



Lymph node, peritoneal and bone marrow micrometastases in gastric cancer: Their clinical significance

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Abstract

The 7th TNM classification clearly states that micrometastases detected by morphological techniques (HE stain and immunohistochemistry) should always be reported and calculated in the staging of the disease (pN1mi or M1), while patients in whom micrometastases are detected by non-morphological techniques (e.g., flow cytometry, reverse-transcriptase polymerase chain reaction) should still be classified as N0 or M0. In gastric cancer patients, micrometastases have been detected in lymph nodes, the peritoneal cavity and bone marrow. However, the clinical implications and/or their prognostic significance are still a matter of debate. Current literature suggests that lymph node micrometastases should be encountered for the loco-regional staging of the disease, while skip lymph node micrometastases should also be encountered in the total number of infiltrated lymph nodes. Peritoneal fluid cytology examination should be obligatorily performed in pT3 or pT4 tumors. A positive cytology classifies gastric cancer patients as stage IV. Although a curative resection is not precluded, these patients face an overall dismal

prognosis. Whether patients with a positive cytology should be treated similarly to patients with macroscopic peritoneal recurrence should be evaluated further. Gastric cancer cells are detected with high incidence in the bone marrow. However, the published results make comparison of data between groups almost impossible due to severe methodological problems. If these methodological problems are overcome in the future, specific target therapies may be designed for specific groups of patients.

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Key words: Gastric cancer; D2 lymphadenectomy; Lymph node micrometastases; Peritoneal micrometastases; Bone marrow micrometastases

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INTRODUCTION

Histologically confirmed metastatic infiltration of peri- and extra-gastric lymph nodes has been defined as the strongest independent dismal prognostic factor for both early^[1] and advanced^[2] gastric cancer patients. It could be proposed that, by performing a D2 lymphadenectomy, coexisting micrometastases, skip metastases and skip micrometastases are resected and thus more R0 resec-

tions are achieved, facts probably leading to locoregional control of the disease, better outcome and increased survival^[3,4].

However, recurrences are very common, even after an oncological R0 resection, and the peritoneum represents the most frequent site of recurrence of the disease^[5]. This fact is probably related to the intraperitoneal presence of free cancer cells shed from the serosal surface of the primary tumor^[6]. It is uniformly accepted that peritoneal metastasis constitutes the most frequent cause of death, with a mean survival of only a few mo following peritoneal recurrence^[7].

Moreover, even after an aggressive surgical approach and extended lymphadenectomy, a significant proportion of patients will eventually develop metastatic disease despite the potentially curative surgery, indicating the presence of early disseminated disease not apparent at the time of primary treatment. Since epithelial cells are not present in bone marrow under normal circumstances, identification of micrometastases in the bone marrow has been proposed as evidence of systemic micrometastatic disease^[8]. It is likely that this group of patients is understaged, probably because of the presence of occult metastatic disease at the time of initial surgery^[9].

The 7th TNM classification^[10] defines micrometastases as a metastatic focus between 0.2 and 2 mm and clearly states that, if they are detected by morphological techniques (HE stain and immunohistochemistry), they should always be reported and calculated in the staging of the disease (pN1mi or M1). On the other hand, patients in whom micrometastases are detected by non-morphological techniques [e.g., flow cytometry, reverse-transcriptase polymerase chain reaction (RT-PCR)] should still be classified as N0 or M0. Obviously, the previously mentioned terminology, classification and clarifications can also be applied in gastric cancer.

Herein, we review the current knowledge and evidence of the prognostic significance of lymph node, peritoneal and bone marrow micrometastases detectable by morphological techniques in gastric cancer patients.

LYMPH NODE MICROMETASTASES

Three methods have been used for the identification of lymph node micrometastasis: serial sectioning, immunohistochemical staining and RT-PCR. Serial sectioning constitutes a histological method, which can detect metastasis previously missed by the conventional technique, but may still fail to identify isolated tumor deposits^[11]. RT-PCR has been reported as highly sensitive^[12] but it is compromised by false-positive results caused by biological contamination^[13]. Positive RT-PCR results indicate the presence of tumor DNA; however, they may not indicate the presence of viable tumor cells^[14]. Thus, immunohistochemistry with human anti-CK antibodies represents the most accurate method for micrometastasis detection^[15] and the most frequently applied technique in research^[16].

Lymph node micrometastases have been reported as

immunohistochemically detectable in 10% of early gastric cancer patients^[16], in 52.6% of T2N0 patients^[17], and in 21%^[18] to 49%^[19] of all node-negative gastric cancer patients.

Particularly in the subgroup of level I lymph node negative patients, the incidence of histologically detected metastases in the level II lymph nodes (skip metastases) ranges between 2.8% in cases of early^[20] and 5%^[21] to 17.4%^[22] in all other gastric cancers. Moreover, in patients who had been histologically classified as level I lymph node negative, the incidence of micrometastases detected by immunohistochemistry in the level II lymph nodes (skip micrometastases) ranges between 10% in cases of early^[23] and 17%^[24] in cases of T1-2N0 gastric cancers.

Although other reports^[25,26] failed to show any relationship between micrometastases presence and recurrence rate or outcome, Cai *et al.*^[27] reported a 5 years survival of 100% for the micrometastasis negative, compared to an 85% for the micrometastasis positive *sm* gastric cancer patients. Maehara *et al.*^[28] reported a 50% shorter survival for the micrometastases positive, compared to the micrometastases negative, early gastric cancer patients who died from recurrence of the disease. Saito *et al.*^[29] reported the presence of micrometastases in 50% of the early gastric cancer patients who presented with recurrence of the disease classified as pN0 on the initial conventional histology. Finally, experimental data^[30] addressed that micrometastases in lymph nodes have high proliferative activity, thus potentially can develop metastases. Based on the above, micrometastases undoubtedly cannot be ignored.

The clinical significance of the skip metastases and skip micrometastases remains controversial and the controversies are mainly related to the small number of patients enrolled in skip metastasis studies^[31], the probable different prognosis of patients with histologically *vs* micrometastatically detected skip metastases^[15] and the concern that patients with histologically detected skip metastasis may represent cases of overlooked histological metastasis or micrometastasis in level I lymph nodes, thus being misclassified as patients with skip metastasis^[32].

Saito *et al.*^[32] compared gastric cancer patients with skip metastasis to patients with metastasis in the level I and level II lymph nodes and concluded that both the clinicopathological characteristics as well as the prognosis of patients with skip metastasis were similar to patients with level I lymph node metastases, but not to patients with level II lymph node metastases. Li *et al.*^[22] reported that the cumulative survival rate was not statistically different between gastric cancer patient with solitary skip lymph node metastases compared to patients with solitary level I lymph node metastases. Park *et al.*^[31] reported that in patients with positive nodes extending into the level II lymph nodes, the survival curves did not show statistical differences between skip(+) and skip(-) groups of patients, further supporting the theory that the number but not the level of lymph node metastases has prognostic significance.

A last issue regards the clinical significance of the possible micrometastatic infiltration of the nos 7 and 9 lymph node stations complex. It has been proposed that the most likely route for para-aortic lymph node metastases is from the left gastric artery nodes, passing by the celiac artery^[33]. Thus, these lymph nodes should be always evaluated, regardless the mode of operation, even in cases of minimally invasive surgery. Yanagita *et al*^[34] investigated the clinical significance of the morphological distribution of metastatic foci (metastasis, micrometastasis or isolated tumor cells) in sentinel lymph nodes with gastric cancer and concluded that in patients with non-marginal sinus type sentinel node metastasis, attention should be paid to the possibility of non sentinel node or even pN2 metastases presence. Thus, if the sentinel node cannot be identified in the perigastric lymph nodes, around the celiac artery lymph nodes should be always explored to reduce the likelihood of false negative results in sentinel node mapping^[20].

PERITONEAL MICROMETASTASES

It has been postulated that the majority of gastric cancer patients with intraperitoneal free cancer cells (IFCCs) do not escape postoperative peritoneal recurrence^[35]. For more than three decades, IFCCs have been assumed to play an important role in the development of peritoneal metastases, which is the foremost pattern of failure after potentially curative resection for gastric cancer^[6,36-40], while the peritoneal cavity can be a route for dissemination of malignant cells, either by direct continuity with the lesion or acting as a receptacle for lymphatic spread^[38,40].

Peritoneal lavage cytology is widely accepted as the gold standard for diagnosis of IFCCs. Upon entering the abdominal cavity, prior to manipulating the tumor, 200 mL of warm normal saline is introduced and manually dispersed in the Douglas cavity, paracolic gutters and in the right and left subphrenic cavity. At least 50 mL of fluid is subsequently recovered, after gentle stirring, from several regions of the abdominal cavity. The fluid is then centrifuged for 5 min at 1500 r/min. The sediment is smeared onto one or more glass slides and stained using Papanicolaou's method. Cytological findings are classified as positive, negative or suspicious. The following cell characteristics are used to determine the presence of malignant cells: presence of aggregate, size, shape, type of cytoplasm, cytoplasmic vacuoli, mainly nuclear abnormalities, nuclear chromatin, nuclear-cytoplasmic ratio, mitotic figures and nucleolar prominence. When necessary, the glass slide containing the nucleated cell layer is further analyzed by immunohistochemistry using the CEA antigen antibodies^[41].

Cytology can be easily and safely performed; it requires approximately 15 min for a cytopathologist to analyze the patient's slides and its estimated cost is \$60.20^[42]. The method has a sensitivity of 90% to 96.7% and nearly 100% specificity in the diagnosis of IFCCs^[43]. False-positive results have been recognized with a rate of 4.5% to

5%, probably secondary to reactive mesothelial cells^[38,39,44], but this problem can be eliminated by the use of immunohistochemistry^[45].

The detection rate of IFCC ranges between 14% and 47%, depending upon the cohort of patients included^[6,35-40,44], while, when only potentially curative resections were included, the rate of IFCC varied from 4.4% to 11%^[46-48], and from 22% to 30% in gastric carcinoma involving the serosa^[49,50].

Mezhir *et al*^[51] demonstrated that a positive peritoneal cytology, even in the absence of gross peritoneal disease, indicates a poor outcome. In the Dutch Gastric Cancer Group^[48], positive cytological findings were found in 4.4% of the patients and were indicative of a poor prognosis, with a median survival of 13 mo. In Bentrem *et al*'s study^[46], which included 371 patients with gastric carcinoma undergoing diagnostic laparoscopy and peritoneal washing cytology prior to any attempt for R0 resection, IFCCs were detected in 6.5% of the patients and this finding was an independent dismal prognostic factor, correlating with a median survival of 14.8 mo *vs* 98.5 mo for patients with negative cytology. Thus, the Japanese Society for Gastric Cancer has included peritoneal cytology as part of the staging procedure^[52], while the TNM classification system has classified cytology-positive gastric cancer patients as stage IV patients since 1997^[53].

In cases of gross peritoneal recurrence, the promising results which have been reported^[54,55] following cytoreductive surgery and intraperitoneal chemotherapy suggest that an aggressive approach to the peritoneum may improve survival. However, randomized studies examining the effectiveness of cytoreductive surgery and intraperitoneal chemotherapy as a standard treatment strategy in select patients with peritoneal carcinomatosis from gastric cancer are required^[54]. On the other hand, the management of patients with positive peritoneal cytology as the only evidence of M1 disease is largely unknown^[50,51,54]. However, results from Western series suggest that a positive peritoneal cytology is related to a poor outcome regardless of treatment^[46,56].

BONE MARROW MICROMETASTASES

Several studies in upper gastrointestinal tract cancer patients disclosed the presence of bone marrow micrometastases, both in the preoperative period as well as up to twelve mo after surgery. When micrometastases are detected preoperatively, some do not persist and may represent transient shed cells that the host can clear^[57]. For micrometastases detected at the time of surgery, it is believed that these tumor cells represent systemic residual disease, while single clonogenic tumor cells are the reason for later clinical relapse^[58].

Bone marrow micrometastases do not represent "true" micrometastases based on the TNM terminology. However, in gastric cancer patients, the incidence of epithelial cells detected by immunohistochemistry at the time of the curative resection in the bone marrow of the iliac crest

ranges between 25%^[59] and 53%^[60], although tumor cells appear more frequently (79%) in resected rib marrow^[61].

Compared to clinicopathological factors of the primary tumor, bone marrow micrometastases have been reported to be related to the depth of invasion of the gastric wall, cytological differentiation, lymph node spread and increased tumor microvessel density^[62].

The clinical or the prognostic significance of bone marrow micrometastases are still a matter of debate. Schlimok *et al.*^[63] found that epithelial cells were detectable in the bone marrow of 35% of gastric cancer patients without distant metastases and, in univariate analysis, their presence has adverse prognostic significance for the relapse-free survival. Jauch *et al.*^[60] found that epithelial cells were detectable in the bone marrow in 53% of gastric cancer patients without distant metastases and, in multivariate analysis, the presence of three or more cells had adverse prognostic significance for disease free survival for patients with T1-2N0 tumors while it did not reach significance for more advanced tumors or for patients with less than three epithelial cells in bone marrow. Heiss *et al.*^[58] concluded that a high expression of plasminogen activator inhibitor type 1 in tumor cells in bone marrow represents an independent dismal prognostic factor for the disease free survival of T1-2 tumors. On the other hand, Schott *et al.*^[64] and de Manzoni *et al.*^[65] clearly stated that the presence of cytokeratine-positive cells in the bone marrow of curatively resected gastric cancer patients did not affect outcome. However, bone marrow micrometastases in the rib were detected in 67% of esophagogastric cancer patients treated with surgery alone, but only in 39% of post-neoadjuvant chemoradiotherapy patients^[66].

The inconsistency among the previously mentioned reports can be explained either by methodological problems related to the immunohistochemical techniques or by the clinical behavior of the gastric cancer itself.

All authors agree that the published results make comparison of data between groups almost impossible due to severe methodological problems such as: the lack of consensus about the preferable detection method; the lack of standardization of the methods used; whether immunohistochemistry was chosen as the method of choice and cytokeratins as the optimal antigens; the selection of the antibody; the number of cell analyzed; and the selection of the control specimens which can all influence the results. Finally how many cells are needed in order establish a positive result needs to be clarified^[67].

Unlike the hypothesis that the contact of gastric cancer cells with peritoneal cells supports their ability to develop the full metastatic phenotype, cancer cells in the bone marrow might have been positively selected during early stages of metastasis and the majority of these cells appear to be in a dormant state of cell growth^[68].

evaluated for detection of lymph node micrometastases, which should be further encountered in the loco-regional staging of the disease. The detection of skip lymph node micrometastases should also be encountered in the total number of infiltrated lymph nodes. If there is a suspicion of gastric serosa invasion (pT3 or pT4), peritoneal fluid cytology examination should be obligatorily performed in order to verify the presence of IFCCs. A positive cytology classifies gastric cancer patients as stage IV, indicating a poor prognosis even for those who underwent a potentially curative resection. Whether patients with a positive cytology should be treated similarly to the patients with macroscopic peritoneal recurrence should be evaluated further. Since the prevalence of gastric cancer cells in the bone marrow is high, neoadjuvant or adjuvant therapies should not be precluded in selected groups of patients. Which patients will be classified as high-risk should be determined by future studies. If a uniformly accepted methodological technique for bone marrow micrometastases detection is established in the future, specific target therapies may be applied in specific groups of patients.

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CONCLUSION

A D2-lymphadenectomy specimen should always be

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