# Variation in diagnosis, treatment and outcome in colon and rectal cancer

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# Chapter 3

# Variation in lymph node evaluation

# 3.1
# 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 2000-2006 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 analyse the association with overall survival.

**Results**

The number of evaluated LNs was determined for 10,788 (91%) of 11,818 tumours. 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. Males, younger patients, tumours 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 12 or more 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.

# Introduction

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 about 1,000 patients died of rectal cancer.[1;2]

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.[3] 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 (UICC) advise to evaluate at least 12 LNs. The Dutch guidelines for rectal cancer recommend a minimum of 10 evaluated LNs, because one of the criteria for patients with high risk stage II is less than 10 evaluated LNs.[4;5]

In several studies, a large variation in number of evaluated LNs is found.[6;7] These differences in retrieval of LNs have been attributed to, among other things, extent of the lymphadenectomy and accuracy of pathologic examination.[6;8;9] Furthermore, individual differences in biological behaviour of tumour and host may affect the number of evaluated LNs.[10] Because LNs are 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.[11] Furthermore, in patients with rectal cancer, preoperative radiotherapy results in fewer LNs evaluated.[12;13] 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 analyse 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 (NCR)**

The nationwide population-based NCR includes all newly diagnosed malignancies. Notification is obtained from the automated pathology archive (PALGA),[14] haematologi-

cal departments and the National Registry of Hospital Discharge Diagnosis, which accounts for up to 8% of new cases.[15]

All data are obtained from patient files in the hospital. Specially trained registration assistants collect patient, tumour and treatment characteristics. Topography and morphology are coded according to the International Classification of Diseases for Oncology (ICD-O) and staging according to the TNM classification.[5;16] Data quality is high and data completeness is estimated to be at least 95%.[17;18] 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-III (pT1-4NanyM0), and who were diagnosed in the period 2000-2006, 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 tumours were excluded, because treatment strategies differed widely in this group, e.g. sometimes these tumours were treated as colon tumours and in other cases as rectum tumours. 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 categorised 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 centre 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 organised via a national quality assurance program including visits of laboratories by professionals. Surgery was performed in 97 different hospitals and LN evaluation was done in 58 different pathology laboratories.

**Statistical analyses**

Differences in LN retrieval between groups were tested using a chi-square 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 cut-off points at 0.167, 0.332 and 0.599.

The influence of gender, age at diagnosis, year of diagnosis, depth of invasion, LN involvement, tumour grade, type of radiotherapy, type of surgery, type of hospital and type of pathology laboratory on adequate LN evaluation (≥12 evaluated LNs) was evaluated using 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 intra-class correlation coefficient (ICC) is an estimation of the dependency of observations within a level.[19] First, a null model was estimated. Second, patient and tumour characteristics were added stepwise into the model.

The relationship between number of evaluated LNs and overall survival was analysed and adjusted for gender, age at diagnosis, year of diagnosis, depth of invasion, tumour grade, number of positive nodes, type of radiotherapy, type of surgery and adjuvant chemotherapy, using Cox proportional hazard modelling. Patients with a history of another malignancy were excluded from the multivariate survival analyses. The Kaplan-Meier method was used to analyse 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 1 January 2008, the date of linking with the municipality registry.

P values were considered significant at the 0.05 level. For all analyses, STATA version 10.0 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+, 12 or more LNs were evaluated. This improved over time, from 12% in 2000 to 30% in 2006, and from 20% in 2000 to 41% in 2006, respectively. The median number of evaluated LNs was 6 among patients with pN0 and 8 among patients with pN+; the mean number of evaluated LNs was 7.4 and 9.5, respectively. Among patients with pN0, the median number of evaluated LNs was highest in patients operated in a non-teaching hospital, whose LNs were evaluated in an academic pathology laboratory. Among patients with pN+, this

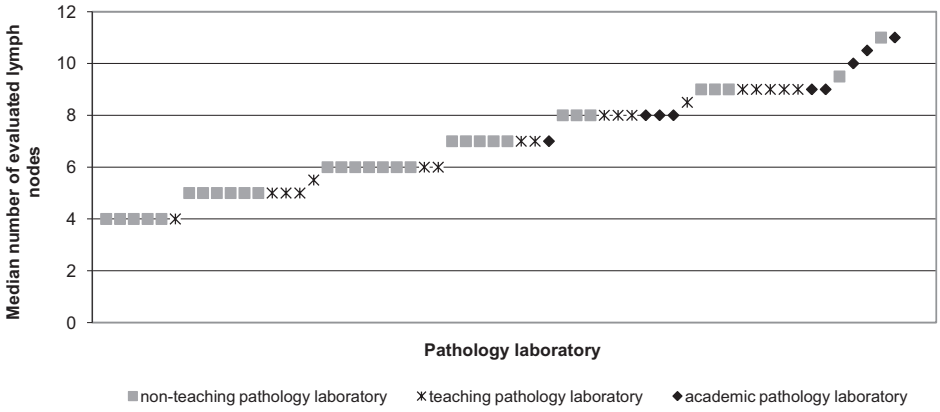



*Figure 1 Median number of evaluated lymph nodes according to (type of) pathology laboratory*

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 (Figure 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).

*Table 1 Number of evaluated lymph nodes and characteristics of study population (with surgical treatment, pT1-4NanyM0, 2000-2006) according to LN involvement*

| | pN0 (N=7,500) | | pN+ (N=4,318) | |
|---|---|---|---|---|
| | N | % | N | % |
| 1-3 | 1,718 | 23 | 385 | 9 |
| 4-6 | 1,832 | 24 | 979 | 23 |
| 7-9 | 1,362 | 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 |
| Number of examined LN unknown, but ≥1 | 556 | 7 | 325 | 8 |
| Unknown whether LN were examined / Not registered in the NCR | 126 | 2 | 23 | 1 |

(to be continued on the next page)

*Table 1 (continuation)*

| | pN0 (N=7,500) | | | pN+ (N=4,318) | | |
|---|---|---|---|---|---|---|
| Total of patients with exact number of examined LNs | N | % | Median examined LNs | N | % | Median examined LNs |
| (≥1 LN) | 6,818 | 100 | 6 | 3,970 | 100 | 8 |
| Gender | | | | | | |
| Male | 4,144 | 61 | 6 | 2,314 | 58 | 8 |
| Female | 2,674 | 39 | 6 | 1,656 | 42 | 8 |
| Age at diagnosis (yrs) | | | | | | |
| <50 | 438 | 6 | 8 | 342 | 9 | 10 |
| 50-69 | 3,436 | 50 | 6 | 2,064 | 52 | 8 |
| ≥70 | 2,944 | 43 | 6 | 1,564 | 39 | 8 |
| Year of diagnosis | | | | | | |
| 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 | 1,078 | 16 | 6 | 606 | 15 | 8.5 |
| 2005 | 1,082 | 16 | 7 | 654 | 16 | 9 |
| 2006 | 1,184 | 17 | 8 | 670 | 17 | 10 |
| Depth of invasion | | | | | | |
| pT1 | 685 | 10 | 5 | 113 | 3 | 6 |
| pT2 | 2,886 | 42 | 6 | 825 | 21 | 8 |
| pT3 | 3,033 | 44 | 7 | 2,771 | 70 | 9 |
| pT4 | 214 | 3 | 6 | 261 | 7 | 9 |
| Tumour grade | | | | | | |
| Well differentiated | 337 | 5 | 6 | 143 | 4 | 8 |
| Moderately differentiated | 4,362 | 64 | 6 | 2,342 | 59 | 8 |
| Poorly differentiated/ Undifferentiated | 674 | 10 | 7 | 768 | 19 | 9 |
| Unknown | 1,445 | 21 | 6 | 717 | 18 | 9 |
| Type of RT | | | | | | |
| Preoperative RT | 4,248 | 62 | 6 | 2,602 | 66 | 8 |
| Preoperative RT and CT | 334 | 5 | 6 | 181 | 7 | 9 |
| Postoperative RT | 181 | 3 | 6 | 265 | 5 | 9 |
| No RT | 2,055 | 30 | 7 | 922 | 23 | 9 |
| Type of surgery | | | | | | |
| Low anterior resection | 3,575 | 52 | 6 | 2,056 | 52 | 8 |
| Abdominoperineal resection | 2,519 | 37 | 6 | 1,498 | 38 | 8 |
| Other | 724 | 11 | 7 | 416 | 10 | 9 |
| Adjuvant chemotherapy | | | | | | |
| No | 6,725 | 99 | 6 | 3,087 | 78 | 8 |
| Yes | 93 | 1 | 6 | 883 | 22 | 9 |
| Type of hospital and type of pathology laboratory | | | | | | |
| Non-teaching hospital and lab. | 1,562 | 23 | 6 | 882 | 22 | 8 |
| Teaching hospital and non-teaching lab. | 1,696 | 25 | 6 | 967 | 24 | 8 |
| Non-teaching hospital and teaching lab. | 1,179 | 17 | 6 | 717 | 18 | 9 |
| Teaching hospital and lab. | 1,693 | 25 | 6 | 999 | 25 | 8 |
| Non-teaching hospital and academic lab. | 105 | 2 | 9 | 69 | 2 | 9 |
| Academic hospital and lab. | 583 | 9 | 8 | 336 | 8 | 10 |

LN, lymph node; RT, radiotherapy; CT, chemotherapy

The proportion of patients who had any LNs evaluated, but of whom the number of LNs was not stated in the pathology report, was 7% for patients with pN0 and 8% for patients with pN+. This proportion decreased over time, from 10% in 2000 to 2% in 2006 for patients with pN0, and from 13% to 2% for patients with pN+, respectively. 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 more than 10%.

The proportion of patients with pN+ increased with a rising number of evaluated LNs (Figure 2a). The increase becomes less steep around 9 evaluated LNs. The highest proportion of patients with pN+ 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. Males, younger patients and tumours with nodal involvement were more likely to have 12 or more LNs evaluated. Tumours with a deeper invasion had a higher odds of having 12 or more LNs evaluated compared to patients with a T1 tumour. 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 12 or more LNs evaluated compared to patients who received preoperative radiotherapy (respectively OR 1.33 (95% CI 1.04-1.71); OR 1.54 (95% CI 1.37-1.74)), and patients who received preoperative radiotherapy and chemotherapy had a lower odds of having 12 or more LNs evaluated (OR 0.77, 95% CI 0.60-0.98). Patients who underwent an abdominoperineal resection were less likely to have 12 or more 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 odds ratio of having 12 or more 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, the overall survival was lower among patients with less than 10 evaluated LNs compared to patients with 10-12 evaluated LNs after adjustment for relevant factors (Table 3). The survival was also lower for patients with an unknown number of evaluated LNs in both groups; HR 1.42 (95%

*Table 2 Multilevel logistic regression with odds ratios of having had 12 or more lymph nodes evaluated (multivariate analysis)*

| | OR | 95% CI | P-value |
|---|---|---|---|
| Gender | | | |
| Male | 1.00 | Reference | |
| Female | 0.85 | 0.77-0.94 | 0.002 |
| Age at diagnosis (yrs) | | | |
| <50 | 1.00 | Reference | |
| 50-69 | 0.68 | 0.57-0.82 | <0.001 |
| ≥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 |
| Lymph node involvement | | | |
| pN0 | 1.00 | Reference | |
| pN+ | 1.61 | 1.45-1.79 | <0.001 |
| Tumour 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 | | | |
| Non-teaching hospital and non-teaching laboratory | 1.00 | Reference | |
| Teaching hospital and non-teaching laboratory | 0.82 | 0.62-1.07 | 0.143 |
| Non-teaching 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 |
| Non-teaching 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; 95% CI, 95% confidence interval; RT, radiotherapy; CT, chemotherapy

*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[1] | pN0 | | | pN+ | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | P-value | HR | 95% CI | P-value |
| 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 |
| Number of examined LNs 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 |
| Number of examined LNs 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 |

[1] Adjusted for age at diagnosis, gender, year of diagnosis, depth of invasion, tumour grade, number of positive nodes, type of RT, type of surgery and adjuvant chemotherapy
LN, lymph node; RT, radiotherapy; HR, hazard ratio; 95% CI, 95% confidence interval; NCR, Netherlands Cancer Registry

CI 1.11-1.81) among patients with pN0 and HR 1.52 (95% CI 1.22-1.90) among patients with pN+.

Among patients who received preoperative radiotherapy, the survival was not statictical significantly lower for patients with negative LNs with 4-9 evaluated LNs and with an unknown number of evaluated LNs compared to patients with 10-12 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+, the survival of patients with less than 10 evaluated LNs is not statistically significantly lower compared

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

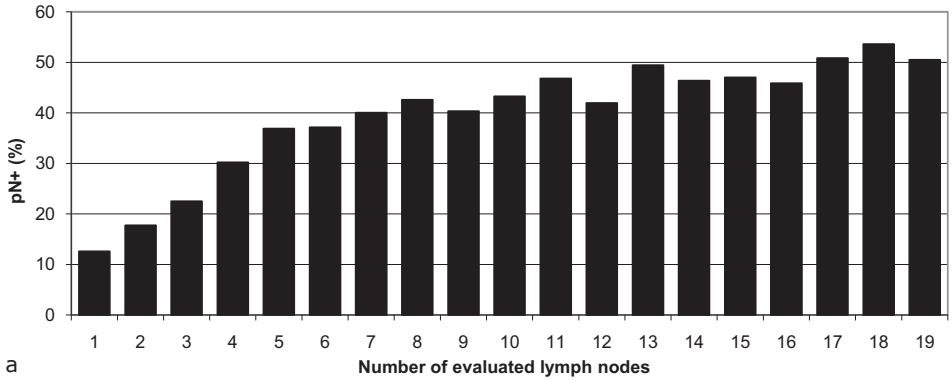
| | HR | 95% CI | P-value |
|---|---|---|---|
| No. of LNs evaluated[1] | | | |
| 1-3 | 0.86 | 0.65-1.14 | 0.293 |
| 4-9 | 1.03 | 0.87-1.21 | 0.752 |
| 10-12 | 1.00 | Reference | |
| 13-15 | 0.97 | 0.79-1.20 | 0.804 |
| 16-18 | 0.95 | 0.73-1.25 | 0.734 |
| 19-21 | 1.19 | 0.86-1.65 | 0.302 |
| ≥ 22 | 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[2] | | | |
| 1st quartile (0 – 0.167) | 1.00 | Reference | |
| 2nd quartile (0.168 – 0.332) | 1.07 | 0.89-1.29 | 0.462 |
| 3rd quartile (0.333 – 0.599) | 1.53 | 1.25-1.87 | <0.001 |
| 4th quartile (0.600 – 1) | 2.03 | 1.60-2.58 | <0.001 |

[1] Adjusted for gender, age at diagnosis, year of diagnosis, depth of invasion, tumour grade, type of radiotherapy, type of surgery and adjuvant chemotherapy
[2] LN ratio was the number of positive lymph nodes divided by the number of evaluated lymph nodes
HR, hazard ratio; 95% CI, 95% confidence interval; LN, lymph node

to patients with 10-12 evaluated LNs (Table 4). The 3rd and 4th quartile of the LNR had a lower survival compared to the 1st 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.

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

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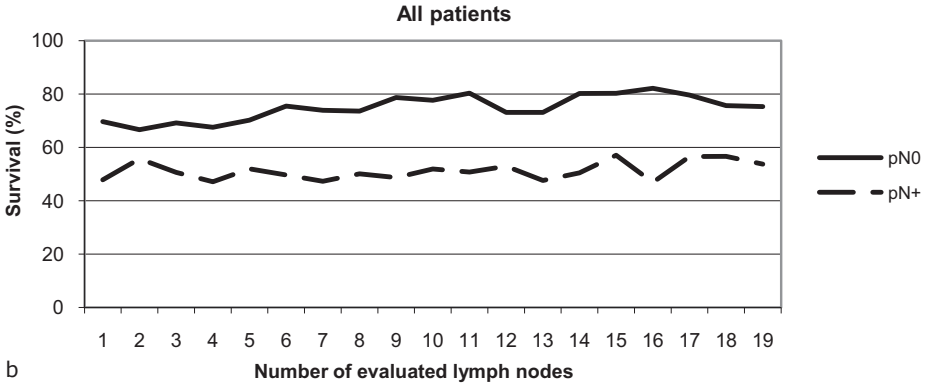


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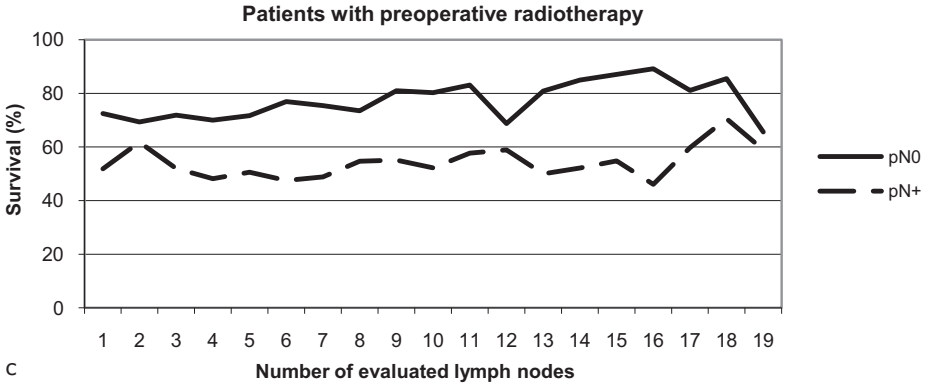


c

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

## 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 2000-2006. Although the UICC recommends a minimum of 12 evaluated LNs for accepting the N0 status, in only 17% of the pN0 patients 12 or more LNs were 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.[20]

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.[11] 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 gender.[21] Positive LNs are slightly larger than negative LNs.[22;23] 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.[13;24] Low anterior resections include often high ligation of the inferior mesenteric artery, leading to an increased LN retrieval.[25]

Two single-institution studies reported a lower LN retrieval after preoperative chemoradiotherapy compared to surgery alone.[13;24] 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 odds ratio of having an inadequate LN evaluation in patients who received preoperative radiotherapy.[12;26] Radiotherapy will decrease the number and size of, involved and uninvolved, LNs and consequently, it may be more difficult to find them.[27]

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 labour-intensive and time-consuming process, suggesting that academic pathology laboratories can provide greater scrutiny, as may expected from engaging in research activities. However, there were also non-academic 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.[8;9;28] 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.[4] 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, since the presence of nodal metastases is an 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.[11;29] An explanation could be that surgeons may have performed an incomplete resection without, or with a less thorough, nodal dissection leading to a worse survival.[11] Another clarification for this relation in patients with negative LNs could be understaging of the disease due to falsely categorising node positive patients as node negative. When more LNs were analysed, 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 tumour. Patients with fewer LNs may be patients with a reduced immune response to their cancer leading to smaller, more difficult to detect, LNs.[30]

The LNR also plays an important role in the survival of patients with positive LNs. Several studies, including our study, showed a better survival for patients with a low LNR.[31-33] 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 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.[34-36] According to the Dutch guidelines, 10 LNs have to be evaluated for accepting N0 status.[4] In our study after around 9 evaluated LNs, the proportion of patients with pN+ still increased when evaluating more LNs, but it was less sharp. However, 5-year overall survival flattened at about 9 evaluated LNs, suggesting the cut off 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 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 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

1. Comprehensive Cancer Centres. http://www.cancerregistry.nl Accessed on 18-5-2009.
2. Statistics Netherlands. http://statline.cbs.nl/StatWeb Accessed on 3-5-2009.
3. Derwinger K, Carlsson G, Gustavsson B. Stage migration in colorectal cancer related to improved lymph node assessment. Eur J Surg Oncol 2007; 33(7):849-853.
4. National Working Group on Gastrointestinal Cancers. Guidelines rectal cancer: http://www.oncoline.nl Accessed on 10-6-2009.
5. Wittekind C, Greene FL, Hutter RVP, Klimpfinger M, Sobin LH, (eds). TNM Atlas. Fifth ed. Berlin: Springer-Verlag; 2004.
6. Baxter NN, Virnig DJ, Rothenberger DA, Morris AM, Jessurun J, Virnig BA. Lymph node evaluation in colorectal cancer patients: a population-based study. J Natl Cancer Inst 2005; 97(3):219-225.
7. Miller EA, Woosley J, Martin CF, Sandler RS. Hospital-to-hospital variation in lymph node detection after colorectal resection. Cancer 2004; 101(5):1065-1071.
8. 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(2):157-164.
9. Johnson PM, Malatjalian D, Porter GA. Adequacy of nodal harvest in colorectal cancer: a consecutive cohort study. J Gastrointest Surg 2002; 6(6):883-888.
10. 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(4):666-672.

11. Tepper JE, O'Connell MJ, Niedzwiecki D, Hollis D, Compton C, Benson AB, III et al. Impact of number of nodes retrieved on outcome in patients with rectal cancer. J Clin Oncol 2001; 19(1):157-163.
12. 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(2):426-431.
13. Wichmann MW, Muller C, Meyer G, Strauss T, Hornung HM, Lau-Werner U et al. Effect of preoperative radiochemotherapy on lymph node retrieval after resection of rectal cancer. Arch Surg 2002; 137(2):206-210.
14. Casparie M, Tiebosch AT, Burger G, Blauwgeers H, van de Pol A, van Krieken JH 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(1):19-24.
15. Visser O, Coebergh JWW, Van Dijck JAAM, Siesling S. Incidence of cancer in the Netherlands 1998. Utrecht: Vereniging van Integrale Kankercentra; 2002.
16. Fritz A, Percy C, Jack A, Shanmugaratnam K, Sobin L, Parkin DM et al. International Classification of Diseases for Oncology. Third edition. Geneva: WHO; 2000.
17. 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(5):974-977.
18. 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(3):369-376.
19. Twisk JWR. Applied Multilevel Analysis. New York: Cambridge University Press; 2006.
20. Van Steenbergen 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(2):135-140.
21. Sarli L, Bader G, Iusco D, Salvemini C, Mauro DD, Mazzeo A et al. Number of lymph nodes examined and prognosis of TNM stage II colorectal cancer. Eur J Cancer 2005; 41(2):272-279.
22. Wong JH, Severino R, Honnebier MB, Tom P, Namiki TS. Number of nodes examined and staging accuracy in colorectal carcinoma. J Clin Oncol 1999; 17(9):2896-2900.
23. Monig SP, Baldus SE, Zirbes TK, Schroder W, Lindemann DG, Dienes HP et al. Lymph node size and metastatic infiltration in colon cancer. Ann Surg Oncol 1999; 6(6):579-581.
24. Rullier A, Laurent C, Capdepont M, Vendrely V, Belleannee G, Bioulac-Sage P et al. Lymph nodes after preoperative chemoradiotherapy for rectal carcinoma: number, status, and impact on survival. Am J Surg Pathol 2008; 32(1):45-50.
25. 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(2):148-157.
26. Tekkis PP, Smith JJ, Heriot AG, Darzi AW, Thompson MR, Stamatakis JD. A national study on lymph node retrieval in resectional surgery for colorectal cancer. Dis Colon Rectum 2006; 49(11):1673-1683.
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(9):1108-1120.
28. Leung AM, Scharf AW, Vu HN. Factors Affecting Number of Lymph Nodes Harvested in Colorectal Cancer. J Surg Res 2009; DOI: 10.1016/j.jss.2009.09.001.
29. Pocard M, Panis Y, Malassagne B, Nemeth J, Hautefeuille P, Valleur P. 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(7):839-845.
30. Klintrup K, Makinen JM, Kauppila S, Vare PO, Melkko J, Tuominen H et al. Inflammation and prognosis in colorectal cancer. Eur J Cancer 2005; 41(17):2645-2654.
31. Peng J, Xu Y, Guan Z, Zhu J, Wang M, Cai G et al. Prognostic significance of the metastatic lymph node ratio in node-positive rectal cancer. Ann Surg Oncol 2008; 15(11):3118-3123.
32. Peschaud F, Benoist S, Julie C, Beauchet A, Penna C, Rougier P et al. The ratio of metastatic to examined lymph nodes is a powerful independent prognostic factor in rectal cancer. Ann Surg 2008; 248(6):1067-1073.

33. Kim YS, Kim JH, Yoon SM, Choi EK, Ahn SD, Lee SW 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(3):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(2):63-69.
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(2):209-216.
36. Hernanz F, Revuelta S, Redondo C, Madrazo C, Castillo J, Gomez-Fleitas M. Colorectal adenocarcinoma: quality of the assessment of lymph node metastases. Dis Colon Rectum 1994; 37(4):373-376.