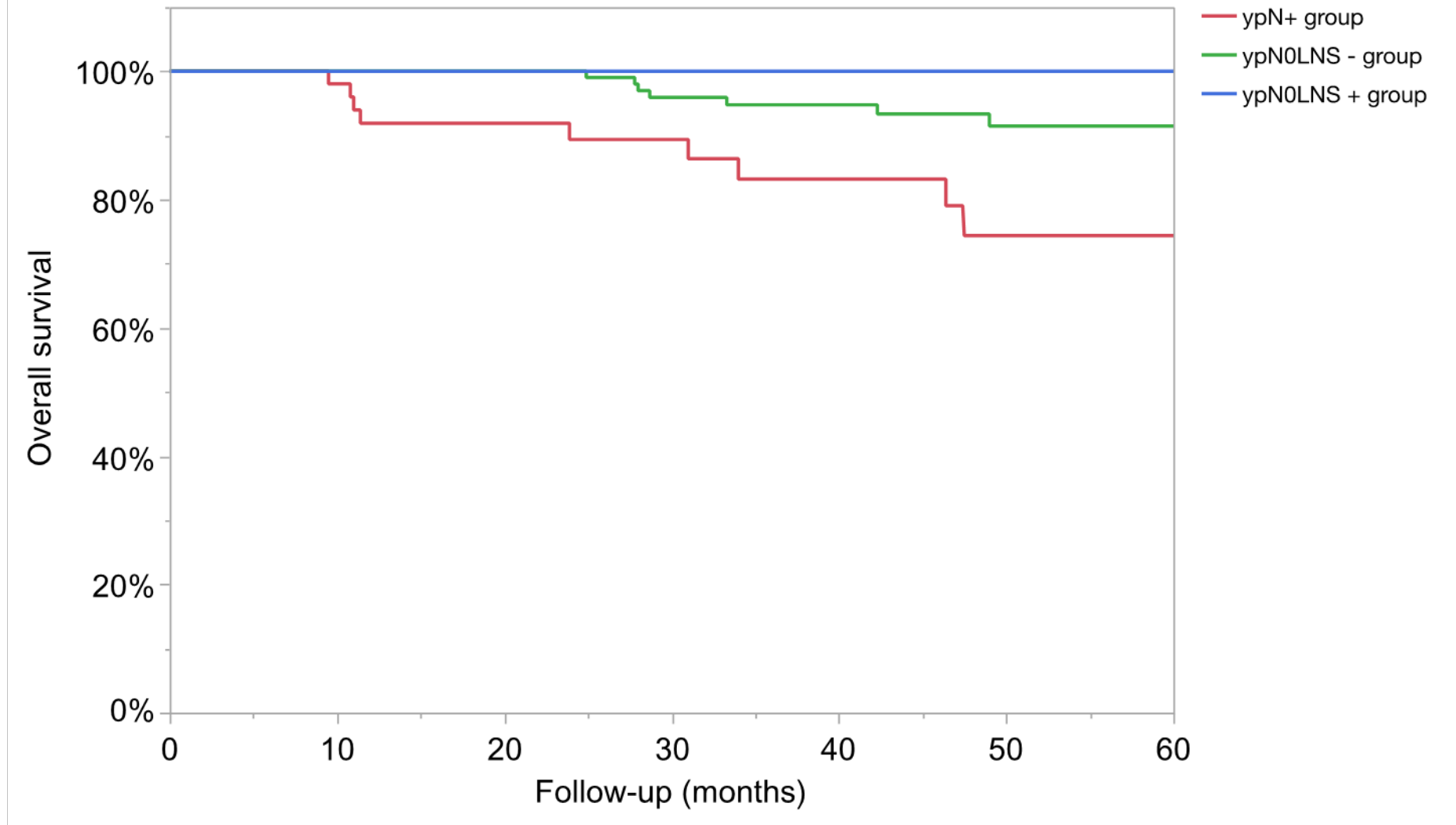
Figure 1: A) Sterilized lymph node with no sign of tumor infiltration, showing signs of sterilization with fibrosis, sheets of macrophages and giant cells; Low magnification (HES x2); B) Higher magnification (HES x10) of the same sterilized lymph node. Macrophages and giant cells are clearly identified.

Figure 2: Overall survival curves in patients with ypN+ disease, and in patients with ypN0 disease according to the presence of sterilized lymph nodes

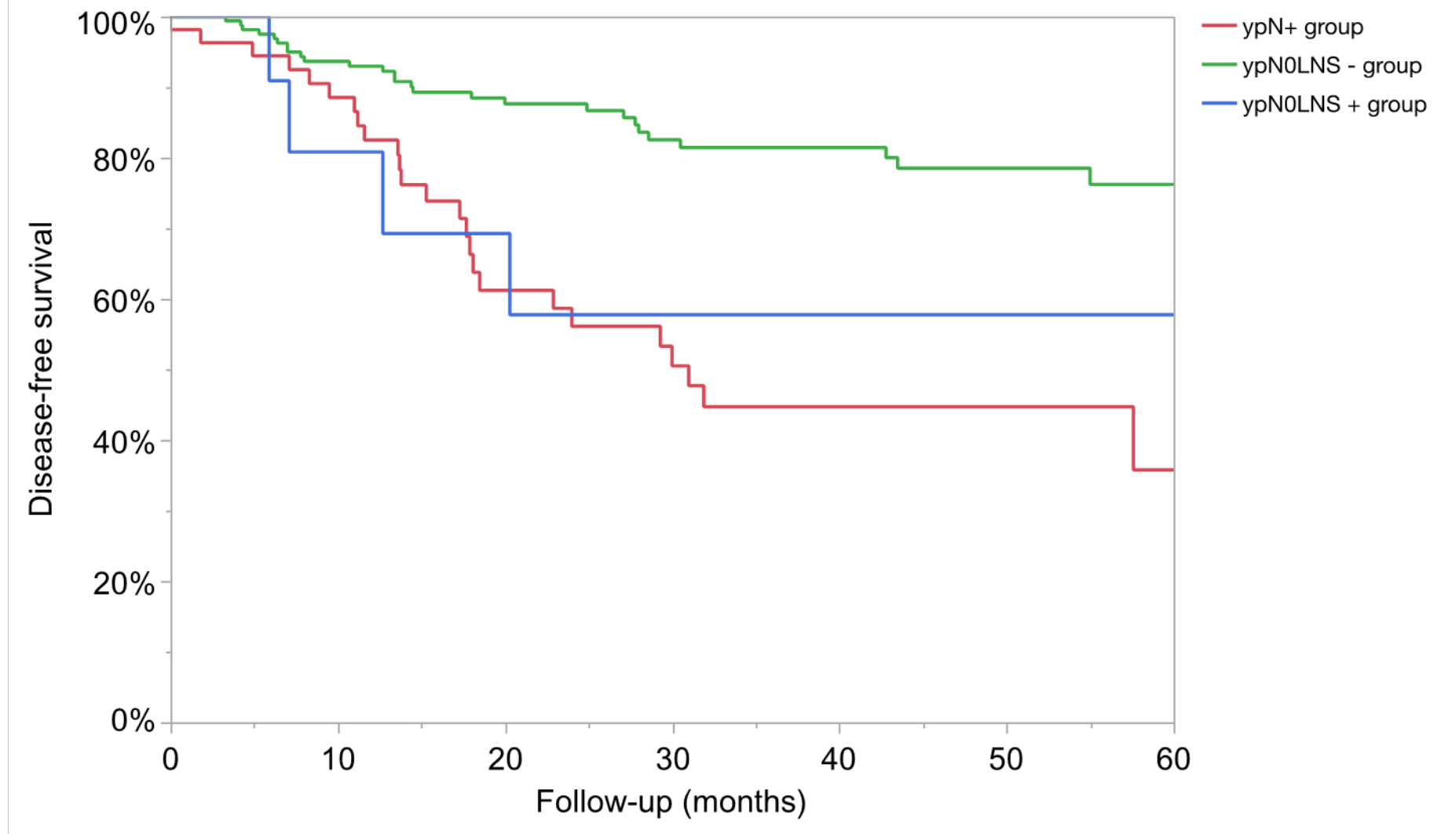
Figure 3: Disease-free survival curves in patients with ypN+ disease, and in patients with ypN0 disease according to the presence of sterilized lymph nodes







ypN+ group	55	50	37	32	23	15	9
ypN0LNS - group	168	149	119	92	69	48	31
ypN0LNS + group	12	10	9	6	6	3	2



ypN+ group	55	45	25	19	12	10	5
ypN0LNS - group	168	139	106	78	61	44	30
ypN0LNS + group	12	9	7	4	4	3	2

Table 1: Clinical characteristics of ypN0 patients according to the presence or not of sterilized lymph nodes

Characteristics	ypN0/LNS+ n=12	ypN0/LNS- n=168	P-value
Sex:			0.060
Male / Female	4 (33) / 8 (67)	108 (64) / 60 (36)	
Age, years:			0.74
≥ 70 / < 70	4 (33) / 8 (67)	44 (26) / 124 (74)	
Body mass index, kg/m ² :			0.76
≥ 25 / < 25	5 (45) / 6 (55)	64 (41) / 92 (59)	
ASA score:			0.046
1-2 / ≥ 3	4 (36) / 7 (64)	109 (68) / 51 (32)	
Tumour location:			0.53
Mid / Low	8 (67) / 4 (33)	96 (57) / 71 (43)	
Pretreatment CEA level, ng/ml:			1
> 5 / ≤ 5	1 (20) / 4 (80)	24 (32) / 51 (68)	
Clinical T stage at presentation:			0.62
cT1-2 / cT3-4	2 (18) / 9 (82)	16 (11) / 130 (89)	
Clinical N stage at presentation:			0.35
cN0 / cN+	4 (33) / 8 (67)	71 (45) / 87 (55)	
Chemotherapy regimens during CRT:			
FOLFOX / XELOX			
5-fluorouracil / Capecitabine			
Delay between neoadjuvant CRT and surgery:			0.76
≥ 7 weeks / < 7 weeks	7 (64) / 4 (36)	93 (57) / 69 (43)	
Surgical procedure:			1
Abdominoperineal resection	2 (17)	32 (19)	
Sphincter-preserving surgery	10 (83)	136 (81)	

ASA: American Society of Anaesthesiologists; CEA: carcinoembryonic antigen; CRT: Chemoradiotherapy

Table 2: Pathological characteristics of ypN0 patients according to the presence or not of sterilized lymph nodes

Characteristics	ypN0/LNS+ n=12	ypN0/LNS- n=168	P-value
Tumour size, mm:			0.36
≥ 20 / < 20	6 (50) / 6 (50)	103 (64) / 58 (36)	
Mesorectal grading:			0.40
Near complete or incomplete	3 (25)	22 (15)	
Complete	9 (75)	126 (85)	
Tumour differentiation:			0.078
Well / moderate	4 (50)	116 (79)	
Poor	4 (50)	31 (21)	
ypT stage:			0.75
ypT0-2 / ypT3-4	9 (75) / 3 (25)	110 (65) / 58 (35)	
T-downstaging:			0.17
Yes / No	10 (91) / 1 (9)	86 (67) / 43 (33)	
Number of lymph nodes harvested:			0.81
≥ 12 / < 12	5 (42) / 7 (58)	76 (45) / 92 (55)	
Stage downstaging:			0.36
Yes / No	10 (100) / 0	118 (86) / 19 (14)	
R1 resection:			0.24
Yes / No	2 (17) / 10 (83)	12 (7) / 156 (93)	
Tumor regression grade:			0.013
0-1 / 2-3	10 (83) / 2 (17)	76 (46) / 89 (54)	
Lymphovascular emboli:			0.65
Yes / No	2 (17) / 10 (83)	20 (12) / 146 (88)	

Table 3: Predictive factors of disease-free survival at univariate and multivariate analyses in patients with ypN0 disease

Characteristics	Univariate analysis		Multivariate analysis			
	5-year disease-free survival	Log-rank P-value	n	Odds Ratio	95%CI	P-value
Sex:		0.56				
Male	74%					
Female	83%					
Age, years:		0.28				
≥ 70	69%					
< 70	79%					
Tumour location:		0.077				0.79
Mid rectal tumour	80%			1.19	0.35-5.52	
Low rectal tumour	72%			1		
Pretreatment CEA level, ng/ml:		0.46				
> 5	82%					
≤ 5	90%					
Clinical T stage at presentation:		0.41				
T1-2	86%					
T3-4	76%					
Clinical N stage at presentation:		0.95				
N0	74%					
N+	82%					
Chemotherapy regimens during CRT:		0.77				
FOLFOX / XELOX	80%					
5-fluorouracil / Capecitabine	71%					
Delay between neoadjuvant CRT and surgery:		0.51				
≥ 7 weeks	77%					
< 7 weeks	78%					
Surgical procedure:		0.0031	119			0.063
Abdominoperineal resection	64%			3.68	0.94-18.44	
Sphincter-preserving surgery	80%			1		
Tumor size, mm:		0.14				0.33
≥ 20	70%			1.74	0.59-6.33	
< 20	89%			1		
Mesorectal grading:		0.083				0.033
Near complete or incomplete	60%			3.14	1.10-8.34	
Complete	79%			1		
ypT stage:		0.061				0.49
ypT0-2	81%			1.74	0.36-8.02	
ypT3-4	68%			1		
T-downstaging:		0.12				0.19
Yes	82%			0.37	0.08-1.65	
No	67%			1		
Presence of LNS:		0.043				0.042
Yes	58%			3.93	1.06-11.81	
No	78%			1		
Number of lymph nodes harvested:		0.14				0.14
≥ 12	80%			0.48	0.16-1.27	
< 12	72%			1		
Stage downstaging:		0.85				
Yes	80%					
No	69%					
R1 resection:		0.91				
Yes	76%					
No	83%					

Tumor regression grade		0.58			
0-1	80%				
2-3	75%				
Lymphovascular emboli:		0.0054			0.071
Yes	64%		2.96	0.90-8.48	
No	79%		1		
Adjuvant chemotherapy:		0.62			
No	77%				
Yes	79%				

CEA: carcinoembryonic antigen; CRT: chemoradiotherapy; LNS: sterilized lymph node; TRG: Tumor Regression Grade