

# Identifying additional lymph nodes in radical cystectomy lymphadenectomy specimens

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**Lymph node count has prognostic implications in bladder cancer patients who are treated with radical cystectomy. Lymph nodes that are too small to identify grossly can easily be missed, potentially leading to missed nodal metastases and inaccurate nodal counts, resulting in inaccurate prognoses. We investigated whether there is a benefit to submitting the entire lymph node packet for histological examination to identify additional lymph nodes. We prospectively assessed 61 pelvic lymphadenectomy specimens in 14 consecutive patients undergoing radical cystectomy. The specimens were placed in Carnoy's solution overnight, then analyzed for lymph nodes. The residual tissue was entirely submitted to assess for additional lymph nodes. In 61 specimens, we identified 391 lymph nodes, ranging from 4–44 nodes per patient. We identified 238 (61%) lymph nodes with standard techniques and 153 (39%) lymph nodes in submitted residual tissue. The number of additional lymph nodes found in the residual tissue ranged from 0 to 26 (0–75%) per patient. These lymph nodes ranged in size from 0.05 to 1 cm. All additional lymph nodes were negative for metastatic disease. Submitting the entire specimen for histological examination allowed for identification of more lymph nodes in radical cystectomy pelvic lymphadenectomy specimens. However, as none of the additional lymph nodes contained metastatic disease, it is unclear if there is a clinical benefit in evaluating lymph nodes that are neither visible nor palpable in lymphadenectomy specimens.**

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Lymph node count has been shown to be clinically important in the management of bladder cancer patients treated by radical cystectomy and pelvic lymph node dissection.<sup>1–5</sup> Lymph node numbers have considerable prognostic value; however, lymph node count can depend upon multiple factors including extent of lymph node dissection and surgical technique. Separate from intraoperative effort, surgical pathologic processing may not identify all lymph nodes in pelvic lymph node dissection specimens. Missed lymph nodes could potentially lead to missed nodal metastases and inaccurate nodal counts with the associated inaccurate prognostic information. If a small number of lymph nodes are identified, the remainder of the

original specimen may be entirely submitted in an attempt to identify additional nodes. However, this requires additional effort and expense for report completion. We investigated whether there is a benefit in submitting the entire lymphadenectomy specimen for histological examination in terms of identifying additional lymph nodes.

## Materials and methods

Study approval was obtained from the Institutional Review Board. Cystectomy and pelvic lymph node dissections were performed by three surgeons, using their choice of standard or extended lymph node dissection technique. The number of submitted lymph node packets per patient was left up to the discretion of the surgeon as was the extent of the lymphadenectomy. Using our department's surgical pathology lymph node grossing protocol, each submitted lymph node packet was placed in Carnoy's solution overnight to help identify lymph

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nodes in the specimen.<sup>6</sup> Specimens were then analyzed for lymph nodes by visual inspection and palpation. After submitting all identified lymph nodes, the residual fibroadipose tissue was entirely submitted for histological processing to microscopically detect additional lymph nodes. Lymph nodes identified microscopically in the residual tissue were labeled as ‘additional lymph nodes’. One pathologist was responsible for all lymph node packet processing in an attempt to control for inter-user variability. The histological analysis and counting of lymph nodes was performed by one pathologist. The presence of a capsule and sinus histiocytes was necessary to distinguish between a true lymph node vs a lymph node fragment.

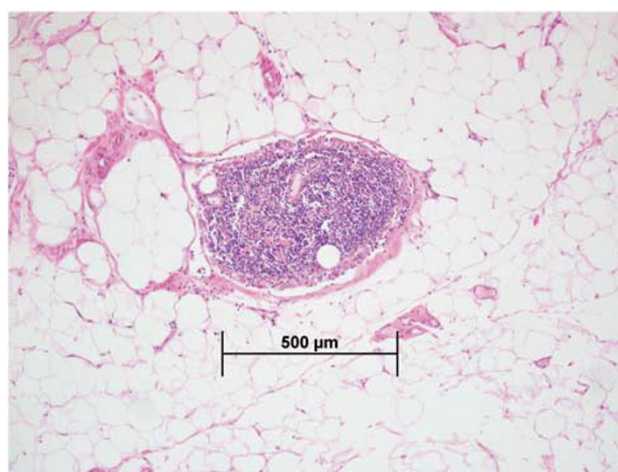
### Statistical Analysis

Pearson’s correlation analysis was performed on the number of initial nodes recovered, the number of total nodes and the proportion of additional nodes (per specimen) as a function of specimen size (cm). We also computed the average number of nodes recovered and the proportion of additional nodes for each additional centimeter of specimen size, using a linear marginal model estimated by generalized estimating equations (GEE) with compound symmetry covariance structure. Confidence intervals were based on empirical (sandwich) variance estimator. The GEE estimators were used instead of ordinary least squares to account for dependence of observations within patients (clustered design).<sup>7</sup> We also performed exploratory analyses to examine the association of the total number of specimens submitted per patient with the node yield, after controlling for the combined volume of all specimens per patient. These analyses were based on a linear model estimated by ordinary least squares (rather than GEEs), because data were examined at the level of individual patients (rather than individual specimens). All analyses were performed in SAS 9.2 (SAS Institute Inc., Cary, NC, USA). All reported *P*-values are two-sided.

### Results

We prospectively assessed 61 pelvic lymph node dissection specimens (packets) in 14 consecutive patients undergoing radical cystectomy. The final pathologic stage of the tumors present in the cystectomy specimens were as follows: T0=3, Tis=3, T1=2, T2b=2, T3a=3, T4a=1. In 61 specimens, we identified 391 lymph nodes, ranging from 4 to 44 nodes per patient. We identified 238 (61%) lymph nodes with standard processing techniques and 153 (39%) additional lymph nodes with submission of residual fibroadipose tissue. The dimensional range of the lymph nodes retrieved using standard techniques was 0.1–6.5 cm. Four lymph nodes identified by standard processing were

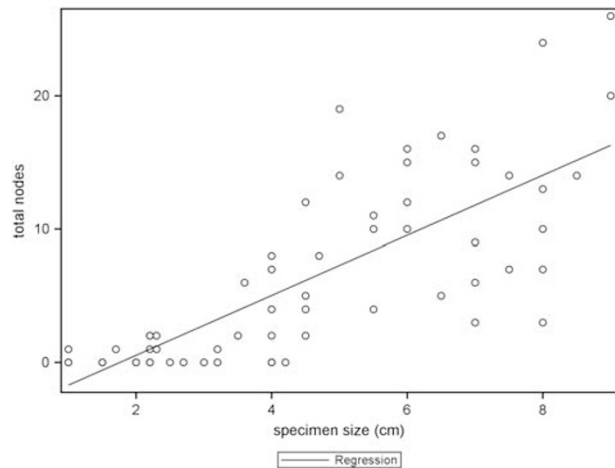
positive for metastatic urothelial carcinoma. The number of additional lymph nodes ranged from 0 to 26 (0–75%) per patient. For the initial standard evaluation, the mean number of nodes identified per patient was 17 and the median per patient was 14. For the additional processing evaluation, the mean number of nodes identified per patient was 10.9 and the median was 10.5. Additional lymph nodes ranged in size from 0.05 to 1 cm, with a median size of 0.2 cm (Figures 1 and 2). All additional lymph nodes were negative for metastatic disease by standard microscopic examination. Figure 3 shows a linear relationship between the total number of nodes recovered (initial recovery and submission of remaining tissue) and specimen size. On average, one may expect to find 2.2 lymph nodes for each additional centimeter in greatest dimension of the specimen (95% CI (1.6, 2.9), *P*<0.001). Figure 4 shows the relationship between the initial number of lymph nodes recovered and specimen size, on average 1.5 lymph nodes for each additional centimeter in greatest dimension of the specimen (95% CI (0.9, 2.1), *P*<0.001). The relationship between the proportion of additional lymph nodes and specimen size is shown in Figure 5. The number of additional nodes relative to the total number of nodes also increases with increasing specimen size by an average of 3% for each additional cm in greatest dimension (95% CI (0.3%, 5.9%), *P*=0.03). We also analyzed whether the number of submitted lymph node packets impacted total lymph node count. The number of lymph nodes retrieved per patient did not depend on the number of packets submitted, after controlling for the combined volume of all packets per patient (*P*=0.48). That is, for a given total specimen volume, the number of packets submitted had no influence on node yield. In addition, when looking at specimen volume, lymph node count increased by an average of 1.2 nodes for every 10 cm<sup>3</sup> of specimen volume after controlling for the total number of specimens per



**Figure 1** Lymph node, 0.5 mm in size, shown at ×10 magnification.



**Figure 2** Gross representation of a lymph node, 2 mm in size.

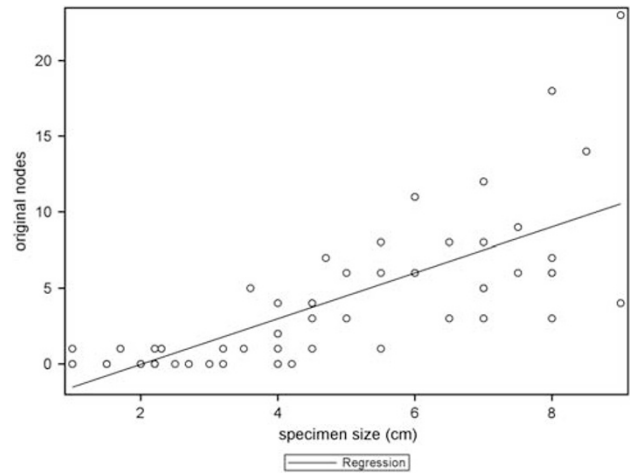


**Figure 3** Association of the total number of lymph nodes and specimen size (cm). Pearson's  $R = 0.76$ ,  $P < 0.001$ .

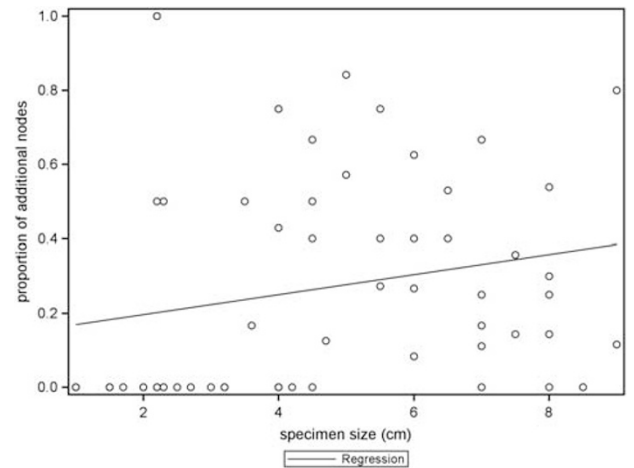
patient ( $P = 0.049$ ). The total number of nodes recovered per patient did not differ significantly between the three surgeons ( $P = 0.59$ ).

## Discussion

There are pros and cons to submitting an entire lymph node packet for histological examination. One benefit is that, in theory, submitting the entire packet would eliminate missed lymph nodes. Missed lymph nodes could potentially lead to missed nodal metastases and a change in pathologic stage, clinical prognosis, and an opportunity to administer adjuvant therapy. Another benefit would be an increase in the total lymph node count per patient. The cons include the additional expense of processing and examining the entire specimen. In addition, many of the additional lymph nodes identified would be extremely small. It is unclear if there is a clinical benefit of evaluating lymph



**Figure 4** Association of the number of initial lymph nodes identified and specimen size (cm). Pearson's  $R = 0.74$ ,  $P < 0.001$ .



**Figure 5** Association of the proportion of additional lymph nodes with specimen size (cm). Pearson's  $R = 0.21$ ,  $P = 0.03$ .

nodes that are neither visible nor palpable in lymphadenectomy specimens.

A large amount of literature has stressed the importance of lymph node count in radical cystectomy pelvic lymphadenectomy specimens. Koppie *et al*<sup>1</sup> looked at 1121 patients who underwent radical cystectomy for clinically localized urothelial carcinoma of the bladder, demonstrating that the number of lymph nodes removed was a predictor of survival. Further, the probability of survival did not plateau, but instead continued to rise as the number of lymph nodes removed increased.<sup>1</sup> Similarly, Wright *et al*<sup>2</sup> looked at long-term survival in 1260 patients with lymph node-positive bladder cancer, who underwent cystectomy. The number of positive and total lymph nodes removed remained independent predictors of survival. Removal of greater than 10 lymph nodes was associated with increased overall survival.<sup>2</sup> Shirotake *et al*<sup>3</sup> examined 169 patients who underwent radical cystectomy with pelvic lymphadenectomy for bladder cancer and

found that the removal of less than nine lymph nodes was an independent factor of worse cancer-specific survival. Lymph node count being associated with improved prognosis for bladder cancer patients has been supported by multiple other studies.<sup>4,5</sup> The concept of lymph node density as a prognostic factor has also been well studied in the literature. Lymph node density is defined by two variables: the number of positive lymph nodes divided by the total number of lymph nodes removed. Lymph node density has been correlated with recurrence-free survival and has been found in some studies to be a better surrogate end point than total number of positive nodes.<sup>4,8,9</sup> Five-year survival rates have been listed as high as 64% for a lymph node density of less than 20% compared with only 8% for a lymph node density greater than 20%.<sup>8,9</sup>

Some potential factors influencing lymph node count have been examined in bladder cancer patients treated by cystectomy and pelvic lymphadenectomy. Ather *et al*<sup>10</sup> looked at separate submission of standard lymphadenectomy in six packets vs en bloc lymphadenectomy in bladder cancer. Thirty-four patients were treated with standard lymph node dissection, submitted in six packets, and 43 were treated with en bloc lymphadenectomy, submitted in two packets. The proportion of patients with positive lymph nodes was not significantly different between the two groups; however, standard lymphadenectomy submitted in six different containers significantly improved the nodal yield over en bloc resection.<sup>10</sup> Our study did not find a statistically significant difference between the number of packets submitted per patient and lymph node yield, after controlling for the combined volume of all packets per patient. However, this analysis was limited by a small number of patients ( $n = 14$ ).

Factors influencing lymph node count have been more thoroughly examined in other solid tumor malignancies, such as colon cancer. Jakub *et al*<sup>11</sup> found no statistical difference in the number of lymph nodes retrieved, based on the surgeon, pathologist or pathology technician. Age of the patient, primary site of the tumor, stage and year of surgery were all significantly associated with number of lymph nodes retrieved.<sup>11</sup> Ostadi *et al*<sup>12</sup> found that most of the variation in the number of lymph nodes identified in surgical specimens from colorectal cancer operations was accounted for by differences between pathology assistants, suggesting that the number of lymph nodes identified is dependent on the experience of the person processing the specimen. Other studies have found the role of the surgeon, pathologist, and specimen size to be significant factors in lymph node count.<sup>13,14</sup>

Although past studies have looked at nodal yield in pelvic lymphadenectomy specimens in bladder cancer patients, to our knowledge, none have examined the amount of additional lymph nodes found by submitting the residual fibroadipose tissue.

A recent study by Phan *et al*<sup>15</sup> investigated the ability to identify hilar lymph nodes in radical nephrectomy specimens by entirely submitting the hilar fat region for microscopic evaluation. Additional lymph nodes were found in only 3 out of 50 nephrectomy specimens and none contained metastatic disease. Our study suggests that up to 39% additional lymph nodes may be found by submitting the entire lymphadenectomy specimen in radical cystectomy cases. We found that the number of total nodes present in the specimen was directly proportional to the size of the specimen. Similarly, the number of initial nodes identified increased with specimen size. When evaluating total nodes identified, one may expect to find 2.2 lymph nodes for each centimeter in greatest specimen dimension. As the number of additional nodes relative to the total number of nodes also increased with increasing specimen size, our data suggests a benefit in submitting the remaining fibroadipose tissue in larger specimens in terms of maximum lymph node yield.

Our study suggests that in pelvic lymphadenectomy specimens, up to 39% additional lymph nodes may be identified by submitting all fibroadipose tissue. The total number of lymph nodes identified in a specimen directly impacts the concept of lymph node density (ratio of positive nodes to total number of examined nodes). Nearly all studies performed on this issue have confirmed the independent prognostic significance of lymph node density upon multivariate analysis, with a lymph node density of 20% being the most commonly used cut-off in the literature.<sup>4,8,9</sup> Underreporting total number of lymph nodes would change the denominator of the calculated lymph node density and thereby impact the statistical analysis of these studies. However, it is unclear how the pathologic processing was performed on the lymphadenectomy specimens in these studies or whether the entire specimen was submitted or not.

None of the additional lymph nodes we identified in the residual tissue contained metastatic disease. Thus, there was no clinical impact of identifying the additional nodes in terms of tumor staging or patient management. In addition, the majority of additional lymph nodes were sub-centimeter in size, with a median size of 0.2 cm (Figures 1 and 2). Because of the small size of this lymph node population, it is possible that some of these lymph nodes are actually fragments of larger, previously sampled lymph nodes from the specimen. In this case, there would be an overestimation of the true lymph node count by submission of all fibroadipose tissue. However, additional lymph nodes identified could be whole lymph nodes that were simply too small to appreciate grossly, and therefore were not sampled. The decision as to whether lymphoid tissue is counted as a lymph node vs a lymph node fragment can be difficult and is dependent on the examining pathologist. We limited the examination of all lymph

nodes to one pathologist to control for inter-user variability. In addition, the presence of a capsule and sinus histiocytes was necessary to distinguish between a true lymph node vs a lymph node fragment.

For our institution, standard lymph node packet processing includes palpation and visual inspection for lymph nodes. The remaining fibroadipose tissue is then separated and saved for a period of time until the pathology report is signed out. If very few lymph nodes are identified, the pathologist may choose to return to the specimen to try to identify additional lymph nodes. The entire lymph node packet is not routinely submitted for histological examination as a large amount of the specimen is fibroadipose tissue. The assumption is that there would be few or no lymph nodes present in the remaining tissue. In our institution, we use Carnoy's solution to help identify lymph nodes.<sup>1</sup> Other institutions use a variety of other chemicals to aid in lymph node identification. The protocol for lymph node specimen processing may differ between institutions, and this method is rarely commented on in papers. The number of lymph nodes reported per specimen may vary between institutions, depending on their particular processing method, whether they submit the entire specimen or not, and on the experience of the individual processing the specimen.

Although we examined 61 specimens and 391 lymph nodes, our study was limited to a small number of patients ( $n = 14$ ). In addition, our patient population came from three separate surgeons, with some specimens including an extended lymph node dissection. We found no statistical difference in lymph node count between the three surgeons. However, standardizing the lymph node template between surgeons, or limiting the study to one surgeon would better control for the variation of lymph node numbers between patients. The number of lymph nodes identified per specimen is likely dependent on the experience of the person processing the lymph node packet. We attempted to control for inter-user variability by having all specimens processed by the same experienced pathologist. For the same reason, the microscopic examination of lymph nodes was completed by one pathologist.

The current literature supports the concept that higher lymph node numbers impart a clinical and prognostic benefit for patients undergoing radical cystectomy with lymphadenectomy. Our findings suggest that more lymph nodes can be identified by submitting the entire lymph node packet in radical cystectomy specimens. However, it is unclear if there is a clinical benefit of evaluating lymph nodes that are neither visible nor palpable in lymphadenectomy specimens.

## Disclosure/conflict of interest

The authors declare no conflict of interest.

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