Clinicopathological characteristics predict lymph node metastases in ypT0-2 rectal cancer after chemoradiotherapy

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Abstract

Background: Changes in rectal cancer treatment include increasing emphasis on organ preservation. Local excision after chemoradiotherapy (CRT) for rectal cancer with excellent clinical response reduces morbidity and mortality compared to total mesorectal excision, although residual lymph node metastases (LNM) may cause local recurrence. Our aim is to identify clinicopathological factors predicting the presence of residual LNM in rectal cancer patients with ypT0-2 tumours after neo-adjuvant CRT. These risk factors may help select patients who can be spared radical surgery without compromising oncological outcomes.

Methods: Rectal cancer patients with ypT0-2 tumours after CRT and radical resection from five centres treated between June 1999 and February 2012 were included. Histopathology was extensively reviewed. Clinicopathological characteristics and their association with residual LNM were investigated.

Results: Out of 657 consecutive CRT treated rectal cancer patients 210 with ypT0-2 disease were included. Residual nodal disease was found in 44 cases (21.0%). Independent predictors of LNM were clinical nodal involvement (cN+) (OR 2.79 [95% CI 1.04-7.48], p=0.042), “high grade” histopathology assessed in the post-CRT resection specimen (OR 6.46 [95% CI 1.23-34.02], p=0.028), and residual tumour diameter ≥10mm (OR 2.54 [95% CI 1.06-6.09], p=0.036). An algorithm combining these factors adequately stratified patients according to LNM risk, independently of ypT category.

Conclusions: Clinical nodal involvement, “high grade” histopathology, and residual tumour diameter ≥10mm are strong and independent predictors of residual nodal disease in rectal cancer patients with ypT0-2 tumours after CRT. Risk stratification based on these factors may help identify patients suitable for organ preserving therapy and should be validated in appropriately selected populations.

Introduction

For locally advanced rectal cancer patients, neo-adjuvant chemoradiotherapy (CRT) consisting of long course radiotherapy and concurrent chemotherapy followed by total mesorectal excision (TME) is the standard of care. In 8-24% of patients, neo-adjuvant CRT results in a pathological complete response (pCR). These patients have been reported to have an excellent prognosis with a five-year local recurrence rate of 0-2.8% and 83.3-96.9% five-year disease free survival.[1-3]

Radical resection may therefore be superfluous in selected patients with a good clinical response and postoperative morbidity and mortality associated with TME[4, 5] could be avoided. Indeed, local recurrence rates as low as 2.8% and 4.7% have been reported after a "wait-and-see" policy for selected patients with a clinical complete response after CRT.[6, 7] Nevertheless, several other studies showed worse outcomes (local failure rates of 23-60%).[8-10] Critics of "wait-and-see" point out that criteria for clinical complete response cited in the literature are not consistent and the evidence is based on highly selected patient groups.[11]

A full thickness local excision of the residual tumour or scar area is an attractive alternative to "wait-and-see", since it removes possible tumour remnants in the bowel wall. It also provides additional information on tumour response and can be used to identify histopathological risk factors for locally recurrent disease. Especially for early tumours in the distal part of the rectum, this strategy has been successfully used in several small international studies.[12] However, identification of patients who are most likely to benefit from an organ preserving procedure remains difficult. A validated set of histopathological risk factors could help to stratify patients according to local recurrence risk. Unfortunately, studies on local excision after CRT are relatively scarce and often lack sufficient numbers of patients to perform a risk stratification based on histopathological factors.

An alternative approach is to investigate tumour characteristics associated with residual lymph node metastases (LNM) in the mesorectal fat of CRT treated radical TME specimens. Residual LNM are a potential source of recurrent disease after local excision and predictors of LNM in radical resection specimens are therefore likely to overlap with predictors of local

recurrence after local excision.[13, 14] In addition, patients with ypT0-2 rectal cancers after CRT may be cured with a full thickness local excision in the absence of LNM. We therefore investigated possible predictors of LNM in this group of patients in a multicenter study with central review of histopathology.

Materials and methods

Patients and study design

This report describes a pooled analysis of consecutive rectal cancer patients from five independent centres with ypT0-2 tumours who received neo-adjuvant CRT followed by TME surgery between June 1999 and February 2012. Patients considered for CRT either had evidence of locally advanced disease on pre-operative MRI (defined as a cT4 tumour, a cT3 tumour with threatened mesorectal fascia, a cT3 tumour less than 5cm from the anal verge, and/or clinical N2 disease), or were otherwise expected to benefit from CRT during a multidisciplinary team meeting (e.g. attempt to preserve the sphincter in case of a very low T2 tumour). Patients received external beam long course radiotherapy consisting of 45-50Gy in 25-28 fractions of 1.8-2.0Gy and concomitant fluoropyrimidine based chemotherapy (with or without oxaliplatin). The clinical target volume included the primary tumour and the mesorectum with vascular supply, containing the perirectal, presacral and internal iliac nodes. For this purely restrospective study ethics approval and informed consent were not required.

Histopathology

Routine histopathological evaluation of the resection specimens was performed in the laboratories of the participating hospitals according to international guidelines. For the study, haematoxylin and eosin (H&E) stained glass slides or high resolution digitally scanned slides as well as histopathology reports were retrieved and centrally reviewed by a single investigator (SLB). Difficulties and discrepancies with the original histopathology report were resolved by consulting an expert gastro-intestinal pathologist (IDN).

Cases were excluded if a tumour was determined to be >ypT2 at review or the histopathological slides (glass or digital) were unavailable.

The pathological tumour category (ypT) and pathological nodal category (ypN) were evaluated according to the 5th edition of TNM[15] classifying mesorectal tumour deposits of ≥3mm without evidence of residual lymph node tissue, as positive lymph nodes regardless of

their contour. Lymph nodes with fibrosis or acellular mucin lakes, but without viable tumour cells were considered to be negative for tumour.

In addition to ypT category the evaluated tumour related characteristics included residual tumour diameter (RTD), histopathological type and differentiation grade, tumour regression grade (TRG), extent of tumour necrosis, and presence of intramural venous and lymphatic invasion, perineural growth, budding, intramural acellular mucinous lakes, calcifications, and peritumoural inflammatory infiltrate.

RTD was defined to be the largest distance between viable tumour cells in the mucosa, submucosa or muscularis propria. In case of tumour regression with fibrosis and scattered residual tumour cells and glands, this was the largest distance between individual tumour cells in the slide. In case tumour cells were present in two slides, RTD was estimated to be at least 4mm, since a block of paraffin embedded tissue was estimated to be 4mm thick. This was at least 8mm in case of tumour in three slides etc. However, due to the retrospective nature of the study it was not possible in every case to reliably reproduce the position of the various tissue blocks and associated slides relative to each other.

Histopathological type and differentiation grade of the tumour were assessed in the post-CRT resection specimen and defined according to WHO 2010 criteria.[16] For analytical purposes the cases were subsequently categorized as having “high grade” histopathology (including poorly differentiated adenocarcinoma, undifferentiated carcinoma and signet ring cell carcinoma) vs. “other” histopathology (including low grade adenocarcinoma, mucinous carcinoma, and pathological complete response).

For TRG, a four-tier grading scale adjusted from Dworak’s system[17] was used. Grades are defined as follows: grade 1 (no significant response) “no fibrosis or significant fibrosis outgrown by cancer”; grade 2 (partial response) “residual cancer outgrown by fibrosis”; grade 3 (near complete response) “scattered single tumour cells or small groups of tumour cells”; grade 4 (pathological complete response) “no viable tumour cells”.

Lymphatic invasion was defined as tumour cells in a space covered with endothelial cells in the absence of erythrocytes.[18] Venous invasion was diagnosed in case of tumour within a

smooth muscle-lined space or in an endothelial-lined space with additional fibrin clots, erythrocytes or both, without erythrocyte extravasation into the surrounding tissue.[18, 19] Budding was defined as “presence of at least five foci of up to five tumour cells in a microscopic field using a 20x objective and evaluated in the area where such foci are most dense” as described by Ueno et al.[20] Grade of tumour necrosis was evaluated according to Pollheimer et al.[21] Acellular mucinous lakes were determined to be present or absent in the specimen regardless of tumour cells in the surrounding tissue. Peritumoural inflammatory infiltrate was determined to be conspicuous or non-conspicuous as originally described in the Jass classification.[22]

Statistical analysis

SPSS version 20 was used to perform the analyses. For RTD a receiver operating characteristic curve (ROC) was created to estimate the cut-off value with optimal sensitivity and specificity for predicting presence of LNM. Mann-Whitney U test or independent samples Kruskal-Wallis test was used for non-parametrical continuous variables. Categorical variables were analyzed using the χ^2 test, Mann-Whitney U test or independent samples Kruskal-Wallis test where appropriate. Factors with a statistically significant association with LNM or a statistical trend were subsequently included in a multivariate analysis using binary logistic regression. A p-value of <0.05 was considered statistically significant whereas a p-value of <0.1 was taken to reflect a trend towards significance.

Results

Patient selection

Out of 657 consecutive rectal cancer patients from five centres, who received long course CRT and TME, 211 (32.1%) were found to have ypT0-2 disease. One patient was excluded for lack of the histopathological slides, resulting in 210 patients who were included in the analysis.

Lymph nodes

Median number of examined lymph nodes per patient was 7 (range 0-39). Residual nodal disease was found in 44 patients (21.0%). Presence of LNM was not related to number of lymph nodes sampled in the current population (median number of examined lymph nodes: 6.5 vs. 7.0 respectively in patients with vs. without residual LNM (p=0.439). Of the patients without LNM there were 34 who showed signs of tumour regression in lymph nodes including acellular mucin in 7 cases.

Clinical characteristics

Table 1 shows clinical characteristics and their association with presence of LNM. Centre of origin, gender, clinical nodal status (cN), and type of chemotherapy (fluoropyrimidine only vs. capecitabine + oxaliplatin) were significantly associated with presence of LNM.

Histopathological tumour characteristics

Changes in classification of histopathological characteristics compared with the original pathology reports were made in 18 cases after slide review (8.6%). This included either a T-category downgrade (n=8), T-category upgrade (n=6), N-category downgrade (n=1), or N-category upgrade (n=3). Tumour type was not changed. Other factors investigated in this study (e.g. tumour differentiation grade, lymphatic invasion, tumour regression grade,

budding etc.) were not consistently described in the original reports and were therefore primarily scored at the time of slide review.

Table 2 shows the investigated histopathological characteristics and the associated LNM rate. The ypT category did not significantly predict residual nodal disease (LNM rate 17.4%, 14.8% and 25.8% for ypT0, ypT1, and ypT2 respectively; p=0.159; and LNM rate 16.8% vs. 25.8% for ypT0-1 vs. ypT2 respectively; p=0.112). RTD had a strong association with presence of LNM. Initial analysis of histopathological characteristics revealed that mean RTD was significantly higher in the ypN+ compared to the ypN0 group (11.2mm and 6.0mm respectively, p=0.022). A ROC curve showed a RTD of ≥10mm to be the optimal cut-off value to predict LNM (sensitivity 43.2%; specificity 81.9%; AUC 0.598). Therefore, this value was used in the subsequent analyses which showed LNM in 16.0% vs. 38.3% for RTD <10mm and ≥10mm respectively (p=0.001).

Out of 24 patients with a near complete response (TRG 3) there were 3 with a RTD of ≥10mm and 1 of those showed residual nodal disease.

“High grade” histopathology (assessed after neo-adjuvant therapy) was found in 8 patients including 6 with poorly differentiated adenocarcinoma and 2 with undifferentiated carcinoma. There were no cases with signet ring cell carcinoma. The majority of patients had “other” histopathology (n=202) including low grade adenocarcinoma (n=103), mucinous carcinoma (n=13) and pathological complete response (n=86). “High grade” histopathology was a statistically significant predictor for the presence of LNM (LNM rate 62.5% vs. 19.3% for “high grade” vs. “other” histopathology respectively (p=0.003)).

Routine histopathology details

The median number of tissue blocks available for re-evaluation per case was 15 (range 5-52) and the median number of blocks from the tumour area was 6 (range 2-43). The proportion of cases in which the entire tumour area was embedded could not be reliably determined retrospectively, since this was not consistently described in the original reports. Additional tumour area blocks were embedded in 23 cases (11.0%) that lacked residual viable tumour

in the initial slides, and this included 14 patients with ypT0 (16.3%). Three additional levels from the tumour blocks were cut in 5 cases (2.4%), including 2 cases with ypT0 (2.3%). Immunohistochemistry with cytokeratins was performed in 12 cases (5.7%) including 5 patients (5.8%) with ypT0.

Multivariate analysis

Factors with a statistically significant association with residual nodal disease or a statistical trend were included in a multivariate analysis (table 3). Independent predictive value was shown for clinical nodal involvement (OR 2.79, 95%CI 1.04-7.48 for cN+ vs. cN0; p=0.042), residual tumour diameter ≥10mm (OR 2.54, 95%CI 1.06-6.09 for RTD ≥10mm vs. <10mm; p=0.036), and “high grade” histopathology (OR 6.46, 95% CI 1.23-34.02 for “high grade” vs. “other” histopathology; p=0.028). Centre of origin, gender, and type of chemotherapy did not show an independent association with ypN category.

Combining independent risk factors

The independent risk factors identified in the multivariate analysis were subsequently combined to investigate their potential for risk stratification in the current study population (table 4). Patients without clinically detectable LNM (cN0) and with “other” histopathology had the lowest LNM risk (7.7%), whereas patients with “high grade” histopathology had a high risk regardless of clinical nodal status and RTD. RTD was of additional value for stratification of patients who had both clinical nodal involvement (cN+) and “other” histopathology (17.8% vs. 47.8% for RTD <10mm and ≥10mm respectively; p=0.002).

Based on these data we devised an algorithm which stratifies patients in three subgroups (low, intermediate, and high risk) according to risk of residual LNM (figure 1). LNM risk was 7.7%, 17.8%, and 51.6% for the low, intermediate, and high risk categories respectively (p<0.001; figure 2).

Role of ypT category

The ypT category did not reach statistical significance or a statistical trend in this study. In a subgroup analysis of patients with a ypT2 tumour (n=88), the algorithm described in the previous paragraph was able to adequately stratify patients according to LNM risk (7.7%, 14.7%, and 50.0% for patients in the low, intermediate and high risk categories respectively; p<0.001). For patients with a ypT0-1 tumour this was 7.7%, 19.4%, and 66.7% (p=0.024). Patients with a pathological complete response of the primary tumour (ypT0) had residual nodal disease in 10.3% and 20.8% of cases depending on clinical nodal status (cN0 and cN+ respectively; p=0.231).

Discussion

In this study including 210 TME specimens of consecutive rectal cancer patients with ypT0-2 tumours after CRT, we showed that clinical nodal involvement (cN+), "high grade" histopathology (i.e. poorly differentiated or undifferentiated carcinoma), and residual tumour diameter (RTD) of ≥10mm are strong independent risk factors for residual LNM. We devised an algorithm based on these risk factors, which adequately stratifies patients according to risk of residual nodal disease in the current population. Moreover, we showed that the predictive value of this algorithm was independent of pathological tumour category after neo-adjuvant treatment (ypT).

Clinically suspected nodal disease was the strongest independent risk factor for residual LNM at histopathological examination. Residual LNM risk in cN+ patients was 24.6%, which explains why clinical trials investigating feasibility of local excision after CRT generally exclude patients with clinical evidence of nodal involvement.[23, 24] However, LNM rate was 10.4% in the cN0 group showing that clinical imaging is relatively inaccurate for the prediction of nodal disease.[25 26]

RTD was useful only in cN+ patients. RTD can be regarded as a footprint of the original tumour which reflects its level of therapy resistance, similar to tumour regression grade. TRG correlates with the therapy resistance of associated LNM, with similar levels of regression in both the primary tumour and the lymph nodes.[27] The predictive value of RTD is most likely based on the same principle. In case of a local excision the advantage of RTD over TRG is that it is based on the amount of microscopically detectable residual tumour in the specimen, whereas for TRG, an estimate of the amount of "tumour mass turned fibrosis" is essential.[17] Estimates of TRG are therefore not feasible after local excision, since an important part of the fibrotic areas are located in the mesorectal fat and therefore missing in the specimen.

"High grade" histopathology was a strong and independent risk factor associated with a 62.5% risk of LNM, although it was found in relatively few cases in the current population. This result is in accordance with previous series on early colorectal cancer.[20, 28]

Differentiation grade was determined in the CRT treated resection specimens, since pre-

therapy biopsies are notoriously unreliable for grading purposes with substantial variation between grade of differentiation determined on biopsy and after definitive surgery,[29] probably due to sampling error. Indeed, WHO criteria define type and grade according to the relative dominance of specific tumour components (e.g. more or less than 50% gland formation; more or less than 50% mucin production),[16] and a superficial biopsy may miss a relevant component entirely. On the other hand, CRT may induce significant morphological changes including disappearance of tumour tissue with fibrosis and mucinous degeneration.[30] This may change the proportion of various tumour components and may yield a different grade than would have been the case without neo-adjuvant treatment. However, since both the primary tumour and LNM have been reported to undergo similar levels of regression with loss of the most susceptible tumour components[27] it may be hypothesized that the post-CRT morphology is likely to reflect the risk of residual LNM most adequately.

The relatively low number of examined lymph nodes is a limitation to this study, since a minimum of 12 nodes is generally recommended for adequate nodal staging.[31] However, lymph node yield is known to decrease after chemoradiation and the median number of 7 nodes found in this study is comparable with results described in several previous reports after neo-adjuvant therapy.[32-35] Lymph node yield was not associated with nodal positivity in the current population. However, this may be related to a lack of statistical power to detect a correlation, since previous studies found LNM rate to increase with number of examined lymph nodes.[33, 34]

Furthermore, the multicenter design of this study implies some inherent variations between centres in distribution of patient and treatment characteristics, such as gender, clinical stage, and type of chemotherapy. However, the included rectal cancer patients constitute an adequate reflection of the case-mix encountered in clinical practice, and results may therefore be widely applicable. Moreover, the multivariate analysis showed the identified risk factors to be independent of centre.

However, the current results cannot be extrapolated directly to a local excision setting. For example, pathological tumour category may be underestimated in local excision specimens

due to the often discontinuous nature of residual tumour foci after neo-adjuvant CRT, since some residual tumour cells may remain undetected in the mesorectal fibrosis. Furthermore, our study is based on a relatively unfavourable population including many patients with unfavourable clinical characteristics such as T4 tumours or clinical N2 disease, and many of them would in practice never be considered for rectal preservation. Therefore, our results are hypothesis generating, and the identified risk factors, as well as their association with local recurrence risk, should be investigated and validated in appropriately selected populations. In summary, this study shows that clinical nodal involvement, "high grade" histopathology, and residual tumour diameter are strong and independent predictors for the presence of residual nodal disease in rectal cancer patients with ypT0-2 tumours after neo-adjuvant CRT. An algorithm combining these risk factors to stratify patients according to low, intermediate, or high LNM risk was shown to be accurate, regardless of ypT category. If validated in appropriately selected populations these factors may contribute to an effective stratification of patients according to risk of LNM and local recurrence. This may improve decision making regarding local or radical surgery, and may help save selected patients from undergoing an unnecessary, yet potentially harmful TME, while ensuring oncological safety.

Disclosure/Conflict of interest

No conflict of interest declared.

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Author contributions

SB and IDN designed the study and reviewed the histopathological slides. SB performed the initial statistical analysis and wrote the first manuscript draft. TAV , NPW, HAMS, CAMM, AC,

CV, IL, JHWW, and HJR contributed to acquisition of cases and clinical data, as well as analysis/interpretation of data. All authors provided critical input on the subsequent versions and approved the final manuscript.

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Table 1. Association of clinical characteristics with residual lymph node metastases.

Clinical characteristics	Univariate analysis				
Total N=210	ypN0		ypN+		P-value *
	N	%	N	%	
Total no. of patients	166	79.0	44	21.0	
Age (years) #	62 (55-68)		59.5 (54.25-69.75)		0.665 ^
Total number of examined lymph nodes #	7 (4-11)		6.5 (5-12.75)		0.439 ^
Centre of origin					
Centre 1	64	86.5	10	13.5	0.004
Centre 2	27	61.4	17	38.6	
Centre 3	21	70.0	9	30.0	
Centre 4	6	75.0	2	25.0	
Centre 5	48	88.9	6	11.1	
Gender					
Male	125	83.9	24	16.1	0.007
Female	41	67.2	20	32.8	
cT					
cT2	7	87.5	1	12.5	0.184^
cT3	99	75.6	32	24.4	
cT4	60	85.7	10	14.3	
Missing	-	-	1	-	
cN					
cN0	60	89.6	7	10.4	0.018
cN+	98	75.4	32	24.6	
Missing	8	-	5	-	
Type of chemotherapy					
FP only ‡	121	75.6	39	24.4	0.033
CAPOX	44	89.8	5	10.2	
Missing	1	-	-	-	
Distance to anal verge					
<5cm	92	82.9	19	17.1	0.148
≥5cm	74	74.7	25	25.3	

FP: fluoropyrimidine; CAPOX: capecitabine + oxaliplatin; cT: clinical primary tumour category; cN: clinical nodal category; ypT: pathological primary tumour category after multimodality therapy; ypN: pathological nodal category after multimodality therapy

* Chi-square test is used unless stated otherwise

^ Mann-Whitney U test

Median and interquartile range

‡ Capecitabine (n=139) or bolus 5FU + leucovorin (n=21; centre 1 and 4)

Table 2. Association of pathological characteristics with residual lymph node metastases.

Histopathological characteristics	Univariate analysis				
Total N=210	ypN0		ypN+		P-value *
	N	%	N	%	
Total no. of patients	166	79.0	44	21.0	
ypT					
ypT0	71	82.6	15	17.4	0.159^
ypT1	23	85.2	4	14.8	0.112 ‡
ypT2	72	74.2	25	25.8	
RTD					
<10mm	137	84.0	26	16.0	0.001
≥10mm	29	61.7	18	38.3	
Histopathological type/grade #					
“Other”	163	80.7	39	19.3	0.003
“High grade”	3	37.5	5	62.5	
TRG					
pCR	71	82.6	15	17.4	0.769^
Near complete response	16	66.7	8	33.3	
Partial response	76	80.9	18	19.1	
No significant response	3	50.0	3	50.0	
Intramural venous invasion					
Present	0	-	0	-	N/A
Absent	166	79.0	44	21.0	
Intramural lymphatic channel invasion					
Present	11	68.8	5	31.2	0.292
Absent	155	79.9	39	20.1	
Intramural perineural growth					
Present	0	-	0	-	N/A
Absent	166	79.0	44	21.0	
Budding					
Positive	8	61.5	5	38.5	0.117
Negative	155	79.9	39	20.1	

	Missing	3	-	-	-	
Necrosis						
	Absent	136	81.0	32	19.0	0.117^
	Focal (<10%)	17	81.0	4	19.0	
	Moderate (10-30%)	4	44.4	5	55.6	
	Extensive (>30%)	8	72.7	3	27.3	
Peritumoural inflammatory infiltrate						
	Conspicuous	27	73.0	10	27.0	0.317
	Other	139	80.3	34	19.7	
Acellular mucinous lakes						
	Present	40	75.5	13	24.5	0.459
	Absent	126	80.3	31	19.7	
Calcification						
	Present	42	85.7	7	14.3	0.190
	Absent	124	77.0	37	23.0	

ypT: pathological primary tumour category after multimodality therapy; ypN: pathological nodal category after multimodality therapy; RTD: residual tumour diameter; TRG: tumour regression grade; pCR: pathological complete response

* Chi-square test is used unless stated otherwise

^ Mann-Whitney U test

‡ ypT0-1 vs. ypT2

“Other” histopathology includes low grade adenocarcinoma (n=103), mucinous carcinoma (n=13) and pathological complete response (n=86). “High grade” histopathology includes poorly differentiated carcinoma (n=6), and undifferentiated carcinoma (n=2).

Table 3. Multivariate analysis.

		Odds ratio (95% CI)	P-value
Centre of origin	Centre 1 Centre 2 Centre 3 Centre 4 Centre 5	1.00 1.42 (0.37-5.42) 1.32 (0.32-5.46) 0.0 (0.0-∞) 0.48 (0.11-2.07)	0.426
Gender	Male Female	1.00 1.98 (0.86-4.59)	0.110
cN	cN0 cN+	1.00 2.79 (1.04-7.48)	0.042*
Type of chemotherapy	FP only CAPOX	1.00 0.45 (0.10-2.01)	0.298
RTD	<10mm ≥10mm	1.00 2.54 (1.06-6.09)	0.036*
Histopathology	“Other” “High grade”	1.00 6.46 (1.23-34.02)	0.028*

cN: clinical nodal category; RTD: residual tumour diameter; FP: fluoropyrimidine; CAPOX: capecitabine + oxaliplatin

*statistically significant ($p<0.05$)

Table 4. Independent risk factors and lymph node metastases rate (N=197^).

cN0			cN+		
	"Other" histopathology	"High grade" histopathology		"Other" histopathology	"High grade" histopathology
RTD <10mm	8.0% (4/50)	100.0% (1/1)	RTD <10mm	17.8% (18/101) *	25.0% (1/4) #
RTD ≥10mm	6.7% (1/15)	100.0% (1/1)	RTD ≥10mm	47.8% (11/23)	100.0% (2/2)
Total	7.7% (5/65)	100.0% (2/2)	Total	23.4% (29/124)	50.0% (3/6)

Figures represent patients with LNM in each subgroup: % (N/Ntotal).

cN: clinical nodal category; RTD: residual tumour diameter;

^ Cases with at least one missing value (n=13) were excluded

* p=0.002 for RTD <10mm vs. RTD ≥10mm

p=0.083 for RTD <10mm vs. RTD ≥10mm

Titles and legends to figures

Figure 1. Flow chart depicting algorithm for risk stratification.

LNM: lymph node metastases; cN: clinical nodal category

Figure 2. Risk of residual LNM based on the flow chart algorithm* (n=197^).

* Risk factors are: clinical nodal involvement (cN+), residual tumour diameter ≥10mm, and "high grade" histopathology (including poorly differentiated and undifferentiated carcinoma).

^ Cases with at least one missing value (n=13) were excluded.