New insights into the prognostic value of the tumor-stroma ratio in patients with breast cancer

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The prognostic value of the tumor-stroma ratio in tumor-positive axillary lymph nodes of breast cancer patients

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ABSTRACT

Purpose

The tumor-stroma ratio (TSR) has previously been found to be a strong prognostic parameter in primary breast cancer tumors. Since the presence of tumor cells in lymph nodes is important for clinical decision making, the influence of the TSR in the primary breast tumor combined with the TSR in tumor-positive lymph nodes on prognosis was evaluated.

Methods

Women with invasive breast cancer without distant metastasis who underwent an axillary lymph node dissection between 1985 and 1994 at the Leiden University Medical Center were analyzed retrospectively. TSR assessment was performed on hematoxylin and eosin stained tissue slides.

Results

In total, 87 (45.5%) primary tumors were scored as stroma-low and 104 (54.5%) as stroma-high. Patients with a high stromal percentage in the primary tumors had a statistically significant worse relapse-free period (RFP) compared to stroma-low tumors (HR 1.97, 95% CI 1.37-2.82, $p < 0.001$). A total number of 915 lymph nodes were assessed for the TSR. In 101 (52.9%) patients, heterogeneity was observed between stroma percentage category in the primary tumor and lymph nodes. The combination of the TSR of the primary tumor and the TSR of tumor-positive lymph nodes strengthened each other as an independent prognostic parameter for RFP ($p = 0.019$). Patients with primary tumor stroma-low/lymph nodes stroma-low tumors showed strongly improved RFP rates compared to patients with primary tumor stroma-high/lymph node stroma-high tumors with 10-year percentages of 58% versus 8%, respectively.

Conclusions

Assessing the TSR on tumor-positive lymph nodes can provide additional prognostic information. Stromal activation strongly differs between primary tumors and lymph node metastases.

INTRODUCTION

In patients with invasive breast cancer, the presence of a regional lymph node (LN) metastasis is one of the most important prognostic parameters for long-term prognosis (1). Careful evaluation of LN status is crucial to decide whether patients should undergo an axillary lymph node dissection (ALND) or axillary radiotherapy and also plays a large role in deciding on adjuvant chemotherapy. As breast cancer is a heterogeneous disease (2), distinguishing patients who need more aggressive therapy from patients who would benefit from a more conservative approach remains a difficult challenge. Prognostic parameters derived from the stromal compartment might provide an important tool. The interaction between tumor cells and cells in the tumor microenvironment has gained significant interest in the last two decades. The tumor stroma consists of inflammatory cells, capillaries, fibroblasts and extracellular matrix (3). Fibroblasts that surround and infiltrate the primary tumor (PT), the so-called cancer-associated fibroblasts (CAFs), are believed to play a key role in tumor progression by secreting chemokines and growth factors. This may lead to increased cancer cell proliferation, promoting motility and invasiveness, enhanced angiogenesis and tumor-promoting inflammation (4, 5). Based on the analysis of hematoxylin and eosin (H&E) stained histologic slides, our research group developed an internationally validated prognostic tool, the tumor-stroma ratio (TSR). This tool assesses the amount of stromal proliferation within the borders of the PT. This parameter has shown to be of high prognostic value in several types of epithelial neoplasms, including breast cancer (6-10), colon cancer (11-14), gastric cancer (15) and esophageal cancer (16). These studies have invariably shown a worse prognosis in patients with so-called stroma-high tumors compared to patients with stroma-low tumors.

The additional prognostic value of TSR assessment in metastatic LNs for disease-free survival (DFS) in patients with stage III colorectal cancer was published by Van Pelt et al. (17). By our knowledge, the influence of stromal growth in LNs affected by breast cancer has not yet been investigated. The objective of this current study was to evaluate the prognostic value of the TSR in the primary tumor combined with the TSR in tumor-positive LNs in primary breast tumors compared to the TSR in primary breast tumors alone.

MATERIAL AND METHODS

Study population

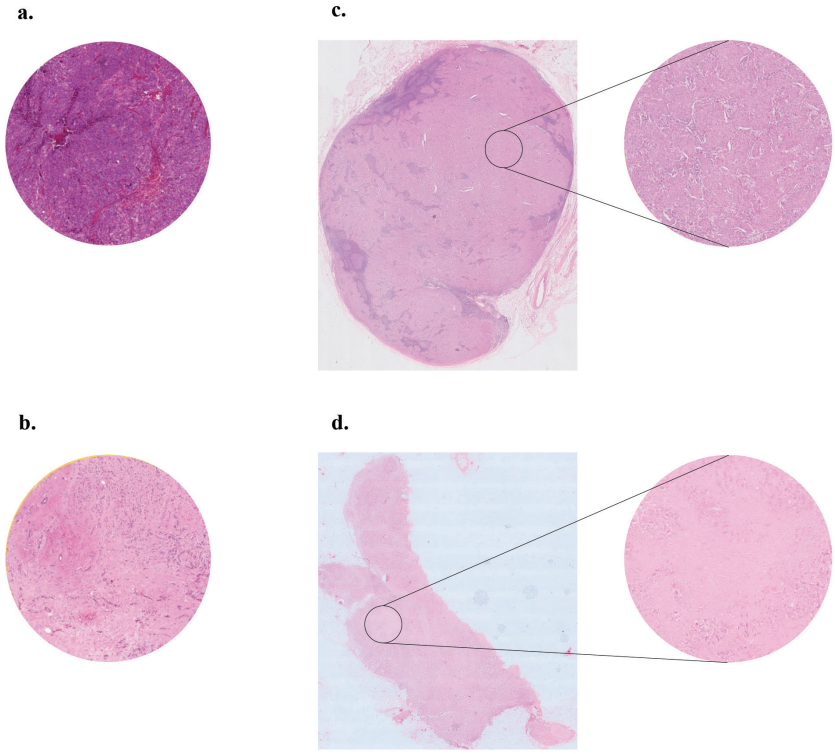
The patients included in this study were selected from a database consisting of patients with invasive breast cancer without distant metastasis, who were primarily treated with surgery between 1985 and 1994 at the Leiden University Medical Center. Patient data were assessed retrospectively (n = 677). Only patients who underwent an axillary lymph node dissection were included in this study. Patients with a history of cancer (other than basal cell carcinoma or cervical carcinoma in situ), bilateral breast cancer or absence of resected tissue slides were excluded, leaving 193 patients for analysis. The resected tumors were graded by an experienced breast cancer pathologist using the current pathological standards. TSR assessment of the primary breast tumors was described earlier (9). All samples were handled in a coded fashion, according to national ethical guidelines ("Code for Proper Secondary Use of Human Tissue", Dutch Federation of Medical Scientific Societies).

TSR assessment

The TSR was visually assessed by conventional light microscopy on 5 µm routine H&E stained slides. First, the PT and LNs were evaluated with a 5x objective to identify the most stroma-rich tissue area(s). The most stroma-abundant area was selected and assessed with a 10x objective. Only tumor fields with tumor cells present at all borders of the image field were eligible. The stroma percentage was scored by increments of 10%. A stroma percentage ≤50% was categorized as stroma-low and a stroma percentage >50% was considered stroma-high (Figure 1). Positive LNs were identified as stroma-high if at least one of the LNs had a stroma percentage of >50% (figure 1). Lymph node metastases of >0.2 mm but ≤2 mm were defined as micrometastases. In the case of micrometastases, the TSR was evaluated in a smaller image field as long as tumor cells were present at all borders.

FIGURE 1. Examples of the tumor-stroma ratio in breast cancer. Lymph nodes were scanned with an automated scanning system (Philips Ultra Fast Scanner 1.6 RA) at 20x magnification.

a. Primary tumor stroma-low **b.** Primary tumor stroma-high **c.** Stroma-low tumor-positive lymph node **d.** Stroma-high tumor-positive lymph node.



Statistical analyses

SPSS software version 23.0 (SPSS Inc., IBM Company Chicago, IL, USA) was used to perform the statistical analyses. Cohen's kappa value was used to assess the inter-observer agreement. A value above 0.6 was considered as valid. The χ^2 test was used for the evaluation of statistically significant differences for categorical variables between patients with stroma-high or stroma-low tumors. For numerical variables (lymph node yield), distribution was tested for normality using the Shapiro-Wilk

test. Statistically significant differences of non-parametric variables were analyzed using the Mann-Whitney U test. The primary endpoint was the relapse-free period (RFP), which was defined as the time from date of surgery until local, regional or distant recurrence of breast cancer. Patients who died or were lost to follow-up were censored at the last date on which they were known to be recurrence-free and/or alive. The definition of secondary endpoint overall survival (OS) was the time from date of surgery until death from any cause. Kaplan-Meier curves were compared with log-rank tests to assess differences in RFP. Univariate and multivariate Cox regression analyses were calculated for RFP and OS. Parameters with a *p*-value of less than 0.10 in univariate analysis were entered in multivariate analysis. For all analyses, a *p*-value of less than 0.05 was considered statistically significant. Effect modification was evaluated by adding interaction in the Cox regression analysis.

RESULTS

Patients

In total, H&E slides derived from 193 breast cancer patients could be evaluated for the TSR. Two patients were excluded due to poor quality of LN tissue slides, leaving 191 patients for analysis. The study group consisted of women with a median age at time of diagnosis of 57.4 years (range 27.5-87.6 years). The median follow-up period was 7.3 years (range 0.2-23.0 years). Table 1 provides a detailed overview of patient characteristics.

TABLE 1. Patient characteristics and statistically significant differences between stroma-low and stroma-high primary tumors calculated with the χ^2 test.

		Stroma-low		**Stroma-high**		
	n	n = 87	*%*	n = 104	*%*	*p*-value
Age (in years)						
<40	15	9	10.3	6	5.8	0.364
>40-60	94	39	44.8	55	52.9	
>60	82	39	44.8	43	41.3	
Grade						
I	18	5	5.7	13	12.5	0.170
II	85	37	42.5	48	46.2	
III	88	45	51.7	43	41.3	

TABLE 1. Continued.

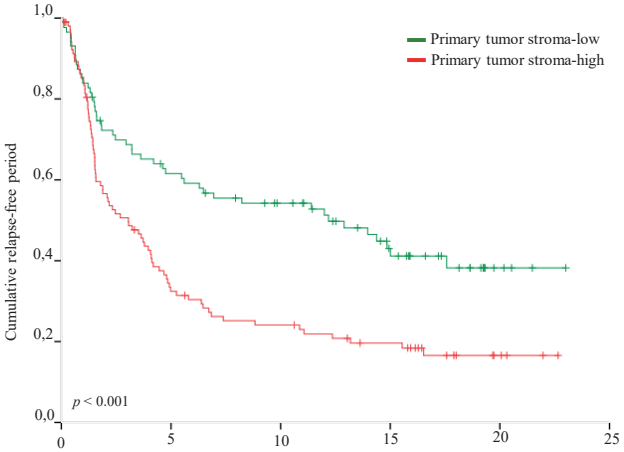
		Stroma-low		Stroma-high		
	n	n = 87	%	n = 104	%	*p*-value
Histological type						
Ductal carcinoma	171	83	96.5	88	85.4	0.010
Lobular carcinoma	18	3	3.5	15	14.6	
Tumor stage						
pT1	42	16	18.6	26	26.3	0.449
pT2	109	54	62.8	55	55.6	
pT3/4	34	16	18.6	18	18.2	
Nodal stage						
pN1	148	75	86.2	73	70.2	0.011
pN2	11	1	1.1	10	9.6	
pN3	32	11	12.6	21	20.2	
ER status						
Negative	83	40	47.1	43	44.8	0.760
Positive	98	45	52.9	53	55.2	
PR status						
Negative	86	36	42.4	50	51.0	0.241
Positive	97	49	57.6	48	49.0	
HER2 status						
Negative	118	57	82.6	61	82.4	0.978
Positive	25	12	17.4	13	17.6	
Surgery with or without radiotherapy						
MST without RT	62	30	34.5	32	30.8	0.860
MST with RT	63	28	32.2	35	33.7	
BCS without RT	0	0	0	0	0	
BCS with RT	76	29	33.3	37	35.6	
Chemotherapy						
No	127	52	59.8	75	72.1	0.072
Yes	64	35	40.2	29	27.9	
Hormonal therapy						
No	136	61	70.1	75	72.1	0.761
Yes	55	26	29.9	29	27.9	

Abbreviations: BCS = breast conserving therapy, ER = estrogen receptor, HER2 = human epidermal growth factor receptor 2, MST = mastectomy, PR = progesterone receptor, RT = radiotherapy

Prognostic value of the TSR in the primary tumor

In total, 87 (45.5%) PTs were determined to be stroma-low and 104 (54.5%) as stroma-high. Patients with stroma-high PTs had a statistically significant worse RFP compared to stroma-low tumors (HR 1.97, 95% CI 1.37-2.82, $p < 0.001$) (figure 2). After 10 years of follow-up, 75% of patients with stroma-high tumors developed a recurrence compared to 46% of patients with stroma-low tumors. The multivariate analysis showed that the TSR in the PT is a statistically significant independent prognostic factor for RFP (HR 1.70, 95% CI 1.16-2.49, $p = 0.006$) (table 2) and OS (HR 1.49, 95% CI 1.04-2.14, $p = 0.029$) (supplementary table 1). In the stroma-high group, statistically significant more patients had a tumor of lobular type and a higher nodal stage (table 1). The TSR assessment of the PTs in the total group of patients was previously published by our group (9). The tissue slides were scored in a blinded fashion by a second observer with a Cohen's kappa of 0.85 (almost perfect agreement).

FIGURE 2. Kaplan-Meier analysis for relapse-free period of patients with stroma-low primary tumors and stroma-high primary tumors.



Numbers at risk				
Primary tumor stroma-low	86	49	35	14
Primary tumor stroma-high	102	32	22	11

The TSR in tumor-positive lymph nodes

In total, 915 LNs were analyzed (range 1-18 per patient). LNs were categorized as stroma-high if at least one of the LNs had a stroma percentage of >50%. The LNs of 160 (83.8%) patients were scored as stroma-low and 31 as stroma-high (16.2%). Stroma-low PTs and stroma-low LNs were seen in 73 patients (38.2%). Stroma-high PTs and stroma-high LNs were seen in 17 patients (8.9%). In 101 (52.9%) patients, heterogeneity was observed between the stroma percentage category in the primary tumor and in the lymph nodes. No interaction between the TSR in the PTs and LNs was found, as well as between the TSR in LNs and nodal status. The Mann-Whitney U test did not show a statistically significant difference between lymph node yield (not normally distributed) and the TSR category of LNs. In 10 patients, only micrometastases were observed. These small tumor fields consisted of tumor cells for more than 90%. Thirty percent of the LNs were scored in a blinded fashion by a second observer with a Cohen's kappa of 0.79.

Prognostic value of the TSR in primary tumor combined with tumor-positive lymph nodes

The TSRs of the PT and positive LNs were combined to evaluate the possibility of an additional prognostic effect. The four different combinations of the TSR (PT stroma-low/LNs stroma-low, PT stroma-low/LNs stroma-high, PT stroma-high/LNs stroma-low and PT stroma-high/LNs stroma-high) were plotted for the RFP with an overall *p*-value of 0.001 (figure 3). The patient characteristics of these four groups were described in supplementary table 2. Patients with PT stroma-low/LNs stroma-low showed better 10-year RFP rates compared to patients with PT stroma-high/LNs stroma-high with percentages of 58% versus 8%, respectively. These analyses showed a strong prognostic impact of high amounts of stroma in the PT as well as LNs with regard to RFP. Multivariate analysis showed that the combination of the TSR in PT and LNs is an independent prognostic factor for RFP ($p = 0.019$) (table 2). A non-statistically significant trend was seen in favor of stroma-low PT/stroma-low LNs for OS ($p = 0.084$) (supplementary table 1)

TABLE 2. Univariate and multivariate analyses for the relapse-free period calculated by Cox regression analysis.

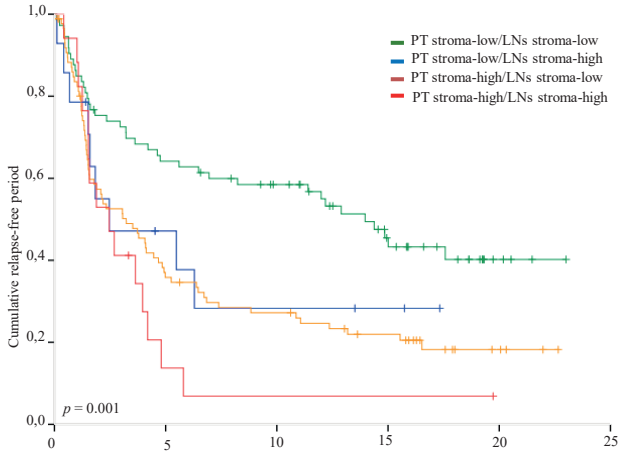
	Relapse-free period									
	Univariate analysis				*Multivariate analysis: TSR in PT*			*Multivariate analysis: TSR in PT and LNs*		
	n	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age (in years)										
<40	15			0.868						
>40-60	94	1.17	0.62-2.22							
>60	82	1.10	0.57-2.12							
Grade										
I	18			0.745						
II	85	0.99	0.53-1.85							
III	88	1.14	0.61-2.11							
Histological type										
Ductal carcinoma	171			0.131						
Lobular carcinoma	18	1.52	0.88-2.60							
Tumor stage										
pT1	42			0.472						
pT2	109	0.88	0.58-1.34							
pT3/4	34	1.17	0.69- 1.98							
Nodal stage										
pN1	148			0.001			0.610			0.674
pN2	11	2.46	1.27-4.77		1.42	0.71-2.84		1.35	0.67-2.71	
pN3	32	1.90	1.23-2.93		1.11	0.68-1.82		1.13	0.69-1.84	
ER status										
Negative	83			0.311						
Positive	98	1.21	0.84-1.73							
PR status										
Negative	86			0.311						
Positive	97	0.83	0.59-1.19							

TABLE 2. Continued.

	Relapse-free period									
		Univariate analysis			*Multivariate analysis: TSR in PT*			*Multivariate analysis: TSR in PT and LNs*		
	n	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value
HER2 status										
Negative	118			0.331						
Positive	25	0.76	0.43-1.33							
Surgery with or without radiotherapy										
MST without RT	62			0.017			0.039			0.050
MST with RT	63	1.62	1.05-2.48		1.65	1.04-2.63		1.64	1.03-2.62	
BCS without RT	0									
BCS with RT	66	0.94	0.61-1.47		0.99	0.63-1.55		1.02	0.64-1.61	
Chemotherapy										
No	127			<0.001			0.004			0.004
Yes	64	0.47	0.32-0.70		0.53	0.35-0.82		0.53	0.35-0.82	
Hormonal therapy										
No	136			0.488						
Yes	55	0.87	0.59-1.29							
TSR										
Stroma-low	87			<0.001			0.006			
Stroma-high	104	1.97	1.37-2.82		1.70	1.16-2.49				
TSR PT combined with LNs										
PT low/LN low	73			0.001						0.019
PT low/LN high	14	1.78	0.86-3.68	0.120				1.58	0.76-3.30	0.223
PT high/LN low	87	2.04	1.37-3.04	<0.001				1.75	1.15-2.65	0.009
PT high/LN high	17	2.86	1.56-5.24	0.001				2.41	1.29-4.49	0.006

Abbreviations: BCS = breast conserving therapy, ER = estrogen receptor, HER2 = human epidermal growth factor receptor 2, LN = lymph nodes, MST = mastectomy, PR = progesterone receptor, PT = primary tumor, RT = radiotherapy, TSR = tumor-stroma ratio

FIGURE 3. Kaplan-Meier analysis for relapse-free period of patients with PT stroma-low/LNs stroma-low, PT stroma-low/LNs stroma-high, PT stroma-high/LNs stroma-low, PT stroma-high/LNs stroma-high.



Numbers at risk				
PT stroma-low/LNs stroma-low	73	44	33	14
PT stroma-low/LNs stroma-high	13	5	3	1
PT stroma- high/LNs stroma-low	89	30	21	11
PT stroma-high/LNs stroma-high	17	2	1	1

Abbreviations: LN = lymph node, PT = primary tumor

DISCUSSION

This is the first study investigating the TSR in tumor-positive LNs in patients with invasive breast cancer. Patients with LN metastases were previously considered to be immediately eligible for adjuvant chemotherapy, irrespective of other clinic-pathological parameters. As studies have shown that patients with 1-3 positive LNs do not necessarily have a worse prognosis compared to node-negative tumors, subsequent guidelines have since stated that LN involvement in itself is not a reason for adjuvant chemotherapy (18). However, further research is needed to refine the

prognosis of lymph node-positive patients further, both to omit chemotherapy in some cases or possibly to escalate chemotherapy for others.

Analogous to our work regarding the prognostic implication of stromal proliferation in PTs, we investigated the added significance of assessing stroma in breast cancer positive LNs. We found that incorporating the TSR of LNs combined with the TSR of the corresponding PT provided a superior prediction of RFP compared to the TSR of the PT alone. When the TSR is solely evaluated in the PT, the disease recurrence rate after 10 years is 75% in primary stroma-high tumors, whereas the number is 46% in primary stroma-low tumors. When the TSR of the LNs is added to these two groups, a group of patients with high risk can be identified, namely PT stroma-high/LNs stroma-high. Considering that this patient group has a recurrence rate of 92% after 10 years, this method seems capable of identifying a group of patients with a worse prognosis.

An interesting result is a strong discrepancy between the TSR in the PT and the LNs of the same patients. In 101 (52.9%) patients, heterogeneity was observed between the stroma percentage category in the PT and LNs. Only a small proportion of patients was scored as stroma-high when evaluating the LNs (n = 31), which is in stark contrast with the fairly large amount of stroma-high PTs (n = 104). Consequently, a high number of patients with stroma-high tumors presented with stroma-low LN metastases. This finding might be reflective of differential activity of signaling processes across primary and metastatic tumors. The formation of genetically and transcriptionally distinct subclones of tumor cells that arise during tumor evolution might influence the activation of tumor-associated stroma as well as tumor cell dissemination. In the current study, we found that at least one LN with a high amount of stroma was predictive for a statistically significant decreased RFP. A previously published study by Van Pelt et al. also showed the additional value of the TSR in lymph nodes. The authors concluded that the assessment of the TSR in the PT combined with the TSR in metastatic LNs has an additional value with regards to the prediction of DFS in patients treated with adjuvant therapy for stage III colon cancer (17). Incorporating the TSR in clinical practice has certain advantages compared to other potential biomarkers. TSR scoring can be carried out on standard H&E slides and is performed by visually eyeballing the tissue area during the standard pathological assessment. TSR scoring takes less than a

minute and requires no additional costs. Implementation of this method in daily practice is, therefore, an easy and non-expensive option. The concordance of the inter-observer variability has been high between researchers from our group, which is also confirmed in the current study (6, 10, 14).

The patients for this study were primarily treated with surgery between 1985 and 1994 and are part of a well-characterized treatment cohort with long-term follow-up. However, this obviously means that modern-day adjuvant chemotherapy and hormonal regimens and selection of these treatment modalities according to current guidelines were not applied to this dataset. This is reflected by the relatively poor prognosis of the included patients compared to currently treated patient groups. Therefore, before definitive conclusions can be drawn regarding the prognostic and therapeutic implication of tumoral LN fibrosis, validation of the current results in modern-day cohorts should be undertaken.

Lastly, according to treatment guidelines, breast cancer patients first undergo a sentinel lymph node biopsy (SLNB) in case of no suspicion of positive lymph nodes by ultrasound or clinical examination (1). Depending on the presence of LN metastasis, an ALND will be performed. Evaluation of the TSR in a tumor-positive LN dissected during sentinel node procedure is interesting. A recent publication from Giuliano et al. showed that a less invasive SLNB alone was non-inferior to predicting overall survival compared to ALND in women with T1 or T2 tumors, no palpable axillary lymphadenopathy and 1 or 2 positive sentinel LNs (19). Evaluation of the TSR in sentinel nodes could be an important next step to evaluate if this clinical prognostic marker can select patients who will benefit from ALND or axillary radiotherapy.

CONCLUSIONS

The TSR is a simple, fast and cheap method. Assessing the TSR on tumor-positive LNs can provide further prognostic stratification in breast cancer patients. Stromal activation strongly differs between PTs and LN metastases, likely reflecting heterogeneity of the tumor stroma metastatic process.

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SUPPLEMENTARY DATA

SUPPLEMENTARY TABLE 1. Univariate and multivariate analyses for overall survival calculated by Cox regression analysis.

	Overall Survival									
	Univariate analysis				*Multivariate analysis: TSR in PT*			*Multivariate analysis: TSR PT and LNs*		
	n	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age (in years)										
<40	15			<0.001			0.296			0.305
>40-60	94	1.25	0.62-2.51		0.70	0.33-1.48		0.73	0.34-1.55	
>60	82	2.40	1.20-4.80		0.91	0.41-2.02		0.95	0.42-2.15	
Grade										
I	18			0.835						
II	85	1.06	0.59-1.88							
III	88	1.15	0.65-2.05							
Histological type										
Ductal carcinoma	171			0.274						
Lobular carcinoma	18	1.34	0.79-2.25							
Tumor stage										
pT1	42			0.384						
pT2	109	1.17	0.78-1.77							
pT3/4	34	1.44	0.86- 2.42							
Nodal stage										
pN1	148			<0.001			0.269			0.280
pN2	11	2.74	1.46-5.16		1.69	0.88-3.27		1.67	0.86-3.22	
pN3	32	1.94	1.29-2.92		1.20	0.75-1.91		1.21	0.76-1.93	
ER status										
Negative	83			0.809						
Positive	98	1.04	0.75-1.46							
PR status										
Negative	86			0.006			0.504			0.523
Positive	97	0.63	0.45-0.88		0.89	0.62-1.26		0.89	0.62-1.27	
HER2 status										
Negative	118			0.736						
Positive	25	0.92	0.55-1.52							

SUPPLEMENTARY TABLE 1. Continued.

	Overall Survival								
	Univariate analysis				*Multivariate analysis: TSR in PT*			*Multivariate analysis: TSR PT and LNs*	
	n	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Surgery with or without radiotherapy										
MST without RT	62			0.001			0.021			0.033
MST with RT	63	1.04	0.71-1.53		1.02	0.66-1.58		1.02	0.66-1.59	
BCS without RT	0									
BCS with RT	66	0.51	0.34-0.77		0.58	0.37-0.91		0.60	0.38-0.94	
Chemotherapy										
No	127			<0.001			<0.001			<0.001
Yes	64	0.35	0.23-0.52		0.41	0.26-0.66		0.42	0.26-0.68	
Hormonal therapy										
No	136			0.126						
Yes	55	1.31	0.93-1.86							
TSR										
Stroma-low	87			0.003			0.029			
Stroma-high	104	1.65	1.86-2.29		1.49	1.04-2.14				
TSR PT combined with LNs										
PT low/LN low	73			0.002						0.084
PT low/LN high	14	2.14	1.11-4.14	0.023				1.56	0.78-3.14	0.209
PT high/LN low	87	1.73	1.20-2.49	0.003				1.55	1.05-2.29	0.029
PT high/LN high	17	2.50	1.41-4.42	0.002				1.91	1.03-3.52	0.039

Abbreviations: BCS = breast conserving therapy, ER = estrogen receptor, HER2 = human epidermal growth factor receptor 2, LN = lymph nodes, MST = mastectomy, PR = progesterone receptor, PT = primary tumor, RT = radiotherapy, TSR = tumor-stroma ratio

SUPPLEMENTARY TABLE 2. Patient characteristics categorized in patients with stroma-low PTs/stroma-low LNs, stroma-low PTs/stroma-high LNs, stroma-high PTs/stroma-low LNs and stroma-high PTs/stroma-high LNs.

	Stroma-low PT/ stroma-low LNs		**Stroma-low PT/ stroma-high LNs**		**Stroma-high PT/ stroma-low LNs**		**Stroma-high PT/ stroma-high LNs**		
	n = 73	%	n = 14	%	n = 87	%	n = 17	%	*p*-value
Age (in years)									
<40	8	11.0	1	7.1	4	4.6	2	11.8	0.281
>40-60	35	47.9	4	28.6	49	56.3	6	35.3	
>60	30	41.1	9	64.3	34	39.1	9	52.9	
Grade									
I	5	6.8	0	0	10	11.5	3	17.6	0.475
II	32	43.8	5	35.7	41	47.1	7	41.2	
III	36	49.3	9	64.3	36	41.4	7	41.2	
Histological type									
Ductal carcinoma	69	95.8	14	100	72	83.7	16	94.1	0.034
Lobular carcinoma	3	4.2	0	0	14	16.3	1	5.9	
Tumor stage									
pT1	15	20.8	1	7.1	22	26.8	4	23.5	0.248
pT2	46	63.9	8	57.1	43	52.4	12	70.6	
pT3/4	11	15.3	5	35.7	17	20.7	1	5.9	
Nodal stage									
pN1	63	86.3	12	85.7	62	71.3	11	64.7	0.095
pN2	0	0	1	7.1	8	9.2	2	11.8	
pN3	10	13.7	1	7.1	17	19.5	4	23.5	
ER status									
Negative	33	45.8	7	53.8	36	45.0	7	43.8	0.943
Positive	39	54.2	6	46.2	44	55.0	9	56.3	
PR status									
Negative	28	38.9	8	61.5	41	50.0	9	56.3	0.278
Positive	44	61.1	5	38.5	41	50.0	7	43.8	
HER2 status									
Negative	49	83.1	8	80.0	52	83.9	9	75.0	0.895
Positive	10	16.9	2	20.0	10	16.1	3	25.0	
Surgery with or without radiotherapy									
MST without RT	23	31.5	7	50.0	29	33.3	3	17.6	0.268
MST with RT	22	30.1	6	42.9	27	31.0	8	47.1	
BCS without RT	0	0	0	0	0	0	0	0	
BCS with RT	28	38.4		7.1	31	35.6	6	35.3	

SUPPLEMENTARY TABLE 2. Continued.

	Stroma-low PT/ stroma-low LNs		**Stroma-low PT/ stroma-high LNs**		**Stroma-high PT/ stroma-low LNs**		**Stroma-high PT/ stroma-high LNs**		
	n = 73	%	n = 14	%	n = 87	%	n = 17	%	*p*-value
Chemotherapy									
No	42	57.5	10	71.4	63	72.4	12	7.06	0.233
Yes	31	42.5	4	28.6	24	27.6	5	29.4	
Hormonal therapy									
No	53	72.6	8	57.1	63	72.4	12	70.6	0.686
Yes	20	27.4	6	42.9	24	27.6	5	29.4	

Abbreviations: BCS = breast conserving therapy, ER = estrogen receptor, HER2 = human epidermal growth factor receptor 2, LNs = lymph nodes, MST = mastectomy, PR = progesterone receptor, PT = primary tumor, RT = radiotherapy