Micrometastasis is defined as microscopical deposit of malignant cells, less than 2mm in diameter, separated from the primary tumor. This does not include discontinuous growth in peritumoral region, but include microinvolvement of regional lymph nodes. Its potential ability to escape immune supervision, blood and lymphatic vessel invasion, and progression to macroscopical malignant growth distinguish this concept as one of the most prominent scientific problems today.

In the patients with gastric and cardiac adenocarcinoma micrometastasis could be detected in regional lymph nodes, bone marrow and peritoneal fluid. Approximately 40% of patients classified as pN0 according to traditional staining method with hemotoxylin and eosin (HE) have nodal micrometastasis. Using a technique of lymph node serial sections, detection of nodal metastasis and micrometastasis is markedly increased, but more sensitive immunostaining and molecular techniques further increase it.

Micrometastases have also been detected in bone marrow in as much as one third of patients with upper digestive malignancies, and stomach as well. There is strong correlation between prevalence of micrometastatic bone marrow disease and depth of tumor penetration and differentiation of primary tumor.

Using immunostaining occult and disseminated tumor cells could be detected in the abdominal fluid after peritoneal washing in 20-70% patients, and prevalence also depends on the stage of the disease.

Micrometastatic involvement or perigastric lymph nodes, in cases with unclear malignant lymph node involvement is routinely examined at the Institute of Digestive Disease in Belgrade, from March 2000.

BIOLOGY OF MICROMETASTASIS

The important feature of micrometastasis is the absence of its own stroma, including the lack of neovascularization. Since cells in micrometastatic nest receive oxygen and nutrients by passive diffusion, tumor size could not exceed more than 2-3mm.

Tumor cells probably could behave as dormant metastatic cells for quite a long time. However, absence of tumor growth, or progression, does not mean absence of proliferative neoplastic activity within tumor, but balance between tumor proliferation and cell death. This balance...
Cytokeratin is protein ingredient of intracytoplasmatic filaments which take part in formation of cytoskeleton in normal and malignant epithelial cells. It could be used as a reliable mark of epithelial cell presence in ectopic localizations, as it is in lymph nodes. Epithelial cells could be detected either with specific anticytokeratin antibodies or using immunological staining. Molecular biology techniques could be more sensitive in detection of micrometastasis than immunohistochemical techniques. Most of tumor cell lines produce specific proteins not present in normal cells. These cells could be detected via detection of specific genes encoding for synthesis of oncoproteins, using RT-PCR method (reverse transcriptase-polymerase chain reaction). For example, detection of carcinoembryonic antigen (CEA), specific for many adenocarcinomas, can detect ectopic CEA-positive cells. It is believed that diagnostic sensibility of serial sections with HE staining alone is lowest, although partly effective in diagnosis of micrometastatic disease.

Using HE technique micrometastases could be detected in 20% of pN0 patients. Sensibility of immunohistochemical techniques is significantly higher and is about 40%, even higher. Using RT-PCR method micrometastasis could be detected in as much as 60% of pN0 patients. RT-PCR is more sensitive than method of immunohistochemical detection, but method is compromised with false positive results caused by various sources of biological contamination.

**PREDICTORS OF MICROMETASTATIC NODE INVOLVEMENT**

Correlation between histological and molecular biological tumor characteristics and micrometastatic node disease, thus predicting of micrometastatic node involvement is the topic of a few scientific papers, but still not recognized in clinical practice. Analyzing endoscopic samples Natsugoe et al. found positive correlation of E-cadherin and -cathenin expression and micrometastatic node disease.

Analyzing early gastric carcinoma Kashimura et al. found positive correlation not only with biological tumor characteristics, but also with the extent of submucosal invasion. Cai et al. emphasized that occult lymph node involvement could be influenced with histological grade of tumor, and tumor size. Kashimura et al. put attention to invasion of lymphatic vessels within gastric wall, and depth of tumor penetration among other prognostic fac-

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**TABLE 1**

<table>
<thead>
<tr>
<th>Types of occult lymph node involvement</th>
<th>Grade 0 (G0)</th>
<th>Grade 1 (G1)</th>
<th>Grade 2 (G2)</th>
<th>Grade 3 (G3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mi-</td>
<td>No positive cells within lymph node</td>
<td>Four of less positive separated malignant cells (“single cells metastasis”)</td>
<td>Five or more single malignant cells or cell cluster less than 200u in diameter</td>
<td>Histologically overt micrometastasis measuring from 200u to 2mm in diameter</td>
</tr>
</tbody>
</table>

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persists due to poorly understood biological system which includes immune surveillance in relation of tumorogenesis and angiogenic factors in advanced growth, invasion and metastatic dissemination. Multistep process of active process of angiogenesis include extracellular matrix degradation, proliferation of endothelial cells, migration and neotubular formation.

Most important angiogenic factors are hypoxia, presence of inflammatory mediators and growth factors, such as endothelial growth factors (VEGF) and fibroblast growth factor (FGF), and other factors, i.e. trauma. Stimulatory effect of trauma could explain prominent proliferative activity of residual tumour cells after surgical intervention. In contrast, postoperative angiogenesis could be positively influenced by surgical removal of primary tumor.

Micrometastases show significant increase of apoptotic activity after treatment with angiogenic inhibitors. There is belief that antiangiogenic mediators against VEGF and FGF, already tested in vitro and in vivo, could be used in the clinical treatment of patients with various malignant tumors, including gastric adenocarcinoma. Nevertheless the concept of minimal residual diesase in lymph nodes has more questions than answers.

There are controversies about most apropriate diagnostic technique, clinical significance of nodal micrometastasis, possibility of preoperative prediction of micrometastatic nodal disease and role of micrometastasis in sentinel lymph node (SLN) biopsy.

**DETECTION OF MICROMETASTASIS**

At present, micrometastatic nodal disease could be detected by three procedures: serial hematoxylin and eosin staining (HE) on light microscopy, immunohistochemical detection using specific antibodies and molecular biological techniques.

The evaluation HE stained serial sections of lymph nodes on light microscopy is standard technique of histological method of examination but it is limited due to fact that many small tumor cell clusters and single cells could be overlooked. Although the sensibility of this technique is increasing with increasing numbers of sections through the lymph node, it has been accompanied or repressed with more sensitive procedures.

Many studies confirmed significantly higher sensivity of immunohistochemical method in the detection of micrometastatic lymph node involvement than, HE staining.
Clinical significance of nodal micrometastasis

Numerous studies revealed lymph node micrometastasis as an independent prognostic factor in patients with gastric adenocarcinoma. Some of them showed that presence of nodal micrometastasis does not correlate with survival in the patients with advanced gastric adenocarcinoma (study from National Cancer Center Hospital, Tokyo).

Furthermore, Siwert et al. found that micrometastatic nodal disease has no importance in HE node positive patients. Siye and morphological subclassification of occult lymph node involvement put light into initial confusion related to clinical significance, separating histologically overt micrometastasis as bigger cluster of tumor cells without surrounding stromal reaction from smaller or submicroscopical microinvolvement. Kikuchi et al. distinguished three categories of occult lymph node involvement, and showed their prognostic significance (Table 1.). Fellbaum et al. found clinical significance in single cell cathegrity of micrometastases also, with the requirement of more than three cancer cells in one of sections through lymph node in at least 10% analyzed lymph nodes. It seems that some tumor cells could escape immune supervision of the host more probable with the increase of tumor cellularity within perigastric lymph nodes.

Also, the relationship between tumor cellularity and number of cells presented as nodal micrometastatic involvement could determine the existence of occult lymph node involvement and relate to prognosis of the patients.

The importance of single cell metastasis in bone marrow is controversial too. Although survival rates in patients with bone marrow single cell micrometastasis is generally worse than in those without, not all of them have dissemination during 5 year’s follow-up after surgical procedure.

It seems that behaviour of single cell micrometastasis in bone marrow depends on numerous factors including cell viability, characteristics of host immune system, and characteristics of microenvironment. G2 type of microinvolvement after Kikuchi criteria seems to be less controversial, so it is recommended to classify it as clinical significant micrometastasis (dissemination).

Detection of micrometasis in sentinel lymph node

The importance of sentinel lymph node (SLN) biopsy is related to detection of the malignant cells in the first and/or the closest peritumoral lymph node (or lymph nodes). Histologically proven malignant involvement on frozen sections, might enable minimally invasion, possible up-staging of the tumor disease and lead to optimal surgical treatment. Localization of SLN is mapped using vital staining or injection of radiolucent coloid in the tumor or peritumoral tissue. Using this strategy diagnostic process is focused to one, or a group of lymph nodes, thus reducing overall amount of nodal tissue to be evaluated.

The concept of sentinel lymph node biopsy is controversial in respect to difficult selection of first lymph node and the type of diagnostic procedure for fast intraoperative evaluation. Gershenwald et al. claimed that type of diagnostic procedure is essential for accurate intraoperative detection of SLN involvement.

In most patients with local recurrence after negative SLN biopsy using HE staining, the use of more sensitive diagnostic procedures later demonstrated micrometastatic disease. Mori et al. developed procedure of fast RT-PCR reaction and used it in frozen section examination.

If concept of SLN biopsy guided surgery of gastric cancer could prove as reliable and clinical important, the detection of micrometasis in would be essential part of diagnostic procedure.

Conclusion

Clinical significance of occult lymph node involvement, in the patients with gastric adenocarcinoma, is still unknown as a result of relatively small series of analyzed cases and limited follow up. At present, the role of occult lymph node involvement proved its significance in two major fields: defining criteria for limited surgical dissection in the patients with early (sm) carcinoma in respect to detection of micrometastatic tissue in sentinel lymph node, and distinguishing the category of pN0 (Mi+) patients with potential benefit of postoperative adjuvant therapy.

Rezime

Značaj limfonodalnih mikrometastaza kod bolesnika sa adenokarcinomom želuca

Mikrometastaza je mikroskopski (manji od 2 mm) depozit malignih celija koji je odvojen od primarnog tumora. Analizirana je dostupna medicinska literatura o mikrometastatskoj bolesti i njenom značaju, uz poseban osvrt na nodalne mikrometastaze kod bolesnika sa adenokarcinomom želuca.

Imunohistohemijska dijagnostika omogućava najtačniju detekciju okultnog zahvatanja perigastričnih limfnih nodusa. I poređenje navedenih rezultata sa promotivanim radiokolloidom u suhima i jos bolje sa RT-PCR testom.

Većina autora se slaže da, trenutno, ne postoji saglasnost niti oko faktora rizika, niti oko kliničkog značaja detekcije nodalnih mikrometastaz kod bolesnika sa adenokarcinomom želuca.

Trenutni značaj detekcije nodalnih mikrometastaza kod bolesnika sa adenokarcinomom želuca ograničen je na dve oblasti; definisanje kriterijuma za ograničenu nodalnu dissekciju kod bolesnika sa ranim (sm) karcinomima, u zavisnosti od pregleda stražarskog limfnog nodusa ili grupe stražarskih limfnih nodusa, kao i definisanje kate-
gorije pN0 (Mi+) bolesnika koji potencijalno mogu imati koristi od postoperativne adjuvantne terapije.

Ključne reči: neoplazije želuca, adenokarcinom, metastaze, nodalne metastaze, okultno zahvatanje, mikrometastaze

Abreviations

early (sm) carcinoma; early (submucosal) carcinoma; Mi+/- micrometastatic dissease (present/absent); pN0-nodal staging according to International Union Against Cancer; HE staining-staining method with hemotoxylin and eosin; VEGF - endothelial growth factor; FGF- fibroblast growth factor; SLN - sentinel lymph node RT-PCR - reverse transcriptase-polymerase chain reaction; CEA - carcinoembryonic antigen G0-G3- types of occult lymph node involvement (Modified Kikuchi criterions)