

Occurrence of No.12 lymph node micrometastasis in gastric cancer and its effect on clinicopathological parameters and prognosis*

Tianzeng Dong¹ (✉), Lirong Zhang²

¹ Emergency Department, Handan People's Hospital, Handan 056001, China

² Department of Neurology, General Hospital of Jizhong Energy Fengfeng Group Co. LTD., Handan 056200, China

Abstract

Objective This study aimed to investigate the occurrence of No.12 lymph node micrometastasis in patients with gastric cancer and its relationship with clinicopathological parameters and prognosis.

Methods A cohort of 160 gastric cancer patients who underwent gastrectomy and lymph node dissection were selected as the research subjects. The immunohistochemical method was used to detect the micrometastasis of No.12 lymph node sections with negative routine pathological detection. At the same time, the clinical data of patients were collected and followed up to analyze the clinical significance of No.12 lymph node micrometastasis.

Results A total of 370 No.12 lymph nodes were detected in 160 surgical specimens. Among 160 patients, 27 patients were found to be positive for No.12 lymph nodes during routine pathological examination, with a positive rate of 16.8%. A total of 308 lymph nodes from 133 patients with negative routine pathological examinations were stained by immunohistochemistry. A total of 17 lymph nodes from 10 patients were found to be positive. The results showed that 37 of the 160 patients had No.12 lymph node metastasis, and the positive rate was 23.1%, which was 6.3% higher than that of routine pathological examination. Logistic multivariate analyses showed that the depth of invasion, lymph node metastasis in other groups, and clinical stage were independent risk factors for No.12 lymph node metastasis. The average follow-up time was 79.3 months, and the overall median survival time was 47.9 months. The survival time of the No.12 lymph node-negative group was 67.3 ± 2.5 months, the median survival time was 73.2 months; the survival time of the No.12 lymph node-positive group was (28.4 ± 5.4) months, and the median survival time was 31.3 months. The survival time of the No.12 lymph node-negative group was significantly longer than that of the positive group ($\chi^2 = 12.75, P = 0.000$).

Conclusion No.12 lymph node micrometastasis is a signal affecting the prognosis of patients with gastric cancer. Standardized dissection of No.12 lymph nodes is recommended for patients with gastric cancer who can undergo radical resection.

Key words: gastric tumor; lymphatic metastasis; micrometastasis

Received: 28 September 2021

Revised: 11 November 2021

Accepted: 21 January 2022

Gastric cancer is one of the most common gastrointestinal malignancies. Compared with Japan, South Korea, and other countries, the detection rate of early gastric cancer in China is low. Many patients are first diagnosed with gastric cancer when they have already progressed to the advanced stage and already lost the best treatment opportunity^[1]. At present, the scientific nature of radical surgery based on D2 lymph

node dissection is widely recognized, but there are still some debates on the scope and strategy of some local dissection^[2]. The hepatoduodenal ligament is an important anatomical marker in radical gastrectomy for gastric cancer. It has important structures, such as the biliary tract, portal vein, and proper artery. It is prone to complications such as lymphatic leakage and bile leakage after surgery. Therefore, cleaning the tissue

✉ Correspondence to: Tianzeng Dong. Email: dongtianzeng23@163.com

* Supported by a grant from the Hebei Medical Science Research Project (No. 20191831).

© 2022 Huazhong University of Science and Technology

in this area has high technical requirements for the operator^[3]. Regional lymph node metastasis is the main factor affecting the prognosis of patients with gastric cancer. Accurate determination of lymph node status is of great significance for the rational selection of surgical methods and accurate judgment of patient prognosis^[4]. Lymph node micrometastasis (LNM) is a special form of metastasis and its clinical significance in patients with gastric cancer remains controversial^[5]. In this study, 160 patients with gastric cancer who underwent D2 or above radical surgery were studied to explore the occurrence law of No.12 lymph node micrometastasis and its impact on clinicopathological parameters and prognosis.

Materials and methods

Research object

From January 2012 to January 2016, we collected the clinical data of gastric cancer patients in the General Hospital of Jizhong Energy Fengfeng Group Co., Ltd. The inclusion criteria were: (1) Radical lymphadenectomy with D2 or above; (2) None of the patients received radiotherapy or neoadjuvant chemotherapy before surgery; (3) All patients were diagnosed with gastric cancer for the first time; (4) Their ages ranged from 30 to 70 years. Exclusion criteria: (1) Patients with residual gastric cancer; (2) Combined with other malignant tumors or a history of malignant tumors; or (3) The clinical and follow-up data were incomplete. Finally, 160 patients were included in the study, including 154 patients with advanced gastric cancer (96.2%) and 6 patients with early gastric cancer (3.8%) comprising 88 males and 72 females. There were 99 elderly patients aged above 60 years. The tumor was mainly located in the lower 1/3, with a total of 94 cases. The tumors were located in the upper 1/3, middle 1/3, trans regional, or whole stomach in 32 cases, 27 cases, and 7 cases, respectively. According to the Borrmann classification, there were 9 cases of type I, 43 cases of type II, 90 cases of type III, and 18 cases of type IV. The clinical stages were generally delayed. There were 36 cases of stage I + II and 124 cases of stage III and IV. Serum carcinoembryonic antigen (CEA) level was normal in 142 cases, increased in 18 cases; serum CA19-9 level was normal in 145 cases, and increased in 15 cases.

No.12 lymph node dissection steps

The omentum was cut close to the lower edge of the liver, the visceral peritoneum of the hepatoduodenal ligament was cut horizontally, and the proper hepatic artery, the front of the common bile duct, and the right gastric artery were dissected individually. The duodenal peritoneum was opened by Kocher incision to reach the rear of the duodenum, the right peritoneum of the hepatoduodenal ligament was cut, the right edge of the

common bile duct was exposed, and the No.12b and No.12a lymph nodes were cleaned. The posterior wall was then stripped from the back of the portal vein, the cleaned tissue was pulled to the upper left, the No.12p lymph nodes around the portal vein were cleaned, and then stripped to the bifurcation of the common hepatic artery and gastroduodenal artery. So far, the No.12 lymph node has been cleaned.

Detection of clinical metastasis and micrometastasis of lymph nodes

According to the operation specifications of the kit, No.12 lymph node sections with negative routine pathological examination were detected for micrometastasis by immunohistochemical analysis. Cytokeratin CK20 was detected using a two-step method. The main operation steps include slicing, dewaxing, antigen repair, elimination of endogenous peroxidase activity by hydrogen peroxide, dropping primary antibody (mouse anti-human CK20 monoclonal antibody, Bode company), secondary antibody incubation, DAB color development, counterstaining, dehydration, transparency, sealing, and microscopic examination.

Follow up

Follow-up was mainly conducted through telephone and outpatient visits. The duration of follow-up was once every 3 months in the first year, once every half a year in the second year, and once every year in the third year and after. The follow-up period was until January 2021. According to the condition of the patients, routine blood examination, biochemical examination, or thoracoabdominal basin CT and endoscopy were performed. Nutritional deficiencies, such as those of vitamin B12 and iron were closely monitored and treated, if necessary.

Statistical analyses

All data were statistically analyzed using SPSS software (version 20.0). The relationship between No.12 lymph node metastasis and clinicopathological parameters was analyzed using the chi square test, Fisher's exact probability method, and multivariate analyses was performed using the logistic risk model. The log-rank test was used to compare the effect of No.12 lymph node metastasis on the prognosis of patients with gastric cancer. Inspection level $\alpha = 0.05$. Statistical significance was set at $P < 0.05$.

Results

Routine pathological examination and immunohistochemical staining were used to detect lymph node metastasis and

micrometastasis

A total of 370 No.12 lymph nodes were detected in 160 surgical specimens. Among the 160 patients, 27 patients were positive for No.12 lymph nodes during routine pathological examination, with a positive rate of 16.8%. A total of 308 lymph nodes from 133 patients with negative routine pathological examinations were stained by immunohistochemistry. A total of 17 lymph nodes from 10 patients were found to be positive. The results showed that 37 of the 160 patients had No.12 lymph node metastasis, and the positive rate was 23.1%, which was 6.3% higher than that of routine pathological examination (Fig. 1).

Univariate analysis of the effects of No.12 lymph node metastasis and micrometastasis on clinicopathological parameters

Univariate analysis showed that the occurrence of No.12 lymph node clinical metastasis was related to Borrmann classification, lymph node metastasis, and clinical stage of gastric cancer ($P < 0.05$). The results showed that the occurrence of No.12 lymph node micrometastasis was only related to the Borrmann classification, lymph node metastasis, and clinical stage of gastric cancer ($P < 0.05$) (Table 1).

Multivariate analyses of the influence of No.12 lymph node metastasis on clinicopathological parameters

The pathological factors, including tumor location, No.12 lymph node metastasis, lymph node metastasis in other groups, clinical stage, degree of differentiation, and tumor diameter were included in the logistic risk model for multivariate analyses. The results showed that the depth of invasion, lymph node metastasis in other groups, and clinical stage were independent risk factors affecting No.12 lymph node metastasis (Table 2).

Follow up results

The average follow-up time was 79.3 months, and the overall median survival time was 47.9 months. The survival time of the No.12 lymph node-negative group was 67.3 ± 2.5 months, the median survival time was 73.2 months; the survival time of the No.12 lymph node-positive group was 28.4 ± 5.4 months, and the median survival time was 31.3 months; and the difference between the two groups was statistically significant ($\chi^2 = 12.75$, $P = 0.000$). The survival time of the No.12 lymph node-negative group was significantly longer than that of the positive group (Fig. 2).

Discussion

In 1971, Huvos discovered the phenomenon of lymph node micrometastases in breast cancer for the first time. It was defined as a non-metastatic tumor with a diameter less than 2 mm, which is difficult to detect by routine pathological examination. In 1998, the international alliance against cancer (UICC) updated this concept and defined a single tumor cell or cell cluster with a diameter of < 0.2 mm as “isolated tumor cell nest” (ITC). This concept is still used today. Therefore, the most accurate definition of LNM is the tumor cell cluster with $0.2 \text{ mm} \leq r < 2 \text{ mm}$ ^[6].

In China, D2 lymph node dissection is generally recognized as the standard surgical method for patients with gastric cancer. The Japanese Gastric Cancer Association divides the lymph nodes into three stations and 16 groups of lymph nodes according to the gastric omentum and large blood vessels around the stomach^[7]. Groups 1 to 6 belong to the first stop, including the left and right of the cardia, large and small curvatures of the stomach, and upper and lower pylorus. The second and third stations, that is, groups 7–16, include the total liver, pancreas, posterior duodenum, celiac trunk, left stomach, splenic artery and hilar lymph nodes, superior mesenteric artery lymph nodes, and para-aortic lymph nodes^[8].

The hepatoduodenal lymph nodes are no.12a along the hepatic artery, no.12b along the bile duct, and no.12p behind the portal vein^[9]. There are many methods for detecting LNM. The serial section method was first used in the clinic, but it is difficult to detect a small number of cancer cells because of its heavy workload. RT-PCR has high specificity and sensitivity, but it has high requirements for experimental technology, is uneconomical, and has not been fully popularized. Therefore, at present, the most commonly used method for detecting LNM is the immunohistochemical method, which is economical, easy to perform, and widely used^[10].

A total of 370 No.12 lymph nodes were detected in 160 surgical specimens. Among the 160 patients, 27 patients were positive for No.12 lymph nodes during routine pathological examination, with a positive rate of 16.8%. Furthermore, cytokeratin CK20, with high sensitivity, was used to detect micrometastases. A total of 308 lymph nodes from 133 patients with negative routine pathological examinations were stained by immunohistochemistry. A total of 17 lymph nodes in 10 patients were found to be positive. The results showed that 37 of the 160 patients had No.12 lymph node metastasis, and the positive rate was 23.1%, which was 6.3% higher than that of routine pathological examination. As an important defense line of human immune function, the lymphatic system will be significantly damaged by the expansion of intraoperative

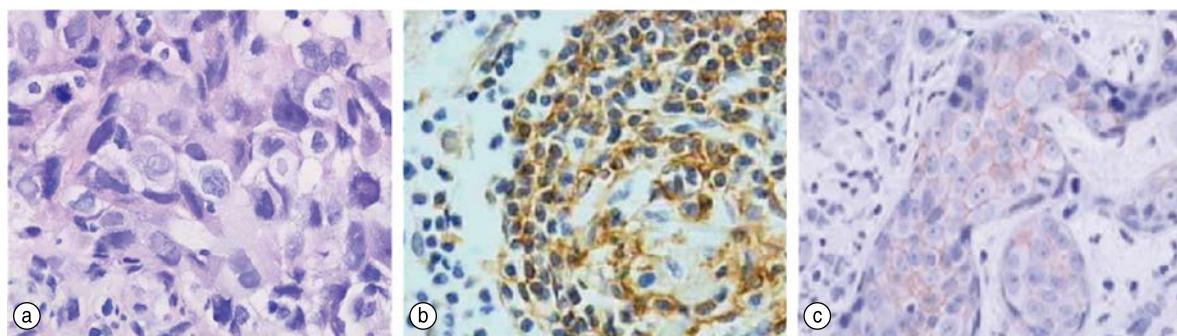


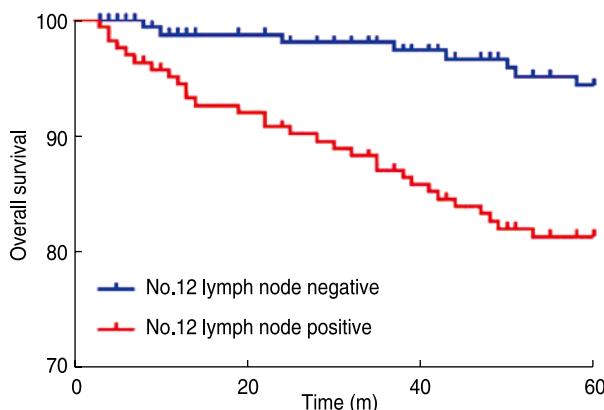
Fig. 1 Routine pathological examination and immunohistochemical staining of the lymph nodes

Table 1 Comparison of the effects of No.12 lymph node metastasis and micrometastasis on each clinicopathological parameter

Index	No.12 Conventional pathology (+)	No.12 Conventional pathology (-)	χ^2	P	No.12 (+)	No.12 (-)	χ^2	P
Gender			0.004	0.949			0.784	0.376
Female	15	73			18	70		
Male	12	60			19	53		
Age (years)			0.316	0.574			0.182	0.669
< 60	9	52			13	48		
≥ 60	18	81			24	75		
Tumor site			3.543	0.315			6.319	0.097
Upper 1/3	5	27			6	26		
Middle 1/3	4	23			5	22		
Down 1/3	15	79			17	77		
Whole stomach	3	4			4	3		
Differentiation			0.157	0.692			0.041	0.839
Low	17	89			24	82		
Middle-high	10	44			13	41		
Borrmann			15.944	0.001			41.581	0.000
I	1	8			1	8		
II	6	37			6	37		
III	11	79			15	75		
IV	9	9			15	3		
Lauren			0.077	0.781			2.664	0.103
intestinal	15	70			24	61		
diffuse	12	63			13	62		
Size (cm)			0.054	0.816			0.065	0.799
< 5	16	82			22	76		
≥ 5	11	51			15	47		
Lymphatic metastasis			5.391	0.020			6.407	0.011
Yes	26	102			35	93		
No	1	31			2	30		
Stage			4.243	0.039			5.717	0.017
I + II	2	34			3	33		
III + IV	25	99			34	90		
CEA			0.001	0.980			0.009	0.923
Normal	24	118			33	109		
Rise	3	15			4	14		
CA19-9			0.148	0.700			0.091	0.763
Normal	25	120			34	111		
Rise	2	13			3	12		

Table 2 Multivariate analyses of the impact of patient clinicopathological parameters

Index	SE	Wald	df	95%CI	P
Tumor site	0.575	1.566	1	0.182–1.731	0.226
T stage	0.864	5.159	1	0.191–5.661	0.024
Lymphatic metastasis	0.661	6.026	1	0.163–2.177	0.013
Clinical stages	0.961	4.026	1	0.091–3.920	0.037
Differentiation	0.927	1.217	1	0.091–3.452	0.305
Size	0.763	2.562	1	0.126–2.503	0.063

**Fig. 2** No.12 Comparison of survival curves between patients in the lymph node-negative and positive groups

lymph node dissection, which greatly reduces the immune ability of patients to tumors and affects the long-term prognosis of patients. In addition, the anatomy of the hepatoduodenal ligament is complex, and the surgical risk is high. Cleaning this area will significantly improve the occurrence of postoperative complications such as bile leakage, gallbladder injury, hepatic artery stenosis, portal vein injury, and lymphoma. Therefore, there is still controversy regarding whether No.12 lymph nodes should be routinely cleaned in radical gastrectomy. However, in this study, combined with the results of routine pathological examination and micrometastasis detection, it was found that the metastasis rate of No.12 lymph nodes could be as high as 23.1%, which proved the necessity of cleaning No.12 lymph nodes during radical gastrectomy.

Feng *et al.* [11] reported that the depth of tumor invasion and Borrmann classification are the most important factors affecting No.12 lymph node metastasis, which is consistent with the conclusion of this study. It further shows that tumor infiltration into the serosa and Borrmann type IV are important factors for lymph node metastasis of gastric cancer No.12. This suggests that patients with poor Borrmann classification, lymph node metastasis in other groups, and late clinical stage should pay more attention to the significance of No.12 lymph

node dissection. However, no correlation was observed between No.12 lymph node metastasis and tumor location ($P > 0.05$). The relationship between No.12 lymph node metastasis and tumor location in gastric cancer needs to be further studied.

Regarding the significance of lymph node micrometastasis on the prognosis of patients, some researchers believe that there is a correlation between them, while others believe that micrometastasis has no effect on the prognosis of patients. The patients in this group were followed up for an average of 79.3 months. It was found that the survival time of patients in No.12 lymph node negative group and positive group were (67.3 ± 2.5) months and (28.4 ± 5.4) months respectively. The survival time of patients in the No.12 lymph node-negative group was significantly longer than that in the positive group. This more intuitively illustrates the impact of No.12 lymph node metastasis on patient prognosis. Therefore, we believe that it is necessary to perform No.12 lymph node metastasis in patients with gastric cancer. As a relatively controllable factor, postoperative complications should follow the principle of No.12 lymph node dissection to perform vascular choroidization dissection to avoid the shedding and planting of tumor cells. Careful identification of duct structures, such as the bile duct, portal vein, and hepatic artery, is an important measure to prevent postoperative complications.

In conclusion, No.12 lymph node metastasis is a strong indicator of poor prognosis. Standardized lymph node dissection is recommended for patients with advanced gastric cancer who can undergo radical resection.

Acknowledgments

Not applicable.

Funding

This study was supported by a grant from the Hebei Medical Science Research Project (No. 20191831).

Conflicts of interest

The authors indicated no potential conflicts of interest.

Author contributions

Not applicable.

Data availability statement

Not applicable.

Ethical approval

This study was approved by Handan People's Hospital ethics committee (SWYX:No. 2020-107).

References

1. Zhou Y, Zhang GJ, Wang J, et al. Current status of lymph node micrometastasis in gastric cancer. *Oncotarget*. 2017;8(31):51963-51969.
2. Liang H. The Precised Management of Surgical Treatment for Gastric Cancer: Interpretation of the 5th edition of Japanese Gastric Cancer Treatment Guideline and the 15th edition of Japanese Classification for Gastric Cancer. *Zhonghua Zhong Liu Za Zhi*. 2019;41(3):168-172.
3. Dang XD, He Y, Lai BQ. Efficacy of Bispectral index-monitored closed-loop targeted-controlled infusion of propofol for laparoscopic radical operation for gastric cancer. *J Hainan Med Univ*. 2019;25(1): 58-62.
4. Shen S, Cao S, Jiang H, et al. The short-term outcomes of gastric cancer patients based on a proposal for a novel classification of perigastric arteries. *J Gastrointest Surg*. 2020;24(11):2471-2481.
5. Lou GC, Dong J, Du J, et al. Clinical significance of lymph node micrometastasis in T1N0 early gastric cancer. *Math Biosci Eng*. 2020;17(4):3252-3259.
6. Tavares A, Wen X, Maciel J, et al. Occult tumour cells in lymph nodes from gastric cancer patients: should isolated tumour cells also be considered? *Ann Surg Oncol*. 2020;27(11):4204-4215.
7. Zhang P, Lan TH, Zhou YM, et al. Risk factor analysis of perioperative complications in patients with radical gastrectomy for gastric cancer. *Zhonghua Wei Chang Wai Ke Za Zhi (Chinese)*. 2019;22(8):736-741.
8. Wang JW, Chen CY. Prognostic value of total retrieved lymph nodes on the survival of patients with advanced gastric cancer. *J Chin Med Assoc*. 2020;83(8):691-692.
9. Takeuchi M, Takeuchi H, Kawakubo H, et al. Risk factors for lymph node metastasis in non-sentinel node basins in early gastric cancer: sentinel node concept. *Gastric Cancer*. 2019;22(1):223-230.
10. Shimada A, Takeuchi H, Nishi T, et al. Utility of the one-step nucleic acid amplification assay in sentinel node mapping for early gastric cancer patients. *Gastric Cancer*. 2020;23(3):418-425.
11. Feng JF, Huang Y, Liu J, et al. Risk factors for No. 12p and No. 12b lymph node metastases in advanced gastric cancer in China. *Ups J Med Sci*. 2013;118(1):9-15.

DOI 10.1007/s10330-021-0526-6

Cite this article as: Dong TZ, Zhang LR. Analysis of the adverse reactions of atezolizumab: a real-world study based on FAERS database. *Oncol Transl Med*. 2022;8(3):115–120.