

Preoperative Tattooing and Improved Lymph Node Retrieval Rates From Colectomy Specimens in Patients With Colorectal Cancers

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Hypothesis: India ink tattooing at the time of colonoscopy increases the yield of lymph nodes found in pathological analysis of colectomy specimens.

Design: Retrospective study.

Setting: Virginia Mason Medical Center, Seattle, Washington.

Patients: Two hundred nine patients with colorectal cancers underwent surgical resections between April 5, 2006, and June 25, 2009, at one institution.

Main Outcome Measures: A retrospective review of a prospectively collected database was performed, with review of pathology reports for all cases. Adequate lymph node analysis was defined as evaluation of at least 12 lymph nodes.

Results: Of 209 patients undergoing resections, 174 had colonic neoplasms, and 35 had rectal neoplasms. Sixty-

two of 174 patients with colon cancer had India ink tattooing at the time of colonoscopy. The mean (range) numbers of lymph nodes examined in tattooed and nontattooed specimens were 23 (7-77) and 19 (2-74), respectively ($P=.03$). At least 12 lymph nodes were analyzed for 87.1% of the tattooed specimens compared with 72.3% of the nontattooed specimens ($P=.02$). Eight of 35 patients with rectal cancer had India ink tattooing at the time of colonoscopy. Fifty-four percent of patients with rectal cancer had undergone neoadjuvant chemoradiotherapy. The median numbers of lymph nodes examined in tattooed and nontattooed specimens were 19 and 16, respectively.

Conclusions: Tattooing of colonic lesions at the time of preoperative colonoscopy seems to increase the quality of lymph node analysis. We advocate routine tattooing of all suspicious neoplasms at the time of colonoscopy.

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COLON CANCER IS THE SECOND leading cause of cancer death in the United States.¹ However, over the last 2 decades, effective adjuvant therapeutic strategies have been developed that have resulted in increased cure rates among patients with nodal metastases. National Comprehensive Cancer Network guidelines (<http://www.nccn.org>) recommend adjuvant chemotherapy in all

(ie, the ratio of the number of positive nodes to the total number of lymph nodes examined) has been proposed as a better measure of nodal (or N) staging.⁸⁻¹⁰ That is, the number of negative nodes is a more precise predictor of survival than the current N staging.

There are 2 likely explanations for this observation. The first explanation is the potential therapeutic benefit gained from extensive lymphadenectomy in patients with colorectal cancer. That is, surgical resection of metastatic nodes likely results in prolonged survival. The evidence for this is strongest in patients with rectal cancer who undergo total mesorectal excision. Therefore, increased numbers of lymph nodes examined likely represent a surrogate for a more thorough lymphadenectomy in many patients. The second explanation is stage migration. Examination of more lymph nodes increases the odds of finding positive nodes and “downstages” disease in some patients to stage III. These pa-

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patients with nodal metastases (stage III), whereas patients with node-negative stage I or stage II disease are usually managed with surgery alone. Study findings have suggested that the number of lymph nodes examined is correlated with long-term survival.²⁻⁷ Furthermore, the lymph node ratio

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tients, in turn, are treated with adjuvant chemotherapy or chemoradiotherapy and will have improved survival compared with a cohort of patients with node-positive disease who are inaccurately staged as having node-negative disease. Regardless of the explanation, the consistent observation by many investigators is that higher numbers of lymph nodes examined in patients with colorectal cancer are associated with increased long-term survival.^{2-7,11}

Although the number of lymph nodes required for accurate staging has been controversial, ranging from 6 to 20 nodes,^{3,4,7,12,13} the American Society for Clinical Oncology, the National Comprehensive Cancer Network, and the American Joint Committee on Cancer have endorsed a 12-node minimum as a consensus standard for hospital-based performance in colectomies performed for colon cancer.¹⁴ Unfortunately, there are many factors that may lead to variation in the number of lymph nodes examined. Patient variables (eg, central obesity), location of tumor (rectum vs right colon), surgeon factors (extent of surgical resection), preoperative radiotherapy, and diligence of the pathologist or pathology technician affect lymph node retrieval rates.^{3-7,15-20} Evidence suggests that the latter explanation may be the single most important factor.¹⁹ In casual conversation with our pathology technicians, we were informed that lymph node retrieval was facilitated in specimens with preoperative tattooing: even tiny lymph nodes are black and more easily identified (**Figure**). The objective of this study was to confirm this empirical observation.

METHODS

DATA SOURCE

Patients undergoing colectomy or proctectomy for cancer between April 5, 2006, and June 25, 2009, at the Virginia Mason Medical Center were identified from a prospectively collected database. Pathology and endoscopy reports were reviewed. Tumor-specific data were confirmed in the pathology reports, including tumor location, tumor stage and grade, nodal staging (number of lymph nodes examined and number of positive nodes), and presence of India ink tattooing.

PATIENT SELECTION

Patients who did not undergo surgery for possible colorectal cancer were excluded. Other excluded patients included those operated on for fistulas, ovarian cancer, diverticulitis, and inflammatory bowel disease. Patients were also excluded if an endoscopy report was unable to be obtained. Adequate lymph node analysis was defined as evaluation of at least 12 lymph nodes. Rectal cancers were analyzed separately from colon cancers.

DATA ANALYSIS

Patients with colorectal cancer were categorized into 2 cohorts depending on whether India ink tattooing had been performed at the time of colonoscopy. The mean and median numbers of lymph nodes were determined, as well as whether adequate numbers of lymph nodes (defined as ≥ 12) were evaluated. Data were analyzed using 1-tailed Fischer exact test or 1-tailed *t* test with unequal variance as appropriate. $P < .05$ was considered statistically significant.



Figure. Fresh specimen before pathological dissection demonstrates the black dye easily visible in small (<5-mm) lymph nodes.

RESULTS

A total of 209 patients undergoing colectomies for cancer were identified, 174 with colon cancer and 35 with rectal cancer. There were fewer lymph nodes identified in rectal cancer specimens, especially those from patients receiving neoadjuvant chemoradiotherapy. Patient data in those with colon cancers and rectal cancers treated with or without radiotherapy are summarized in **Table 1**. There was a modest but significant difference between study groups in the number of lymph nodes retrieved. The mean (range) numbers of lymph nodes examined in tattooed and nontattooed specimens were 23 (7-77) and 19 (2-74), respectively ($P = .03$). In contrast to some previous reports,^{8,9} colon cancer site was not associated with the number of lymph nodes analyzed. The nontattooed group had 69.9% right colon resections, while the tattooed group had 46.0% right colon resections.

There was a significant difference between patients having colon cancers with tattooed vs nontattooed specimens in the observed compliance with the proposed quality metric (≥ 12 lymph nodes examined). As summarized in Table 1, there was about a 15% absolute difference in compliance, 87.1% vs 72.3%, between the 2 groups. This represents a 53.4% improvement in compliance with the quality metric among the tattooed cases. **Table 2** summarizes the consistency of our findings across all T stages and N stages. For each T stage except T4, there was at least a 15% improvement in compliance with the process measure of at least 12 lymph nodes examined. Of non-tattooed, node negative (NO) specimens, 30.2% had fewer than 12 nodes examined. In contrast, only 12.2% of tattooed NO specimens had fewer than 12 nodes examined. Therefore, the patients in whom stage migration is most likely (those with NO disease) seem to benefit the most from tattooing.

There is little evidence that our findings are explained by differences between patients with tattooed vs nontattooed specimens. Data in Table 2 summarize that our patient groups are similar for T stage. As summarized in **Table 3**, right colectomies were more common among patients with nontattooed specimens. There was no apparent surgeon effect on our findings. Sixteen

Table 1. Number of Lymph Nodes Analyzed With and Without India Ink Tattooing at the Time of Colonoscopy

| Specimen | <12 Lymph Nodes Retrieved | ≥12 Lymph Nodes Retrieved | % Adequate | No. of Lymph Nodes | | P Value |
|-----------------------------------|---------------------------|---------------------------|------------|--------------------|--------|---------|
| | | | | Mean | Median | |
| Colon Cancers | | | | | | |
| Nontattooed | 31 | 81 | 72.3 | 19 | 17 | .02 |
| Tattooed | 8 | 54 | 87.1 | 23 | 20 | |
| Rectal Cancers^a | | | | | | |
| Nontattooed | 7 | 20 | 74.1 | 14 | 14 | .41 |
| Tattooed | 3 | 5 | 62.5 | 17 | 15 | |
| Total | | | | | | |
| Nontattooed | 38 | 101 | 72.7 | 18 | 16 | .04 |
| Tattooed | 11 | 59 | 84.3 | 22 | 19 | |

^aAmong patients having rectal cancers, 59.3% with nontattooed specimens vs 37.5% with tattooed specimens received neoadjuvant chemotherapy.

Table 2. Number of Lymph Nodes Analyzed by T and N Stages

| Variable | T Stage | | | | |
|------------------------------|---------|------|-------|------|------|
| | 0 | 1 | 2 | 3 | 4 |
| Nontattooed Specimens | | | | | |
| <12 Lymph nodes retrieved | 6 | 3 | 5 | 11 | 3 |
| ≥12 Lymph nodes retrieved | 8 | 6 | 16 | 35 | 14 |
| % Adequate | 57.1 | 66.8 | 76.2 | 76.1 | 82.4 |
| Tattooed Specimens | | | | | |
| <12 Lymph nodes retrieved | 2 | 3 | 0 | 1 | 1 |
| ≥12 Lymph nodes retrieved | 9 | 10 | 10 | 19 | 6 |
| % Adequate | 81.8 | 76.9 | 100.0 | 95.0 | 85.7 |
| Variable | N Stage | | | | |
| | 0 | 1 | 2 | | |
| Nontattooed Specimens | | | | | |
| <12 Lymph nodes retrieved | 19 | 7 | 2 | | |
| ≥12 Lymph nodes retrieved | 44 | 20 | 15 | | |
| % Adequate | 69.8 | 74.1 | 88.2 | | |
| Tattooed Specimens | | | | | |
| <12 Lymph nodes retrieved | 5 | 3 | 0 | | |
| ≥12 Lymph nodes retrieved | 36 | 13 | 5 | | |
| % Adequate | 87.8 | 81.3 | 100.0 | | |

surgeons contributed cases to this series. However, 5 surgeons accounted for 80.5% of cases (range, 15-37 cases per surgeon); 8 surgeons had 3 or fewer cases. The ratio of tattooed specimens to nontattooed specimens was similar across all surgeons, and the ratio of tattooed specimens to nontattooed specimens for high-volume surgeons was similar for all but 1 surgeon. That is, our findings cannot be accounted for by different percentages of tattooed specimens among surgeons. The retrieval compliance rate of at least 12 lymph nodes was slightly higher (90.2%) among high-volume surgeons, with 100.0% compliance in 3 of 5.

COMMENT

Our data suggest that the routine performance of India ink tattooing during diagnostic colonoscopy would result in

Table 3. Location of Colon Cancers

| Specimen | Colectomy | | | | Laparoscopy Assisted | % From Right Colectomy |
|-------------|-----------|------|------------|-------|----------------------|------------------------|
| | Right | Left | Transverse | Total | | |
| Nontattooed | 79 | 25 | 6 | 3 | 8 | 79.9 |
| Tattooed | 29 | 23 | 7 | 4 | 6 | 46.0 |

increased rates of adequate lymph node analysis in colorectal cancer specimens. Authors of previous studies²⁻⁷ have concluded that higher numbers of lymph nodes analyzed are correlated with longer survival among patients with colon cancer. Intergroup Trial INT-0089 investigators reported this observation in a 2003 study.² That trial randomized patients with high-risk stage II and all patients with stage III colon cancers to adjuvant fluorouracil, levamisole hydrochloride, and leucovorin calcium in 4 different combinations. The investigators found that survival increased in association with more analyzed lymph nodes. Even when no lymph nodes were involved with metastatic disease, overall and disease-free survival improved with more analyzed nodes. Data from the Surveillance, Epidemiology and End Results database also support the conclusion that adequate lymphadenectomy (≥15 lymph nodes) is correlated with increased survival independent of cancer stage.⁷ In this database, the authors found that (1) the mean number of lymph nodes analyzed was 9 and (2) that evaluation of at least 15 nodes was associated with a 20% improvement in survival compared with evaluation of 7 or fewer nodes.

The validity of at least 12 nodes examined as a process measure for surgical quality has been challenged.^{14,21} Whereas surgical technique affects nodal harvest, some investigators have concluded that the number of lymph nodes analyzed is dependent on the quality of specimen analysis by the pathology laboratory.^{17,19,22} Johnson et al¹⁹ ascertained that the most significant variable associated with the number of lymph nodes harvested is analysis of the specimen by a pathologist. Similarly, our data suggest that tattooing (and facilitated nodal identification) is most strongly correlated with adequate lymph node analysis. If fewer than 12 lymph nodes are identified, the College of American Pathologists recommends the use of visual enhancement techniques such

as fat stretching or various solvents (ether, alcohol, or oil based). However, these techniques are time and labor intensive.

Therefore, it is likely that the association between survival and number of lymph nodes harvested is at least in part a reflection of stage migration and of more appropriate use of adjuvant treatment. Our data support this hypothesis. The patients in whom stage migration most affects treatment are those with N0 disease. With inadequate lymphadenectomy or inadequate specimen examination, patients with true node-positive disease are inaccurately staged as having N0 disease and are not given appropriate adjuvant treatment. Of 63 patients with N0 disease in our series who underwent resection without preoperative tattooing, 19 (30.2%) had fewer than 12 lymph nodes identified. Of 41 patients having N0 disease with preoperative tattooing, 5 (12.2%) had fewer than 12 lymph nodes examined. Therefore, it is likely that many patients having N0 disease without preoperative tattooing had understaged disease because of inadequate numbers of lymph nodes analyzed. However, it is important to emphasize that our study cannot assess the effect of specimen tattooing on treatment or survival.

Swanson et al³ reported that the number of lymph nodes analyzed in patients with N0 disease is correlated with survival. Five-year survival rates ranged from 64% in patients having N0 disease with 1 to 2 nodes examined to 86% in patients having N0 disease with more than 25 nodes analyzed.

In a 2009 population-based study from the Netherlands, Kelder et al⁴ found that the number of lymph nodes analyzed was associated with survival in patients with node-negative disease but not in patients with node-positive disease. This further supports the hypothesis that the correlation between survival and number of lymph nodes analyzed is at least in part a reflection of stage migration.

We found some variation in the results of lymph node harvesting among surgeons. However, the finding of more lymph nodes analyzed in tattooed specimens was consistent across all surgeons. Furthermore, we found no apparent association between tumor variables or patient factors and the placement of tattoos. Therefore, of the variables that contribute to lymph node yield at the time of pathological evaluation—(1) patient factors such as intrinsic number of lymph nodes per patient, (2) surgical factors such as tumor location and extent of resection, and (3) pathological factors, including specimen preparation and node identification ability—we believe that the third factor is most important. According to Johnson et al,¹⁹ variables associated with more lymph nodes analyzed were high surgeon volume, performance of right colectomy, and gross examination by a staff pathologist. Conversely, variables associated with fewer lymph nodes analyzed were low surgeon volume, performance of left colectomy, and gross examination by a pathology resident or a pathology technician. Other researchers have identified hospital volume as a predictor of nodal harvest rates.^{14,15,17} Together, we believe at least 12 nodes examined as a quality process measure is a systems or hospital measure that is affected most significantly by performance in the pathology department but also by the quality of surgical resection.

The role of sentinel node biopsies in retrieval of colorectal cancer specimens is controversial. In our opinion, the literature supports the conclusion that routine sentinel node biopsy is unwarranted.²³⁻²⁹ However, if one assumes that sentinel node staining increases the chances of identifying lymph nodes at highest risk for metastatic disease, then India ink tattooing can enhance the quality of lymph node analysis in colorectal cancer specimens. The probability of identifying the sentinel node is likely increased in tattooed specimens, especially in specimens with small lymph nodes. Anecdotally, we also observed that India ink staining altered the lymphatic resection in several cases, with easy identification of stained nodes at the root of mesentery.

It is unclear why our positive findings between tattooing of colonic lesions and increased quality of lymph node analysis did not apply to rectal cancer. Our numbers are small, and 54.3% of patients with rectal cancer had neoadjuvant chemoradiation, an intervention known to result in fewer nodes retrieved.¹⁶ However, even if our findings with respect to pathological analysis do not apply to patients with rectal cancer, the original indication for tattooing (anatomic localization to facilitate accurate cancer resection margins) has great usefulness. Therefore, we believe that we are justified in recommending India ink tattooing at the completion of all diagnostic colonoscopies that detect colon or rectal cancers.

Increasingly, the lymph node ratio (the ratio of the number of positive nodes to the total number of nodes analyzed) is being advocated as an optimal method to define node status.⁸⁻¹⁰ Current American Joint Committee on Cancer nodal staging, N1 (1-3 positive nodes) and N2 (≥ 4 positive nodes), is an imprecise measure of metastatic burden. Using that staging system, patients with metastases in 3 of 4 nodes have the same disease stage as patients with metastases in 3 of 12 or more lymph nodes. Studies⁸⁻¹⁰ have shown that the lymph node ratio is a better measure of metastatic burden and predictor of long-term disease-free survival than the current American Joint Committee on Cancer TNM system for colorectal cancer. Therefore, even if the association between survival and number of lymph nodes examined is largely a reflection of stage migration and does not truly affect survival in the entire population of patients with colorectal cancer, the enhanced staging and prognostic information provided by better pathological evaluation adds value.

In conclusion, this study confirms our hypothesis that India ink tattooing of cancers during diagnostic colonoscopy enhances the quality of pathological examination of specimens. Therefore, we propose that India ink tattooing of suspicious colorectal neoplasms should become routine practice during diagnostic colonoscopy.

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INVITED CRITIQUE

Is It Time to Move Beyond Lymph Node Evaluation in the Staging of Colon Cancer?

The focus on regional nodal evaluation in colon cancer as a staging measure, prognostic variable, and quality indicator is stronger now than ever before, with more than 300 peer-reviewed publications in 2009 specifically about lymph node staging, which emphasizes the critical role of this surgical and pathological factor. That lymph node evaluation as a staging measure is clinically relevant and that it is considered one of the most important prognostic markers in colorectal cancer are indisputable, yet only recently has the National Quality Forum with the American College of Surgeons and the American Society of Clinical Oncology endorsed the 12-node mini-

imum as a colon cancer treatment quality measure. This benchmark is not to be underestimated, as it has had a profound effect, with now more than 94% of National Comprehensive Cancer Network–designated cancer centers not only pursuing but also achieving this new standard in surgical oncology. As with most advances in medicine, the 12-node minimum has engendered considerable controversy, which our group presented at this year's annual meeting of the American Surgical Association. One related and provocative question emerges from this debate. Is it the surgeon, the pathologist, or possibly even the patient who is the prognostic linchpin in colorectal cancer?