Original article

The value of intraoperative imprint cytology in the assessment of lymph node status in gastric cancer surgery

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Abstract

Background. The intraoperative assessment of lymph node status is important when performing limited surgery in gastric cancer patients. Currently available techniques for this assessment are frozen section, imprint cytology, and other molecular methods; most current studies use the frozen-section method. In the present study, we focused on the accuracy and feasibility of imprint cytology as a tool to assess lymph node status intraoperatively in gastric cancer surgery.

Methods. Between April 2001 and March 2003, we performed imprint cytology of the sentinel nodes in 260 consecutive patients. After review by an experienced cytopathologist, the sensitivity, specificity, and overall accuracy of the method were determined.

Results. The time required for the intraoperative imprint cytology was 8 min, and the sensitivity, specificity, and overall accuracy were 52.2%, 88.8%, and 73.8%, respectively.

Conclusion. Imprint cytology could be a useful technique for the assessment of lymph node status intraoperatively if the sensitivity and specificity can be improved to an acceptable level.

Key words Gastric cancer · Cytodiagnosis · Intraoperative · Sentinel lymph node biopsy · Lymphatic metastasis

Introduction

Pathologic confirmation and accurate staging of the surgical specimen is critical in surgical oncology to determine the extent of surgery, to estimate the prognosis of individual patients, and to establish optimal plans for adjuvant therapy. In the treatment of gastric cancer, the traditional surgery in Korea and Japan has included

gastrectomy with safe margins and routine lymphadenectomy, mostly more than D2 according to the Japanese classification [1]. So the evaluation of lymph node status was only possible in the late postoperative period through the histologic examination of formalin-fixed surgical specimens, which means that surgeons had to perform lymphadenectomy without an exact knowledge of the lymph node status.

From the development of the sentinel node concept in melanoma and breast cancer, numerous techniques have evolved to assess lymph node status intraoperatively, such as frozen section, imprint cytology, rapid immunostaining, and other molecular methods. Through these techniques, surgeons have been able to avoid unnecessary lymph node dissection with an acceptable risk of an additional trip to the operation room.

As more patients are now being diagnosed in the early stages of gastric cancer, many attempts are being made to reduce both the extent of lymph node dissection and the extent of gastric resection itself. Even though many studies have proposed risk factors which predict the likelihood of lymph node metastasis in gastric cancer, no single risk factor can perfectly predict lymph node metastasis preoperatively. So performing limited surgery in gastric cancer patients without exact knowledge of lymph node status always carries a risk of residual nodal disease. Because reoperation to complete lymph node dissection carries the risk of additional morbidities, the need for accurate intraoperative diagnosis of lymph node metastasis is greater than ever.

Here, we report the result of our study of the accuracy and usefulness of imprint cytology as a tool to assess lymph node status intraoperatively in gastric cancer surgery.

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Patients and methods

We performed imprint cytology to assess lymph node status in 298 gastric cancer patients who underwent curative surgery at our institution from April 2001 to March 2003. Patients with T4 lesions and those with grossly enlarged perigastric lymph nodes were excluded from the study. Also, patients who were treated by laparoscopic surgery were excluded. With these exclusion criteria, 260 of the 298 patients were enrolled in this study, and informed consent was obtained from all 260 patients.

After laparotomy was performed and the gastric lesions were identified, the sentinel nodes were mapped by the subserosal injection of 4cc of indocyanine green dye peritumorally. Five minutes after the dye injection, green-stained perigastric nodes were collected, and these were considered to be sentinel nodes. The dissected sentinel nodes were immediately bisected along the longest axis and each side was imprinted onto a slide glass. The slides were fixed in 95% ethanol and then stained with hematoxylin and eosin. The remaining lymph nodes were fixed in formaldehyde for permanent section. The imprinting and staining procedures were performed in the operation room by surgeons, and the results of the imprint cytology were recorded in each patient's operation record. The slides were reviewed by an experienced cytopathologist, and all patients with gastric cancer underwent standard lymphadenectomy, D2 lymphadenectomy at minimum, regardless of their imprint cytology results. The data from the study were assessed by determining the sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy of the method. The calculations for determining positive and negative predictive values were as follows:

Positive predictive value = $\frac{\text{true positive}}{\text{true positive} + \text{false positive}}$

Negative predictive value = $\frac{\text{true negative}}{\text{true negative} + \text{false negative}}$

Results

The number of enrolled patients was 260. There were more male patients, with the sex ratio being 1.8 to 1. The median age of the patients was 53.7 years. When we classified our patients by T stage, 81 patients (31.2%) were classified as pT1 stage, 117 patients (45%) were pT2 stage, and 62 patients (23.8%) were pT3 stage. The time required for the imprint cytology was 8 min, and the mean operation time was 152 min. The time interval between the performance of imprint cytology and the permanent histopathology report was about 5 days.

Table 1. Results of imprint cytology

Permanent histopathology	Imprint cytology (IC)		
	Positive	Negative	Total
Positive	76	69	145
Negative	22	181	203

Sensitivity of IC, 52.2%; specificity of IC, 88.8%; positive predictive value, 77.6%; negative predictive value, 70.6%; accuracy, 73.8%

The mean number of identified sentinel nodes (i.e., the nodes that were stained green after the dye injection) was two for each patient (range, none to six nodes). But the overall detection rate was 66.9%; that is, we could detect stained nodes in only 174 of the 260 patients. The sentinel node detection rate was 79% (64 of 81 patients) in the pT1 group, 68.4% (80 of 117 patients) in the pT2 group, and 48.4% (30 of 62 patients) in the pT3 group. We used 348 lymph nodes for imprint cytology; the results are shown in Table 1. The sensitivity and specificity of imprint cytology were 52.2% and 88.8%, respectively. The positive predictive value and negative predictive value were 77.6% and 70.6%, respectively, and the overall accuracy was 73.8%.

Discussion

In Korea and Japan, and also in many other countries, more patients are being diagnosed while in the early stages of gastric cancer, and many centers are adopting the strategies of limited surgery, mostly laparoscopic, in the treatment of gastric cancer. The two most commonly used techniques in the limited surgery for gastric cancer are laparoscopic partial gastrectomy and laparoscopic distal gastrectomy with D1+ α lymphadenectomy. All the limited surgery techniques leave more lymphatic tissue than the standard D2 lymphadenectomy; therefore, there is always some risk of residual nodal disease. To reduce the extent of lymph node dissection, the operating surgeon must be able to assess the status of the lymph node that carries the highest chance of tumor cell metastasis, i.e., the sentinel node, and the surgeon must decide the extent of lymph node dissection based on accurate information on sentinel node status. Many studies have reported risk factors which predict the likelihood of lymph node metastasis in early gastric cancer, such as tumor size, depth, and differentiation, but none of these factors alone is perfectly predictive of lymph node metastasis [2,3]. So the accurate intraoperative assessment of lymph node status in gastric cancer surgery is essential, especially when performing limited surgery for early

Table 2. Advantages and disadvantages of the imprint cytology and frozen-section methods

	Imprint cytology	Frozen section
Advantages	Easy preparation of tissues Less time requirement Minimal loss of tissue No artifacts caused by freezing Serial multisection is easy	Fewer indeterminate results Widely used in clinical practice
Disadvantages	Need for an experienced cytopathologist May yield indeterminate results	More time and equipment needed Tissue loss and artifacts caused by freezing Difficult to perform serial sections

gastric cancer. Even though the frozen-section method is the most widely used technique for intraoperative diagnosis, imprint cytology has many advantages over this method [4–6]. The advantages and disadvantages of both methods are listed in Table 2.

In the present study, the time required for a single procedure of sentinel node imprint cytology was 8 min, which was very short compared to the time of 15–20 min required for frozen section. It takes about 2 min to prepare a harvested lymph node and make an imprint slide, and about 4 min for the staining. Currently available techniques for intraoperative diagnosis, apart from frozen section and imprint cytology, are rapid immunohistochemistry and other nonmorphologic methods, such as flow cytometry and molecular analysis [7]. To date, there is no single ideal method for the intraoperative diagnosis of lymph node status. The ideal method should be accurate and easy to perform and require minimal time and minimal personnel.

To establish the sentinel node concept in gastric cancer, there are many obstacles to overcome. First, most important of all, there is no universal agreement on the sentinel node concept in the field of gastric cancer surgery. The stomach has very rich and complex lymphatic channels [8]. Unlike the relatively simple and orderly lymphatic pathways of the breast, sentinel nodes in gastric cancer can be detected in multiple numbers and in unpredictable locations. Also, skip metastasis is reported in 3%–10% of gastric cancer patients with lymph node metastasis, with some reports being as high as 15%–20%. Sometimes lymph node metastasis is not confined to the N1 group, even in patients with solitary lymph node metastasis. Kosaka et al. [9] reported a high incidence of skip metastasis, especially in tumors of the middle and upper areas of the stomach, and Ichikura et al. [10] reported that 4% of lymph node metastases were located in N2 stations, in patients with early gastric cancer with a single lymph node metastasis. Secondly, even though it has not been confirmed in many western series, a survival benefit after standard D2 lymphadenectomy is well documented at specialized centers and the morbidity is quite minimal. Thirdly,

the clinical impact of nodal micrometastasis is not clear yet. And, last, which is the most limiting step in clinical practice, neither imprint cytology nor frozen sections yield satisfactory results as they do for breast cancer and melanoma [5,11,12]. In breast cancer and melanoma, many centers report that intraoperative diagnosis with these methods shows high sensitivity and high specificity, both being higher than 90% for each method [6,13].

In our study, the sentinel node detection rate, and the sensitivity and specificity of intraoperative imprint cytology were lower than we expected, and it is not possible to recommend applying this technique as standard procedure in gastric cancer at this time. But there are many ways to overcome the poor result of the present study. Our overall detection rate of sentinel nodes was 66.9% with the dye method alone, but the detection rate was increased to 79.0% and 68.4% in the subgroups of patients with pT1 and pT2 lesions, respectively. Considering that the main patients subjected to sentinel node navigation surgery would be patients in the early stages of the disease, the detection rate would be improved in actual practice. Also, our study was conducted with the single dye method alone, and we would expect to have a better detection rate with a dual method, using both a radioactive isotope and vital dye. The low sensitivity of sentinel node imprint cytology could be increased by making serial multisections of the node and using both imprint cytology and frozen sections. The imprint cytology method has the advantages of saving tissue without the need for tissue freezing, and imprint cytology can easily be combined with frozen sections. Also, the concomitant use of rapid immunohistochemistry could increase the positive rate. The low specificity of sentinel node imprint cytology could be increased by lowering the incidence of indeterminate results.

In conclusion, imprint cytology is a valuable technique for the evaluation of lymph node status intraoperatively, and it could be a future solution in gastric cancer surgery if the sensitivity and specificity were to be increased to an acceptable level. This could be done

by the use of combined diagnostic methods and by making serial multisections of sentinel nodes. Also, the idea of lymphatic mapping and the sentinel node concept should be more precisely understood to support the sentinel node concept in gastric cancer surgery.

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