The development of new treatment strategies for oesophageal cancer

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Chapter 3

Clinical significance of immunohistochemically detected micrometastases in histologically negative lymph nodes of patients with adenocarcinoma of the distal oesophagus or gastric cardia is the most important prognostic factor in patients with oesophageal adenocarcinoma.

Previous studies have demonstrated that immunohistochemical examination of lymph nodes may improve the pathological staging of oesophageal carcinoma. In daily practice is still debated due to contradictory results. This study analyses the clinical significance of immunohistochemically detected micrometastases in histologically negative lymph nodes of patients with adenocarcinoma of the distal oesophagus or gastric cardia.
INTRODUCTION

Oesophageal cancer is a highly aggressive carcinoma with poor long-term outcome. Surgery is generally considered to offer the best chance for cure, but even after resection with curative intent long-term survival is only 25%.\(^1\)\(^2\) This is due to the development of locoregional recurrences, distant metastases or a combination of both.\(^2\)

Controversy exists on how to improve survival. One strategy aiming to reduce locoregional recurrences is to perform a transthoracic resection with en bloc lymph node dissection in the posterior mediastinum and the upper abdomen, since an extended resection removing more (possibly tumour positive) lymph nodes might improve patient outcome when compared to a more limited transhiatal resection.\(^3\)

Another strategy to improve survival and to reduce locoregional and distant recurrence is the addition of non-surgical treatment to surgery. Recent studies suggest that preoperative chemoradiotherapy improves overall survival and disease specific survival.\(^4\)

To optimize treatment strategies for individual patients, prognostic factors can help to identify patients who will not be cured by surgery alone. Lymph node status as assessed by conventional histological examination is the most important prognostic factor in patients with oesophageal cancer.\(^5\) However, even when histological examination does not show lymphatic dissemination, tumour recurrence is not rare after potentially curative oesophagectomy. Locoregional recurrence or distant relapse after radical resection in node negative patients indicates that disseminated tumour cells, undetectable by current methods, must already have been present at the time of surgery. Immunohistochemical assays with monoclonal antibodies against tumour-associated antigens or epithelial-cell proteins can be used to detect isolated tumour cells or micrometastases (i.e. small cluster of tumour cells) in lymph nodes that are tumour-free on routine histological examination. However, the clinical significance of these immunohistochemical assays is still controversial with different prognostic values in different studies.\(^6\)\(^7\) This is probably due to variations in techniques, antibodies, and interpretations.

For clinical application, a marker for micrometastases has to be both highly specific and sensitive. With respect to specificity, the marker should be able to distinguish tumour cells from normal (esp. haematopoietic) cells. For sensitivity, the marker has to detect at least a large majority of the tumour cells. In addition, the question has to be answered whether all disseminated tumour cells are precursors of clinically relevant metastases or that they are just transiently shed cells with limited life span.
The aim of the present study was to analyse the incidence of nodal isolated
tumour cells and micrometastases (as assessed by three different
immunohistochemical assays) in patients with histologically node negative
adenocarcinoma of the distal oesophagus or gastric cardia after transthoracic
oesophageal resection, and to determine the clinical significance of these
immunohistochemical assays.

METHODS

Patients
Between April 1994 and February 2000, 161 patients were included in a
randomized controlled trial comparing limited transhiatal oesophagectomy to
transthoracic oesophagectomy with extended en bloc lymphadenectomy for
high-grade dysplasia (HGD) or adenocarcinoma of the distal oesophagus or
gastro-oesophageal junction (GOJ), in the Academic Medical Center,
Amsterdam, The Netherlands. Six of these 161 patients did not undergo
resection due to locoregional irresectability and/or distant dissemination, as
detected during the operation. One hundred thirteen patients (73.91%) were
excluded since they showed lymph node metastasis detected by routine
pathologic examination with hematoxylin and eosin staining. Since it is
acknowledged that a transhiatal oesophagectomy with limited lymph node
dissection is not an optimal staging procedure, another 20 patients who
underwent transhiatal resection were also excluded. The remaining 22
patients represent the study population. None of the patients received
chemo- and/or radiotherapy preoperatively, and no adjuvant treatment was
administered postoperatively. A limited number of patients received palliative
external radiotherapy for symptomatic tumour recurrence.
The study was done in accordance with the guidelines of the local ethics
committee.

Operative procedure
All 22 patients underwent subtotal oesophagectomy and resection of the lesser
curvature of the stomach through a right-sided thoracotomy and a midline
laparotomy, followed by a left-sided cervical oesophagostomy. The
thoracic lymphadenectomy comprised the lower and middle mediastinal,
subcarinal, and right-sided paratracheal lymph nodes dissected en bloc, and the
ortapulmonary-window nodes dissected separately. The paracardiac, lesser
curvature, left gastric artery (along with the lesser curvature), celiac trunk, common
hepatic artery, and splenic artery nodes were dissected via the laparotomy.
In all resection specimens, the origin of the left gastric artery was marked. Subcarinal nodes were marked separately.

Follow-up
All patients were seen at the outpatient clinic at intervals of three to four months during the first two years and every six months for three more years. After five years, follow-up data were obtained by telephone from the patient or his/her family practitioner. Recurrence of disease was diagnosed on clinical grounds. However, when relapse was suspected, radiologic, endoscopic, or histologic confirmation was sought for. Recurrent disease was classified as locoregional (including lymphogenic recurrence in the upper abdomen, mediastinum or cervical region) or distant (occurring as haematogenic recurrent disease). None of the patients were lost to follow-up.

Conventional pathologic examination
Processing of the resection specimens was done using a standardized protocol. Pathologic examination was performed by or under supervision of an experienced gastro-intestinal pathologist. Tumours were staged according to the UICC TNM classification 2002.11 Carcinoma of the gastric cardia and distal oesophagus were considered one clinical entity.14,15 All lymph nodes identified by the pathologist were collected in separate boxes and marked according to location, then cut in two with both sides stained with hematoxylin and eosin and evaluated for tumour involvement.

Immunohistochemistry
Three serial sections of 5 μm were cut at two separate levels from the formalin-fixed and paraffin-embedded archival tissue blocks. Specimens were deparaffinised, and pre-treated with 1% pronase (Dako, Hamburg, Germany) for antigen retrieval. To block unspecific binding sites, the slides were immersed in blocking solution (1:10 normal horse serum in Tris-saline). The antibody reactions for the anti-epithelial cell monoclonal antibody Ber-EP4 (dilution 1:200) and the monoclonal anticytokeratin antibody cocktail AE1/AE3 (dilution 1:150) (both Dako, Hamburg, Germany) were developed with the alkaline phosphatase-antialkaline phosphatase technique combined with the new fuchsin stain (Sena, Heidelberg, Germany), as described previously.6 Ber-EP4 is an antibody against two glycopolypeptides of 34 and 49 kD on the surface and in the cytoplasm of all epithelial cells (except parietal cells, hepatocytes, and the superficial layers of squamous epithelium). This antibody does not react with mesenchymal tissue, including lymphoid tissue.16 The antibody cocktail AE1/AE3 is specific for a range of human cytokeratins in
epithelial cells and does not react with lymphoid tissue. Immunohistochemical stainings for BerEP4 and AE1/AE3 were performed at the surgical laboratory of the University Hospital Eppendorf, Hamburg, Germany. The mouse monoclonal antibody CAM 5.2 (Becton-Dickinson, San Jose, CA, USA) is specific for intracellular cytokeratin-8 and -18 and does not react with haematopoietic and lymphoid cells. This staining was performed at the department of pathology, Academic Medical Center, Amsterdam, The Netherlands, according to the routine PAP/Giemsa method. In representative slides of the original adenocarcinoma, expression of the marker molecules was assessed within the original tumour, hereby demonstrating the presence of the specific markers for each tumour. Formalin fixed, paraffin embedded tissue sections of normal colonic mucosa served as positive staining controls, and isotype-matched irrelevant murine monoclonal antibodies served as negative controls (purified immunoglobulin mouse myeloma protein for IgG1; Sigma, Deisenhofen, Germany).

Detection of micrometastases
Micrometastasis was defined as the presence of a cluster of at least two tumour cells and was distinguished from the presence of an isolated positive cell (Figure 2A and B). Isolated tumour cells were discriminated from micrometastases since they seem to show a different level of clinical significance. False-positive non-neoplastic haematopoietic cells (e.g. reticular cells and plasma cells which can also show staining for cytokeratins), were discriminated from isolated tumour cells by microscopic morphological and nuclear differences (Figure 1C). Clusters of positive cells with malignant characteristics were designated as micrometastases when detected in the sinuses or lymphoid interstitium. In contrast, tumour cells surrounding the lymph node were considered as contamination occurred during the processing of the resection specimen (Figure 2D). The immunostained slides were evaluated by an experienced GI-pathologist (FJWtK), who was unaware of the clinical data.

Statistical analysis
Results are expressed as mean ± SD. All statistical analyses were performed using the Statistical Software Package version 11.5 (SPSS INC., Chicago, IL, USA). The association between clinicopathological features and the presence of micrometastases was analysed using Student's t-test (continuous data) or Chi-squared test (categorical data). Overall survival was estimated according to the Kaplan-Meier method and compared using the log-rank test. P-values of 0.05 or less were considered statistically significant.
RESULTS

Patient and tumour characteristics
There were 17 males (77.3%) and 5 females (22.7%) with a median age of 64 years (range 48 - 76 years). Two patients underwent oesophageal resection for HGD. Fourteen patients had an adenocarcinoma of the distal oesophagus developed in a Barrett segment, whereas the other eight patients had an adenocarcinoma of the GOJ or gastric cardia without Barrett’s metaplasia. The majority of patients (64%) had an early lesion (HGD or T1).

Detection of nodal micrometastases
Lymph node micrometastases (Figure 1B) were detected in five of the 22 patients (22.7%). They were found in 16 of the 636 lymph nodes examined (2.5%). Lymph nodes containing micrometastases were widely distributed, but the truncal nodes (M1a nodes) were most frequently involved (1x subcarinal, 4x distal oesophagus, 10x celiac trunk, 1x lesser omentum). The BerEP-4 and EA1/AE3 antibody showed an intense staining in all primary tumours and detected micrometastases in all five patients (15 and 13 out of 16 positive lymph nodes, respectively). In contrast, the CAM5.2 antibody with a moderate to strong staining in the primary tumours, only detected two micrometastases in two separate lymph nodes from one patient. In four lymph nodes, an isolated tumour cell was identified in the lymphoid interstitium (Figure 1A), but only in one patient this finding was not accompanied by the presence of micrometastases in other lymph nodes. In 124 of the 616 negative lymph nodes (20.1%), positive single cells were found that did not demonstrate malignant characteristics. These false-positively immunostained cells predominantly possessed haematopoietic cell morphology (e.g. plasma cells, lymphoid cells or mast cells) with a nucleus size comparable to surrounding cells and large cytoplasm (Figure 2C) and were more frequently found with the AE1/AE3 staining. In addition, in five micrometastases negative lymph nodes (0.8%) there were positive epithelial cells with malignant characteristics found at the edge of a lymph node which were considered false-positive due to contamination (Figure 2D).

Correlation between micrometastases and clinicopathological parameters
The presence of micrometastases was not significantly correlated with clinicopathological parameters at the time of operation (esp. age, gender, location of tumour, depth of tumour invasion, tumour differentiation grade and radicality of resection), although a trend was seen towards a positive
association with lymph-angio invasion \( (p=0.07) \) (Table 1A). A significant association was found between the presence of micrometastases and the development of locoregional recurrences \( (p=0.01) \) and distant metastases \( (p=0.006) \) (Table 1b). Of the 17 patients without immunohistochemically detected micrometastases no patient developed a locoregional recurrence and only one patient developed liver metastases after two years. In contrast, of the five patients with micrometastases one patient developed a locoregional recurrence, two a distant metastasis, and one patient developed both a locoregional recurrence and a distant metastasis. The one patient with a single isolated tumour cell in the lymphoid interstitium without a cluster of tumour cells did not develop recurrent disease after four and half years.
**TABLE 1A**
Correlation of micrometastases and clinicopathological findings

<table>
<thead>
<tr>
<th>Lymph node micrometastasis</th>
<th>Absent (n=17)</th>
<th>Present (n=5)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yrs, mean ± SD)</td>
<td>64±8</td>
<td>65±8</td>
<td>0.7</td>
</tr>
<tr>
<td>Gender</td>
<td>male (17)</td>
<td>female (5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 (71)</td>
<td>5 (100)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 (29)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td><strong>Tumour characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumour location</td>
<td>Oesophagus (16)</td>
<td>12 (71)</td>
<td>4 (80)</td>
</tr>
<tr>
<td></td>
<td>gastric cardia (6)</td>
<td>5 (29)</td>
<td>1 (20)</td>
</tr>
<tr>
<td>Depth of invasion&lt;sup&gt;a&lt;/sup&gt;</td>
<td>T0 (2)</td>
<td>2 (12)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>T1 (12)</td>
<td>10 (59)</td>
<td>2 (40)</td>
</tr>
<tr>
<td></td>
<td>T2 (1)</td>
<td>1 (6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>T3 (7)</td>
<td>4 (23)</td>
<td>3 (60)</td>
</tr>
<tr>
<td>Differentiation grade</td>
<td>well (5)</td>
<td>5 (29)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>moderate (9)</td>
<td>6 (35)</td>
<td>3 (60)</td>
</tr>
<tr>
<td></td>
<td>poor (8)</td>
<td>6 (35)</td>
<td>2 (40)</td>
</tr>
<tr>
<td>Vascular/lymphatic invasion</td>
<td>absent (14)</td>
<td>12 (71)</td>
<td>2 (40)</td>
</tr>
<tr>
<td></td>
<td>present (8)</td>
<td>5 (29)</td>
<td>3 (60)</td>
</tr>
<tr>
<td><strong>Operation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radicality of resection&lt;sup&gt;b&lt;/sup&gt;</td>
<td>R0 (18)</td>
<td>14 (82)</td>
<td>4 (80)</td>
</tr>
<tr>
<td></td>
<td>R1 (4)</td>
<td>3 (18)</td>
<td>1 (20)</td>
</tr>
</tbody>
</table>

<sup>a</sup>T0: carcinoma in situ, T1: tumour limited to the (sub)mucosa, T2: tumour infiltrates muscularis propria, but not adventitia, T3: tumour infiltrates adventitia.<br><sup>b</sup>R0: microscopically radical, R1: microscopically radical.

**TABLE 1B**
Correlation of micrometastases and clinical outcome parameters.

<table>
<thead>
<tr>
<th>Lymph node micrometastasis</th>
<th>Absent (n=17)</th>
<th>Present (n=5)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical outcome</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locoregional recurrence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (20)</td>
<td>17 (100)</td>
<td>3 (60)</td>
<td>0.01</td>
</tr>
<tr>
<td>Yes (2)</td>
<td>0 (0)</td>
<td>2 (40)</td>
<td></td>
</tr>
<tr>
<td>Distant metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (18)</td>
<td>16 (94)</td>
<td>2 (40)</td>
<td>0.006</td>
</tr>
<tr>
<td>Yes (4)</td>
<td>1 (6)</td>
<td>3 (60)</td>
<td></td>
</tr>
</tbody>
</table>
Association of micrometastases with overall survival
The median overall survival for the 22 patients with histologically node negative adenocarcinoma was 82.8 months (95% CI 70.9 - 94.6), which was significantly higher than the median overall survival of 21.0 months (95% CI 12.5 - 29.5) for the 113 pN1 patients (p<0.001, log-rank test)
A significant difference in overall survival was observed between patients with (n=5) and without (n=17) micrometastases (p=0.005; log-rank test) (Figure 2).
After 2 years the probability for overall survival was 94% (95% CI 82 - 100) for the micrometastases negative group which remained unchanged up to 5 years, while for the patients with micrometastases, the 2 year survival was 60% (95% CI 39 - 100) which declined after 5 years to 40% (95% CI 23 - 88). The overall survival of these pNO micrometastases positive patients was not significantly different from the overall survival of the 113 pN1 patients (p=0.3; log-rank test).

DISCUSSION
Lymphatic dissemination is known to be the most important prognostic factor for patients with oesophageal carcinoma. In this study it was demonstrated that immunohistochemically detected micrometastases of at least two tumour cells in lymph nodes also have prognostic significance for patients operated upon for adenocarcinoma of the distal oesophagus or gastric cardia. Several other studies have demonstrated the frequent presence of micrometastatic tumour cells in bone marrow and lymph nodes in these patients with an incidence varying from 25% to 65% (Table 2). However, whether these micrometastases have clinical significance remains controversial. Two studies which assessed the prognostic value of micrometastases in bone marrow, showed a similar significant impact on survival and recurrent disease in a combination of patients with pN0 or pN1 oesophageal cancer. However, three of the six studies analysing the prognostic value of micrometastases in lymph nodes could not demonstrate such an adverse effect on patient outcome. Interestingly, these three negative studies all included exclusively pN0 patients. This is in contrast with the results of our study in which the presence of micrometastasis in histologically negative lymph nodes was significantly associated with the development of locoregional recurrences, distant metastases, and a reduced overall survival.
A possible reason for these contradictory results involves differences in the number of lymph nodes examined between studies. Oesophageal carcinomas are known to metastasize frequently to lymph nodes at considerable distance
FIGURE 2
Kaplan-Meier curves of 22 patients with histologically node negative adenocarcinoma of the distal oesophagus or gastric cardia. There were 17 patients without micrometastases and five patients with micrometastases. A statistically significant difference was observed between the two groups (p=0.005; log-rank test).

Probability of overall survival

Survival in months

from their primary sites, even at an early stage of tumour invasion, while leaving lymph nodes in the immediate vicinity of the tumour unaffected (skip-metastases). Therefore, the extent of lymphadenectomy and number of nodes examined influences staging accuracy. In previous studies the mean number of lymph nodes examined varied widely, ranging from less than 10 to 379 per patient. To exclude variable results due to suboptimal staging procedures, only patients who underwent transthoracic resection with extended lymphadenectomy were included in this study with a mean of 29 lymph nodes examined per patient. Consistent with other studies, the lymph nodes containing micrometastases were widely distributed. Skip-micrometastases were frequently found in truncal nodes (stage M1a for distal oesophageal carcinoma) without positive cells in peri-oesophageal lymph nodes.

Another explanation for these conflicting data is that comparison of results
between studies is hampered by the substantial variation in methods with respect to staining protocols and antibodies. Even in our small series, the use of three different antibodies yielded variable results. In contrast to the anti-cytokeratin marker CAM5.2, the Ber-EP4 and AE1/AE3 antibodies both seem sensitive enough to detect the majority of clinically relevant micrometastases although both antibodies failed to identify the presence of micrometastases in one and three lymph nodes respectively. With respect to specificity, the AE1/AE3 antibody did stain more false-positive haematopoietic cells, which would make the Ber-EP4 antibody the marker of first choice. Moreover, the variation in terminology and definitions of micrometastases between studies is reason for confusion. It has been suggested that the finding of isolated tumour cells should be distinguished from micrometastases since it is unclear whether these single cells are all precursors of clinically relevant metastases or that they are just transiently shed cells with limited life span. Although O’Sullivan et al. showed that cultured single metastatic cells from rib marrow of patients with oesophagogastric cancer were found to be tumourigenic when inoculated subcutaneously in athymic nude mice, it has been demonstrated that the formation of a metastasis is a complex process.

### Table 2

**Micrometastases in oesophageal cancer: reported data on incidence and prognostic significance in bone marrow and lymph nodes.**

<table>
<thead>
<tr>
<th>Author</th>
<th>Patients (n)</th>
<th>Tumour type*</th>
<th>N-stage</th>
<th>Antibody</th>
<th>Presence of positive cells (%)</th>
<th>Overall survival</th>
<th>Local relapse</th>
<th>Distant metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thorban</td>
<td>30 SCC</td>
<td>N0/1</td>
<td>CK2</td>
<td>Ber-EP4</td>
<td>37</td>
<td>nd</td>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Macadam</td>
<td>31 AC/SCC</td>
<td>N0/1</td>
<td>Ber-EP4</td>
<td>AE1/AE3</td>
<td>36</td>
<td>p=0.02</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

**Lymph node micrometastases**

<table>
<thead>
<tr>
<th>Author</th>
<th>Patients (n)</th>
<th>Tumour type*</th>
<th>N-stage</th>
<th>Antibody</th>
<th>Presence of positive cells (%)</th>
<th>Overall survival</th>
<th>Local relapse</th>
<th>Distant metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Izbicki</td>
<td>63 AC/SCC</td>
<td>N0/1</td>
<td>Ber-EP4</td>
<td>AE1/AE3</td>
<td>65</td>
<td>p&lt;0.001</td>
<td>p=0.08</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Natsugoe</td>
<td>69 SCC</td>
<td>N0/1</td>
<td>AE1/AE3</td>
<td>42</td>
<td>P&lt;0.05</td>
<td>nd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glickman</td>
<td>78 AC/SCC</td>
<td>N0</td>
<td>AE1/AE3</td>
<td>25</td>
<td>ns</td>
<td>ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sato</td>
<td>50 SCC</td>
<td>N0</td>
<td>AE1/AE3</td>
<td>40</td>
<td>ns</td>
<td>ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nakamura</td>
<td>53 SCC</td>
<td>N0</td>
<td>AE1/AE3</td>
<td>26</td>
<td>ns</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Komukai</td>
<td>104 SCC</td>
<td>N0/1</td>
<td>AE1/AE3</td>
<td>45</td>
<td>p&lt;0.01</td>
<td>P&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present study</td>
<td>22 AC</td>
<td>N0</td>
<td>Ber-EP4 &amp; AE1/AE3</td>
<td>23</td>
<td>P=0.005</td>
<td>0.01</td>
<td>0.006</td>
<td></td>
</tr>
</tbody>
</table>

*Histological type of tumour: SCC = squamous cell carcinoma, AC = adenocarcinoma; *not determined; *not significant.
and only a small percentage of circulating tumour cells (0.05%) survive and initiate a metastatic focus. In addition, data on patients with breast cancer show that the finding of isolated tumour cells in sentinel lymph nodes has no impact on outcome and recurrent disease in these patients is very low. Therefore, it was decided not to consider isolated tumour cells as early dissemination in this study. This hypothesis would be in line with the finding that the one patient with an isolated tumour cell without micrometastases did not develop recurrent disease. Interestingly, exclusion of isolated tumour cells in lymph nodes as micrometastasis was not applied in the three studies in which lymph node micrometastases are not a prognostic factor for pNO patients, which could be an explanation for the discrepant results.

Despite the demonstrated prognostic significance of micrometastases in oesophageal carcinoma in this study, the question remains whether clinical implementation of this immunohistochemical analysis is feasible and useful. Immunohistochemical examination of lymph nodes is time-consuming and costly. In the literature there is still no consensus about how many slides should be considered as representative samples for the detection of micrometastases. As more sections are cut from tissue blocks, more micrometastases might be identified, but examination of numerous consecutive sections is not practical as a routine procedure. The results of this study, however, show that examining two levels is sufficient to detect the presence of the majority of clinically relevant micrometastases, since none of the pNO micrometastasis negative patients developed a locoregional recurrence and only one patient developed a distant metastasis.

Another problem is the frequent presence of false-positive cells. The possibility of non-specific reactions has particular importance when the detection of isolated tumour cells also would have therapeutic consequences. This study shows that haematopoietic cells can be falsely immunostained with anti-cytokeratin markers, implicating the need for morphological evaluation to exclude these false-positive cells. However, this evaluation is subject to inter-observer variation and a study analysing non-specific staining in bone marrow of breast cancer patients by double immunolabelling revealed false-positive cells in 5.4% of the patient samples even after morphological evaluation. Moreover, positive tumour cells can sometimes be identified at the edge of the lymph node, which should be considered as contamination. Therefore, histological verification of positive cells would always be of crucial importance. These methodological difficulties, and the possibility of false-negative results due to heterogeneous expression of a marker molecule within and between tumours, make the clinical application of this technique less useful for daily practice.
In conclusion, this study demonstrates that immunohistochemically detected micrometastases in lymph nodes have prognostic significance for patients operated upon for adenocarcinoma of the distal oesophagus or gastric cardia. We demonstrated that even minimal lymphatic dissemination is associated with a high incidence of systemic and/or locoregional recurrence. Extensive transthoracic resection with 2-field lymphadenectomy does not always prevent locoregional recurrences. Therefore, even for these early disseminated carcinomas, systemic (neo-)adjuvant treatment and/or additional radiotherapy is needed to improve long-term survival. In this context, immunohistochemical assessment of lymph nodes has the potential to refine the staging system for oesophageal cancer and to help identification of patients who will not be cured by surgery alone. However, the clinical application of this technique is still hampered because of the risk of false-positivity, false-negativity and high costs.
REFERENCES


