Original paper

Prognostic impact of micrometastasis in patients with esophageal cancer

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Squamous cell carcinoma (SCC) of the esophagus and adenocarcinoma of the esophago-gastric junction (AEG) are diseases with poor prognosis. Despite radical surgery having been carried out, many patients are at risk of cancer recurrence, especially with the presence of metastases in the lymph nodes.
The study involved 60 patients suffering from SCC and AEG who had lymph nodes surgically removed between 2012 and 2018. Only lymph nodes with N0 status were subjected to immunohistochemistry examination. Histopathological criteria were used for the diagnosis of micrometastases (MM), defined as tumor cells or cell clusters of 0.2–2 mm diameter in the lymph node and tumor cell microinvolvement defined as free-floating neoplastic cells or cell clusters within the sub-capsular sinus or intramedullary sinuses of the lymph node.
A total of 1130 lymph nodes were removed during surgery, with an average of 22 lymph nodes per patient (range 8–58). Micrometastases were found in 7 (11.66%) patients: 6 (10.0%) with AEG and 1 (1.66%) with SCC, representing a statistically significant difference $p = 0.017$. Multivariate analysis of the study group did not confirm the dependence of the MM on the T features ($p = 0.7$) or G ($p = 0.5$). In a Cox regression analysis, MM were not a risk factor for death, HR: 2.57 (0.95; 7.00), $p = 0.064$.
There was no difference in overall survival for patients with MM (N (+)) and those without (N0), $p = 0.055$, but there was a statistically significant difference in time of relapse between patients with and without MM ($p = 0.049$).
Patients with the N (+) status are at high risk of cancer recurrence, and therefore we believe that complementary treatment should be considered in this group.

Key words: esophagus, cancer, micrometastasis.

Introduction

Squamous cell carcinoma (SCC) of the esophagus and adenocarcinoma of the esophago-gastric junction (AEG) are diseases with poor prognosis, with surgery being the leading therapeutic treatment. In the previous studies, the 5-year survival of patients after surgical treatment ranges from 10 to 30% [1, 2]. However, the 5-year survival rate is significantly better in patients with N0 lymph node status, which is confirmed by other reports where 70% of patients survived over 5 years [3]. Despite radical surgery having been carried out, many patients are at risk of cancer recurrence, especially in the presence of lymph node metastases [4, 5].

The classic hematoxylin and eosin staining (H+E) does not always allow detection of this pathology in the lymph nodes. Diagnostic methods that can effectively detect them include immunohistochemis-

try (IHC) using monoclonal anti-cytokeratin bodies (monoclonal anti-cytokeratin CK antibody cocktail AE1/AE3) and molecular examination with reverse transcription-polymerase chain reactions (RT-PCR). Immunohistochemistry uses the presence of epithelial markers to detect micrometastases (MM), as these markers are absent in healthy lymph nodes. This method is characterized by high specificity of 100% and sensitivity of 40% [6]. The use of IHC and molecular methods as well as PCR allows the identification of MM and the identification of a group of patients at risk of cancer recurrence. The aim of the study was to detect MM and tumor cell involvement (TCM) in regional lymph nodes and their impact on the survival and cancer recurrence of patients treated surgically due to SCC and adenocarcinoma of AEG.

Material and methods

Patients

This study included 60 patients suffering from SCC and AEG treated surgically with removal of lymph nodes in the period 2012–2018 (Table I). The study included patients after radical surgery with the N0, R0 status who did not receive pre-operative and post-operative chemotherapy and/or radiotherapy.

Patients with SCC underwent esophageal resection with two- or three-field lymphadenectomy from laparotomy, right-sided thoracotomy and neck access and with esophago-gastric anastomosis in the chest or in the neck. Patients with AEG underwent upper resection of the stomach with the esophago-gastric anastomosis in the chest (Ivor Lewis operation) or gastrectomy with an esophago-intestinal anastomosis in the abdominal cavity or in the chest with lymphadenectomy D2 (Roux-en-Y operation).

Immunohistochemical staining

Immunohistochemistry

The expression of epithelial antigens was determined in formalin-fixed paraffin embedded slides. The sections (4 μm thick) were deparaffinized in xylenes and hydrated in alcohols. Endogenous peroxidase was blocked with 3% hydrogen peroxide for 10 min. Protein blocking was done with a protein blocker to reduce non-specific binding of primary and secondary antibodies. Next, the slides were incubated with an anti-cytokeratins cocktail (AE1/AE3, Cell Marque, Rocklin, CA) at 1 : 200 dilution. This was followed by visualization of the antigen-antibody complex using chromogen 3,3 diaminobenzidine.

Histopathologic criteria used for diagnosis of MM in lymph nodes [6]:

- MM were defined as tumor cells or cell clusters of 0.2–2 mm diameter in the lymph node,
- TCM is defined as free-floating neoplastic cells or cell clusters within the sub-capsular sinus of the lymph node.

Follow-up

Patients were subject to post-operative check-ups every 3 months in the first year and every 6 months in subsequent years. During the check-ups, images were routinely taken from the patient's chest examination, chest tomography, endoscopic examination (gastroscopy, EUS bronchoscopy, EBUS with microscopic verification), and some PET-CT.

Statistical analyses

All analyses were done in the statistical software SPSS, ver. 27 with $\alpha = 0.05$. The Cox regression coefficient (hazard ratio) was given with 95% confidence intervals. Kaplan-Meier analysis was conducted. Patients from different groups were compared in terms of survival time and time free of relapse using the log-rank test. Dependency between the presence of MM and selected characteristics was calculated using Fisher's exact test.

Ethical

This study was supported by a grant from Jagiellonian University Collegium Medicum and ethical approval was obtained from the Ethics Committee of Jagiellonian University – project number K/ZDS/005078.

Results

Patients

This study included 60 patients (mean age 64.7 years, range: 41–83; 46 males): 29 with AEG (AEG1 and AEG3) and 31 with SCC of the esophagus. Patients with AEG1 tumors underwent transmediastinalesophagectomy, proximal stomach resection and D2 lymphadenectomy. An extended total gastrectomy with resection of the distal esophagus and D2 lymphadenectomy was performed on patients with AEG3 tumors. Among patients with AEG, 6 underwentgastrectomy (AEG3) while an upper gastrectomy with anastomosis in the chest was performed on the remaining patients. In 3 patients, esophagointestinal anastomosis was performed in the abdominal cavity and in the chest of 3 patients. All of these patients were reconstructed by the Roux-en-Y method (Table I). For patients undergoing an upper gastrectomy, the esophago-gastric anastomosis was performed in the chest. D2 lymphadenectomy was performed in all patients. Among patients operated on due to SCC, 24 had an Ivor Lewis resection with two-field lymphadenectomy and 7 had a McKeown resection with three-field lymphadenectomy (Table II).

Table I. Clinical and pathomorphological data of patients with squamous cell carcinoma and esophago-gastric junction adenocarcinoma

Group	SCC	AEG	Total
N	31	29	60
Sex M/F	25/6	23/6	60
Age (years)	45–75	33–78	33–78
Follow-up (months)	27.7 (1–96.7)	26.6 (1–91.7)	27.4 (1 –96.7)
Differentiation grade (G1 : G2 : G3)	1 : 16 : 15	0 : 16 : 13	1 : 32 : 28
Lauren type (I : M : D)		19 : 4 : 6	
TNM	T1N0M0 11 T2N0M0 3 T3N0M0 2 T1N1M0 1 T2N1M0 6 T3N1M0 6 T2N2M0 2	T1N0M0 5 T2N0M0 6 T3N0M0 1 T1N1M0 1 T2N1M0 8 T3N1M0 1 T4N1M0 1 T2N2M0 1 T3N2M0 5	T1N0M0 16 T2N0M0 9 T3N0M0 3 T1N1M0 2 T2N1M0 14 T3N1M0 7 T4N1M0 1 T2N2 M0 3 T3N2 M0 5
	31	29	60

AEG 1 – esophago-gastric junction adenocarcinoma type 1, AEG 3 – esophago-gastric junction adenocarcinoma type 3, I : M : D – intestinal : mixed : diffuse, SCC – squamous cell carcinoma, TNM – tumor-node-metastasis

Table II. Distribution of patients with esophago-gastric junction adenocarcinoma according to T grade

Category	AEG1			AEG3		
PN0 cases with MM TCM		MM	TCM		MM	TCM
T1	11	1		5	1	
T2	3	1	1	6	2	1
T3	2			2	1	
Total	16	2	1	13	4	1

AEG 1 – esophago-gastric junction adenocarcinoma type 1, AEG 3 – esophago-gastric junction adenocarcinoma type 3, MM – micrometastasis, TCM – tumor cell microinvolvement

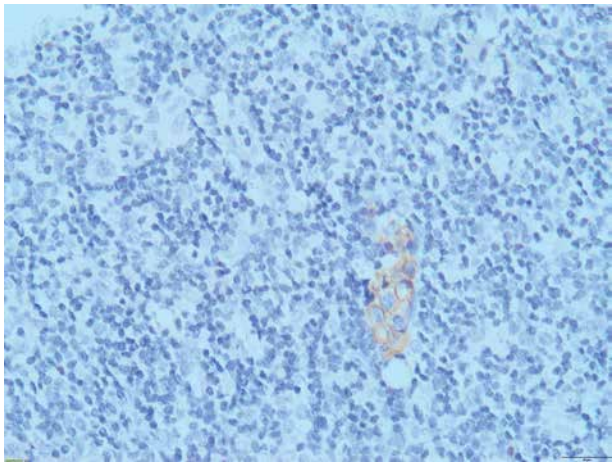

Fig. 1. Micrometastasis in a lymph node from a patient with esophago-gastric junction adenocarcinoma. This section was stained with the antibody cocktail AE1/AE3

A cluster of positively stained tumor cells in medulla of the lymph node.

Correlation between clinicopathological features and micrometastasis

A total of 1130 lymph nodes were surgically removed (R0), with an average of 22 lymph nodes per patient (range 8–58). Only lymph nodes with N0 status were subjected to IHC examination. The mean follow-up time for the 60 patients was 27.4 months (range 1–96.7 months) (Table I).

Micrometastases were found in 7 (11.66%) patients: 6 (10.0%) patients with AEG and 1 (1.66%) with SCC, representing a statistically significant difference, $p = 0.017$. In 5 (6.55%) patients, MM of sizes 0.2–2 mm were found (Fig. 1), and in 2 (3.27%) patients, MM were below 0.2 mm (TCM) (Table II). Patients diagnosed with TCM (Fig. 2) were included in the group of patients with MM and subjected to analysis. Patients with an MM status were included in the group of patients with the characteristic N (+).

Micrometastasis was predominant in patients with AEG, representing 20.7%, whereas the prevalence of MM was lower in the SCC group at 3.33%. Multivariate analysis did not demonstrate the dependence of MM on T features ($p = 0.7$) or G features ($p = 0.5$) (Table II). In a Cox regression analysis, MM were not a risk factor for death, HR: 2.57 (0.95; 7.00) $p = 0.064$.

Survival

Impact of micrometastasis on overall survival

Mean time of survival equaled M = 63.20 (SD = 5.56) months for patients without MM and M = 24.96 (SD = 2.39) months for patients with MM – this difference was not statistically significant ($p = 0.055$). The cumulative proportion of patients who survived at the end of follow-up was 46% (SD = 8%) for subjects without MM and 29% (SD = 17%) for subjects with MM (Fig. 3).

Impact of micrometastasis on disease free-survival

There was a statistically significant difference in time without a relapse between patients with and without MM ($p = 0.049$). Mean number of months without a relapse equaled M = 62.31 (SD = 5.69) for patients without MM and M = 21.43 (SD = 3.13) for patients with MM. The cumulative proportion of subjects who did not relapse at the end of follow-up was 47% (SD = 7%) for the negative MM group and 29% (SD = 17%) for the positive MM group (Fig. 4).

Discussion

According to both the tumor-node-metastasis and American Joint Committee on Cancer classifications, tumor grading does not always give a definitive answer as to the prognosis of patients; hence attempts are made to publish analyses indicating prognostic and predictive factors [7, 8]. Rice *et al*. indicated that metastasis in the lymph nodes in patients with esophageal cancer is an independent prognostic factor [9]. Lymph node metastases occur more frequently in AEG than in esophageal SCC, and they depend on the T feature, where in the T3 stage with a tumor infiltration length above 4 cm, their occurrence is estimated at 60–80%, and in relation to the G3 feature, the range is 30–80% [9]. In our report, no correlation was found between the T and G features and the occurrence of MM.

Immunohistochemistry is a standard technique in the detection of MM and uses the presence of cy-

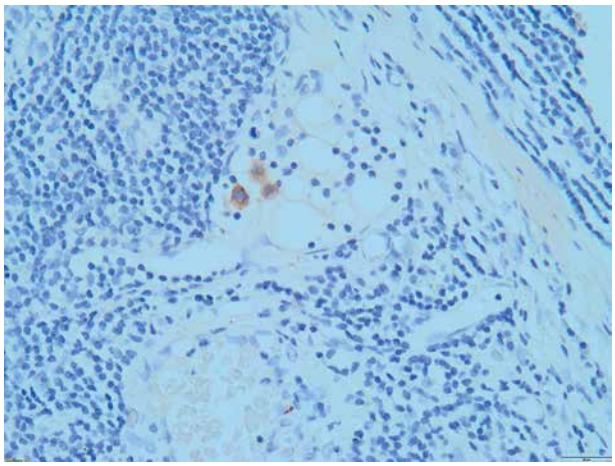

Fig. 2. Tumor cell involvement in a lymph node of a patient with esophago-gastric junction adenocarcinoma

This section was stained with the antibody cocktail AE1/AE3. A tumor cell without a surrounding sinus of the lymph node.

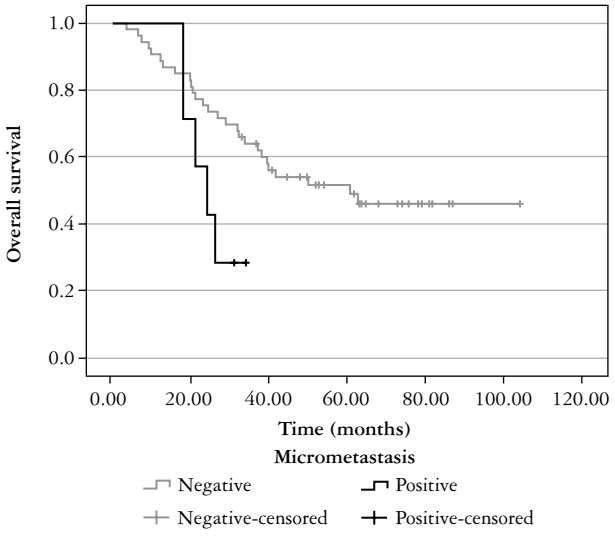

Fig. 3. Overall survival curve for patients with or without micrometastasis by the log-rank test of Kaplan-Meier

The survival of patients with micrometastasis shows a downward trend on the verge of statistical significance ($p = 0.055$).

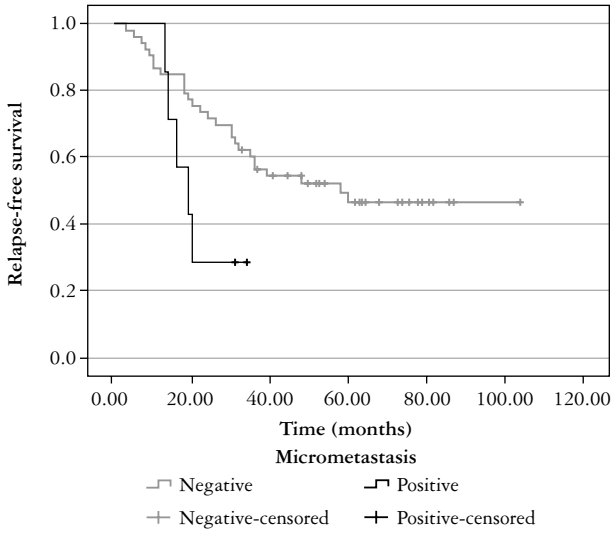

Fig. 4. Relapse-free survival curve for patients with or without micrometastasis by the log-rank test of Kaplan-Meier

tokeratins to detect tumor cells. However, detection of MM is dependent on both the method and the researcher's experience. In the present study, we detected MM in 7 cases (11.66%) in the lymph nodes of patients in whom the H+E investigations were negative. In the published literature, detection by IHC accounts for 5–55% of patients treated for esophageal cancer [5, 6, 9–11]. In the examined group, MM dominated among patients with AEG.

Our report confirms that MM are a factor of poor prognosis for patients with esophageal cancer. All patients diagnosed with them died. They were a factor in the relapse of the cancer disease. Although the presented analysis did not confirm their occurrence as a risk factor for death and the effect on overall survival, the data may suggest it because the results are close to statistical significance. This situation may be associated with the small group of analyzed patients.

The detection of MM in the lymph nodes is not new, but their impact on survival is not well known [10, 12]. Fukagawa *et al*., together with Harrison *et al*., reported no effect on long-term survival in patients with gastric cancer; hence, there is no definite agreement on the risk and impact on survival of MM in gastric cancer [13, 14]. Gray *et al*. reported no effects on survival in a 10-year follow-up of patients undergoing surgery for esophageal cancer with bone marrow MM diagnosed during esophageal resection [12]. The authors believe that these patients are potentially burdened with the risk of recurrence of cancer disease, but that this did not affect the long-term survival [10]. Moreover, Glickman *et al*. did not determine survival among patients with esophageal cancer. However, Natsugoe *et al*. published data highlighting the negative impact on survival among patients with esophageal SCC [15–17]. Rahbari *et al*. conducted a meta-analysis of 39 studies in a group of more than 4,000 patients with colorectal cancer, and demonstrated that the detection of MM was associated with adverse effects on overall survival (HR: 2.20, 95% CI: 1.43–3.40), disease-specific survival (HR: 3.37, 95% CI: 2.31–4.93), and disease-free survival (HR: 2.24, 95% CI: 1.57–3.20) [18]. Sun *et al*. reported the correlation between the T feature and the influence of MM on the recurrence of disease in patients following surgical treatment of esophageal cancer and resection using the Ivor Lewis technique [19]. Finally, Koenig *et al*. and Prenzel *et al*. presented evidence of poor prognosis among patients treated for esophageal cancer, not only with N0 features but also at advanced T1 stage [20, 21].

The process of MM and metastasis at the molecular level is not precisely understood. It is believed that they are involved in the micro-reef formation, circulating tumor cells as well as circulating tumor micro-embolus and disseminated tumor cells, and therefore it is proposed to consider them as prognostic factors that determine the survival of patients. However, only 0.01–0.1 circulating cancer cells have the ability to metastasize, and these must have the ability to, at minimum, detach from the primary tumor, grow in the extracellular matrix and induce new blood vessel formation. It is also known that during cancer progression there is a mutual correlation between cells that are already metastatic and the cancerous tumor [22, 23]. The role of isolated tumor cells (ITC) is also unclear. Isolated tumor cells can be defined as either single cells or a small cluster of cells having a size of ≤ 0.2 mm, which are normally detected by IHC and RT-PCR. These cells do not all become MM and do not always activate cancer, and therefore have no metastatic potential. Such a condition may be dependent on the genotypic differences of tumor cells, or the body's immune system, which may not always lead to the development of active cancer [22, 23]. However, Yonemura *et al*. are of a different opinion, having observed an adverse effect on survival in patients with ITC [24]. Yanagita *et al*. also analyzed patients with the ITC trait, and detected an adverse effect on patient survival. The authors conclude that this is found especially in patients who have not undergone radical lymphadenectomy [25].

Our study has a few limitations. Patients with SCC and AEG carcinoma constitute a diverse group in terms of tumor biology. The analyzed groups of patients are small, so the power of statistical calculations may be at risk of type 2 error. Larger groups of patients and differentiation of the above-mentioned factors may lead to different results in the conducted studies. Detailed molecular studies could allow us to better understand the biology of both cancers.

Conclusions

Our research suggests that patients with MM in the course of esophageal cancer, particularly in AEG, are at risk of cancer recurrence and one should consider introducing complementary treatment and intensive post-operative management.

The authors declare no conflict of interest.

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