Review Article · Übersichtsarbeit

ONKOLOGIE

Onkologie 2000;23:330–333

Lymph Node 'Micrometastases' and 'Microinvolvement' in Esophageal Carcinoma

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Key Words
Esophageal carcinoma · Adenocarcinoma · Squamous cell carcinoma · Lymph node · Micrometastasis

Summary
The development of new sensitive immunohistochemical methods allows the detection of single tumor cells or cell clusters in lymph nodes staged as tumor free on routine histologic examination. The prevalence and prognostic impact of these so-called lymph node 'micrometastases' has been studied in a variety of different tumor types. Only limited and still somewhat conflicting data are available for esophageal carcinoma. A differentiation between 'tumor cell microinvolvement' and true 'micrometastases' may help to clarify these controversies. While lymph node micrometastases are common even in patients with pT1 or pT2 squamous cell esophageal cancer, they appear to occur late in patients with esophageal adenocarcinoma. In contrast, tumor cell microinvolvement of lymph nodes in the absence of micrometastases seems to be more common in patients with adenocarcinoma. Periesophageal inflammation and scarring, due to the underlying chronic gastroesophageal reflux disease in patients with adenocarcinoma of the distal esophagus, may account for the apparent differences in the biology and pattern of lymph node metastases between these two esophageal tumor entities. A prognostic effect, similar to that of frank lymph node metastases, has been convincingly shown for lymph node micrometastases but not for lymph node microinvolvement. Although preliminary, these observations support the use of different strategies in lymphadenectomy for squamous cell and adenocarcinoma of the esophagus.

Schlüsselwörter
Ösophaguskarzinom · Adenokarzinom · Plattenepithelkarzinom · Lymphknoten-Metastasen · Mikrometastasen

Zusammenfassung
Die Entwicklung neuer sensitiver immunhistochemischer Methoden ermöglicht es, einzelne disseminierte Tumorzellen und Tumorzellcluster in Lymphknoten von Patienten nachzuweisen, die in der Standardhistologie mit dem Befund pN0 diagnostiziert wurden. Die Prävalenz und die prognostische Bedeutung dieser «Mikrometastasen» wurde bislang für mehrere Tumortypen untersucht. Nur wenige und teilweise widersprüchliche Untersuchungen liegen für das Ösophaguskarzinom vor. Eine Differenzierung des «Tumorzell-Mikroinvolvements» von eigentlichen «Mikrometastasen» könnte zu einer Klärung beitragen. Während Mikrometastasen in Lymphknoten beim Plattenepithelkarzinom des Ösophagus ein häufiges Phänomen darstellen und auch bei mehr als 20% der Patienten mit pT1-Karzinom nachgewiesen werden können, sind Mikrometastasen in Lymphknoten beim frühen Adenokarzinom des Ösophagus selten. Im Gegensatz dazu läßt sich beim Adenokarzinom des Ösophagus ein Mikroinvolvement von Lymphknoten häufiger als beim Plattenepithelkarzinom nachweisen. Lymphknoten-Mikrometastasen, aber nicht das Mikroinvolvement, scheinen einen negativen Effekt auf die Prognose zu haben. Diese offensichtlichen Unterschiede in der Biologie und dem Muster der Lymphknotenmetastasierung legen ein differenziertes Vorgehen bei der Lymphadenektomie nahe.

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Introduction

In contrast to the decreasing rate of gastric cancer, the prevalence of esophageal cancer currently is rising significantly in the United States and Western Europe [1, 2]. This is primarily due to a dramatic increase in the incidence of adenocarcinoma, which usually arises from metaplastic columnar epithelium in the distal esophagus as a consequence of chronic gastroesophageal reflux. The only potentially curative therapeutic approach to esophageal carcinoma is a complete surgical removal of the tumor. Nevertheless, independent of the histologic subtype, the overall prognosis for patients with carcinoma of the esophagus remains poor. Even after a complete tumor resection most patients die within 5 years from metastatic disease [3–5].

In patients without evident systemic metastases and who undergo a potentially 'curative resection', the presence of lymph node metastases on routine histologic assessment has been identified as the single most important and independent predictor of survival for both squamous cell and adenocarcinoma of the esophagus [1, 5]. In most studies the overall 5-year survival rate of patients with frank lymph node metastases is smaller than 20% [5, 6]. However, local and/or systemic recurrence of esophageal cancer is often also observed in patients who had a complete tumor resection and show no evidence of lymph node metastases on routine histologic assessment. The presence of occult tumor cell clusters or single tumor cells in lymph nodes, that have not been detected by routine histological examination, has recently been implicated as an explanation for this observation [7–10]. This would suggest that tumor spread is by far greater than assumed on the basis of routine histopathologic studies [3, 9, 11].

The prevalence and prognostic significance of lymph node micrometastases has been studied in a variety of different tumor types, including breast [12, 13], colon [14–16], lung, and stomach cancer [17, 18], and has been correlated with poor prognosis in some of these studies. Few studies assessed lymph node micrometastases in patients with esophageal carcinoma, with controversial results regarding the prevalence and clinical role of this phenomenon [6, 9, 11]. One possible explanation for this controversy is the observation that by some investigators deposits of immunohistochemically positive epithelial cells in lymph nodes have been classified as metastatic or nonmetastatic based on the accompanying stromal reaction.

Techniques for the Detection of Lymph Node Micrometastases

Cytoceratin proteins are essential constituents of the cytoskeleton of normal and malignant epithelial cells. They can thus serve as a potential marker for tumor cells in nonepithelial tissue. With the development of sensitive immunhistochemical techniques relying on specific markers for cytoceratin proteins, it became possible to detect small clusters or even single tumor cells in tissue sections which were considered tumor-free on routine histology [15, 19]. Several groups have evaluated the prevalence of micrometastases in lymph nodes of a variety of solid tumors, using the cytoceratin expression as an epithelial marker for the presence of individual tumor cells [6, 9, 20]. Because AE1/AE3 positivity has been reported in mesothelial as well as in epithelial cells, an anti epithelial cell antibody against two glycopolypeptides of 34 and 49 kD on the surface and in the cytoplasm of epithelial cells has also been used for the detection of tumor cells in lymph nodes. The monoclonal antibody Ber-EP4, as an example for this antibody type, does not react with mesenchymal tissue including lymphoid tissue [8, 9, 11].

In a variety of solid organ tumors lymph node micrometastases detected by these techniques did not always correlate with survival or recurrence patterns. Consequently, it is still discussed controversially whether tumor cells detected immunohistochemically truly represent lymph node metastases or only constitute an 'epiphenomenon' [9]. A differentiation between 'tumor cell microinvolvement' and 'true micrometastases' may help to clarify this dispute. According to this concept, a positive immunohistochemical reaction alone is not sufficient to define lymph node micrometastases. Therefore, we have adopted the following definition for the diagnosis of a lymph node micrometastases: Individual tumor cells or tumor cell clusters less than 0.2 mm in greatest dimension, with a stromal reaction like granulation tissue or desmoplastic connective tissue, which indicate that the tumor cells are resident within the lymph node. Accordingly, lymph node microinvolvement is defined as individual tumor cells or clusters without this stromal reaction (fig. 1, 2).

More recently, attempts have been made to detect individual tumor cells in lymph nodes by utilizing the polymerase chain reaction. We have used this technique in the detection of lymph node micrometastases in patients with colon carcinoma (fig. 3). With the application of a mutant allele-specific amplification method, 1 cancer cell in 10^3 normal lymph node cells in was detectable [6, 3, 16]. This appears to be a very promising approach. However, there are remarkable discrepancies reported between the results of immunohistochemistry and polymerase chain reaction (PCR). Often tumor cell documentation within lymph nodes by PCR does not correlate with histopathological or immunohistochemical findings. As a result it is still unclear whether PCR techniques detect DNA derived from degraded and nonviable cancer cells or from real micrometastases [15, 21].

Lymph Node Micrometastases and Microinvolvement in Patients with Esophageal Cancer

Only few studies examined lymph node micrometastases and microinvolvement in patients with esophageal cancer [6, 9, 11]. All studies used immunohistochemical methods on paraffin-embedded or fresh frozen lymph node tissue. The results of these studies differ markedly.

Izbicki et al. [11] reported on an investigation of 68 patients with esophageal carcinoma, including 19 adenocarcinomas and 49 squamous cell carcinomas. None of the patients had neoadjuvant or adjuvant treatment and all underwent a complete (R0) tumor resection. A total of 399 lymph nodes were negative on routine histology examination and were included in the immunohistochemical screening using the Ber-EP4 antibody.

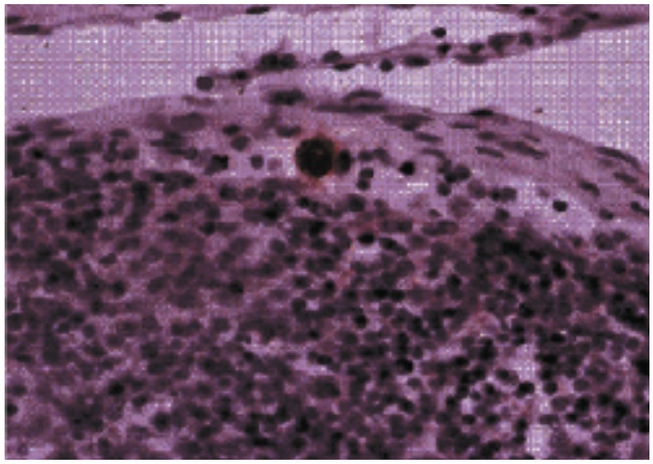

Fig. 1. Tumor cell microinvolvement in a section of a lymph node in a patient with squamous cell carcinoma, stained with AE1/AE3 antibody cocktail. No reaction with the surrounding stroma was observed, an essential criterion for micrometastases.

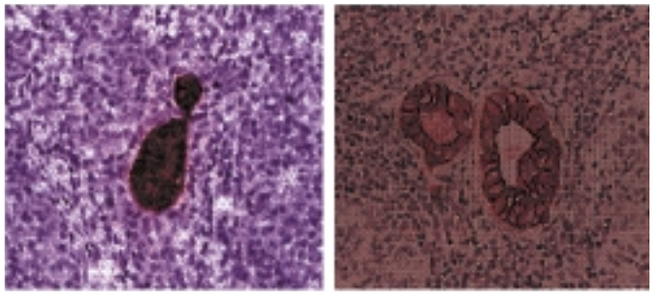

Fig. 2. Micrometastases in sections of lymph nodes of patients with esophageal squamous cell carcinoma (left panel) and esophageal adenocarcinoma (right panel). Stained with antibody cocktail AE1/AE3. Strong stromal reaction and clusters of tumor cells were observed.

Cryostat sections of three different levels from each lymph node were investigated. In 50% of the patients a positive reaction with the Ber-EP4 antibody was described but no differentiation was made between micrometastases and microinvolvement. Nevertheless, detection of Ber-EP4-positive cells within lymph nodes was an independent predictor for overall and relapse-free survival. In this study 5 patients with Ber-EP4-positive nodes had early tumor stages.

Glickman et al. [6] investigated lymph nodes of 49 adenocarcinomas and 29 squamous cell carcinomas of the esophagus, all without histologically identifiable lymph node metastases. 64% of patients had undergone preoperative radiation and chemotherapy. A total of 574 lymph nodes embedded in paraffin were sectioned serially to obtain 5 representative slides for immunohistochemical investigation with cytoceratin antibody cocktail AE1/AE3. In 31% of the patients with adenocarcinoma and in 17% of patients with squamous cell carcinoma of the esophagus, positive cells were detected in the resected lymph nodes. Micrometastases were, however, not differentiated from microinvolvement.

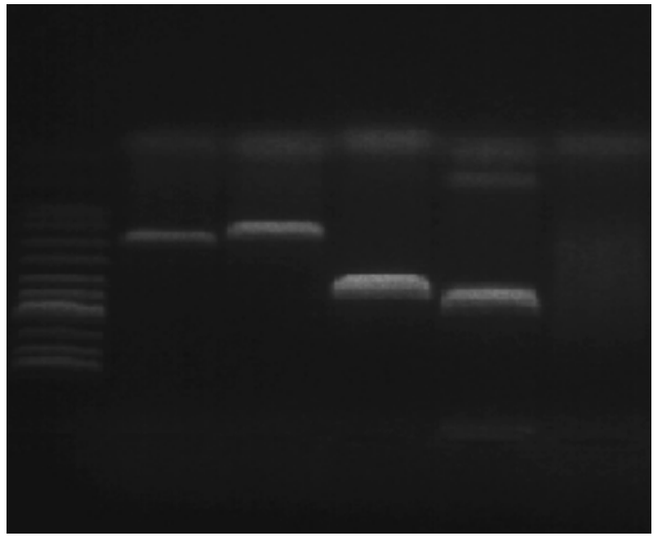

Fig. 3. Example for detection of micrometastases in pN0 lymph nodes of patients with colon carcinoma by polymerase chain reaction, using a fresh frozen lymph node and primer against CEA and Cytoceratin 20. The CEA-specific fragments were 177 bp (a) and 160 bp (b), the Cytoceratin 20-specific fragment was 350 bp (c). Lane d: Control for efficient RNA amplification using a β-microglobulin-specific fragment.

Natsugoe et al. [9] from our institution investigated lymph nodes of 69 R0-resected patients with squamous cell carcinoma of the esophagus. Immunohistochemistry was performed with a cytoceratin antibody cocktail, positive reactions were confirmed with the Ber-EP4 antibody. Findings were divided into tumor cell 'microinvolvement' and 'micrometastases' as described above. A total of 1,954 lymph nodes were investigated. In 13/41 patients (31.7%) staged negative for lymph node metastases on routine histology, lymph node micrometastases were found. An additional 2 patients showed tumor cell microinvolvement of lymph nodes without evidence of micrometastases. On univariate analysis lymph node micrometastases were a prognostic factor with an impact on survival equal to that of frank lymph node metastases.

In a subsequent study we compared the prevalence of lymph node micrometastases and lymph node microinvolvement between 41 patients with squamous cell and 41 patients with adenocarcinoma of the esophagus, who had a complete tumor resection and showed no evidence of lymph node metastases on routine histopathologic evaluation [22]. Lymph node micrometastases were significantly less common in patients with adenocarcinoma of the esophagus as compared to patients with squamous cell esophageal cancer ($p < 0.05$), while lymph node microinvolvement was more common in patients with adenocarcinoma. None of 30 the patients with pT1 adenocarcinoma had evident lymph node micrometastases as compared to 6/27 patients with pT1 squamous cell esophageal cancer ($p < 0.05$). Periesophageal inflammation and scarring due to underlying chronic gastroesophageal reflux disease in patients with adenocarcinoma of the distal esophagus may account for these apparent differences in the biology and pattern of lymph

node metastases of these two esophageal tumor entities. Lymph node micrometastases, but not microinvolvement, had a significant negative effect on survival.

Conclusion and Outlook

With immunohistochemical techniques, individual epithelial cells and cell clusters suggesting tumor cells can be detected in lymph nodes of a substantial portion of patients with esophageal cancer staged as pN0 on routine histological assessment. The prognostic impact of this finding in patients with esophageal carcinoma is still unclear. In the studies discussed above different populations were examined. Therefore the results are difficult to compare. In contrast to others, Glickman et al. [6] included patients who had undergone preoperative radiochemotherapy and reported a markedly higher prevalence of micrometastases than in most other studies. This could be explained by a bias in favor of selecting patients with more advanced tumor stages for neoadjuvant therapy protocols.

Whether epithelial cells detected by immunohistochemical methods in lymph nodes truly represent metastases or are an 'epiphenomenon' is therefore still controversial. Recent studies indicate that a differentiation between adenocarcinoma and squamous cell carcinoma of the esophagus and a separation of tumor cell 'microinvolvement' from 'micrometastases' may help to clarify these issues in future studies. Recent data from our institution indicate a very low probability of lymph node metastases and micrometastases in patients with early adenocarcinoma of the distal esophagus. In these patients a modification of the surgical approach towards a more limited resection and lymphadenectomy may be justified [23]. However, more studies with long-term follow-up are required, before the proven principles of surgical therapy for esophageal cancer are abandoned.

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