Peer Review File

Article Information: https://dx.doi.org/10.21037/jgo-22-147

Reviewer A

In this retrospective Western study, the authors in investigate lymph node station involvement for patients with gastric cancer. Although relatively small compared to Eastern studies, the findings add to the existing literature supporting extended lymph node dissection for more advanced cancers. The authors are correct that Western studies are needed to support lymph node classification systems. It is relatively surprising that regional lymph nodes were involved in 25% of patients with cT1 cancer, but there were no involved lymph nodes in a D2 distribution. The limitation of potential downstaging due to preoperative therapy is addressed. However, due to the known inaccuracy of EUS and clinical staging, I would be hesitant to omit a D2 lymph node dissection in any patient - especially since it can be done without any added morbidity.

Reply:
Thank you for this comment and your time for reviewing our manuscript. We agree with the reviewer that difficulties exist with preoperative staging and accurate clinical staging is known to be unreliable, especially with regard to lymph node metastases. We also agree with the reviewer that a D2 lymphadenectomy cannot be omitted based on these data. In addition, previous trials comparing D1/D2 had significant homogenization (especially the Dutch trial), which reduces the probability of detecting a potential advantage of D2. Further research is very much needed. We have added the following sentences to the discussion

Changes in the text (changes underlined):
Page 15, line 11-14 (discussion):
One of these trials; Bonenkamp et al. showed a higher morbidity (43% versus 25%) and mortality (10% versus 4%) after D2 lymphadenectomy (28). 5-year follow-up showed no significant difference in overall survival (D2: 47% versus D1: 45%). 15-year follow-up showed a significant difference in loco-regional recurrence after D1 lymphadenectomy (D2: 21.8% versus D1: 40.7%) (5). In addition, there was a high cross-over in this study: 52% of patients in de D1 group underwent a more extended lymphadenectomy than D1, while in de D2 group, 84% underwent a more limited dissection than D2 (29).
Page 16, line 12-15 (discussion):
This controversy in current literature between the superiority of either, D1 or D2 lymphadenectomy, and a trend toward improved survival for D1 or D2 depending on T-stage and N-stage suggest the necessity for a more tailored approach. In addition, based on the data of the current study, in combination with unreliable staging, a D2 lymphadenectomy for cT1 may not be omitted, and further, larger and prospective studies are needed to confirm these study results.

Reviewer B

I have read with pleasure your article dealing with the retrospective analysis of the rate of lymph node metastases in station 8-12 for each cT-stage in gastric cancer.
This manuscript is well written and interesting.

1. Please describe the rate of indication EUS and diagnostic laparoscopy.

Reply 1:
We agree that these are important figures. The rate of EUS indication was 41.1% (46/112), the rate of diagnostic laparoscopy was 18.8% (21/112). We have added these data to the text.

Changes in the text (changes underlined):
Page 9, line 14-18 (results):
Eight patients (7.1%) were diagnosed with a cT1 gastric tumor, 18 patients (16.1%) with a cT2 tumor, 57 patients (50.9%) with a cT3 tumor and 5 patients (4.5%) with a cT4 tumor. Clinical T-stage was not recorded in 24 patients (21.4%). 46 patients (41.1%) underwent EUS screening and 21 patients (18.8%) underwent a diagnostic laparoscopy previous to treatment. 34 patients (30.4%) were operated minimally invasively and 78 (69.6%) open. A total gastrectomy was performed in 54 patients (48.2%) and a subtotal gastrectomy in 58 patients (51.8%), with a Roux-Y or Billroth-II reconstruction.

2. Endoscopic diagnosis and imaging are critical to this study. Is the diagnostic capability ensured? For example, endoscopy was performed by an expert endoscopist with at least 10 years of experience.

Reply 2:
Diagnostic screening was performed by an experienced radiologist and/or experienced endoscopist. We have added this to the methods section of the manuscript.

Changes in the text: (changes underlined):
Page 7, line 14-16 (methods):
Diagnostic screening was performed by an experienced radiologist dedicated to GI care and/or an experienced endoscopist dedicated to upper GI cancer care.

3. Please add significant differences to the table.
Reply 3: we agree with the reviewer on this point. The table is adjusted based on the previous comments of the reviewer.

Changes in text:
Table 1 was adjusted (page 18)

Table 1. Patients characteristics (N (%)

Characteristics	Total (N=112)	cT1 (N=8)	cT2 (N=18)	cT3 (N=57)	cT4 (N=5)	cTx (N=24)	P value**
Age at time of surgery - Yr							0.45
Median	66	74	62	66	63	67	
Range	32-86	33-83	39-78	41-86	55-73	32-82	
Sex - no. (%)							0.08
Male	79 (70.5)	4 (50.0)	10 (55.6)	46 (80.7)	2 (40.0)	17 (70.8)	
Female	33 (32.5)	4 (50.0)	8 (44.4)	11 (19.3)	3 (60.0)	7 (29.2)	
Additional diagnostic screening– no. (%)							
EUS	46 (41.1)	5 (62.5)	4 (22.2)	24 (42.1)	3 (60.0)	10 (41.7)	0.30
Diagnostic laparoscopy	21 (18.8)	1 (12.5)	2 (11.1)	13 (22.8)	3 (60.0)	2 (8.3)	0.07
Neo-adjuvant therapy - no. (%)							0.24
Chemotherapy	78 (69.6)	2 (25.0)	15 (83.3)	40 (70.2)	4 (80.0)	17 (70.8)	
None	34 (30.4)	6 (75.0)	3 (16.7)	17 (29.8)	1 (20.0)	7 (29.2)	
Surgical approach - no. (%)							0.53
Minimal invasive	34 (30.4)	3 (37.5)	5 (27.8)	14 (24.6)	3 (60.0)	9 (37.5)	
Open	78 (69.6)	5 (62.5)	13 (72.2)	43 (75.4)	2 (40.0)	15 (62.5)	
Resection - no. (%)							0.19
Total gastrectomy	54 (48.2)	5 (62.5)	5 (27.8)	27 (47.4)	5 (100)	12 (50.0)	
Subtotal gastrectomy	58 (51.8)	3 (37.5)	13 (72.2)	30 (52.6)	0	12 (50.0)	
Tumor location - no. (%)							0.37
Fundus	11 (9.8)	0	1 (5.6)	7 (12.3)	1 (20.0)	2 (8.3)	
Corpus	24 (21.4)	4 (50.0)	3 (16.7)	11 (19.3)	2 (40.0)	4 (16.7)	
Antrum	34 (30.4)	3 (37.5)	10 (55.6)	13 (22.8)	0	8 (33.3)	
Pylorus	15 (13.4)	0	1 (5.6)	11 (19.3)	0	3 (12.5)	
Whole rumen	11 (9.8)	1 (12.5)	1 (5.6)	4 (7.2)	1 (20.0)	4 (16.7)	
Oesophagus and gaster	9 (8.0)	0	2 (11.1)	5 (8.8)	1 (20.0)	1 (4.2)	
Unknown	8 (7.1)	0	0	6 (10.5)	0	2 (8.3)	
Tumor size - cm							0.25
Median	4	2	4	4	5	4	
Range	0-15.0	0-5.0	0.2-6.5	0-15.0	3.5-7.0	1.0-8.0	
Differentation no. (%)							0.744
Well to moderate differentiated	23 (20.5)	2 (25.0)	2 (11.1)	13 (22.8)	2 (40.0)	4 (16.7)	
Poorly to undifferentiated	59 (52.7)	4 (50.0)	11 (61.1)	31 (54.4)	2 (40.0)	11 (45.8)	
Unknown	30 (26.8)	2 (25.0)	5 (8.3)	13 (22.8)	1 (20.0)	9 (37.5)	
Lauren classification no. (%)							0.746
Intestinal type	39 (34.8)	2 (25.0)	8 (44.4)	20 (35.1)	2 (40.0)	7 (29.2)	
Diffuse type	36 (32.1)	3 (37.5)	5 (27.8)	15 (26.3)	1 (20.0)	12 (50.0)	
Mixed	6 (5.4)	0	1 (5.6)	3 (5.3)	1 (20.0)	1 (4.2)	
Unknown	31 (27.7)	3 (37.5)	4 (16.7)	19 (33.3)	1 (20.0)	4 (16.7)	
Pathological T-stage - no. (%)							0.01*
(y)pT1	23 (20.5)	4 (50.0)	8 (44.4)	6 (10.5)	1 (20.0)	4 (16.7)	
(y)pT2	22 (19.6)	1 (12.5)	4 (22.2)	10 (17.5)	0	7 (29.2)	
(y)pT3	33 (29.5)	1 (12.5)	2 (11.1)	21 (36.8)	1 (20.0)	8 (33.3)	
(y)pT4	27 (24.1)	0	4 (44.4)	15 (26.3)	3 (60.0)	5 (20.8)	
(y)pT0***	7 (6.3)	2 (25.0)	0	5 (8.8)	0	0	
Clinical node status - no. (%)							< 0.01*
Positive	45 (40.2)	1 (12.5)	6 (33.3)	32 (56.1)	3 (60.0)	3 (12.5)	
Negative	56 (50.0)	7 (87.5)	9 (50.0)	22 (38.6)	1 (20.0)	17 (70.8)	
Unknown	11 (9.8)	0	3 (16.7)	3 (5.3)	1 (20.0)	4 (16.7)	
Pathological node status - no. (%)							0.03*
Positive	60 (53.6)	2 (25.0)	11 (61.1)	33 (57.9)	5 (100)	9 (37.5)	
Negative	52 (46.4)	6 (75.0)	7 (38.9)	24 (42.1)	0	15 (62.5)	
No. of lymph nodes dissected							0.21
Median	25	21	25	27	27	22	
Range	4-72	4-32	6-47	4-72	23-39	8-45	
No. of positive lymph nodes							0.15
Median	1	0	1	2	5	0	
Range	0-40	0-1	0-40	0-17	1-12	0-10	

Reviewer C

The status of lymph node metastasis in gastric cancer is important when considering surgical treatment of gastric cancer. There are many articles that examine the rate of lymph node metastasis of gastric cancer in detail, however, most of them are conducted on patients in East

Asia, and this study on Western patients is of certain significance.
However, this article has the following problems, and unfortunately, the value of the article is not high.

1. The number of patients is too small. In a study of lymph node metastasis rate of T1 2368 patients by Tanaka et al., the rate of metastasis to No. 7 nodes was 1.4%, to No. 8a 0.63%, to No. 9 0.72%, and to No. 11p 0.42%. The metastasis rate of pT1 cancer to suprapancreatic nodes is originally low, and at least 200 cases must be examined for accurate calculation.
(Tanaka N, Katai H, Taniguchi H, Saka M, Morita S, Fukagawa T, et al. Surgical Treatment for Early Gastric Cancer (in Japanese With English Abstract). Stomach Intest (2009) 44: 700–6. Doi: 10.11477 / mf.1403101642)

Reply 1:
We agree with the reviewer that numbers of patients is not very high but this is a case series of patients from a single expert center in the Netherlands, where only 600 gastrectomies are performed each year in the whole country. In addition, this is the first study to report on this in a Western gastric cancer population, which is an important first step, as results from Asian studies cannot be directly extrapolated to the Western gastric cancer population, where patients are more obese and all get neoadjuvant chemotherapy before surgery. The results of this study, with its relatively small sample size, seem to justify the application of the Japanese guideline to this Western population with regard to lymphadenectomy. We very much agree that this is only a first small step and that larger, prospective studies are needed to follow-up on this subject.

Changes to text (changes underlined):
Page 17-18, line 21-4 (discussion):
This study has several limitations. Firstly, the small sample size; only eight patients with a cT1-stage could be included. <u>In the Netherlands, gastric cancer is a quite rare disease with only approximately 1100 new patients per year, and only 600 gastrectomies (33, 34). Therefore, there is no screening for gastric cancer, and most patients present with advanced and even incurable disease. We realize that this study is very much limited by the small sample size but this is only a first step in investigating the distribution of lymph node metastases in a Western gastric cancer population. This preliminary work will need to be followed up with prospective and multicenter studies.</u>

2. The Japanese guidelines recommend nodal dissection up to D1+ for cT1 cancer because the preoperative diagnostic ability is usually about 70% and some pT2 cases contaminated. When examining the relationship between the depth of invasion and the rate of nodal metastasis, the preoperative diagnostic ability for depth of invasion must be examined simultaneously.

Reply 2:
We agree with the reviewer that this is very important to investigate. In this study, all patients with cT ≥ 1 or cT1N+ were neoadjuvantly treated with chemotherapy unless there was a contra-indication (frailty or emergency surgery). Unfortunately, therefore, the number of patients to

investigate by comparing their cT stage with their pT stage is too small to perform reliable analysis and draw definite conclusions. We have added this to methods, results, and the to the discussion. In addition, supplementary table 1 was added.

Changes to text (changes underlined):
Page 7, lines 6-8 (methods):
Secondary outcomes were to compare the rate of lymph node metastases in station 1-7 between different cT-stages, to compare the rate of lymph node metastases in station 1-7 and 8-12 between different (y)pT-stages, to investigate the accuracy of cT and cN in patients without neoadjuvant therapy, up- or downstaging in patients following neoadjuvant therapy, and to compare the lymph node metastases pattern; percentage of pathological positive lymph nodes per lymph node station, between different cT and (y)pT-stages.

Page 9-10, lines 20-4 (results):
Correct clinical staging could only be investigated in the small group of patients without neoadjuvant therapy (supplementary table 1). The results show in the six patients with cT1: four pT1, one pT2 and one pT3 tumors. In the three patients with cT2: one pT1, one pT2 and one pT4 tumor. In the 17 patients with cT3: three pT2, seven pT3 and seven pT4 tumors. In the one patient with cT4: one patient with pT4 tumor. Finally, in the seven patients with cTx: one pT1, one Pt2, one pT3 and one pT4 tumor. In supplementary table 1 results are depicted for cN compared to pN and cT and cN compared to ypT and ypN.

Page 14, lines 19-21 (discussion):
In addition, for analysis of accuracy of cT-stage and cN-stage in patients without neoadjuvant therapy, sample size was limited. The results show poor accuracy, however, these analyses should be repeated in larger studies with prospective data.

Page 24, supplementary table 1:

Supplementary table 1a
cT-stage compared to (y)pT-stage

	cT1 (n=8)	cT2 (n=18)	cT3 (n=17)	cT4 (n=1)	cTx (n=7)
pT1	4	1	-	-	1
pT2	1	1	3	-	1
pT3	1	-	7	-	4
pT4	-	1	7	1	1
ypT1	-	7	6	1	3
ypT2	-	3	7	-	6
ypT3	-	2	14	1	4
ypT4	-	3	8	2	4
ypT0	2	-	5	-	-

Supplementary table 1b
cN-stage compared to (y)pN-stage

	cN0 (n=56)	cN1 (29)	cN2 (14)	cN3 (2)	cNx (11)
pN0	16	2	-	-	-
pN1	6	5	-	-	1
pN2	2	3	-	-	1
pN3	6	1	3	-	1
ypN0	11	11	6	1	5
ypN1	8	3	2	-	2
ypN2	2	1	1	-	1
ypN3	5	3	2	1	-

---

3. There exist some difficulties to handle patients with preoperative chemotherapy. If chemotherapy is effective, the foci of lymph node metastases may disappear. If these foci are not regarded as metastasis, the rate of metastasis cannot be examined accurately.

Reply 3: thank you for this valuable comment. We agree that this needs more nuancing in the manuscript. We have added this to methods, results and also to part of the discussion. In addition, supplementary table 1 was added.

Changes to text (changes underlined):
Page 7, lines 8 (methods):
Secondary outcomes were to compare the rate of lymph node metastases in station 1-7 between different cT-stages, to compare the rate of lymph node metastases in station 1-7 and 8-12 between different (y)pT-stages, to investigate the accuracy of cT <u>and cN</u> in patients without neoadjuvant therapy, <u>up- or downstaging in patients following neoadjuvant therapy,</u> and to compare the lymph node metastases pattern; percentage of pathological positive lymph nodes per lymph node station, between different cT and (y)pT-stages.

Page 10, line 3-4 (results):
<u>In supplementary table 1 results are depicted for cN compared to pN and cT and cN compared to ypT and ypN.</u>

Page 14, line 19-21 (discussion):

In addition, for analysis of accuracy of cT-stage and cN-stage in patients without neoadjuvant therapy, sample size was limited. The results show poor accuracy, however, these analyses should be repeated in larger studies with prospective data.

Page 14, line 16-18 (discussion):
Possible downstaging was also shown for the N-stage, as cN was generally higher than ypN stage in neoadjuvantly treated patients. However, clinical lymph node staging is known to be even more unreliable than clinical tumor staging.

Page 24, Supplementary table 1:

Supplementary table 1a
cT-stage compared to (y)pT-stage

	cT1 (n=8)	cT2 (n=18)	cT3 (n=17)	cT4 (n=1)	cTx (n=7)
pT1	4	1	-	-	1
pT2	1	1	3	-	1
pT3	1	-	7	-	4
pT4	-	1	7	1	1
ypT1	-	7	6	1	3
ypT2	-	3	7	-	6
ypT3	-	2	14	1	4
ypT4	-	3	8	2	4
ypT0	2	-	5	-	-

Supplementary table 1b
cN-stage compared to (y)pN-stage

	cN0 (n=56)	cN1 (29)	cN2 (14)	cN3 (2)	cNx (11)
pN0	16	2	-	-	-
pN1	6	5	-	-	1
pN2	2	3	-	-	1
pN3	6	1	3	-	1
ypN0	11	11	6	1	5
ypN1	8	3	2	-	2
ypN2	2	1	1	-	1
ypN3	5	3	2	1	-

---

4. The methods for diagnosing pathological nodal metastases are not described in this article. It must be clearly stated whether the sectioning of the lymph node is a single maximal plane or multiple sectioning, whether the staining method is H&E only or with the addition of cytokeratin immunostaining, and how micrometastasis and isolated tumor cells are handled.

Reply 4: Thank you for this important point. All lymph nodes under 5 mm were totally embedded for microscopic evaluation, larger lymph nodes were totally embedded in slices of 3-4 mm thick. Initial microscopic evaluation was performed by standard H&E staining. In case of suspicion of micrometastasis (0.2-2.0 mm) or isolated tumor cells in a lymph node, or in case of suspicion of residual tumor cells in patients with extensive response to neoadjuvant therapy, additional keratin stains were performed.

Changes to text (changes underlined):

Page 7 line 20-23 (methods)
Lymph nodes < 5 mm were totally embedded and H&E stained. Larger lymph nodes were embedded in slices of 3-4 mm thick. If micrometastatic disease was suspected, or if extensive response to neoadjuvant therapy was present, additional keratin stains were performed.

5. The quality control of this study is questionable. Some cases with an extremely small number of searched lymph nodes were included. The reliability is low unless at least 15 nodes are harvested.

Reply 5:
We agree with the reviewer that more than 15 lymph nodes should be examined in gastric cancer. In 8.0% (9/112) of the cases less than 15 lymph nodes were harvested. To prevent selection bias, these cases were not excluded. Some of these cases had previous gastric or abdominal surgery

Changes to text (changes underlines):
Page 9, lines 18-20 (results):
Patients with less than 15 lymph nodes in the resection specimen (9 patients) were patients that had previous gastric surgery or acute/semi-acute surgery.

Page 18, lines 8-10 (discussion):
Furthermore, there were 9 patients with < 15 lymph nodes examined, these patients were probably understaged, which may have influenced results. These patients however, were not excluded from analyses to prevent selection bias.

6. Even if cT1 patients do not have metastases to suprapancreatic nodes, whether the extent of dissection can be reduced from D1+ to D1 is another question. A prospective study comparing the prognoses of D1 and D2 is needed to conclude that D1 is sufficient for cT1 patients.

Reply 6:
Thank you for this comment. We agree with the reviewer that this needs to be investigated in a large and prospective study. We have added text about this in the discussion section of the manuscript.

Changes to text (changes underlined):
Page 18, lines 4-7 (discussion):
Especially whether a limited D1 dissection is justified in patients with cT1 disease needs to be further investigated, also considering the accuracy of both clinical T and N staging. With this in mind, studies investigating the value of sentinel node navigation surgery seem very promising (35).