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Ex-vivo lymphadenectomy during gastrectomy for adenocarcinoma optimizes lymph node yield

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Abstract

Introduction—Variability in surgical and pathological techniques in Western centers may lead to inconsistency in lymph node staging in patients with gastric adenocarcinoma. We hypothesize that ex-vivo dissection (EVD) after gastrectomy for adenocarcinoma increases lymph node yield.

Methods—We retrospectively reviewed 222 consecutive patients who underwent gastrectomy with curative intent for adenocarcinoma between November 2010 and June 2014. In August of 2012, we began performing EVD of nodes in surgical specimens (EVD group, N=111), as opposed to submitting specimens en-bloc with lymph node basins attached to the specimen (No EVD group, N=111). Primary end-point was lymph node yield.

Results—The median number of lymph nodes procured was significantly higher in the EVD compared to the No EVD group (30 vs 21 lymph nodes, respectively; $P<0.0001$). Moreover, 28% of the No EVD group were not adequately staged (defined by ≤ 15 nodes), compared to 5% of the EVD group ($P<0.0001$). Stage-for-stage overall survival was not significantly different.

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CA: Participated in research design, performance of the research, data analysis, and writing of the paper, no conflict of interest

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Conclusion—EVD may be a useful tool to maximize lymph node yield. However, this had no impact on staging or survival. This is an interesting finding that warrants further investigation.

Keywords

ex-vivo dissection; gastric cancer; gastrectomy; lymph node; staging

Introduction

Gastric cancer is the fourth leading cause of cancer and cancer-related deaths worldwide [1]. Curative treatment of gastric cancer is surgical resection and lymphadenectomy [2]. Given the high recurrence rates from operation alone, a multimodality approach is preferred for locoregionally advanced non-metastatic gastric cancer [3]. Advances in neoadjuvant and adjuvant treatment modalities in combination with surgical resection have optimized disease-free and overall survival rates [4].

Lymph node metastasis is common (>50%) in primary gastric cancer invading beyond the submucosa (T2) [5] and lymph node status is a key prognostic factor in gastric cancer [6]. According to the 2010 7th edition of the American Joint Committee on Cancer (AJCC) staging manual, at least 16 lymph nodes must be examined to obtain proper oncologic staging [7]. Performance of a D2 lymphadenectomy, which removes perigastric and the first and second tier of nodes along the branches of the celiac axis, splenic vessels, and splenic hilum, optimizes the potential to obtain the recommended number of nodes required for staging. Multiple studies have demonstrated the feasibility of this technique by Western surgeons on Western patients, who typically have higher body mass indices (BMI) and more co-morbidities than Eastern patients [8,9]. Failure to obtain sufficient lymph nodes may lead to under-staging, especially when ten nodes or fewer are examined [10]. Conversely, some studies demonstrated improved prognostic ability in gastric cancer patients who had more than 16 lymph nodes examined [11,12]. We routinely perform D2 lymphadenectomy at our institution, and have previously reported the increase in lymph node yield by a fat clearing technique [13]. Nevertheless, we have not always procured the recommended 16 lymph nodes to properly stage patients. Thus, we sought to examine other techniques to optimize our lymph node counts. Over the last two years, we have adopted the technique of surgeon *ex vivo* dissection (EVD) to further optimize the number of lymph nodes examined. The purpose of this study is to examine the effect of EVD on the number of lymph nodes examined. Our hypothesis is that EVD optimizes the lymph nodes counts and thus improves staging. In patients who did not receive neoadjuvant therapy, improved nodal staging has the potential to impact decisions about postoperative adjuvant therapy.

Methods

Patients

All patients who underwent a gastrectomy with curative intent for adenocarcinoma at Memorial Sloan Kettering Cancer Center from November 2010 to June 2014 were identified from a prospectively maintained gastric database. The study was conducted according to the institutional human research committee procedures. A total of 222 consecutive patients, who

followed our standard treatment algorithm based on the preoperative stage (either neoadjuvant chemotherapy followed by resection for locally advanced tumors, or proceeding immediately to resection for early stage gastric cancer), underwent gastrectomy for adenocarcinoma via the open or minimally invasive approach. Neoadjuvant radiation therapy is not routinely administered according to our standard treatment algorithm. In August of 2012, we began performing *ex vivo* dissection of nodes in our surgical specimen (EVD group) as opposed to submitting specimens en-bloc with the lymph node basins attached to the specimens (No EVD group). The decision to perform an *ex vivo* dissection was surgeon specific.

All procedures were performed by two surgeons (VES & DC). Patient-related data included gender, age, and BMI. Tumor-related data included oncologic and histopathologic data, including tumor type, histology, Lauren classification, World Health Organization (WHO) classification, tumor location, TNM staging based on the 7th edition AJCC staging on postoperative evaluation, and number of lymph nodes examined. Treatment-related data included use of neoadjuvant chemotherapy, extent of gastric resection, and extent of lymphadenectomy. The primary end-point was the lymph node yield. The secondary end-point was stage specific survival.

Technique of EVD

Specimens in the No EVD group were submitted intact to the pathology department for frozen section examination of the margin and further processing as deemed clinically appropriate. Specimens in the EVD group were processed immediately following gastrectomy on the back table in the OR, prior to being sent to pathology for a frozen section of the proximal or distal margin. On the back table, lymph nodes included in the specimen were divided into 10-12 discrete lymph node packets with accompanying adipose tissue, correlating with lymph node stations (Figure 1). This process takes 3-5 minutes. These labeled stations were then processed separately by our pathologists and reported separately in the final pathology report. The pathological evaluation of the specimens are performed by pathology assistants, surgical oncology fellows, or gastrointestinal subspecialty fellows, which are all overseen by the attending pathologist.

Statistical Analysis

Statistical analyses were performed using Graphpad Prism software version 5.03 (GraphPad Software, Inc. La Jolla, CA). Categorical variables were compared using chi-square or Fisher's exact test, whereas continuous variables were compared using Mann-Whitney *U*-test (two-tailed). Multivariate binary logistic regression analysis was used to identify independent predictors of understaging (procuring <16 lymph nodes). Forward stepwise regression analysis was utilized, including parameters in the model with a $P < 0.10$. Overall survival curves were generated using the Kaplan-Meier method. All results are expressed as mean \pm SD, unless specified otherwise. The null hypothesis was rejected when $\alpha < 0.05$.

Results

All patient-, tumor-, and treatment-related variables are listed in Table 1. There were no significant differences between the EVD and No EVD groups. However, mean [\pm SD] lymph node counts were significantly higher in the EVD group compared to the No EVD group (34 ± 15.4 v. 22 ± 8.4 , respectively; $P<0.0001$) (Figure 2). Mean lymph node counts were significantly higher at each individual N status, including N0 (31 ± 15.6 v. 19 ± 7.5 , respectively; $P<0.0001$), N1 (36 ± 16.3 v. 24 ± 7.3 , respectively; $P=0.01$), and N3 (41 ± 15.2 v. 28 ± 7.1 , respectively; $P=0.003$), but not N2 (32 ± 10.7 v. 25 ± 10.4 , $P=0.09$) (Table 2). Although significantly more patients in the EVD group had at least 16 lymph nodes procured (95% v. 72%, respectively; $P<0.0001$), lymph node positivity rates (positive/total lymph nodes) were not significantly different. Overall AJCC stage distribution was not different comparing the No EVD to the EVD groups.

The median follow-up of all patients was 17 months (range 1-38 months). Median follow-up was significantly longer in the No EVD group (19.5 v. 14 months, respectively; $P<0.0001$). One-year overall survival rates in the EVD and No EVD groups were 97.8% and 99.0% respectively. Two-year overall survival rates were 92.0% and 87.3%, respectively. Three-year overall survival rates for Stage I, II, and III disease were 98.8%, 65.9%, and 43.6%, respectively. There were only three patients with Stage IV disease (all downstaged preoperatively with chemotherapy) and all were alive at last follow-up. Stage-specific overall survival was not significantly different between the two groups for Stage I (log rank $p=0.32$), Stage II (log rank $p=0.18$), and Stage III (log rank $p=0.80$) disease. There was no significant difference in overall survival based on *ex vivo* dissection technique ($P>0.05$) (Figure 2A). Moreover, overall survival based on *ex vivo* dissection technique and nodal positivity were not significantly different ($P>0.05$) (Figure 2B). There was no significant difference in overall survival when comparing node negative patients in the EVD and No EVD groups (log rank $p=0.49$) or node positive patients (log rank $p=0.92$). Finally, there was no significant difference in recurrence-free survival between the EVD and No EVD groups (log rank $p=0.56$).

Variables postulated to affect adequate staging (≥ 16 lymph nodes) in gastric cancer are listed in Table 3. *Ex vivo* dissection was the most significant variable ($P<0.0001$), followed by the use of neoadjuvant chemotherapy ($P=0.02$), obesity ($P=0.03$), and extent of lymphadenectomy ($P=0.03$). On multivariate analysis, performing an *ex vivo* dissection was the most significant predictor of adequate staging (odds ratio [OR]: 7.04; 95% confidence interval [CI]: 2.70-18.18; $P<0.0001$), followed by performing a D2 lymphadenectomy (OR: 5.31; 95% CI: 1.46-19.61; $P=0.01$), and neoadjuvant chemotherapy use (OR: 2.27; 95% CI: 1.02-5.05; $P=0.04$).

Discussion

Ex vivo dissection increased our lymph node counts, on average, by 12 lymph nodes. Moreover, EVD increased the proportion of patients that were adequately staged according to the AJCC guidelines. Nevertheless, despite these improvements, EVD had no impact on staging or survival.

Various histopathologic techniques have been described to maximize lymph nodes counts in gastric cancer specimens. In 1990 we reported on a series of 11 patients with resectable gastric cancer our institution describing a comprehensive fat-clearing method that doubled lymph node counts ($P<0.01$) and identified smaller nodes ($P<0.001$) [13]. Hanna et al reported on a series of 114 gastric cancer specimens that underwent two different techniques to harvest lymph nodes: manual nodal dissection (MND) and systematic fat blocking (SFB) [14]. In SFB all the fat tissue (excluding the greater omentum) and large lymph nodes were removed from the specimen. The remaining fat was divided into blocks of tissue measuring $20\times 10\times 5$ mm and stained with haematoxylin and eosin then examined for lymph nodes by light microscopy. The SFB technique retrieved significantly more lymph nodes than the conventional MND technique (66 ± 21 vs. 50 ± 20 , respectively; $P=0.001$). Additionally, the SFB technique was better in identifying smaller lymph nodes and consistently produced a cumulative higher percentage of positive lymph nodes compared to MND. Lavy and colleagues describe a technique using acetone as a fat dissolving solution [15]. The acetone group had a higher lymph node count compared to MND (26.1 vs. 19.3, respectively; $P=0.003$); however, there was no significant difference in the average number of positive lymph nodes ($P=0.22$).

Other factors affecting lymph node counts in gastric cancer specimens have been studied. For example, Schoenleber et al reviewed 99 consecutive patients who underwent gastrectomy for gastric adenocarcinoma to determine factors that affect lymph node counts [16]. The surgeon ($P=0.16$) did not affect lymph node counts; however, the senior pathologist ($P=0.03$) and, more significantly, the pathology technician ($P<0.001$) had the most significant influence on lymph node counts on multivariate analysis. The study by Cichowitz and colleagues reviewing 88 patients with esophagogastric cancers demonstrated that the pathologist also had a significant influence on lymph node counts, independent of the level of experience [17]. Age, gender, operative approach, and use of neoadjuvant therapy did not impact lymph node counts. Similar findings regarding the influence of the pathologist and lymph node counts have been reported in the colorectal literature [18,19]. In our study we used the same dedicated group of gastric cancer pathologists to review all of our specimens.

Several studies have demonstrated a survival advantage for examining more lymph nodes. This was first described in the East by the Koreans and Japanese [20,21]. Noda and colleagues from Japan demonstrated the importance of examining small lymph nodes (4 mm post-histologic processing) to avoid stage migration [20]. Lee et al from Korea demonstrated a survival advantage associated with obtaining at least 15 nodes [21]. Moreover, stage IIIB patients who had ≥ 35 nodes procured (compared to <20) also had a stage-specific survival advantage. Karpeh and colleagues from our institution also demonstrated a survival advantage by obtaining a minimum of 15 lymph nodes in gastric cancer [22]. Altorki et al retrospectively reviewed a series of 264 patients with esophageal cancer treated by esophagectomy without neoadjuvant therapy [20]. He demonstrated a death hazard reduction of 34% in patients that had more than 16 nodes resected and a 48% reduction in patients that had more than 25 nodes [23].

Adequate staging can be achieved with *ex vivo* dissection. Schmidt et al demonstrated that when D2 lymphadenectomy was combined with *ex vivo* dissection all 331 patients had the recommended minimum of 16 lymph nodes examined [9]. In our study we had 23% more patients meet the minimum required lymph node counts required for proper staging using the *ex vivo* dissection technique. Cichowitz et al demonstrated a median of 11 more lymph nodes counted with *ex vivo* dissection [17]. Nevertheless, both of these studies, as well as our current study, did not demonstrate any stage-specific survival advantage associated with *ex vivo* dissection in gastric cancer.

This study has several limitations, including the inherent biases of a retrospective study. Nevertheless, the sample size is robust with >200 gastric cancer resections that were all done consecutively in a relatively short time period. The short follow-up time, which is under two years in both cohorts, is a limitation that prevents full assessment and comparisons of stage-matched survival. Furthermore, some institutions may have additional costs associated with processing individual lymph node packets. Nonetheless, we've proposed a simplified, modification of the well-described Asian technique to improve lymph node yield for Western patients. It offers the Western surgeon an additional tool that adds no morbidity to patients, and can be done in a relatively short period of time in the operating room. Most importantly EVD allows the surgeon and collaborating pathologist to maximize lymph node yield following lymphadenectomy for gastric cancer.

In summary, our study demonstrates that EVD of regional lymph nodes following curative gastrectomy for gastric cancer is associated with an increase in lymph node counts with surgeon *ex vivo* dissection. Compared to non-EVD processing of the surgical specimen, EVD was associated with an improvement in the proportion of patients with adequate nodal staging, as defined by the AJCC recommendation of >15 nodes. However, this increase in the number of examined nodes and in adequately staged patients had no impact on overall stage distribution or stage-specific survival.

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Primary Discussant

John T. Mullen, MD (Boston, MA)

Thank you Dr. Afaneh for your excellent presentation. As you know, over the years there has been much debate as to the proper extent of lymph node dissection for patients with gastric cancer, and there has been much published about the frequent problem of understaging in the West owing to low numbers of examined lymph nodes, including many proposals for new staging systems (e.g., using lymph node ratios, log odds of metastatic lymph nodes, etc.) designed to compensate for these low nodal yields. You and your co-authors are to be congratulated for adopting a technique known to significantly improve lymph node yields which has long been employed in Asia and has also been employed at my institution over the past several years – ex-vivo dissection (EVD). Surprisingly, though you demonstrated much higher lymph node yields with EVD than without EVD, there were no differences in either the stage-specific stratification of the patients or in overall survival. I have two questions for you:

- 1) The first and most obvious question is how can you explain the fact that examining 12 more lymph nodes in the EVD group than in the non-EVD group led to absolutely zero difference in nodal staging? The percentages of patients in each of the N groupings (i.e., N0, N1, etc.) are virtually identical (there isn't even a trend!), and the P value is 1.0.
- 2) In light of your findings, will you and your colleagues continue to take the extra time to do ex-vivo dissections as you currently do on patients with gastric cancer, will you modify the procedure in some way to achieve even higher nodal yields (e.g., dissect out individual lymph nodes as opposed to entire packets), or will you abandon it altogether?

Closing Discussant

Dr. Afaneh

- 1) Dr. Mullen, thank you taking the time to carefully review our manuscript. I think you raise an important question. I believe the reason we found no difference in nodal staging is two-fold. First, at baseline, we are performing an extended lymphadenectomy (D2). The additive effect of *ex vivo* dissection may be less substantial in these patients. The number of patients undergoing a D1 lymphadenectomy was small and therefore we are unable to make any conclusions in those patients. In hospitals where the median lymph node yield is often below 8-10 nodes, the effect may be more demonstrable. Second, our follow-up is relatively short. The current study has a follow-up period of less than three years, therefore it may be difficult to assess any impact on stage distribution or even survival. The true staging of those patients with inadequate nodal dissection will never really be known.
- 2) This is an important question given the negative finding of our study. It may be premature to fully abandon this technique as our colleagues in the East have clearly demonstrated a benefit of *ex vivo* dissection. Reviewing the data

at three years would be necessary to assess any impact on survival. Other techniques we are currently considering includes submitting the lymph node stations as they are removed *in vivo* as separate nodal stations or having the pathologists take additional slides of the packets submitted.

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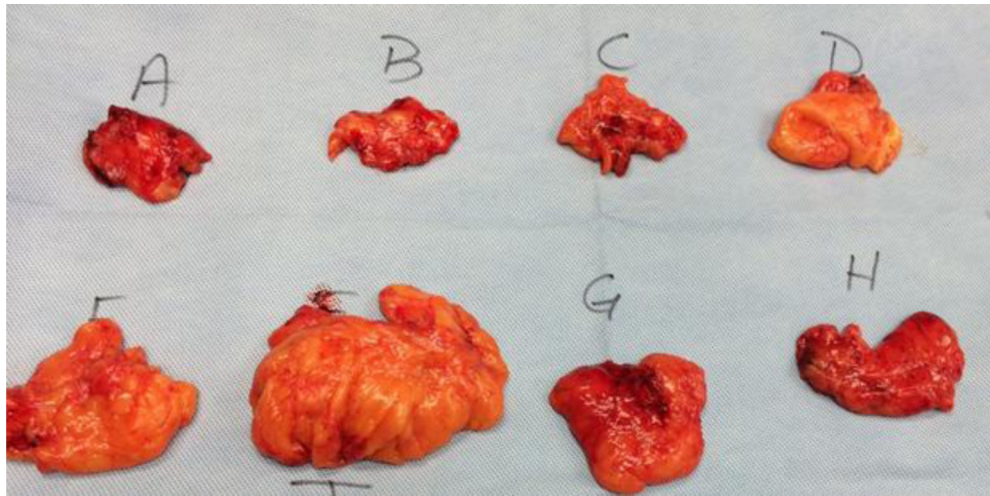


Figure 1.
Ex vivo dissection of a specimen into lymph node stations.

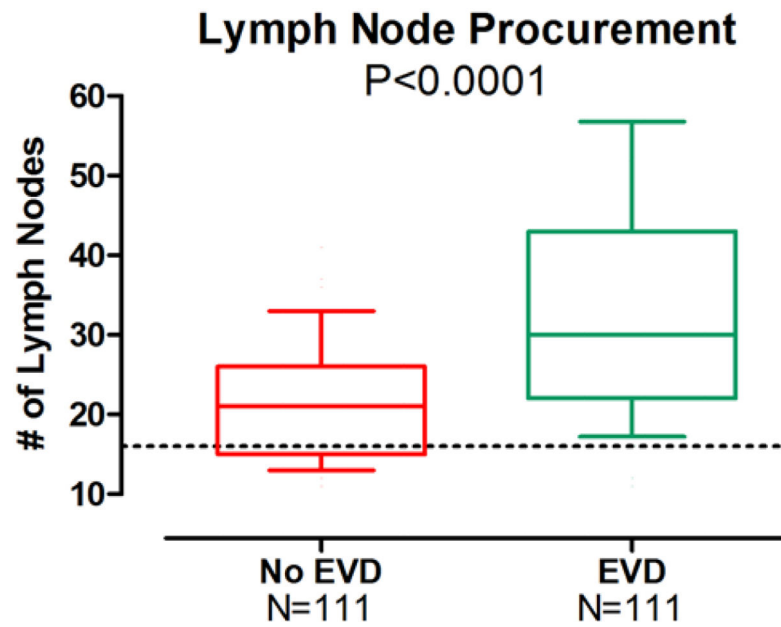


Figure 2. The box and whisker plots illustrate the 10th, 25th, 50th (median), 75th, and 90th percentiles. The dotted line represents the minimum number of lymph nodes (16) required for proper staging of a patient with adenocarcinoma. The ex-vivo lymphadenectomy technique maximized the number of lymph nodes procured ($P < 0.0001$).

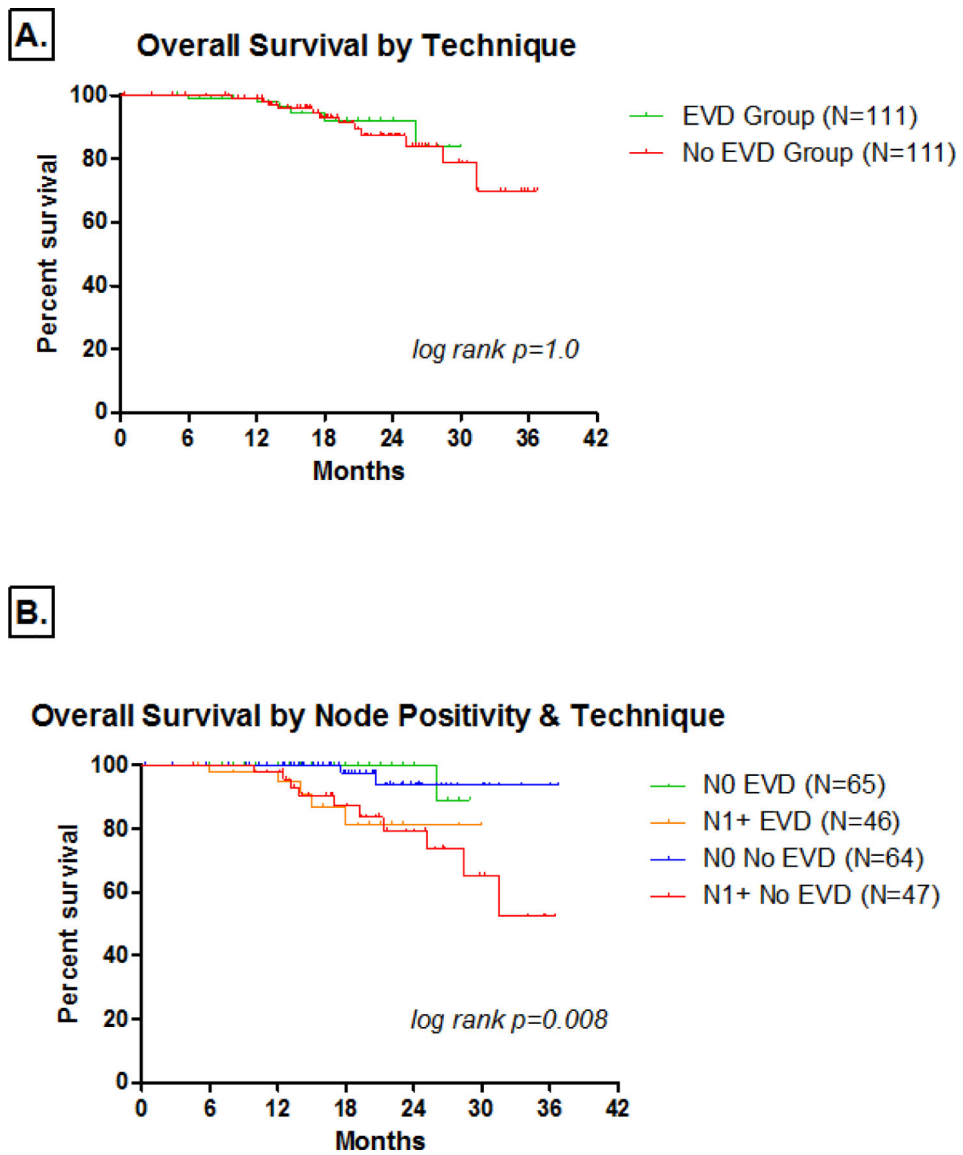


Figure 3. The Kaplan-Meier demonstrate the overall survival rates based on [A] *ex vivo* dissection technique and [B] lymph node positivity and *ex vivo* dissection technique.

Table 1Patient parameters stratified by *ex vivo* dissection

	No EVD Group N=111	EVD Group N=111	P Value
Patient-related Variables			
Male, n (%)	59 (53%)	60 (54%)	0.83
Female, n (%)	52 (47%)	51 (46%)	
Age, years	62±13.6	61±15.6	0.97
Body mass Index [BMI] (kg/m ²)	26.9±5.3	26.6±4.8	0.81
Tumor-related Variables			
Histology, n (%):			
Well-differentiated	3 (3%)	7 (6%)	0.07
Moderately differentiated	48 (43%)	60 (54%)	
Poorly differentiated	60 (54%)	44 (40%)	
Lauren classification, n (%):			
Intestinal	48 (43%)	58 (52%)	0.38
Mixed	17 (15%)	16 (14%)	
Diffuse	46 (42%)	37 (34%)	
WHO classification, n (%)			
Tubular	65 (59%)	57 (51%)	0.47
Signet ring	43 (39%)	47 (42%)	
Other/unknown	4 (4%)	7 (6%)	
Location, n (%):			
Lower third	51 (46%)	46 (41%)	0.32
Middle third	34 (31%)	29 (26%)	
Upper third	26 (23%)	36 (33%)	
T status, n (%):			
T0	6 (5%)	7 (6%)	0.97
T1a	19 (17%)	22 (20%)	
T1b	20 (18%)	19 (17%)	
T2	16 (14%)	12 (11%)	
T3	30 (27%)	31 (28%)	
T4a	18 (16%)	19 (17%)	
T4b	2 (2%)	1 (1%)	
N status, n (%):			
N0	64 (58%)	63 (57%)	1.0
N1	18 (16%)	19 (17%)	
N2	13 (12%)	13 (12%)	

	No EVD Group N=111	EVD Group N=111	P Value
N3	16 (14%)	16 (14%)	
Stage, n (%):			
<i>in situ</i>	6 (5%)	6 (5%)	
I	47 (43%)	45 (41%)	0.98
II	29 (26%)	31 (28%)	
III	28 (25%)	27 (24%)	
IV	1 (1%)	2 (2%)	
Treatment-related Variables			
Neoadjuvant Chemotherapy, n (%):			
Yes	50 (45%)	61 (55%)	0.18
No	61 (55%)	50 (45%)	
Total/Subtotal Gastrectomy, n (%)			
Distal Gastrectomy, n (%)	44 (40%)	44 (40%)	1.0
	67 (60%)	67 (60%)	
Lymphadenectomy, n (%):			
D1+	7 (6%)	9 (8%)	1.0
D2	104 (94%)	102 (92%)	

WHO: World Health Organization

Table 2Lymph node counts stratified by *ex vivo* dissection

	No EVD Group	EVD Group	P Value
Lymph Nodes Examined	22±8.4	34±15.4	<0.0001
Male	20±7.4	32±14.8	<0.0001
Female	24±8.8	35±16.1	0.0002
Age<60 years	23±7.2	31±11.5	0.0009
Age ≥60 years	21±9.0	35±17.4	<0.0001
BMI<30 kg/m ²	23±8.3	33±15.8	<0.0001
BMI ≥30 kg/m ²	19±8.2	37±14.0	<0.0001
Well-Moderately differentiated	19±7.0	26±13.5	0.02
Poorly differentiated	24±9.0	37±15.0	<0.0001
Intestinal/Mixed type	21±8.6	31±14.7	<0.0001
Diffuse type	23±7.9	37±15.9	<0.0001
Signet ring classification	23±8.0	37±14.7	<0.0001
Lower third	20±8.1	31±13.5	<0.0001
Middle third	22±7.5	33±14.9	<0.0001
Upper third	24±8.8	36±15.0	<0.0001
T status lymph node yield:			
T0	21±5.5	27±12.4	0.52
T1a	16±6.0	31±16.4	0.003
T1b	22±9.4	27±12.4	0.12
T2	24±10.4	36±14.5	0.01
T3	23±6.7	35±16.2	0.003
T4	23±9.2	41±14.7	0.0001
N status lymph node yield:			
N0	19±7.5	31±15.6	<0.0001
N1	24±7.3	36±16.3	0.01
N2	25±10.4	32±10.7	0.09
N3	28±7.1	41±15.2	0.003
Stage:			
I	19±7.6	29±14.6	<0.0001
II	23±9.3	36±16.8	0.001
III	25±7.8	39±14.4	<0.0001
Neoadjuvant chemotherapy	23±8.0	33±14.2	<0.0001
No neoadjuvant chemotherapy	20±8.5	34±16.9	<0.0001

	No EVD Group	EVD Group	P Value
Total/Subtotal Gastrectomy	22±7.0	35±14.9	<0.0001
Distal Gastrectomy	22±9.2	33±15.9	<0.0001
D1+ Lymphadenectomy	10±5.7	32±11.2	0.002
D2 Lymphadenectomy	23±8.0	34±15.8	<0.0001
Lymph Node Positivity Rate (Positive /Total Lymph Nodes) (%)	9.6±18.4	8.8±17.6	0.82

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Table 3

Adequate Staging

	Percent of patients adequately stage (16 lymph nodes)	P Value
Male	98/119 (82%)	0.43
Female	88/103 (85%)	
Age<60 years	72/83 (87%)	0.29
Age 60 years	113/139 (81%)	
BMI<30 kg/m ²	145/169 (86%)	0.03
BMI 30 kg/m ²	39/53 (74%)	
Neoadjuvant chemotherapy	99/111 (89%)	0.02
No Neoadjuvant chemotherapy	85/111 (77%)	
Total/Subtotal Gastrectomy	76/88 (86%)	0.33
Distal Gastrectomy	109/134 (81%)	
D1 Lymphadenectomy	10/16 (63%)	0.03
D2 Lymphadenectomy	175/206 (85%)	
No EVD	80/111 (72%)	<0.0001
EVD	105/111 (95%)	

BMI: body mass index; EVD: *ex vivo* dissection