

Factors Influence Lymph Node Retrieval after Resection for Rectal Cancer

Somaia Elsheikh and
Abed Zaitoun M

Department of Histopathology, Nottingham
University Hospitals, NHS Trust, United
Kingdom

Abstract

Introduction: Total lymph node (TLN) count is an important qualifier of cancer evaluation and extensive nodal resection has been associated with lower rates of cancer recurrence; allows for more accurate cancer staging and improved survival following resection for rectal cancer.

Aim: To examine the adequacy of lymph node sampling in rectal cancer and the influence of the clinicopathological factors and treatment modalities on the total lymph node yield.

Methods: This is a retrospective study includes 409 cases of rectal cancer treated by surgical intervention only (126 cases), or surgery after short or long course chemotherapy (75 and 208 cases respectively).

Results: TLN count range from (2-50, mean 12.38). Twelve lymph nodes were found in 49.4% of the specimens with or without neoadjuvant chemo radiotherapy (CRT). Patient treated with surgery only have a significant high total LN yield (mean is 15.53) in comparison with (12.99 and 10.25 for short course CRT and long course CRT respectively, P value <0.001). The number of lymph nodes was significantly correlated with the modality of treatment, anterior quadrant involvement, vascular and perineural invasion, pathological staging, Duke's and Mandard staging. On multivariate only perineural invasion, high tie lymph node involvement, Dukes stage, and tumour load were found to be the main predictors of the number of lymph node yield.

Conclusion: The neoadjuvant CRT has a significant effect on the adequacy of lymph node sampling and a negative impact on the lymph nodes retrieved. This negative effect was more prominent with the long term rather than the short term CRT. This should be taken in consideration at the N staging of the tumour to avoid down-staging.

Keywords: Rectal cancer; Lymph node; Chemo-Radiotherapy

Corresponding author: Somaia Elsheikh

✉ soelsheikh@yahoo.com

Department of Histopathology, Nottingham
University Hospitals, NHS Trust, United
Kingdom.

Tel: 04401159249924

Citation: Elsheikh S, Zaitoun MA.
Factors Influence Lymph Node Retrieval
after Resection for Rectal Cancer. J.
Adenocarcinoma. 2016, 1:2.

Received: May 12, 2016; **Accepted:** May 27, 2016; **Published:** June 02, 2016

Introduction

Bowel cancer is the fourth most common cancer in the UK (2013), accounting for 13% of all new cases. It is the third most common cancer in both males (14% of the male total) and females (11%) separately. There are over 14000 new cases of rectal cancer and 6000 deaths with the disease within the UK every year [1]. Preoperative chemo radiotherapy is now considered standard treatment for patients with a locally advanced rectal cancer and a threatened circumferential resection margin (CRM) [2]. The primary purpose of chemo radiotherapy is to improve local

control and resectability and the response to preoperative therapy has been shown to be linked to long-term outcome in several studies [3-5].

Pathological staging of colorectal cancer requires adequate resection of the main tumour with the regional lymph nodes by total mesorectal excision (TME). An accurate examination of the surgical specimens is essential for the correct assessment of the lymph node status as inadequate lymph node removal may result in understaging of rectal cancer. The National Cancer Institute (NCI) recommends that at least 12 lymph nodes to be removed

for adequate staging of colorectal cancer [6]. This minimum number of retrieved lymph nodes was initially recommended by the World Congress of Gastroenterology held in Sydney in 1990 [7]. Removal of fewer lymph nodes can result in understaging, hence, some patients who would benefit from receiving adjuvant therapy, therefore, would not be offered any [6]. Nevertheless, low number of retrieved lymph nodes is associated with adverse overall survival, higher rates of local recurrence and significant morbidity [8-10].

Preoperative radiotherapy is thought to decrease the lymph node yield after surgical excision [11-13]. This is probably caused by the immune response and fibrosis in lymph nodes exposed to radiotherapy, which results in diminution in their size, making their identification in the pathology specimen difficult. This study aims to look at the effect of preoperative chemo radiotherapy (CRT) on the pathological criteria of the rectal cancer including the number of lymph nodes retrieved in the mesorectal specimen.

Materials and Methods

Patient data

This study was performed on a cohort of 409 cases of consecutive, unselected primary operable rectal cancer diagnosed between 2009 and 2010 at Nottingham University Hospital. Patient's clinical history and tumour characteristics including patients' age, sex, site of tumour, histologic grade, tumour necrosis, or fibrosis, depth of tumour invasion beyond the muscularis propria, high tie lymph node involvement, and the total lymph node (LN) status, vascular (VI) and perineural invasion were obtained from patient's reports.

Hundred and twenty six patients (29%) were treated with surgery only while 282 were treated with long-course preoperative chemo-radiotherapy before surgery for rectal cancer as described previously [14]. The latter group had locally advanced rectal cancer, cT3/4 and/or N+ and were considered inoperable or of borderline resectability due to potential circumferential resection margin (CRM) involvement. Pre-treatment work-up consisted of digital rectal examination, sigmoidoscopy and biopsy, barium enema, pelvic magnetic resonance imaging (MRI)/ computed tomography and computed tomography of the chest and abdomen. All patients were discussed at a multidisciplinary team meeting consisting of specialist colorectal surgeons, oncologists, radiologists and pathologist before being referred for neoadjuvant treatment.

The study was approved by the Nottingham University Hospital ethics committee. This group was divided to short and long term CRT that included 75 and 208 patients respectively. Dissection of the specimen was performed according to the departmental standard operating procedure in accordance with the recommendation of the Royal College of pathologist. Lymph nodes were identified by direct inspection and manual palpation through serial transverse slicing of the mesorectum and sigmoid mesentery. All the identifiable lymph nodes present in the specimen are dissected for histological examination. If less than 12 lymph nodes identified representative sampling from the

mesenteric fat surrounding the tumour was performed. Neither fat clearance, nor sentinel node mapping methods were used in this series of patients.

Details of the characterisation of this cohort of the patients according to the treatment modality were described in (Table 1). Tumours were staged (pT stage) according to the TNM staging criteria and Duck's staging system [15].

The response to radiotherapy was graded using a rectal radiotherapy grading system adapted from Mandard et al. grading system [16]. This comprised the following:

Table 1 The clinicopathological criteria for each treatment modality group.

Variables	Modalities of treatment			Total
	Surgery	Short term RT	Long term RT	
Sex				
Male	69(26.5%)	55(21.2%)	136(52.3%)	260
Female	57(38.3%)	20(13.4%)	72(48.3%)	149
Tumour Site				
Upper rectum	93(60.4%)	33(21.4%)	28(18.2%)	145
Middle rectum	21(16.5%)	35(27.6%)	71(55.9%)	127
Lower rectum	12(9.4%)	7(5.5%)	109(85.2%)	128
Tumour perforation				
Present	7(21.2%)	1(3%)	25(75.8%)	33
Absent	119(31.6%)	74(19.7%)	138(48.7%)	376
Anterior quadrant involvement				
Yes	81(34.5%)	44(18.7%)	111(46.8%)	236
No	44(25.6%)	31(18%)	97(56.4%)	172
Tumour grade				
Well differentiated	19(44.4%)	11(23.4%)	17(36.2%)	47
Moderately differentiated	103(34.6%)	60(20.1%)	135(45.3%)	298
Poorly differentiated	4(16.7%)	3(12.5%)	17(70.8%)	24
pT stage				
pT0	0	0	46(100%)	46?
pT1	7(26.9%)	3(11.5%)	16(61.5%)	26
pT2	32(42.1%)	12(15.8%)	32(42.1%)	76
pT3	70(30.8%)	54(23.8%)	103(45.4%)	227
pT4	17(50.0%)	5(14.7%)	12(35.3%)	34
Vascular invasion				
Absent	63(25.5%)	39(15.8%)	145(58.7%)	247
Present	63(38.5%)	36(22.4%)	63(39.1%)	161
Perineural Invasion				
Absent	102(30.4%)	59(17.6%)	175(52.1%)	336
Present	24(32.9%)	16(21.9%)	33(45.2%)	73
High tie lymph node				
Negative	120(30.6%)	72(18.4%)	200(51.0%)	392
Positive	6(40%)	3(13.3%)	7(46.7%)	16
Mandard Grading				
1	0 (0.0%)	0	4342(10097.7%)	43
2	0(0.0%)	01	67(100%)	67
3	0(1.4%)	0(1.4%)	72(100%)	72
4	0(0.0%)	0(0.0%)	27(100%)	27
Dukes Staging				
A	33(39.8%)	12(14.5%)	38(45.8%)	83
B	29(26.6%)	22(20.2%)	58(53.2%)	109
C1	59(37.6%)	38(24.2%)	60(38.2%)	157
C2	5(31.3%)	2(12.5%)	9(56.3%)	16

RT: Radiotherapy

TRG 1: complete response with absence of residual cancer and fibrosis extending through the wall.

TRG 2: presence of residual tumour cells scattered through the fibrosis.

TRG 3: increase in the number of residual cancer cells, with fibrosis predominant.

TRG 4: residual cancer outgrowing fibrosis.

TRG 5: absence of regressive changes.

Statistical analysis

Statistical analysis was performed using SPSS 21 statistical software. Chi-square test was used to compare clinic-pathological parameters among different treatment modalities. One way ANOVA was used to access the relation between the number of retrieved nodes and categorical variable while spearman correlation test was used to compare the former with numerical variables. Multivariate regression model was used to determine the most significant factors affecting the lymph node yield. All p-values were two-sided, and $p < 0.05$ was considered significant.

Results

This is a retrospective study of a cohort of 409 consecutive cases. The age of the patients was between 20 and 89 years (mean 65.4), which included 260 male and 149 female. The management included surgical excision alone 126 (29%), or a preoperative short or long term chemo-radiotherapy 75 (17.2%), and 260 (59.8%) respectively prior to surgery. Most of the rectal tumours included in the study were moderately differentiated adenocarcinoma (68.5%), with a predominant upper rectal location (35.4%). Forty six patients (10.6%) with a known pT stage were found to have stage pT0 after radiotherapy, with the pT3 stage represent the most predominate stage regard less of the treatment modality (55.5%). It was found that some adverse prognostic parameters as vascular invasion and perineural infiltration and high tie lymph node involvement were more frequent in the patient received long course CRT in comparison with those who went for the surgery or the preoperative short term CRT.

Considering a minimum of 12 lymph node as a measurement of adequacy of lymph node sampling, it was found that from the 409 cases, 202 cases were adequately sampled (49.4%). When cases were categorized according to the modality of treatment, the adequacy was 68.9%, 58.7%, 33.7% in surgery, short term CRT and long term CRT respectively.

Total lymph node (TLN) count ranged from (2-50, mean 12.38). Patient treated with surgery only have a significant high total LN yield (mean is 15.53, 12.99, and 10.25 in surgery, short chemo radiotherapy and long course chemo radiotherapy respectively, P value < 0.005). High lymph node retrieval was found to be significantly associated with adverse prognostic factor in rectal cancer as vascular invasion, perineural infiltration, high tie lymph node involvement, high tumour stage and high Duke's staging (p value are < 0.005 , 0.002, 0.026, 0.006, and 0.003 respectively) (Table 2).

Table 2 Factors affecting lymph node retrieval in rectal cancer.

Parameters	Lymph nodes (mean)	SD	P value
Treatment modality			
Surgery	15.5	6.6	< 0.005
SCRT	12.9	5.8	
LCRT	10.2	6.2	
Sex			
Male	11.99	6.826	0.12
Female	13.07	6.485	
Tumour Site			
Upper rectum	14.32	6.530	< 0.005
Middle rectum	12.26	6.361	
Lower rectum	10.16	6.622	
Tumour perforation			
Present	12.48	6.489	0.29
Absent	11.21	8.954	
Anterior quadrant involmment			
No	10.80	5.301	< 0.005
Yes	13.55	7.390	
Tumour grade			
Well differentiated	12.64	6.219	0.76
Moderetly differntiated	12.83	6.498	
Poorly differentiated	13.83	10.627	
Vascular invasion			
Absent	11.19	6.089	< 0.005
Present	14.19	7.225	
Perineural Invasion			
Absent	11.91	6.321	0.002
Present	14.53	8.000	
High Tie LN			
Negative	12.25	6.476	0.026
Positive	16.06	10.680	
Mandard Grading			
1	7.95	3.697	0.01
2	9.34	4.433	
3	11.38	6.628	
4	11.54	6.895	
5	13.00	3.697	
Tumour pT stage			
pT1	9.00	4.630	0.003
pT2	12.00	6.427	
pT3	13.43	6.830	
pT4	14.71	7.837	
Dukes Staging			
A	10.98	6.208	0.006
B	13.02	7.324	
C1	13.41	5.869	
C2	16.56	11.431	
Circumferenrial margin Involvement			
Negative	15.13	12.404	0.079
Positive	12.62	6.165	

A significant positive correlation was also found between high lymph node yield and upper rectal tumour with anterior quadrant involvement (p value<0.005).

For the group of patient who received neo-adjuvant CRT it was found that the highest number of lymph node dissection was found in those with a poor response to treatment as defined by Mandard grading system (p value is 0.01).

The number of lymph node yield was compared with the number of blocks used for the microscopic assessment of each case. It was found that the using large number of small blocks was associated with high total lymph node (p=0.1). Surprisingly, a reverse relation was identified between using large blocks and the total lymph node yields (p=0.04). However the sum of both the large and the small block used wasn't seems to affect the total lymph nodes (Table 3).

Multivariate analysis of the factors that have a significant correlation with the number of lymph node showed that only perineural invasion, high tie lymph node involvement, Dukes stage, and tumour load are the factors that can predict the number of lymph node retrieved from an excision specimen (Table 4).

The lymph nodes were categorized according to their size into five groups. The relation between the size of the node and the number of lymph node yield in different treatment modalities was assessed (surgery only versus neoadjuvant). It was found that in two groups (>0-2 mm and >10-15 mm), the number of lymph node in patient treated with surgery alone is significantly higher than those treated with preoperative CRT (Table 5).

Table 3 Factors affecting lymph node retrieval.

Parameters	Correlation Coefficient	P-value
Age	-0.059	0.23
Percentage of Tumour necrosis	0.174	0.012
Percentage of Tumour fibrosis	-0.238	0.001
Tumour load	0.255	<0.005
Small blocks	0.117	0.01
Large blocks	-0.100	0.04
Total blocks	0.72	0.14

Table 4 Factors influence lymph node yield on Multivariate analysis.

Parameters	B- Coefficient	P-value
Treatment	0.063	0.270
Site tumour	0.052	0.377
Anterior quadrant	-0.053	0.367
Vascular invasion	0.056	0.499
Perineural invasion	0.205	0.006
High tie lymph node involmnet	0.351	0.000
Duke's staging	0.350	0.000
Percentage of necrosis	0.284	0.067
Percentage of fibrosis	0.864	0.006
Tumour load	0.731	0.005
Number of small blocks	0.082	0.184
Number of large blocks	-0.016	0.799

Table 5 The relation between treatment modalities and lymph node size.

Lymph nodes size groups	Treatment modalities	Number of patients	Mean	Std. Deviation	Significance
0>-2mm	S [®]	126	6.0397	3.59	0.015
	RT*	283	4.9258	2.97	
>2-5 mm	S	126	5.8333	3.18	0.321
	RT	283	4.0283	3.03	
>5-10 mm	S	126	2.9683	2.58	0.226
	RT	283	1.8516	2.34	
>10-15 mm	S	126	0.7460	1.52	<0.005
	RT	283	0.2650	0.831	
>15 mm	S	126	0.0556	0.317	0.74
	RT	283	0.0283	0.264	

[®]Surgery; *Radoithrapy

Discussion

Previous studies and guidelines recommended examination of greater number of nodes to provide an adequate staging of tumour [6,17-19]. False negative classification of the lymph node stage will affect patients who might benefit from adjuvant treatment [19].

The number of lymph nodes dissected from rectal specimens was found to be directly proportional to the probability of detecting disease in them [20]. Nevertheless, adequate sampling of lymph nodes decreases the incidence of local recurrences [21,22] and improves the patient overall survival [23,24].

The adequacy of lymph node sampling was defined as a minimum of 12 lymph nodes according to the recommendation of the World Congress of Gastroenterology in Sydney [6] and the NCI guidelines [7].

The number of lymph nodes that should be examined in order to be considered adequate is not universally agreed upon. Various studies have suggested numbers ranging from 7 to 20, based on their individual series [9,20,25,26]. The last edition of TNM classification of the American Joint Committee on Cancer recommended at least 10 to 14 lymph nodes in radical colon and rectum resections without neoadjuvant treatment [27].

In this series the lymph node sampling was adequate in 69.8% of patient treated with surgery alone, and was decreasing from 58.7% to 33.7% with the increase of the course of the neoadjuvant CRT from short term to long term.

In the current study the mean number of lymph nodes excised from the rectal specimen ranged between 2 and 50, mean 12.38, which is in agreement with previous studies and international and national guidelines mentioned above. The wide range of variation may be related to the low number of lymph node retrieval associated with the neoadjuvant CRT. Other factors related to the ability of both the surgeon and the pathologist in excision and assessing the rectal cancer respectively are found to influence the number of lymph node retrieval [28,29].

The Dutch Colorectal Cancer study, which randomly subdivides the patients to receive preoperative short radiotherapy course

followed by TME surgery or TME surgery only, found out that there was a significant difference in the number of retrieved lymph nodes [9]. In agreement with previous studies, in the current study the number of lymph node yield after surgery alone overweighed the number retrieved with upfront CRT (mean is 15.53). Interestingly, the effect of the CRT on the number of lymph nodes was time dependent where patient received short term CRT had more lymph nodes than those who received long term CRT (12.99, and 10.25 in short term CRT and long term CRT respectively, P value <0.005). This contradicts Marijnen et al. who found no down staging after short-term preoperative radiotherapy in rectal cancer patients [11].

Our findings are compatible with Sermier et al. [30] and other studies, that prove the adverse effect of the long term in comparison to the short term CRT on the lymph node retrieval and hence the adequate staging of rectal [31-33].

Other clinic-pathological parameters that found to have a significant positive impact on the lymph node yield included upper rectal location which is compatible Marcos et al. finding [20]. Thorn found a higher number of lymph nodes in the mid rectal tumours [34]. Other significant positive association was found with involvement of the anterior quadrant, intravascular and perineural invasion, positive high tie lymph node, high Duke's and pT stages and poor response to neoadjuvant CRT as defined by Mandar, these findings are in agreement with some studies [12,20] but not with others [34,35]. Other histological

features as the percentage of tumour load and tumour necrosis had a positive effect of the lymph node number while tumour fibrosis has a significant negative impact.

In this series, the age of the patient did not have any effect on the number of lymph node which come in agreement with previous studies [20,26] but in contrast with others that found higher lymph node yield in younger patients [12,36]. The sex of the patient did not have any association with the number of lymph nodes, however the effect of sex on lymph node number is not consistent in the literature [26,36].

On multivariate only perineural invasion, high tie lymph node involvement, Dukes stage, and tumour load were found to be the main predictors of the number of lymph node yield.

In conclusion, our result provides further evidence to the effect of the widely used neoadjuvant CRT on the number of lymph node retrieved in rectal cancer which was found to be time dependent. Furthermore, the current TNM classification of rectal cancers needs to be improved and take in consideration the effect of neoadjuvant CRT on the number of lymph node retrieved [37]. Despite tumour down staging, pathological postoperative staging remained a very strong prognostic factor for both T and N stages and remains pivotal for selection of patients who may benefit from additional chemotherapy [38]. Nevertheless, the pathological N staging of irradiated specimen should be interpreted with caution.

References

- 1 Cancer Research UK (2011) Cancer statistics.
- 2 Gerard JP, Conroy T, Bonnetain F, Bouche O, Chapet O, et al. (2006) Preoperative radiotherapy with or without concurrent fluorouracil and leucovorin in T3-4 rectal cancers: results of FFCD 9203. *Journal of clinical oncology* 24: 4620-4625.
- 3 Bouzourene H, Bosman FT, Seelentag W, Matter M, Coucke P (2002) Importance of tumor regression assessment in predicting the outcome in patients with locally advanced rectal carcinoma who are treated with preoperative radiotherapy. *Cancer* 94: 1121-1130.
- 4 Bozzetti F, Andreola S, Baratti D, Mariani L, Stani SC, et al. (2002) Preoperative chemoradiation in patients with resectable rectal cancer: results on tumor response. *Annals of surgical oncology* 9: 444-449.
- 5 Rodel C, Martus P, Papadopoulos T, Fuzesi L, Klimpfinger M, et al. (2005) Prognostic significance of tumor regression after preoperative chemoradiotherapy for rectal cancer. *Journal of clinical oncology* 23: 8688-8696.
- 6 Nelson H, Petrelli N, Carlin A, Couture J, Fleshman J, et al. (2001) Guidelines 2000 for colon and rectal cancer surgery. *J Natl Cancer Inst* 93: 583-596.
- 7 Fielding LP, Arsenault PA, Chapuis PH, Dent O, Gathright B, et al. (1991) Clinicopathological staging for colorectal cancer: an International Documentation System (IDS) and an International Comprehensive Anatomical Terminology (ICAT). *Journal of gastroenterology and hepatology* 6: 325-344.
- 8 Cianchi F, Palomba A, Boddi V, Messerini L, Pucciani F, et al. (2002) Lymph node recovery from colorectal tumor specimens: recommendation for a minimum number of lymph nodes to be examined. *World J Surg* 26: 384-389.
- 9 Goldstein NS, Sanford W, Coffey M, Layfield LJ (1996) Lymph node recovery from colorectal resection specimens removed for adenocarcinoma. Trends over time and a recommendation for a minimum number of lymph nodes to be recovered. *American journal of clinical pathology* 106: 209-216.
- 10 Goldstein NS (2002) Lymph node recoveries from 2427 pT3 colorectal resection specimens spanning 45 years: recommendations for a minimum number of recovered lymph nodes based on predictive probabilities. *Am J Surg Pathol* 26: 179-189.
- 11 Marijnen CA, Nagtegaal ID, Klein Kranenbarg E, Hermans J, van de Velde CJ, et al. (2001) No downstaging after short-term preoperative radiotherapy in rectal cancer patients. *Journal of clinical oncology* 19: 1976-1984.
- 12 Baxter NN, Morris AM, Rothenberger DA, Tepper JE (2005) Impact of preoperative radiation for rectal cancer on subsequent lymph node evaluation: a population-based analysis. *Int J Radiat Oncol Biol Phys* 61: 426-431.
- 13 Nagtegaal ID, van de Velde CJ, van der Worp E, Kapiteijn E, Quirke P, et al. (2002) Macroscopic evaluation of rectal cancer resection specimen: clinical significance of the pathologist in quality control. *Journal of clinical oncology* 20: 1729-1734.
- 14 Dhadda AS, Dickinson P, Zaitoun AM, Gandhi N, Bessell EM (2011) Prognostic importance of Mandard tumour regression grade following pre-operative chemo/radiotherapy for locally advanced rectal cancer. *European journal of cancer* 47: 1138-1145.
- 15 Dukes CE, Bussey HJ (1958) The spread of rectal cancer and its effect on prognosis. *Br J Cancer* 12: 309-320.
- 16 Dhadda AS, Bessell EM, Scholefield J, Dickinson P, Zaitoun AM (2014) Mandard tumour regression grade, perineural invasion, circumferential resection margin and post-chemoradiation nodal status strongly predict outcome in locally advanced rectal cancer treated with preoperative chemoradiotherapy. *Clin Oncol* 26: 197-202.
- 17 Deo SV, Shukla NK, Srinivas G, Mohanti BK, Raina V, et al. (2001) Colorectal cancers--experience at a regional cancer centre in India. *Tropical gastroenterology: official journal of the Digestive Diseases Foundation* 22: 83-86.
- 18 Tepper JE, O'Connell MJ, Niedzwiecki D, Hollis D, Compton C, et al. (2001) Impact of number of nodes retrieved on outcome in patients with rectal cancer. *Journal of clinical oncology* 19: 157-163.
- 19 Tsai HL, Lu CY, Hsieh JS, Wu DC, Jan CM, et al. (2007) The prognostic significance of total lymph node harvest in patients with T2-4N0M0 colorectal cancer. *Journal of gastrointestinal surgery* 11: 660-665.
- 20 Morcos B, Baker B, Al Masri M, Haddad H, Hashem S (2010) Lymph node yield in rectal cancer surgery: effect of preoperative chemoradiotherapy. *European journal of surgical oncology* 36: 345-349.
- 21 O'Dwyer ST, Haboubi NY, Johnson JS, Gardy R (2001) Detection of lymph node metastases in colorectal carcinoma. *Colorectal disease* 3: 288-294.
- 22 Scott KW, Grace RH (1989) Detection of lymph node metastases in colorectal carcinoma before and after fat clearance. *Br J Surg* 76: 1165-1167.
- 23 Sarli L, Bader G, Iusco D, Salvemini C, Mauro DD, et al. (2005) Number of lymph nodes examined and prognosis of TNM stage II colorectal cancer. *Eur J Cancer* 41: 272-279.
- 24 Caplin S, Cerottini JP, Bosman FT, Constanda MT, Givel JC (1998) For patients with Dukes' B (TNM Stage II) colorectal carcinoma, examination of six or fewer lymph nodes is related to poor prognosis. *Cancer* 83: 666-672.
- 25 Withers HR, Haustermans K (2004) Where next with preoperative radiation therapy for rectal cancer? *International journal of radiation oncology, biology, physics* 58: 597-602.
- 26 Scabini S, Montecucco F, Nencioni A, Zoppoli G, Sartini M, et al. (2013) The effect of preoperative chemoradiotherapy on lymph nodes harvested in TME for rectal cancer. *World J Surg Oncol* 11: 292.
- 27 Ihnat P, Delongova P, Dvorackova J, Horacek J, Vavra P, et al. (2013) Improving the quality of histopathological examination of colorectal cancer specimens through standard protocol implementation. *Rozhl Chir* 92: 703-707.
- 28 Sprenger T, Rothe H, Langer C, Becker H, Liersch T (2009) Comment on "lymph nodes after preoperative chemoradiotherapy for rectal carcinoma: number, status, and impact on survival". *The American journal of surgical pathology* 33: 1107.
- 29 Sprenger T, Rothe H, Becker H, Beissbarth T, Homayounfar K, et al. (2013) Lymph node metastases in rectal cancer after preoperative radiochemotherapy: impact of intramesorectal distribution and residual micrometastatic involvement. *The American journal of surgical pathology* 37: 1283-1289.
- 30 Sermier A, Gervaz P, Egger JF, Dao M, Allal AS, et al. (2006) Lymph node retrieval in abdominoperineal surgical specimen is radiation time-dependent. *World J Surg Oncol* 4: 29.
- 31 Moore HG, Gittleman AE, Minsky BD, Wong D, Paty PB, et al. (2004) Rate of pathologic complete response with increased interval between preoperative combined modality therapy and rectal cancer resection. *Dis Colon Rectum* 47: 279-286.

- 32 Francois Y, Nemoz CJ, Baulieux J, Vignal J, Grandjean JP, et al. (1999) Influence of the interval between preoperative radiation therapy and surgery on downstaging and on the rate of sphincter-sparing surgery for rectal cancer: the Lyon R90-01 randomized trial. *Journal of clinical oncology* 17: 2396.
- 33 Gerard JP, Baulieux J, Francois Y, Grandjean JP, Romestaing P, et al. (1998) The role of radiotherapy in the conservative treatment of rectal carcinoma--the Lyon experience. *Acta Oncol* 37: 253-258.
- 34 Thorn CC, Woodcock NP, Scott N, Verbeke C, Scott SB, et al. (2004) What factors affect lymph node yield in surgery for rectal cancer? *Colorectal disease* 6: 356-361.
- 35 Maschuw K, Kress R, Ramaswamy A, Braun I, Langer P, et al. (2006) Short-term preoperative radiotherapy in rectal cancer patients leads to a reduction of the detectable number of lymph nodes in resection specimens. *Langenbecks Arch Surg* 391: 364-368.
- 36 Wright FC, Law CH, Last L, Khalifa M, Arnaout A, et al. (2003) Lymph node retrieval and assessment in stage II colorectal cancer: a population-based study. *Annals of surgical oncology* 10: 903-909.
- 37 Burke HB (2004) Outcome prediction and the future of the TNM staging system. *J Natl Cancer Inst* 96: 1408-1409.
- 38 Stipa F, Zerneck A, Moore HG, Minsky BD, Wong WD, et al. (2004) Residual mesorectal lymph node involvement following neoadjuvant combined-modality therapy: rationale for radical resection? *Annals of surgical oncology* 11: 187-191.