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The clinical importance of micrometastases within the lymphatic system in patients after total gastrectomy

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ABSTRACT

Background: In spite of radical gastrectomy with resection of the lymphatic system, where no metastases are found during histopathological examination, about 30% of patients have relapse of the neoplastic process. This situation may be caused by micrometastases or isolated neoplastic cells in the lymphatic system which were not identified during a standard histopathological examination.
Aim: The aim of the study was to evaluate the clinical importance of micrometastases within the lymphatic system in patients with gastric cancer.
Materials and methods: A group of 20 patients treated for gastric cancer were subjected to retrospective analysis. Of all the patients who underwent surgery, a group with tumours classified as T1 or T2 was selected. No metastases within the lymphatic system were found in the standard evaluation – N0 mark. Paraffin-embedded blocks of lymph nodes were cut and new specimens were made, which were then stained again by means of immunohistochemistry. Antibodies against cytokeratin AE1/AE3 were used.
Results: A total of 319 lymph nodes were assessed in 20 patients in an H+E examination. After the immunohistochemical examination, micrometastases within the lymphatic system were found in 4 (20%) patients and isolated neoplastic cells in other 4 (20%) patients.
Conclusion: On the basis of numerous publications and our own material, we think that the presence of micrometastases may be related to a worse prognosis. The clinical importance of micrometastases within the lymphatic system in patients after total gastrectomy.

1. Background

The incidence of gastric cancer in Europe and Poland shows a falling tendency. Nevertheless, gastric cancer continues to be a significant clinical problem. It is the second malignancy causing most deaths around the world.[1] The results of treatment of advanced gastric cancer are unsatisfactory and leave much to be desired.

In patients with gastric cancer, the most important prognostic factor is the state of the regional lymphatic system. In spite of radical gastrectomy with resection of the lymphatic system, where no metastases are found during a histopathological examination, about 30% of patients have

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relapse of the neoplastic process.[2] This situation may be caused by micrometastases or isolated neoplastic cells in the lymphatic system, which were not identified during a standard histopathological examination. A standard procedure is to subject a lymph node to section in one plane and to collect two or three specimens, which are subsequently stained with haematoxylin and eosin. Small concentrations of gastric cancer cells located in the subcapsular sinuses of lymph nodes may be overlooked during a standard histopathological evaluation. The application of more sensitive methods of histopathological evaluation such as immunohistochemical examination or molecular biology techniques enables identification of micrometastases and isolated tumour cells within the lymphatic system, which were not found during a standard evaluation. Similarly, in the case of other tumours such as: breast cancer, melanoma, lung cancer or colorectal cancer, the application of immunohistochemical staining for evaluation of the lymphatic system enables identification of micrometastases in up to 20–45% of patients on average.[3–5] At present, there is no definite opinion concerning the importance of micrometastases within the lymphatic system.

2. Aim

The aim of the study was to evaluate the clinical importance of micrometastases within the lymphatic system in patients with gastric cancer.

3. Material and methods

A group of 20 patients treated for gastric cancer at the Department I of General Surgery and Surgical Oncology, Greater Poland Cancer Centre, Poznań, Poland, from 2002 to 2007 were subjected to a retrospective analysis. All consecutive, eligible patients treated in this period were enrolled for the study. No patient received neoadjuvant or adjuvant therapy. On the basis of prior diagnosis of adenocarcinoma, all the patients underwent total gastrectomy with resection of the lymphatic system, inclusive the second compartment (D2 resection). The continuity of the alimentary tract was reconstructed by means of the Roux-Y method. After the surgery, all the patients underwent a standard histopathological evaluation of the entire resected specimen. The evaluation of the lymphatic system consisted in the section of the node and collection of one or two specimens. After fixing, the specimens were stained with haematoxylin and eosin and then subjected to microscopic assessment. Of all the patients who underwent surgery, a group with tumours classified as T1 or T2 was selected. No metastases within the lymphatic system were found in the standard evaluation – N0 mark. Paraffin-embedded blocks of lymph nodes were excised and new specimens were made, which were then stained again by means of immunohistochemistry. Antibodies against cytokeratin AE1/AE3 were used. The obtained specimens were assessed by a histopathologist. According to the definition, the cellular deposits with diameter range between 0.2 and 2 mm were recognised as micrometastases. On the other hand, the concentrations of neoplastic cells with the diameter smaller than 0.2 mm were recognised as isolated tumour cells.

4. Results

A total of 319 lymph nodes were assessed in 20 patients in an H + E examination, i.e. 15.96 nodes per patient, median 15.5. After the immunohistochemical examination, micrometastases within the lymphatic system were found in 4 (20%) patients and isolated neoplastic cells in other 4 (20%) patients. Thus, the application of the immunohistochemical examination enabled identification of neoplastic cells within the lymphatic system in 8 (40%) patients (Table 1). In all the patients with diagnosed micrometastases, tumour was located subcardially and the micrometastases were found in the lymph nodes around the cardia, i.e. groups 1 and 2 in the Japanese classification. In two of the four patients apart from the micrometastases there were isolated tumour cells identified in another nodal station along the lesser curvature station 3. The survival time of the patients with identified micrometastases was lower than that of the patients without neoplastic cells found within the lymphatic system after immunohistochemical examination. It was 34.69 months vs. 49.86 months, respectively. In the case of isolated neoplastic cells located in the lymphatic system, in comparison with the N0 group after immunohistochemical examination, the difference in survival time was 45.33 vs. 49.86, respectively. On the other hand, in the group of patients with identified micrometastases, the survival time was shorter in the patients with isolated tumour cells identified in other nodal stations besides the micrometastases, i.e. 16.15 months vs. 53.22 months. The statistical analysis was not performed due to the group of patients being too small.

5. Discussion

Radical resection of the whole organ together with the lymphatic system still remains a standard method of treatment of gastric cancer. Unfortunately, even as much as 13.8% of patients who underwent radical resection due to early gastric cancer infiltrating only the mucosa or submucosa have a relapse of the neoplastic process. In a multi-factor analysis of the causes of relapse of early gastric cancer, which comprised a group of 3883 patients, Youn et al. recognised the following negative risk factors: age, size of tumour, number of focuses and state of lymph nodes. Of the elements listed above, it was the state of the regional lymphatic system that proved to be the most significant factor of relapse. In the group subjected to analysis, the most frequent occurrences were: the formation of distant metastases (55.7%), local relapse (34%) and invasion of the peritoneum (10.3%).[6] Lai et al. presented similar conclusions on the basis of an analysis of 2923 patients with early gastric cancer. The authors regarded the presence of metastases in the lymphatic system to be the first independent risk factor of relapse of the neoplastic process.[7] It is very likely that relapse of the neoplastic process is caused by unidentified micrometastases of the gastric cancer which are present in the lymphatic system.

Table 1 – Patients with micrometastases (MM) and isolated tumour cells (ITC).

	Age	Sex	T	Grade	Lauren	Number of examined lymph nodes	IMH	Site of MM/ITC
1	72	Male	T2a	G2	Intestinal	11	MM/ITC	1,2/3
2	53	Male	T1b	G3	Mixed	20	MM	1,2
3	65	Male	T2a	G1	Intestinal	26	ITC	1,2
4	74	Male	T2a	G2	Intestinal	30	ITC	4
5	72	Male	T2a	G3	Intestinal	14	MM/ITC	1,2/3
6	80	Male	T2a	G2	Mixed	24	MM	1,2
7	80	Female	T2a	G2	Mixed	12	ITC	3
8	50	Male	T1a	G3	Mixed	14	ITC	1,2

IMH – immunohistochemical staging; MM – micrometastases; ITC – Isolated tumour cells.

The introduction of new methods of histopathological assessment, such as immunohistochemistry or molecular biology techniques, enabled detection of micrometastases and isolated tumour cells of gastric cancer within the lymphatic system. In the available literature, micrometastases within the lymphatic system were identified in a group of 10% up to 68.1% of patients with diagnosed gastric cancer, regardless of the degree of progression.[8–12] In early gastric cancer, the frequency of occurrence of micrometastases ranges between 10% and 25%. In our research, the presence of micrometastases within the lymphatic system was diagnosed in 20% of the patients with T1 and T2 tumours. Cao et al. present similar results in a studied group of patients with gastric cancer after immunohistochemical examination of specimens of lymph nodes with cytokeratin AE1/AE3. The presence of micrometastases was diagnosed in a group of 21.3% of patients.[13] Lee reports that the application of immunohistochemical examination increased the percentage of identified metastatic lymph nodes from 13% to 34%.[10] In all of the presented publications, the evaluation of the lymphatic system was made by means of immunohistochemical examination with the application of AE1/AE3 antibodies against cytokeratin.[8,13–15] Identification of micrometastases in the lymphatic system is also possible when molecular biology techniques are applied. The application of the technique of reverse transcriptase in a polymerase chain reaction with the use of cytokeratin 20 gene as a marker also enabled identification of micrometastases in a 46.7% of patients after resection of gastric cancer. According to Matsumoto et al., the application of RT-PCR is a more sensitive examination than immunohistochemical staining, 28% vs. 14% in identification of micrometastases in patients with gastric cancer.[8] However, as Yamamoto suggests, the application of RT-PCR may detect not only the cells of gastric cancer but also the DNA of the tumour, simultaneously resulting in a high percentage of falsely positive results.[16]

The clinical importance of micrometastases and their influence on patients' survival rate continues to raise arguments and controversies (Table 2). Both the reports by Ishii and Lee show that the group of patients with micrometastases in the lymphatic system had a worse prognosis than an identical group without micrometastases. The differences in the 5-year survival rate were statistically significant and they reached 49% for the group with micrometastases, whereas for the group without micrometastases they amounted to 76%.[14,17] In another publication by Ze-Yu Wu, a group of patients with micrometastases had a shorter mean survival rate– (18 ± 7.4 months) in comparison with the group without micrometastases (22.86 ± 3.17 months). The difference was statistically significant $p < 0.05$. The authors present similar results for cancers of the cardia and distal part of the oesophagus, where the presence of micrometastases was a significant factor of local relapse and dissemination of the neoplastic process.[18] Cao et al. showed in their latest publication that in comparison with patients without micrometastases patients' survival rate was longer and statistically significant ($p < 0.001$), regardless of the single-cell or clustered-cell type of micrometastasis.[13] Fukagawa et al. present different results. They did not observe statistical significance in a 5-year and 10-year period of observation of patients with identified isolated neoplastic cells in the lymphatic system, as compared with a group of patients without isolated neoplastic cells. The differences were 94% vs. 79% and 89% vs. 74% ($p > 0.05$), respectively.[15] Fukagawa's publication was based on an analysis of the results of patients treated in two centres, American and Japanese. The study

Table 2 – Clinical impact of micrometastases found in lymph nodes of patients with gastric cancer.

Author	Year	Method	Antibody	Upstaging	Impact on prognosis
Siewert	1996	IMH	AE1/AE3	90%	Negative
Ishida	1997	IMH	CEA, CAM 5.2	17.6%	Negative
Fukagawa	2001	IMH	AE1/AE3	36%	None
Nakajo	2001	IMH	AE1/AE3	15%	Negative
Lee	2002	IMH	AE1/AE3	24%	Negative
Ishigami	2003	IMH	AE1/AE3	9%	Negative
Doekhie	2005	IMH	CAM 5.2	49%	Negative
Wu	2007	RT-PCR	CK-20	46.7%	Negative
Cao	2010	IMH	AE1/AE3	21.3%	Negative

IMH – immunohistochemical staging; RT-PCR – reverse transcription polymer chain reaction.

concerned only isolated neoplastic cells. In the authors' opinion isolated neoplastic cells do not exhibit a tendency to make metastases by proliferation, they do not react with the stroma, do not infiltrate the vessels or structures of lymph nodes. This might explain why such a small amount of metastasis in the lymph node is not a risk factor of relapse and worse prognosis. In our material, the biggest difference in patients' survival time was observed in the patients who had micrometastases within the nodal station nearest the tumour and isolated neoplastic cells in other stations in comparison with the group without any metastases. On average, the patients with invaded lymphatic systems survived 16.15 months, whereas those without metastases survived 40.75 months.

In the process of metastatic formation in the lymphatic system, tumourous cells migrate via the lymph vessels to the lymph nodes, where the following stages of settlement, invasion and proliferation occur. However, individual tumourous cells are detected by means of immunohistochemical examinations or molecular biology techniques. They are eliminated in the lymph nodes by the immune system in the process of irreversible cell cycle arrest or induced apoptosis. According to the theory of cancer stem cells, gastric cancer progenitor cells must be present within the lymph node to form a metastasis in that place.[19] The absence of progenitor cells in the lymph node may account for the observation by Nakajo et al. and Siewert et al., who found that isolated neoplastic cells did not exhibit proliferative activity and did not infiltrate the node structure.[20,21] Individual non-progenitor tumourous cells found in the lymph node should not have clinical significance. In the case of micrometastases, which according to the definition have a diameter of more than 0.2 mm in light microscopy, they are more likely to contain progenitor cells. This would account for worse prognosis and shorter survival rate of this group of patients in comparison with patients without metastases in the lymph nodes. The determination of clinical importance of micrometastases and isolated neoplastic cells may contribute to a better knowledge of the mechanisms of relapse of the neoplastic disease. Recently, new drugs have appeared, e.g. lapatinib, which is used in the adjuvant therapy of patients with gastric cancer. In combination with standard chemotherapy, these drugs significantly increase the 5-year survival rate. It is necessary to precisely specify the indications for this type of therapy. The application of chemotherapy, radiotherapy as an adjuvant therapy (and immunotherapy in the future) may be of utmost significance, especially in patients with minimal deposits of neoplastic cells.[22–24]

6. Conclusion

The introduction of new methods of assessment of the lymphatic system resulted in more detections of micrometastases and isolated neoplastic cells. At present, there is no final consensus concerning their clinical importance. However, on the basis of numerous publications and our own material, we think that their presence may be related to a worse prognosis. They are an important factor of relapse of the neoplastic process. They may play a crucial role in the process of qualifying for adjuvant chemotherapy, which offers a chance to improve the effects of treatment.

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