Prognostic Value of Bone Marrow Micrometastasis in Patients with Operable Esophageal Squamous Cell Carcinoma

A Long-Term Follow-Up Study

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Introduction: Detection of bone marrow micrometastasis (BMM) has been focused on as a prognostic parameter in various malignant neoplasms recently. This study was designed to evaluate the prognostic significance of BMM detection in patients with operable esophageal squamous cell carcinoma (ESCC) after long-term follow-up.

Methods: In 61 consecutive patients with ESCC who had undergone radical surgical resection, BMM was detected through reverse transcriptase-polymerase chain reaction (RT-PCR). Correlation between BMM detection and prognosis of the 61 patients was analyzed.

Results: BMM was found in 13 patients (21.3%). No significant correlation between BMM detection and tumor, node, metastasis (TNM) stage was found. The median survival time, 5-year overall survival rate, 5-year disease-free survival rate, and 5-year distant disease-free survival rate for cases with positive BMM were 13.0 months, 15.4%, 7.7%, and 34.2%, respectively, compared with that of 66.0 months, 59.7%, 49.1%, and 60.6% for cases with negative BMM ($p < 0.05$). In multivariate analysis, BMM were found to be an independent factor in the prediction of overall survival (odds ratio [OR] 3.928, $p = 0.001$), disease-free survival (OR 4.285, $p < 0.001$), and distant disease-free survival (OR 3.270, $p = 0.013$).

Conclusions: BMM is an independent prognostic factor in the prediction of the subsequent development of metastatic disease and disease outcome for operable ESCC patients, and may be a useful adjunct to conventional tumor staging. Further studies are required to evaluate the value of neoadjuvant or adjuvant systemic therapy in ESCC patients with BMM.

Key Words: Bone marrow micrometastasis, Esophageal squamous cell carcinoma, Prognosis

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Esophageal cancer is the eighth most common cancer worldwide.[1] Surgery remains the mainstay of treatment for resectable disease, but the prognosis for patients treated by surgery alone is poor. A significant proportion of patients undergoing potentially curative (R0) resections subsequently go on to relapse indicating the presence of early disseminated disease not apparent at the time of primary treatment.[2] Although the conventional tumor-staging parameters can provide reliable information about the proportion of a population of patients who will experience a recurrence of the disease, these measures cannot predict which individuals will relapse after primary therapy. Thus, new parameters need to be defined to improve the identification of patients at a high risk for relapse, so that adjuvant therapy may be added to these patients.

One of the most promising parameters in this regard is the detection of micrometastases in bone marrow. Micrometastases are defined as microscopic deposits of malignant cells distinct from primary tumors, which have the capacity to develop into macroscopic disease.[3] Consequently, detection of malignant cells in sampled bone marrow is suggestive of, but not conclusive evidence of, the presence of a metastatic phenotype.[4] The detection of bone marrow micrometastasis (BMM) has been described in patients of many cancers, such as breast cancer, gastric cancer, prostate cancer, colorectal cancer, lung cancer, oral cancer, esophageal cancer, and so on.[5–15] Most of the previous studies revealed a positive correlation of BMM detection with relapse and metastases. Recently, Domschke et al.[5] reported the largest single-center cohort of BMM detection in breast cancer patients ($n = 1378$) with the longest observation time (median 82.0 months), and found that patients with BMM had a significantly higher incidence of distant metastases, poorer overall survival, and disease-free survival.

We have previously reported the detection of BMM in a group of 61 patients with esophageal squamous cell

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carcinoma (ESCC), and found that BMM was an independent predictor of a poor outcome after a short-term follow-up.[16] To date, only one study has reported the long-term outcome of BMM detected at the time of surgery in operable esophageal cancer.[15] However, most of their patients had adenocarcinomas (78.6%, 33 of 42). No study has been concerned with the long-term outcome of BMM in patients with ESCC. The aim of this study was to investigate the impact of BMM detected at the time of surgery on long-term survival in patients with operable ESCC.

PATIENTS AND METHODS

This study was undertaken at the Cancer Center of Sun Yat-Sen University and was approved by its Ethics Committee. Informed consent was obtained from all patients before they entered the study.

Patients

Sixty-one consecutive patients with ESCC who underwent radical surgical resection between October 2006 and May 2007 were enrolled in this study. The medical history was obtained from all patients who then underwent a physical examination. A chest radiograph, barium meal, contrast enhanced computed tomography scan of the chest and abdomen, esophagoscopic biopsy, complete blood count, blood biochemistry analyses, and liver and renal function evaluations were also performed.

Methods

The methodology relating to the detection of BMM has been described previously and is briefly summarized below.[16] Bone marrow was aspirated from a rib at the beginning of surgery. Mononuclear cells were isolated by density-gradient centrifugation using a Ficoll gradient, and then stored at −86°C until they were used for analysis. To detect tumor cells in bone marrow, we used CK19 mRNA as the marker, which is epithelium-specific. Expression of CK19 mRNA in the bone marrow was assessed through the RT-PCR, using the specific oligonucleotide primers which have previously been used to detect CK19 in other cancers.

Follow-Up

Follow-up was performed every 3 months for the first year and every 6 months thereafter. During each follow-up visit, the patients received a clinical evaluation, blood biochemistry examination, including that of tumor markers (squamous-cell carcinoma antigen, carcinoembryonic antigen), ultrasonography, and radiograph examination. Computed tomography was performed every 6 months. Endoscopic examinations were performed when necessary. Follow-up was continued up to November 2013 or until death, if this occurred earlier.

Statistical Analysis

Statistical analysis was performed using SPSS 13.0 software (SPSS Inc., Chicago, IL). Overall survival, disease-free survival, and distant disease-free survival rates were analyzed. Overall survival time was calculated from the date of operation, to the date of death or most recent follow-up. Disease-free survival was defined as survival without the development of local recurrences or distant metastases. Distant disease-free survival was defined as survival without the development of distant metastases. Univariate analysis of survival was performed using the Kaplan-Meier method to estimate survival probabilities in patient subgroups, with the entry factors of gender, age (≤60 years versus >60 years), tumor location, histologic grade, pT category, pN category, pTNM stage, and BMM. The log-rank test was used to assess differences in survival between groups. Multivariate analysis was performed to investigate the prognostic factors by the Cox proportional hazard regression model. All statistical tests were performed two-sided, and a p value less than 0.05 was considered to be statistically significant.

RESULTS

Patient Characteristics

This study group contained 42 men and 19 women ranging in age from 35 to 75 years (median 58 years). The primary lesions were most often found in the middle third of the thoracic esophagus, with 21 well-differentiated tumors, 33 moderately differentiated tumors, and 7 poorly differentiated tumors. Twenty-two of the sixty-one patients (36.1%) proved postoperatively to have histologically confirmed lymph node metastases. According to the seventh edition of the American Joint Committee on Cancer (AJCC) staging system for ESCC, the study included 3 stage Ia patients, 8 stage Ib patients, 10 stage IIa patients, 17 stage IIb patients, 14 stage IIIa patients, 8 stage IIIb patients, and 1 stage IIIc patients.

None of the 61 patients underwent chemotherapy or radiotherapy before surgery, and none had prior malignant disease or distant metastases on routine examination before surgery. All 61 patients underwent transthoracic esophagectomy with two-field lymphadenectomy (the mediastinal and perigastric lymph nodes), and all had radical resection (R0). No patient died during treatment in hospital and 30 days after surgery.

TABLE 1. Correlation of BMM Detection with Tumor, Node, Metastasis Stage According to the Seventh Edition of American Joint Committee on Cancer Staging System for ESCC

Category	Number of Patients	BMM		p Value
		Positive (%)	Negative (%)	
pT category				0.319
pT1-2	17	2 (11.8)	15 (88.2)	
pT3-4	44	11 (25.0)	33 (75.0)	
pN category				0.193
pN0	39	6 (15.4)	33 (84.6)	
pN1-2	22	7 (31.8)	15 (68.2)	
pTNM stage				0.059
I–II	38	5 (13.2)	33 (86.8)	
III	23	8 (34.7)	15 (65.2)	

BMM, bone marrow micrometastasis; ESCC, esophageal squamous cell carcinoma.