Purpose: We postulated that the pathologic evaluation of the lymph nodes of surgical specimens from patients with rectal cancer can have a substantial impact on time to relapse and survival.

Patients and Methods: We analyzed data from 1,664 patients with T3, T4, or node-positive rectal cancer treated in a national intergroup trial of adjuvant therapy with chemotherapy and radiation therapy. Associations between the number of lymph nodes found by the pathologist in the surgical specimen and the time to relapse and survival outcomes were investigated.

Results: Patients were divided into groups by nodal status and the corresponding quartiles of numbers of nodes examined. The number of nodes examined was significantly associated with time to relapse and survival among patients who were node-negative. For the first through fourth quartiles, the 5-year relapse rates were 0.37, 0.34, 0.26, and 0.19 ($P = .003$), and the 5-year survival rates were 0.68, 0.73, 0.72, and 0.82 ($P = .02$). No significant differences were found by quartiles among patients determined to be node-positive. We propose that observed differences are primarily related to the incorrect determination of nodal status in node-negative patients. Approximately 14 nodes need to be studied to define nodal status accurately.

Conclusion: These results suggest that the pathologic assessment of lymph nodes in surgical specimens is often inaccurate and that examining greater number of nodes increases the likelihood of proper staging. Some patients who might benefit from adjuvant therapy are misclassified as node-negative due to incomplete sampling of lymph nodes.

Surgery is the mainstay of therapy in the treatment of adenocarcinoma of the rectum. For most patients, either an abdominoperineal resection (APR) or a low anterior resection (LAR) is standard management. There has been a gradual evolution of surgical management based on the concept that a more complete resection of the perirectal soft tissue will produce lower local recurrence rates and perhaps higher survival than more conventional surgical approaches. This technique of total mesorectal resection has gained wide acceptance among surgical oncologists and gastrointestinal surgeons experienced in the treatment of rectal cancer. There is a significant body of single-institution data to suggest that the use of total mesorectal excision may produce lower local recurrence rates and perhaps higher survival than less aggressive surgical procedures. Less information is available on the importance of the extent of pathologic assessment on outcome.

Adjuvant therapy of rectal cancer at most centers in the United States is given to patients with tumor extension through the bowel wall or with positive lymph nodes and consists of a combination of fluorouracil (5-FU)-based chemotherapy and pelvic radiation therapy. As is the case with surgical technique, the aggressiveness of the pathologist in determining which patients have positive lymph nodes or local tumor extension could have a major impact on the management of these patients and could markedly influence the outcome of clinical trials designed to assess the value of adjuvant therapy.

The number of lymph nodes assessed pathologically is thus a combination of the aggressiveness of the surgeon in resecting widely around the primary tumor and of the pathologist in searching the specimen for lymph nodes. More positive lymph nodes can also be found if the pathologist uses specialized techniques to evaluate the nodes, such as step sectioning of the node, using immunohistochemistry to determine nodal metastases, or the use of the polymerase chain reaction to find molecular correlates of tumor presence.

In an attempt to determine the impact of insufficient sampling on time to relapse and survival, we evaluated a cohort of patients who were entered onto a GI Intergroup...
trial of adjuvant therapy for patients with resected, high-risk (T3, T4, or node-positive) rectal cancer. This study began in 1990 and randomized 1,792 patients to one of four adjuvant therapeutic regimens, all of which used 5-FU and radiation therapy after potentially curative surgical resection with an LAR or APR. The preliminary data from this study have been reported and did not show any definite advantage to any of the individual treatment regimens.

**PATIENTS AND METHODS**

**Eligibility**

Patients were eligible for the treatment trial if they had histologic proof of adenocarcinoma of the rectum and a potentially curative resection had been performed with neither gross nor microscopic evidence of residual disease. If the tumor was adherent to an adjacent structure, the margins of the specimen had to be specifically documented to be free of malignant cells. A regional metastasis that could not be resected en bloc with the primary tumor rendered the patient ineligible, as did evidence of peritoneal metastases. The tumor had to have a pathologic indicator of a poor prognosis, ie, either extension of the primary tumor through the bowel wall or positive lymph nodes without evidence of distant metastatic disease (T3, T4, or N1 to N3, and M0). Tumor nodules in the perirectal fat without definite nodal structure were considered to be positive nodes. For the purposes of this study, a tumor was considered to be a rectal (and not a colon) cancer if a portion of the tumor was located below the level of the peritoneal reflection. Alternatively, a tumor whose lower margin was within 12 cm of the anal verge on endoscopy was assumed to be rectal in origin unless the tumor was surgically found to be entirely above the peritoneal reflection.

All patients had to be older than 18 years of age, have a performance status (Zubrod) of 0 to 2, and be nonpregnant and nonlactating. Patients could not have received prior radiation therapy to the pelvis, could have had no prior chemotherapy or immunotherapy, and must have recovered adequately from the acute effects of the surgery. There could be no previous or concurrent malignancy within 5 years of the diagnosis of rectal cancer. The presence of a synchronous colonic cancer (tumor-node-metastasis stage I) that had been completely resected was not grounds for protocol exclusion. There could not have been other serious medical illnesses that would have limited the ability of the patient to receive protocol therapy, the leukocyte count had to be more than 4,000/μL, and the platelet count had to be more than 130,000/μL.

Patients were entered onto the study and begun on therapy no sooner than 3 weeks and no later than 10 weeks after surgery. Patients were eligible for the treatment trial if they had histologic proof of adenocarcinoma of the rectum and a potentially curative resection had been performed with neither gross nor microscopic evidence of residual disease. If the tumor was adherent to an adjacent structure, the margins of the specimen had to be specifically documented to be free of malignant cells. A regional metastasis that could not be resected en bloc with the primary tumor rendered the patient ineligible, as did evidence of peritoneal metastases. The tumor had to have a pathologic indicator of a poor prognosis, ie, either extension of the primary tumor through the bowel wall or positive lymph nodes without evidence of distant metastatic disease (T3, T4, or N1 to N3, and M0). Tumor nodules in the perirectal fat without definite nodal structure were considered to be positive nodes. For the purposes of this study, a tumor was considered to be a rectal (and not a colon) cancer if a portion of the tumor was located below the level of the peritoneal reflection. Alternatively, a tumor whose lower margin was within 12 cm of the anal verge on endoscopy was assumed to be rectal in origin unless the tumor was surgically found to be entirely above the peritoneal reflection.

Patients were stratified according to type of operation (APR or LAR), extent of nodal involvement (none, one to three, or > three involved regional nodes), and extent of invasion of perirectal fat or adjacent structures (none, extension into perirectal fat, and adherence to or invasion of adjacent organs or structures).

**Follow-Up**

Patients were had follow-up visits at 3-month intervals for 2 years after the completion of therapy, every 6 months for 3 years, and then yearly. At follow-up, patients gave their medical histories and underwent physical examinations, complete blood and platelet counts, and liver chemistry analyses. Chest x-rays and proctoscopies were performed every 6 months. Barium enemas or colonoscopies were performed at 1, 3, and 5 years after surgery.

**Statistical Methods**

The statistical methods for the treatment trial are described elsewhere. This article describes an exploratory analysis of the previously published data.

The statistical distributions of the numbers of nodes examined were described in the following subgroups: the overall eligible patient population (N = 1,664); node-negative patients (n = 527); and node-positive patients (n = 1,137). The distributions between patients with negative and positive nodal status were compared using graphical techniques and hypothesis testing. The Kolmogorov-Smirnov test was used to statistically compare the distributions overall; means were compared using the Wilcoxon test.

The χ² test was used to determine the association between nodal status (negative, positive) and the following categorical variables: performance status (0, 1, 2); operative procedure (LAR, APR); obstruction status (no, yes); invasion of perirectal fat or organs (none, microextension to fat, macroextension to fat); grade (well differentiated, moderately differentiated, poorly differentiated, other); vascular invasion (no, yes), and treatment arm (describe treatments). Tumor stage was defined as T3 versus T4 among node-negative patients and ≤ T2 versus > T3 among node-positive patients. Age was compared between patients with negative and positive lymph node status using the t test.

Spearman’s rank correlation coefficient was used to estimate the correlation between the number of nodes examined and the number of positive nodes identified among node-positive patients.

The method of Kaplan and Meier was used to estimate the survival and time-to-relapse curves by quartiles of the distributions of the numbers of nodes examined within subgroup. Univariate comparisons were made using the log-rank test. Cox regression was used to...
determine associations between survival and time to relapse and the following potentially prognostic variables simultaneously: number of nodes examined (continuous and by quartiles), treatment, age (continuous), performance status (0, 1, 2), operative procedure (LAR, APR), obstruction (no, yes), invasion of perirectal fat (none, microextension to fat, macroextension to fat, adherence to adjacent organs, invasion of adjacent organs), tumor stage ($\leq$ T3, T4) among node-negative patients, grade (well differentiated, moderately differentiated, poorly differentiated, other), and vascular invasion (no, yes). Survival was measured from study entry to death from any cause. Time to relapse was measured from study entry to documented relapse of disease or death from any cause.

RESULTS

A total of 1,664 patients (527 node-negative patients and 1,137 node-positive patients) were studied in this analysis. Nodal status was significantly associated with grade ($P = .002$), tumor stage ($P = .005$), and the presence of vascular invasion ($P = .001$) and was marginally associated with performance status ($P = .08$). Mean age was also significantly different, although the absolute difference was very small (node-negative patients, 61.9 years [$n = 549$]; node-positive patients, 60.7 years [$n = 1,151$]; $t$ test, $P = .03$). The following variables were not found to be significantly associated with nodal status: treatment ($P = .99$), operative procedure ($P = .41$), and obstruction ($P = .29$).

The distributions of the number of nodes examined by nodal status (negative, positive) are similar in shape but are statistically different (Kolmogorov-Smirnov test, $P = .01$). The means were also found to be significantly different (Wilcoxon test, $P = .001$).

The median follow-up for this cohort was approximately 7.5 years. Kaplan-Meier curves of time to relapse and survival by the number of nodes examined (in quartiles corresponding to each nodal status group) are given in Figs 1 through 6. For node-negative patients, the data were categorized into the following quartiles (average of 132 patients per quartile; Table 2): (1) $\leq$ zero and less than five nodes; (2) $\leq$ five and less than nine nodes; (3) $\leq$ nine and less than 14 nodes; and (4) $\leq$ 14 nodes. For node-positive patients (average of 284 patients per quartile; Table 3), these quartiles were as follows: (1) $\leq$ zero and less than six nodes; (2) $\leq$ six and less than 10 nodes; (3) $\leq$ 10 and less than 15 nodes; and (4) $\leq$ 15 nodes, based on the actual distribution of nodes in these patients.

Among the node-negative patients, there were significant differences in both the time to relapse and survival related to the number of lymph nodes that were examined by the pathologist. Improved survival was associated with a larger number of nodes examined. The $P$ values associated with the log-rank test were .003 for time to relapse and .02 for

![Fig 1. Failure-free survival for N0 patients by nodes-examined quartiles.](image1)

![Fig 2. Survival for N0 patients by nodes-examined quartiles.](image2)

![Fig 3. Failure-free survival for N1 patients by nodes-examined quartiles.](image3)
survival. No significant differences were found in the node-positive group, where the \( P \) values associated with the log-rank test were .37 for time to relapse and .44 for survival. Five-year relapse and survival rates by quartiles are given for all patients in Table 1, for node-negative patients in Table 2, and for node-positive patients in Table 3.

Cox proportional hazards models were developed for time to relapse and survival among node-negative patients. The final model for time to relapse included the number of nodes examined (continuous, \( P = .0001; \) relative risk [RR], 0.948) and tumor stage (\( \geq T3, T4 \) \( P = .0068; \) RR, 1.8). For survival, the final model included the number of nodes examined (continuous, \( P = .014; \) RR, 0.97), age (continuous, \( P = .0001; \) RR, 1.04), performance status (0, 1, 2) \( P = .0002; \) RR, 1.684), and tumor stage (\( \geq T3, T4 \) \( P = .003; \) RR, 1.86).

Each of these models was also considered using the number of nodes examined by quartiles. For time to relapse, node-negative patients in the fourth quartile for number of nodes examined (\( \geq 14 \)) had significantly improved survival over those in the first (\( \geq 0 \) and \( < 5 \) nodes) and second (\( \geq 5 \) and \( < 8 \) nodes) quartiles but not those in the third (\( \geq 8 \) and \( < 14 \) nodes) quartile \( P = .0005, P = .0024, \) and \( P = .16, \) respectively). Patients in the second quartile did not differ significantly from those in the first \( P = .59, \) whereas patients in the third quartile had a prolonged time to relapse \( P = .03. \)

For the survival analysis, node-negative patients in the fourth quartile for number of nodes examined (\( \geq 14 \)) had significantly improved survival over node-negative patients in the first (\( \geq 0 \) and \( < 5 \) nodes), second (\( \geq 5 \) and \( < 8 \) nodes), and third (\( \geq 8 \) and \( < 14 \) nodes) quartiles \( P = .008, P = .04, \) and \( P = .02, \) respectively). Patients in the second and third quartiles did not differ significantly from those in the first \( P = .48 \) and \( P = .59, \) respectively. This exploratory analysis suggests that for predicting survival the number of nodes be dichotomized as less than 14 versus \( \geq 14 \) nodes examined.

Among the patients who were determined to be node-positive, the correlation between the number of nodes

<table>
<thead>
<tr>
<th>Table 1. Outcome by Number of Nodes Examined (quartiles) in All Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Nodes Examined</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>( \geq 0 ) and ( &lt; 5 )</td>
</tr>
<tr>
<td>( \geq 5 ) and ( &lt; 9 )</td>
</tr>
<tr>
<td>( \geq 9 ) and ( &lt; 14 )</td>
</tr>
<tr>
<td>( \geq 14 )</td>
</tr>
</tbody>
</table>
examined and the number of positive nodes was estimated to be 0.34. Little data were available on measurement of the depth of invasion (although good information was available on tumor stage) (n = 224), nearest radial surgical margin (n = 101), and minimum distance to the nearest radial margin (n = 153). Therefore, we were not able to adequately assess the impact of these variables on time to relapse and survival.

**DISCUSSION**

It has been well demonstrated in many studies that the number of lymph nodes involved with tumor has a strong impact on outcome of patients treated for rectal cancer. Indeed, the American Joint Committee on Cancer staging system divides nodal status based on whether one to three nodes are involved or four or more. Gastrointestinal pathologists believe that it is important to analyze a moderate number of nodes in order to adequately determine the nodal status for the patient. It has been suggested that a total of six to 17 nodes be identified in the specimen to have an adequate evaluation, although the wide variation shows that there is little agreement on what is adequate. Some pathologists have routinely performed fat clearance on resected specimens, and the yield in terms of total number of nodes found and evaluated by the pathologist has been much higher than that reported in series without fat clearance techniques. Although there is clearly no absolute cutoff, we found that examining 14 or more nodes was an appropriate number to assure an adequate assessment of the nodal status.

Other recent series have shown higher numbers of nodes found than in the present series and have also shown that node-negative patients had fewer nodes examined than node-positive patients. For example, Wong et al reported that a mean 14 of nodes was found in node-negative patients compared with 20 in node-positive patients. In the current series, we also found on average that fewer nodes were examined among node-negative patients than among node-positive patients, although the differences were small (mean, 10.1 ± 11.3). The large sample size allowed us to detect such small differences; this is true for age and the comparison of the distributions of numbers of nodes examined as well.

<table>
<thead>
<tr>
<th>No. of Nodes Found in Node-Negative Patients</th>
<th>No. of Patients</th>
<th>5-Year Relapse Rate (%)</th>
<th>5-Year Survival Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 0 and &lt; 5</td>
<td>127</td>
<td>37</td>
<td>68</td>
</tr>
<tr>
<td>≥ 5 and &lt; 9</td>
<td>138</td>
<td>34</td>
<td>73</td>
</tr>
<tr>
<td>≥ 9 and &lt; 14</td>
<td>129</td>
<td>26</td>
<td>72</td>
</tr>
<tr>
<td>≥ 14</td>
<td>133</td>
<td>19</td>
<td>82</td>
</tr>
</tbody>
</table>

**Table 3. Outcome by Number of Nodes Examined (quartiles) in Node-Positive Patients**

<table>
<thead>
<tr>
<th>No. of Nodes Examined</th>
<th>No. of Patients</th>
<th>5-Year Relapse Rate (%)</th>
<th>5-Year Survival Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 0 and &lt; 6</td>
<td>270</td>
<td>41</td>
<td>61</td>
</tr>
<tr>
<td>≥ 6 and &lt; 10</td>
<td>306</td>
<td>49</td>
<td>58</td>
</tr>
<tr>
<td>≥ 10 and &lt; 15</td>
<td>264</td>
<td>47</td>
<td>57</td>
</tr>
<tr>
<td>≥ 15</td>
<td>297</td>
<td>46</td>
<td>61</td>
</tr>
</tbody>
</table>

The percentage of patients with positive nodes would be expected to be higher in the present series than in some others in the literature because our patients were selected to be at high risk for relapse based on tumor or nodal stage for entry onto an adjuvant therapy study. Thus, we cannot easily compare our data with those studies in the literature that have evaluated all patients treated surgically with colon or rectal cancer. However, to our knowledge, this is the only study that specifically evaluates outcome by tumor and nodal stage based on the number of nodes in the specimen in a group of patients treated in a consistent manner. It is also distinct in that most other series have evaluated patients with both colon and rectal cancer. It is easier to find lymph nodes in patients with colon cancer than in patients with rectal cancer, so the data on colon cancer may not be applicable to rectal cancer patients. This is especially true since colon cancer is more common than rectal cancer, so the data will generally be skewed.

The demonstration of a substantial difference in outcome for node-negative patients based on the number of nodes examined in the specimen can be explained in two ways. One is that some surgeons may have performed a more complete mesorectal resection with more soft tissue and associated nodes excised or a more thorough nodal dissection, thereby improving survival. Although a possible explanation, this does not seem likely for the node-negative patients. This could only be true if many of the node-negative patients were truly node-positive in second-elevation nodes that were not resected, while the primary drainage areas, which were resected, were negative. In addition, if this explanation were true, one would expect to see a survival benefit from resecting more nodes in patients who were node-positive, since in those individuals there would be a much higher likelihood of leaving residual positive nodes behind after a poor resection than in the patients who were otherwise node-negative. The fact that our results did not improve in the node-positive patients with more nodes being evaluated supports the contention that the improvement was not related primarily to the surgical procedure.

A more likely explanation is that the pathologists’ determination of node positivity was incorrect and that patients who were in fact node-positive were falsely categorized as...
node-negative. When more nodes were analyzed, these patients were more likely to be correctly classified as node-positive. This explanation is supported by the data of Goldstein et al., who found that the number of patients classified as node-positive continued to increase until 17 to 20 nodes were found. Scott and Grace found that 13 nodes needed to be examined to have a high confidence in pathologic node negativity. Recent studies by Ratto et al. support the contention that a more careful pathologic technique increases the number of nodes found in the specimen and the percentage of patients with positive nodes or with N2 (rather than N1) disease.

There are other data to support the contention that a full pathologic evaluation can provide valuable data that have an impact on outcome. Adam et al. have studied the effect of circumferential margin on local recurrence. They demonstrated a major influence of margin involvement on local recurrence rate and survival. This is consistent with other data from this same group, which has shown by multivariate analysis that tumor involvement of the circumferential margin is the single most critical factor in predicting local recurrence. The circumferential margin, which is historically created by blunt dissection at operation, is the retroperitoneal soft tissue over the nonperitonealized surface of a rectal resection specimen. Because of the biologic importance of this margin, routine assessment of circumferential margins is usually recommended, along with measurement of the distance from the tumor to the circumferential margin, the “surgical clearance” around the tumor. Nevertheless, many pathologists and surgeons alike remain unaware of the importance of the circumferential margin, and at present, it is not routinely assessed on pathologic examination of colorectal resection specimens. On attempting to analyze the prognostic importance of circumferential margin status in our study, we found that few pathology reports (< 10%) contained information referable to this parameter.

Lymph node collection from rectal resection specimens is both time-consuming and problematic. The radiologic and clinical criteria for diagnosis of nodal metastasis are based on size alone, despite the fact that large, easily identified lymph nodes in resection specimens are often reactive. In fact, one study showed that most nodal metastases in colorectal cancer are found in lymph nodes less than 5 mm in diameter. However, small lymph nodes are difficult to find, especially amid large amounts of perirectal soft tissue, and on gross examination, they are similar in color and consistency to blood vessels. Due to the lack of widely accepted pathology practice standards for lymph node examination in colorectal cancer specimens, there are many variations in the basic pathologic techniques used for lymph node collection and submission for microscopic analysis. Some of these variations include (1) the use of “clearing” solutions to improve visualization of small lymph nodes in the pericolonic or perirectal fat, (2) the submission of one half versus both halves of each node for microscopic examination, and (3) the preparation of one versus more than one tissue level per paraffin block of submitted nodal tissue. Thus, even by acceptable practices of “routine” lymph node examination, the thoroughness of the assessment varies. In an effort to reduce this variation, the College of American Pathologists has recently recommended that all grossly negative or equivocal lymph nodes be submitted in their entirety for microscopic examination. For grossly positive lymph nodes, it is recommended that a representative sample be submitted for microscopic confirmation. However, if fewer than 12 to 15 nodes are found after careful gross examination, it is suggested that additional visual enhancement techniques that aid in the microscopic identification of lymph nodes, such as fat clearing, be considered. Unfortunately, the purchase and disposal costs for chemicals required for these techniques are high. Clearly, the labor-intensive and otherwise expensive nature of optimal lymph node collection from colorectal cancer specimens is at odds with the economic constraints of the present health care environment.

There is at present a great deal of interest in the use of sentinel nodes to determine true nodal positivity more accurately. This approach has been used extensively for malignant melanoma and breast cancer, with good results. There is at present substantial interest in using sentinel nodes in colon cancer, but the studies have not yet confirmed the implications of the sentinel node determination of positivity. For rectal cancer, the sentinel node evaluation techniques have not been established, and they are likely to be more difficult than those for colon cancer. However, sentinel nodes may offer a much more accurate determination of nodal positivity, especially if they allow for intensive study of one or two nodes with immunohistochemistry and polymerase chain reaction. The clinical implications of this approach are not known.

The implications of these data are substantial. Eligibility and stratification for the large intergroup clinical trials in rectal cancer have relied on nodal status. Although there is no risk of including patients inappropriately on these studies based on the number of nodes examined, it is likely that some T2 patients who could have been included on study and who may have benefited from adjuvant therapy were not accrued and not treated because they were erroneously thought to be node-negative.

In addition, it may be that the substantial relapse rate among patients with node-negative disease is strongly
correlated with the pathologic evaluation. If an improved pathologic assessment were performed, it might be, for example, that selected patients with true T3N0 disease (in contrast to true T3N1/2) do not need to be treated. It is at least worth considering the possibility that more carefully evaluated subsets of patients would better define the patients who are truly at high risk. In support of this, Willett et al\(^\text{28}\) have suggested that careful pathologic evaluation can define a group of patients with T3N0 disease who may not need adjuvant therapy, although they did not evaluate the number of nodes found by the pathologist.

The major importance of this study is the demonstration of how critical a complete pathologic assessment of nodal status is in determining therapy and impact on prognosis. This study demonstrates the need for more uniformity in pathologic assessment, both for the benefit of the individual patient and for determination of effective national databases. It is clear that although it is likely that pathologic misclassification has produced many of the results shown here, they could be partially due to the extent of surgery. As mentioned earlier, there are good data to show that extent of surgery is important in optimizing local control and, perhaps, survival. Improving the quality of medical care in both of these areas could have a major impact on overall outcome, which likely will exceed those that will be produced in the near future by improvements in adjuvant chemotherapy and radiation therapy.

REFERENCES