

## Variation in Lymph Node Evaluation in Rectal Cancer: A Dutch Nationwide Population-Based Study

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### ABSTRACT

**Background.** For adequate staging and subsequent accurate estimation of prognosis, a sufficient number of lymph nodes (LNs) has to be evaluated. This study aimed to identify factors associated with adequate nodal evaluation and to determine its relationship with survival.

**Methods.** Data from all patients with stage I to III rectal carcinoma who underwent surgical treatment and who were diagnosed in the period 2000 to 2006 were retrieved from the Netherlands Cancer Registry. Multilevel logistic analysis was performed to examine the influence of relevant factors on the number of evaluated LNs. Kaplan–Meier and Cox regression analyses were used to analyze the association with overall survival.

**Results.** The number of evaluated LNs was determined for 10,788 (91%) of 11,818 tumors. Median number of evaluated LNs was 7, ranging from 4 to 11 between pathology laboratories. The proportion of patients with positive LNs increased with increasing number of evaluated LNs. Men, younger patients, tumors with deeper invasion and nodal involvement, patients without preoperative radiotherapy who underwent a low anterior resection, and patients whose LNs were evaluated in an academic pathology

laboratory were more likely to have  $\geq 12$  LNs evaluated. After adding these factors to the model, unexplained variation between pathology laboratories and between hospitals remained. The overall survival increased with increasing number of evaluated LNs.

**Conclusions.** A large variation in LN evaluation among patients with rectal cancer was revealed. Improvement in LN evaluation by both hospitals and pathology laboratories could improve staging, leading to more reliable estimation of prognosis.

Colorectal cancer is a common disease in the Netherlands. In 2007, almost 12,000 new patients were diagnosed, among whom approximately 3,300 have a rectal cancer, whereas approximately 1,000 patients died of rectal cancer.<sup>1,2</sup>

Stage of disease at diagnosis is an important prognostic factor in patients with rectal cancer, in particular the presence of nodal and distant metastases are associated with worse survival.<sup>3</sup> For adequate staging and subsequently an accurate estimation of prognosis, a sufficient number of lymph nodes (LNs) must be evaluated. However, a widely accepted standard of the number of evaluated LNs required is still lacking. The guidelines of the International Union Against Cancer advise the evaluation of at least 12 LNs. The Dutch guidelines for rectal cancer recommend a minimum of 10 evaluated LNs because one of the criteria for patients at high risk for stage II disease is  $<10$  evaluated LNs.<sup>4,5</sup>

In several studies, a large variation in number of evaluated LNs was found.<sup>6,7</sup> These differences in retrieval of LNs have been attributed to, among other things, extent of lymphadenectomy and accuracy of pathologic examination.<sup>6,8,9</sup> Furthermore, individual differences in the biological behavior of tumor and host may affect the number of evaluated LNs.<sup>10</sup>

Because LNs are more easier to collect among patients with colon cancer compared to patients with rectal cancer, this could lead to more adequate LN evaluation in these patients.<sup>11</sup> Furthermore, in patients with rectal cancer, preoperative radiotherapy results in fewer LNs evaluated.<sup>12,13</sup> Therefore, only patients with rectal cancer were included in this study.

The purposes of this study were: (1) to describe variation in LN evaluation in patients with rectal cancer; (2) to identify factors associated with adequate LN evaluation, and (3) to analyze the relationship between number of evaluated LNs and survival. The hypothesis to be tested was that both hospitals and pathology laboratories have an influence on the quality of LN evaluation and that a higher number of evaluated LNs is associated with better staging and improved overall survival.

## METHODS

### *Netherlands Cancer Registry*

The nationwide population-based Netherlands Cancer Registry (NCR) includes all newly diagnosed malignancies. Notification is obtained from the automated pathology archive (PALGA), hematological departments, and the National Registry of Hospital Discharge Diagnosis, which accounts for up to 8% of new cases.<sup>14,15</sup>

All data are obtained from patient files in the hospital. Specially trained registration assistants collect patient, tumor, and treatment characteristics. Topography and morphology are coded according to the International Classification of Diseases for Oncology (ICD-O) and staging according to the tumor, node, metastasis system (TNM) classification.<sup>5,16</sup> Data quality is high, and data completeness is estimated to be at least 95%.<sup>17,18</sup> Follow-up of all patients is completed up to January 2008 by linking the NCR to the municipality registry. Death certificates are not available in an identifiable form to the NCR.

### *Patients*

From the NCR, all patients who underwent surgical resection for rectal cancer (C20.9), stages I to III (pT1–4NanyM0), and who were diagnosed in the period 2000 to 2006 were selected ( $N = 11,818$ ). Patients with

rectosigmoid cancer and patients who only underwent polypectomy or another kind of local resection were excluded. Patients with rectosigmoid tumors were excluded because treatment strategies differed widely in this group—sometimes these tumors were treated as colon tumors and in other cases as rectum tumors. Furthermore, patients with 0 evaluated LNs ( $N = 629$ ) were excluded from all analyses because the registration of 0 evaluated LNs was not unambiguous over time and between regions. It could also mean that it was unknown whether LNs were evaluated or that the number of evaluated LNs was not registered in the NCR. Patients in whom all evaluated LNs were negative were considered as pN0, irrespective of the number of evaluated LNs. Type of radiotherapy was categorized into preoperative radiotherapy, preoperative chemoradiation, postoperative radiotherapy, and no radiotherapy.

### *Hospitals and Pathology Laboratories*

Type of hospital was linked to the hospital where the surgery was performed, including three categories: non-teaching, teaching, and university hospitals. A teaching hospital was defined as a hospital that provided medical training to surgical residents. A university hospital was defined as a teaching hospital affiliated with a university. The one categorical oncology center in the Netherlands was classified as university hospital. Most pathology laboratories served more than one hospital. The pathology laboratories used different methods for pathological review. Since the 1980s, regional guidelines have been developed that include compulsory items for a pathology report, including TNM classification and number of evaluated LNs. All pathology reports are submitted to a national database (PALGA) that gives yearly feedback on the quality of reporting and coding of diseases. Quality assessment is organized via a national quality assurance program, including visits of laboratories by professionals. Surgery was performed in 97 different hospitals, and LN evaluation was performed in 58 different pathology laboratories.

### *Statistical Analyses*

Differences in LN retrieval between groups were tested by  $\chi^2$  test. Lymph node ratio (LNR), determined by dividing the number of positive nodes by the total number of evaluated nodes, was split into quartiles with cutoff points at 0.167, 0.332, and 0.599.

The influence of sex, age at diagnosis, year of diagnosis, depth of invasion, LN involvement, tumor grade, type of radiotherapy, type of surgery, type of hospital, and type of pathology laboratory on adequate LN evaluation ( $\geq 12$

evaluated LNs) was evaluated by logistic multilevel analysis. Multilevel analysis takes into account a hierarchical structure. In this study, the data had a three-level structure: patients with rectal cancers were clustered within hospitals of surgery, and hospitals of surgery were clustered within pathology laboratories. The intraclass correlation coefficient (ICC) is an estimation of the dependency of observations within a level.<sup>19</sup> First, a null model was estimated. Second, patient and tumor characteristics were added stepwise into the model.

The relationship between number of evaluated LNs and overall survival was analyzed and adjusted for sex, age at diagnosis, year of diagnosis, depth of invasion, tumor grade, number of positive nodes, type of radiotherapy, type of surgery, and adjuvant chemotherapy by Cox proportional hazard modeling. Patients with a history of another malignancy were excluded from the multivariate survival analyses. The Kaplan–Meier method was used to analyze the relation between the number of evaluated LNs and 5-year overall survival. Follow-up time was calculated as the time from diagnosis to death or January 1, 2008, the date of linking with the municipality registry.

*P* values were considered significant at the 0.05 level. For all analyses, STATA software, version 10.0 (Stata-Corp, College Station, TX), was used.

## RESULTS

The number of LNs could be determined for 10,788 (91%) of the 11,818 patients (Table 1). In 17% of all patients with pN0 and in 27% of all patients with pN+ disease,  $\geq 12$  LNs were evaluated. This improved over time, from 12% in 2000 to 30% in 2006, and from 20% in 2000 to 41% in 2006. The median number of evaluated LNs was 6 among patients with pN0 and 8 among patients with pN+ disease; the mean number of evaluated LNs was 7.4 and 9.5, respectively. Among patients with pN0 disease, the median number of evaluated LNs was highest in patients operated in a nonteaching hospital, whose LNs were evaluated in an academic pathology laboratory. Among patients with pN+ disease, this median was highest in patients operated in a university hospital, whose LNs were evaluated in an academic pathology laboratory.

The median number of evaluated LNs by pathology laboratory ranged from 4 to 11 LNs (Fig. 1). The academic pathology laboratory had the highest median number of evaluated LNs. The quartile of pathology laboratories with the lowest median number of evaluated LNs (median  $< 6$  LNs) had a proportion of patients with positive LNs of 34%. Among the quartile of pathology laboratories with the highest median number (median  $> 8$  LNs), this proportion was 39% ( $P < 0.001$ ).

The proportion of patients who had any LNs evaluated, but for whom the number of LNs was not stated in the pathology report, was 7% for patients with pN0 disease and 8% for patients with pN+ disease. This proportion decreased over time, from 10% in 2000 to 2% in 2006 for patients with pN0 disease, and from 13 to 2% for patients with pN+ disease. The proportion of patients with an unknown number of evaluated LNs ranged from 0 to 46% between pathology laboratories. In 11 pathology laboratories, this proportion was  $> 10\%$ .

The proportion of patients with pN+ disease increased with a rising number of evaluated LNs (Fig. 2a). The increase becomes less steep at approximately 9 evaluated LNs. The highest proportion of patients with pN+ disease was found in patients with 18 evaluated LNs.

In the null model of the multilevel analysis, both the variance of the hospital level and the variance of the pathology laboratory level was statistically significant. The ICC of the hospital level was 0.040 and of the pathology laboratory level was 0.104, meaning that 4.0% of the total variance could be attributed to the hospital level and 10.4% to the pathology laboratory level. Table 2 shows the results of the logistic multilevel analysis. Men, younger patients, and tumors with nodal involvement were more likely to have  $\geq 12$  LNs evaluated. Tumors with a deeper invasion had a higher odds of having  $\geq 12$  LNs evaluated compared to patients with a T1 tumor. The odds ratio (OR) increased by year of diagnosis, up to 3.57 (95% CI 2.93–4.34) in 2006. Patients who received postoperative radiotherapy or no radiotherapy had a higher odds of having  $\geq 12$  LNs evaluated compared to patients who received preoperative radiotherapy (respectively, OR 1.33, 95% confidence interval [95% CI] 1.04–1.71; and OR 1.54, 95% CI 1.37–1.74), and patients who received preoperative radiotherapy and chemotherapy had a lower odds of having  $\geq 12$  LNs evaluated (OR 0.77, 95% CI 0.60–0.98). Patients who underwent an abdominoperineal resection were less likely to have  $\geq 12$  LNs evaluated compared to patients who underwent a low anterior resection. Patients whose LNs were evaluated in an academic pathology laboratory, irrespective of type of hospital of surgery, had a statistically significant higher OR of having  $\geq 12$  LNs. After adding these variables to the model, the variances of both levels remained significant, meaning that there was still unexplained variation within the group of hospitals and within the group of pathology laboratories. Of the total variance, 5.0% (ICC 0.050) could be attributed to the hospital level and 6.9% (ICC 0.069) to the pathology laboratory level.

Both among patients with pN+ and among patients with pN0 disease, the overall survival was lower among patients with  $< 10$  evaluated LNs compared to patients with 10 to 12 evaluated LNs, after adjustment for relevant factors (Table 3). The survival was also lower for patients with an

**TABLE 1** Number of evaluated LNs and characteristics of study population (with surgical treatment, pT1–4NanyM0, 2000–2006) according to LN involvement

Variable	pN0 (N = 7500)		pN+ (N = 4318)			
	N	%	N	%		
<b>No. of evaluated LNs</b>						
1–3	1718	23	385	9		
4–6	1832	24	979	23		
7–9	1362	18	946	22		
10–12	887	12	698	16		
13–15	480	6	438	10		
16–18	252	3	248	6		
19–21	146	2	124	3		
≥22	141	2	152	4		
Unknown, but ≥1	556	7	325	8		
Unknown whether LNs were examined/not registered in the NCR	126	2	23	1		
Characteristic	N	%	Median examined LNs	N	%	Median examined LNs
Total no. of patients with exact number of examined LNs (≥1 LN)	6818	100	6	3970	100	8
<b>Sex</b>						
Male	4144	61	6	2314	58	8
Female	2674	39	6	1656	42	8
<b>Age at diagnosis (y)</b>						
<50	438	6	8	342	9	10
50–69	3436	50	6	2064	52	8
≥70	2944	43	6	1564	39	8
<b>Year of diagnosis</b>						
2000	783	11	6	452	11	8
2001	811	12	5	478	12	7
2002	935	14	5	559	14	8
2003	945	14	6	551	14	7
2004	1078	16	6	606	15	8.5
2005	1082	16	7	654	16	9
2006	1184	17	8	670	17	10
<b>Depth of invasion</b>						
pT1	685	10	5	113	3	6
pT2	2886	42	6	825	21	8
pT3	3033	44	7	2771	70	9
pT4	214	3	6	261	7	9
<b>Tumor grade</b>						
Well differentiated	337	5	6	143	4	8
Moderately differentiated	4362	64	6	2342	59	8
Poorly differentiated/undifferentiated	674	10	7	768	19	9
Unknown	1445	21	6	717	18	9
<b>Type of RT</b>						
Preoperative RT	4248	62	6	2602	66	8

TABLE 1 continued

Characteristic	N	%	Median examined LNs	N	%	Median examined LNs
Preoperative RT and CT	334	5	6	181	7	9
Postoperative RT	181	3	6	265	5	9
No RT	2055	30	7	922	23	9
Type of surgery						
Low anterior resection	3575	52	6	2056	52	8
Abdominoperineal resection	2519	37	6	1498	38	8
Other	724	11	7	416	10	9
Adjuvant chemotherapy						
No	6725	99	6	3087	78	8
Yes	93	1	6	883	22	9
Type of hospital and type of pathology laboratory						
Nonteaching hospital and laboratory	1562	23	6	882	22	8
Teaching hospital and nonteaching laboratory	1696	25	6	967	24	8
Nonteaching hospital and teaching laboratory	1179	17	6	717	18	9
Teaching hospital and laboratory	1693	25	6	999	25	8
Nonteaching hospital and academic laboratory	105	2	9	69	2	9
Academic hospital and laboratory	583	9	8	336	8	10

LN lymph node, NCR Netherlands Cancer Registry, RT radiotherapy, CT chemotherapy

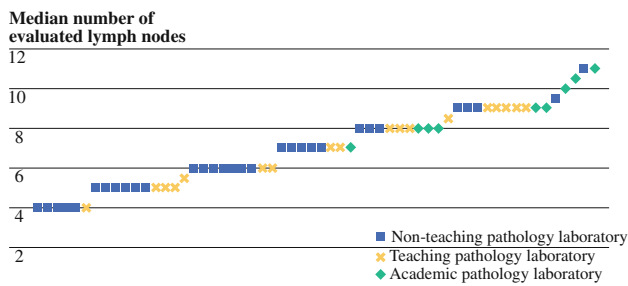


FIG. 1 Median number of evaluated lymph nodes according to type of pathology laboratory

unknown number of evaluated LNs among patients with pN0 disease (hazard ratio [HR] 1.42, 95% CI 1.11–1.81) and among patients with pN+ disease (HR 1.52; 95% CI 1.22–1.90).

Among patients who received preoperative radiotherapy, the survival was not statistically significantly lower for patients with negative LNs with 4 to 9 evaluated LNs and with an unknown number of evaluated LNs compared to patients with 10 to 12 evaluated LNs. Patients with positive nodes who received preoperative radiotherapy of whom the number of evaluated LNs was not registered in the NCR had a statistically significant worse survival (HR 3.57, 95% CI 1.21–10.53), but the number of patients was very small.

After adding LNR to the survival analyses of the patients with pN+ disease, the survival of patients with

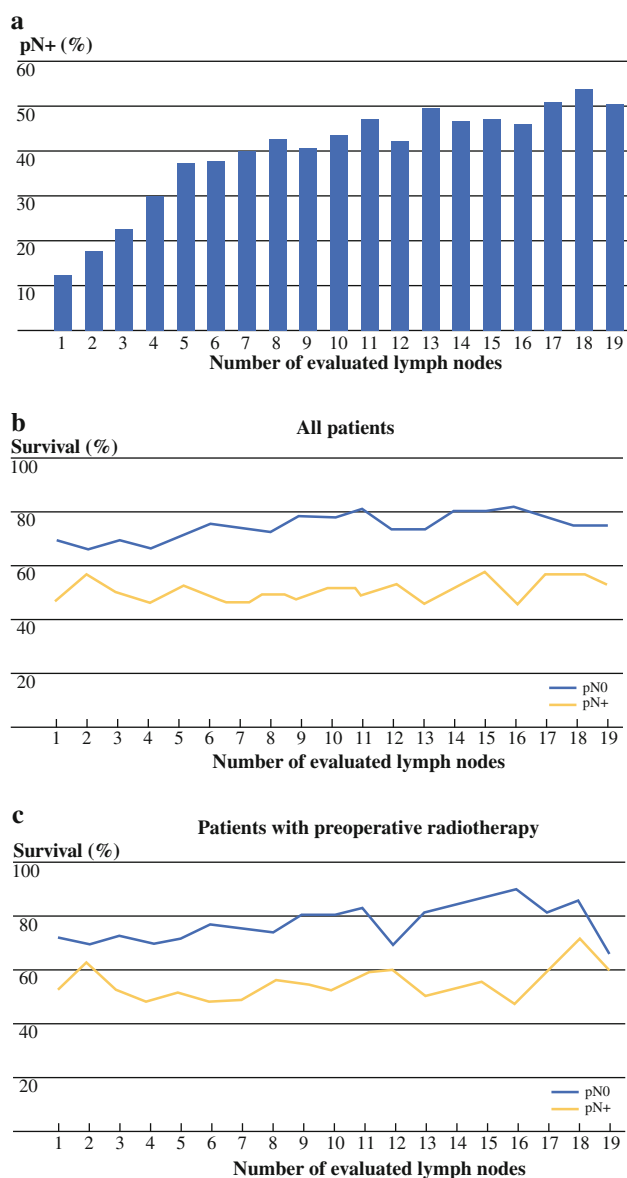
<10 evaluated LNs is not statistically significantly lower compared to patients with 10 to 12 evaluated LNs (Table 4). The third and fourth quartile of the LNR had a lower survival compared to the first quartile of the LNR, respectively (HR 1.53, 95% CI 1.25–1.87; and HR 2.03, 95% CI 1.60–2.58).

Figure 2b demonstrates 5-year overall survival according to number of evaluated LNs and nodal involvement for the whole study population. Figure 2c demonstrates the same for patients who received preoperative radiotherapy. Both figures show an improved survival by an increasing number of evaluated LNs for patients with pN0 disease.

In Fig. 2b, the line flattened from approximately 9 evaluated LNs. The difference between survival of patients with pN0 and pN+ disease became larger from approximately 6 evaluated LNs. In Fig. 2c, survival increased until 16 evaluated LNs, but then there was a decline at 12 evaluated LNs among patients with pN0 disease.

## DISCUSSION

The present population-based study showed a large variation between pathology laboratories and between hospitals in the number of evaluated LNs in patients with rectal carcinoma in the Netherlands in the period 2000 to 2006. Although the International Union Against Cancer recommends a minimum of 12 evaluated LNs for accepting the N0 status, in only 17% of the pN0 patients were  $\geq 12$



**FIG. 2** Proportion of patients with positive nodes (pN+ disease) according to number of evaluated lymph nodes (a) and 5-year overall survival rates (Kaplan–Meier method) in all patients (b) and in patients who received preoperative radiotherapy (c)

LN evaluated. However, a steady and marked improvement was observed over time from 12% in 2000 to 30% in 2006. A population-based study in the southern part of the Netherlands suggested that this improvement in LN evaluation over time might be due to feedback to medical specialists.<sup>20</sup>

This study described a large variation in LN evaluation between pathology laboratories. After adjustment in the multilevel analysis for pathology laboratories, variation between the hospitals remained, suggesting a role for both surgeons and pathologists. The extent of the resection of surgeons and the diligence of pathologists in searching the

specimen for LNs both have an influence on the LN yield.<sup>11</sup> Collaboration between surgeons and pathologists, including giving feedback to each other, could lead to improvement in LN evaluation.

Several factors affected adequate LN evaluation. Differences in immune response of patients may clarify the effect of age and sex.<sup>21</sup> Positive LNs are slightly larger than negative LNs.<sup>22,23</sup> As a consequence, positive LNs are easier to identify by a pathologist, clarifying the influence of LN status on adequate LN evaluation. Two other studies reported, similar to our result, a lower LN retrieval in patients who underwent an abdominoperineal resection compared to patients who underwent a low anterior resection.<sup>13,24</sup> Low anterior resections include often high ligation of the inferior mesenteric artery, leading to an increased LN retrieval.<sup>25</sup>

Two single-institution studies reported a lower LN retrieval after preoperative chemoradiotherapy compared to surgery alone.<sup>13,24</sup> We compared the LN retrieval after preoperative chemoradiotherapy with the LN retrieval after preoperative radiotherapy and found a markedly lower LN yield as well.

We revealed, similar to other studies, a higher OR of having an inadequate LN evaluation in patients who received preoperative radiotherapy.<sup>12,26</sup> Radiotherapy will decrease the number and size of, involved and uninvolved LNs, and consequently, it may be more difficult to find them.<sup>27</sup>

Patients whose LNs were evaluated in an academic pathology laboratory, irrespective of the type of hospital where they were operated, had a higher chance of adequate LN evaluation. This suggests that the academic status of the pathology laboratories has a large effect. An explanation could be workload. The examination and detection of LNs is a labor-intensive and time-consuming process, suggesting that academic pathology laboratories can provide greater scrutiny, as may be expected from engaging in research activities. However, there were also nonacademic pathology laboratories with a high median number of evaluated LNs, pointing to the influence of other factors as well. Several studies demonstrated variation between individual surgeons and pathologists.<sup>8,9,28</sup> Unfortunately, no detailed information on surgeon or pathologist level was available on a national basis.

Adjuvant chemotherapy for patients with rectal cancer is in some countries recommended or standard therapy. In the Netherlands, some hospitals administer adjuvant chemotherapy to patients with rectal cancer, but it is not recommended in the guidelines.<sup>4</sup> Therefore, although LN evaluation does not have implications in determining treatment strategies, it is essential for including eligible patients in trials concerning adjuvant treatment. Furthermore, because the presence of nodal metastases is an

**TABLE 2** Multilevel logistic regression with ORs of having had  $\geq 12$  LNs evaluated (multivariate analysis)

Characteristic	OR	95% CI	P
Sex			
Male	1.00	Reference	
Female	0.85	0.77–0.94	0.002
Age at diagnosis (y)			
<50	1.00	Reference	
50–69	0.68	0.57–0.82	<0.001
$\geq 70$	0.51	0.42–0.62	<0.001
Year of diagnosis			
2000	1.00	Reference	
2001	1.01	0.81–1.26	0.917
2002	1.13	0.91–1.40	0.265
2003	1.26	1.02–1.56	0.036
2004	1.60	1.30–1.97	<0.001
2005	2.15	1.76–2.63	<0.001
2006	3.57	2.93–4.34	<0.001
Depth of invasion			
pT1	1.00	Reference	
pT2	1.95	1.53–2.49	<0.001
pT3	2.57	2.02–3.27	<0.001
pT4	2.38	1.72–3.27	<0.001
LN involvement			
pN0	1.00	Reference	
pN+	1.61	1.45–1.79	<0.001
Tumor grade			
Well differentiated	1.00	Reference	
Moderately differentiated	0.91	0.71–1.17	0.472
Poorly differentiated/undifferentiated	1.04	0.79–1.37	0.779
Unknown	0.88	0.67–1.15	0.343
Type of RT			
Preoperative RT	1.00	Reference	
Preoperative RT and CT	0.77	0.60–0.98	0.035
Postoperative RT	1.33	1.04–1.71	0.023
No RT	1.54	1.37–1.74	<0.001
Type of surgery			
Low anterior resection	1.00	Reference	
Abdominoperineal resection	0.77	0.69–0.86	<0.001
Other	1.07	0.91–1.26	0.412
Type of hospital and type of pathology laboratory			
Nonteaching hospital and nonteaching laboratory	1.00	Reference	
Teaching hospital and nonteaching laboratory	0.82	0.62–1.07	0.143
Nonteaching hospital and teaching laboratory	1.17	0.82–1.65	0.388
Teaching hospital and teaching laboratory	1.41	0.99–1.99	0.056
Nonteaching hospital and academic laboratory	2.59	1.08–6.21	0.033
Academic hospital and academic laboratory	2.59	1.70–3.95	<0.001

OR odds ratio, LN lymph node, 95% CI 95% confidence interval, RT radiotherapy, CT chemotherapy

important prognostic factor, adequate LN evaluation remains important for the estimation of prognosis.

Several studies have, similar to our study, demonstrated that a low number of evaluated LNs is associated with

worse prognosis of patients.<sup>11,29</sup> An explanation could be that surgeons may have performed an incomplete resection without, or with a less thorough, nodal dissection, leading to worse survival.<sup>11</sup> Another clarification for this relation in

**TABLE 3** Multivariate survival analyses of overall survival according to LN involvement among all patients and among patients with preoperative radiotherapy

No. of LNs evaluated <sup>a</sup>	pN0			pN+		
	HR	95% CI	<i>P</i>	HR	95% CI	<i>P</i>
All patients						
1–3	1.52	1.24–1.85	<0.001	1.43	1.15–1.78	0.008
4–9	1.25	1.04–1.52	0.020	1.23	1.06–1.78	0.001
10–12	1.00	Reference		1.00	Reference	
13–15	1.10	0.83–1.48	0.504	0.94	0.76–1.16	0.574
16–18	0.91	0.62–1.34	0.640	0.86	0.66–1.12	0.273
19–21	0.82	0.49–1.37	0.445	1.03	0.74–1.42	0.881
≥22	0.78	0.44–1.38	0.396	0.91	0.64–1.29	0.607
Unknown	1.42	1.11–1.81	0.005	1.52	1.22–1.90	<0.001
Unknown whether LNs were examined/not registered in NCR	0.72	0.39–1.31	0.281	0.72	0.38–1.38	0.320
Patients with preoperative RT						
1–3	1.36	1.05–1.77	0.021	1.54	1.17–2.03	0.002
4–9	1.18	0.92–1.52	0.192	1.43	1.17–1.74	<0.001
10–12	1.00	Reference		1.00	Reference	
13–15	0.81	0.53–1.22	0.303	0.97	0.73–1.28	0.805
16–18	0.65	0.35–1.23	0.191	0.83	0.60–1.16	0.274
19–21	0.95	0.47–1.89	0.874	0.76	0.48–1.21	0.246
≥22	0.91	0.44–1.88	0.791	0.90	0.56–1.45	0.663
Unknown	1.27	0.92–1.76	0.146	1.84	1.39–2.45	<0.001
Unknown whether LNs were examined/not registered in NCR	0.63	0.27–1.43	0.265	3.57	1.21–10.53	0.021

LN lymph node, RT radiotherapy, HR hazard ratio, 95% CI 95% confidence interval, NCR Netherlands Cancer Registry

<sup>a</sup> Adjusted for age at diagnosis, sex, year of diagnosis, depth of invasion, tumor grade, number of positive nodes, type of RT, type of surgery, and adjuvant chemotherapy

patients with negative LNs could be understaging of the disease as a result of falsely categorizing node-positive patients as node negative. When more LNs were analyzed, these patients were more likely to be correctly classified as node positive. The relation between number of evaluated LNs and survival was also found in patients with positive LNs, suggesting the influence of other factors. It may reflect the variability of the host response to the tumor. Patients with fewer LNs may be patients with a reduced immune response to their cancer, leading to smaller, more difficult to detect, LNs.<sup>30</sup>

The LNR also plays an important role in the survival of patients with positive LNs. Several studies, including ours, showed a better survival for patients with a low LNR.<sup>31–33</sup> The prognosis of patients with the same number of positive LNs, but with variation in number of evaluated LNs, differs. The LNR distinguishes between these subgroups and is therefore an important prognostic factor. After adding LNR to the survival analyses, there were no longer any statistically significant differences between number of

evaluated LNs, whereas patients with a higher LNR had far worse survival, indicating the LNR was a more important prognostic factor than LN count.

Recommendations about the minimum number of evaluated LNs vary in literature from 6 to 17 to as many as possible.<sup>34–36</sup> According to the Dutch guidelines, 10 LNs have to be evaluated for accepting N0 status.<sup>4</sup> In our study, after approximately 9 evaluated LNs, the proportion of patients with pN+ disease still increased when evaluating more LNs, but it was less sharp. However, 5-year overall survival flattened at approximately 9 evaluated LNs, suggesting the cutoff of 10 evaluated LNs in the Dutch guidelines was well chosen.

In conclusion, this population-based study reported a large variation in LN evaluation between pathology laboratories and between hospitals in patients with rectal cancer in the Netherlands, thus leading to understaging of patients. Patients whose LNs were evaluated in an academic pathology laboratory had a higher chance of adequate LN evaluation. Survival decreased by decreasing number of



**TABLE 4** Multivariate survival analysis of overall survival among patients with pN+ disease

Characteristic	HR	95% CI	P
No. of LNs evaluated <sup>a</sup>			
1–3	0.86	0.65–1.14	0.293
4–9	1.03	0.87–1.21	0.752
10–12	1.00	Reference	
13–15	0.97	0.79–1.20	0.804
16–18	0.95	0.73–1.25	0.734
19–21	1.19	0.86–1.65	0.302
≥22	1.07	0.75–1.53	0.718
No. of positive nodes			
1–3	1.00	Reference	
4–6	1.10	0.92–1.31	0.306
>6	1.46	1.13–1.87	0.003
LN ratio <sup>b</sup>			
First quartile (0–0.167)	1.00	Reference	
Second quartile (0.168–0.332)	1.07	0.89–1.29	0.462
Third quartile (0.333–0.599)	1.53	1.25–1.87	<0.001
Fourth quartile (0.600–1)	2.03	1.60–2.58	<0.001

HR hazard ratio, 95% CI 95% confidence interval, LN lymph node

<sup>a</sup> Adjusted for sex, age at diagnosis, year of diagnosis, depth of invasion, tumor grade, type of radiotherapy, type of surgery and adjuvant chemotherapy

<sup>b</sup> LN ratio was the number of positive lymph nodes divided by the number of evaluated lymph nodes

evaluated LNs. Both surgeons and pathologists are responsible for improvement in LN yield, leading to better staging and more accurate estimation of prognosis for patients with rectal cancer.

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## REFERENCES

- Comprehensive cancer centres. <http://www.cancerregistry.nl>. Accessed 18 May 2009.
- Statistics Netherlands. <http://statline.cbs.nl/StatWeb>. Accessed 3 May 2009.
- Derwinger K, Carlsson G, Gustavsson B. Stage migration in colorectal cancer related to improved lymph node assessment. *Eur J Surg Oncol*. 2007;33:849–53.
- National Working Group on Gastrointestinal Cancers. Guidelines for rectal cancer. <http://www.oncoline.nl>. Accessed 10 May 2009.
- Wittekind C, Greene FL, Hutter RVP, Klimpfinger M, Sobin LH, editors. TNM atlas. 5th ed. Berlin: Springer-Verlag; 2004.
- Baxter NN, Virnig DJ, Rothenberger DA, et al. Lymph node evaluation in colorectal cancer patients: a population-based study. *J Natl Cancer Inst*. 2005;97:219–25.
- Miller EA, Woosley J, Martin CF, Sandler RS. Hospital-to-hospital variation in lymph node detection after colorectal resection. *Cancer*. 2004;101:1065–71.
- Evans MD, Barton K, Rees A, Stamatakis JD, Karandikar SS. The impact of surgeon and pathologist on lymph node retrieval in colorectal cancer and its impact on survival for patients with Dukes' stage B disease. *Colorectal Dis*. 2008;10:157–64.
- Johnson PM, Malatjalian D, Porter GA. Adequacy of nodal harvest in colorectal cancer: a consecutive cohort study. *J Gastrointest Surg*. 2002;6:883–8.
- Caplin S, Cerottini JP, Bosman FT, Constanda MT, Givel JC. For patients with Dukes' B (TNM stage II) colorectal carcinoma, examination of six or fewer lymph nodes is related to poor prognosis. *Cancer*. 1998;83:666–72.
- Tepper JE, O'Connell MJ, Niedzwiecki D, et al. Impact of number of nodes retrieved on outcome in patients with rectal cancer. *J Clin Oncol*. 2001;19:157–63.
- Baxter NN, Morris AM, Rothenberger DA, Tepper JE. Impact of preoperative radiation for rectal cancer on subsequent lymph node evaluation: a population-based analysis. *Int J Radiat Oncol Biol Phys*. 2005;61:426–31.
- Wichmann MW, Muller C, Meyer G, et al. Effect of preoperative radiochemotherapy on lymph node retrieval after resection of rectal cancer. *Arch Surg*. 2002;137:206–10.
- Casparie M, Tiebosch AT, Burger G, et al. Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. *Cell Oncol*. 2007;29:19–24.
- Visser O, Coebergh JWW, Van Dijck JAAM, Siesling S. Incidence of cancer in the Netherlands, 1998. Utrecht: Vereniging van Integrale Kankercentra; 2002.
- Fritz A, Percy C, Jack A, et al. International classification of diseases for oncology. 3rd ed. Geneva: World Health Organization; 2000.
- Schouten LJ, Jager JJ, van den Brandt PA. Quality of cancer registry data: a comparison of data provided by clinicians with those of registration personnel. *Br J Cancer*. 1993;68:974–7.
- Schouten LJ, Hoppener P, van den Brandt PA, Knottnerus JA, Jager JJ. Completeness of cancer registration in Limburg, The Netherlands. *Int J Epidemiol*. 1993;22:369–76.
- Twisk JWR. Applied multilevel analysis. Cambridge: Cambridge University Press; 2006.
- Van Steenberghe LN, Van Lijnschoten G, Rutten HJ, Lemmens VE, Coebergh JW. Improving lymph node detection in colon cancer in community hospitals and their pathology department in southern Netherlands. *Eur J Surg Oncol*. 2010;36:135–40.
- Sarli L, Bader G, Iusco D, et al. Number of lymph nodes examined and prognosis of TNM stage II colorectal cancer. *Eur J Cancer*. 2005;41:272–9.
- Wong JH, Severino R, Honnebler MB, Tom P, Namiki TS. Number of nodes examined and staging accuracy in colorectal carcinoma. *J Clin Oncol*. 1999;17:2896–900.
- Monig SP, Baldus SE, Zirbes TK, et al. Lymph node size and metastatic infiltration in colon cancer. *Ann Surg Oncol*. 1999;6:579–81.
- Rullier A, Laurent C, Capdepon M, et al. Lymph nodes after preoperative chemoradiotherapy for rectal carcinoma: number, status, and impact on survival. *Am J Surg Pathol*. 2008;32:45–50.
- Titu LV, Tweedle E, Rooney PS. High tie of the inferior mesenteric artery in curative surgery for left colonic and rectal cancers: a systematic review. *Dig Surg*. 2008;25:148–57.
- Tekkis PP, Smith JJ, Heriot AG, et al. A national study on lymph node retrieval in resectional surgery for colorectal cancer. *Dis Colon Rectum*. 2006;49:1673–83.

27. Wheeler JM, Warren BF, Jones AC, Mortensen NJ. Preoperative radiotherapy for rectal cancer: implications for surgeons, pathologists and radiologists. *Br J Surg.* 1999;86:1108–20.
28. Leung AM, Scharf AW, Vu HN. Factors affecting number of lymph nodes harvested in colorectal cancer. *J Surg Res.* doi: [10.1016/j.jss.2009.09.001](https://doi.org/10.1016/j.jss.2009.09.001).
29. Pocard M, Panis Y, Malassagne B, et al. Assessing the effectiveness of mesorectal excision in rectal cancer: prognostic value of the number of lymph nodes found in resected specimens. *Dis Colon Rectum.* 1998;41:839–45.
30. Klintrup K, Makinen JM, Kauppila S, et al. Inflammation and prognosis in colorectal cancer. *Eur J Cancer.* 2005;41:2645–54.
31. Peng J, Xu Y, Guan Z, et al. Prognostic significance of the metastatic lymph node ratio in node-positive rectal cancer. *Ann Surg Oncol.* 2008;15:3118–23.
32. Peschaud F, Benoist S, Julie C, et al. The ratio of metastatic to examined lymph nodes is a powerful independent prognostic factor in rectal cancer. *Ann Surg.* 2008;248:1067–73.
33. Kim YS, Kim JH, Yoon SM, et al. Lymph node ratio as a prognostic factor in patients with stage III rectal cancer treated with total mesorectal excision followed by chemoradiotherapy. *Int J Radiat Oncol Biol Phys.* 2009;74:796–802.
34. Cserni G, Vinh-Hung V, Burzykowski T. Is there a minimum number of lymph nodes that should be histologically assessed for a reliable nodal staging of T3N0M0 colorectal carcinomas? *J Surg Oncol.* 2002;81:63–9.
35. Goldstein NS, Sanford W, Coffey M, Layfield LJ. 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. *Am J Clin Pathol.* 1996;106:209–16.
36. Hernanz F, Revuelta S, Redondo C, et al. Colorectal adenocarcinoma: quality of the assessment of lymph node metastases. *Dis Colon Rectum.* 1994;37:373–6.