Circulating Tumor Cells as a Biomarker for Preoperative Prognostic Staging in Patients With Esophageal Cancer

Matthias Reeh, MD, Katharina E. Effenberger, PhD,*† Alexandra M. Koenig, MD,* Sabine Riethdorf, PhD,† Dominique Eichstädt, MD,† Eik Vettorazzi, MSc,‡ Faik G. Uzunoglu, MD,* Yogesh K. Vashist, MD,* Jakob R. Izbicki, FACS,* Klaus Pantel, PhD,† and Maximilian Bockhorn, MD**

Objective: We evaluated the prognostic significance of circulating tumor cells (CTCs) in patients with esophageal cancer (EC).

Background: Despite the availability of several preoperative diagnostic techniques, accurate pretreatment staging of EC remains challenging.

Methods: In this single-center, prospective study, peripheral blood samples for CTC analyses were obtained preoperatively from 100 patients who were judged to have resectable EC. CTC detection was performed using the CellSearch System. Data were correlated with clinicopathological parameters and patient outcomes.

Results: CTCs were detected in 18% (18/100) of all eligible patients. Patients with CTCs showed significantly shorter relapse-free ($P < 0.001$) and overall survival ($P < 0.001$) than CTC-negative patients. Even in patients with lymph node invasion and without distant metastases (pN+, M0, N = 45), CTC detection indicated significantly worse relapse-free ($P < 0.001$) and overall survival ($P = 0.007$). Multivariate analyses of eligible patients identified CTCs as a strong, independent, prognostic indicator of tumor recurrence (hazard ratio, 5.063; 95% confidence interval, 2.233–11.480; $P < 0.001$) and overall survival (hazard ratio, 3.128; 95% confidence interval, 1.492–6.559; $P = 0.003$).

Conclusions: This is the first study to report that CTCs detected by an automated immunomagnetic detection system are independent, prognostic indicators of patients' outcome in EC. Thus, implementation of CTCs may improve accuracy of preoperative staging in EC.

Keywords: CellSearch, circulating tumor cells, esophageal cancer, staging, surgery

(*Ann Surg* 2015;261:1124–1130)

ClinicalTrials.gov Identifier: NCT01858805

Esophageal cancer (EC) is one of the most aggressive tumors, with a median survival of less than 2 years and long-term survival rates below 15%.[1,2] More than two thirds of all patients with EC develop local recurrence or distant metastases and die despite complete resection of the primary tumor and multimodal treatments. Recurrence or metastasis supposedly results from clinically occult, minimal residual disease caused by circulating tumor cells (CTCs) or disseminated tumor cells.[3,4] CTCs and disseminated tumor cells can derive from the same primary tumor, either circulating in the blood stream (CTCs) or disseminating to the bone marrow (disseminated tumor cells). Both groups of tumor cells have the potential to be precursors of metastases from various tumors, including EC.[5–10] Although, most shed tumor cells may die within the circulatory system due to physical and anatomic conditions, some CTCs seem to display an especially malignant potential, acquire stem cell characteristics, and finally evolve into metastases. Large studies investigating the metastatic potential and prognostic value of CTCs in EC are yet to be conducted. CTC detection by the CellSearch system is used in patients with metastatic breast, prostate, and colon cancer.[7,8,11] The clinical relevance of this system for nonmetastatic cancers is yet to be proven.

Here we assessed CTCs as a staging tool for nonmetastatic EC to stratify patients into defined prognostic subgroups. An appropriate staging system is essential for determining treatment strategies, especially those involving neoadjuvant treatments, in patients with EC. Despite the availability of several preoperative diagnostic techniques, accurate pretreatment staging remains inconsistent.[12] Therefore, a novel tool for early tumor detection, adequate prognostic staging, and accurate therapy monitoring in EC is urgently needed. The key aim of this study was to determine whether preoperative CTC detection can accurately indicate prognosis in patients with EC and may improve preoperative staging.

PATIENTS AND METHODS

Study Design

This prospective, single-institution study conducted at the University Hospital Hamburg-Eppendorf (Hamburg, Germany) enrolled 123 patients with ECs that were initially considered resectable. Informed consent was obtained from all patients. The study was approved by the medical ethics committee of the Chamber of Physicians of Hamburg. Only patients with histologically proven EC were included. None of the patients received neoadjuvant treatment. Peripheral blood samples for CTC analysis were collected immediately before surgery. Demographic, clinical, operative, and postoperative data were gathered for each patient.

All patients underwent thoracoabdominal esophagectomy by means of a right thoracotomy and a median, inverse T-shaped laparotomy. Peritumoral resection included *en bloc* subtotal esophageal resection with dissection of the right paratracheal, aortopulmonary window, subcardial, and mediastinal lymph nodes (LNs) and the azygos vein, and a collar or high intrathoracic anastomosis. Extensive lymphadenectomy of the upper abdominal compartment was performed (D2 lymphadenectomy, including pericardial and prepyloric LNs and those along the left gastric artery, lesser and greater gastric curvatures, coeliac trunk, common hepatic artery, hepatoduodenal ligament, and splenic artery). In 3 patients, esophagectomy was not performed due to local or distant tumor spread not detected during preoperative staging and only proven during abdominal exploration.

From the Departments of *General, Visceral, and Thoracic Surgery, †Tumor Biology, and ‡Biometry and Epidemiology, University Medical Centre, Hamburg-Eppendorf, Hamburg, Germany.

Disclosure: Supported by investigational grants from the "Hamburger Stiftung zur Foerderung der Krebsbekämpfung" and "B. Braun-Stiftung" (to M.R. and K.E.E.), and funding from The European Research Council, Brussels, (Advanced Investigator grant DISSECT, to K.P.).

The manuscript has been seen and approved by all authors.

The authors declare that the material has not been previously published or submitted for publication elsewhere.

All authors declare that they have no potential conflicts (financial, professional, or personal) to disclose that are relevant to the manuscript.

Reprints: Matthias Reeh, MD, General, Visceral, and Thoracic Surgery, University Medical Centre of Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany. E-mail: mreeh@uke.de.

ISSN: 0003-4932/15/26106-1124

DOI: 10.1097/SLA.0000000000001130

Moreover, 2 patients were found to have lung metastases after abdominal exploration and preparation of the gastric tube. Therefore, these 2 patients with lung metastases underwent esophagectomy.

Histopathological analyses were performed by a senior specialist in gastrointestinal pathology. All resected LNs were counted, identified by location and assessed separately. Standard histopathological analysis of paraffin-embedded LNs was performed by preparing 5-μm–thick serial sections, and hematoxylin-eosin staining and van Gieson staining. The analysis included tumor type, stage and grade, as determined according to the seventh edition of the tumor, node and metastasis classification.[13]

Postoperative follow-up was conducted at 3-month intervals for the first 2 years, including physical examination, plain chest radiography, abdominal ultrasonography, endoscopy, endoscopic ultrasonography, and computed tomography of the chest and abdomen. Studies of tumor markers (carcinoembryonic antigen and cancer antigen 19–9) and bone scans were also performed. Recurrence was diagnosed using biopsy or if unequivocal evidence of tumor masses with a tendency to grow was present during follow-up. Events considered were death, local recurrence, and distant metastasis. Overall survival was the time from operation to death or last follow-up; and progression-free survival was defined as the time from operation to diagnosis of tumor recurrence.

CTC Analysis

CTC analysis was performed using CellSearch as previously described.[14] Blood samples (7.5 mL) were collected in CellSave preservative tubes, stored at room temperature and processed within 48 hours of collection, according to the manufacturer's instructions. The accuracy and reproducibility of the CellSearch system have been described previously.[14,15] Presence of a nucleus, cytokeratin expression, round or oval cell morphology, and absent CD45 expression were the criteria for CTCs.[14] Cutoff value for CTC positivity was 1 CTC.[16]

Statistical Analysis

PASW Statistics 18 software (SPSS Inc., Chicago, IL) was used. Histological characteristics were expressed as descriptive statistics. The χ^2 test was used to investigate the association between CTCs and histopathological parameters. Univariate survival analysis was plotted by the Kaplan–Meier method and analyzed using the log-rank test. The results were presented as the median survival in months with the 95% confidence interval (CI) and number of patients at risk. For the multivariate analysis, the Cox regression model was used. The results were presented as hazard ratio with 95% CI. Significance was indicated by P values of 2-tailed tests less than 0.05.

All authors had access to the study data and had reviewed and approved the final manuscript.

RESULTS

Patient Characteristics and CTC Detection

Of the 123 study patients, 4 were excluded because of different postoperative diagnoses. Another 19 patients were excluded because of technical problems with the CellSearch system. Thus, eventually CTC analyses were performed for 100 patients, including 29 with squamous cell carcinoma (SCC), 68 with adenocarcinoma (AC), 1 with a mixed-type cancer and 2 with anaplastic carcinoma. In-hospital mortality was 6%. These and the 3 patients with mixed-type cancer and anaplastic carcinoma were excluded from survival analyses (N = 91). Fifty-one patients showed LN involvement; 46 showed no LN involvement. LN resection was not performed in 3 patients due to distant tumor spread.

Of the 100 patients, 77 were men and 23 were women, and their median age was 66 years (range, 32–85 years). The overall CTC detection rate was 18.0%. The CTC counts ranged from 1 to 56 cells/7.5 mL blood. Interestingly, only 3 of the 29 patients (10.3%) with SCCs showed 1 CTC or more, whereas 14 of the 68 patients (20.6%) with AC showed 1 CTC or more ($P = 0.261$). Among the 46 LN-negative patients, 8 (17.4%) showed 1 or more. Among the 51 LN-positive patients, 9 (17.6%) showed 1 or more.

We assessed the correlation of CTC positivity with sex and the following histopathological parameters: tumor size, nodal status, metastatic stage, Union for International Cancer Control stage, tumor grade, and resection margin status. The detection of 1 CTC or more showed a trend toward larger tumors ($P = 0.054$) that turned out significant in patients with AC ($P = 0.024$; Table 1). Presence of CTCs was significantly correlated with metastatic stage ($P = 0.013$; Table 1). Among patients with AC, the detection of CTCs was significantly correlated with tumor size ($P = 0.024$), metastatic stage ($P = 0.006$) and Union for International Cancer Control stage ($P = 0.049$). Among patients with SCC, women showed significantly more CTCs than men ($P = 0.006$). Other parameters were not significantly correlated with CTC positivity.

Univariate Survival Analysis

The median survival time was 26 months (95% CI, 22.18–29.82 months). The median follow-up time of surviving patients was 37.5 months. Patients with CTCs did significantly suffer from worse overall (median overall survival $P < 0.001$) and relapse-free survival ($P < 0.001$) compared with patients without CTCs (Fig. 1 A, B). Survival analysis of patients without distant metastases (M0) showed significant survival stratification by CTC status as well ($P < 0.001$) (Fig. 2). In a subgroup analysis including only LN-positive patients (pN+) without distant metastases (M0) (N = 45), CTC-positive patients had significantly worse overall ($P = 0.007$) and relapse-free survival ($P < 0.001$) than pN+, M0 patients without CTCs (Fig. 3 A, B). In LN-negative patients, CTC detection showed prognostic impact on overall ($P = 0.001$) and relapse-free survival ($P < 0.001$) as well.

Multivariate Survival Analysis

Data from 86 M0 patients were used in a multivariate analysis to test the effect of CTCs on overall and relapse-free survival, independent from other risk factors, namely, age, sex, tumor size, LN stage, and tumor grading. The risk of tumor recurrence was 5.1 times higher if CTCs were detected ($P < 0.001$; hazard ratio, 5.063; 95% CI, 2.233–11.480) (Table 2). In addition, the presence CTCs was an independent prognostic marker for overall survival ($P = 0.003$; hazard ratio, 3.128; 95% CI, 1.492–6.559) (Table 2).

DISCUSSION

This study proves the clinical significance of CTCs as a preoperative staging parameter in EC. Despite the availability of several preoperative diagnostic tools, for example, computed tomography, endoscopy, and endoscopic ultrasonography, pretreatment staging remains inaccurate. Furthermore, no adequate tool exists for patient stratification for multimodal treatments.

To our knowledge, this is the largest prospective study on the prognostic significance of CTCs in patients with EC judged preoperatively to be nonmetastatic. Overall and relapse-free survivals were similarly influenced by the presence of CTCs and were significantly shorter, irrespective of other risk factors such as tumor stage, LN invasion, tumor grade, and histological subtype. A similar influence of CTCs was observed in patients with AC; the impact of these cells in patients with SCC must be investigated by larger patients' cohorts with SCC. Several studies have reported that LN status is the most

TABLE 1. Patient Characteristics and Correlation of CTCs With Clinicopathological Parameters

Variables	All	CTC-Positive	P*	SCC	CTC-Positive	P*	Adenocarcinoma	CTC-Positive	P*
All	100†	18 (18.0)		29	3 (10.3)		68	14 (20.6)	
Age (yr)			0.669			0.099			0.902
≤65	49	8 (16.3)		13	0		35	7 (20.0)	
>65	51	10 (19.6)		16	3 (18.8)		33	7 (21.2)	
Sex			0.595			0.006			0.247
Male	77	13 (16.9)		20	0		56	13 (23.2)	
Female	23	5 (21.7)		9	3 (33.3)		12	1 (8.3)	
Tumor size			0.054			0.660			0.024
pT1	23	3 (13)		6	0		17	3 (17.6)	
pT2	20	1 (5)		8	1 (12.5)		12	0	
pT3	42	8 (19)		12	2 (16.7)		27	5 (18.5)	
pT4	12	5 (41.7)		3	0		10	5 (50.0)	
Missing‡	3	1 (33.3)		1	0		2	1 (50.0)	
Nodal status			0.253			0.472			0.268
pN0	46	8 (17.4)		16	3 (18.8)		29	5 (17.2)	
pN1	19	1 (5.3)		7	0		11	1 (9.1)	
pN2	16	3 (17.6)		2	0		13	2 (15.4)	
pN3	16	5 (33.3)		3	0		13	5 (38.5)	
Missing‡	3	1 (33.3)		1	0		2	1 (50.0)	
Lymph node invasion			0.974			0.112			0.657
Yes	51	9 (17.6)		12	0		37	8 (21.6)	
No	46	8 (17.4)		16	3 (18.8)		29	5 (17.2)	
Missing‡	3	1 (33.3)		1	0		2	1 (50.0)	
Metastatic stage			0.013			0.730			0.006
M0	95	15 (15.8)		28	3 (10.7)		64	11 (17.2)	
M1	5	3 (60.0)		1	0		4	3 (75.0)	
UICC stage			0.091			0.621			0.049
I	30	4 (13.3)		9	1 (11.1)		21	3 (14.3)	
II	23	4 (17.4)		11	2 (66.7)		11	2 (18.2)	
III	42	7 (16.7)		8	0		32	6 (18.8)	
IV	5	3 (60.0)		1	0		4	3 (75.0)	
Tumor grade			0.610			0.816			0.871
1	9	1 (11.1)		3	0		6	1 (16.7)	
2	39	6 (15.4)		17	2 (11.8)		22	4 (18.2)	
3/4	49	11 (22.4)		8	1 (12.5)		38	9 (23.7)	
Missing‡	3	0		1	0		2	0	
Resection margins			0.572			0.525			0.371
R0	84	14 (16.7)		25	3 (12.0)		56	10 (17.9)	
R1	13	3 (23.1)		3	0		10	3 (30.0)	
Missing‡	3	1 (33.3)		1	0		2	1	
Resection			0.452			1.000			0.296
Yes	97	17 (17.5)		28	3 (10.7)		66	13 (19.7)	
No	3	1 (33.3)		1	0		2	1 (50.0)	
Histological type			0.261						
AC	68	14 (20.6)							
SCC	29	3 (10.3)							

*Indicates significance according to χ^2 test when CTC-negative patients are compared with CTC-positive patients.
Round parentheses indicate percentages.
†Twenty-nine SCCs, 68 ACs, 1 SCC and AC, and 2 anaplastic carcinomas.
‡The missing values refer to patients that did not undergo resection as a result of distant metastases or local tumor infiltration.
UICC indicates Union for International Cancer Control.

significant risk and prognostic factor in patients with EC.[17,18] We found that CTC detection seems to be a stronger indicator of overall and relapse-free survival than pathological LN stage shown by the results of the multivariate analysis.

The release of CTCs may be an important step in the metastatic cascade.[19] Klein[20] showed that CTC detection in patients with early-stage tumors indicates that hematogenous spread occurs at an early stage of tumor progression, even in the absence of lymphatic spread. Our results are consistent with these findings. In 8 patients with stage I or II tumors, CTCs were found. These results contribute to our understanding of the metastatic cascade, especially that in EC, in which CTC involvement detected by an immunomagnetic system has not been reported before.

CTC detection rates in patients with EC from other studies that used different tests (eg, reverse transcriptase-polymerase chain reaction)[21] ranged from 2% to 32.9%.[22–24] Lack of methodological uniformity, nucleic acid-extraction protocols, and molecular marker selection, as well the inconsistent definition of CTC positivity in polymerase chain reaction-based methods may account for this large variation. The CellSearch system was designed to detect CTCs in peripheral blood samples by using immunomagnetic enrichment (EpCAM) and immunocytochemical (cytokeratin, CD45, DAPI)

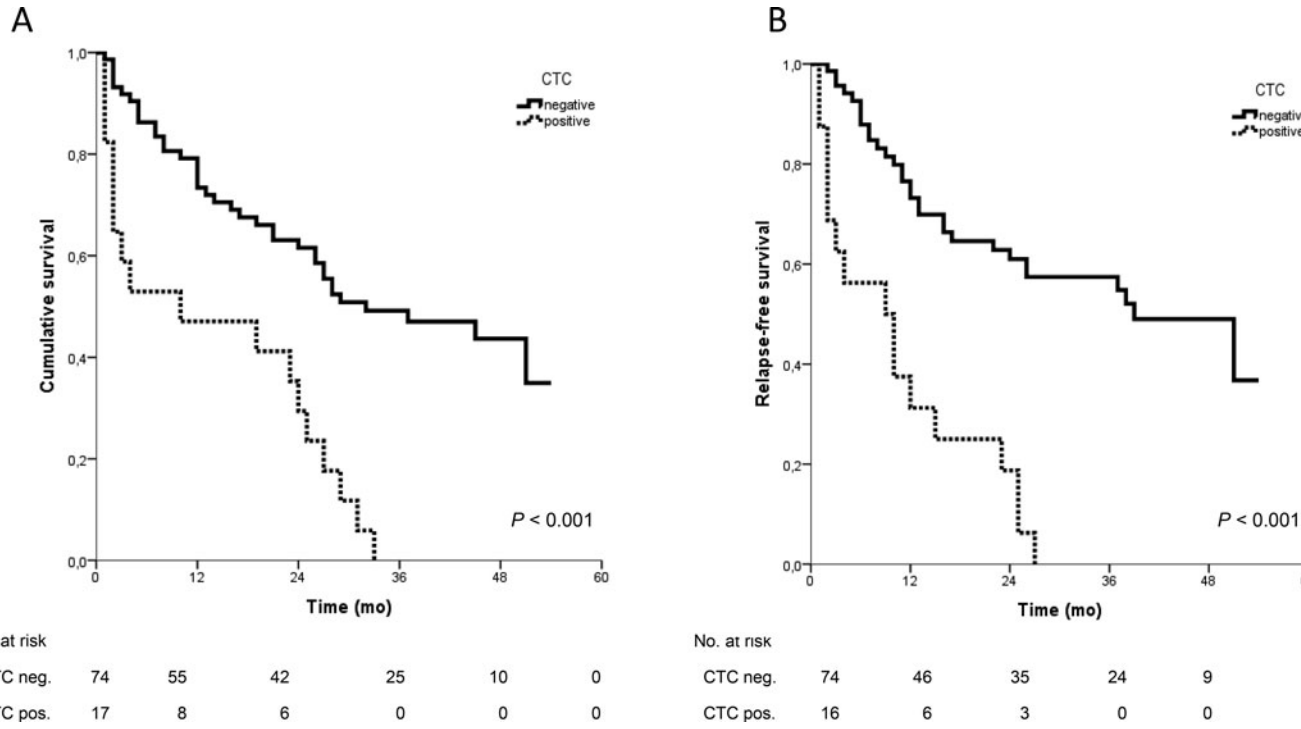

FIGURE 1. A, B, Outcomes of patients with EC with CTCs compared with patients without CTCs. Correlation of overall (A) and relapse-free survival (B) with CTCs in patients with EC (N = 91).

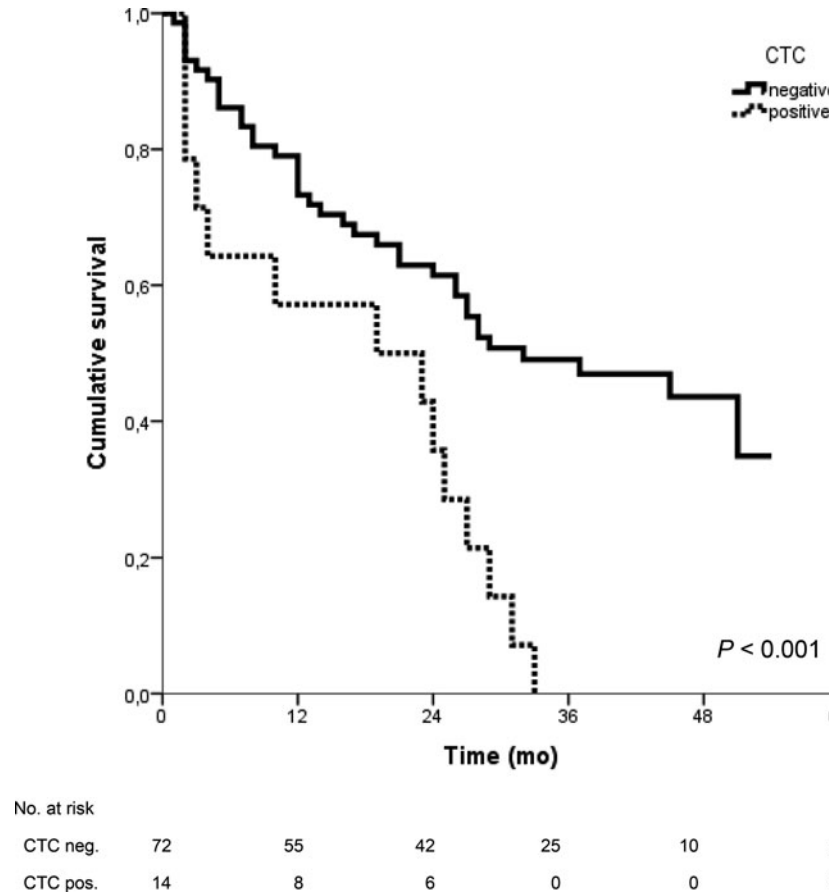

FIGURE 2. Outcomes of patients with EC with CTCs compared with patients without CTCs. Correlation of overall survival with CTCs in patients without distant metastases (M0) (N = 86).

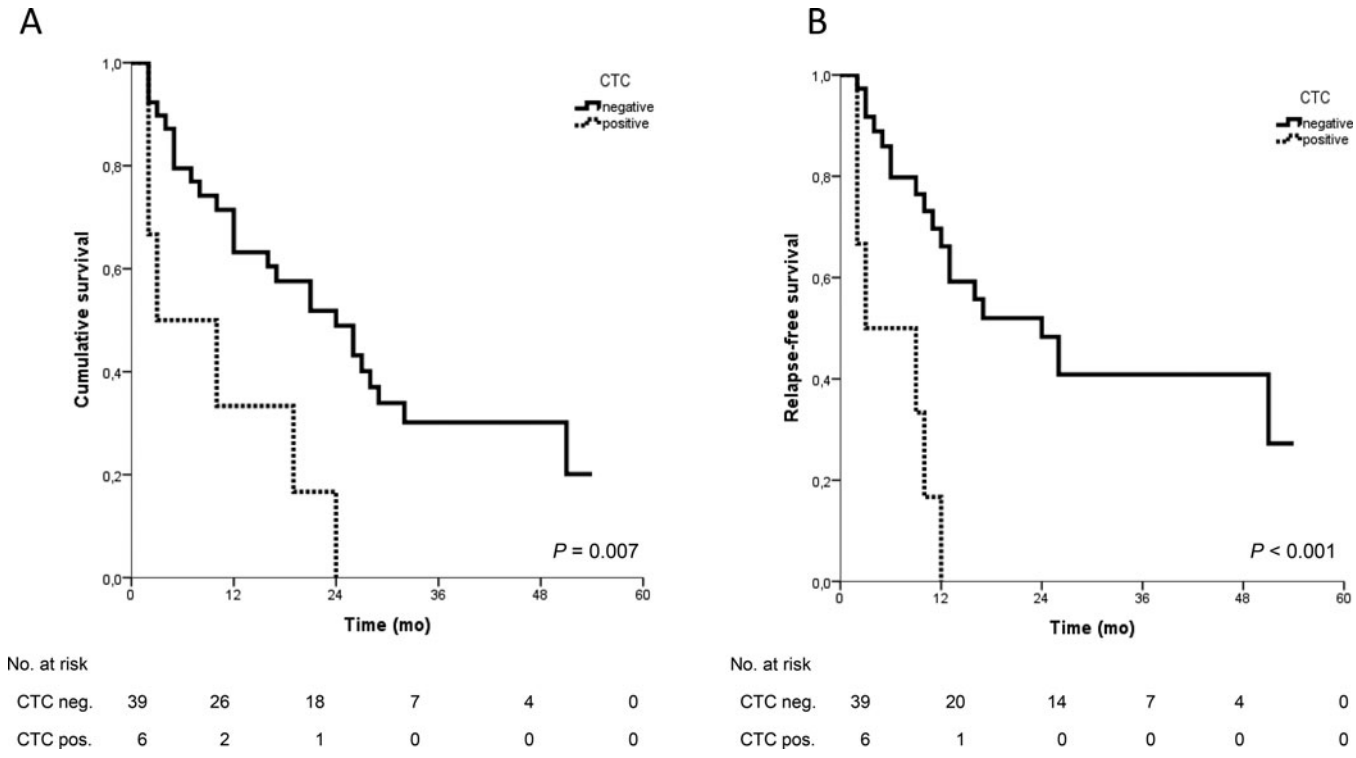

FIGURE 3. A, B, Outcomes of patients with EC with CTCs compared with patients without CTCs. Correlation of overall (A) and relapse-free survival (B) with CTCs in patients with lymph node invasion but without distant metastases (N = 45).

TABLE 2. Multivariate Analyses of Overall Survival and Relapse-free Survival of Eligible Patients (M0) in This Study

	Overall Survival			Relapse-free Survival		
Variables	**HR**	**95% CI**	***P****	**HR**	**95% CI**	***P****
Age						
≤65 vs > 65 yr	2.526	1.331–4.795	0.005	2.615	1.269–5.388	0.009
Sex						
Female vs male	0.913	0.447–1.866	0.803	0.634	0.279–1.440	0.276
Circulating tumor cells						
Negative vs positive	3.128	1.492–6.559	0.003	5.063	2.233–11.480	<0.001
Tumor stage						
pT1 vs pT2	2.196	0.724–6.664	0.165	1.989	0.644–6.145	0.232
pT1 vs pT3	3.788	1.394–10.296	0.009	4.079	1.490–11.165	0.006
pT1 vs pT4	3.988	1.124–14.149	0.032	3.749	1.043–13.479	0.043
Lymph node stage						
pN0 vs pN1	1.495	0.594–3.766	0.393	1.455	0.546–3.877	0.453
pN0 vs pN2	2.880	1.192–6.958	0.019	2.553	0.928–7.021	0.069
pN0 vs pN3	2.144	0.852–5.396	0.105	1.428	0.459–4.445	0.538
Grading						
G1 vs G2/G3	0.629	0.337–1.173	0.145	0.726	0.356–1.484	0.380

*Indicates significance according to Cox regression analysis comparing the specified variables.
HR indicates hazard ratio.

analyses, providing high accuracy, sensitivity, and reproducibility.[14,15] Polymerase chain reaction-based methods lack visual confirmation of CTCs and can yield false-positive results.[25,26] The clinical significance of the CellSearch system and its suitability for therapeutic monitoring has been proven for various cancer entities.[5,8,11,27–29] Large, prospective, multicenter studies have validated CTC detection as the strongest prognostic factor for overall and recurrence-free survival, supporting our hypothesis that CTCs can be used as biomarkers to improve current preoperative tumor staging.[11,28] Most studies using CellSearch used a cutoff of more than 5 CTCs. Tibbe *et al*[16] reported that errors due to interobserver variability were the main reason for selecting 5 CTCs as the threshold value. Elimination of such errors may reduce the cutoff from 5 CTCs to 1 CTC per 7.5 mL of blood. Recent studies analyzing the stages of nonmetastatic

tumors have reduced this cutoff to 2 or 1 CTC.[9,30] We used a strict cutoff of 1 CTC or more, and found that CTC-positive patients with nonmetastatic disease had a significantly shorter overall and relapse-free survival than patients without CTCs. This finding confirms the use of CTCs as a reliable biomarker in patients with EC. Hiraiwa *et al*[30] reported that 5 of 23 CTC-positive patients with metastatic EC showed a significantly shorter overall survival than CTC-negative patients. However, the number of patients with nonmetastatic tumors in their study (n = 10) was too small to indicate clinical significance.

Regardless of whether a multimodal treatment approach is used, surgery remains the only chance for curative treatment in EC, even though the operation is one of the most invasive thoracic and visceral surgical procedures, with morbidity rates exceeding 50%.[31] Therefore, accurate preoperative diagnosis and prognostic staging are imperative before surgical treatment. The use of CTC detection might improve prognostic staging. Currently, up to two thirds of patients develop local or distant tumor recurrence after intended curative resection, and more than 90% die of tumor recurrence and distant metastases. There is a lack of validated serum biomarkers for EC that can be used for tumor staging, prognostic stratification and post-therapy tumor monitoring. Radioimaging techniques are of limited use for this purpose. Budd et al[32] proposed that CTC assessment is superior to current imaging methods in patients with metastatic breast cancer. CTC detection could be used in the future for preoperative treatment decisions and immediate assessment of the success/failure of adjuvant treatments.[27] However, this hypothesis needs to be tested in future clinical trials.

Although our results show that CTC detection in patients with EC is a strong, independent indicator for overall and relapse-free survival, it must be mentioned that detection systems based on epithelial markers, like the CellSearch system, may underestimate the number of CTCs in patients with cancer. The single enrichment technique of CellSearch involving antibodies against EpCAM may partially explain this underestimation. Several studies have reported EpCAM-negative tumors in different epithelial cancers.[33–35] Another reason may be that most ECs are undifferentiated, which can lead to a lower expression of epithelial surface antigens. During the epithelial-to-mesenchymal transition, some tumor cells that separate from the primary tumor lose their epithelial characteristics, whereas in the bloodstream, and express mesenchymal markers like vimentin or fibronectin on their surface.[19,36,37] Epithelial-to-mesenchymal transition is associated with a high malignant potential and chemotherapy resistance.[38,39] Such tumor cells may be responsible for distant metastases and tumor relapse. Moreover, there may also be a platelet cloak phenomenon of single tumor cells or microemboli including CTCs likely to have enhanced metastatic potential. Thus, the CellSearch system may miss single cloaked CTCs due to EpCAM epitopes being physically covered. The CellSearch system may not detect all CTCs, especially those with high malignant potential.[40] Nevertheless, the EpCAM-based system has provided significant prognostic data in several prospective trials, including this study. CTCs detected by CellSearch play a significant role in disease progression and patient survival. We found that CTC detection had a strong prognostic significance in patients with AC; the impact of these cells in squamous cell patients with carcinoma needs to be investigated. Although EpCAM expression is present in about 80% of SCCs, this expression is low or moderate in approximately 50% of these patients.[41] In addition, cytokeratins used by the CellSearch system may not be optimal for detection of this histotype.[42]

CONCLUSIONS

To our knowledge, this is the first study to report that CTC detection by the CellSearch system is an independent prognostic indicator in patients with EC judged preoperatively to be non-metastatic. CTC-positive patients had significantly shorter overall and progression-free survival rates. CTCs were an independent and strong prognostic marker of overall and recurrence-free survival in EC. Our results suggest that CTC detection can enable accurate preoperative staging in EC, and thereby may give an opportunity to improve treatment that strongly needs to be examined in the future by large, multicenter trials.

ACKNOWLEDGMENTS

The authors thank Cornelia Coith and Susanne Hoppe, for their excellent assistance during the CTC detection by the CellSearch system. M.R., K.E.E., and A.M.K. contributed equally to this work and therefore share first authorship. All authors had a substantial contribution to the conception and design, acquisition of data, or analysis and interpretation of data, drafting the article, or revising it critically for important intellectual content and final approval of the version published.

REFERENCES

1. Rice TW, Rusch VW, Apperson-Hansen C, et al. Worldwide esophageal cancer collaboration. *Dis Esophagus*. 2009;22:1–8.
2. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2009. *CA Cancer J Clin*. 2009;59:225–249.
3. Izbicki JR, Hosch SB, Pichlmeier U, et al. Prognostic value of immunohistochemically identifiable tumor cells in lymph nodes of patients with completely resected esophageal cancer. *N Engl J Med*. 1997;337:1188–1194.
4. Vashist YK, Effenberger KE, Vettorazzi E, et al. Disseminated tumor cells in bone marrow and the natural course of resected esophageal cancer. *Ann Surg*. 2012;255:1105–1112.
5. Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med*. 2004;351:781–791.
6. Braun S, Vogl FD, Naume B, et al. A pooled analysis of bone marrow micrometastasis in breast cancer. *N Engl J Med*. 2005;353:793–802.
7. Cohen SJ, Punt CJ, Iannotti N, et al. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. *J Clin Oncol*. 2008;26:3213–3221.
8. de Bono JS, Scher HI, Montgomery RB, et al. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. *Clin Cancer Res*. 2008;14:6302–6309.
9. Rink M, Chun FK, Minner S, et al. Detection of circulating tumour cells in peripheral blood of patients with advanced nonmetastatic bladder cancer. *BJU Int*. 2011;107:1668–1675.
10. Thorban S, Rosenberg R, Busch R, et al. Epithelial cells in bone marrow of oesophageal cancer patients: a significant prognostic factor in multivariate analysis. *Br J Cancer*. 2000;83:35–39.
11. Cristofanilli M, Hayes DF, Budd GT, et al. Circulating tumor cells: a novel prognostic factor for newly diagnosed metastatic breast cancer. *J Clin Oncol*. 2005;23:1420–1430.
12. Kutup A, Link BC, Schurr PG, et al. Quality control of endoscopic ultrasound in preoperative staging of esophageal cancer. *Endoscopy*. 2007;39:715–719.
13. Sobin LH, Gospodarowicz MK, Wittekind C, et al. *TNM Classification of Malignant Tumours*. 7th ed. Chichester, West Sussex, UK; Hoboken, NJ: Wiley-Blackwell; 2010.
14. Allard WJ, Matera J, Miller MC, et al. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. *Clin Cancer Res*. 2004;10:6897–6904.
15. Riethdorf S, Fritsche H, Muller V, et al. Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the CellSearch system. *Clin Cancer Res*. 2007;13:920–928.
16. Tibbe AG, Miller MC, Terstappen LW. Statistical considerations for enumeration of circulating tumor cells. *Cytometry A*. 2007;71:154–162.
17. Kunisaki C, Makino H, Kimura J, et al. Impact of lymph-node metastasis site in patients with thoracic esophageal cancer. *J Surg Oncol*. 2010;101:36–42.
18. Bogoevski D, Onken F, Koenig A, et al. Is it time for a new TNM classification in esophageal carcinoma? *Ann Surg*. 2008;247:633–641.
19. Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. *Science*. 2011;331:1559–1564.
20. Klein CA. Parallel progression of primary tumours and metastases. *Nat Rev Cancer*. 2009;9:302–312.

21. Lurje G, Schiesser M, Claudius A, et al. Circulating tumor cells in gastrointestinal malignancies: current techniques and clinical implications. *J Oncol*. 2010:392652.
22. Kaganoi J, Shimada Y, Kano M, et al. Detection of circulating oesophageal squamous cancer cells in peripheral blood and its impact on prognosis. *Br J Surg*. 2004;91:1055–1060.
23. Ito H, Kanda T, Nishimaki T, et al. Detection and quantification of circulating tumor cells in patients with esophageal cancer by real-time polymerase chain reaction. *J Exp Clin Cancer Res*. 2004;23:455–464.
24. Hashimoto T, Kajiyama Y, Tsutsumi-Ishii Y, et al. Circulating micrometastases of esophageal cancer detected by carcinoembryonic antigen mRNA reverse transcriptase-polymerase chain reaction: clinical implications. *Dis Esophagus*. 2008;21:690–696.
25. De Luca A, Pignata S, Casamassimi A, et al. Detection of circulating tumor cells in carcinoma patients by a novel epidermal growth factor receptor reverse transcription-PCR assay. *Clin Cancer Res*. 2000;6:1439–1444.
26. de Cremoux P, Extra JM, Denis MG, et al. Detection of MUC1-expressing mammary carcinoma cells in the peripheral blood of breast cancer patients by real-time polymerase chain reaction. *Clin Cancer Res*. 2000;6:3117–3122.
27. Hayes DF, Cristofanilli M, Budd GT, et al. Circulating tumor cells at each follow-up time point during therapy of metastatic breast cancer patients predict progression-free and overall survival. *Clin Cancer Res*. 2006;12:4218–4224.
28. Cohen SJ, Punt CJ, Iannotti N, et al. Prognostic significance of circulating tumor cells in patients with metastatic colorectal cancer. *Ann Oncol*. 2009;20:1223–1229.
29. Krebs MG, Sloane R, Priest L, et al. Evaluation and prognostic significance of circulating tumor cells in patients with non-small-cell lung cancer. *J Clin Oncol*. 2011;29:1556–1563.
30. Hiraiwa K, Takeuchi H, Hasegawa H, et al. Clinical significance of circulating tumor cells in blood from patients with gastrointestinal cancers. *Ann Surg Oncol*. 2008;15:3092–3100.
31. Metzger R, Bollschweiler E, Vallbohmer D, et al. High volume centers for esophagectomy: what is the number needed to achieve low postoperative mortality? *Dis Esophagus*. 2004;17:310–314.
32. Budd GT, Cristofanilli M, Ellis MJ, et al. Circulating tumor cells versus imaging–predicting overall survival in metastatic breast cancer. *Clin Cancer Res*. 2006;12:6403–6409.
33. Konigsberg R, Obermayr E, Bises G, et al. Detection of EpCAM positive and negative circulating tumor cells in metastatic breast cancer patients. *Acta Oncol*. 2011;50:700–710.
34. Mikolajczyk SD, Millar LS, Tsinberg P, et al. Detection of EpCAM-negative and cytokeratin-negative circulating tumor cells in peripheral blood. *J Oncol*. 2011;2011:252361.
35. Maetzel D, Denzel S, Mack B, et al. Nuclear signalling by tumour-associated antigen EpCAM. *Nat Cell Biol*. 2009;11:162–171.
36. Bonnomet A, Brysse A, Tachsidis A, et al. Epithelial-to-mesenchymal transitions and circulating tumor cells. *J Mammary Gland Biol Neoplasia*. 2010;15:261–273.
37. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144:646–674.
38. Mego M, Mani SA, Lee BN, et al. Expression of epithelial-mesenchymal transition-inducing transcription factors in primary breast cancer: The effect of neoadjuvant therapy. *Int J Cancer*. 2012;130:808–816.
39. Gradilone A, Raimondi C, Nicolazzo C, et al. Circulating tumour cells lacking cytokeratin in breast cancer: the importance of being mesenchymal. *J Cell Mol Med*. 2011;15:1066–1070.
40. Wicha MS, Hayes DF. Circulating tumor cells: not all detected cells are bad and not all bad cells are detected. *J Clin Oncol*. 2011;29:1508–1511.
41. Stoecklein NH, Siegmund A, Scheunemann P, et al. Ep-CAM expression in squamous cell carcinoma of the esophagus: a potential therapeutic target and prognostic marker. *BMC Cancer*. 2006;6:165.
42. Makino T, Yamasaki M, Takeno A, et al. Cytokeratins 18 and 8 are poor prognostic markers in patients with squamous cell carcinoma of the oesophagus. *Br J Cancer*. 2009;101:1298–1306.