Clinical Significance of Metastasis or Micrometastasis to the Lymph Node Along Superior Mesenteric Vein in Gastric Carcinoma: a Retrospective Analysis

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Abstract

Background: The validity of lymphadenectomy of the lymph node along the superior mesenteric vein (LN14v) in gastric cancer remains controversial. The study investigated the characteristics and prognosis of gastric cancer with metastasis or micrometastasis to LN14v.

Methods: A retrospective study of 626 patients receiving radical gastrectomy in our center from January 2003 to December 2015 was analyzed. Totally, 303 patients receiving lymphadenectomy of 14v and lymph node micrometastasis was evaluated by immunohistochemical staining for cytokeratin nodes CK8/18. Logistic regression model was applied to confirm the predictive factors of micrometastasis. Survival analysis was performed to evaluate the effect of micrometastasis or metastasis on prognosis.

Results: The metastatic rate of No.14v lymph node was 15.8% and the micrometastatic rate was 3.9%. Multivariate analysis showed site, Borrmann classification, postoperative lymph node metastasis (pN), the metastasis of LN6 and LN9 were predictive factors of LN14v micrometastasis or metastasis ($P < 0.05$). The 5-year survival rate of positive group (14v micrometastasis or metastasis) was 12.4%. The prognosis of patients without micrometastatic 14v lymph node was better than positive group. While the difference between group of LN14v micrometastasis and LN14v metastasis was not obvious. In matched analysis, patients with gastric cancer of stage $\geq$ II, U/M area, pN2-3 and LN 6(+) underwent lymphadenectomy of 14v suffered better survival than those without lymphadenectomy of 14v.

Conclusion: Lymph node micrometastasis could provide accurate prognostic information for patients with GC. Thus, lymphadenectomy of LN14v should be recommended for patients with gastric cancer of stage $\geq$ II, U/M area, pN2-3 and LN 6(+).

Introduction

Gastric cancer (GC) was one the most common cancer-related deaths carcinoma throughout the world [1, 2]. Radical gastrectomy is the optimal choice to cure locally advanced resectable gastric cancer. As the 15 years long-term result of Dutch trial showed that D2 lymphadenectomy was associated with lesser rate of recurrence and improved the overall survival than D1 dissection, gastrectomy plus D2 lymph node dissection has been increasingly considered as the standard surgical procedure for advanced resectable gastric cancer [3]. However, over 50% patients suffering from worse survival would subsequently relapse or die after radical surgery, due to node, distant metastasis or locoregional recurrence [4]. Among the metastatic routes in GC (direct infiltration or spread, lymph node metastasis, hematogenous metastasis and implant metastasis), lymph node metastasis remains the most common pathway. However, the extent of lymphadenectomy during the surgical procedure to improve the survival to the utmost extent with less complications remains controversial.

According to 6th edition of International Union Against Cancer (UICC), lymph node metastasis (LNM) is classified into isolated tumor cells (ITC), micrometastasis (MI) and macrometastasis (MA) depending on the size of metastatic deposit [5]. Methods to investigate the presence of MI varies from serial slices with

Hematoxylin and eosin (HE) staining, immunohistochemical (IHC) staining to real-time reverse transcription-polymerase chain reaction (RT-PCR) [6–9]. Cytokeratin is one component of the cytoskeleton of epithelial cell, which is not present in normal lymph nodes. And its antibody was widely used to detect the minute deposit of tumor cells in lymph node by IHC staining. There are still controversies about the clinical features of MI and its prognostic significance on GC [10–13].

The JCOG9501 trial didn't reach the conclusion that D2 lymphadenectomy plus para-aortic lymph node dissection obtains the superiority of improving the survival in comparison to D2 lymphadenectomy [14, 15]. There were controversies that whether it was necessary for patients with GC to receive 14v dissection and how to identify the subgroup for whom the benefit was maximized from 14v dissection. The study was mainly designed to analyze the characteristics associated with 14v metastasis or micrometastasis and the clinical features and prognostic significance of MI for GC. Otherwise, oncological outcomes were also analyzed.

Methods And Materials

Patients

From January 2003 to December 2015, 626 patients with GC (including esophagogastric junction carcinoma) in Cancer Hospital of China Medical University, Liaoning Cancer Hospital were prospectively enrolled in this study (Supplementary Figure 1). The study was conducted in accordance with the Declaration of Helsinki and the study protocol was reviewed and approved by the Ethics Committee of Liaoning Cancer Hospital. Informed consent was waived by the Ethics Committee of Liaoning Cancer Hospital. To analyze the characteristics associated with 14v metastasis or micrometastasis, the inclusion criteria included: ☐ Age ranged from 18 to 75 years old; ☐ Histopathological examination diagnosis of gastric carcinoma on well-established criteria; ☐ Intraoperative exploration and postoperative TNM stage revealed no distant metastases (M0); ☐ No unresective factors were founded; ☐ Radical gastrectomy plus the No.14v lymph node dissection was performed (D2 or D2+). The study excluded cases for which: ☐ R0 surgery after conversion therapy; ☐ Gastric remnant carcinoma; ☐ 14v lymph node biopsy; ☐ Suffering from other malignancies; ☐ Without complete follow-up data. A total of 303 patients receiving radical gastrectomy plus the No.14v lymph node dissection were retrospectively collected to make comparisons between groups. Patients with clinical or pathologic TNM stage (☐-☐) or with positive lymph nodes received a perioperative chemotherapy regimen of SOX or XELOX, divided into 2-4 preoperative and several postoperative cycles. 255 cases without No.14v lymph node metastasis underwent CK8/18 immunohistochemistry to detect the minute deposit of tumor cells in lymph nodes. Patients without micrometastatic lymph node were classified into negative group, and patients with micrometastatic or metastatic lymph nodes were classified into positive group.

No.14v lymph node dissection

The No.14v lymph nodes were classified as lymph nodes along the superior mesenteric vein. All radical gastrectomy procedures plus the No.14v lymph node dissection were performed by 3 experienced

surgeons. The dissection criteria of the No.14v lymph node were as follow: Firstly, omentobursectomy and lymphadenectomy of No.6 was performed. And then No.14v lymph nodes were completely removed from the root of nodes. In order to expose the superior mesenteric vein, Henle trunk and middle colic vein, the soft tissue around the superior mesenteric vein was completely removed as well.

Clinicopathological features

Patients' clinicopathological characteristics included tumor size, location (U, M, L area), grade of differentiation (differentiated, undifferentiated), Borrmann type, histological type (adenocarcinoma, signet ring or mucinous carcinoma), pathologic TNM stage (postoperative category), extent of resection (total, distal or proximal), the number of harvested lymph node, etc.

Immunohistochemical stain

A total of 576 lymph nodes without No.14v metastasis from 255 patients were reexamined by one pathologist to confirm the absence of lymph node metastasis by HE staining section. Lymph nodes were stored in 261 paraffin-embedded blocks and 3 serial tissue sections were made from each block with 4 mm in thickness and immunohistochemical stain (CK8/18, 1:200 dilution) were performed to evaluate metastasis of lymph nodules. The tumor tissues of GC were used as the positive control to stain in the same manner as experiment group (Supplementary Figure 2).

Assessing staining results

We evaluated the staining results according the location, structure, morphology and staining color. CK8/18 was mainly located in the cytoplasm at the marginal sinus of lymph nodes (Figure 2). Positive staining cells were filled with brown-yellow color component, while the negative cells are unstained. Furthermore, positive samples were reconfirmed by detecting the structure and morphology of the cells. Only the size of the cell nest was 0.2-2 mm, it was defined as MI and the sample was classified as positive sample. For any one of serial sections was positive staining, we would categorize it as positive group. All of the slices were reviewed by two experienced pathologists by independently observing the CK staining sections under the microscope ($\times 100$ and $\times 200$). When disagreements between pathologists happened, a consensus was sought by pathologists rereviewing the slices and discussing it together.

Statistical analysis

Mean $\pm$ standard deviation was used to represent continuous values and categorical data were expressed as percentage. Chi-square test or Fisher's test was applied to evaluate the relationship between clinicopathological characteristics and 14v metastasis or micrometastasis variance. Independent factors associated with 14v metastasis or micrometastasis were analyzed in Logistic regression method. Variables with P value of ≤ 0.05 in univariate analysis were selected for the multivariate analysis. To predict prognostic risk factors, we use the multivariable Cox proportional hazard model and univariate analysis. The Kaplan–Meier method and log-rank test was applied to distinguish difference of survival

data between two groups. Data was proceeded by SPSS 23.0 software. The P value $\geq$ 0.05 was considered to be statistically significant.

Results

Clinicopathological features and clinical outcomes

A total of 303 patients underwent radical surgery plus 14v dissection in the study and clinicopathological features were shown in Table 1. Most patients were male (66.0%) and their age ranged from 28 to 75 years old (mean: 55.92 y). A majority of tumors were located in L area (67.7%), presented with poor differentiation (77.2%) and canalicular adenocarcinoma histological type (73.9%). A minority of patients underwent total or proximal gastrectomy surgery (24.1%). And cases were classified into stage I (17.5%), II (20.1%), III (62.4%) (postsurgical pathological stage). The metastatic incidence of No.14v lymph node was 15.8% and the micrometastatic incidence was 3.9%. The total metastatic rate of 14v was 19.1%. The median number of overall harvested lymph nodes and harvested 14v lymph nodes was 28.48 and 2.03, respectively. Patients were followed up every 3 months during the first 3 years, subsequently every 6 months for the following 2 years and once a year after 5 years until the time of death or the deadline, December 31th, 2018. The following-up time ranged from 3 to 178 months (median: 46m). And the 3-, 5-year overall survival rate of patients with radical gastrectomy plus 14v dissection was 71.2% and 50.7%, respectively.

Table 1
Clinicopathological features of patients with gastric cancer undergoing radical
gastrectomy plus 14v dissection

Clinicopathological characteristics	Value	Percentage(%)
Age (y)	55.92 ± 10.74	
Gender		
Male	200	66.0
Female	103	34.0
Tumor size (cm)	6.02 ± 10.74	
Location		
U	14	4.6
M	84	27.7
L	205	67.7
Histological type		
Adenocarcinoma	224	73.9
Signet-ring or mucinous carcinoma	79	26.1
Grade of differentiation		
Well or moderate	69	22.8
Poor	234	77.2
Borrmann type		
ⅠⅠⅠ	178	58.7
Ⅱ	108	35.6
Ⅲ	17	5.6
Postoperative T category (pT)		
T1-2	81	26.7
T3-4	222	73.3
Postoperative N category(pN)		
N0	89	29.4
N1	41	13.5

Location, U/M/L, the upper/middle/lower third of stomach

Clinicopathological characteristics	Value	Percentage(%)
N2	63	20.8
N3	110	36.3
Extent of resection		
Total or proximal	73	24.1
Distal	230	75.9
Pathological stage(pTNM)		
I	53	17.5
II	61	20.1
III	189	62.4
The number of harvested lymph nodes	28.48 ± 10.52	
The number of metastatic lymph nodes	6.28 ± 7.51	
Without 14v micrometastasis	245	80.9
With 14v micrometastasis	10	3.3
With 14v metastasis	48	15.8
The number of harvested lymph nodes of 14v	2.03 ± 1.439	
Location, U/M/L, the upper/middle/lower third of stomach		

Clinicopathological characteristics associated with 14v metastasis or micrometastasis

We sought to identify subgroups that were likely to be presented with 14v metastasis or micrometastasis. It revealed the metastasis or micrometastasis of 14v was associated with tumor size ($P=0.001$), location ($P=0.027$), Borrmann type ($P=0.003$), pT category ($P=0.001$), pN category ($P=0.000$), pTNM stage ($P=0.000$) and the number of metastatic lymph node ($P=0.000$) (Table 2.). Logistic regression analysis demonstrated that location ($P=0.004$, RR: 0.320, 95%CI: 0.146-0.700), Borrmann type ($P=0.010$, RR: 0.010, 95%CI: 1.104–2.089) and pN category ($P=0.000$, RR: 3.709, 95%CI: 2.326–5.914) were significantly correlated with 14v metastasis or micrometastasis (Table 3.).

Table 2
Univariate analysis of clinicopathological features associated with 14v metastatic status

Variable	14v micrometastasis (-)	14v micrometastasis or metastasis (+)	<i>P</i> value
Age (y)			
<60	159	36	0.686
≥60	86	22	
Gender			
Male	162	38	0.930
Female	83	20	
Tumor size (cm)			
<5.0	127	16	0.001
≥5.0	118	41	
Location			
U	14	0	0.027
M	73	11	
L	158	47	
Histological type			
Adenocarcinoma	179	45	0.480
Signet-ring or mucinous carcinoma	66	13	
Grade of differentiation			
Well or moderate	57	12	0.674
Poor	118	46	
Borrmann type			
ⅢⅢⅢ	154	24	0.003
ⅢⅢ	81	27	
Ⅲ	10	7	
Postoperative T category (pT)			
T1-2	77	4	0.000
Location, U/M/L, the upper/middle/lower third of stomach			

Variable	14v micrometastasis (-)	14v micrometastasis or metastasis (+)	<i>P</i> value
T3-4	168	54	
Postoperative N category(pN)			
N0	89	0	0.000
N1	37	4	
N2	55	8	
N3	64	46	
Extent of resection			
Total or proximal	62	11	0.310
Distal	183	47	
Pathological stage(pTNM)			
I	52	1	0.000
II	59	2	
III	134	55	
The number of harvested lymph nodes	28.90 ± 10.419	26.72 ± 10.843	0.157
The number of metastatic lymph nodes	4.56 ± 6.034	13.55 ± 8.728	0.000
The number of harvested lymph nodes of 14v			
1	120	33	0.120
2	57	15	
≥3	67	8	
Location, U/M/L, the upper/middle/lower third of stomach			

Table 3
Univariate analysis of regional lymph nodes associated with 14v metastatic status

Lymphatic metastasis	14v micrometastasis (-)	14v micrometastasis or metastasis (+)	<i>P</i> value
No.1			
(+)	23	17	0.000
(-)	179	30	
No.2			
(+)	10	1	0.001
(-)	41	3	
No.3			
(+)	90	39	0.000
(-)	142	11	
No.4			
(+)	79	37	0.000
(-)	154	14	
No.5			
(+)	46	29	0.000
(-)	103	9	
No.6			
(+)	96	50	0.000
(-)	135	6	
No.7			
(+)	63	29	0.000
(-)	167	20	
No.8a			

No.1, right paracardial lymph node; No.2, left paracardial lymph node; No.3, lymph node along the lesser curvature; No.4 (4sa, 4sb, 4sd), lymph node along the short gastric vessels, the left gastroepiploic vessels and the right gastroepiploic vessels; No.5, the suprapyloric lymph node; No.6, the infrapyloric lymph node; No.7, lymph node along the left gastric artery; No.8a, lymph node along the common hepatic artery; No.9, lymph node around the celiac artery; No.10, lymph node at the splenic hilum; No.11 (11p and 11d); lymph node along the proximal splenic artery and distal splenic artery; No.12a, lymph node in the hepatoduodenal ligament (along the hepatic artery); No.14v, lymph node along the superior mesenteric vein.

Lymphatic metastasis	14v micrometastasis (-)	14v micrometastasis or metastasis (+)	<i>P</i> value
(+)	42	40	0.000
(-)	189	11	
No.9			
(+)	15	23	0.000
(-)	178	22	
No.10			
(+)	1	2	0.005
(-)	10	0	
No.11			
(+)	16	17	0.000
(-)	164	17	
No.12a			
(+)	14	17	0.000
(-)	127	19	
No.1, right paracardial lymph node; No.2, left paracardial lymph node; No.3, lymph node along the lesser curvature; No.4 (4sa, 4sb, 4sd), lymph node along the short gastric vessels, the left gastroepiploic vessels and the right gastroepiploic vessels; No.5, the suprapyloric lymph node; No.6, the infrapyloric lymph node; No.7, lymph node along the left gastric artery; No.8a, lymph node along the common hepatic artery; No.9, lymph node around the celiac artery; No.10, lymph node at the splenic hilum; No.11 (11p and 11d); lymph node along the proximal splenic artery and distal splenic artery; No.12a, lymph node in the hepatoduodenal ligament (along the hepatic artery); No.14v, lymph node along the superior mesenteric vein.			

Regional lymph nodes associated with 14v metastasis or micrometastasis

In order to investigate 14v lymphatic drainage pathway, the study included lymph node, No.1, No.2, No.3, No.4 (4sa, 4sb and 4sd), No.5, No.6, No.7, No.8a, No.9, No.10, No.11, No.12a to conduct univariate and multivariable analysis. It demonstrated that the metastatic status of LN14v was significantly correlated with that all regional nodes (all $P < 0.05$, Table 4.). Multivariable analysis results revealed that the metastasis of LN6 and LN9 to be independent variables associated with LN14v metastasis or micrometastasis ($P = 0.003$, RR: 0.101, 95%CI: 0.022–0.496; $P = 0.013$, RR: 0.093, 95%CI: 0.014–0.608) (Table 5.). Of 146 patients with LN6 metastasis, 34.2% cases had the metastasis or micrometastasis of LN14v. And LN6 status showed the low false negative rate (10.7%) to predict the absence of metastasis or micrometastasis of LN14v.

Table 4
Multivariate analysis of clinicopathological features associated with 14v metastatic status

Variable	β	RR(95%CI)	Pvalue
Tumor size	0.331	1.392(0.649–2.988)	0.396
Location	-0.139	0.320(0.146-0.700)	0.004
Borrmann type	0.418	1.519(1.104–2.089)	0.010
Postoperative T category (pT)	0.624	1.866(0.565–6.160)	0.306
Postoperative N category(pN)	1.311	3.709(2.326–5.914)	0.000
Pathological stage(pTNM)	-0.747	0.474(0.097–2.313)	0.356

Table 5
Multivariate analysis of regional lymph nodes associated with 14v metastatic status

Lymphatic metastasis	β	RR(95%CI)	Pvalue
No.1	1.378	3.968(0.340-46.257)	0.271
No.3	-0.172	0.842(0.054–13.085)	0.902
No.4	1.464	4.325(0.206–90.878)	0.346
No.5	-0.189	0.150(0.010–2.203)	0.167
No.6	-2.294	0.101(0.022–0.496)	0.003
No.7	1.062	2.891(0.118–71.054)	0.516
No.8a	-1.395	0.248(0.037–1.681)	0.153
No.9	-2.38	0.093(0.014–0.608)	0.013
No.11	2.048	7.750(0.203-295.209)	0.270
No.12a	-0.345	0.708(0.029–17.523)	0.833
No.1, right paracardial lymph node; No.3, lymph node along the lesser curvature; No.4 (4sa, 4sb, 4sd), lymph node along the short gastric vessels, the left gastroepiploic vessels and the right gastroepiploic vessels; No.5, the suprapyloric lymph node; No.6, the infrapyloric lymph node; No.7, lymph node along the left gastric artery; No.8a, lymph node along the common hepatic artery; No.9, lymph node around the celiac artery; No.11 (11p and 11d); lymph node along the proximal splenic artery and distal splenic artery; No.12a, lymph node in the hepatoduodenal ligament (along the hepatic artery); No.14v, lymph node along the superior mesenteric vein.			

Prognostic value of metastatic status of 14v in gastric cancer

The 5-year overall survival rate of patients with LN14v metastasis and LN14v micrometastasis was 12.9% and 10.0%. And the 5-year survival rate of patients of positive group (LN14v micrometastasis or metastasis) was 12.4%. The negative group (neither LN14v metastasis nor micrometastasis) had a more favorable survival in comparison to the positive group (LN14v micrometastasis or metastasis) ($P = 0.000$, $HR = 4.001$, $95\%CI = 2.789-5.739$, Fig. 1). In stratified analysis, the negative group showed higher overall survival rate (60.1%) than that of LN14v micrometastasis or metastasis group ($P = 0.000$, $HR = 2.093$, $95\%CI = 1.480-2.961$; $P = 0.000$, $HR = 3.931$, $95\%CI = 2.671-5.787$, Fig. 2). But the difference between patients with 14v micrometastasis and 14v metastasis was not significant ($P = 0.901$, $HR = 1.047$, $95\%CI = 0.501-2.171$). Univariate analysis results showed age, gender, tumor size, Borromann type, pT stage, pN stage, pTNM stage, and pathological type were correlated to the prognosis. Furthermore, multivariable Cox proportional hazard model analysis demonstrated that LN14v metastatic status ($P = 0.001$, $HR = 1.936$, $95\%CI = 1.323-2.834$), pT stage ($P = 0.003$, $HR = 2.725$, $95\%CI = 1.416-5.244$), pN stage ($P = 0.000$, $HR = 2.090$, $95\%CI = 1.688-2.588$), pathological type ($P = 0.043$, $HR = 1.448$, $95\%CI = 1.012-2.072$) and Borromann type ($P = 0.000$, $HR = 1.341$, $95\%CI = 1.148-1.566$) were significant prognostic variables (Table 6.). Even having underwent radical gastrectomy plus the LN14v dissection, patients with 14v metastasis or micrometastasis had worse survival than that of patients of stage I, II and III neither with LN14v metastasis nor micrometastasis ($P = 0.000$, Fig. 3).

Table 6

Univariate and multivariate analysis of overall survival in patients with gastric cancer undergoing radical gastrectomy plus 14v dissection

Characteristics	Univariate analysis			Multivariate analysis		
	β	HR (95%CI)	<i>P</i> value	β	HR (95%CI)	<i>P</i> value
Gender	0.354	0.702(0.503–0.980)	0.037	0.081	0.922(0.773–1.110)	0.366
Age	0.378	1.459(1.052–2.023)	0.024	0.268	1.307(0.932–1.832)	0.12
Tumor size	0.919	2.506(1.761–3.567)	0.001	0.04	1.040(0.695–1.557)	0.847
Location	0.09	1.094(0.823–1.454)	0.537			
Borrmann type	0.409	1.505(1.294–1.751)	0.001	0.293	1.341(1.148–1.566)	0.000
Postoperative T category (pT)	1.757	5.797(3.267–10.286)	0.001	1.003	2.725(1.416–5.244)	0.003
Postoperative N category(pN)	0.908	2.480(2.058–2.989)	0.001	0.737	2.090(1.688–2.588)	0.000
Micro- or metastasis Status of 14v	1.386	4.001(2.7894–5.739)	0.000	0.661	1.936(1.323–2.834)	0.001
Histological type	0.582	1.789(1.269–2.524)	0.001	0.37	1.448(1.012–2.072)	0.043
Grade of differentiation	0.542	1.719(1.101–2.684)	0.017	0.102	1.107(0.679–1.806)	0.684
Extent of resection	0.461	1.586(1.107–2.271)	0.012	0.264	1.302(0.883–1.921)	0.183

The benefit of lymphadenectomy of LN14v in gastric cancer

Having established that 14v metastatic status was prognosis significance for adequately staged patients treated by radical gastrectomy plus the 14v dissection, we sought to identify patient subgroups for whom the benefit was maximized and those for whom TRG was not of prognostic significance. In matched analysis, patients with gastric cancer of stage $\geq$ II, U/M area, pN2-3 and LN 6(+) underwent lymphadenectomy of 14v suffered better survival than those without lymphadenectomy of 14v ($P=0.006$, Fig. 4).

Discussion

Immunohistochemical staining for cytokeratin nodes CK8/18 to evaluate micrometastases has been reported in other fields. But the study firstly introduced CK8/18 to evaluate micrometastases of lymph nodes to formulate clinicopathological characteristics and prognosis of gastric cancer with metastasis or micrometastasis to LN14v, repressing the largest numbers cases associated with LN14v metastasis or micrometastasis. It demonstrated location, pN stage, Borrmann type and the LN6 metastatic status were predictive factor of LN14v metastasis or micrometastasis, which implied that tumor located at M or L area, presented with pN3a or N3b stage, Borrmann I or II subtype and 6 metastasis were likely to present with 14v metastasis or micrometastasis. It screened out patient subgroup for whom the benefit may be maximized from LN14 dissection and those for whom LN14v dissection seemed to be not prognostic significant. Secondly, it revealed that micrometastatic status of LN14v lymph node was one of important prognostic factors. Lymph node micrometastasis could provide accurate prognostic information for patients with GC. Thus, immunohistochemical detection of micrometastasis of lymph nodes should be recommended.

It was a contentious issue whether LN14v metastasis was categorized to regional lymph node (local disease) or systemic disease [16]. When compared to those with locoregional lymph nodes metastasis, patients with LN14v metastasis had the worse 5-year survival rate (< 10%), and it was similar to that with LN16 metastasis, which was categorized to stage III, implying its systemic disease role. However, several studies demonstrated some patients with 14v metastasis would benefit from LN14v dissection and prolonged their survival, indicating at least some patients with 14v metastasis were at the state of local disease rather than systemic disease [16]. According to the second edition of Japanese Classification of Gastric Carcinoma, LN14v dissection was included in N2 group for tumors located at the lower third of the stomach [17]. But it was once classified as M1 status in the third edition of Japanese Classification of Gastric Carcinoma, which recommended it was unnecessary to dissect LN14v for patients with gastric cancer [17]. Furthermore, the 3th edition of Japanese gastric cancer treatment guidelines 2010 proposed patients with tumor located at the lower third of the stomach with No.6 metastasis need dissect LN14v [18]. When we evaluate whether it is essential for patients with GC to dissect LN14v, the 14v metastatic rate, clinicopathological features associated with 14v metastasis, security and feasibility of 14v dissection, the significance of dissection should be taken into account.

Lymphatic metastasis is considered to spread from lymphatic flows of the primary tumor site and the lymphatic flows from any special point have some preferred pathway [19–24]. There mainly exist three pathways in the region of lower stomach. The lymphatic drainage from LN6 directly flows to LN14v, and then lymphatic flows reach LN16, which finally joins to thoracic duct. In terms of lymphatic flow pathway, LN6 is anatomically upstream of LN14v, whose metastatic status is stringently correlated to that of LN6. The previous study investigated the impact of regional nodes metastatic status on LN14v metastasis [16]. It revealed that the metastatic status of LN6 and LN9 were predictive factors of LN14v metastasis. Our study was consistent with the previous research and the stepwise lymphatic metastasis theory. In our study, we found LN6 metastatic status was one significant independent variable for the metastatic status of LN14v. Of 146 patients with LN6 metastasis, 34.2% cases were presented with the metastasis or micrometastasis of LN14v. Similar to previous studies, the LN6 status predicted the absence of LN14v

metastases with a low false-negative rate (10.7%). Otherwise, the study also demonstrated tumor site, Borrmann classification and pN stage were also correlated with LN14v metastasis or micrometastasis. Tumor located at region of low or middle stomach, presented with Borrmann I/II subtype and pN2-3 stage were likely to be presented with metastasize or micrometastasize to 14v.

Various interactions factors promoted the occurrence and development of GC, presenting with complicated biological characteristics, high heterogeneity and undesirable prognosis [25]. One study reported that the 5-year survival rate of patients with GC with lymph node metastasis to LN14v was extremely low (11.3%), which was also described in another study with an undesired 5-year survival rate (9.0%) [26, 27]. Our study provided a comparable result that patients with LN14v metastasis or micrometastasis had unfavorable prognosis and the 5-year survival rate was 12.4%. It should be noted that patients with LN14v metastasis or micrometastasis presented with a lower 5-year survival rate in comparison with that with regional lymph nodes metastasis. But according to Sasako's therapeutic index theory that the therapeutic index of LN14v dissection was 2.1 in lower third GC, which was comparable with that of LN12a dissection (2.7), the N2 group lymph node dissection [28]. There is no denying that some patients would obtain some or less benefits from LN14v dissection. It is important to distinguish those would benefit from LN14v dissection. Eom et al deemed even if patients with LN14v metastasis presented with unfavorable prognosis, LN14v dissection could improve overall prognosis, especially in those with tumor site located at middle or low area, positive LN6 lymph node and clinical stage II/III gastric cancer [28]. Our study revealed tumor site, Borrmann classification and pN stage were significantly correlated with LN14v metastasis or micrometastasis, which kept consistent with the previous one. In matched analysis, we suggested patients with gastric cancer of stage II , U/M area, pN2-3 and LN 6(+) underwent lymphadenectomy of 14v suffered better survival than those without lymphadenectomy of 14v.

At present, detection methods of lymph node micrometastase mainly include serial section, PCR, and immunohistochemistry. Serial sections can significantly improve the positive rate of lymph node micrometastasis, but it is difficult to promote it in clinical practice in large scale because of its heavy workload. PCR is characterized by high sensitivity and specificity for lymph node micrometastasis detection, but the requirement of fresh samples, the relatively complicated operation process and high costs hindered the routine application in clinical pathological diagnosis. Compared with previous one, immunohistochemical method seems to be more useful in clinical work. Cytokeratin makes up one component of the cytoskeleton of epithelial cell, which is not present in normal lymph nodes. Ishii et al reported that the usage of CK8/18 monoclonal antibody represented one accurate method to detect lymph node micrometastasis in gastric cancer [7]. For 35 cases underwent lymph node micrometastatic detection, the positive rate was 11.4%. Yun et al also confirmed that CK8/18 monoclonal antibody is one reliable method in detecting lymph node micrometastasis, and its positive rate is as high as 31.3% [13]. Our study suggests that lymph node micrometastatic rate of 14v is only 3.9%. The discrepancy may result from different interval between serial sections, the harvested number of sections from different paraffin-embedded blocks, the included cases and the focused lymph node. In this study, patients with gastric cancer stage III accounted for 62.4%.

Although a majority of studies demonstrated that patients with lymph node micrometastasis would be presented with worse prognosis, the questions whether lymph node micrometastasis resulted in postoperative recurrence or metastasis and it consequently affected patients' prognosis remained controversial [10, 12, 29, 30]. For the micrometastatic status can be promoted or inhibited by various factors, such as the host's immune status, postoperative radiotherapy or chemotherapy, tumor microenvironment and so on. In accord with previous study, we revealed that the micrometastatic status is a significant variable associated with patients' survival. But the difference between survival of patients with LN14v micrometastasis and that of LN14v metastasis was not obvious, which may result from the limited samples of positive micrometastatic cases. So more multi-center, randomized trials are required to further investigate the extent of the impact of micrometastatic status on survival for GC. There is no doubt that micrometastatic status can be considered as one promising prognostic predictor in GC, which can provide accurate pathological staging and favorable treatment guidelines. We recommend patients with LN14v micrometastasis or on suspect of LN14v micrometastasis receive LN14v dissection.

Our study has some limitations, mainly for its retrospective case-control design and the limited participants. The small sample size may produce selection bias. Secondly, in comparison with the previous study, different interval between serial sections and the harvested number of sections from different paraffin-embedded blocks would influence the micrometastatic rate of LN14v. Thirdly, although the study revealed that LN14v metastatic or micrometastatic status was an independent risk factor associated with survival, randomized studies were required to clarify the advantages of LN14v dissection on survival for patients with LN14v metastasis or micrometastasis.

In conclusion, locally advanced gastric carcinoma, located at middle or low stomach area with LN6 metastasis were likely to be presented with metastasis or micrometastasis of LN14v and the D2 gastrectomy surgery plus LN14v dissection would be recommended for patient with gastric cancer of stage $\geq$ II, U/M area, pN2-3 and LN 6(+), if the serious complication of LN14v dissection can be sufficiently controlled. Micrometastatic status of LN14v can be considered as one promising prognostic predictor for GC.

Declarations

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Ethical approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and the study protocol was reviewed and approved by the Ethics Committee of Liao Ning Cancer Hospital. Informed consent was waived by Ethics Committee of Liao Ning Cancer Hospital.

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Competing interests

There were no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Author contribution

Xing Xu searched and reviewed the literature, designed the research and wrote the paper. Guoliang Zheng designed the research. Yan Zhao performed statistical analysis. Tao Zhang reviewed the literature and interpreted the paper. Zhichao Zheng participated in reviewing the literature, designing the research, revising and writing the paper.

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Figures

Figure 1

Kaplan-Meier curves was employed to compare the overall survival data between the negative group (without 14v micrometastasis and metastasis) and the positive group (with micrometastasis or metastasis) ($P=0.000$, $HR=4.001$, $95\%CI=2.789-5.739$)

Figure 2

Comparisons of overall survival by 14v metastasis or micrometastasis status in patients with GC. Kaplan-Meier curves were employed to analyze the difference between groups (the group without 14v micrometastasis or metastasis vs the group with 14v micrometastasis, $P=0.000$, $HR=2.093$, $95\%CI=1.480-2.961$; the group without 14v micrometastasis or metastasis vs the group with 14v metastasis, $P=0.000$, $HR=3.931$, $95\%CI=2.671-5.787$; the group with 14v micrometastasis vs the group with 14v micrometastasis, $P=0.901$, $HR=1.047$, $95\%CI=0.501-2.171$).

Figure 3

Overall survival after R0 resection categorized by tumor stage and 14v metastatic status (14v (+), 14v metastasis or micrometastasis; stage I, II, III). $P = 0.000$ (the group of 14v (+) vs the group of all stages, 14v (-), log-rank test).

Figure 4

Overall survival after R0 resection categorized by lymphadenectomy of 14v in patients with gastric cancer of stage $\geq$ U or Middle area, pN2-3 and LN 6(+), $P = 0.006$.

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