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DISEASES OF THE ESOPHAGUS

Original article

Analysis of micrometastatic disease in histologically negative lymph nodes of patients with adenocarcinoma of the distal esophagus or gastric cardia

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SUMMARY. **Lymphatic dissemination is the most important prognostic factor in patients with esophageal carcinoma. However, the clinical significance of lymph node micrometastases is still debated due to contradictory results. The aim of the present study was to identify the incidence of potentially relevant micrometastatic disease in patients with histologically node-negative esophageal adenocarcinoma and to analyze the sensitivity and specificity of three different immunohistochemical assays. From a consecutive series of 79 patients who underwent a transthoracic resection with extended 2-field lymphadenectomy, all 20 patients with pN0 esophageal adenocarcinoma were included in this study. A total of 578 lymph nodes were examined for the presence of micrometastases by immunohistochemical analysis with the antibodies Ber-EP4, AE1/AE3 and CAM 5.2. Lymph node micrometastases were detected in five of the 20 patients (25%). They were identified in 16 of the 578 lymph nodes examined (2.8%) and most frequently detected with the Ber-EP4 and AE1/AE3 antibody (sensitivity 95% and 79% respectively). In 114 of the 559 negative lymph nodes (20.4%), positive single cells were found that did not demonstrate malignant characteristics. These false-positive cells were more frequently found with the AE1/AE3 staining (specificity of the Ber-Ep4 and AE1/AE3 antibody 94% and 84% respectively). The presence of nodal micrometastases was associated with the development of locoregional recurrences ($P = 0.01$), distant metastases ($P = 0.01$), and a reduced overall survival (log rank test, $P = 0.009$). For the detection of clinically relevant micrometastatic disease in patients operated upon for adenocarcinoma of the distal esophagus or gastric cardia, Ber-EP4 is the antibody of first choice because of its high sensitivity and specificity. Immunohistochemically detected micrometastases in histologically negative lymph nodes have potential prognostic significance and are associated with a high incidence of both locoregional and systemic recurrence. Therefore, this technique has the potential to refine the staging system for esophageal cancer and to help identify patients who will not be cured by surgery alone.**

KEY WORDS: **esophageal cancer, immunohistochemistry, lymph node, micrometastasis.**

INTRODUCTION

Esophageal cancer is a highly aggressive carcinoma with poor long-term outcome.[1,2] Lymph node status, as assessed by conventional histological examination, is the most important prognostic factor in patients with esophageal cancer.[2,3] However, 30–40% of patients with histologically N0 carcinoma develop locoregional recurrences, distant metastases or both within 5 years.[2] This suggests that in these patients (lymph node) metastastic disease was initially present but remained unidentified by conventional preoperative work-up and standard histopathological examination.

Metastatic nodes can be missed if an insufficient number of lymph nodes is examined. One strategy aiming to improve staging (and survival) is to perform a transthoracic resection with extended lymph node dissection in the posterior mediastinum and the upper abdomen, since with an extended resection more (possibly tumor positive) nodes are removed when compared to a more limited transhiatal resection.[4–6]

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Another possibility to improve the staging of N0 esophageal carcinoma patients involves detection of clinically relevant micrometastastic disease undetectable by current methods. By current American Joint Committee on Cancer criteria, lymph nodes are positive if they contain deposits of tumor cells visible by hematoxylin and eosin (H&E) staining that are at least 0.2 mm in diameter.[7,8] Pathologists have long recognized the presence of smaller volume disease within regional lymph nodes. The term micrometastastic disease describes evidence of tumor metastases within regional lymph nodes that are not scored as positive by conventional histopathologic criteria. Immunohistochemical assays with monoclonal antibodies against tumor-associated antigens or epithelial-cell proteins can be used to detect smaller clusters of tumor cells or isolated tumor cells in lymph nodes that are tumor-free on routine H&E examination. However, the clinical significance of these immunohistochemical assays is still controversial with different prognostic values in different studies.[9–16] Retrospective studies using a variety of techniques, antibodies, and disease definitions do not consistently demonstrate a correlation between the presence of micrometastases and tumor recurrence. Prospective studies are hampered by the large numbers of patients required, the substantial time and expense involved in the methodologies to identify micrometastases. In addition, the lack of a clear definition hampers research into the clinical significance of this micrometastatic disease.

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 tumor cells from normal (especially hematopoietic) cells. For sensitivity, the marker has to detect at least a large majority of the tumor cells. In addition, the question has to be answered whether all disseminated tumor 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 identify the incidence of micrometastatic disease in patients with histologically node-negative adenocarcinoma of the distal esophagus or gastric cardia after transthoracic esophageal resection and to analyze the sensitivity and specificity of three different immunohistochemical assays. In addition, the clinical significance of these micrometastases was reviewed and the results compared to existing literature.

PATIENTS AND METHODS

Patients

Between April 1994 and February 2000, 161 patients were included in a randomized controlled trial comparing limited transhiatal esophagectomy to transthoracic esophagectomy with extended *en bloc* lymphadenectomy for high-grade dysplasia (HGD) or adenocarcinoma of the distal esophagus or gastro-esophageal junction (GOJ), in the Academic Medical Center, Amsterdam, the Netherlands.[6] Six of these 161 patients did not undergo resection due to locoregional irresectability and/or distant dissemination, as detected during the operation. One hundred and thirteen patients were excluded since they showed lymph node metastasis detected by routine pathologic examination with H&E staining. Since it is acknowledged that a transhiatal esophagectomy with limited lymph node dissection is not an optimal staging procedure, 20 patients who underwent transhiatal resection were excluded. Another two patients with HGD were also excluded since it is accepted that this lesion is not invasive and will not metastasize. The remaining 20 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 tumor recurrence.

Post-operatively, all patients were seen at the outpatient clinic at intervals of 3–4 months during the first 2 years and every 6 months for 3 more years. After 5 years, follow-up data were obtained by telephone from the patient or his/her family practitioner. Recurrent disease was classified as locoregional (including lymphogenic recurrence in the upper abdomen, mediastinum or cervical region) or distant (occurring as hematogenic recurrent disease). None of the patients were lost to follow-up.

The study was done in accordance with the guidelines of the local ethics committee.

Operative procedure

All 20 patients underwent subtotal esophagectomy and resection of the lesser curvature of the stomach through a right-sided thoracotomy and a midline laparotomy, followed by a left-sided cervical esophagogastrostomy. The thoracic lymphadenectomy in the left lateral position comprised the lower and middle mediastinal, subcarinal, and right-sided paratracheal lymph nodes dissected en bloc. The aortapulmonary-window nodes were dissected separately; after mobilization of the esophagus and primary tumor, the aortic arch was approached from below after identification of the left vagal nerve in order not to damage the left recurrent nerve. 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.

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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. Tumors were staged according to the International Union Against Cancer (UICC) TNM classification 2002.[8] Carcinoma of the gastric cardia and distal esophagus were considered one clinical entity.[17] All lymph nodes identified by the pathologist were marked according to location, then cut in two with both sides stained with H&E and evaluated for tumor involvement with H&E staining.

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 deparaffinized, and pretreated 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.[9] Ber-EP4 is an antibody against two glycopolypeptides of 34 and 49 kDa 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.[18] The antibody cocktail AE1/AE3 is specific for a range of human cytokeratins in epithelial cells and does not react with lymphoid tissue.[19] Immunohistochemical stainings for Ber-EP4 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 hematopoietic and lymphoid cells.[20] 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 tumor, hereby demonstrating the presence of the specific markers for each tumor. 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).

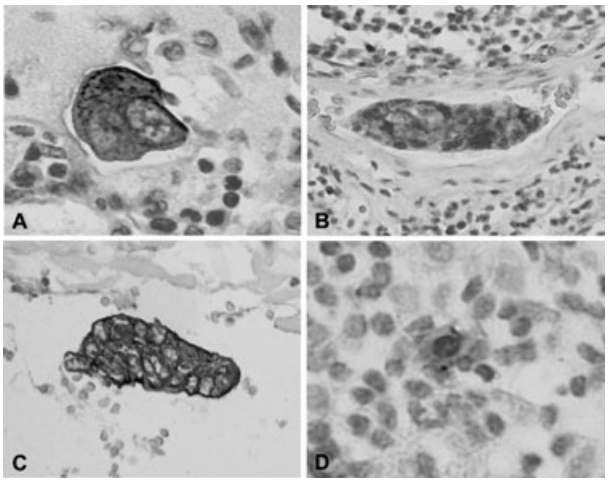

Fig. 1 Representative examples of immunohistochemically detected isolated tumor cells (A), micrometastases (B) and false positive cells (C, D) in lymph nodes. A: single positive cell with malignant characteristics (e.g. double nucleus); B: cluster of positive cells with malignant characteristics in lymph node sinus. C: single positive cell with hematopoietic characteristics (e.g. nuclear size comparable to surrounding cells) D: cluster of positive cells with malignant characteristics but suspect for contamination due to location.

Definition of micrometastatic disease

Tumor deposits within lymph nodes were classified and staged according to the revised guidelines set by the Americal Joint Committee on Cancer (AJCC).[8] Isolated tumor cells and small clusters of positive cells (< 0.2 mm) were discriminated from micrometastases (0.2–2 mm) (Fig. 1a,b).[7,21] False-positive non-neoplastic hematopoeitic cells (e.g. reticular cells and plasma cells which can also show staining for cytokeratins), were discriminated from isolated tumor cells by microscopic morphological differences (Fig. 1c). Clusters of positive cells with malignant characteristics were only designated as micrometastases when detected in the sinuses or lymphoid interstitium. In contrast, tumor cells surrounding the lymph node were considered as contamination that probably had occurred during the processing of the resection specimen (Fig. 1d). 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

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Table 1 Comparison of Ber-Ep4 (glycopolypeptides) and AE1/AE3 (cytokeratins) immunohistochemical analyses of micrometastatic disease. Agreement for staining of micrometastases and isolated tumor cells occurred in 14 (74%) of 19 nodes. False positively stained hematopoetic cells were more frequently found with AE1/AE3

	Micrometasases ($n = 16$)		Isolated tumor cells ($n = 3$)		Non-tumor cells ($n = 114$)	
	Ber-Ep–	Ber-Ep+	Ber-Ep–	Ber-Ep+	Ber-Ep–	Ber-Ep+
AE1/AE3+	12	1	2	0	11	79
AE1/AE3–	3		1		24	

features and the presence of micrometastases was analyzed 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 tumor characteristics

There were 16 males (73%) and four females (27%) with a median age of 64 years (range 48–76 years). Thirteen patients had an adenocarcinoma of the distal esophagus developed in a Barrett segment, whereas the other seven patients had an adenocarcinoma of the GOJ or gastric cardia without Barrett's metaplasia. The majority of patients (60%) had an early lesion (pT1). The mean follow-up was 81.2 months.

Detection of nodal micrometastatic disease

A mean of 29 lymph nodes per patient were examined. Micrometastases (0.2–2 mm) were found in 16 of the 578 histologically N0 lymph nodes examined (2.8%). These micrometastases (Fig. 1b) were detected in five of the 20 patients (25%). Two patients with disease stage I (according to AJCC classification) were upstaged to stage IIb, and three patients with disease stage IIa were upstaged to stage III.

The 16 lymph nodes containing micrometastases were widely distributed, but the truncal nodes (M1a nodes) were most frequently involved ($1\times$ subcarinal, $4\times$ distal esophagus, $10\times$ celiac trunk, $1\times$ lesser omentum). Skip metastases were found in two of the five carcinomas with M1a celiac trunk node involvement without positive lymph nodes around the tumor at the distal esophagus.

In three lymph nodes derived from two of these 20 patients (10%), isolated tumor cells with malignant characteristics were identified in the lymphoid interstitium but were not considered indicative for micrometastatic disease (Fig. 1a). In addition, in four micrometastatically negative lymph nodes there were positive epithelial cells with malignant characteristics found at the edge of a lymph node which were considered false-positive due to contamination (Fig. 1d).

Sensitivity and specificity of immunohistochemical analyses

The Ber-EP4 and AE1/AE3 antibody showed an intense staining in all primary tumors and detected micrometastatic disease in all five patients. The sensitivity of Ber-EP4 and AE1/AE3 for the detection of micrometastatic disease was 95% and 79%, respectively. Agreement between Ber-EP4 and AE1/AE3 stains on a node-to-node basis was 74% (Table 1). In contrast, the CAM5.2 antibody with a moderate to strong staining in the primary tumors, only detected two micrometastases in two separate lymph nodes from one patient.

In 114 of the 559 negative lymph nodes (20.4%), positive single cells were found that did not demonstrate malignant characteristics. These false-positively immunostained cells predominantly possessed hematopoeitic cell morphology (e.g. plasma cells, lymphoid cells or mast cells) with a nucleus size comparable to surrounding cells and large cytoplasm (Fig. 1c) and were more frequently found with the AE1/AE3 staining (Table 1). The specificity of the Ber-Ep4 and AE1/AE3 antibody was 94% and 84% respectively.

Correlation between micrometastastic disease and clinicopathological parameters

The presence of micrometastastatic disease was not significantly correlated with clinicopathological parameters at the time of operation (especially age, gender, location of tumor, depth of tumor invasion, tumor differentiation grade and radicality of resection), but a correlation with lymph-angio invasion could be demonstrated ($P = 0.04$) (Table 2).

A significant association was also found between the presence of micrometastases and the development of locoregional recurrences ($P = 0.01$) and distant metastases ($P = 0.01$) (Table 3). Of the 15 patients without immunohistochemically detected micrometastases no patient developed a locoregional recurrence and only one patient died due to liver metastases after 2 years. In contrast, of the five patients with micrometastases, four patients died due to recurrent disease, one patient developed a locoregional recurrence, two a distant metastasis, and one patient developed both a locoregional recurrence and a distant metastasis.

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Table 2 Correlation of micrometastases and clinicopathological findings

Patient characteristics	(*n*)	Lymph node micrometastasis		*P*-value
		Absent (*n* = 15) No. (%)	Present (*n* = 5) No. (%)	
Age (year, mean ± SD)	64 ± 8	64 ± 9	65 ± 8	0.7
Gender	male (16)	11 (73)	5 (100)	0.2
	female (4)	4 (27)	0 (0)	
Tumor characteristics				
Tumor location	Esophagus (15)	10 (67)	3 (60)	0.9
	gastric cardia (5)	5 (33)	2 (40)	
Depth of invasion†	T1 (12)	10 (67)	2 (40)	0.4
	T2 (1)	1 (7)	0 (0)	
	T3 (7)	4 (27)	3 (60)	
Differentiation grade	well (3)	3 (20)	0 (0)	0.5
	moderate (9)	6 (40)	3 (60)	
	poor (8)	6 (40)	2 (40)	
Vascular/lymphatic invasion	absent (13)	11 (73)	2 (40)	0.04
	present (7)	4 (27)	3 (60)	
Operation				
Radicality of resection‡	R0 (18)	13 (87)	4 (80)	0.7
	R1 (4)	2 (13)	1 (20)	

†T0: carcinoma *in situ*, T1: tumor limited to the (sub)mucosa, T2: tumor infiltrates muscularis propria, but not adventitia, T3: tumor infiltrates adventitia; ‡R0: microscopically radical, R1: microscopically irradical.

Table 3 Correlation of micrometastases and clinical outcome parameters

Clinical outcome	(*n*)	Lymph node micrometastasis		*P*-value
		Absent (*n* = 15) No. (%)	Present (*n* = 5) No. (%)	
Locoregional recurrence	No (18)	15 (100)	3 (60)	0.01
	Yes (2)	0 (0)	2 (40)	
Distant metastasis	No (18)	14 (93)	2 (40)	0.01
	Yes (4)	1 (6)	3 (60)	

Correlation between micrometastatic disease and overall survival

The median overall survival for the 20 patients with histologically node-negative adenocarcinoma was 73.6 months (95% CI 76.9–94.3), 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-lank test).

A significant difference in overall survival was observed between patients with ($n = 5$) and without ($n = 15$) micrometastases ($P = 0.009$; log-rank test, Fig. 2). After 2 years the probability for overall survival was 93% (95% CI 82–100) for the micrometastases-negative group which remained unchanged up to 5 years, while for the patients with micrometastases, the 2 years survival was 60% (95% CI 39–100) which declined after 5 years to 40% (95% CI 23–88). The overall survival of these pN0 micrometastases-positive patients was not significantly different from the overall survival of the 113 pN1 patients ($P = 0.3$; log-rank test).

When the definition of micrometastatic disease was changed and isolated tumor cells were included

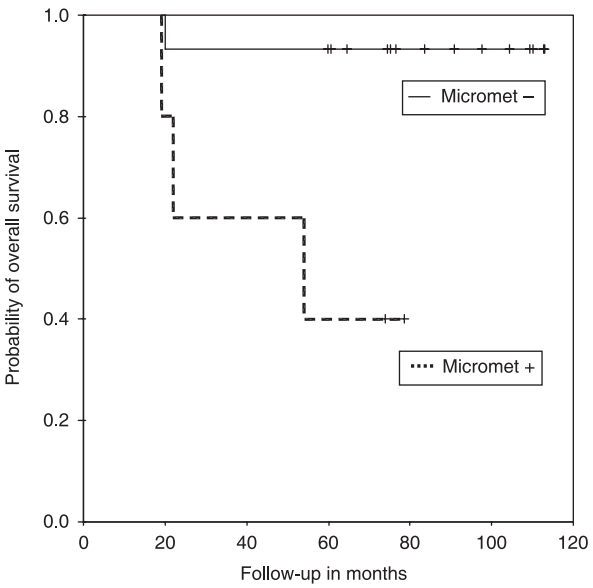

Fig. 2 Kaplan-Meier curves of 20 patients with histologically node-negative adenocarcinoma of the distal esophagus or gastric cardia. There were 15 patients without micrometastases and five patients with micrometastases. A statistically significant difference was observed between the two groups ($P = 0.009$; log-rank test).

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Table 4 Micrometastases in esophageal cancer: reported data on incidence and prognostic significance in bone marrow and lymph nodes

Author	Patients (*n*)	Tumor type†	N-stage	Antibody	Presence of positive cells (%)	Overall survival	Local relapse	Distant metasasis
Bone marrow micrometastases								
Thorban[22]	30	SCC	N0/1	CK2	37	nd‡	$P < 0.01$	$P < 0.01$
Macadam[23]	31	AC/SCC	N0/1	Ber-EP4	36	$P = 0.02$	nd	
Lymph node micrometastases								
Izbicki[9]	63	AC/SCC	N0/1	Ber-EP4	65	$P < 0.001$	$P = 0.08$	$P < 0.001$
Natsugoe[10]	69	SCC	N0/1	AE1/AE3	42	$P < 0.05$	nd	
Glickman[11]	78	AC/SCC	N0	AE1/AE3	25	ns§	ns	
Sato[12]	50	SCC	N0	AE1/AE3	40	ns	ns	
Mueller[13]	30	AC	N0	AE1/AE3	17	ns		
Nakamura[14]	53	SCC	N0	AE1/AE3	26	ns	0.04	ns
Komukai[15]	104	SCC	N0/1	AE1/AE3	45	$P < 0.01$	$P < 0.01$	
Schurr[16]	85	AC	N0/1	Ber-Ep4	49	$P < 0.01$		
Present study	20	AC	N0	Ber-EP4 & AE1/AE3	25	$P = 0.009$	0.01	0.006

†Histological type of tumor: SCC = squamous cell carcinoma, AC = adenocarcinoma; ‡not determined; §not significant.

as micrometastases, no difference in overall survival was observed between patients with and without micrometastases ($P = 0.16$; log-rank test).

DISCUSSION

Lymphatic dissemination is known to be the most important prognostic factor for patients with esophageal carcinoma.[3] Several studies have demonstrated that immunohistochemically detected micrometastic disease in lymph nodes or bone marrow, can upstage 25% to 65% of these patients (Table 4).[9–16,22,23] However, whether these micrometastases have clinical significance remains controversial. Comparison between studies is difficult since immunohistochemistry techniques are difficult to standardize with differences in antibodies, staining techniques and scoring systems.

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 esophageal cancer.[22,23] In contrast, four of the eight studies analyzing the prognostic value of micrometastases in lymph nodes could not demonstrate such an adverse effect on patient outcome.[11–14] Interestingly, these four negative studies all included exclusively pN0 patients. This is the first study which correlates the presence of micrometastatic disease in histologically negative lymph nodes to the development of locoregional recurrences, distant metastases, and a reduced overall survival. The four studies that could demonstrate a decreased overall survival with lymphatic microinvolvement all included macrometastastatic N1 patients.[9,10,15,16] The study of Schurr *et al*. even revealed an independent prognostic value of nodal microinvolvement.[16]

A possible reason for these contradictory results involves differences in the number of lymph nodes examined between studies. Esophageal carcinomas are known to metastasize frequently to lymph nodes at considerable distance from their primary sites, even at an early stage of tumor invasion, while leaving lymph nodes in the immediate vicinity of the tumor unaffected (skip-metastases). Therefore, the extent of lymphadenectomy and number of nodes examined influences staging accuracy.[24,25] In previous studies the mean number of lymph nodes examined varied widely, ranging from less than 10[9,11] to 37[12] 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.

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 anticytokeratin 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 hematopoietic 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 tumor cells should be distinguished from micrometastases since it is unclear

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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 metastastic cells from rib marrow of patients with esophagogastric cancer were found to be tumorigenic when inoculated subcutaneously in athymic nude mice[26] it has been demonstrated that the formation of a metastasis is a complex process[27] and only a small percentage of circulating tumor cells (0.05%) survive and initiate a metastastic focus.[28] In addition, data on patients with breast cancer show that the finding of isolated tumor cells in sentinel lymph nodes has no impact on outcome and recurrent disease in these patients is very low.[29] Therefore, it was decided not to consider isolated tumor cells as early dissemination in this study as is suggested by the AJCC.[15] When this definition of micrometastatic disease was used, there was no significant discrepancy in survival between patients with micrometastases and patients with histologically N1 carcinomas. Interestingly, when isolated tumor cells were included in the definition of micrometastatic disease, the prognostic clinical significance of micrometastases disappeared. This could be an explanation for the discrepant results since exclusion of isolated tumor cells in lymph nodes as micrometastasis was not applied in three of the four studies in which lymph node micrometastases were not a prognostic factor for pN0 patients.[11–13]

Despite the demonstrated potential prognostic significance of micrometastases in esophageal 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 and examination of numerous consecutive sections is not practical as a routine procedure. It could be hypothesized that immunohistochemical staining of micrometastasic disease is only necessary in a limited number of lymph nodes when this technique is combined with sentinel lymph node mapping. However, a sentinel node procedure in esophageal and cardiac cancer is hampered by the anatomical location of the esophagus and its lymphatic drainage in the closed space of the mediastinum.[30] So far, this technique is still considered experimental although preliminary data indicate that it is reliable in laparoscopically resected early adenocarcinoma.[31,32]

Another problem is the frequent presence of false-positive cells. The possibility of non-specific reactions has particular importance when the detection of isolated tumor cells also would have therapeutic consequences. This study shows that hematopoietic cells can be falsely immunostained with anticytokeratin markers, implicating the need for morphological evaluation to exclude these false-positive cells. However, this evaluation is subject to interobserver variation[33] and a study analyzing non-specific staining in bone marrow of breast cancer patients by double immunolabeling revealed false-positive cells in 5.4% of the patient samples even after morphological evaluation.[34] These methodological difficulties, with the possibility of false-negative results due to heterogeneous expression of a marker molecule within and between tumors, in combination with the time-consuming method make the clinical application of the technique used in this study less useful for daily practice.

In analyzing the clinical significance of micrometastatic disease, we acknowledge that a study of 20 patients has its limitations. The patients were retrieved in a high-volume center over a 7-year period. They all underwent an extended transthoracic resection for N0 carcinomas which is a minority of all patients undergoing surgical resection. However, this is the first study with strict inclusion criteria with respect to optimal lymph node mapping and histologically node-negative patients. The small number of patients precludes a multivariate analysis to identify micrometastases as an independent prognostic variable. However, our data demonstrate that the immunohistochemical staining of micrometastases is hampered by methodological difficulties with heterogeneous expression of a marker molecule within and between tumors, and that this staining is a time-consuming method. Another limitation of this study is that the identification of micrometastatic disease was analyzed by immunohistochemical stainings after conventional histological assessment of only a limited number of slides. It might be hypothesized that histological examination of additional slides also results in the detection of more micrometastatic disease. In the literature there is still no consensus about how many slides should be considered as representative samples for the detection of micrometastases. The results of this study show that the immunohistochemical examination at two levels can be sufficient to detect the presence of the majority of clinically relevant micrometastases.

In conclusion, this study demonstrates that for the detection of lymph node micrometastatic disease in patients operated upon for adenocarcinoma of the distal esophagus or gastric cardia, Ber-EP4 is the antibody of first choice. Our data support the currently used definition of micrometastatic disease (0.2–2 mm)[7,8] and suggest that these immunohistochemically detected micrometastases have potential prognostic significance since they are associated with a high incidence of both locoregional and systemic recurrence in these patients. In this context, immunohistochemical assessment of lymph nodes has the potential to refine the staging system for esophageal cancer and to help identify patients who

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have not been cured by surgery alone. However, the clinical application of this technique is still hampered because of the risk of false-positivity and high costs.

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