

Lymphatic Routes of Gastric Cancer Metastasis Elucidated by Triple Lymph Node Analysis

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Abstract

Background: Although the development of lymphatic invasion by gastric cancer has been solved using 'solitary lymph node metastasis' research, few facts are known about the sequential flow following the sentinel station.

Aim: In this study, we investigate the lymphatic spread of gastric cancers using 'triple lymph nodes metastases' analysis.

Methods: During January 2001 and December 2015, 402 subsequent patients with gastric cancer underwent gastrectomy with standardized lymph node dissection at our institute. Among them, lymph node involvement was revealed in 234 patients by a histopathological study. The lymphatic spread of gastric cancer was analyzed theoretically based on the probability of additional lymph node metastases from each station.

Results: The numbers of cases were 64, 41, 17, 23, 17, 13 and 59 according to the number of metastases of 1, 2, 3, 4, 5, 6 and 7 or more, respectively. The lesser curvature lymph node (#3) was the key station and skip metastasis was sometimes seen in lymph nodes of the intermediate region at the solitary lymph node metastasis stage. Some lymphatic flows were demonstrated by double and triple lymph node metastasis analysis, especially many flows from perigastric toward intermediate regions.

Conclusion: This study demonstrated theoretically a lymphatic route for gastric cancer cells using a novel methodology. The results may be applicable to making preoperative diagnosis and for deciding on optimal lymph node dissection in gastric cancer surgery.

Keywords Gastric cancer; Lymph node; Lymphatic flow

Introduction

The physics of motion of one or two objects has been elucidated by Newton using the 'Newtonian equation of motion'.

However, when three or more objects are present, it is very difficult to describe the state of the movements. This problem is called the 'three-body problem'.

It was proved by Poincaré that the motion of three objects could not be described by the usual differential and integral equations, and that the results could become very different according to the initial status. This proof has later led to the 'chaos theory'.

Although the development of lymphatic invasion by gastric cancer has been clarified using sentinel node biopsy technique [1] and 'solitary lymph node metastasis' research [2], little is known about the sequential flow from the sentinel station. In this study, we investigated the lymphatic spread of gastric cancers using a novel methodology, named 'triple lymph node metastases' analysis.

Methods

Data

During January 2001 and December 2015, 402 consecutive patients with gastric cancer underwent gastrectomy with standardized lymph node dissection at our institute. The distribution and frequency of lymph node metastases at each station were analysed. The position of the lymph node station (LNS) and the numbering were defined according to the Japanese Classification of Gastric Cancer (Table 1) [3].

This study was approved by the local ethics committees of St. Luke's International Hospital, and informed consent was obtained from all patients prior to surgery for the use of data for publication.

No.	Definition*	Involved LN
Perigastric region		
#1	Right paracardial LNs	4
#2	Left paracardial LNs	2
#3	Lesser curvature LNs	27

#4sa	Left greater curvature LNs along the short gastric arteries	1
#4sb	Left greater curvature LNs along the left gastroepiploic artery	1
#4d	Right greater curvature LNs	8
#5	Suprapyloric LNs	0
#6	Infrapyloric LNs	5
Intermediate region		
#7	LNs along the trunk of left gastric artery	9
#8a	Anterosuperior LNs along the common hepatic artery	3
#9	Celiac artery LNs	0
#10	Splenic hilar LNs	0
#11p	Proximal splenic artery LNs	3
#11d	Distal splenic artery LNs	0
#12a	Hepatoduodenal ligament LNs	1

Table 1: Anatomical definitions of lymph node stations and numbers of involved lymph node in the solitary lymph node metastasis analysis (*According to the Japanese Classification of Gastric Cancer [3]; LN: lymph node).

Statistical analysis and characterization of lymph node metastases

We hypothesized that pairwise and sequential relationships between LNSs might represent lymphatic cancer cell flows. In order to assess these relationships, we used the following approach. We evaluated the probability for each LNS metastasis occurring initially given that specific other LNS metastases had occurred previously as follows:

1) The quantity $N(A)$ represents the number of involved lymph nodes on station #A and the quantity $T(N)$ represents the given number of studied lymph nodes. In this condition, the quantity $P(A)=N(A)/T(N)$ represents the probability that lymph node metastasis on station #A would be detected in a given number of studied lymph nodes.

2) In addition, we determined the following parameters for multiple lymph node metastasis analysis. We determined $N(A\ B)$ as the number of paired involved lymph nodes existing simultaneously on stations #A and #B in a certain patient. We also determined $TP(N)$ as the number of possible pairings in a certain patient. In this condition, we defined the quantity $P(A\ B)=N(A\ B)/TP(N)$ as the probability that lymph node metastases on #A and #B were simultaneously detected in a certain patient.

3) Under these conditions, $P(A\ | \ B)=P(A\ B)/P(B)$ represents the probability that a #A LNS metastasis will be detected if a lymph node metastasis was detected on station #B. To assess whether there was a sequential relationship between the pair of LNSs from #A towards #B, we considered the ratio of $P(A\ | \ B)$.

4) In practice, $P(A\ | \ B)$ is calculated as $P(A\ | \ B)=P(A\ B)/P(B)=(N(A\ B)/TP(N))/(N(B)/T(N))$, where $T(N)$ and $TP(N)$ are constants, so we adopted $P'(A\ | \ B)=N(A\ B)/N(B)$ as the representation of the

probability that #A LNS metastasis would be detected if lymph node metastasis was detected on station #B.

5) This analysis was done for all analyses in this study. In the double lymph node metastases analysis, $P'(A\ | \ B)=N(A\ B)/N(B)$ indicates the lymphatic flow from #A towards #B. In the triple lymph node metastasis analysis, $P'(A\ B|C)=N(A\ B\ C)/N(C)$ indicates the lymphatic flow from a block of stations #A and #B towards #C.

Using the statistical analysis described above, solitary, double, and triple lymph node metastases were analyzed.

Results

Study population profile

The background data of the studied patients are shown in Table 2. The characteristics of the study population were almost the same as that in the nationwide survey by the Japanese Gastric Cancer Association [4]. Among the 402 patients who underwent gastrectomy, lymph node involvement was revealed in 234 (58%) patients by a histopathological study. Overall, 12,167 lymph nodes were dissected and examined, among which 1,309 (11%) lymph nodes were found to be involved with cancer cells. The numbers of cases were 64, 41, 17, 23, 17, 13 and 59 according to the number of metastases of 1, 2, 3, 4, 5, 6 and 7 or more, respectively.

Age (years)	
Median	67.5
Range	28-94
Gender	
Male	257
Female	145
Tumor location*	
U	89
M	152
L	161
Surgical procedures	
Distal	277
Total	103
Proximal	22
pT**	
T1	159
T2	63
T3	99
T4	81
pN**	
N0	168
N1	105

N2	70
N3	59
pM**	
M0	361
M1	41
pStage**	
I	167
II	104
III	90
IV	41
Histological findings	
Differentiated	161
Undifferentiated	235
Others	6

Table 2: Patient characteristics (*: U: upper third, M: middle third, L: lower third; **: According to Japanese Classification of Gastric Carcinoma [3])

In these 234 patients with positive lymph nodes, the median size of primary tumors was 47 mm (range, 19-155 mm). Among the patients with distant metastasis (M1), a simple gastrectomy without standardized lymph node dissection was performed for the patients with only cytological positive cancer cells in the peritoneum (CY1, P0). Some patients in the M1 group underwent gastrectomy with palliative intent because of bleeding or stenosis.

Solitary lymph node prevalence

Single lymph node involvement was seen in 64 patients (Table 1). Among them, 48 (75%) patients in perigastric stations (#1-#6), including 27 (42%) in #3, which may be the key station. Skip metastasis to intermediate stations (#7, #8, #11p, #11d, #12a) was seen in 16 (25%) patients in this solitary lymph node metastasis stage.

Double lymph node prevalence

We then sought to assess the sequential relationship between LNSs. Figure 1 demonstrates the extent to which the probability of an LNS metastasis occurring changes if another specific LNS metastasis occurred previously. In this heat map figure, red indicates the increased probability that an LNS metastasis is found if a specific prior LNS metastasis was present, and the extent of the probability change is indicated by the intensity of the colour. A careful analysis of these data indicates that some LNS metastases occur in isolation whereas others are likely to follow a previous LNS metastasis. For example, #1, #4sb, #9 and #11p are most likely to follow #3. Other cancer cell flows revealed were from #1 to #2, #11p and from #4d to #4sa, #4sb. In some LNSs where relatively rare lymph node involvement was detected (#10, #11d and #12a), the probability of lymphatic cancer cell flow was decreased by one rank. When a similar analysis was performed in the limited number of patients with relatively few lymph node involvement (2 to 6 lymph nodes involved, number of patients=111), some other

probable lymphatic cancer cell flows appeared (Figure 2). This subgroup analysis additionally showed other lymphatic flows from #3 towards #2, #5, #7, #8a and from #6 to #8a.

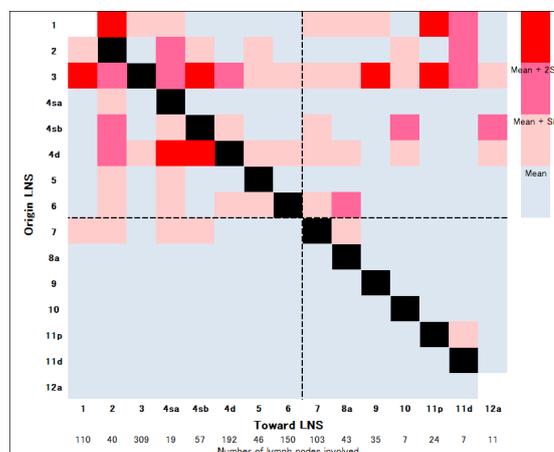


Figure 1: A heat map showing the sequential relationships between LNSs. The red color indicates the probability that an LNS metastasis is present if a specific prior LNS metastasis was found, and the extent of the probability is indicated by the intensity of the color. In some LNSs, where relatively rare lymph node involvement was detected (#10, #11d and #12a), the probability of lymphatic cancer cell flow was decreased by one rank. The dashed lines indicate the border between the perigastric and intermediate regions. LNS: Lymph Node Station.

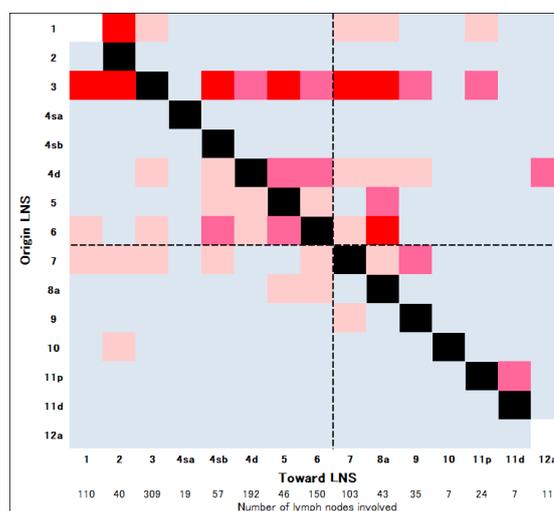


Figure 2: A heat map showing the sequential relationship between LNSs among the limited number of patients with relatively few lymph node involvements. Some additional probable lymphatic flows appeared in the analysis of the total number of cases shown in Figure 1.

Triple lymph node prevalence

If two LNSs are related in the double lymph node metastasis analysis, they are regarded as one block (LNS block) in the triple lymph node metastasis analysis. This paired comparison shows which LNS block influences the risk of other LNS metastases occurring and the weight of each relationship. The heat map figure indicates that #9 LNS metastasis is most likely to follow #3-4sb LNS block metastasis. Other cancer cell flows revealed were from the #1-3 block to #2, #11p and from the #1-2 blocks to #11p (Figure 3).

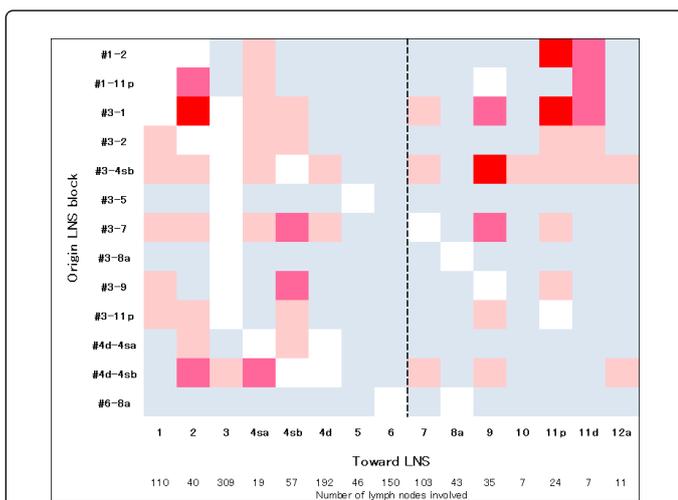


Figure 3: A heat map showing the triple lymph node metastasis analysis. It shows which LNS block influences the risk of other LNS metastases occurring and the weight of each relationship.

Discussion and Conclusion

The present study has demonstrated the probable cancer cell flow via lymphatic vessels in gastric cancer using a novel and unique approach. This study revealed the following: at an early stage, not only lymph nodes in the perigastric region but also some lymph nodes in intermediate regions are involved by gastric cancer cells. At the more advanced stage, intermediate lymph node-involvement increased much more than perigastric lymph node-involvement. This means that inter-regional flows toward the para-aortic area (from perigastric to intermediate region) are more prevalent than intra-regional flows (from perigastric to perigastric region and from intermediate to intermediate region).

Lymphatic routes in the stomach have been intensively investigated by many researchers historically. Sato carried out a detailed study of the lymphatic around the stomach based on minute macroscopic dissections of the lymphatic [5]. The results have strongly influenced the Japanese Classification of Gastric Carcinoma [3]. Kinami et al. [6-8] have advocated a lymphatic basin theory of the stomach as the proper pattern of lymphatic in early gastric cancer, and demonstrated the physiological lymphatic flow in the stomach. Although our results supported the lymphatic basin theory to some extent, it should be pointed out that the lymphatic flow might be different from these physiological flows when many lymph nodes are involved by gastric cancer cells. It is necessary to analyse cases with many lymph nodes involved by gastric cancer, such as those in our present study.

Kinami et al. [7] utilize their lymphatic basin theory in function-preserving gastrectomy by omitting the standard lymph node dissection. From a prognostic perspective in gastric cancer, the omission of lymph node dissection is problematic because preoperative or intraoperative lymph node staging in gastric cancer patients is difficult regardless of various efforts [9,10]. At present, supported by the results of our present study, lymph node dissection including intermediate regions seems to be necessary in patients with a possible lymph node involvement. Japanese gastric cancer treatment guidelines recommend D2 standard gastrectomy in almost all stages of gastric cancer [11].

Sentinel node biopsy is now widely used to predict the nodal state in early gastric cancer after it was demonstrated to be feasible in a prospective multicenter trial [1]. The recent inventions of useful devices and computer-aided diagnosis are being utilized in gastric cancer surgery [12,13]. More recently, some novel and unique methodological approaches have been widely used in various medical fields [14,15]. Solitary lymph node metastasis analysis and our present study might be included among these innovative research advances [2].

The limitations of our study included the primitive methodology and the small number of patients. If we can acquire much more data, it may be possible to perform more extensive analyses such as lymphatic flow analysis at each tumor location (upper, middle and lower third) in gastric cancer. We would then be able to utilize the data for practical use, such as function-preserving surgery with the omission of the standard lymph node dissection,

In conclusion, this study demonstrated theoretically a lymphatic route for metastatic spreading of gastric cancer cells using a novel methodology. The results may be useful in allowing more accurate preoperative diagnosis of lymph node involvement in gastric cancer and may be applicable in deciding the optimal lymph node dissection.

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