

ORIGINAL ARTICLE

## Towards a tailored lymphadenectomy for gastric cancer based on the correlation between the primary tumor location and the first lymphatic drain basin: Preliminary data

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### Summary

**Purpose:** The contradictory long-term results following D2 lymphadenectomy have revealed the necessity for a more tailored lymphadenectomy in cases of gastric cancer. Among the patients who had undergone a modified D2 lymphadenectomy for gastric cancer, we further analyzed the subgroup in which histologically and immunohistochemically solitary lymph node metastases were detected. Classifying the primary tumors as towards the lesser and towards the greater curvature, we propose possible routes of lymphatic spread and possible clinical implications.

**Method:** Between January 2007 and December 2016, 212 patients suffering from gastric adenocarcinoma underwent a modified D2 lymphadenectomy. Solitary lymph node metastases were detected by histology in 14 patients (7 skip metastases) and by immunohistochemistry in an additional 10 patients (5 skip micrometastases).

**Results:** The incidence of the histologically detected solitary lymph node metastases was 6.6% for the whole cohort, in-

creasing to 11.3% with the use of immunohistochemistry. The incidence of the histologically detected skip solitary lymph node metastases was 3.3% for the whole cohort, increasing to 5.7% with the use of immunohistochemistry. Tumors of the lower and middle third of the stomach were equally drained both to the level I and II lymph node stations. However, tumors towards the lesser curvature were mainly drained in the level II lymph node stations (12 out of 19; 63%), while tumors towards the greater curvature were all drained in the level I lymph node stations (5 out of 5; 100%).

**Conclusion:** Primary gastric tumors towards the lesser curvature should be treated by a modified D2 lymphadenectomy. However, for tumors towards the greater curvature, a D1(+) lymphadenectomy always including the no. 7 & 9 lymph node stations complex, might be enough.

**Key words:** gastric cancer, D2 lymphadenectomy, micrometastases, skip metastases, solitary lymph node metastasis, transverse metastases

### Introduction

Histologically confirmed metastatic infiltration of peri- and extra-gastric lymph nodes has been defined as the strongest independent dismal prognostic factor for both early [1] and advanced [2] gastric cancer patients.

Thus, it could be proposed that by performing a D2 lymphadenectomy, apart from the removal of

the gross disease in the stomach and in the regional lymph nodes (LN), coexisting micrometastases are also resected [3], and coexisting skip metastases and micrometastases are resected as well [4], thus more R0 resections are achieved [5], facts probably leading to locoregional control of the disease, better outcome and increased survival [6].

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Received: 14/04/2017; Accepted: 26/04/2017

However, prospective randomized studies [7-11] as well as a meta-analysis [12] revealed higher statistically significant postoperative morbidity and mortality rates (mainly related to the surgical technique) following D2 lymphadenectomy and no 5-year and 11-year survival benefit compared to the D1. Only one study [13] disclosed that after a median follow-up of 15 years, D2 lymphadenectomy was associated with lower locoregional recurrence and gastric cancer-related death rates, compared to D1 operation.

Due to the disappointing early postoperative results published in the Western countries following D2 lymphadenectomy, as well as the contradictory long-term results after D2 lymphadenectomy published by Eastern and Western authors, the necessity for a more tailored lymphadenectomy was emerged. On that field, literature addresses the central and crucial role of the solitary lymph node metastasis (which represents the first lymphatic drain basin), in correlation to the location of the primary tumor. Although the sentinel lymph node concept has severe limitations due to the high percent of false-negative results [14], the research is continued.

In the present study, among the patients who had undergone D2 lymphadenectomy for gastric cancer, we further analyzed the subgroup in which histologically and immunohistochemically solitary lymph node metastases were detected. Classifying the primary tumors as towards to the lesser and towards to the greater curvature, we propose possible routes of lymphatic spread and possible clinical implications.

## Methods

From 2007 onwards, we prospectively collected all patients who were referred to our Department, for further investigation and treatment, having been diagnosed with gastric tumors. Demographics, clinical data, adjuvant or neo-adjuvant therapies, type of operation, postoperative complications, histological findings, follow-up and time elapsed to either local or distant recurrence were prospectively recorded.

Excluding patients (i) who were diagnosed with histological types others than adenocarcinoma, (ii) with pathologically serosa-positive gastric cancer, (iii) who had undergone neoadjuvant therapies, (iv) who were operated on for palliation, (v) who suffered from concomitant metastatic disease and (vi) who had undergone D0 or D1 lymphadenectomy, a total of 212 adenocarcinoma patients were submitted to D2 lymphadenectomy, as first therapeutic option with curative intent, between January 2007 and December 2016.

Postoperatively, standard histological examination by hematoxylin and eosin (HE) staining, disclosed metastatic infiltration of at least two lymph nodes in

the level I and/or II peri- and extragastric lymph node stations in 152 patients.

Solitary lymph node metastases were histologically detected in 14 patients, while the remaining 46 patients were classified as pN0.

All lymph nodes of the 14 patients with histological solitary lymph node metastases were further submitted to immunohistochemistry for micrometastases detection, but no micrometastases were revealed.

All lymph nodes of the 46 patients who had been classified as pN0 by histology, were further submitted to immunohistochemistry for micrometastases detection. No patient with more than one micrometastasis were detected. In 5 patients micrometastases were detected in the level I perigastric lymph nodes stations and in an additional 5 patients micrometastases were detected in the level II extragastric lymph nodes stations (skip micrometastases).

Overall, 24 patients, with histologically (n=14) and immunohistochemically (n=10) detected solitary lymph node micrometastases, constituted the material of the present study and were further analyzed.

### *Surgical technique*

The dissection of the regional lymph nodes was based on the Japanese Classification of Gastric Carcinoma [15]. Thus, for D1 lymphadenectomy, the appropriate (depending on the location of the primary tumor) no. 1-6 lymph node stations were included in the gastrectomy specimen, while in the modified D2 lymphadenectomy, the no. 7, 8a, 9, 11p, 11d and 12a lymph node stations were routinely dissected (Figure 1). The level II lymph node stations were recognizable as they had been sent separately to the Pathology Department with special indices demonstrating their exact location. Dissection of the no.10 lymph node station, splenectomy or distal pancreatectomy was not performed in any of the patients. For staging of the tumors, the TNM classification system according to the AJCC Staging Manual, 7<sup>th</sup> edition, was used [16].

### *Histopathology and immunohistochemistry*

Primary tumors and lymph nodes were fixed in formalin and embedded in paraffin. The presence or absence of lymph node metastasis was examined routinely by HE staining using a representative cut section through the largest diameter of the lymph nodes.

One additional section of 4- $\mu$ m thickness from each node was prepared for immunohistochemical staining with a monoclonal anti-cytokeratin (CK) antibody cocktail (AE1/AE3; Dako, Glostrup, Denmark) that reacts with a broad spectrum of human CKs, to detect micrometastases and/or clusters of isolated tumor cells. Briefly, for AE1/AE3 immunostaining paraffin-embedded sections were de-paraffinized in xylene and rehydrated through gradual ethanol dilutions. Endogenous peroxidase activity was blocked by incubation for 30 min with 1% hydrogen peroxide, and antigen retrieval was performed by autoclaving sections in 0.01 mol/L citrate buffer, pH 6.0 for 20 min at 800 W. A monoclonal mouse anti-human CK antibody (clone AE1/AE3) was

applied at a dilution of 1:50. The Dako Real Envision kit was then used. Diaminobenzidine was used as chromogen. Lymphoid tissue was used as an internal negative control, while additional sections from the primary tumors were used as positive controls.

### Statistics

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS), version 17.0 (SPSS Inc., Chicago, Ill, USA). Fisher's test was used for calculating the association between clinicopathological characteristics. A  $p$  value  $<0.05$  was considered statistically significant. Because of the small number of patients, multivariate analysis was not performed.

## Results

Solitary lymph node metastases were detected by histology in 14 patients and by immunohistochemistry in an additional 10 patients. There were 13 males with a median age of 70 years (IR 60-75.5) and 11 females with a median age of 69 years (IR 56-80). The mean tumor size was  $45.6 \pm 25.17$  mm and the mean number of nodes resected was  $25.21 \pm 12.2$ . The majority of tumors ( $n=12$ ) were of enteric subtype, whereas 6 tumors were of diffuse subtype and 6 had mixed histology. Table 1 summarizes the data of the present study. The majority of tumors ( $n=14$ ) were graded as moderate, whereas 9 tumors were graded as low and only 1 case as high grade malignancy.

Solitary metastases were detected in 12 patients in the level I lymph node station (7 by histology and 5 by immunohistochemistry) and in the level II lymph node stations in 12 patients (7 by histology and 5 by immunohistochemistry). With regard to frequency, the solitary lymph node metastases were located in the no 7 ( $n=7$ ), no 6 ( $n=6$ ), no 5 ( $n=4$ ), no 9 ( $n=2$ ), no 8 ( $n=2$ ), no 3 ( $n=1$ ), no 4 ( $n=1$ ), and no 12 ( $n=1$ ) lymph node stations (Figure 2).

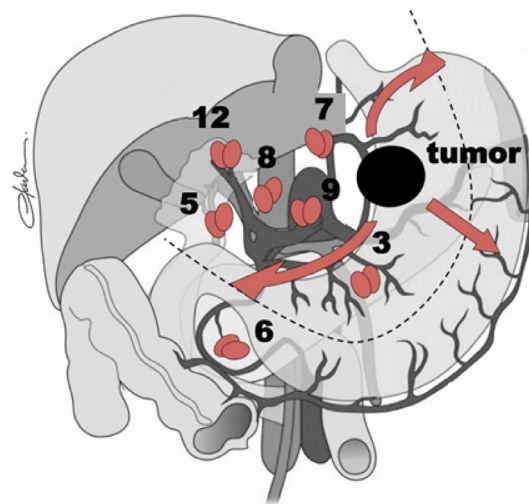
The above findings showed that the incidence of the histologically detected solitary lymph node metastases was 6.6% for the whole cohort (14 out of 212), while this incidence increased to 11.3% (24 out of 212) with the use of immunohistochemistry ( $p=0.05$ ).

The incidence of the histologically detected skip solitary lymph node metastases was 3.3% for the whole population (7 out of 212), while this incidence increased to 5.7% (12 out of 212) with the use of immunohistochemistry. Skip metastases represented the 50% (7 out of 14) of the histologically detected, 50% (5 out of 10) of the immunohistochemically detected and 50% (12 out of 24) of all solitary lymph node metastases. Of interest, the majority of skip metastases were found at the

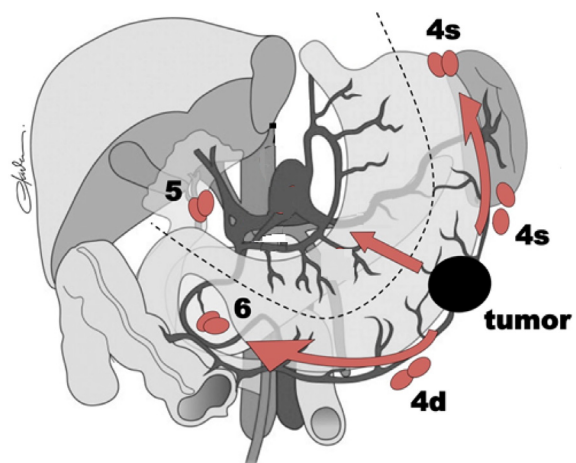
no. 7 lymph node station, indicating a tropism towards this specific nodal station compared to the other nodal stations ( $p=0.043$ ).

The majority of solitary metastases was found along the lesser curvature (17 vs 7 patients;  $p=0.003$ ). Solitary metastases in the level I lymph node stations were mainly located along the greater curvature (7 out of 12 patients), while solitary metastases in the level II lymph node stations were mainly located in the no. 7-9 lymph node stations complex (9 out of 12 patients).

Low T stage tumors (T1-2) were correlated with increased incidence of solitary metastases (16 vs 8;  $p=0.04$ ). Especially for these patients with T1-2 tumors ( $n=16$ ), solitary lymph node metastases were detected in the level II lymph node



**Figure 1.** Correlation between primary tumors towards the lesser curvature (black spot) and the location of their solitary lymph node metastasis (red spots). Note that tumors towards the lesser curvature drained both in level I and level II LN stations.



**Figure 2.** Correlation between primary tumors towards the greater curvature (black spot) and the location of their solitary lymph node metastasis (red spots). Note that tumors towards the greater curvature all drained in the level I LN stations.

**Table 1.** Profile of the patients included in this study

Gender/ Age	Tumor location		Tumor size (mm)	T	Type of LN infiltration	(+) LN station	No of LN retrieved	Histological type	Grade	Type of operation
M/84	Middle	Lesser/ Posterior	15	T1	Histol. Metastasis	6	18	Enteric	Moderate	Subtotal gastrectomy
F/80	Lower	Lesser/ Anterior/ Posterior	65	T2	Histol. Metastasis	6	12	Mixed	Low	Subtotal gastrectomy
F/83	Middle	Lesser	70	T3	Histol. Metastasis	6	23	Diffuse	Moderate	Subtotal gastrectomy
M/72	Middle	Greater	48	T2	Histol. Metastasis	4	19	Enteric	Moderate	Subtotal gastrectomy
F/79	Lower	Lesser/ Posterior	80	T3	Histol. Metastasis	5	24	Enteric	Moderate	Subtotal gastrectomy
M/81	Middle	Lesser/ Posterior	20	T1b	Histol. Metastasis	6	16	Enteric	High	Subtotal gastrectomy
M/74	Upper	Lesser/ Anterior	35	T3	Histol. Metastasis	3	32	Mixed	Low	Total gastrectomy
M/70	Lower	Lesser/ Posterior	14	T3	Histol. Skip Metastasis	8	26	Diffuse	Low	Subtotal gastrectomy
F/64	Lower	Lesser/ Anterior	21	T1	Histol. Skip Metastasis	12	13	Enteric	Moderate	Subtotal gastrectomy
F/56	Lower	Lesser/ Anterior/ Posterior	30	T1	Histol. Skip Metastasis	7	14	Diffuse	Moderate	Subtotal gastrectomy
M/62	Middle	Lesser/ Posterior	85	T2	Histol. Skip Metastasis	7	14	Enteric	Low	Total gastrectomy
F/47	Upper	Lesser/ Posterior	30	T2	Histol. Skip Metastasis	9	33	Mixed	Low	Total gastrectomy
M/64	Middle	Lesser/ Anterior/ Posterior	60	T2	Histol. Skip Metastasis	9	34	Enteric	Moderate	Subtotal gastrectomy
M/53	Upper	Lesser/ Posterior	35	T3	Histol. Skip Metastasis	8	54	Enteric	Moderate	Total gastrectomy
M/70	Lower	Lesser/ Anterior	35	T3	Micrometastasis	5	16	Diffused	Low	Subtotal gastrectomy
M/77	Lower	Greater/ Anterior	50	T2	Micrometastasis	5	15	Enteric	Moderate	Subtotal gastrectomy
F/83	Lower	Greater	100	T3	Micrometastasis	6	15	Mixed	Low	Subtotal gastrectomy
F/69	Lower	Greater/ Posterior	65	T2	Micrometastasis	5	43	Enteric	Low	Subtotal gastrectomy
M/70	Lower	Greater	45	T3	Micrometastasis	6	27	Mixed	Moderate	Subtotal gastrectomy
F/62	Lower	Lesser/ Posterior	4	T2b	Skip micrometastasis	7	51	Mixed	Moderate	Subtotal gastrectomy
F/56	Middle	Lesser	30	T1	Skip micrometastasis	7	16	Diffuse	Moderate	Subtotal gastrectomy
M/54	Middle	Lesser/ Anterior	17	T2a	Skip micrometastasis	7	38	Enteric	Low	Subtotal gastrectomy
F/71	Lower	Lesser/ Posterior	26	T2a	Skip micrometastasis	7	29	Diffuse	Moderate	Subtotal gastrectomy
M/58	Lower	Lesser/ Anterior/ Posterior	66	T2b	Skip micrometastasis	7	23	Enteric	Moderate	Subtotal gastrectomy



**Table 2.** Summary of significant findings of the present study

Parameters	Values	p value
Skip metastases (LN 7 station vs all other LN stations)	7/12 vs 5/12	0.043
Solitary metastases along the lesser curvature	17/24 vs 7/24	0.0001
Low T tumors (T1-2) and incidence of solitary metastases	16/24 vs 8/24	0.04
Tumors along the lesser curvature mainly drained in the level II LN stations, while tumors along the greater curvature always drained in the level I LN stations	12/19 vs 5/5	0.037

LN: lymph node

stations in 10 of them, and mainly in the no. 7-9 lymph node station complex (9 out of 10).

Tumors of the lower third of the stomach drained equally both to the level I (7 out of 13) and level II (6 out of 13) lymph node stations. Tumors of the middle third of the stomach also drained equally both to level I (4 out of 8) and level II (4 out of 8) lymph node stations.

However, tumors towards the lesser curvature mainly drained in the level II lymph node stations (12 out of 19; 63%; Figure 1), while tumors towards the greater curvature all drained in the level I lymph node stations (5 out of 5; 100%;  $p=0.037$ ; Figure 2).

Table 2 summarizes the factors significantly correlated with the presence of solitary nodal metastases in patients with gastric cancer.

## Discussion

The present study discovered that by using immunohistochemistry, both the incidence of the solitary lymph node metastases, as well as the incidence of skip metastases nearly doubled (11.3 vs 6.6% and 5.7 vs 3.3%, respectively), compared to histology alone.

The 7<sup>th</sup> TNM classification [16] clearly stated for the first time that the presence of micrometastases (N1mi) should be mentioned, although did not include them in the staging of the disease and did not correlate their presence to prognosis yet.

However, experimental data [17] have shown that micrometastases in lymph nodes have high proliferative activity, thus they have metastatic potential, while clinical data have shown a 5-year survival of 100% for the micrometastasis-negative compared to 85% for micrometastasis-positive gastric cancer patients [18], a 50% shorter survival for the micrometastases-positive compared to the micrometastases-negative early gastric cancer patients who died of disease recurrence [19] and presence of micrometastases in 50% of the early gastric cancer patients who presented with disease recurrence having been classified as pN0 on

the initial conventional histology [20]. Based on the above data, undoubtedly micrometastases cannot be ignored.

The clinical significance of skip metastases remains controversial. On one hand, Saito et al. comparing gastric cancer patients with skip metastasis to patients with metastasis in the level I and II lymph node stations, concluded that the prognosis of patients with metastasis in the level II was significantly worse than that of patients with either skip metastasis or metastasis in the level I lymph nodes [21]. Moreover, gastric cancer patients with metastatic lymph nodes extending to the extraperigastric area, had significantly unfavorable prognoses compared with patients with metastatic lymph nodes within the perigastric area, while lymph node metastasis to the extraperigastric area was an independent dismal prognostic factor [22]. On the other hand, Li et al. reported that the cumulative survival rate was not statistically different between gastric cancer patient with solitary skip lymph node metastases compared to patients with solitary level I lymph node metastases [23]. Park et al. reported that in patients with positive nodes extending into the level II lymph node station, the survival curves did not show statistical differences between skip(+) and skip(-) groups of patients [3]. Finally, Ma et al. concluded that there was no survival difference between the skip metastasis group and the other solitary lymph node metastasis group [24].

An interesting finding of the present study is that primary gastric tumors towards the greater curvature never metastasized into the level II lymph node stations, even in the presence of transverse metastases, while primary gastric tumors towards the lesser curvature were characterized by a more unpredictable lymphatic spread.

Traditionally, studies on the lymphatic stream from gastric tumors were based on their classification as tumors of the upper, middle and lower third of the stomach. According to this classification, no.1 and 3 lymph node stations were the most common first metastasized stations in upper-third tumors, while no. 3, 4 and 6 lymph node stations

were more frequently metastasized in lower and middle-third tumors [25,26].

Tokunaga et al. reported that primary gastric cancer in the upper half of the stomach and posterior wall accord skip metastasis in the no.11p lymph node station, proposing the presence of a lymphatic drainage route along the posterior gastric artery directly into the no. 11p lymph node station [27].

Liu et al. found that in patients with cancer of the upper stomach at the lesser curvature region, the no. 7 and no. 8 lymph nodes should be treated as N1 disease, in patients with cancer of the middle stomach at the lesser curvature region, the no. 7 lymph nodes should be also treated as N1 disease, whereas in patients with cancer of the lower stomach at the lesser curvature region, the no. 1, no. 7 and no. 8 lymph nodes should be inspected more carefully [28]. In patients with cancer of the middle stomach at the greater curvature region, the no.10 lymph nodes should be inspected more carefully and if no. 10 lymph nodes are questionable, resection of the spleen should be undertaken, although no. 10 lymph nodes can be considered as a N3 lymph node station for the middle area gastric cancer.

Lee et al. found 18% transversal metastases rate (mainly to the no. 6 lymph node station) for tumors of the lesser curvature, but only 6.3% transversal metastases rate (mainly to the no. 3 lymph node station) for tumors of the greater curvature. The authors concluded that a lesser curvature tumor was a risk factor for skip metastasis, while tumors located circumferentially in the lesser curvature or longitudinally in the lower

third of the stomach were independent risk factors for transversal metastasis [29].

Based on the traditional division of gastric cancer in upper, middle and lower third disease, we have previously proved [30] that without a modified D2 lymphadenectomy, a true R0 resection was not achieved in almost 12% of the patients, while since the most likely route for para-aortic lymph node metastases is from the left gastric artery nodes passing by the celiac artery [31], these lymph nodes should be always evaluated, regardless of the location of the primary tumor and the type of operation.

## Conclusions

The results of the present study have shown that primary gastric tumors towards the lesser curvature mainly drained in the level II lymph node stations, thus these tumors should be treated with a modified D2 lymphadenectomy. On the other hand, tumors towards the greater curvature always drained in the level I lymph node stations, thus a D1(+) lymphadenectomy, always including the no. 7 & 9 lymph node stations complex, might be enough.

## Acknowledgement

Eleftherios Spartalis made the artistic work in both Figures 1 and 2.

## Conflict of interests

The authors declare no conflict of interests.

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