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Number of lymph nodes assessed has no prognostic impact in node-negative rectal cancers after Neoadjuvant Therapy.
Results of the "Italian Society of Surgical Oncology (S.I.C.O.) Colorectal Cancer Network"(SICO-CCN) multicentre collaborative study.

Maurizio Degiuli[1], Simone Arolfo[2], Andrea Evangelista[3], Laura Lorenzon[4], Rossella Reddavid[1], Carlo Staudacher[5], Paola De Nardi[6], Riccardo Rosati[6], Ugo Elmore[6], Claudio Coco[7], Gianluca Rizzo[7], Claudio Belluco[8], Marco Forlin[8], Marco Milone[9], Giovanni Domenico De Palma[9], Daniela Rega[10], Paolo Delrio[10], Mario Guerrieri[11], Monica Ortenzi[11], Andrea Muratore[12], Patrizia Marsanich[12], Angelo Restivo[13], Simona Deidda[13], Matteo Zuin[14], Salvatore Pucciarelli[14],Raffaele De Luca[15], Roberto Persiani[4], Alberto Biondi[4], Franco Roviello[16], Daniele Marrelli[16], Giovanni Sgroi[17], Luca Turati[17], Mario Morino[2]

[1]University of Torino, School of Medicine Department of Oncology Digestive Surgery and Surgical Oncology, San Luigi University Hospital, Orbassano, Torino
[2]Digestive and Oncological Surgery, Center for Minimal Invasive Surgery, Department of Surgical Sciences, Molinette Hospital and University of Torino School of Medicine, Italy
[3]AOU Città della Salute e della Scienza University Hospital, Unit of Clinical Epidemiology and CPO, Torino, Italy
[4]Division of General Surgery, Fondazione Policlinico Universitario A Gemelli, Universita' Cattolica del Sacro Cuore, Rome, Italy
[5]Department of Surgery, San Raffaele Hospital, Milan, Italy.
[6]Division of Gastrointestinal Surgery, San Raffaele Hospital, Milan, Italy.
[7]Polo Apparato Digerente e Sistema Endocrino-Metabolico - Area Chirurgica Addominale, Fondazione Policlinico Universitario "Agostino Gemelli" - Università Cattolica del Sacro Cuore, Rome, Italy.
[8]Department of Surgical Oncology, CRO-IRCCS, National Cancer Institute, Aviano, Italy
[9]Advanced Biomedical Sciences Department, "Federico II" University, AOU "Federico II", Naples Italy
[10]Colorectal Surgical Oncology, National Cancer Institute - IRCCS - G. Pascale Foundation, Napoli, Italy
[11]Clinica Chirurgica, Azienda Ospedaliero-Universitaria Torrette di Ancona, Italy
[12]Division of General Surgery" E. Agnelli Hospital, Pinerolo (Torino), Italy
[13]Colorectal Surgery, A.O.U. Cagliari, Department of Surgical Science, University of Cagliari, Italy
[14]Department of Surgical, Oncological and Gastroenterological Sciences, University of Padova, Italy
[15]National Cancer Institute, Research Centre, "Giovanni Paolo II, Surgery Unit, Bari, Italy
[16]Department of General Surgery, Azienda Ospedaliero-Universitaria Senese, Nuovo Policlinico Le Scotte, Siena, Italy
[17]Surgical Oncology, Ospedale Treviglio - ASST Bergamo Ovest Piazza Meneguzzo, 1 - 24047 Treviglio (BG), Italy

Corresponding Author. Prof Maurizio Degiuli, Head, Digestive Surgery and Surgical Oncology, San Luigi University Hospital (SLUH), Regione Gonzole 10, Orbassano Torino Italy; office: +39 011 9026525; mobile +393358111286; e-mail: maurizio.degiuli@unito.it

Running head. Impact of number of nodes assessed in irradiated rectal cancer

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Abstract

Introduction. We retrospectively investigated the impact of number or complete absence of nodes retrieved on survival of patients with rectal cancer (RC) treated with neoadjuvant radiation-therapy (NAT).

Methods. All patients with RC treated with NAT followed by curative surgery from 2000 to 2014 in 14 Italian referral Centres for Colorectal Surgery were enrolled. Information about number of nodes harvested, node ratio, type of radiation therapy schedule and tumour stage were recorded. Impact of number or complete absence of nodes retrieved on overall survival (OS) and on cumulative incidence of death for disease (CIDD) was assessed and factors influencing node yield were investigated.

Results. In total, 1407 patients were included. Mean number of nodes retrieved was 12.9, while no lymph nodes were found in only 32 patients (2%, ypNnull). Definite nodal stage was ypN0 in 1001 patients (71%) and ypN+ in 372 patients (27%). In multivariable analysis ypNnull patients showed worse OS and CIDD compared to both ypN0 and ypN+. In ypN0 patients, number of nodes assessed, stratified in 4 groups (<5, 5-10, 11-15 and >15), did not significantly influence OS and CIDD. Long-course radiation schedule and early T stages negatively affected node assessment.

Conclusion. Complete absence of nodes assessed was associated with worse prognosis compared to node-negative and node-positive patients. In node-negative patients number of nodes was not associated to OS and CIDD. Based on data from this large population of irradiated RC, number of nodes assessed has no prognostic impact in node-negative patients

Introduction

Total Mesorectal Excision (TME) in combination with preoperative chemo-radiation therapy (CRT) is the standard of care of locally advanced extra-peritoneal rectal cancer[1]. Metastatic lymph nodes (ypN+) represent an independent negative prognostic factor for survival. Node-negative (ypN0) patients show a better prognosis, but a minimum number of 12 nodes retrieved is actually required by current guidelines to certify the adequacy of nodal staging [2]. In absence of at least 12 nodes evaluated (ypNx), patients should be treated with adjuvant chemotherapy. Several factors related to the patient (sex, obesity), to the surgeon (specimen size and extent of lymphadenectomy) and to the tumour (size, stage, and site), may influence the number of nodes retrieved [3-7]. Moreover, neoadjuvant CRT (NAT) significantly reduces the yield of LNs in the specimen [8-9]. The impact of nodal assessment on ypN0 patients' survival is not clear. Habr Gama et al showed even a significant 5-year disease free survival (DFS) benefit in patients who underwent NAT when no lymph nodes were found in the surgical specimen (ypNnull)[10]. Anyway, impact of LNs yield on oncologic outcome in node-negative cancers has been studied in several single-institution experiences with conflicting results and also other authors reported that the absence of LNs is not a negative predictor of survival[11] or that the current 12-LN threshold is not relevant in neoadjuvant settings[12]. Other reports didn't find a cut-off value of nodal yield influencing survival in node-negative irradiated rectal cancers[13-14]. The aim of this large sample size retrospective multicenter Italian series was to investigate whether the absence of nodes harvested in the specimen really improves patients' survival as documented by several authors in smaller studies; to establish if a relevant threshold lower than 12 nodes can be identified in ypN0 patients and to investigate which factors may influence nodal yield after NAT.

Material and Methods

*Study population and design***.**

This was a retrospective cohort study of patients with locally advanced rectal cancers consecutively treated with NAT followed by curative surgery, between January 2000 and December 2014 in 14 Italian high-volume Referral Centres for Colorectal Surgery. The study was approved by the institutional review board of all participating centres. All items required by STROBE checklist for reports of observational studies have been included.

*Treatment***.**

All patients were treated with preoperative CRT or RT alone followed by Rectal Anterior Resection (RAR) or abdomino-perineal resection (APR) or Hartmann procedure (HP) with proper TME. Inclusion criteria were age between 18 and 80 years, histologically proven rectal adenocarcinoma with indication to NAT, no previous history of cancer and no other coexisting tumour. Tumor distance from the anal verge was 3 to 12 cm. Total mesorectal excision was performed removing the rectum along with the mesorectum up to the level of the levators. Patients with distant metastases and/or peritoneal carcinomatosis were excluded from the enrollment. Only patients with a minimum 2 years follow up (FU) were included in the analysis. Neoadjuvant treatment was chosen by a multidisciplinary team based on clinical staging of the disease given by histology, endorectal US, abdominal CT scan and pelvic MRI. According to these information, radiation therapy was delivered to Stage II or III tumours with one of the following schedules: a) long course conventionally fractionated RT (1.8-2.0 Gy × 25-28 days) with fluorouracil (FU)-based single-agent radiosensitization; and b)short course RT (5 Gy X 5 days). Eight to twelve weeks after NAT patients were submitted to either RAR or APR or HP with TME and ligation of the inferior mesenteric vessels at their origin. A complete 12 cycles course of adjuvant FU-based chemotherapy was delivered after surgery in node-positive patients. A standard data-form was created and provided to each centre for collection of information on patients' characteristics and outcomes.

Study Variables.

Data fields included demographics, number of nodes harvested, LN ratio, tumour distance from the anal verge, serum CEA level (ng/ml), type of radiation therapy schedule (long versus short course), interval between the end of NAT and surgery, vascular invasion, length of the specimen (cm), tumour stage (UICC) and administration of adjuvant therapy. (Table 1)

*Outcomes***.**

The primary outcome was impact of number of nodes assessed on OS. Secondary outcomes were OS measured from the date of surgery to the date of death due to any cause or the date of last contact, and cumulative incidence of death for disease (CIDD) (death for disease or for other/unknown causes) and factors influencing LN yield.

Statistical Analysis.

OS was estimated by the Kaplan–Meier product-limit method whereas the CCID was estimated using the competing risks approach proposed by Gooley et al[15]. Impact of number of nodes assessed on OS was investigated using a Cox proportional hazard model adjusting for age, gender, percentage of positive lymph nodes, distance from the anal verge, serum CEA level, type of CRT schedule, interval of time between the end of CRT and surgery, vascular involvmemnt, specimen lenght, TNM stage and administration of adjuvant CT. To evaluate a potential non-linear effect on OS, the number of lymph nodes retrieved was included in the model using a restricted cubic spline transformation. The same approach was used to evaluate the effect on CCID using the Fine & Gray model for competing risk outcomes[16]. Factors associated with the number of lymph nodes assessed were investigated using a linear regression model. In all models fitted in this cohort study, missing data were multiple imputed using the method of chained equations [17] and combined estimates were obtained from five imputed datasets. The statistical analysis was performed using STATA version 11.1 (ice command for multiple imputation).

Results

Between January 2000 and December 2014, 1737 patients from 14 National referral centres underwent TME (RAR, 1494 pts, or APR, 226 pts, or HP, 17 pts) after NAT. For 31 patients the information about number of lymph nodes retrieved was not available while 299 pts (17%) were lost at follow up. Finally 1407 patients with complete available data were included in the analysis, with a median follow up time of 4 years. Ninety-two percent of patients underwent a long course CRT schedule, while a short course RT schedule was administered in the remnant 8% of patients. The median interval between the end of RT/CRT

and surgery was 8 weeks (IQR 7-8.5). 15% of patients had a pathologic complete response (pCR) and did not undergo any postoperative adjuvant treatment, which was administered in 47% of patients. (Table 1)

Mean number of nodes retrieved was 12.9 (range 0-69), while no lymph nodes in the specimen were found in only 32 patients (2% of cases, ypNnull); definitive patients' nodal stage was ypN0 in 1001 patients (71%) and ypN+ in 372 pts (27%). All ypNnull had been given long course conventionally fractionated RT with FU-based single-agent chemotherapy. The 5-year OS and CIDD rates were respectively 82.8% and 10.2%, while 5-year cumulative incidence of death for other/unknown cause rate was 6.9% (Figure 1). In a multivariable proportional hazard Cox model, adjusted for all investigated variables, only a LN ratio higher than 30% and the length of specimen were reported as independent factors associated with OS and CIDD with results reported as hazard ratios (HRs) and 95 per cent confidence intervals (Table 2).

Kaplan-Meyer plots examined with log-rank test to determine statistical differences across groups showed that ypN+ patients had a worse 5-year OS (Adjusted HR=1.76 (1.28 to 2.74), p=0.001) and CIDD (Adjusted HR=1.92 (1.27 to 2.89), p=0.002) as compared with both ypN0 and ypNnull patients (Figure 2); but, unlike Habr Gama et al, the complete absence of lymph nodes in the specimen (ypNnull) negatively affected both OS and CIDD as compared to ypN0 patients. Furthermore, in a multivariable Cox model adjusted for all investigated variables, ypNnull patients had a higher HR both for OS and CIDD as compared not only to ypN0 but also to ypN+ patients (Table 3). Among ypN0 patients, the lowest OS HR related to the number of nodes retrieved derived from a Cox model with non linear transformation, was associated to a cut off number of 10 (Figure 3), and the lowest CIDD HR was associated to a cut off number of 9 nodes retrieved. In other words, the risk of mortality related to LN yield seems to decrease until a number of 10/9 nodes harvested, with a HR that remains constant over these cut-off points. Nevertheless, Kaplan-Meyer plots examined with log-rank test showed that OS rates of ypN0 patients with more or less than 10 lymph nodes retrieved were comparable as well as those with more or less than 9. A multivariable Cox model showed that, overall, the number of lymph nodes assessed, stratified in 4 groups (<5, 5-10, 11-15, >15) didn't significantly influence patients' OS and CIDD (Table 2).

In the last multivariable analysis we have investigated which factors significantly affect lymph nodes yield after NAT. Duration of radiotherapy and pT stage were the only variables related to the number of lymph nodes assessed. According to estimates of the linear regression model, patients with a Short Course CRT and advanced pT stage were associated with a significantly higher mean number of nodes retrieved.

Discussion

Neoadjuvant treatment has become a standard in locally advanced RC over the last 20 years. The impact of NAT on number of lymph nodes assessed is well known. In a recent meta-analysis of 34 studies, CRT resulted in a mean reduction of 3.9 nodes and RT in a mean reduction of 2.1 nodes yielded as compared with patients who did not receive any radiation treatment[18]. However the prognostic value of this reduction has not yet been clarified.

Interestingly, in a retrospective study conducted in two collaborating centers from Brazil, Habr Gama et al [10] reported that complete absence of LNs in resected specimen of 32 (11%) out of 281 irradiated patients was associated with favorable pathologic features (ypT and perineural invasion status) and with 5-year DFS similar to patients with ypN0 and significantly better than ypN+ patients. These observations were interpreted as the result of increased sensitivity to CRT and tumor down-staging .

A non-inferior oncologic value of absence of nodes in neoadjuvant setting was observed by Kim et al In this retrospective study of 258 patients, with the limits of population size, absence of nodes (only 2.7% of patients) was associated with cancer specific and recurrence free survival rates comparable to those of node-negative patients, regardless of LN yield[19].

The same conclusions were drawn by Lee at al[12] in a single Institution analysis of 132 patients. Absence of nodes assessed was observed in 7.6% of patients and was associated with OS similar to that of ypN0 group (p=0.032) and significantly better than that of ypN+ patients (p=0.002).

In contrast, Raoof et al[13] in a recent retrospective high volume study on 3995 patients obtained from the Surveillance, Epidemiology and End Results (SEER) website showed that ypNnull patients had worse OS and DSS than patients with 'one to eight' or 'at least nine' LNs assessed.

In this retrospective large sample size study, the pathologists found no lymph nodes (ypNnull) in only 2% of resected specimens, making any comparison with the remaining 98% of the sample rather statistically irrelevant. This very low rate of specimens without nodes assessed is in line with the current literature [18] and can probably be explained by the skills of CRC surgeons and by the high level of expertise of the pathologists working in the referral centers participating into the study. Nevertheless, with the limit described, the conclusions of our data analysis are in contrast with those of Habr-gama, Kim and Lee: in the present series patients with no LNs after NAT showed a worse OS and a higher CIDD as compared both to node-negative and node-positiv patients.

The second question addressed in this study was the impact of chemoradiation-therapy on the number of nodes assessed and the prognostic value of LNs yield in irradiated patients. Considering the decrease of LNs yield observed by several authors and the dissimilarity of the reports concerning its prognostic impact in ypN0 patients, it is obvious to question if the 12-LNs benchmark reported in current guidelines should be

mandatory also after neoadjuvant treatment adopting radiation therapy regimens, and, more generally, if number of LNs assessed should continue to play a prognostic role in irradiated patients.

In a retrospective cohort study of 4790 patients published by Gill et al[20], no association between reduced LNs yield and DSS was seen when the analysis was restricted to ypN0 patients.

Hall et al, in a retrospective analysis on 4565 patients, suggested that eight LNs was the threshold for adequate lymph node dissection after NAT[21].

Kim WR et al[19], in a series of 302 patients, showed that, overall, the long-term outcomes of patients with more or less than 12 LNs harvested were not significantly different and that, in a subgroup analysis of ypN0, the group with <5 nodes had the most favorable oncologic outcomes.

Govindarajan et al[9], in a single-institute series of 708 patients, showed that number of nodes assessed was significantly lower in NAT group. They reported that the 12-LNs threshold was not relevant and often not achievable in patients treated with NAT. Furthermore, a lower LN count after NAT was not associated with under-staging or inferior survival.

Han et al [22] recently reported that a retrieval of LNs ≥ 8 and LNs ≥ 12 should be achieved to obtain accurate staging and optimal treatment for patients with and without NAT respectively.

Also Kim YW et al[11], in a study of 258 patients, described similar 5-years DSS rates among ypN0 patients with 1-11, 12-25 and 26-65 nodes examined and that, in ypN0 patients, a reduced number of nodes retrieved, as compared to >12, did not represent an inferior oncologic outcome. They concluded that in ypN0 patients, number of nodes assessed does not impact survival and recurrence.

In contrast Raoof et al[13] observed that the group with ≥ 9 nodes examined had significantly better OS (p <0.001; 5-year OS 83.2 versus 78.0%) and DSS (p =0.004; 5 year DSS 87.9% versus 85.1%) than the group with 1-8.

Tsai et al[23], in a single-centre study of 372 ypN0 patients from MD Anderson Cancer Center, found that median number of LN examined was 7 and that, compared with patients who had ≤ 7 LNs, patients who had >7 LNs had significantly higher 5-year rates of freedom from relapse and DFS, without any significant difference of OS. They concluded that number of LNs assessed is associated independently with recurrence and DSS in ypN0 patients after NAT and that therefore number of negative nodes represents a prognostic factor in irradiated patients.

In favor of minimum number of 12 nodes assessed, Lykke et al[24], in a study of 6793 patients with or without NAT derived from the national database of the Danish Colorectal Cancer Group, found that a LNs yield ≥ 12 was associated with better OS as compared to a LNs yield <12, irrespective of NAT. They even concluded

that it is uncertain whether number of 12 LNs is the most sensitive cut-off value or whether a higher number should be aimed for.

In our study the lowest Hazard Ratio for OS related to number of nodes harvested was associated to a cut-off number of 10, whereas the lowest HR for CIDD was related to a cut off number of 9. Nevertheless, Kaplan Meyer plots examined with log-rank test showed that OS and CIDD of ypN0 patients with more or less than 10 and 9 nodes assessed were comparable. Hence a cut-off value of nodes assessed able to significantly affect survival was not observed.

Moreover, a further multivariate Cox model documented that number of nodes harvested did not significantly affect patients' survival and cumulative risk of death from disease. Since CRT was the prevalent schedule used, our findings are similar to the results of the PROCARE study[25]: after NAT LN count has no prognostic value.

The third question addressed in the study was concerning factors that could potentially reduce number of nodes harvested in specimens of patients with RC treated with NAT. These factors have already been investigated in a literature review[26]. Tumor regression grade with radiation-induced lymphocyte destruction and stromal fibrosis were the most important factors for the decrease of nodes retrieval.

Sermier et al[27] reported that the longer is the delay between radiotherapy and surgery, the lower is node yield in the mesorectum. These data were confirmed in the Stockolm III trial[28].

In our study, the only factors significantly reducing nodes yield were long-course chemoradiation schedule and early T-stages (pT1 and pT2; respectively $p<0.001$ and $p=0.013$). As the delay of surgery and radiation therapy dose were the same in short and long-course schedules, the association of chemotherapy rather than time to surgery represented the strongest factor reducing the number of nodes assessed (Table 4).

This study has few limitations: first, retrospective nature of the design may generate a series of uncontrolled bias. Furthermore, data coming from 14 different centers over a long period of time cannot warrant a complete homogeneity of treatment administered. Despite these limitations, the study seems to respond to our introductive questions. First, complete absence of LNs assessed, despite its low rate, does not favorably affect patients' survival as previously reported in single centers and/or low volume retrospective series and rather is associated with worse prognosis as compared with both node negative and positive irradiated patients.

Second, data analysis on ypN0 patients showed that number of nodes is not associated to overall and cancer specific survival. A relevant threshold of nodes assessed was not observed in this series. In neoadjuvant setting number of LNs examined in node-negative patients is not a significant independent prognostic factor.

Last, the association of chemotherapy to long-course RT and early pT stages (pT1 and pT2) are independent factors associated to a lower number of LNs retrieved in the specimen.

Based on our data, differently from other recent reports, it is not possible to define a threshold of nodes assessed below which node negative patients have a worse prognosis; number of nodes assessed has no prognostic impact on irradiated RC patients. Further prospective studies are needed to confirm these findings, but probably the 12-LN threshold should be questioned in these patients.

References

1. van Gijn W, Marijnen CA, Nagtegaal ID, Kranenbarg EM, Putter H, Wiggers T *et al*; Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer: 12-year follow-up of the multicentre, randomised controlled TME trial. *Lancet Oncol* 2011; **12**:575-582.
2. Sobin LH, Greene FL; TNM classification: clarification of number of regional lymph nodes for pN0. *Cancer* 2009; **92**:452.
3. Baxter NN, Virnig DJ, Rothenberger DA, Morris AM, Jessurun J, Virnig BA; Lymph node evaluation in colorectal cancer patients: a population-based study. *J Natl Cancer Inst* 2005;**97**:219-225.
4. Dworak O; Number and size of lymph nodes and node metastases in rectal carcinomas. *Surg Endosc* 1989;**3**:96-99.
5. Gorok D, Nagy P, Peter A, Perner F; Influence of obesity on lymph node recovery from rectal resection specimens. *Pathol Oncol Res* 2003;**9**:180-183.
6. Thorn CC, Woodcock NP, Scott N, Verbeke C, Scott SB, Ambrose NS; What factors affect lymph node yield in surgery for rectal cancer? *Colorect Dis* 2004;**6**:356-361.
7. Shen SS, Haupt BX, Ro JY, Bailey HR, Schwartz MR; Number of lymph nodes examined and associated clinicopathologic factors in colorectal carcinoma. *Arch Pathol Lab Med* 2009;**133**:781-786.
8. Wichmann MW, Müller C, Meyer G, Strauss T, Hornung HM, Lau-Werner U *et al*; Effect of preoperative radiochemotherapy on lymph node retrieval after resection of rectal cancer. *Arch Surg* 2002;**137**:206-10.
9. Govindarajan A, Gonen M, Weiser MR, Shia J, Temple LK, Guillem JG *et al*; Challenging the feasibility and clinical significance of current guidelines on lymph node examination in rectal cancer in the era of neoadjuvant therapy. *J Clin Oncol* 2011; **29**:4568-4573.
10. Habr-Gama A, Perez RO, Proscurshim I, Rawet V, Pereira DD, Sousa AH *et al*; Absence of lymph nodes in the resected specimen after radical surgery for distal rectal cancer and neoadjuvant chemoradiation therapy: what does it mean? *Dis Colon Rectum* 2008;**51**:277-283.
11. Kim YW, Kim NK, Min BS, Lee KY, Sohn SK, Cho CH *et al*; The prognostic impact of the number of lymph nodes retrieved after neoadjuvant chemoradiotherapy with mesorectal excision for rectal cancer. *J Surg Oncol* 2009;**100**:1-7.
12. Lee WS, Lee SH, Baek JH, Lee WK, Lee JN, Kim NR *et al;* What does absence of lymph node in resected specimen mean after neoadjuvant chemoradiation for rectal cancer. *Radiat Oncol* 2013;**19**:8-202.
13. Raoof M, Nelson RA, Nfonsam VN, Warneke J, Krouse RS; Prognostic significance of lymph node yield in ypN0 rectal cancer. *Br J Surg* 2016; **103**:1731-1737.

14. Rullier A, Laurent C, Capdepont M, Vendrely V, Belleannée G, Bioulac-Sage P *et al*; Lymph nodes after preoperative chemoradiotherapy for rectal carcinoma: number, status, and impact on survival. *Am J Surg Pathol* 2008;**32**:45-50.
15. Gooley TA, Leisenring W, Crowley J*,* Storer BE; Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med* 1999; **18**:695-706.
16. Fine JP, Gray RJ; A Proportional Hazards Model for the Subdistribution of a Competing Risk. *Journal of the American Statistical Association* 1999; **94**(446):496-509**.**
17. Royston P; Multiple imputation of missing values: update of ice. *Stata Journal* 2005; **5**(4):527-536.
18. Mechera R, Schuster T, Rosenberg R*,* Speich B: Lymph node yield after rectal resection in patients treated with neoadjuvant radiation for rectal cancer: a systematic review and meta-analysis. *Eur J Cancer* 2017;**72**:84-94.
19. Kim WR, Han YD, Cho MS, Hur H, Min BS, Lee KY *et al*; Oncologic impact of fewer than 12 lymph nodes in patients who underwent neoadjuvant chemoradiation followed by total mesorectal excision for locally advanced rectal cancer. *Medicine* (Baltimore) 2015; **94**:e1133.
20. Gill A, Brunson A, Lara P Jr, Khatri V, Semrad TJ; Implications of lymph node retrieval in locoregional rectal cancer treated with chemoradiotherapy: a California Cancer Registry Study. *Eur J Surg Oncol* 2015;**41**:647-52.
21. Hall MD, Schultheiss TE, Smith DD, Fakih MG, Kim J, Wong JY *et al*; Impact of total lymph node count on staging and survival after neoadjuvant chemoradiation therapy for rectal cancer. *Ann Surg Oncol* 2015;**22 Suppl 3:**S580-587.
22. Han J, Noh GT, Yeo SA, Cheong C, Cho MS, Hur H *et al*; The number of retrieved lymph nodes needed for accurate staging differs based on the presence of preoperative chemoradiation for rectal cancer. *Medicine* (Baltimore) 2016; **95**: e4891.
23. Tsai CJ, Crane CH, Skibber JM, Rodriguez-Bigas MA, Chang GJ, Feig BW *et al*: Number of lymph nodes examined and prognosis among pathologically lymph node-negative patients after preoperative chemoradiation therapy for rectal adenocarcinoma. *Cancer* 2011;**117**:3713-3722.
24. Lykke J, Jess P, Roikjaer O, Danish Colorectal Cancer Group; Increased lymph node yield is associated with improved survival in rectal cancer irrespective of neoadjuvant treatment: results from a national cohort study. *Dis Colon Rectum* 2015;**58**:823-30.
25. Ceelen W, Willaert W, Varewyck M, Libbrecht S[3] Goetghebeur E, Pattyn P *et al*; Effect of neoadjuvant radiation dose and schedule on nodal count and its prognostic impact in Stage II-III rectal cancer. *Ann Surg Oncol* 2016;**23**:3899-3906.
26. Bustamante-Lopez L, Nahas CS, Nahas SC, Ribeiro U Jr, Marques CF, Cotti G *et al*; Understanding the factors associated with reduction in the number of lymph nodes in rectal cancer patients treated by neoadjuvant treatment. *Int J Colorectal Dis* 2016;**32**:925-927.

27. Sermier A, Gervaz P, Egger JF, Dao M, Allal AS, Bonet M *et al*; Lymph node retrieval in abdominoperineal surgical specimen is radiation time-dependent. *World J Surg Oncol* 2006;**4**:29.
28. Pettersson D, Lörinc E, Holm T, Iversen H, Cedermark B, Glimelius B *et al*; Tumour regression in the randomized Stockholm III Trial of radiotherapy regimens for rectal cancer. *Br J Surg* 2015;**102**:972-978.

Figure Legends

Figure 1. Overall Survival and Cumulative Incidence of Death for Disease and for other/unknown cause.

Figure 2. Overall Survival and cumulative incidence of death from disease in patients with no harvested lymph nodes (ypNnull), negative lymph nodes (ypN0) and positive lymph nodes (ypN+) after neoadjuvant treatment followed by surgery.

Figure 3. Cox model with non-linear transformation.

Table 1. Patients' characteristics

	N Non Missing	(N=1364)
Age, median (IQR)	1354	64 (56;71)
Sex (males)	1364	64% (899)
Distance from the anal verge, median (IQR)	967	6 (4;8)
CEA, median (IQR)	849	2.7 (1.7;5.0)
RT Long Course	1192	92% (1099)
Interval between RT and surgery, median (IQR)	822	8.0 (7.0;8.5)
Vascular invasion	994	7% (76)
Stage	849	
0		15% (130)
I		32% (288)
II		25% (221)
III		28% (248)
IV		0% (3)
Peritumoral lymphocytes	351	31% (110)
Adjuvant CT	871	47% (428)
Number of lymph nodes retrieved	1364	
<=5		20% (287)
6-10		27% (375)
11-15		22% (313)
>15		31% (432)
Lymph node ratio	1364	
0		73% (1033)
<=10%		7% (103)
10%-20%		6% (89)
20%-30%		3% (48)
>30%		10% (134)
Nodal stage		
ypN0		71% (1001)
ypN+		27% (374)
ypNnull		2% (32)

RT: radiation therapy; CT: chemotherapy; ypN0: negative lymph nodes after neoadjuvant treatment; ypN+: positive lymph nodes after neoadjuvant treatment; ypNnull: no retrieved lymph nodes after neoadjuvant treatment

Table 2. Association between patients' characteristics and Overall Survival (Cox proportional Hazard models) and Cumulative Incidence of Death from Disease (Fine and Gray models).

	Overall Survival		Cumulative incidence of death from Disease	
	HR (95%CI)	p	HR (95%CI)	p
Age (for 5 years increase)	1 (0.99 to 1.01)	0.694	1 (0.99 to 1)	0.633
Sex (males)	1.04 (0.77 to 1.43)	0.783	0.92 (0.61 to 1.38)	0.692
Nr of nodes retrieved in node negative rectal cancer				
<=5	1	-	1	-
6-10	0.71 (0.43 to 1.19)	0.195	0.71 (0.36 to 1.43)	0.341
11-15	0.77 (0.45 to 1.35)	0.366	0.96 (0.49 to 1.89)	0.905
>15	0.89 (0.52 to 1.53)	0.675	0.93 (0.45 to 1.89)	0.835
Percentage of positive nodes				
None	1	-	1	-
<=10%	1.08 (0.54 to 2.13)	0.831	1.11 (0.47 to 2.62)	0.806
10%-20%	1.18 (0.59 to 2.37)	0.637	1.19 (0.46 to 3.09)	0.724
20%-30%	0.82 (0.31 to 2.12)	0.677	0.87 (0.23 to 3.28)	0.833
>30%	3.28 (1.94 to 5.57)	<0.001	3.59 (1.83 to 7.03)	<0.001
Distance from the anal verge	0.94 (0.89 to 1)	0.054	0.93 (0.86 to 0.99)	0.035
Serum CEA level	1.01 (1 to 1.02)	0.197	1.01 (0.99 to 1.02)	0.376
Radiation therapy schedule				
Short Course	1	-	1	-
Long Course	1.02 (0.55 to 1.91)	0.942	1.41 (0.54 to 3.69)	0.481
Interval between Radiotherapy and Surgery (weeks)	0.96 (0.91 to 1.02)	0.185	0.97 (0.88 to 1.06)	0.433
Vascular Invasion	0.87 (0.45 to 1.7)	0.677	0.95 (0.41 to 2.18)	0.899
Length of the specimen	1.03 (1.01 to 1.05)	0.006	1.02 (1.00 to 1.05)	0.056
TNM Stage	1.15 (0.9 to 1.48)	0.255	1.12 (0.84 to 1.49)	0.444
Peritumoral lymphocytes	1.29 (0.5 to 3.34)	0.53	1.54 (0.72 to 3.3)	0.233
Adjuvant Chemotherapy	0.98 (0.67 to 1.43)	0.909	1.16 (0.76 to 1.77)	0.479

Table 3. Multivariable Cox model adjusted for all investigated variables*

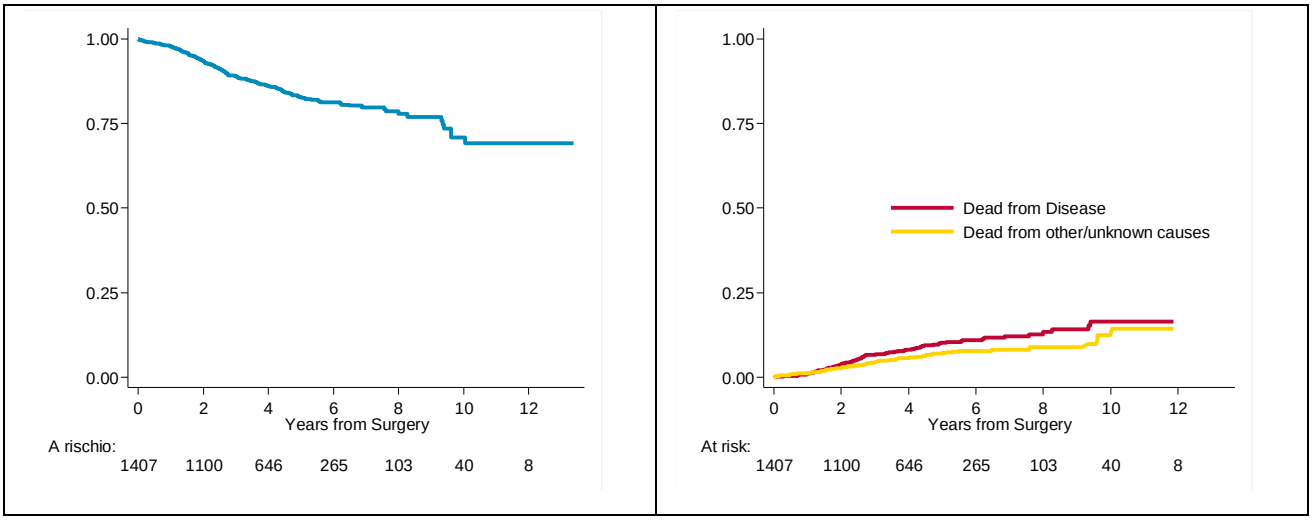
	Overall Survival		Cumulative incidence of death from Disease	
	HR (95%CI)	*p*	HR (95%CI)	*p*
Nodal stage				
ypN0	1	-		
ypN+	1.76 (1.28 to 2.44)	0.001	1.92 (1.27 to 2.89)	0.002
ypNnull	2.14 (0.96 to 4.75)	0.063	1.99 (0.7 to 5.67)	0.197

ypN0: negative lymph nodes after neoadjuvant treatment; ypN+: positive lymph nodes after neoadjuvant treatment; ypNnull: no retrieved lymph nodes after neoadjuvant treatment
*Variables included in Cox model adjustment: age,sex, T stage, tumor distance from the anal verge, type of radiation therapy schedule, interval of time from the end of radiation therapy and surgery, tumor vascular invasion, lenght of the specimen, adjuvant treatment

Table 4. Factors influencing lymph nodes yield. Coefficients indicate the average change of lymph nodes number with the variation of the reference variable adjusted for all factors of the model.

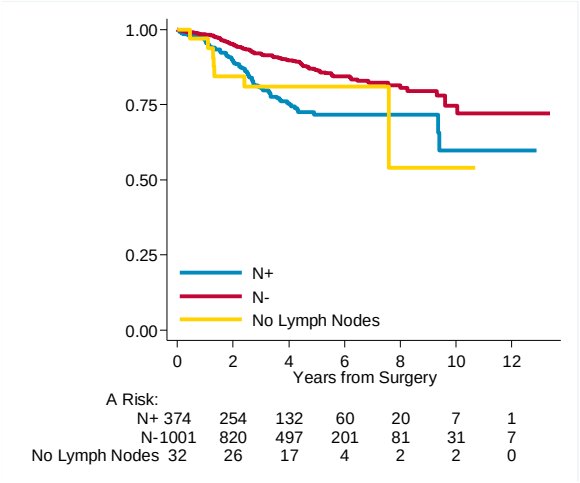
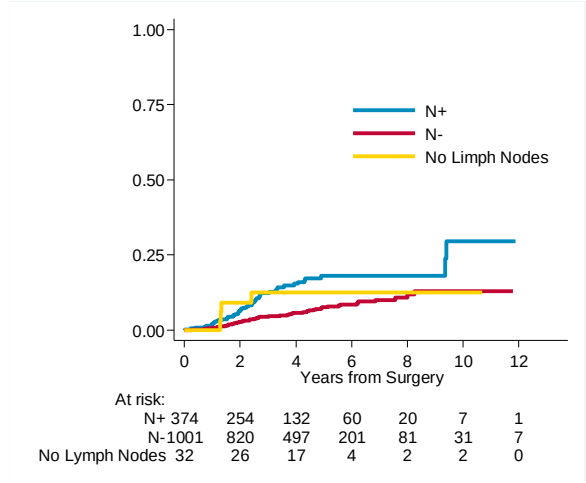
	Coefficient (IC95%)	p
Age (for 5 years increase)	0 (-0.01 to 0.02)	0.583
Males	0.36 (-0.66 to 1.38)	0.492
Distance from the anal verge	0.09 (-0.15 to 0.32)	0.446
CEA	0.02 (-0.03 to 0.07)	0.359
Long vs Short RT schedule	-6.35 (-8.73 to -3.98)	<0.001
Interval between Radiotherapy and Surgery (weeks)	-0.13 (-0.31 to 0.05)	0.147
Vascular Invasion	-1.06 (-3.19 to 1.06)	0.323
Length of the specimen	0.04 (-0.02 to 0.1)	0.21
T Stage		
1	-0.37 (-2.35 to 1.62)	0.718
2	-0.14 (-1.73 to 1.45)	0.862
3	1.85 (0.39 to 3.3)	0.013
4	3.43 (0.28 to 6.58)	0.033
Peritumoral lymphocytes	-1.96 (-5.61 to 1.7)	0.231

Figure 1



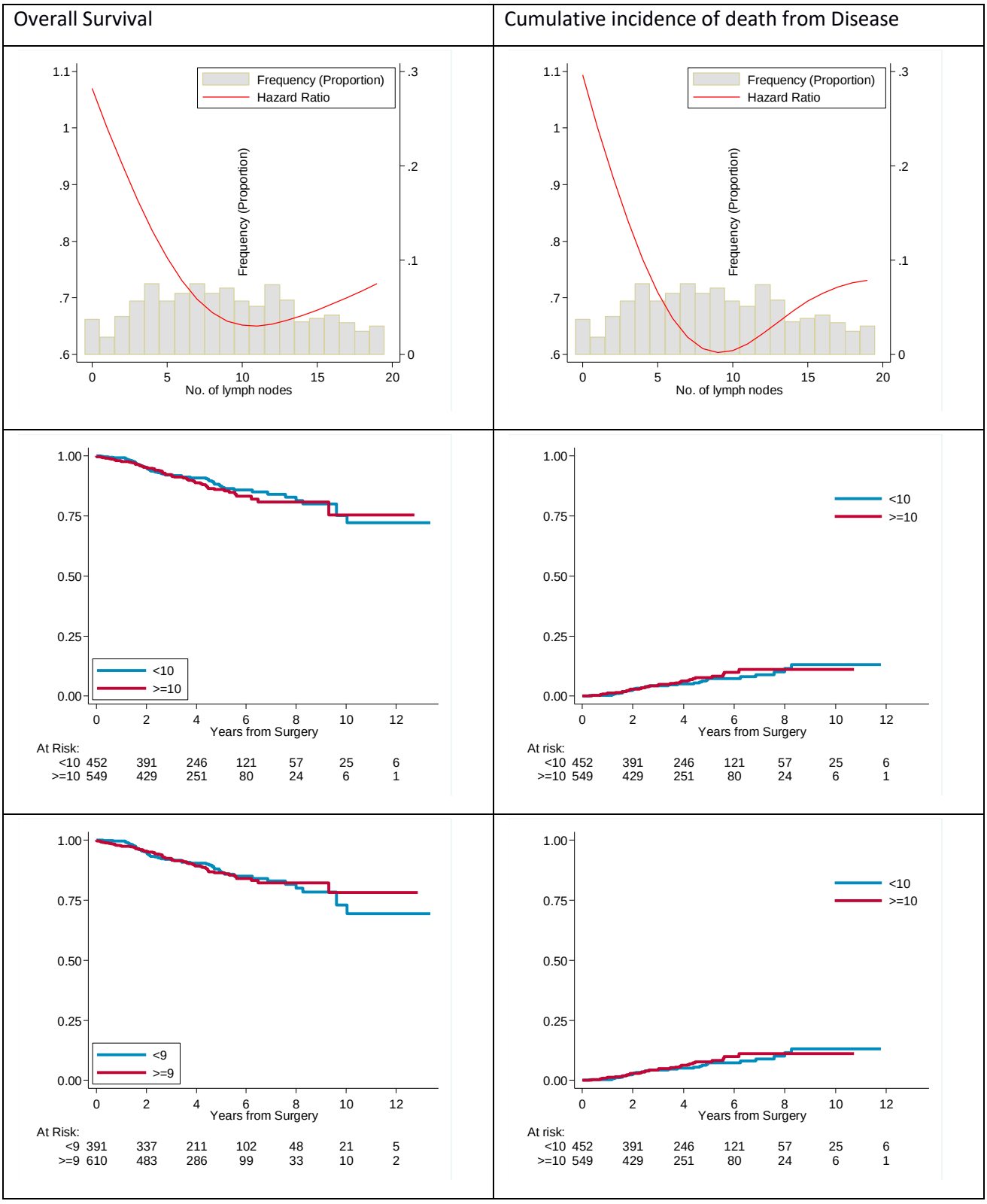
Outcome	Estimate (95%CI)
5-year Overall Survival	82.8 (80.3 to 85.1)
5-year Cumulative Incidence of death for disease	10.2 (8.4 to 12.3)
5-year Cumulative Incidence of death for other/unknown cause	6.9 (5.5 to 8.7)

Figure 2

Overall Survival	Cumulative incidence of death from Disease
	

Outcome	5-year Overall Survival	5-year Cumulative Incidence of death for disease
ypN+	71.7 (65.3 to 77.1)	18.1 (13.4 to 23.4)
ypN0	86.5 (83.7 to 88.9)	7.6 (5.7 to 9.8)
ypNnull	81 (62.5 to 91)	12.8 (4 to 26.7)

Figure 3



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