Establishment of pathological quantitative method for determining undifferentiated component ratio in patients with differentiated/undifferentiated mixed-type early gastric cancer and clinical significance of this ratio

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Abstract

Purposes The purpose of this study was to establish a pathological quantitative method for determining the undifferentiated components ratio (UCR) in patients with differentiated/undifferentiated mixed-type (Mixed-type) early gastric cancer (EGC) and to examine the clinical significance.

Methods The subjects were 410 patients who underwent surgical resection for EGC with the invasion limited to m or sm1. Analysis 1: In 12 randomly selected patients with Mixed-type cancer, we calculated the area ratio and the ratio of the length ratio using ImageJ and analyzed the correlation between them. Analysis 2: We generated ROC curves, and determined the cutoff UCR on the basis of the predictive risk factors for lymph node metastasis (LNM). Analysis 3: We analyzed the relationship between clinicopathological factors including UCR/length of undifferentiated component (LUC = maximum dimensions of tumor × UCR) and LNM.

Results Analysis 1: The length ratio can be used as a substitute parameter for the UCR ($r = 0.996$). Analysis 2: The cutoff UCR as a risk factor for LNM was 58% (sensitivity = 1, 1 – specificity = 0.404). Analysis 3: Lymphovascular invasion ($p < 0.0001$), UCR $\geq 58\%$ ($p = 0.023$), and LUC ≥ 25 mm ($p = 0.005$) were identified as significant risk factors for LNM. No LNM was observed in patients with invasion limited to m or sm1 and negativity for lymphovascular invasion and UCR $< 58\%$ (0/215).

Conclusions In the patients with Mixed-type EGC, the length ratio of undifferentiated components can be a substitute parameter for the UCR. LNM rarely occurs in patients without lymphovascular invasion and with an UCR $< 58\%$. The analysis of the UCR has great significance in determining whether additional surgical resection is required after endoscopic resection.

Keywords Early gastric cancer · Mixed-type · Pathological quantitative method

Introduction

Gastric cancer is roughly classified into differentiated-type cancer, which shows a clear tubular formation, and undifferentiated-type cancer, which does not show tubular formation. The Japanese Classification of Gastric Carcinoma (14th edition) [1] states, “where a malignant epithelial tumor consists of more than one histopathological subtype, the different histopathological components should be recorded in descending order of the surface area occupied”. Therefore, in the classification of histopathological types of gastric cancer, the differentiated/undifferentiated mixed type (differentiated-type components >

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undifferentiated components or vice versa) accounts for a large percentage in addition to the purely differentiated and undifferentiated types. However, the determination of the predominance of certain histopathological components is often dependent on the subjective impression and discretion of the pathologists, because quantitative and objective criteria have not yet been established.

On the other hand, treatment for early gastric cancer (EGC) greatly depends on whether the cancer is of the differentiated or undifferentiated type. For undifferentiated-type cancer, gastrectomy with lymph node dissection is generally selected rather than local treatment by endoscopic resection because undifferentiated-type cancer often metastasizes to lymph nodes. Also, “the presence of undifferentiated components in a mixed state” is suggested as one of the risk factors for lymph node metastasis (LNM). These factors are considered in determining whether additional surgical resection is required after the endoscopic resection of differentiated-type EGC [2–7]. The treatment strategy has been established on the basis of a very large amount of accumulated data on EGC, and the difference in histopathological type has been considered a significant factor for LNM. However, the target of assessment was only the predominant histopathological components [2–10], and the mixed state of different histopathological components was not assessed in most analyses. Namely, there has been no fundamental analysis to fully clarify the proportion of undifferentiated components, which can be regarded as a risk factor for LNM and an indication for surgical resection. Therefore, the establishment of objective criteria is desirable.

The purposes of this study using surgical specimens are (1) to develop a quantitative method of determining the undifferentiated component ratio (UCR), (2) to establish the objective criteria for determining the ratio of undifferentiated components to differentiated components (hereafter, UCR) in mixed-type EGC, and (3) to examine clinical significance of the ratio.

Subjects and methods

Subjects

A total of 1651 patients with gastric cancer underwent gastrectomy with lymph node dissection during the period from January 1990 to December 2013. Of these patients, 410 having EGC with an invasion depth of m or sm1 were recruited as the subjects of this study. In the case of multicentric cancer, the tumor with deeper invasion depth was analyzed. When the invasion depth was the same, the tumor with larger diameter was analyzed.

Methods

Classification of histopathological types

Differentiated-type cancer was further classified into well-differentiated tubular adenocarcinoma (tub1), moderately differentiated tubular adenocarcinoma (tub2), and papillary adenocarcinoma (pap). Undifferentiated-type cancer was further classified into poorly differentiated adenocarcinoma (por), signet-ring cell carcinoma (sig), and mucinous adenocarcinoma (muc) [11].

Surgical specimens were classified into purely differentiated type, purely undifferentiated type, and differentiated/undifferentiated (mixed-type) based on the histopathology report. The Mixed-type was defined as a tumor having different histological components with more than 1%.

Analysis 1: pilot analysis of area ratio and length ratio of undifferentiated components in randomly selected patients ($n = 12$)

Forty-seven patients had Mixed-type cancer with a tumor diameter of 40 mm or less. Of these patients, 12 in whom the separate evaluation of differentiated and undifferentiated components was definite were randomly selected. A whole tumor tissue specimen was stained with hematoxylin and eosin and cut into 4- μ m-thick sections at 5-mm intervals. The sections used in the following assessments were sections from 2 to 8 (median, 3 sections).

To obtain the ratio of the area of undifferentiated components to that of whole tumor tissue specimen (hereafter, area ratio), images of all thin sections containing lesions were obtained using an Olympus upright optical microscope (objective, $\times 4$), saved as TIFF files, and printed in color in A4 size. Differentiated and undifferentiated components were marked on the color-printed images. Then, the images were scanned and saved as TIFF or JPG files. These files were imported into the image analysis software ImageJ [12, 13] (NIH, USA), and the area ratio was calculated by measuring and summing the areas of differentiated and undifferentiated components separately (Fig. 1a). When differentiated (or undifferentiated) components overlapped with each other, the overlapped region was assumed as two separate differentiated-type (or undifferentiated-type) cancer regions. When differentiated components overlapped with undifferentiated components, both components were marked and measured separately. When differentiated (or undifferentiated) components were scattered among undifferentiated (or differentiated) components, first the entire area was marked and measured. Then, the area of undifferentiated (or differentiated)

Fig. 1 **a** Representative histological imaging of area mapping (case 7, Table 1). Areas encircled by solid line, undifferentiated components (poorly differentiated adenocarcinoma intermingled with signet-ring cell carcinoma); areas encircled by dotted line, differentiated component (moderately differentiated tubular adenocarcinoma). Areas were obtained using Image J imaging software and added. Then, the area ratio of undifferentiated components was calculated as 71%. **b** Representative histological imaging of area mapping (case 3, Table 1). Areas encircled by solid line, undifferentiated components (poorly differentiated adenocarcinoma); areas encircled by dotted line, dif-

ferentiated component (well differentiated tubular adenocarcinoma). Areas were obtained using Image J imaging software and added. Then, the area ratio of undifferentiated components was calculated as 92%. **c** Representative histological imaging of length mapping (case 7, Table 1). Solid lines, undifferentiated components (poorly differentiated adenocarcinoma intermingled with signet-ring cell carcinoma); dotted lines, differentiated component (moderately differentiated tubular adenocarcinoma). Lines were obtained using Image J imaging software and added. Then, the length ratio of undifferentiated components was calculated as 68%

components was calculated by subtracting the area of differentiated (or undifferentiated) components from the total area (Fig. 1b).

To obtain the ratio of the largest diameter of undifferentiated components to that of the whole tumor tissue specimen (hereafter, length ratio), the largest diameter of differentiated and undifferentiated components was marked on all thin sections containing lesions. The images of the lesions were obtained using an Olympus upright optical microscope (objective, $\times 4$). Similarly to the area ratio already described, the length ratio was obtained by measuring and summing the largest diameters of differentiated and undifferentiated components separately using ImageJ [12, 13] (NIH, USA) (Fig. 1c). When differentiated (or undifferentiated) components overlapped with each other, the overlapped region was assumed as part of either of the differentiated-type (or undifferentiated-type) cancer regions. When differentiated components overlapped with undifferentiated components, the differentiated- and undifferentiated-type cancer regions were marked separately and the largest diameter of each region was measured. When undifferentiated (or differentiated) components were scattered among differentiated (or undifferentiated) components, first the largest diameter of the entire region was marked and measured as the largest diameter of differentiated (or undifferentiated) components. Then, the scattered undifferentiated (or differentiated) components were marked and the largest diameters of those regions were measured and summed to obtain the largest diameter of undifferentiated (or differentiated) components.

The foregoing assessments were performed with guidance from a pathologist (Y.O.) and with the agreement of a surgeon (H.T.) and a pathologist (K.H.) (two authors of this article). The correlation between the area and length ratios was analyzed statistically. When there was a strong correlation between the two, the length ratio, which can be calculated more simply and easily, was used as the parameter representing the UCR in the subsequent analyses.

Analysis 2: determination of cutoff UCR as risk factor for LNM

The relationship between the UCR and their positivity for LNM was examined using receiver operating characteristic (ROC) curves in all patients ($n = 410$) and in the patients who did not have lymphovascular invasion, which is the strongest risk factor for LNM [8, 14–17] (lymphovascular-invasion-negative patients, $n = 369$). The cutoff UCR as a risk factor for LNM was determined using Youden's index (sensitivity + specificity – 1) [18]. The UCR was assessed by an author of this article (H.T.) with guidance from two other authors who are pathologists (K.H. and Y.O.).

Analysis 3: examination of relationship between clinicopathological factors including UCR and presence of LNM

Univariate and multivariate analyses of the relationship between clinicopathological factors and their positivity for LNM were performed to extract independent risk factors for LNM. The clinicopathological factors used in the analyses included patient age and gender, the location of tumor [upper (U), middle (M), or lower (L) third of stomach], macroscopic type (protruded, depressed, or mixed), invasion depth (m or sm1), the presence or absence of ulcer findings, the presence or absence of lymphovascular invasion, maximum dimensions of tumor (≤ 20 or > 20 mm), histopathological type determined by the conventional method (purely differentiated adenocarcinoma, purely undifferentiated adenocarcinoma, or Mixed), UCR (the cutoff value obtained in Analysis 2), and the length of undifferentiated component ($LUC = \text{maximum dimensions of tumor} \times \text{UCR}$).

The deformity of the muscularis propria or the fibrosis in the submucosal layer was considered a positive ulcer finding. The presence of both or either of lymphatic and vein invasions indicated a positive lymphovascular invasion. The clinicopathological factors were assessed according to the Japanese Classification of Gastric Carcinoma (14th edition) [1] and Japanese Gastric Cancer Treatment Guidelines version 3 [11], both established by the Japanese Gastric Cancer Association.

Statistical analyses

Statistical analyses were performed using statistical software SPSS for Mac, version 22.0 (IBM). A correlation coefficient ≥ 0.7 indicated a significantly strong correlation between two groups. Values were expressed as mean $\pm$ SD. The Mann–Whitney U test or the chi-square test was used for comparing two groups. Multivariate analysis was performed by binary logistic regression. $p < 0.05$ was considered to indicate a significant difference in each statistical analysis.

Results

Classification based on histopathological type and LNM positivity rate

The histopathological types of gastric cancer in all 410 patients determined by the conventional method are shown in Table 1. Of these patients, 207 (50.5%) were classified as having purely differentiated-type cancer, 118 (28.8%) were classified as having purely undifferentiated-type cancer, and 85 (20.7%) were classified as having Mixed-type cancer. LNM was found in 16 patients (3.9%). The LNM

Table 1 Histopathological type and incidence of lymph node metastasis in 410 patients with early gastric cancer

Histopathological type	Number of patients (%)	Number of LNM-positive patients (%)	<i>p</i> value
Purely differentiated	207 (50.5)	5 (2.4)	0.124
Purely undifferentiated	118 (28.8)	6 (5.1)	
Mixed	85 (20.7)	5 (5.9)	
Total	410	16 (3.9)	

Differentiated: well- and/or moderately differentiated tubular adenocarcinoma and/or papillary adenocarcinoma

Undifferentiated: signet-ring cell carcinoma and/or poorly differentiated adenocarcinoma and/or mucinous adenocarcinoma

Mixed: intermingled histopathologies of differentiated and undifferentiated types

LNM lymph node metastasis

positivity rate was determined to be 5.9% in the patients with mixed-type cancer (5 patients), 5.1% in those with purely undifferentiated-type cancer (6 patients), and 2.4% in those with purely differentiated-type cancer (5 patients). The highest LNM positivity rate was obtained in the patients with Mixed-type cancer, but the difference did not reach statistical significance among the three groups ($p = 0.124$).

Analysis 1 of area ratio and length ratio of undifferentiated components in Mixed-type cancer

The area and length ratios were evaluated using the average of 11 tissue sections (2–56 sections) for each patient.

In the 12 randomly selected patients with Mixed-type cancer, the mean area and length ratios were 60 ± 32 (8–95%) and $58\% \pm 30\%$ (7–91%), respectively. There was a statistically strong correlation between the area and length ratios (Table 2; $r = 0.996$), indicating that the area ratio of undifferentiated components in Mixed-type cancer can be substituted by the length ratio, which can be more simply calculated.

Analysis 2: determination of cutoff UCR as risk factor for LNM

When the length ratio of undifferentiated components was used in place of the UCR, the UCR were $39\% \pm 45\%$ in all patients ($n = 410$) and $42\% \pm 46\%$ in the lymphovascular invasion-negative patients ($n = 369$).

When all patients were classified according to their positivity for LNM, the mean $\pm$ SD UCR were $54\% \pm 47\%$ in the LNM-positive patients ($n = 16$) and $39\% \pm 45\%$ in the LNM-negative patients ($n = 394$); these were not significantly different ($p = 0.165$) (Fig. 2a). When lymphovascular invasion-negative patients were classified according to their positivity for LNM, the mean $\pm$ SD UCR was significantly higher ($92\% \pm 13\%$) in the LNM-positive patients ($n = 8$) than in the LNM-negative patients ($n = 361$, $40\% \pm 46\%$; $p = 0.004$) (Fig. 2b).

Next, the relationship between the UCR and LNM positivity was analyzed using ROC curves. An area under the curve (AUC) for all cases was 0.594, and it was not statistically significant (95% CI, 0.459–0.730; $p = 0.201$) (Fig. 3a). For lymphovascular invasion-negative cases,

Table 2 Area and length ratios of undifferentiated components in 12 randomly selected patients with early gastric cancer

Patient no.	Area ratio of undifferentiated components, (%)	Length ratio of undifferentiated components, (%)	Histopathological type	Tumor size, mm	Number of sections estimated
1	59	64	sig + tub2	15	2
2	86	81	sig + tub2	7	3
3	92	91	por + tub1	12	3
4	95	90	sig + tub2	8	2
5	72	70	por + tub2	7	2
6	82	75	sig + tub2 + por	20	5
7	71	68	por + sig + tub2	6	3
8	83	79	por + sig + tub2	22	5
9	18	16	tub2 + sig	40	8
10	8	7	tub2 + por	27	8
11	15	17	tub2 + por + tub1	8	2
12	36	34	tub2 + sig + por	15	4

Correlation coefficient ($|r|$) was 0.996

tub1 well-differentiated tubular adenocarcinoma, *tub2* moderately differentiated tubular adenocarcinoma, *por* poorly differentiated adenocarcinoma, *sig* signet-ring cell carcinoma

Fig. 2 a Distributions of undifferentiated component ratio (length ratio of undifferentiated components) according to status of lymph node metastasis (LNM) in all patients. Mean $\pm$ SD undifferentiated component ratios in LNM-positive and LNM-negative patients were $54\% \pm 47\%$ and $39\% \pm 45\%$, respectively. No statistically significant difference was seen between the two groups (Mann–Whitney U test, $p = 0.165$). **b** Distributions of undifferentiated component ratio (length ratio of undifferentiated components) according to status of lymph node metastasis (LNM) in patients without lymphovascular invasion. Mean $\pm$ SD undifferentiated component ratio in LNM-positive patients ($92\% \pm 13\%$) was much higher than that ($40\% \pm 46\%$) in LNM-negative patients (Mann–Whitney U test, $p = 0.004$)

the ROC curve showed a statistically significant AUC of 0.775 [95% CI, 0.684–0.865; $p = 0.008$] (Fig. 3b)], and the cutoff ratio was defined as 58% (sensitivity = 1, $1 - \text{specificity} = 0.404$). The predictive LNM negativity

rate was 100%, the predictive LNM positivity rate was 5.3%, and the LNM positivity rate (prevalence) was 2.2% (Fig. 3b).

Fig. 3 **a** Relationship between lymph node metastasis and undifferentiated component ratio in all patients (ROC curve). AUC = 0.594, 95% CI = 0.459–0.730, $p = 0.201$. AUC, area under curve; CI, confidence interval; ROC, receiver operating characteristic analysis curve. **b** Relationship between lymph node metastasis and undifferentiated-type ratio in patients without lymphovascular invasion (ROC curve). AUC = 0.775, 95% CI = 0.684–0.865, $p = 0.008$

Table 3 Risk factors for lymph node metastasis identified by univariate and multivariate analysis

Variable	LNM positivity rate (%)	Univariate	Multivariate		
		<i>p</i> value	Odds ratio	95% CI	<i>p</i> value
Age					
<65 years	10/184 (5.4)	0.148			
≥65 years	6/226 (2.7)				
Gender					
Male	7/257 (2.7)	0.110			
Female	9/153 (5.9)				
Location					
Upper third	1/42 (2.4)	0.689			
Middle third	10/215 (4.7)				
Lower third	5/153 (3.3)				
Macroscopic type					
Protruded	1/50 (2.0)	0.759			
Depressed	13/313 (4.2)				
Mixed	2/47 (4.3)				
Depth of invasion					
m	8/293 (2.7)	0.052			
sm1	8/117 (6.8)				
Ulcer findings					
Absent	8/261 (3.1)	0.247			
Present	8/149 (5.4)				
Lymphovascular invasion					
Absent	8/369 (2.2)	< 0.0001	16.7	5.2–53.5	< 0.0001
Present	8/41 (19.5)				
Tumor size					
≤20 mm	2/142 (1.4)	0.058			
>20 mm	14/268 (5.2)				
Histopathological type determined by conventional method					
Purely differentiated	5/207 (2.4)	0.124			
Purely undifferentiated	6/118 (5.1)				
Mixed	5/85 (5.9)				
Undifferentiated component ratio					
<58%	7/248 (2.8)	0.162	3.8	1.2–12.2	0.023
≥58%	9/162 (5.6)				
Length of undifferentiated component (maximum dimensions of tumor × undifferentiated component ratio)					
<25 mm	8/311 (2.6)	0.020	4.9	1.6–14.9	0.005
≥25 mm	8/99 (8.1)				

m cancer cell penetration limited to the mucosa, *sm1* cancer cell penetration into the submucosal layer <500 µm from the muscularis mucosae, *CI* confidence interval

Only *p* < 0.25 variables identified by univariate analysis were included in the multivariate analysis

Analysis 3 of relationship between clinicopathological factors including UCR and presence of LNM

Univariate and multivariate analyses of the relationship between clinicopathological factors and presence for LNM were performed to extract the risk factors for LNM (Table 3).

In univariate analysis, only lymphovascular invasion was identified as a significant risk factor for LNM (Table 3). In multivariate analysis, age, gender, invasion depth, presence or absence of ulcer findings, presence or absence of lymphovascular invasion, maximum dimensions of tumor, histopathological type determined by the conventional method, UCR calculated in this study (<58% or ≥58%), and LUC calculated in this study (<25 or ≥25 mm), which were *p* ≤ 0.25

Table 4 Lymph node metastasis (LNM) positivity rate in cases without lymphovascular invasion by two risk factors

Variables	LNM positivity rate		<i>p</i> value
	Undifferentiated component ratio < 58%	Undifferentiated component ratio ≥ 58%	
m	0/156	6/129 (4.7)	0.006
sm1	0/59	2/25 (8.0)	0.028

m cancer cell penetration limited to the mucosa, *sm1* cancer cell penetration into the submucosal layer <500 µm from the muscularis mucosa

in univariate analysis, were used as potential risk factors for LNM. As a result, the presence of lymphovascular invasion (odds ratio = 16.7, 95% CI = 5.2–53.5; $p < 0.0001$), the UCR ≥ 58% (odds ratio = 3.8, 95% CI 1.2–12.2; $p = 0.023$), and the LUC ≥ 25 mm (odds ratio = 4.9, 95% CI 1.6–14.9; $p = 0.005$) were identified as independent risk factors for LNM (Table 3).

Then, cases without lymphovascular invasion were further classified into four groups according to an invasion depth of m or sm1, and UCR < 58% or ≥ 58% (Table 4). Cases of m or sm1 with lower component ratio had LNM rate of 0%. Cases of sm1 and with higher component ratio had LNM rate of 8.0%.

Discussion

According to studies on a large number of surgical cases of EGC by Gotoda et al. [8] and Hirasawa et al. [19], the following four subgroups are regarded as having no or a very little risk of LNM: (1) differentiated intramucosal cancer of any diameter without ulceration, (2) differentiated intramucosal cancer of less than 3 cm diameter with ulceration, (3) differentiated minute submucosal invasive cancer of less than 3 cm diameter without ulceration, and (4) undifferentiated intramucosal cancer less than 2 cm diameter without ulceration. Endoscopic resection is actively performed on patients who meet one of these criteria. However, even in patients diagnosed with differentiated-type gastric cancer before endoscopic resection, undifferentiated components are sometimes found in the resected specimens [2], particularly in tumors larger than 10 mm diameter or submucosal cancers [9]. Therefore, the presence of undifferentiated components is often found as tumors progress beyond the foregoing four conditions after the histopathological diagnosis of a specimen obtained via endoscopic resection. Fujisaki et al. [10] reported that Mixed-type cancer containing 10% or greater undifferentiated components was found in 6.6% of patients who had been diagnosed as having differentiated-type cancer on the basis of biopsy before endoscopic submucosal dissection (ESD).

The presence of undifferentiated components in differentiated-type cancer is recognized as an extremely important factor when determining whether an additional surgical resection, namely, gastrectomy with lymph node dissection, is required. Tanabe et al. [3] reported that the LNM positivity rate for m or sm1 with differentiated-type-predominant mixed-type cancer (8.2%) was higher than in those with purely differentiated-type cancer (0.5%) and was as high as that in those with purely undifferentiated-type cancer (5.5%). Similar results were obtained in this study. The LNM positivity rate (5.9% vs. 2.4%) was higher in patients with Mixed-type cancer than those with purely differentiated-type cancer, but these differences were not statistically significant. The LNM positivity rate (5.9% vs. 5.1%) was higher in patients with Mixed-type cancer than those with purely undifferentiated-type cancer, but these differences were not statistically significant. However, despite these reports, no appropriate treatment for Mixed-type cancer has yet been established.

Although the issues of Mixed-type cancer have been studied, the relationship between UCR and positivity for LNM has attracted much less attention. Most past research on Mixed-type EGC has focused on the predominant histopathological components [2–10]. However, it is possible that LNM may be largely affected by the proportion of undifferentiated components, which is why we conducted this study.

In this study, we clearly demonstrated that in patients with Mixed-type EGC, the length ratio of undifferentiated components could be a substitute parameter for the UCR. In addition, in a multicenter prospective study on the expanded indication of ESD in undifferentiated-type early gastric cancer conducted by the Japan Clinical Oncology Group (JCOG1009/1010), the diagnosis of Mixed-type cancer was based on the largest diameter of undifferentiated components. [20] In a study by Tanabe et al. [3], the length ratio of undifferentiated components was regarded as the UCR. From these results, it seemed appropriate to use the length ratio obtained in this study as a quantitative measurement of UCR.

The present ROC analysis yielded a 58% cutoff value for predicting LNM and the multivariate analysis of UCR (< 58% or ≥ 58%) and LUC (< 25 or ≥ 25 mm) as independent risk factors for LNM. In contrast, the conventional description for the predominant histological type (< 50% or ≥ 50%) was insufficient to predict LNM in this study (data not shown). The negative predictive value for LNM was 100%; therefore, a Mixed-type EGC with UCR < 58% can be treated as a differentiated-type cancer.

An et al. [21] and Abe et al. [14] reported that tumor size (≥ 20 mm) was an independent risk factor for LN metastasis in EGC. In this study, when all patients were classified according to their positivity for LNM, the mean ± SD tumor size was larger (45 ± 28 mm; range, 15–130 mm) in the

LNM-positive patients ($n = 16$) than in the LNM-negative patients ($n = 394$, 32 ± 22 mm; range, 2–160 mm).

Moreover, it was reported that the rate of lymphovascular invasion was significantly higher, the rate of LNM tended to be higher, and the tumor size was greater in patients with differentiated-type-predominant mixed-type cancer than in those with purely differentiated-type cancer [6, 7], indicating that mixed-type cancer was more malignant than purely differentiated-type cancer. Similarly, it was reported that the lymphovascular invasion rate was significantly higher and the LNM rate tended to be higher in patients with undifferentiated-type-predominant mixed-type cancer than in those with purely undifferentiated-type cancer [7], indicating that mixed-type cancer is more malignant than purely undifferentiated-type cancer.

In this study, LNM was found in none of the 215 cases of early gastric cancer with an invasion depth of m or sm1 without lymphovascular invasion and <58% UCR, regardless of tumor diameter and the presence or absence of ulcer. Abe et al. [15] reported that LNM was not found in patients with sm1 cancer without lymphovascular invasion.

However, when the UCR was $\geq 58\%$, the LNM rates in cancer with invasion depths of m and sm1 were 4.7% and 8.0%, respectively. Furthermore, when the undifferentiated component of the longest lesion diameter was longer than 2 cm, there was a risk of LNM [7]. When the undifferentiated component of the longest lesion diameter was >2 cm with mixed-type cancer, it should be treated with noncurative resection even if the UCR is less than 58%. However, we did not conduct any examination of the undifferentiated component of the greatest lesion diameter.

The LNM rate in patients with UCR $\geq 58\%$ was significantly higher than in those with a UCR <58%. Therefore, the UCR ratio is of high clinical significance as an objective parameter for determining the indication for additional surgical resection after endoscopic resection in cases of m or sm1 gastric cancer.

Conclusions

In cases of Mixed-type EGC, the length ratio of the undifferentiated components can be regarded as a substitute parameter for the area ratio of undifferentiated components. In cases of EGC with an invasion depth of m or sm1, the UCR $\geq 58\%$ is an independent risk factor for LNM. LNM rarely occurs in cases of m or sm1 cancer with no lymphovascular invasion and a component ratio of <58%. Evaluation of the UCR has a high clinical impact on determining the indication for additional surgery after endoscopic resection.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human rights statement All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions.

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