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ORIGINAL ARTICLE

Influence of lymph node micrometastasis on the staging system for gastric cancer*

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Abstract

Objective The aim of this study was to investigate the effect of lymph node micrometastasis on the prognosis of patients with gastric cancer and the necessity of integrating it into the gastric cancer staging system.

Methods In total, 241 patients with gastric cancer were included. Hematoxylin and eosin staining of lymph nodes was performed, and negative lymph nodes were evaluated by immunohistochemistry to detect micrometastases. Differences in survival rates between stages were evaluated.

Results (1) A total of 78 patients (32.4%) had lymph node micrometastases. Compared with the group without micrometastases, the overall recurrence rate, lymph infiltration, vascular invasion, and nerve invasion rate in the micrometastasis group were significantly higher ($P < 0.05$). (2) According to the standard N staging system, the rates of disease-free survival (DFS) for the N0, N1, N2, N3a, and N3b groups were 96.0%, 84.0%, 67.6%, 59.0%, and 21.7%, respectively. There was no significant difference in survival between N2 and N3a. The cumulative survival curves for N2 and N3a intersected. (3) The N stage of 38 patients (15.8%) differed between the traditional system and the new N staging system reflecting micrometastasis. The DFS for N0, N1, N2, N3a, and N3b were 97.0%, 86.3%, 74.2%, 65.4%, and 29.2%, respectively. There was no significant difference in survival between N2 and N3a, but the cumulative survival curves for N2 and N3a did not intersect. (4) Based on a Cox multivariate analysis, various independent risk factors for recurrence were identified ($P < 0.05$).

Conclusion Lymph node micrometastasis is an important risk factor for gastric cancer recurrence. Lymph node micrometastasis should be considered in TNM staging to determine prognosis and optimal treatment strategies.

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Key words: gastric cancer; lymph node micrometastasis; TNM stage

In gastric cancer, lymph node metastasis is recognized as the most important determinant of prognosis [1]. As a special form of lymph node metastasis, the clinical value of micrometastasis is still controversial. Its significance was described in the seventh edition of the TNM classification [2–3]. Many previous studies have supported the prognostic value of lymph node micrometastasis in gastric cancer; however, it is not clear whether it should be considered in the lymph node staging system for gastric cancer [4–5]. In this study, we performed a prospective analysis of 241 patients with gastric cancer, including immunohistochemical (IHC) staining of lymph nodes, analyses of clinical pathological data, and a comparison between the new lymph node staging system incorporating micrometastasis with the traditional lymph node staging system. Our findings provide a basis for determining the significance of lymph node micrometastasis in gastric cancer staging.

Materials and methods

Patient origin and immunohistochemical staining

From February 2010 to December 2016, 241 patients who underwent radical gastrectomy in the Department

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of Gastrointestinal Surgery, Qionglai Medical Center Hospital were selected as the study subjects. Surgical specimens were maintained in the Department of Pathology of our hospital. The patients were divided into two groups according to the detection of lymph node micrometastasis. Clinical and pathological results were obtained, including age, sex, tumor size, WHO classification, Lauren classification, average number of lymph nodes dissected, average number of metastatic lymph nodes, lymph node infiltration, vascular infiltration, peripheral infiltration, and TNM stage (7th AJCC). Clinical pathological results and recurrence rates were compared between the two groups.

Definition of lymph node micrometastasis

Lymph node micrometastasis includes two forms: isolated tumor cells (ITC) and micrometastasis. ITC refers to a single tumor cell with a diameter of less than 0.2 mm [6]. At present, there is no evidence that ITC contributes to tumor metastasis. Therefore, micrometastasis in this study refers to a tumor cell cluster with a size of 0.2–2.0 mm, excluding ITCs.

Immunohistochemical staining

Specimens were stained with hematoxylin & eosin (HE) before anti-CAM5.2 IHC staining. The CAM5.2 antibody can recognize low-molecular-weight cytokeratin expressed in tumor cells and can detect micrometastasis in surgical specimens [7]. To improve the micrometastasis detection rate, two or more lymph node sections were used for IHC staining. Brownish-yellow staining indicated micrometastasis in lymph nodes. The lymph nodes with positive HE staining were defined as macrolymph node metastasis or micrometastasis.

Follow-up

The 3-year disease-free survival (DFS) rates were evaluated according to the N stage determined by AJCC stage 7 and the new staging standard. In the new staging system, lymph node micrometastasis was defined as positive lymph nodes, and the number of metastatic lymph nodes was calculated by the sum of macrometastasis and micrometastasis lymph nodes. DFS was defined as the time from randomization to relapse or death for any reason.

Statistical analysis

All data were analyzed using SPSS 20.0. Counts are presented as the number of cases and were evaluated by the chi-squared test. Measurement data are presented as means ± standard deviation and were evaluated by the *t*-test. The Kaplan–Meier method and log rank test were used for the survival analysis. Factors with statistically significant differences in a single-factor survival analysis were included in a multivariate Cox regression analysis. $P < 0.05$ was considered statistically significant.

Results

Comparison of demographic characteristics and clinicopathological parameters

The average age of 241 patients was 59.3 ± 13.4 years (25–87 years). There were 163 males (67.6%) and 78 females (32.4%). The mean follow-up time was (76.8 ± 2.3) months (2.3–106.8 months), and the 3-year DFS rate was 78.9%. A total of 78 patients (32.4%) had lymph node micrometastasis and 163 (67.6%) had no lymph node micrometastasis. There were significant differences in tumor size, WHO classification, Lauren classification, average number of lymph nodes, average number of metastatic lymph nodes, T stage, and N stage between the two groups ($P < 0.05$). There were no significant differences in age and gender between the two groups (Table 1).

Comparison of recurrence and metastasis between groups

Compared with the group without micrometastasis, the overall recurrence rate was significantly higher in the micrometastasis group ($P < 0.05$). The most common types of recurrence were peritoneal, hematogenous, and local lymph nodes in the micrometastasis group and hematogenous, peritoneal, and local lymph nodes in the non-micrometastasis group. The incidences of lymphatic invasion, vascular invasion, and nerve invasion in the micrometastasis group were significantly higher than those in the non-micrometastasis group ($P < 0.05$; Table 2).

Immunohistochemical staining of lymph node metastasis

Micrometastasis was detected by CAM5.2 immunohistochemistry. The macrometastasis and micrometastases were brownish-yellow on IHC staining. There were significant brownish-yellow masses in the macroscopic metastases and scattered and single cell clusters in the micrometastases (Fig. 1).

Survival curve for the traditional N staging system

According to the traditional N staging standard of AJCC 7th edition, the DFS of patients with N0, N1, N2, N3a, and N3b disease were 96.0%, 84.0%, 67.6%, 59.0%, and 21.7%, respectively. A log rank test showed that the differences between N0 and N1, N1 and N2, and N3a and N3b were statistically significant. However, there was no significant difference between N2 and N3a. In the conventional staging system, the cumulative survival

Table 1 demographic characteristics and clinicopathological results

Index	Microtransmission group (n = 78)	No-Microtransmission group (n = 163)	t/χ^2	P
Age	59.6 ± 12.4	59.8 ± 14.2	-0.106	0.915
male	49	114	1.221	0.269
Tumor diameter (cm)	6.5 ± 3.3	4.3±3.1	5.047	0.000
WHO classification			20.148	0.001
well-differentiated	15	57		
moderately differentiated	39	46		
poorly differentiated	10	21		
mamillary	3	26		
Myxoid carcinoma	4	6		
signet ring cell cancer	7	7		
Lauren classification			10.687	0.001
Intestinal	26	91		
diffuse	52	72		
Operation type			15.611	0.000
Subtotal gastrectomy	45	133		
Total gastrectomy	33	30		
Dissected lymph nodes	44.6 ± 17.2	36.8 ± 17.5	3.255	0.001
Metastatic lymph nodes	11.3 ± 13.6	3.5 ± 11.4	4.661	< 0.001
T stage			72.857	< 0.001
T1	10	109		
T2	7	18		
T3	4	2		
T4a	53	31		
T4b	4	3		
N stage			53.646	< 0.001
N0	10	101		
N1	14	19		
N2	18	14		
N3a	16	15		
N3b	20	14		

Table 2 Comparison of recurrence and metastasis rate between the two groups

Index	Microtransmission group (n = 78)	No-Microtransmission group (n = 163)	χ^2	P
Lymphatic invasion	40	39	17.916	< 0.001
Vascular invasion	32	25	19.279	0.001
Perineural invasion	5	10	0.007	0.934
Recurrence (%)	32 (41.0)	21 (12.8)	24.355	< 0.001
Peritoneal	13	8		
Hematogenous	11	9		
Local lymph node	8	4		

curves for N2 and N3a intersected (Fig. 2).

Survival curve for the N staging system reflecting micrometastasis

The N stages for 38 patients (15.8%) changed with the new N staging system. In addition, 8 cases (3.3%) experienced two or more n-phase increases. In this system, the DFS of patients with N0, N1, N2, N3a, and N3b were 97.0%, 86.3%, 74.2%, 65.4%, and 29.2%, respectively. The differences in survival between N0 and N1, N1 and N2, and N3a and N3b were statistically significant. There was no significant difference in survival between N2 and N3a stages; however, in the new staging system, the cumulative survival curves for N2 and N3a did not cross (Fig.3).

Multivariate analysis of prognostic factors for DFS

Based on univariate analyses, a Cox multivariate analysis showed that the combination of nerve infiltration, pathological T stage, number of lymph nodes dissected, and macrometastasis and micrometastasis of lymph nodes

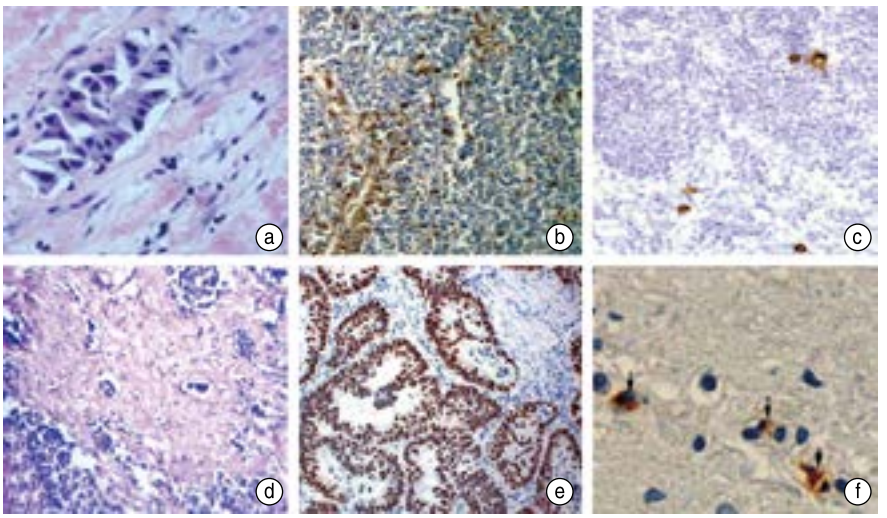

Fig. 1 Immunohistochemical staining of metastases. (a, d) negative staining (a: ×200, d: ×400); (b, e) positive macroscopic metastasis of lymph nodes (b: ×200, e: ×400); (c, f) positive micrometastasis of lymph nodes (c: ×200, f: ×400)

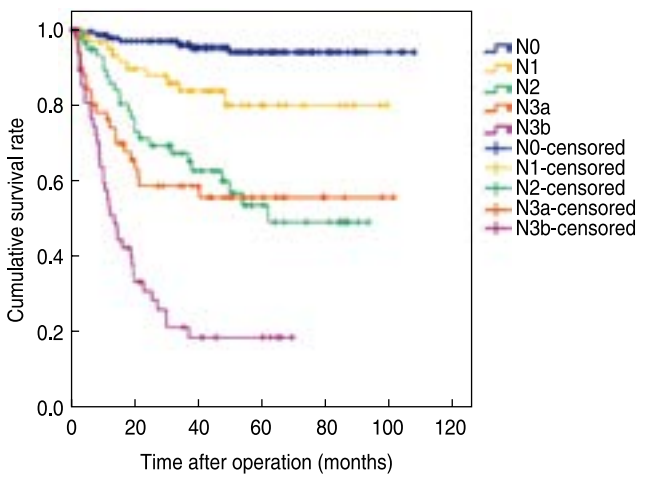

Fig. 2 survival curve of traditional N stage

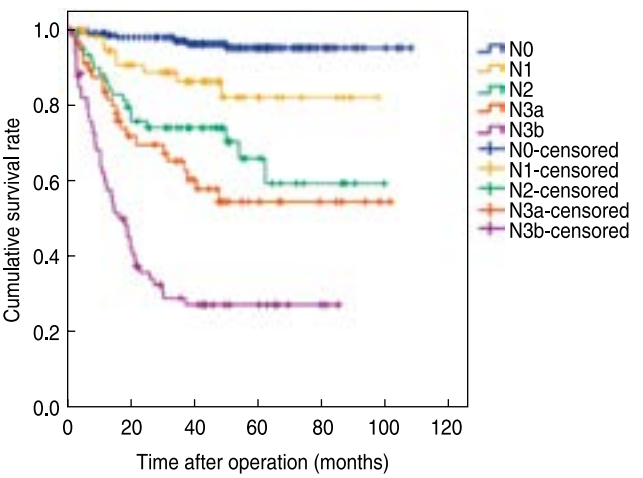

Fig. 3 survival curve of N stage reflecting micrometastasis

were independent risk factors for the recurrence of gastric cancer ($P < 0.05$; Table 3).

Discussion

Many studies have explored the clinical effect of lymph node micrometastasis on pN0 gastric cancer by routine pathological examination, focusing on its role in the minimally invasive treatment of early gastric cancer, such as sentinel lymph node navigation surgery and endoscopic submucosal cleaning surgery [8]. In this prospective study, we focused on the effect of micrometastasis on N-staging. In particular, we evaluated the significance of lymph node micrometastasis in gastric cancer staging. ITCs were also detected by immunohistochemistry but were excluded from analyses owing to the lack of clinical evidence that they affect prognosis [9].

The recurrence rate of micrometastases is related to demographic and clinicopathological factors [10–11]. We hypothesized that lymph node micrometastasis has the same clinical value as lymph node metastasis and constructed a new staging system. By comparing the performance of the traditional N-staging system with that of the new N-staging system, the advantages and disadvantages of each were evaluated. With respect to the performance of staging systems, Ueno proposed three criteria [12–13]: (1) intragroup homogeneity, (2) heterogeneity between group, and (3) monotonicity of the correlation gradient between groups. Compared with the traditional staging system, the new system was more discriminative for the prognosis of each N-phase, and DFS showed more significant differences between

Table 3 multivariate analysis of prognostic factors of DFS

Index	univariate analysis			multivariable analysis		
	HR	95%CI	*P*	HR	95%CI	*P*
Age	1.654	1.354–5.124	0.458	—	—	—
Male	0.968	0.074–1.587	0.500	—	—	—
Tumor diameter	2.865	1.652–4.998	< 0.001	1.018	0.946–1.096	0.652
Operation type	2.144	1.021–3.897	< 0.001	1.033	0.619–1.746	0.912
WHO classification	1.104	0.631–1.934	0.730	—	—	—
Lauren classification	0.658	0.357–1.225	0.189	—	—	—
Lymphatic invasion	3.153	1.398–6.874	< 0.001	1.354	0.438–1.249	0.266
Vascular invasion	3.356	1.287–6.538	< 0.001	1.117	0.679–1.830	0.293
Perineural invasion	4.159	1.874–7.698	< 0.001	2.069	1.190–3.551	0.011
T stage						
T1	1	—	< 0.001	1	—	< 0.001
T2	2.884	1.987–4.521	0.158	3.191	0.697–14.617	0.136
T3	17.654	9.687–36.3254	< 0.001	12.800	3.079–53.654	< 0.001
T4a	35.954	18.554–54.335	< 0.001	30.668	9.879–95.654	< 0.001
T4b	107.632	68.147–198.35	< 0.001	72.697	19.547–276.325	< 0.001
Dissected lymph nodes	1.015	0.236–2.987	0.003	0.971	0.955–0.986	< 0.001
Metastatic lymph nodes	1.035	0.257–2.148	< 0.001	1.049	0.013–1.089	< 0.001
Microtransmission	1.098	0.336–1.987	< 0.001	1.068	1.024–1.179	0.002

different N-phases.

Generally speaking, prognosis is better for stage N3a than for N2. In the conventional N staging system evaluated in this study, the DFS curves for N2 and N3a intersected, suggesting that the survival rate for patients classified as N3a continues to exceed that of patients classified as N2 over time, and the difference grows. This phenomenon may be explained by the inability to detect micro-transfer, which the traditional staging system does not reflect. To account for this difference, we designed a new hypothetical staging system, including the total number of macrometastatic and micrometastatic lymph nodes. In the new N staging system, the survival curves for N2 and N3a no longer crossed. Stages N2 and N3a had stronger discrimination ability with respect to prognosis, and their correlation showed a more monotonous trend.

The inclusion of lymph node micrometastasis in the N staging system would influence treatment strategies; the reclassification of stages will lead to changes in adjuvant treatment, especially radiotherapy and chemotherapy [14–15]. In this study, because the number of metastatic lymph nodes increased with the number of micrometastasis lymph nodes, the N stage for 38 patients (15.8%) was higher in the new system than in the traditional system, and the corresponding TNM stage was adjusted to a later stage. Considering previous reports on micrometastasis proliferation, this phenomenon should not simply be considered an "overestimation" of stages by the new staging system. On the contrary, it can be regarded as an "underestimation" by the traditional staging system. Therefore, in these cases, more aggressive treatment may be needed. In particular, when considering minimally invasive surgery, the operator should cautiously consider the influence of lymph node micrometastasis. Jee *et al* [9] have reported that if endoscopic mucosal resection or ESD is performed according to traditional staging criteria, metastatic lymph nodes may be missed. If micrometastasis is not considered in staging, patients may be at risk of lymph node metastasis after endoscopic mucosal resection or ESD.

To sum up, the results of this study showed that lymph node micrometastasis is an important risk factor for gastric cancer recurrence. Lymph node micrometastasis should be considered in TNM staging to determine prognosis and the best treatment strategy. However, this study had some limitations. (1) The sample size was small. (2) It was a single-center study, and there may be sampling bias. (3) The detection method for micrometastasis needs to be further improved; in the future, RT-PCR with higher sensitivity and specificity can be used.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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