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Lymphovascular Invasion is an Independent Predictor of Survival in Breast Cancer after Neoadjuvant Chemotherapy

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Abstract

Background—Various prognostic indicators have been investigated in neoadjuvant chemotherapy (NAC) treated invasive breast cancer (BC). Our study examines if lymphovascular invasion (LVI) is an independent predictor of survival in women receiving NAC.

Methods—We performed a retrospective analysis in 166 women with operable invasive BC who underwent adriamycin (A) and taxane (T)-based NAC between 2000-2013. Presence of LVI was noted in breast excisions following NAC. Associations between progression-free and overall survival and LVI and other clinicopathologic variables were assessed.

Results—Median follow-up was 31 months (range 1.4-153 months) with a total of 56 events and 24 deaths from any cause. LVI was found in 74 of 166 patients (45%). In univariate analysis, presence of LVI was associated with worse progression-free survival (HR 3.37 95% CI 1.87-6.06, $p<0.01$) and overall survival (HR 4.35, 95% CI 1.61-11.79, $p<0.01$). In multivariate models adjusting for breast cancer subtype, LVI was significantly associated with a decrease in progression-free survival (HR 3.76 95% CI 2.07-6.83, $p<0.01$) and overall survival (HR 5.70 95% CI 2.08-15.64, $p<0.01$). When stratified by subtype, those with hormone receptor or HER2 positive BCs with no LVI had the most favorable progression-free and overall survival. Those with both LVI and triple negative BC had the worst progression-free and overall survival.

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Conclusions—LVI is an important prognostic marker and is associated with worse clinical outcome in breast cancer patients receiving NAC.

Keywords

lymphovascular invasion; neoadjuvant chemotherapy; breast cancer; survival

Introduction

Neoadjuvant chemotherapy (NAC) is a mainstay of treatment for operable and locally advanced breast cancer.[1-4] Several markers have been identified to help predict response to NAC including hormone receptor status, human epidermal growth factor receptor (HER-2) status, histological grade, tumor size, and nodal involvement.[5-10] In addition, response to NAC has been associated with tumor biology, with tumors achieving a pathologic complete response (pCR) being associated with a more favorable clinical outcome compared to those with residual disease.[3,11-15]

Lymphovascular invasion (LVI) is defined as carcinoma cells present within a definite endothelial-lined space (either lymphatic vessels or blood vessels) in the breast.[16,17] While the mechanism of lymphatic metastasis is still largely unknown,[18] the presence of LVI has been extensively studied as a prognostic indicator for progression-free and overall survival in invasive breast cancer. Some studies have shown LVI to be a marker for increased risk of axillary nodal metastases, distant metastases, and death.[19,16,20-22] Yet, others have shown that it is not an independent predictor of overall survival[23] and that its role may be limited to only high-risk groups such as those with positive nodes, tumor size >2cm, high grade, hormone receptor negative tumor, or age <35 years.[24]

The role of LVI as a prognostic marker in NAC treated breast cancer remains unclear. Some studies have shown that LVI is associated with “chemoresistant” cancers[25] and that its absence on core biopsies is associated with a complete pathological response (pCR) and improved survival.[7] However, few studies have examined the role of LVI as an independent predictor of survival with adriamycin (A) and taxane (T)-based NAC regimens.

Our study seeks to evaluate the association of LVI with progression free and overall survival in patients with operable breast cancer treated with NAC. Our hypothesis is that LVI is an independent predictor of survival in NAC treated patients.

Methods

Patient Population

In accordance with Columbia University Medical Center (CUMC) IRB approved protocol (IRB # AAAJ8512), clinical database queries and physician referral were used to identify all women with invasive carcinoma of the breast who received at least part of their care at CUMC and underwent NAC between 2000-2013. Of the 382 patients identified, 33 were excluded for having no electronic/paper chart records (n=9) or incomplete records (n=24) that precluded full data collection. Of the remaining 349, six patients were excluded due to metastatic disease at diagnosis, 109 were excluded as they received no NAC upon further

review, and an additional three were excluded as they received non-traditional NAC regimens (1 Mitomycin/Vinorelbine, 1 Herceptin/Vinorelbine, and 1 Cyclophosphamide/Methotrexate/Fluorouracil). Of the remaining 231 women who received adriamycin (A) or taxane (T)-based NAC, 14 were excluded, as they did not have a surgical pathology report performed at CUMC, 34 were excluded, given that none of their pathology reports addressed LVI, and 17 were excluded as the pathology reports could not confer a clear diagnosis of LVI ("cannot be ruled out"). Thus, a total of 166 women were assessed in this analysis. (Figure 1).

Clinical and Pathological Variables

Clinical and pathological data were abstracted from the medical record by two independent researchers. All data were double-verified, and any discrepancies were resolved by oncologists EC and KK. Age was defined in years at pathological diagnosis and was stratified into <50 years of age and ≥50 years of age. Tumor size was defined as the largest dimension on any imaging modality prior to any treatment and was stratified at 0-5cm and >5cm. Grade was defined as the highest grade seen on any biopsy and was defined as low/intermediate grade (grade 1 and 2) and high grade (grade 3). Estrogen receptor (ER) and progesterone receptor (PR) positivity was defined as 10% or greater expression on any biopsy, as the majority of older pathological reports only specified <10% (negative) or ≥10% (positive). However, in accordance with American Society of Clinical Oncology/College of American Pathologist (ASCO/CAP) guidelines from 2010, a separate analysis was also performed where estrogen receptor (ER) and progesterone receptor (PR) positivity was defined as 1% or greater expression on any biopsy.[26] Tumors were considered HER2-positive if they were 3+ by immunohistochemistry (IHC) or demonstrated gene amplification with a ratio of Her-2 /CEP17 ≥2 by in situ hybridization on either the core biopsy or surgical pathology specimen.[27] Based on prior studies, subtype groups were defined as a) hormone receptor positive (ER and/or PR positive) and HER2 negative, b) HER2 positive regardless of hormonal status, and c) triple negative (ER, PR, and HER2 negative).[28] Clinical and pathological staging was determined based on the American Joint Committee on Cancer (AJCC) TNM Staging Manual, 7th edition. Pathological complete response (pCR) was defined as no residual invasive disease in the breast or lymph nodes on surgical pathology specimens (ypT0/Tis ypN0). To assess pathological response and nodal status after NAC, women were divided into three groups: pathological complete response (ypT0/Tis ypN0), those with invasive disease in the breast only (ypT+ ypN0), and those with any invasive disease in lymph nodes (any T and N+), based on prior studies.[29]

LVI was defined based on the CUMC standard pathological definition as presence of carcinoma cells within a definite endothelial-lined space (either lymphatic or blood vessels). This was rarely verified using D2-40 immuno-histochemical stain for lymphatic endothelium and CD31 for endothelium of all vessels. The presence of LVI was evaluated in post-NAC surgical pathology specimens, as well as pre-therapy core biopsies, although the latter less consistently. As only 70 core biopsies addressed the presence or absence of LVI and absence of LVI on core biopsies may represent sampling error, this data element is less reliable. However, there was some agreement (46 out of 70, 66% k=0.4) between the two with 12 out of 70 surgical pathology specimens showing LVI that was not seen on the core

biopsy and only 12 out of 70 core biopsies showing LVI that was not seen on surgical pathology specimens. Of the 12 where LVI was seen on the core but not surgical pathology biopsies, three surgical pathology biopsies showed pCR, three showed residual node-negative tumor, and six showed nodal disease only with no residual tumor after NAC. As with prior studies[19], 17 were excluded as the pathologist could not rule out LVI. All pathology specimens were interpreted by trained surgical pathologists.

All women received A-based, T-based, or A/T-based NAC. Women were considered to have received radiation therapy (XRT) if they received any type of whole breast radiation with or without nodal radiation. Hormonal therapy was defined as treatment with any selective estrogen-receptor modulator (SERM) or aromatase inhibitor (AI). Surgery type was stratified into lumpectomy or mastectomy with or without lymph node dissection.

Statistical Analysis

Chi square, Fisher's exact and t-tests were used to compare relevant clinical and pathological variables according to presence or absence of LVI. Progression-free survival was based on the STEEP criteria[30], and events were defined as any local/regional or distant metastasis, contralateral invasive breast cancer (excluding in-situ disease), any secondary, non-breast, invasive cancer, and/or death by any cause. Progression free survival (PFS) and overall survival (OS) were calculated in months from date of definitive surgery to date of first event or death (for OS) or last follow-up in those women without events. Kaplan Meier survival analysis and the log-rank statistic were used to estimate survival differences between groups based on clinically relevant variables. Cox proportional hazard models were used to assess the association of LVI and PFS and OS after adjusting for other covariates, including age, tumor size, grade, subtype, and post-surgical nodal status. Stratified analyses were performed using the *a priori* determined variable of subtype (triple negative vs. not triple negative). All analyses were performed using SAS 9.4 and STATA 12.0 with significance defined as a two-sided p-value of 0.05.

Results

Demographics

Of the 166 women, 74 had evidence of LVI on pathology (n=59 with invasion into lymphatics and n=15 with invasion into lymphatics and veins), and 92 had no evidence of LVI on post-NAC surgical pathology samples. Of the 166 women, 18 received A-based, 27 received T-based, and 121 received A/T-based NAC. All women completed the entire course of NAC with the exception of four women who had their NAC terminated early due to progression of disease (n=2), long delays in treatment (n=1) and progression as well as toxicity (n=1).

Mean age was 52 in the LVI group and 51 in the no LVI group (Table 1). In both groups, the majority of women self-reported as non-Hispanic White or Hispanic and had invasive ductal carcinomas, tumors between 0-5cm in size, and high-grade breast cancers. Both groups had a similar distribution of subtypes with hormone receptor positive/HER2 negative being the most common (LVI, n=37; no LVI, n=35), followed by HER2 positive (LVI, n=22; no LVI,

n= 36), then triple negative breast cancer (TNBC) (LVI, n=15; no LVI, n= 21). One patient with HER2 positive breast cancer did not receive neoadjuvant or adjuvant Herceptin as she was lost to follow-up soon after initial medical oncologist visit. The LVI group had no women who achieved pCR, as expected based on our definition of pCR, and in the no LVI group, n=34 (36%) patients achieved pCR (p<0.001). The LVI group also had significantly higher rates of mastectomy (p<0.001) and post-operative radiation therapy (p-0.006).

Survival Analysis

Median follow-up was 31 months (range 1.4-153 months). There were a total of 56 events with 50 of them being recurrence or progression of invasive breast cancer (13 local and 37 distant), 2 being new invasive primary cancers (1 colon and 1 laryngeal) and 4 being death from any cause without evidence of recurrence or progression. Of the 13 local recurrences, six were recurrences in the same breast, three in the lymph nodes, and four in the chest wall. Ten of these women received radiation therapy while two did not (data missing for last woman). There were 24 overall deaths from any cause.

On univariate analysis, presence of LVI was significantly associated with worse PFS (HR 3.37; 95% CI 1.87-6.06; p < 0.01) and OS (HR 4.35; 95% CI 1.61-11.79; p <0.01). Subtype (triple negative as compared to hormone receptor+/HER2- breast cancer) was also significantly associated with worse PFS (HR 2.00; 95% CI 1.06-3.75; p = 0.03) and OS (HR 4.23; 95% CI 1.47-12.17; p < 0.01), (Table 2.1 and 2.2). In addition to presence of LVI and subtype, the presence of lymph node involvement (nodal disease) at the time of definitive surgery (vs. pCR, HR 2.80; 95% CI 1.10-7.11; p = 0.03) was also significantly associated with worse PFS, although post-surgical nodal status itself was not overall significantly associated with either PFS or OS (Table 2.1 and 2.2). Age, size, grade and radiation therapy were not significantly associated with either PFS or OS.

On multivariate analysis, presence of LVI was an independent predictor for a worse PFS survival (HR 3.76, 2.07-6.83, p<0.01) and worse OS (HR 5.70, 2.08-15.64, p<0.01) after adjusting for subtype (Table 3). TNBC was an independent predictor of worse PFS (HR 2.59, 1.37-4.90, p<0.01) and OS (HR 6.06, 2.08-17.68, p<0.01) after adjusting for LVI.

A separate analysis using a cutoff of ≥1% for ER/PR positivity only affected the subtype of 3 women, changing them from TNBC to hormone receptor positive. On univariate analysis using the cutoff of ≥1%, TNBC, as compared to hormone receptor +/HER2- breast cancer, was significantly associated with worse PFS (p=0.05) and a trend towards worse OS (p=0.08). On multivariate analysis both presence of LVI (HR 3.88, 2.13-7.09, p<0.01) and TNBC, as compared to hormone receptor +/HER2- breast cancer, (HR 2.51, 1.31-4.80, p<0.01) were significantly associated with worse PFS. Presence of LVI (HR 5.85, 2.10-16.27, p<0.01) and TNBC, as compared to hormone receptor +/ HER2- breast cancer, (HR 4.41, 1.60-12.21, p<0.01) were also significant predictors of worse OS.

When stratified by triple negative status, those with no TNBC and no LVI had the most favorable PFS and OS. The presence of LVI was associated with a PFS and OS detriment to a similar extent as having TNBC. The presence of both TNBC and LVI was associated with the least favorable survival outcomes (Figure 2.1 and 2.2).

Discussion

In a population of 166 women receiving NAC for newly diagnosed breast cancer, the presence of LVI was associated with worse PFS and OS. In analysis stratified by triple negative status, LVI appeared to affect survival in a similar manner as having triple negative status, and the presence of both pathologic factors together further worsened PFS and OS.

The results of our study are similar to prior studies of LVI in women receiving NAC. In 115 Japanese women receiving NAC, Tamura et al. found that LVI predicted tumor recurrence and death in multivariate models and could be useful in classifying risk.[5] Others found that LVI was a significant component of clinicopathologic scores that predict response and survival in women receiving NAC.[31] In studies looking at various subtypes of breast cancer, LVI was associated with a worse 5-year recurrence free survival rate.[32] Yet, Huang et al. found that in 542 women treated with NAC and radiation therapy, LVI was only associated with survival on univariate analysis, with other factors, such as skin/nodal involvement, tamoxifen use and subtype, being better predictors of overall survival in multivariate models. However, the NAC regimens used in this previously reported study were older than modern regimens, [33] and few studies have focused on the prognostic implications of LVI in surgical pathology specimens after NAC in such a diverse population.

Our study found that LVI, as seen on post-NAC surgical specimens, was an independent predictor of PFS and OS in women receiving modern anthracycline and/or taxane-containing NAC regimens. This is in agreement with the study by Tamura et al. who found that LVI on post-surgical specimens was a better predictor of survival than LVI on biopsy samples pre-NAC in a small population of Japanese women.[5] Interestingly, we found that LVI was associated with PFS and OS, independent of post-surgical stage/nodal status, suggesting that the presence of LVI represents an independent prognostic marker of poor outcome, outside of its potential association with nodal metastasis. Other studies support this finding and have reported that LVI is an independent predictor of survival in multivariate models with size, grade, age and type in those with node negative disease in patients receiving adjuvant chemotherapy.[19] We also found that LVI affected PFS to a similar extent as having a triple negative subtype, a known poor prognostic factor, and that the two variables in conjunction further decreased PFS. This has been seen in other studies as well, and Sakuma et al. found that in 44 women with triple negative breast cancer, presence of LVI was associated with worse disease free survival.[34]

Although the exact mechanism is unknown, LVI may represent an aggressive tumor or tumor environment that could portend a worse prognosis. Lymphangiogenesis is thought to correlate with lymph node metastasis and may be driven by factors secreted by tumor cells and their environment such as vascular endothelial growth factor C (VEGF-C) and matrix metalloproteinase 9 (MMP-9). [35-37] Studies also show that lymphangiogenesis is affected by histology subtype, with TNBC patients having higher densities of lymphatic microvessels and VEGF-C compared with non-TNBC patients, and subsequently worse OS.[38,20,39] The underlying mechanism for LVI as an independent predictor of poor PFS appears independent from existing nodal invasion, and as it appears to vary with subtype, may reflect

not only the aggressiveness of the underlying tumor but also the surrounding tumor micro-environment.

Strengths of this study are that it included a large population of women with breast cancer who received modern regimens of NAC, allowing us to study the prognostic implications of presence of LVI after receiving NAC on survival. Although this was a retrospective study, the data extraction process was double-verified for accuracy. In addition, multiple clinical and pathological covariates were assessed. This is also an ethnically diverse population, which may improve generalizability of results. In addition, we performed our analysis using both the ER/PR positivity cutoff of ≥10% and the newer cutoff of ≥1% with similar results. Limitations include the incomplete assessment of LVI on the core biopsies and the lack of central pathology review. As with all retrospective studies, causality is not possible to assess, and although many potential confounders were accounted for in this study, there is always the possibility of residual confounding. Future research should focus on integrating LVI to delineate this high-risk population, with future studies targeting these patients with new therapeutic approaches.

In conclusion, LVI appears to be an independent predictor of survival in women with invasive breast carcinoma receiving modern NAC regimens. This association also appears to vary by subtype with those with TNBC and LVI having the worst overall prognosis. Further studies in larger cohorts are necessary to determine the prognostic implications of LVI post-NAC in various subtype populations to better inform treatment decisions.

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Abbreviations

LVI	lymphovascular invasion
NAC	neoadjuvant chemotherapy
TNBC	triple negative breast cancer
pCR	pathological complete response
PFS	progression-free survival
OS	overall survival

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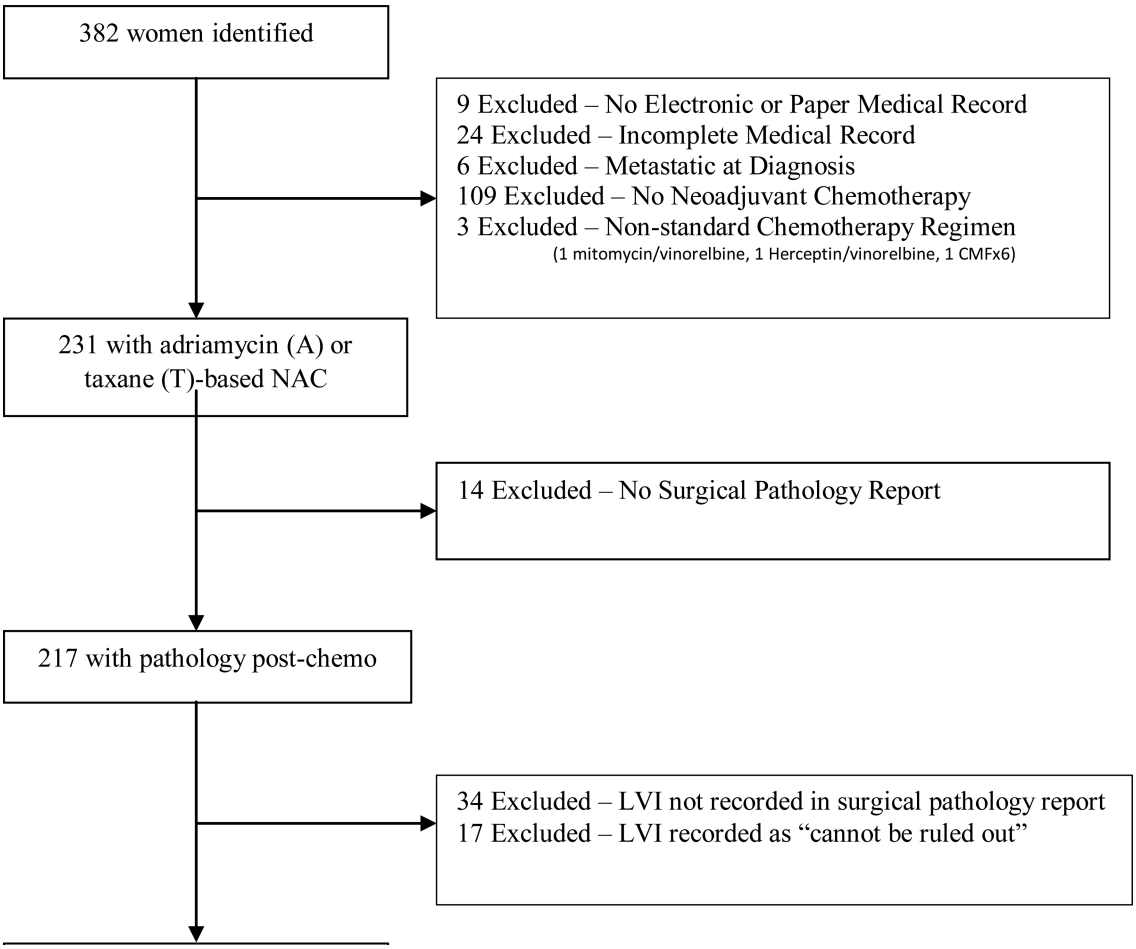

Figure 1.
Selection of Patients

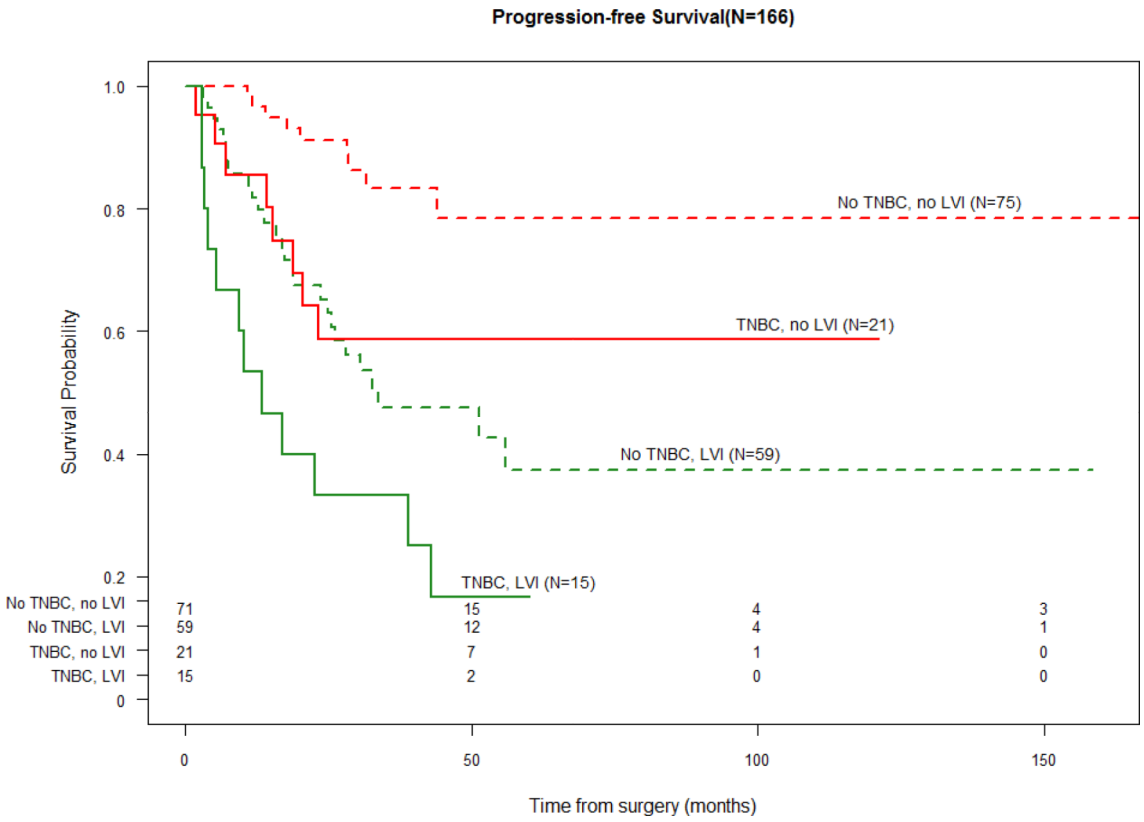

Figure 2.1.

Progression-Free Survival and LVI stratified by “TNBC” vs. “not TNBC” (Number at Risk displayed in table below survival curves)

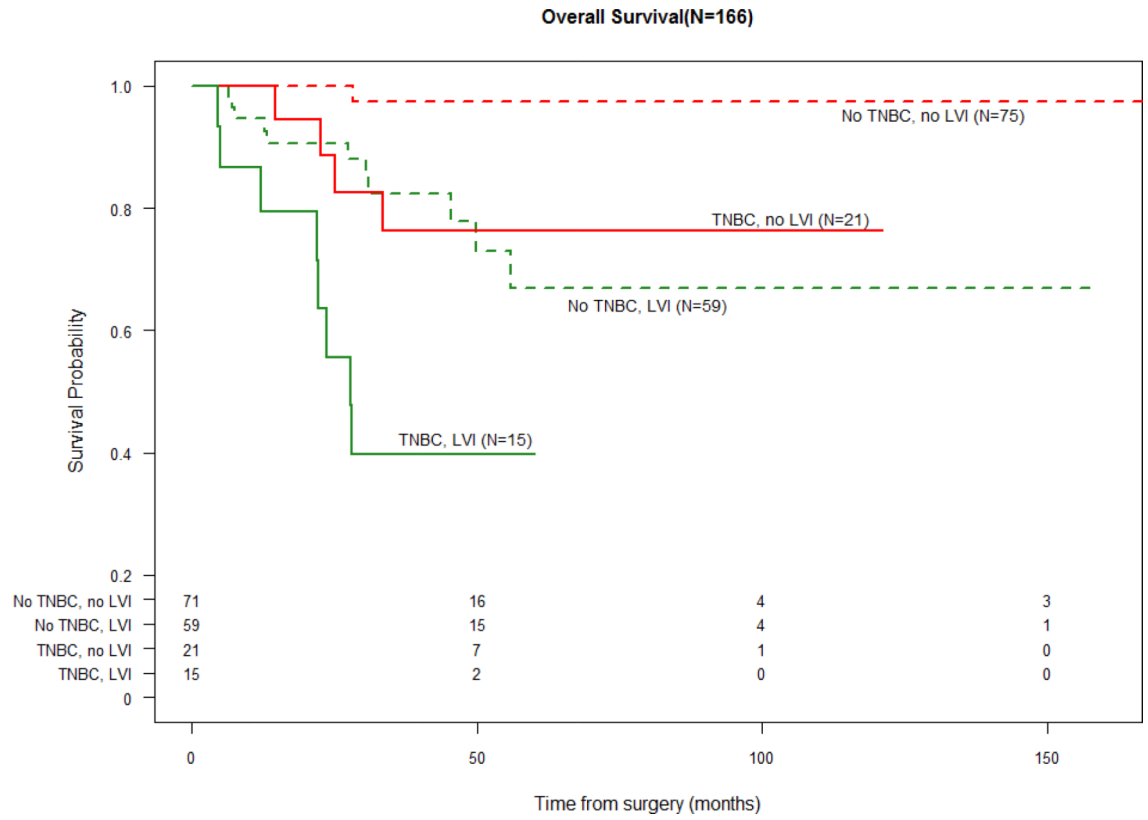

Figure 2.2.

Overall Survival and LVI stratified by "TNBC" vs. "not TNBC" (Number at Risk displayed in table below survival curves)

Table 1

Baseline Characteristics by Lymphovascular (LVI) Status

Variable	LVI (n=74)	No LVI (n=92)	p-value*
Mean Age (SD), years	52 (1.6)	51 (1.1)	0.72
Race			
Non-Hispanic White	29 (39%)	30 (33%)	0.34
Black	10 (14%)	24 (26%)	
Hispanic	30 (41%)	33 (36%)	
Other	1 (1%)	2 (2%)	
Unknown	4 (5%)	3 (3%)	
Type			
Ductal Carcinoma	60 (81%)	77 (86%)	0.33
Lobular Carcinoma	5 (7%)	7 (8%)	
Other	9 (12%)	5 (6%)	
Size			
0-5cm	55 (76%)	74 (80%)	0.53
>5cm	17 (24%)	18 (20%)	
Grade			
Low (I & II)	25 (34%)	29 (33%)	0.911
High (III)	49 (66%)	59 (67%)	
Subtype			
Hormone Receptor+/HER2−	37 (50%)	35 (38%)	0.29
HER2 +	22 (30%)	36 (39%)	
Triple Negative	15 (20%)	21 (23%)	
Pathological Staging			
pCR**	0 (0%)	34 (36%)	<0.001
T+, N −	9 (12%)	29 (32%)	
Node +	65 (88%)	29 (32%)	
Surgery Type			
Lumpectomy	11 (15%)	40 (44%)	<0.001
Mastectomy	63 (85%)	51 (56%)	
Adjuvant Radiation			
Yes	70 (97%)	74 (84%)	0.006
No	2 (3%)	14 (16%)	
Adjuvant Hormonal Therapy			
Yes	53 (75%)	54 (68%)	0.40
No	18 (25%)	25 (32%)	
Neoadjuvant/Adjuvant Herceptin			
Yes	19 (27%)	36 (52%)	0.003

Variable	LVI (n=74)	No LVI (n=92)	p-value*
No	51 (73%)	33 (48%)	

*p-values represent t-tests for continuous and chi square or Fisher's exact tests for categorical variables.

**pCR – pathological complete response

Table 2.1

Univariate analysis of predictors of progression-free survival (time from definitive surgery)

	No event		Event			
Characteristics	**No.**	**%**	**No.**	**%**	**HR (95% CI)**	**P-value**
Total patients	110	66	56	34		
Age (continuous)					1.00 (0.97, 1.02)	0.6306
Age group						
<50 years	46	61	30	39	1	-
50+ years	64	71	26	29	0.75 (0.44, 1.28)	0.2911
LVI						
No	75	82	17	18	1	-
Yes	35	47	39	53	3.37 (1.87, 6.06)	<0.0001*
Subtype						0.0352*
Hormone positive/HER2	51	71	21	29	1	-
HER2 positive	43	74	15	26	0.89 (0.45-1.77)	0.7481
TNBC	16	44	20	56	2.00 (1.06-3.75)	0.0314*
Post-surgical stage and nodal						0.0610
pCR	29	85	5	15	1	-
T+, N negative	29	76	9	24	1.76 (0.59, 5.26)	0.3119
N+, regardless of T	52	55	42	45	2.80 (1.10, 7.11)	0.0303*
Size (continuous)					1.05 (0.92, 1.20)	0.4745
Size						
0-5 cm	83	64	46	36	1	-
>5 cm	27	77	8	23	0.74 (0.35, 1.57)	0.4369
Grade						
low	41	76	13	24	1	-
High	66	61	42	39	1.79 (0.96, 3.35)	0.0682

Abbreviations: HR, Hazard ratio; CI, confidence interval; LVI: lymphovascular invasion; pCR: pathologic response; TNBC: Triple Negative Breast Cancer

Table 2.2

Univariate analysis of predictors of overall survival (time from definitive surgery)

	Alive		Dead			
Characteristics	**No.**	**%**	**No.**	**%**	**HR (95% CI)**	**P-value**
Total patients	142	86	24	14		
Age (continuous)					1.02 (0.99, 1.05)	0.2757
Age group						
<50 years	66	87	10	13	1	-
50+ years	76	84	14	16	1.29 (0.55, 3.01)	0.5620
LVI						
No	87	95	5	5	1	-
Yes	55	74	19	26	4.35 (1.61, 11.79)	0.0039*
Subtype						0.0898
Hormone positive/HER2 negative	67	93	5	7	1	-
HER2 positive	51	88	7	12	1.52 (0.46-4.97)	0.4927
TNBC	24	67	12	33	4.23 (1.47-12.17)	0.0076*
Post-surgical stage and nodal status						0.1434
pCR	33	97	1	3	1	-
T+, N negative	35	92	3	8	2.97 (0.31, 28.59)	0.3470
N+, regardless of T	74	79	20	21	5.89 (0.79, 44.13)	0.0844
Size (continuous)					0.98 (0.79-1.22)	0.8585
Size						
0-5 cm	110	85	19	15	1	-
>5 cm	32	91	3	9	0.65 (0.19-2.18)	0.4802
Grade						
low	49	91	5	9	1	-
High	89	82	19	18	1.96 (0.72, 5.32)	0.1854

Abbreviations: HR, Hazard ratio; CI, confidence interval; LVI: lymphovascular invasion; pCR: pathologic complete response; TNBC: Triple Negative Breast Cancer

Table 3.1

Multivariate analysis of predictors of progression-free survival (time from definitive surgery)

	No event		Event			
Characteristics	**No.**	**%**	**No.**	**%**	**HR (95% CI)**	**P-value**
Total patients	110	66	56	34		
LVI						
No	75	82	17	18	1	-
Yes	35	47	39	53	3.76 (2.07-6.83)	<0.0001*
Subtype						0.0069*
Hormone positive/HER2 negative	51	71	21	29	1	-
HER2 positive	43	74	15	26	1.11 (0.56-2.21)	0.8688
TNBC	16	44	20	56	2.59 (1.37-4.90)	0.0055*

Table 3.2

Multivariate analysis of predictors of overall survival (time from definitive surgery)

	Alive		Dead			
Characteristics	**No.**	**%**	**No.**	**%**	**HR (95% CI)**	**P-value**
Total patients	142	86	24	14		
LVI						
No	87	95	5	5	1	-
Yes	55	74	19	26	5.70 (2.08-15.64)	0.0007*
Subtype						0.0020*
Hormone positive/HER2 negative	67	93	5	7	1	-
HER2 positive	51	88	7	12	1.89 (0.58-6.22)	0.2936
TNBC	24	67	12	33	6.06 (2.08-17.68)	0.0010*

Abbreviations: HR, Hazard ratio; CI, confidence interval; LVI: lymphovascular invasion; TNBC: Triple Negative Breast