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ORIGINAL ARTICLE

Occult disseminated tumor cells in lymph nodes of patients with gastric carcinoma. A critical appraisal of assessment and relevance

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Abstract
Background and aims In gastric cancer, regional lymph node metastasis verified by histopathological examination is the most important prognostic factor after complete surgical tumor resection (R0). However, the prognostic value of immunohistochemically identifiable disseminated tumor cells in lymph nodes without histopathological tumor burden in patients with gastric cancer is still controversially discussed. The aim of the study was to assess the frequency and prognostic impact of minimal tumor cell spread to lymph nodes in these patients.
Patients–methods One hundred sixty lymph nodes judged as "tumor free" on routine histopathology obtained from 58 patients with gastric adenocarcinoma were analyzed immunohistochemically using the monoclonal anti-EpCAM antibody Ber-EP4 for occult disseminated tumor cells.
Results Tumor cells in lymph nodes were detected in 62 (38.8%) of the 160 "tumor-free" lymph nodes obtained from 39 (67.2%) patients. Multivariate Cox regression analysis confirmed the presence of disseminated tumor cells in "tumor-free" lymph nodes as an independent prognostic factor for both a significantly reduced relapse-free survival (p=0.008) and overall survival (p=0.009).
Conclusions The frequent occurrence and prognostic impact of minimal disseminated tumor cells in lymph nodes of patients with gastric carcinoma support the need for a refined staging system of excised lymph nodes, which should include immunohistochemical examination.

Keywords Disseminated tumor cells · Micrometastasis · Minimal residual disease · Lymph nodes · Gastric carcinoma

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Introduction

Early metastatic relapse after the complete resection of an apparently localized primary tumor indicates an occult tumor cell dissemination or micrometastatic disease, undetectable by current staging procedures. Therefore, more sensitive immunohistochemical and immunocytochemical assays have been developed that are able to detect occult disseminated tumor cells in bone marrow [1–4] and lymph nodes classified as "tumor free" by conventional histopathologic examination [5–17].

In gastric cancer, the strongest predictor for long-term survival after complete tumor resection (R0) is the absence of regional lymph node metastases verified by histopathological examination (pN0) [18–21]. Nevertheless, a relevant number of these patients with stages I and II disease (22–66%) die as a result of local or distant relapse despite complete surgical tumor resection with tumor-free resection margins (R0) [22]. Recent studies in a variety of solid tumors could demonstrate a high incidence of immunohistochemically detectable disseminated tumor cells in lymph nodes classified as "tumor free" on routine histopathological examination [15–17, 23]. Moreover, in several

malignant diseases including carcinoma of the esophagus, colorectum, or breast, the presence of disseminated tumor cells in "tumor-free" lymph nodes has been associated with a poorer postoperative prognosis [7–13, 15, 16, 23]. In patients with gastric carcinoma, the prognostic significance of these minimal tumor cell deposits in lymph nodes remains controversial. The wide differences of applied tumor cell detection protocols contribute to this confusion significantly.

In the present study, we performed our standardized immunohistochemical approach with a monoclonal anti-EpCAM antibody for the identification of disseminated tumor cells in lymph nodes in patients with gastric cancer. This antibody has been shown to be more specific for the detection of ectopic epithelial-derived tumor cells in lymphatic tissue compared with anticytokeratin (CK) antibodies, which were normally used in this context [24, 25]. The immunohistochemical findings were correlated with clinical follow-up data to validate the clinical relevance of the detected cells. Moreover, a critical appraisal of the recent literature revealed that there is an urgent need for standardization of the current tumor cell detection protocols to resolve the question of the prognostic value of disseminated tumor cells in lymph nodes in patients with resectable gastric carcinoma.

Materials and methods

Patients

This study was approved by the ethics committee of the chamber of physicians in Hamburg. Informed consent was obtained from all the patients before their inclusion in the study. Between November 1992 and June 2002, lymph nodes were prospectively sampled from 109 consecutive patients with gastric carcinoma who underwent gastric resection or gastrectomy at the Department of Surgery, University Hospital Hamburg-Eppendorf. Patients with histopathologically verified metastasis in all sampled lymph nodes, advanced peritoneal carcinosis, and/or additional malignancies were excluded. Thus, 58 patients with adenocarcinoma of the stomach undergoing intentionally curative (n=48) or palliative (n=10) surgical procedure were enrolled in the present study. None of the 58 study patients received neoadjuvant chemotherapy or radiotherapy.

All tumors were classified according to the TNM classification of the International Union Against Cancer [26, 27]. The histological classification into intestinal-, diffuse-, or mixed-type carcinoma is based on the classification of Laurén [28].

For survival analyses, patients without complete tumor resection (R_1; n=3), patients who died during the hospital stay (n=2), and patients with overt distant metastasis (n=10) were excluded. Four additional patients were lost to follow-up. The median length of postoperative observation period of the remaining 39 patients was 28 months (range, 3 to 111 months).

Lymph node preparation and immunohistochemical tumor cell detection

During the systematic lymphadenectomy, lymph nodes that were macroscopically inconspicuous were randomly collected and were divided into two parts as described previously [23]. One part of each lymph node was embedded in paraffin for routine histopathological examination, whereas the other part was snap-frozen in liquid nitrogen and stored at −80°C for immunohistochemical analysis. If a lymph node was "positive" on routine histopathology, it was excluded from the immunohistochemical analysis. The remaining nodes that were "negative" on routine histopathology were then analyzed immunohistochemically. From each of these nodes, cryostat sections of 6- to 8-μm thickness were cut at three different levels. Two consecutive samples obtained at each level were stained immunohistochemically with the monoclonal antibody (mAb) Ber-EP4 (IgG1; Dako, Hamburg Germany and Carpinteria, CA, USA) for the presence of disseminated tumor cells. This mAb detects the epithelial cell adhesion molecule (EpCAM), also known as 17-1A or EPG40, which is frequently expressed by epithelial cells and epithelial-derived tumors [12, 16, 25]. We and other groups previously demonstrated that immunohistochemical analysis with anti-EpCAM antibodies is a sensitive and specific method for detecting disseminated tumor cells in lymph nodes in a variety of solid epithelial tumors [9, 10, 12, 16, 23, 24]. Visualization of antibody binding was performed by the alkaline phosphatase-antialkaline phosphatase technique as described previously [16]. In addition, one adjacent section of each collected lymph node was incubated instead of the anti-EpCAM antibody with an isotype-matched, irrelevant murine monoclonal antibodies (MOPC-21; Sigma, Germany) for negative control. Sections of normal colonic mucosa consistently expressing EpCAM served as positive controls. The immunostained slides were evaluated in a blinded fashion by two observers working independently. Lymph nodes were interpreted as positive for disseminated tumor cells if they contained EpCAM-positive single cells or cell clusters and were negative on the negative controls.

Statistical analysis

Associations between categorical parameters were assessed via the χ^2 test and whenever appropriate with the Fisher's exact test. To analyze survival and recurrence events, we

Table 1 Patients characteristics in correlation to presence–absence of EpCAM-positive cells in their lymph nodes

Variable	No. (%) of patients without EpCAM+ cells	No. (%) of patients with EpCAM+ cells	*p* value[a]
All patients	19 (32.8)	39 (67.2)	
Male	14 (36.8)	24 (63.2)	
Female	5 (25.0)	15 (75.0)	0.271
Median age 56 years (27–86)			
<60 years	13 (38.2)	21 (61.8)	
≥60 years	6 (25.0)	18 (75.0)	0.221
Primary tumor			
$pT_{1–2}$	18 (42.9)	24 (57.1)	
$pT_{3–4}$	1 (6.2)	15 (93.8)	0.006
Lymph nodes			
pN_0	10 (50.0)	10 (50.0)	
$pN_{1–3}$	9 (23.7)	29 (76.3)	0.042
Distant metastasis			
M_0	19 (39.6)	29 (60.4)	
pM_1	0	10 (100)	0.012
Tumor grade			
Moderately differentiated (G2)	8 (38.1)	13 (61.9)	
Poorly differentiated (G3)	11 (29.7)	26 (70.3)	0.282
Laurén classification			
Intestinal type	9 (33.3)	18 (66.7)	
Diffuse type	8 (30.8)	18 (69.2)	0.582[b]
Mixed type	2 (40.0)	3 (60.0)	
Resection status			
R_0	18 (32.7)	37 (67.3)	
R_1	1 (33.3)	2 (66.7)	0.704

[a] Fisher exact test
[b] Comparison between intestinal and diffuse type

used log rank tests for univariate analysis. For comparison purposes, log-rank tests were performed. Cox's proportional hazards models were fitted for multivariate analysis. Relative risk and 95% confidence limits are presented. Differences between groups are considered to be significant if the *p* values were less than 0.05 for a two-tailed test (software SPSS 10.0, SPSS Inc. 1999).

Results

Routine histopathological examination of the in total 1,413 resected lymph nodes (mean 24.4 lymph nodes per patient; range six to 49 lymph nodes per patient) revealed lymph node metastases in 309 (21.9%) lymph nodes obtained from 38 (65.5%) of the 58 patients. For the immunohistochemical analysis, a total of 225 lymph nodes from these 58 patients were sampled. Of these sampled lymph nodes, 171 (76%) were judged as "tumor free" on routine histopathology and were further analyzed by immunohistochemistry. In 11 (6.4%) of these 171 lymph nodes, immunohistochemical analysis revealed immunostained cells that were also detectable in the negative control stainings. These single lymph nodes were therefore excluded as false positive. In the remaining 160 lymph nodes (mean 2.8 lymph nodes per patient; range one to eight lymph nodes per patient) EpCAM-positive tumor cells were found in 62 (38.8%) of these lymph nodes from 39 (67.2%) of the 58 patients (Table 1). These minimal tumor cell deposits presented as single cells ($n=10$ patients; Fig. 1), small cell clusters ($n=18$ patients), or both single cells and cell clusters ($n=11$ patients). Half of the patients classified as pN_0 on routine histopathology displayed EpCAM-positive tumor cells in lymph nodes (Table 1). The distribution of EpCAM-positive cells in the three lymph node compartments (D1–3) was not statistically different ($p=0.579$) and is summarized in Table 2.

There were significant correlations between the presence of EpCAM-positive cells in lymph nodes and primary tumor classification (pT category; $p=0.006$), lymph node classification (pN category; $p=0.042$), and the presence of intraoperatively detected distant metastases ($p=0.012$; Table 1).

For Kaplan–Meier survival analysis, a total of 39 patients undergoing curative tumor resection (R0) were available. Relapse-free and overall survival was significantly associated with the presence of lymph node metastases ($pN_{1–3}$; $p=0.0215$ and $p=0.0324$, respectively) and higher age ($p=0.0299$ and $p=0.0050$, respectively; Table 3). Moreover, after a median observation period of 28 months (range, 3 to 111 months), the presence of EpCAM-positive cells in lymph nodes was associated with both a significantly reduced relapse-free survival ($p=0.0149$) and overall survival ($p=0.0215$; Table 4, Figs. 2 and 3). Eleven (47.8%) of 23 patients with EpCAM-

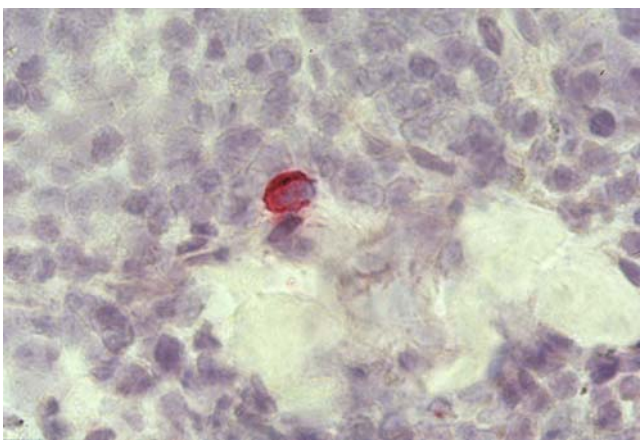

Fig. 1 Kyostate section of a "tumor-free" lymph node with an isolated EpCAM-positive tumor cell (×200 magnification)

Table 2 Distribution of EpCAM-positive cells in the three lymph node compartments

Lymph node compartments	No. of analyzed lymph nodes	No. of EpCAM+ lymph nodes (%)
D1	62	27 (43.5)
D2	72	25 (34.7)
D3	26	10 (38.5)
Total	160	62 (38.8)

p=0.579 (Chi square test)

positive cells in lymph nodes developed recurrence within a mean time of 41 months and 12 (52.2%) of them died within a mean time of 40 months compared with two (12.5%) and three (18.8%) patients without EpCAM-positive cells, respectively, within a mean time of 97 and 92 months, respectively. The estimated 2- and 5-year survival rates were both 81.3% for patients without and 65.2% and 47.8% for patients with EpCAM-positive cells in lymph nodes. Detailed analysis of the relapse-free survival revealed that the presence of EpCAM-positive cells in lymph nodes predicted for both a significantly reduced local recurrence-free survival (p=0.0228) and distant metastasis-free survival (p=0.0359; Table 4). Remarkably, none of the patients without EpCAM-positive cells developed local recurrence compared with five (21.7%) patients displaying EpCAM-positive cells in their lymph nodes. Multivariate Cox regression analysis confirmed the presence of EpCAM-positive cells in "tumor-free" lymph nodes as an independent prognostic factor for both a significantly reduced relapse-free survival (p=0.008) and overall survival (p=0.009). Consequently, patients with EpCAM-positive tumor cells in lymph nodes showed a 8.7 times increased risk for tumor relapse and a 6.1 times increased risk for shorter survival compared to patients without such cells (Table 5).

Discussion

In gastric cancer, the number and the level of lymph node metastasis identified on routine histopathological examination is the most important prognostic factor after curative tumor resection with tumor-free resection margins (R0) [18, 20, 21]. Recent studies in various solid tumors, including gastric cancer, demonstrated high incidences of disseminated tumor cells in the regional lymph nodes previously judged as "tumor free" on routine histopathology using sensitive immunohistochemical assays [5–12, 14–17, 23, 29]. However, especially in gastric cancer, the prognostic relevance of these cells remains unclear. Several studies found no impact of the presence of disseminated tumor cells in lymph nodes on postoperative outcome [30–33], whereas other groups described that the detection of these cells predicted for a significantly reduced postoperative survival [34–40]. However, only two of six published studies that described a prognostic impact of immunohistochemically detected tumor cells in lymph nodes confirmed their results by a multivariate analysis [37, 40]. Our study provides evidence that the presence of disseminated tumor cells in lymph nodes of gastric cancer patients are independent prognostic factors for both a significantly reduced relapse-free survival (p=0.008) and overall survival (p=0.009). Remarkably, none of the patients who were found to be free of disseminated tumor cells in lymph nodes developed local recurrence within the median observation period of 28 months.

Table 3 Kaplan–Meier analysis of relapse-free and overall survival according to clinicopathological factors

Variables	Patient events (%) [months][a]	p value
Relapse		
Male (n=24) vs. female (n=15)	34.8 [73] vs. 31.3 [70]	0.9488
Age ≥60 years (n=18) vs. <60 years (n=21)	50.0 [53] vs. 19.0 [89]	0.0299
$pT_{3–4}$ (n=7) vs. $pT_{1–2}$ (n=32)	28.6 [51] vs. 34.4 [72]	0.6142
$pN_{1–3}$ (n=20) vs. pN_0 (n=19)	50.0 [57] vs. 15.8 [86]	0.0215
G3 (n=24) vs. G2 (n=15)	33.3 [53] vs. 33.3 [72]	0.7508
Diffuse (n=17) vs. intestinal (n=19) vs. mixed type (n=3)	29.4 [73] vs. 36.8 [64] vs. 33.3 [54]	0.8834
Death		
Male (n=24) vs. female (n=15)	39.1 [71] vs. 37.5 [65]	0.8689
Age ≥60 years (n=18) vs. <60 years (n=21)	61.1 [45] vs. 19.0 [90]	0.0050
$pT_{3–4}$ (n=7) vs. $pT_{1–2}$ (n=32)	42.9 [49] vs. 37.5 [71]	0.8992
$pN_{1–3}$ (n=20) vs. pN_0 (n=19)	55.0 [53] vs. 21.1 [82]	0.0324
G3 (n=24) vs. G2 (n=15)	37.5 [52] vs. 40.0 [66]	0.7250
Diffuse (n=17) vs. intestinal (n=19) vs. mixed type (n=3)	41.2 [64] vs. 36.8 [66] vs. 33.3 [55]	0.9320

[a] Mean values of relapse-free and overall survival

Table 4 Prognostic impact of occult disseminated tumor cells in lymph nodes

Event	Patients *without* disseminated tumor cells	Patients *with* disseminated tumor cells	*p* value[a]
Death			
All patient events (%)	3/16 (18.8)	12/23 (52.2)	
Months[b]	92	40	0.0215
Relapse			
All patient events (%)	2/16 (12.5)	11/23 (47.8)	
Months[b]	97	41	0.0149
Local recurrence			
All patient events (%)	0/16 (0)	5/23 (21.7)	
Months[b]		56	0.0228
Distant metastasis			
All patient events (%)	2/16 (12.5)	9/23 (39.1)	
Months[b]	97	45	0.0359

[a] Log-rank test
[b] Mean value of survival in months

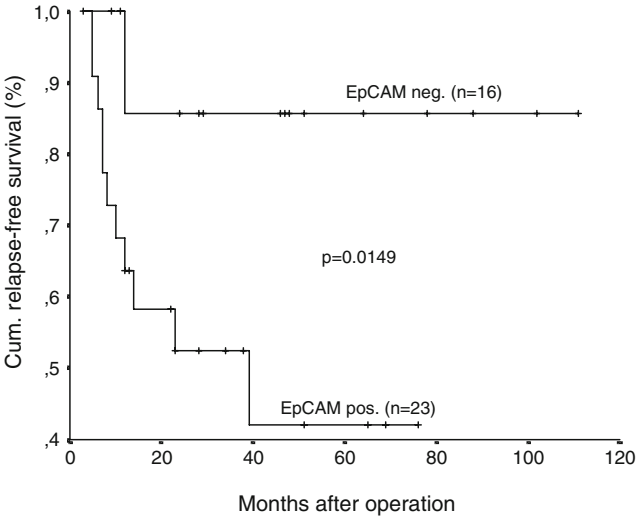

Fig. 3 Relapse-free survival of patients with (EpCAM pos.) and without disseminated tumor cells (EpCAM neg.) in lymph nodes

An explanation for these discrepant results might be due to the lack of any standardization in tumor cell detection protocols (Table 6), which might also explain tumor cell detection rates in lymph nodes of gastric cancer patients that range between 10% [33] and 49% [37] of the analyzed patients.

First, there are differences in the applied tumor cell detection antibodies (Table 4). Although all of these studies applied mAb against CK, different antibody clones against different CK components were used. Some investigators applied the mAb AE1/AE3, which is directed against a broad spectrum of CK components, including CK 1–6, 8, 10, 14–16, and 19 [32, 36, 37, 39], whereas others used the mAb CAM 5.2, which detects the CK components 8 and 18 [34, 38, 40], the mAb MNF 116, which is directed against CK 5, 6, 8, 17, and probably CK 19 [33], a mAb against CK 8 [30], or a cocktail of mAb CAM 5.2 combined with an anti-CEA antibody [35] (Table 6). In this context, it becomes obvious that the application of different antibody clones can result in different tumor cell detection rates due to their different targets. Fukagawa et al. [31] proved this point testing the sensitivities and specificities of three different anti-CK antibodies (AE1/AE3, KL-1, and CAM5.2) using primary tumor tissues of gastric cancer patients and found that mAb AE1/AE3 was the most sensitive one. On the other hand, described irregular CK expression (e.g., CK 18) in normal lymphatic reticulum cells [41], endothelial cells, or extrafollicular dendritic cells [42, 43] can lead to false positive results. Therefore, we used a mAb against the EpCAM, also known as 17-1A or EPG40 [12], which is frequently expressed by epithelial cells and epithelial-derived tumors and which seems to be a more specific target for tumor cell detection in lymph nodes since it is not expressed in lymphatic tissues [12, 16, 24, 25]. Applying this approach, we and other groups were able to demonstrate that detection of EpCAM-positive cells in "tumor-free" lymph nodes was of independent prognostic significance for a worse postoperative prognosis in patients with carcinoma of the pancreas [10, 29], esophagus [9, 10, 23], and lung [12, 16].

In addition, there are differences in the number of immunohistochemically analyzed sections per lymph node (Table 6). Some investigators evaluated only one section per lymph node [30, 32, 34], whereas others analyzed two [31] or three consecutive sections per lymph node [40]. Others [33] performed a serial sectioning of the entire lymph node on three different levels at intervals of 150 μm

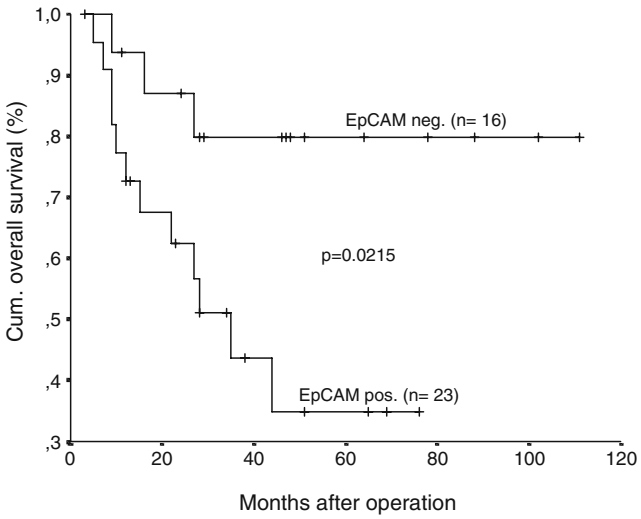

Fig. 2 Overall survival of patients with (EpCAM pos.) and without disseminated tumor cells (EpCAM neg.) in lymph nodes

Table 5 Results of Cox regression survival analysis

	Relative risk	95% confidence interval	*p* value
Overall survival			
$pT_{3–4}$ (n=7) vs. $pT_{1–2}$ (n=32)	0.3	0.084–1.311	0.1
$pN_{1–3}$ (n=20) vs. pN_0 (n=19)	3.5	1.050–11.88	0.04
Grade III (n=24) vs. grade II (n=15)	0.7	0.239–2.090	0.5
Immunohistochemistry positive (n=23) vs. negative (n=16)	6.1	1.566–23.99	0.009
Relapse-free survival			
$pT_{3–4}$ (n=7) vs. $pT_{1–2}$ (n=32)	0.2	0.050–1.201	0.2
$pN_{1–3}$ (n=20) vs. pN_0 (n=19)	4.1	1.067–15.96	0.04
Grade III (n=24) vs. grade II (n=15)	0.8	0.242–2.589	0.7
Immunohistochemistry positive (n=23) vs. negative (n=16)	8.7	1.777–42.37	0.008

or do not give any information about the number of analyzed sections [35, 36, 38].

Another problem is the different criteria in the evaluation of the immunohistochemical findings. Most investigators defined all events of both immunostained single cells or cell clusters that were unidentifiable by routine hematoxylin and eosin staining as disseminated tumor cells or micrometastases (MM) [31, 33–35, 38, 40], whereas others differentiated between "real MM", defined as single tumor cell or tumor cell cluster with a surrounding stromal reaction, and "tumor cell microinvolvement," defined as tumor cells without a stromal reaction [36,39]. Making this differentiation, Nakajo et al. [39] demonstrated that immunostained tumor cells in lymph nodes in the absence of a stromal reaction do not appear to affect survival, whereas the presence of "MM" was significantly correlated with a worse postoperative outcome. However, as described above, these authors did not confirm their results by a

Table 6 Summary of studies reporting on immunohistochemical detection of occult tumor cells in lymph nodes in patients with gastric cancer

Study	No. of patients	No. of analyzed LN	Detection marker (antibody clone)	No. of analyzed sections per LN	Negative controls	Tumor cell detection rates[a]	Impact on survival
Cai et al. [34]	84	2,526	CK (CAM 5.2)	1	No	1.8% (LN) 19% (Pat)	Not stated
Choi et al. [30]	88	2,272	CK 8	1	Yes	2.5% (LN) 31.8% (Pat)	No
Fukagawa et al. [31]	107	4,484	CK (AE1/AE3)	2	No	1.9% (LN) 35.5% (Pat)	No
Ishida et al. [35]	109	2,446	CK (CAM 5.2); CEA	Not specified	Yes	9.4% (LN) 40% (Pat)	Yes
Ishigami et al. [36]	180	4,203	CK (AE1/AE3)	Not specified	No	20% (Pat)	Yes
Kikuchi et al. [32]	51	1,390	CK (AE1/AE3)	1	Yes	4.8% (LN) 43.5% (Pat)	No
Lee et al. [37]	153	3,625	CK (AE1/AE3)	2	No	6.3% (LN) 49% (Pat)	Yes[b]
Maehara et al. [38]	34	420	CK (CAM 5.2)	Not specified	No	3.6% (LN) 23.5% (Pat)	Yes
Morgagni et al. [33]	300	5,400	CK (MNF 116)	Serial sections from three different levels	No	0.6% (LN) 10% (Pat)	No
Nakajo et al. [39]	67	1,761	CK (AE1/AE3)	1	Yes	1.5% (LN) 20.9% (Pat)	Yes
Yasuda et al. [40]	64	2,039	CK (CAM 5.2)	3	No	4% (LN) 32% (Pat)	Yes[b]
Scheunemann et al. (this study)	58	160	EpCAM (Ber-EP4)	Two sections each from three different levels	Yes	38.8% (LN) 67.2% (Pat)	Yes[b]

LN Lymph nodes, *CK* cytokeratins, *EpCAM* epithelial cell adhesion molecule.
[a] Detection rates are stated for immunohistochemically positive lymph nodes (LN) and immunohistochemically positive patients (Pat).
[b] Prognostic value was confirmed by a multivariate analysis

multivariate analysis. Furthermore, negative control immunostainings as described in the present study were only performed by a minority of these investigators [30, 32, 35, 39] (Table 6). Negative control staining, in our experience, is an essential component of immunohistochemistry. It lead to the exclusion of 11 (6.4%) of the analyzed lymph nodes in our study defined as "false positive".

Another difference of our study compared to previously published studies is the study design. All the described studies had a retrospective design reevaluating paraffin-embedded material. This study is prospectively analyzing intraoperatively harvested and randomly collected lymph nodes. We realize that the number of analyzed lymph nodes is low (n=160) and that this could introduce a sampling error. However, we and other groups were able to demonstrate that the described approach applying the anti-EpCAM antibody and analyzing low numbers of prospectively collected lymph nodes provides sufficient information [9, 10, 12, 16, 23, 29]. Furthermore, analyzing all resected "tumor-free" lymph nodes would not be routinely feasible since it is very costly and time-consuming.

As an alternative to immunohistochemistry, nucleic-acid-based detection of occult tumor cells has recently received considerable attention, which allows hypothetically the evaluation of an entire lymph node. In gastric cancer, several groups applied reverse-transcriptase polymerase chain reaction assays for epithelial (e.g., MUC2, CEA, CK20) or tumor marker (e.g., MAGE-3) transcript detection in lymph nodes [44–47]. However, the specificity of these ultrasensitive molecular assays is limited by the lack of any morphological correlate, the heterogeneity of genetic alterations, and the absence of suitable detection markers on mRNA or DNA level exclusively found in carcinoma cells [48–51]. Moreover, up to now, there are no reports evaluating the prognostic impact of occult tumor cells in lymph nodes detected by nucleic-acid-based methods in a valid number of patients with gastric cancer.

Conclusion

Our data indicate that the described immunohistochemical approach using an anti-EpCAM antibody can be used to refine the staging procedure for gastric cancer and help to identify patients at a high risk of tumor recurrence, which cannot be cured by surgery alone. Further studies with larger numbers of patients are needed to resolve the question of the prognostic value of disseminated tumor cells in lymph nodes in patients with resectable gastric carcinoma. However, there is an urgent need for standardization of the current tumor cell detection protocols and study designs in gastric cancer patients. This should include sufficient negative controls and a multivariate survival analysis, before immunohistochemical lymph node staging can be implemented into clinical practice. The detection of the earliest manifestations of tumor cell dissemination is an extremely promising approach, which might enable us to identify suitable candidates for adjuvant treatment strategies, for instance with humanized therapeutic anti-EpCAM antibodies [52].

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