LYMPH NODE MICROMETASTASES OF ADENOCARCINOMA LOCATED IN THE GASTROESOPHAGEAL JUNCTION

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Background: The aim of this study was to evaluate the presence of micrometastases (MM) and tumor cell microinvolvement (TCM) in regional lymph nodes of patients with gastroesophageal junction adenocarcinoma.

Material and methods: 61 patients with adenocarcinoma (stage N0) were included in this study. Lymph nodes removed during surgery were examined using mixed monoclonal antibodies against pan-cytokeratins (AE1/AE3). Micrometastases was defined as single neoplastic cells or infiltration with diameter below 0.5 mm but not involving the interstitium.

Results: Micrometastases were found in 2 cases with adenocarcinoma type I and 4 cases with type III. Tumor cell microinvolvement was found in 2 cases of either adenocarcinoma type I or type III (according to Siewert’s classification). Presence of MM did not influence survival rate.

Conclusions: Despite no influence of MM presence on survival rate, MM came from adenocarcinoma type III more frequently. The presence of MM proved lack of homogeneity in the group of N0 stage and makes evaluation advantageous.

Key words: micrometastasis, adenocarcinoma gastroesophageal junction.

Introduction

Morbidity of adenocarcinoma located in the gastroesophageal junction has increased within the last decade [1, 2]. Radical surgery of tumor with removal of lymph nodes is now routine therapy. Regardless of accepted treatment, the survival rate is still not satisfactory [3]. Metastases to lymph nodes are claimed to be independent and one of the most important prognostic factors in gastroesophageal junction adenocarcinoma [4, 5]. Local recurrences of disease or even disease spread are observed in patients without metastases in routinely examined lymph nodes as well. Thanks to modern immunohistochemical techniques using monoclonal antibodies which expose micrometastasis (MM), diagnosis and treatment of cancer can be improved. These new methods have already been tested in diagnosis of lung cancer, colon cancer, gastric and esophageal cancer [6-8]. Some authors have reported use of monoclonal antibodies for MM assessment as an independent prognostic factor for survival rate in neoplastic disease [9, 10].

Material and methods

Patients

Sixty-one patients with confirmed adenocarcinoma of the gastroesophageal junction were included in the study. According to the Siewert and Stein classification [11] they were divided into three groups: patients with (AEG type I – adenocarcinoma related to Barrett esophagus (AEG type I) (32 cases), adenocarcinoma of cardia (AEG type II), and subcardial adenocarcinoma (AEG type III) (29 cases). Only patients with type I and type III adenocarcinoma were included in this study. The TNM UICC classification was used for assessment of the disease stage [12]. Patients with AEG I tumors underwent transmediastinal esophagectomy, proximal stomach and lymphadenectomy in the posterior me-
diastinum and celiac axis. An extended total gastrectomy with resection of the distal esophagus and D3 lymphadenectomy with transhiatal lymphadenectomy of the inferior mediastinum was the procedure for patients with AEG3 tumors. In total 1130 lymph nodes were removed during surgery, on average 22 lymph nodes per patient (range 8-58 lymph nodes). In 60 cases surgery was radical (R0) while 1 case which was classified as R1. The mean follow-up time for the 61 patients was 27.4 months, with a range 1-96.7 months. Table I shows clinical and histopathologic outcomes of patients.

**Immunohistochemistry**

The expression of epithelial antigens was determined in formalin-fixed paraffin embedded slides. The sections, 4 μm thick, were deparaffinized in xylene and hydrated in alcohol. Endogenous peroxidase was blocked with 3% peroxidase for 10 minutes. Protein blocking was done with a protein blocker to reduce non-specific binding of primary and secondary antibodies. Next, the slides were incubated with anti-cytokeratins cocktail (AE1/AE3, Boehringer, Mannheim, Germany) and anti-Ber-EP4 antibody (DAKO Diagnostica, Hamburg, Germany), at 1 : 500 dilution. The use of a detection system was followed by visualization of the antigen-antibody complex using chromogen 3,3-diaminobenzidine (DAB). Histopathologic criteria used for diagnosis of micrometastasis in lymph nodes:

- Micrometastasis (MM) is defined as tumor cells or cell cluster less than 0.5 mm of maximal diameter within the lymph node.
- Tumor cell involvement (TCM) is defined as free-floating neoplastic cells or cell clusters within the subcapsular sinus or intramedullary sinuses of the lymph node.
- Metastases are defined as neoplastic focus exceeding 0.5 mm in diameter.

**Data analysis**

Relationships between groups were calculated by Cox test. For assessment of morbidity Kaplan-Meier test was used. A P < 0.05 was considered statistically significant.

**Results**

Lymph nodes obtained from 28 patients with N0 stage of disease were examined for the presence of micrometastases. Micrometastasis were found in 6 (21.4%) patients and histopathologic examination showed that 4 (14.3%) patients had adenocarcinoma type III and 2 patients (7.1%) had type I. Tumor cell microinvolvement was found in 4 (14.3%) cases with type I and in 2 cases (7.1%) with type III adenocarcinoma (Table II). Occurrence of MM did not correlate either with T grade or tumor grade or with neoplasm type according to Lauren’s classification. Micrometastases were found more frequently in type III than type I adenocarcinoma, but this difference was not statistically significant. There was no difference in survival time between patients with MM and patients with N0 grade, MM and M1 as well as TCM and pN0. Patients with metastases which exceed 0.5 mm in diameter were included in group N1.

**Discussion**

The assessment of micrometastases in routine HE staining demonstrates false negative rates. Immuno-
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Histochemistry corrects detection outcome of micrometastases in esophageal cancer and identifies them in patients with negative lymph nodes in routine HE (11.9-30%); also molecular examination (RT-PCR) shows a high degree of sensitivity and specificity, 100% and 67%, respectively [13-18].

The impact of micrometastases on the survival and progression of esophageal cancer both in adenocarcinoma (ACC) and squamous cell carcinoma (SCC) remains unclear. A lot of authors conclude micrometastases to be a poor prognostic factor (Table III). Mouriguchi suggests the impact of micrometastases on the survival and recurrence (Table III). Prenzel et al. reported that in patients with negative lymph nodes in routine HE (11.9-30%), also molecular examination (RT-PCR) shows a high degree of sensitivity and specificity, 100% and 67%, respectively [13-18].

The authors suggest that the lamina muscularis mucosa in the early stage of carcinoma of the esophagus, the percentage of micrometastases (SCC and ACC - PT1N0), micrometastases applied to the lamina muscularis mucosa in the early stage of carcinoma of the esophagus, and (67%) respectively, shows a high degree of sensitivity and specificity, 100% and 67%, respectively [13-18].

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Fig. 1. Tumor cell microinvolvement (TCM) in a lymph node of adenocarcinoma of the esophagus. This section was stained with the antibody cocktail AE1/AE3.

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

Fig. 2. Micrometastasis (MM) in a lymph node of adenocarcinoma of the esophagus. This section was stained with the antibody cocktail AE1/AE3.

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

Table III. Survival data of patients with micrometastasis

<table>
<thead>
<tr>
<th>AUTHOR</th>
<th>NO. OF PATIENTS</th>
<th>LYMPH NODES (ptxN0)</th>
<th>LYMPH NODES</th>
<th>MICROMETASTASIS</th>
<th>SURVIVAL* SCC</th>
<th>SURVIVAL* AC</th>
<th>IMPACT OF RECURRENT AC</th>
<th>IMPACT OF RECURRENT SCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prenzel et al. [5]</td>
<td>69</td>
<td>48</td>
<td>1344</td>
<td>4 (15)</td>
<td>3 (33)</td>
<td>57%</td>
<td>79% S</td>
<td>S (poor prognosis)</td>
</tr>
<tr>
<td>Li et al. [21]</td>
<td>93</td>
<td>93</td>
<td>426</td>
<td>32 (93)</td>
<td>18.8%</td>
<td>47% S</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Jiao et al. [13]</td>
<td>106</td>
<td>69</td>
<td>280</td>
<td>6 (280)</td>
<td>5 (280)</td>
<td>13.2%</td>
<td>44% *NS</td>
<td>+ (4 patients)</td>
</tr>
<tr>
<td>Koenig et al. [18]</td>
<td>73</td>
<td>33</td>
<td>1958</td>
<td>8 (18)</td>
<td>1 (15)</td>
<td>24%</td>
<td>53%</td>
<td>poor prognosis</td>
</tr>
<tr>
<td>Zingg et al. [20]</td>
<td>224</td>
<td>86</td>
<td>1204</td>
<td>3.1%</td>
<td>11.3% p=NS</td>
<td>35.7%</td>
<td>73.6% NS (better prognosis with m−)</td>
<td>+ (3×) NS (poor prognosis) + (5×) NS (poor prognosis)</td>
</tr>
<tr>
<td>Izbicki et al. [10]</td>
<td>68</td>
<td>68</td>
<td>399</td>
<td>3.1%</td>
<td>11.3% p=NS</td>
<td>35.7%</td>
<td>73.6% NS (better prognosis with m−)</td>
<td>+ (3×) NS (poor prognosis) + (5×) NS (poor prognosis)</td>
</tr>
<tr>
<td>Mueller et al. [14]</td>
<td>145</td>
<td>75</td>
<td>3987</td>
<td>196</td>
<td>no impact on survival</td>
<td>no impact on survival</td>
<td>no impact on survival</td>
<td>no impact on survival</td>
</tr>
<tr>
<td>Godfrey et al. [17]</td>
<td>30</td>
<td>30</td>
<td>387</td>
<td>3 (4)</td>
<td>8 (26)</td>
<td>S</td>
<td>S</td>
<td>S (poor prognosis) S (poor prognosis)</td>
</tr>
</tbody>
</table>

S – significant, NS – non-significant, M – micrometastasis, SCC – squamous cell carcinoma, AC – adenocarcinoma
cosa represents a lymphatic barrier to the submucosa. The survival of pN0 was significantly worse compared to patients with tumor free lymph nodes, and also the 5-year survival rate of the pN+ group was still inferior compared to the micrometastasis group [5]. Analyzing the patients with SCC and ACC, Koenig et al. claim that the survival of patients in group: N0, low to 11%, and high than 11% of micrometastasis is shorter than the patients in group N0, 43, 27, 11 months respectively [18]. Zingg et al. found that patients with micrometastases in SCC live shorter, which is not observed in patients with ACC. They name four factors that significantly affect disease-free survival: pretreatment (radio-chemotherapy), micrometastasis, UICC stage II, and adenocarcinoma [20]. Li et al. present similar observations. The author observed the appearance of micrometastases in 9.4% of examined lymph nodes but together with the feature T they constitute an independent prognostic factor in the overall 5-year survival rate of the patients [21].

Some authors point out that hidden micrometastasis should be defined as every metastasis found in classic microscopic study [14, 22]. In our study MM were present in 21.4% of cases and were found in adenocarcinoma type III more often than in type I tumors. Additionally, MM did not influence survival time. Glickmann et al. reported MM presence in 15 (31%) cases of adenocarcinoma and in 5 (17%) cases of squamous cell carcinoma (the total number of cases was 78, including 49 cases of adenocarcinomas and 29 cases of squamous cell carcinoma). He found no influence of MM on survival time, which is why he did not recommend the assessment of MM as a method for evaluation of prognosis in oesophageal cancer [15]. Stachura et al. during microscopic assessment of lymph nodes obtained from 40 patients with early stage of gastric cancer reported MM in 3 cases and metastases to lymph nodes in 3 cases as well. Presence of MM was not related to the grade of neoplasm. The authors did not observe an influence of MM on survival time as well but they suggested that MM may be a prognostic factor according to the new concept for distal metastases development [23]. Bonavina et al. studied 46 patients with adenocarcinoma of the gastroesophageal junction. They reported micrometastases in 6 (33.3%) cases from a group of 18 patients with N0 stage of disease. The authors claim that despite the fact of performed radical treatment neoplasm recurrence happened in 3 cases with MM within 12 months [9]. Lee et al. confirmed a detrimental influence of MM on survival rate in patients operated on due to gastric carcinoma [24]. Presence of MM in lymph node indicated by the immunohistochemical method and missed in routine microscopic assessment of HE slides indicates heterogeneity of N0 variable. Patients with MM were reclassified and included in group N0 [15]. In this meaning MM becomes a differentiating factor in neoplasm progression and determines therapeutic decisions. Mueller et al. did not prove an influence of MM on survival time in adenocarcinoma cases depending on carcinoma type (I, II, or III). They reported increased occurrence of MM in type II and III carcinoma in relation to type I. Some authors suggest use of a different strategy of lymphadenectomy in relation to adenocarcinoma type [14]. Meachara et al. think that patients with cytokeratin positive reaction in lymph nodes require monitoring and an individual therapeutic process [25]. In a previous paper, Izbicki et al. reported occurrence of 50% MM in 68 studied carcinoma cases and significant changes of survival time. However, lack of homogeneity in the studied group, difficulties in assessment of patients with adenocarcinoma or squamous cell carcinoma markedly affected final results [10]. The authors suggest that these results may indicate patients who require adjuvant therapy, especially patients with remnant neoplasm who can respond to antineoplastic therapy more effectively than patients with a higher stage of disease. The occurrence of micrometastases is relative to early stage of neoplasm progression. Higashi analyzed 95 cases with submucosal gastric carcinoma and stated that 23 (24%) patients at stage N0 had micrometastases [25]. Tanabe et al. found micrometastases in 34 patients from a group of 78 cases with superficial squamous cell carcinoma of the esophagus and occurrence of neoplastic infiltration of mucosal veins correlated with it [26].

Immunohistochemistry and molecular examination are efficient methods of micrometastasis diagnosis but technically detailed evaluation of all removed lymph nodes is not always possible. That is why the efforts of mapping SN are made. Mapping SN and its immunohistochemical and/or molecular examination can not only have an impact on the detection outcome correction of micrometastases but also on the limitation of lymphadenectomy in patients in the early stage of esophageal cancer [27-29].

Immunohistochemistry and molecular examination are efficient diagnostic tools in the evaluation of the staging of esophageal cancer. Precise examination not only lets us estimate the real stage of neoplasm but also helps in applying the proper therapeutic strategy. Occurrence of micrometastases in lymph nodes is a differentiating feature for stage N0. These alterations were found more often in type III adenocarcinoma than type I and they may suggest biological difference of these neoplasms or give evidence of neoplasm recurrence. Use of cytokeratin for lymph node assessment helps to estimate precisely the grade of progression of neoplastic disease and may be a key in making the therapeutic decision and follow-up for patients. Taking into consideration both the increase of the incidence of carcinoma of the esophago-gastric junction and its still poor prognosis, the precise diagnostics enables the improvement of staging of esophageal cancer. Negative lymph nodes in rou-
tine HE should not assure us of the absence of illness but persuade us to extend the diagnostics with immunohistochemistry. The examination should be the diagnostic canon in the post-treatment evaluation of patients with esophageal cancer.

The authors declare no conflict of interest.

References


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