The Prognostic Importance of Immunohistochemically Detected Node Metastases in Resected Esophageal Adenocarcinoma

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Background. The number or ratio of lymph node metastases detected by hematoxylin & eosin (H&E) staining is the most important predictor of survival in esophageal cancer. The survival effect of lymph node metastases detected on immunohistochemistry (IHC) is controversial. My colleagues and I hypothesized that the extent of nodal disease determined by both H&E and IHC examination would more accurately predict survival than either technique alone.

Methods. The study population consisted of 37 patients who underwent en bloc esophagectomy as primary therapy for esophageal adenocarcinoma 5 or more years ago. All had mediastinal and upper abdominal lymphadenectomy. No patient received neoadjuvant or adjuvant therapy. Tissue blocks were sectioned for H&E staining to confirm the initial histology, and a second slide was stained with monoclonal antibodies AE1 and CAM 5.2, which are directed at a number of cytokeratin antigens. The slides were reviewed by an investigator blinded to clinical outcome. The effect of IHC staining on prognosis was assessed by comparing 5-year survival based on H&E and IHC findings.

Results. A total of 1,970 nodes were examined in the 37 patients. Routine H&E staining detected metastases in 29 patients (78%); the remaining 8 with N0 disease all survived at least 5 years after operation (median not reached). In the 29 patients with N1 disease, survival was 41% at 5 years. In 20 of the 29 N1 patients, metastases were detected by H&E in less than 10% of the nodes removed; 55% of the patients survived 5 years, and 39% survived 8 years. Nine of the 29 patients had metastases detected in more than 10% of the nodes removed, and all died at a median of 17 months. IHC staining was performed on the nodes from the 8 N0 patients and the 20 patients with less than 10% nodal involvement (a total of 28 patients). Additional nodal metastases, not identified on H&E examination, were found in 51 nodes from 17 patients (60.7%). Of the 8 patients who were node negative on H&E examination, 3 had metastases detected by IHC, and all survived 5 years or more free of disease. Of the 20 patients with less than 10% nodal metastases on H&E, 14 (70%) had additional metastases detected by IHC (median, 2 nodes per patient). When combined with the results of H&E staining, the node ratio remained less than 10% in 13 patients and exceeded 10% in 7. Survival in patients whose ratio remained less than 10% was significantly better than in those whose ratio exceeded 10% (actual 5-year survival, 77% vs 14%; \( \chi^2 = 4.662; p = 0.03 \)).

Conclusions. IHC staining techniques can identify nodal metastases missed by routine H&E examination in a large number of patients. The combination of H&E and IHC examination is useful in patients with less than 10% nodal involvement by H&E examination in that IHC detection of micrometastases allows classification into low-risk (> 75% survival) and high-risk (< 15% survival) groups. IHC-detected micrometastases are not of prognostic importance in N0 patients or those with greater than 10% nodal metastases on H&E.

variety of cancer types to detect occult lymph node metastases that have been missed by routine histopathologic assessment. In breast cancer, IHC-detectable occult metastases have been found in up to 39% of node-negative patients, depending on tumor size and histologic subtype [2]. Similarly, occult micrometastases have been detected in as many as 27% of patients with node-negative lung cancer [3]. When patients with lymph node metastases detectable by routine histology are included, the frequency of detection of occult micrometastases increases to as high as 63% [4]. Although nearly all studies to date have shown that lymph node metastases are commonly overlooked with conventional histopathologic techniques, there is surprising disagreement regarding the prognostic implications of these occult micrometastases. Studies in esophageal cancer have consistently shown that micrometastases can be identified in an appreciable number of patients, but once again the prognostic importance of these occult metastases is unclear. Some have suggested that IHC-detected node metastases are of prognostic importance in both node-negative patients and those with N1 disease [5]. Others, however, have not shown such a relationship [6, 7].

Potential explanations for this discrepancy include errors in lymph node staging when a systematic lymph node dissection is not performed, inclusion of patients with various cell types of esophageal cancer, and failure to integrate IHC findings with the number of node metastases found on hematoxylin & eosin (H&E) staining. In this study, my colleagues and I sought to determine the prognostic importance of IHC-detected node metastases with patients classified on the basis of the extent of lymph node involvement. In particular, we hypothesized that the extent of node involvement on both routine H&E and IHC examination would more accurately predict outcome than either technique alone. The study was limited to patients who underwent an en bloc resection with systematic lymph node dissection for esophageal adenocarcinoma.

Patients and Methods
The study population represents a subgroup of 100 consecutive patients who underwent en bloc resection for esophageal adenocarcinoma [1] between January 1982 and November 2000. All patients had a systematic abdominal and mediastinal lymph node dissection at the time of esophagectomy, which was performed as primary therapy, allowing complete assessment of the status of the regional lymph nodes. None of the patients received neoadjuvant chemotherapy or radiation, and none had previous esophageal or gastric resections. Seventy-three of these patients underwent operation before November 1998, a group in which survival can be calculated with a minimum of 5 years of follow-up. Requests were submitted for the tissue blocks corresponding to the lymph nodes for all 73 patients. The study population included the 37 patients for whom all of the tissue blocks could be retrieved from storage in the archives of the Department of Pathology. Demographic information and tumor characteristics for these patients are shown in Table 1.

Surgical Approach
The en bloc procedure was performed through an initial right thoracotomy followed by a midline laparotomy [8]. The thoracic dissection included removal of the azygos vein and its associated nodes; the thoracic duct; and the low paratracheal, subcarinal, paraesophageal, and parahiatal nodes in continuity with the resected esophagus. The block of tissue removed was bounded laterally by the mediastinal parietal pleura, anteriorly by the membranous trachea and pericardium, and posteriorly by the aorta and the vertebral bodies.

The abdominal dissection included removal of the lymph nodes along the hepatic artery and portal vein from the porta hepatis to the celiac trunk, as well as the nodes around the celiac artery, along the left gastric artery, and around the lesser curvature of the stomach. In addition, all of the lymph node–bearing tissue overlying the vena cava and the right crus of the diaphragm were removed in continuity with the resected specimen. On the left side, the lymph node–bearing tissue along the splenic artery and overlying the adrenal gland and the left crus of the diaphragm was also removed.

Histopathologic Assessment
The original H&E-stained slides were obtained for all patients, and the slides were reviewed to confirm the initial diagnosis. A total of 1,970 lymph nodes were examined. The node status by H&E evaluation was reclassified in 5 patients. In 4, re-review of the H&E slides resulted in a change in the number of involved nodes

Table 1. Demographic Information and Tumor Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y, median (IQR)</td>
<td>57 (50.5–67.5)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>32/5</td>
</tr>
<tr>
<td>Tumor depth</td>
<td></td>
</tr>
<tr>
<td>Intramucosal</td>
<td>4 (10.8%)</td>
</tr>
<tr>
<td>Submucosal</td>
<td>4 (10.8%)</td>
</tr>
<tr>
<td>Intramuscular</td>
<td>6 (16.2%)</td>
</tr>
<tr>
<td>Transmural</td>
<td>23 (62.2%)</td>
</tr>
<tr>
<td>Lymph node status</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>8 (21.6%)</td>
</tr>
<tr>
<td>Positive</td>
<td>29 (78.4%)</td>
</tr>
<tr>
<td>Number of nodal metastases</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>8 (21.6%)</td>
</tr>
<tr>
<td>1–4</td>
<td>17 (45.9%)</td>
</tr>
<tr>
<td>&gt; 4</td>
<td>12 (32.4%)</td>
</tr>
<tr>
<td>Lymph node ratio</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>8 (21.6%)</td>
</tr>
<tr>
<td>&lt; 10%</td>
<td>20 (54.1%)</td>
</tr>
<tr>
<td>&gt; 10%</td>
<td>9 (24.3%)</td>
</tr>
<tr>
<td>Nodes removed, median (IQR)</td>
<td>49 (39.5–69)</td>
</tr>
</tbody>
</table>

IQR = interquartile range.
recorded. In 3 patients the number of node metastases was lower, and in 1 the number of node metastases was increased on the second review. One patient had been classified incorrectly as node negative on initial review; 2 node metastases were identified when the slides were re-reviewed. The error rate for the initial H&E evaluation was 13 (0.007%) in 1,970.

On the basis of our previous report of the poor survival associated with a lymph node ratio of more than 10% (17% 5-year survival), IHC assessment of lymph node status was limited to either patients who were node negative by H&E or those with a lymph node ratio of less than 10%. Tissue blocks corresponding to these 1,532 lymph nodes were sectioned to prepare a single 5-μm unstained slide for IHC analysis, by using previously validated techniques [2]. Standard immunoperoxidase staining was performed with antibodies AE1 (Signet Laboratories, Dedham, MA) and CAM 5.2 (Becton Dickinson, San Jose, CA). These antibodies are directed against cytokeratins 8, 10, 14, 15, 16, 18, and 19, allowing identification of epithelial cells. The IHC-stained slides were reviewed by an investigator, blinded to the patients' clinical outcome, who classified each node as positive or negative for micrometastases. Nodes were considered positive for occult metastases when individual immunoreactive cells (ie, expressed cytokeratin antigens) or clusters of cells were present within the substance of the lymph node and only if the morphology of the cells was consistent with cancer [2] (Fig 1).

**Follow-Up**

All patients were followed up by the operating surgeon at 3-month intervals for the first 3 years after operation and every 4 to 6 months thereafter. Follow-up was complete in all patients as of November 1, 2003, and consisted of a history and physical examination, a complete blood count, and a serum liver panel. Computed tomographic scans of the chest and abdomen were obtained at each visit, and suspected recurrent disease was confirmed by either biopsy or additional imaging studies. The median follow-up of surviving patients was 75 months (range, 63 to 100 months).

**Statistical Methods**

Comparison of proportions was performed with Fisher’s exact test. Survival was calculated with the method of Kaplan and Meier [9]. Comparisons between groups were performed with the log-rank test. The end point for these survival calculations was overall survival, including all-cause mortality. Initially the calculations were performed with information regarding the presence of nodal metastases based on H&E staining. The calculations were than repeated by incorporating the additional information regarding nodal involvement obtained by IHC. A p value of less than 0.05 was considered significant. The study was approved by the Institutional Review Board of the University of Southern California School of Medicine.

**Results**

**H&E Evaluation of Lymph Node Status**

A total of 1,970 nodes were examined in 37 patients (median, 49; interquartile range, 39.5 to 69). There was no

<table>
<thead>
<tr>
<th>Tumor Depth</th>
<th>Prevalence of Metastasesa</th>
<th>&gt; 4 Nodal Metastasesb</th>
<th>Lymph Node Ratio &gt; 10%c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intramucosal</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td>Submucosal</td>
<td>2/4</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td>Intramuscular</td>
<td>6/6</td>
<td>1/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Transmural</td>
<td>21/23</td>
<td>11/23</td>
<td>9/23</td>
</tr>
</tbody>
</table>

\(^a\) \chi^2 \text{ test for trend } = 17.16; \ p < 0.0001; \(^b\) \chi^2 \text{ test for trend } = 6.35; \ p = 0.012; \(^c\) \chi^2 \text{ test for trend } = 5.47; \ p = 0.02.

Fig 1. Photomicrograph of an immunohistochemistry-stained slide showing individual tumor cells within the substance of the lymph node and a morphology consistent with cancer.

Fig 2. Actuarial survival based on the presence or absence of lymph node metastases by hematoxylin & eosin examination ($\chi^2 = 8.44; \ p = 0.004$).
Evidence of metastasis by H&E staining in 1,845 of these nodes. Lymph node metastases were identified by H&E staining in 29 patients (78.4%). The frequency of nodal involvement and the number of nodes involved are summarized in Table 1. As expected, the prevalence of node involvement and the frequency of extensive node involvement (> 4 involved nodes or a lymph node ratio of > 10%) increased with increasing depth of tumor invasion (Table 2).

Survival based on the presence or absence of lymph node involvement is depicted in Figure 2. Eight patients had N0 disease, and all survived at least 5 years after operation (median not reached). When lymph node metastases were present, 5-year survival was 41.4% (\( \chi^2 = 8.44; p = 0.0037 \)).

Patients with N1 disease according to the American Joint Committee on Cancer classification system do not represent a homogenous group with respect to survival. When survival is calculated on the basis of the number of involved nodes or on the lymph node ratio (ie, the number of involved lymph nodes divided by the number of nodes removed), 2 distinct subgroups of patients in regard to survival are identified (Fig 3). This suggests that patients should be classified into 3 groups based on the extent of node involvement, as determined by either the number of involved nodes or the lymph node ratio. Seventeen patients had 1 to 4 nodes involved, and 20 had a lymph node ratio of less than 10%. In these patients with an intermediate prognosis, the 5-year survival was 64.7% and 55.0%, respectively.

**IHC Evaluation of Lymph Node Status**

Lymph nodes from the 8 patients with N0 disease and from the 20 patients with a ratio of involved nodes less than 10% were stained with IHC to assess the presence of micrometastases. Nodes from the 9 patients with a ratio of involved nodes more than 10% were not stained because all died of disease at a median of 17 months, indicating that no further prognostic information would be expected from IHC assessment. A total of 1,475 nodes were analyzed with IHC staining. Metastases not identified on H&E assessment were found with IHC in 51 nodes (3.5%) from 17 (60.7%) of the 28 patients. There was no significant difference in survival in patients with and without metastasis detectable by IHC (Fig 4).

In the 8 patients who were N0 on the basis of H&E assessment, 3 (37.5%) had micrometastases detected by IHC. All of these patients have survived 5 years or more, indicating that IHC-detected node metastases may not be of prognostic importance in patients who are node negative by H&E staining when a complete node dissection is performed.

In the 20 patients with a ratio of involved nodes less than 10%, 14 (70%) had additional nodal metastases detected by IHC. A median of 2 (interquartile range, 2 to 3) additional node metastases were identified per patient. When combined with the information obtained by H&E analysis, the lymph node ratio remained less than 10% in 13 patients, and it exceeded 10% in 7. Survival in patients whose ratio of involved nodes remained less than 10% was significantly better than that observed in patients with a combined ratio of involved nodes more than 10% (5-year survival, 76.9% vs 14.3%; \( \chi^2 = 4.66; p = 0.03 \); Fig 5).

Survival probabilities are depicted in Figure 6 for lymph node status with a combination of the information from H&E and IHC assessment. This classification scheme defines 4 subgroups of patients with differing survival probabilities after en bloc resection (Table 3).

**Comment**

It is generally accepted that metastasis to the regional lymph nodes is the most important prognostic factor in patients undergoing resection for cancer. For this reason, the use of IHC to accurately assess the degree of lymph node involvement has attracted considerable attention.
The prognostic value of IHC-detected micrometastasis has been shown in cancers of the breast and lung, and this encouraged us and others to evaluate its use in esophageal cancer. Izbicki and associates [5] reported on 68 patients with esophageal cancer who had a standard resection with IHC analysis of the removed nodes for the presence of micrometastases. Their study showed that IHC-detected nodal metastases were of prognostic importance in patients with and without node metastases on H&E histology. Subsequent publications were in conflict with their results and indicated either that IHC-detected micrometastases were of no prognostic importance [6, 7, 10, 11] or that the prognostic importance was limited only to patients with N0 disease on H&E examination [12–15].

There are several possible explanations for these discrepancies. In some series, the significance of IHC has been assessed without consideration for the H&E node status. This approach assumes that the effect of IHC-detected metastases is independent of the H&E node status, an assumption that seems intuitively incorrect and that was not supported by our data. We have found that the probability of detecting node metastases by IHC in a patient without node involvement by H&E analysis is half that of a patient with involved nodes. This finding suggests that IHC and H&E detection of involved lymph nodes are not independent of each other. In addition, we found no marked difference in survival when patient groups defined only by the presence or absence of node involvement by IHC were compared.

Variability in the extent of lymph node dissection performed is the second possible explanation for the discrepancies in the literature regarding the value of IHC-detected micrometastases. This introduces the possibility that incomplete node removal in some series may result in errors in staging, which may obscure the relationship with survival. In our series, in which a complete node dissection was performed, a benefit of IHC assessment was shown.

The third possible reason for the discrepancy is that most reported series follow the accepted American Joint Committee on Cancer staging system and consider lymph node status to be a dichotomous variable. This results in a staging system that is neither distinctive nor homogenous with regard to survival [1, 16–18]. We and others have suggested that a revision in the staging system for esophageal cancer is necessary to incorporate the relationship between the extent of lymph node disease and prognosis. Our data indicate that in patients who have had an adequate lymphadenectomy, the prognostic importance of IHC-detected micrometastases is restricted to the subgroup of patients with limited node involvement. A similar study by Komukai and colleagues [19] evaluated the prognostic importance of lymph node micrometastases in patients with squamous cell cancer stratified by the extent of nodal involvement. They also concluded that the presence of micrometastases was important in patients with N1 disease only as long as the number of metastatic nodes was limited. If the relationship between extent of nodal involvement and prognosis is not taken into account, there is a risk of missing the potential value of IHC node assessment.

It is also possible that the number of sections examined with IHC may affect the sensitivity of this approach in detecting otherwise occult tumor cells. We chose to perform IHC on a single section as an initial approach for practical reasons. If IHC is to be applied to the staging of esophageal cancer, this approach would be advantageous both in terms of physician acceptance and in terms of cost. It seems that in patients with N1 disease by H&E examination, a single section may be sufficient, because clear prognostic stratification was achieved. Whether
staining additional sections can improve the accuracy of staging in N0 patients requires further study.

The optimal threshold for defining limited and extensive node involvement is not known. In 1982, Skinner and colleagues [20] first suggested a revised classification system in which limited nodal disease was defined by the presence of 2 or fewer node metastases. Skinner and associates revised their recommendations to a threshold of 4 or fewer nodal metastases on the basis of subsequent analysis of additional patients undergoing en bloc resection [21]. Two subsequent studies have been published by DeMeester and associates [22] and Ellis and colleagues [23], who reached similar conclusions. Korst and colleagues [17] and, more recently, Rice and colleagues [16] have recommended a lower threshold, 3 or fewer and 2 or fewer, respectively, to define limited nodal involvement.

Classification according to a lymph node ratio (the number of nodes with metastases divided by the number of nodes removed) has also been proposed as being more accurate for prognostic purposes. Roder and colleagues [18] used a lymph node ratio of 20%, whereas more recent studies [24, 25] would suggest that a lymph node ratio of 10% better stratifies patients with regard to survival. We have recently reviewed our experience with 100 consecutive en bloc resections for esophageal adenocarcinoma in which patients were classified both to the threshold of the number of involved nodes (≤ 4) and the lymph node ratio (≤ 10%) [1]. We found that the lymph node ratio seems to best stratify patients both from the standpoint of 5-year survival and the risk of developing systemic metastatic disease. When N0 disease was present, 5-year survival was 92%. N1 disease with metastases to more than 10% of the nodes removed was associated with 18% survival with operation alone, indicating the need to consider alternatives such as neoadjuvant therapy in these patients. Patients with N1 disease limited to less than 10% of the nodes removed had an intermediate survival of 47%.

We propose that a lymph node classification system be applied to the staging of esophageal carcinoma that combines H&E and IHC findings. This would result in 4 groups of patients with distinctive survival probabilities (Table 3). Although micrometastases can be identified in a third of the patients with no evidence of node involvement by H&E examination, it seems that these IHC-detected node metastases are not of prognostic importance. All of these patients are alive and free of disease at least 5 years after en bloc resection. We are currently studying additional patients to add confidence to this observation. Likewise, IHC detected micrometastases are not of prognostic importance in patients with a ratio of involved nodes that exceeds 10% on the basis of H&E examination, because survival in this group of patients with surgery alone is only 11% at 5 years and because all of these patients died of disease by 6 years. As a result, adding IHC assessment would not be expected to provide any further prognostic information.

The additional information regarding lymph node status provided by IHC analysis has its greatest benefit in the management of patients with a ratio of involved nodes of less than 10% on the basis of H&E assessment. It is important to note that this is the largest of the 3 groups of patients in regard to nodal disease (ie, N0 and N1 ≤ 10% and N1 > 10%). With nearly half of these patients with limited N1 disease surviving long-term after en bloc resection alone, routine administration of adjuvant therapy is difficult to justify. A more precise

Table 3. Results of χ² Analysis for Each Pair of Classification Subgroups

<table>
<thead>
<tr>
<th>Variable</th>
<th>H&amp;E Negative</th>
<th>Combined Ratio &lt; 10%</th>
<th>Combined Ratio &gt; 10%</th>
<th>H&amp;E Ratio &gt; 10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>H&amp;E node negative</td>
<td>NA</td>
<td>χ² = 3.37; p = 0.07</td>
<td>χ² = 9.69; p = 0.002</td>
<td>χ² = 17.07; p &lt; 0.0001</td>
</tr>
<tr>
<td>Combined ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 10%</td>
<td>NA</td>
<td>χ² = 4.66; p = 0.03</td>
<td>χ² = 12.65; p = 0.0004</td>
<td></td>
</tr>
<tr>
<td>Combined ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 10%</td>
<td>NA</td>
<td>χ² = 3.42; p = 0.06</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

H&E = hematoxylin and eosin; NA = not applicable.
prognostic classification of these patients would permit identification of patients who will do well with surgery alone, thus sparing them the risk and inconvenience of unnecessary adjuvant therapy. Our results indicate that the number of IHC-detected lymph node metastases, when combined with the results of H&E assessment, can be used to define 2 distinct subgroups of patients with limited node involvement. When the combined node ratio based on IHC and H&E remains less than 10%, 5-year survival is in excess of 75%, and the routine administration of adjuvant therapy is not likely to be of benefit. However, if IHC identifies additional nodal metastases such that the combined node ratio exceeds 10%, only 14% survive long-term, and this encourages the use of adjuvant therapy.

The refinements in staging of esophageal carcinoma outlined in this study are also of importance in the design of future trials of adjuvant therapy by more precisely identifying patients most at risk for recurrence. Failure to enroll patients likely to be at high risk for systemic metastases would dramatically increase the number of subjects needed to show a benefit. Our results indicate that the ideal study population for such trials would be patients with a ratio of involved nodes of more than 10% either by H&E alone or when IHC and H&E results are combined.

In conclusion, IHC staining techniques can identify nodal metastases missed by routine H&E examination in a large number of patients. The combination of H&E and IHC examination is useful in patients with a ratio of involved nodes less than 10% on H&E examination in that the combined node ratio allows classification into low-risk (>75% survival) and high-risk (14% survival) groups. IHC-detected micrometastases do not seem to be of prognostic importance in N0 patients or in those with a ratio of involved nodes more than 10% on H&E examination. The accepted staging system should be revised not only to include information regarding the extent of nodal involvement, as has been suggested, but also to include the results of IHC staining to provide more accurate postoperative staging for patient management decisions.

References

DISCUSSION

DR JAMES D. LUKEITCH (Pittsburgh, PA): Dr Pairolero, Dr Murray, members, and guests. I would like to thank you for the opportunity to discuss this interesting paper.

Dr Waterman, I enjoyed your presentation and applaud the efforts of you and your surgical scientist colleagues at the University of Southern California for continuing to be leaders in advancing our knowledge of esophageal cancer. The strengths of this paper are numerous, and I would like to outline some of these.

The careful and complete surgical staging of patients who undergo molecular analysis is essential. It is very clear that when an investigator removes an average of close to 50 lymph nodes per patient en bloc with esophageal cancer, an accurate nodal staging has been achieved. This is important when attempting to understand the meaning of occult tumor cells, that is, they must be truly occult, and the only way to exclude histologically positive nodes and a more advanced stage is to perform a careful and systematic analysis of local and regional nodes, which you have shown beautifully.

The follow-up of close to 5 years minimum per patient is also commendable and allows one to make reasonable assumptions regarding the true oncologic outcomes of patients who remain clinically free of disease. The histology was reconfirmed, another important step often omitted from previous studies, and the cytokeratin immunohistochemistry was carefully done. You nicely showed that it may be possible to further stratify survival in some subsets of patients by using this technique, which may be superior to routine histology alone.

I have several other observations and related questions. How did you choose this particular subset of patients from what I assume would be a much larger pool of patients at the University of Southern California, even given the long-term follow-up that you were seeking? Were there statistical methods, issues of significance, and study power at the onset of this study that led you to choose this specific number of patients? Are you currently using this information to direct your adjuvant chemotherapy or radiotherapy in the postoperative setting?

I would like to know how you explain a lack of correlation with disease recurrence in the very small group of patients who were histologically node negative yet were IHC positive. Do you think that these patients were cured by the resection of a few more nodes, and do you think your conclusion in the abstract about the lack of IHC importance in node-negative patients may be too strong given this small number of patients?

Our own studies of node-negative patients performed at the University of Pittsburgh have shown an adverse outcome with reverse transcriptase-polymerase chain reaction for the tumor marker carcinoembryonic antigen (slide). We studied 30 patients who were node negative, and as you can see, the blue bars represent the carcinoembryonic antigen level on a logarithmic scale in patients who remained cancer free. The red bars represent those who experienced recurrence. Although there was some overlap when we looked at survival in this group, there was a clear stratification when we used reverse transcriptase-polymerase chain reaction results in a quantitative fashion to study node-negative patients.

Finally, in our review of the literature on the clinical significance of IHC-positive results in node-negative cancer patients in non-small-cell lung cancer, we found that most favored a prognostic role, and even the 3 largest studies published showed that, clearly, there was a worse overall survival in the group of patients that were node negative but IHC positive. So I wonder how you might consider this in light of your findings, and perhaps future studies may be necessary before we come to conclusions about IHC in esophageal cancer.

Congratulations, again, Dr Waterman, on a very provocative study, and good luck in your surgical career.

DR BRYAN MEYERS (St. Louis, MO): I also enjoyed your presentation and the analysis. I would just caution that the 10% cutoff you suggest might apply only to the patients who are treated surgically in the same manner that yours were. If I ever had 60 lymph nodes reported on a pathology report after one of my esophagectomies, I would probably keel over in shock and surprise. I would also imagine that the lymph nodes that are most likely to be positive are lymph nodes that everyone would take out; they would be next to the esophagus or in the lesser curvature of the stomach. The large number of additional lymph nodes that are obtained by the more en bloc and extensive resections are far more likely to be negative than positive. Thus, for most of us who do not take out 60 lymph nodes, that cut-point of risk must be higher than the 10% figure you derive from your data.

DR WATERMAN: Thank you, Dr Luketic, for your kind comments and for your insightful questions.

We agree that the best way to study the relationship between node status and outcome in cancer is to have the most accurate staging information possible. It is for this reason that we chose to limit this study to patients who underwent en bloc resection with complete local and regional node dissection. As you have correctly pointed out, the study sample was further limited by several other important criteria outlined in the article. As such, the patients reported in this series are in fact a limited subgroup of all patients operated on for esophageal cancer at our institution. First, we limited the analysis to patients with esophageal adenocarcinoma. Second, the study group included only patients operated on more than 5 years ago, so that the true outcome from the oncologic standpoint could be discerned. Third, patients who received preoperative chemotherapy or radiation treatment were excluded because this type of therapy can affect the status of the nodes at the time of resection. From this pool of patients, there were 20 or so for whom it was not possible to locate all of the tissue blocks corresponding to the nodes removed.

Working from this fixed group of study subjects, we are obviously limited in our ability to make definitive conclusions regarding negative outcomes of statistical analyses, for reasons of limited power. In particular, we are less confident of our conclusions regarding the importance of IHC in patients who are node negative on H&E assessment, given the small number of these patients in the study. We are, at present, studying more N0 patients to add confidence to our observations. We are, however, confident in our conclusions regarding patients with limited N1 disease.

In response to your next question, we do not mean to imply that removing the few IHC-positive nodes is what cured the patient. Although we believe that it is likely that local control would be compromised if these involved nodes were left behind, proving that removal of these nodes makes a difference in survival would require a much larger comparative study, which is beyond the scope of this report. Ultimately, the excellent survival observed in these patients treated by surgery alone is more likely a reflection of the absence of systemic disease when node disease is limited. Given the excellent prognosis observed when the lymph node ratio is less than 10%, we currently recommend nothing other than a
complete en bloc resection. However, if the node ratio exceeds 10% with H&E and IHC together, we recommend postoperative chemotherapy.

You may well be correct that the prognosis in N0 patients with IHC metastases may not be as favorable as it is in those who are IHC negative. Your data, as well as those from several other centers both in esophageal cancer and in lung cancer, suggest that IHC-detected node metastases do affect prognosis. We suggest, however, that from a patient management perspective it may not matter a great deal. Consider the fact that survival exceeds 75% with surgery alone in patients with H&E-detected nodal disease (N1 patients) who have a combined node ratio of less than 10% when information from IHC is added. Logically, it seems that patients who have no evidence of nodal involvement on H&E examination (N0 patients) and who have IHC-detected micrometastases should have a survival probability at least this high, unless the combined node ratio exceeds 10%. We have not identified any N0 patients with a sufficient number of IHC-detected micrometastases to bring the ratio of involved nodes to more than 10%. They may of course exist, but this seems unlikely. Although there may be an appreciable difference in survival between truly N0 patients and those who are N0 by H&E but with node disease by IHC, if more such patients were studied, management may not differ because it is hard to recommend additional therapy if complete surgical resection achieves these types of results.

I would like to make one final comment. This N0 group of patients that you have rightly focused on is the hardest group of patients to collect and study. It will take time to collect enough of these patients to perform a definitive study. It would be better if future studies included multiple centers, and if anyone would like to collaborate in this type of study, Dr Hagen would be happy to speak with you after this session.

Thank you, Dr Meyers for your comments. One major limitation in applying these results to other centers is the fact that all of these patients underwent a complete en bloc resection. It remains to be seen whether these same relationships between node status on IHC and survival hold when less extensive resections are performed. You may well be correct that the nodes most likely to be involved are those closest to the tumor. We just do not know at this point. If this is the case, you are correct that the threshold for the node ratio may need to be adjusted higher if the node dissection is less complete. We plan to perform IHC staining on nodes removed from our patients who underwent a transhiatal resection during this time interval to see if these observations hold. Future studies involving patients from multiple centers would also help us to determine whether the staging classification we propose can be applied or extrapolated to other centers.