ORIGINAL ARTICLE

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An easy and practical prognostic parameter: tumor–stroma ratio in Luminal, Her2, and triple-negative breast cancers

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SUMMARY

OBJECTIVE: The stroma surrounding the tumor cells is important in tumor progression and treatment resistance, besides the properties of tumor cells. Studies on the tumor stroma characteristics will contribute to the knowledge for new treatment approaches.

METHODS: A total of 363 breast cancer patients were evaluated for the tumor–stroma ratio. The percentage of stroma was visually assessed on hematoxylin–eosin stained slides. The cases of tumor–stroma ratio more than 50% were categorized as tumor–stroma ratio high, and those less than 50% and below were categorized as tumor–stroma ratio low.

RESULTS: Tumor–stroma ratio-high tumors had shorter overall survival (p=0.002). Disease-free survival tended to be shorter in tumor–stroma ratio-high tumors (p=0.082) compared with tumor–stroma ratio-low tumors. Tumor–stroma ratio was an independent prognostic parameter for the total group of patients (p=0.003) and also axillary lymph node metastasis and tumor–stroma ratio was statistically associated (p=0.004). Also, tumor–stroma ratio was an independent prognostic parameter in node-positive Luminal A and B subgroups for overall survival (p<0.001).

CONCLUSION: Tumor–stroma ratio is an independent prognostic parameter that can be evaluated quite easily in all molecular subtypes of all breast cancers and does not require extra cost and time to evaluate.

KEYWORDS: Stromal Tumor. Breast neoplasms. Survival. Prognosis.

INTRODUCTION

Globally, determining the biological behavior of breast carcinomas (BC), which are the most common cause of cancer deaths in women, is expected to produce essential knowledge for developing new therapeutic approaches[1]. Despite the ever-increasing knowledge accumulation, 30% of patients still develop recurrence after treatment. Therefore, it is thought that not only tumor cells, whose properties are well-known in many respects, but also the stroma surrounding the tumor cells are important in the progression and treatment resistance of the tumor[2,3]. It is a complex issue that how tumor stroma and its components follow in determining tumor behavior. It is thought that tumor stroma affects tumor progression by being affected by a cell to cell, cell to extracellular matrix, genetic, physiological, and environmental factors[4]. Studies on the characteristics of stroma surrounding the tumor cells will contribute to the knowledge for new treatment approaches[5].

Many different components contribute to tumor stroma. It is not always practical to evaluate these components separately, but even determining the tumor–stroma ratio (TSR) gives information about the prognosis of patients. It has been reported that the amount of tumor stroma is an independent prognostic parameter in many tumors[6-9]. In our study, the relation of TSR with prognostic parameters and survival was evaluated in both triple-negative (TN) and ER-positive BC. While the chemotherapy option of patients with TN and PT1/PT2 N0 is controversial, lymph node-positive patients are candidates for adjuvant chemotherapy[10]. We also investigated the role of TSR in survival in these node-positive Luminal A and B groups.

METHODS

Histopathological scoring

The TSR was visually evaluated as previously described by Mesker et al.[11]. The original 4 µm routine hematoxylin and eosin (H&E)-stained slides from formalin-fixed paraffin-embedded blocks of the primary tumor were assessed by conventional light microscopy (Olympus, BX-51, ocular 22 mm). The area with the highest tumor stroma was determined in the 4× objective. The most stroma-abundant area on the slide in which tumor

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cells can be seen on each side was scored using a 10× objective (north–east–southwest). The TSR was scored in multiples of 10 per image field (e.g., 10, 20, and 30%). The cases of TSR more than 50% were categorized as TSR high, and those less than 50% and below were categorized TSR low. Necrosis, in situ tumors, mucus-secreting tumor areas, previous biopsy areas, and peripheral sides of tumors were excluded in evaluating the TSR. Representative examples of microscopic fields selected for TSR quantification from TSR-high and TSR-low tumors are shown in Figures 1 and 2.

Evaluation of the TSR was assessed successfully in all the tumors (100%). Cohen's kappa coefficient revealed an almost perfect agreement in classification (kappa=0.85; 94% concordance in classification) for a set of tumors scored by both observers (ÇÖ and OO).

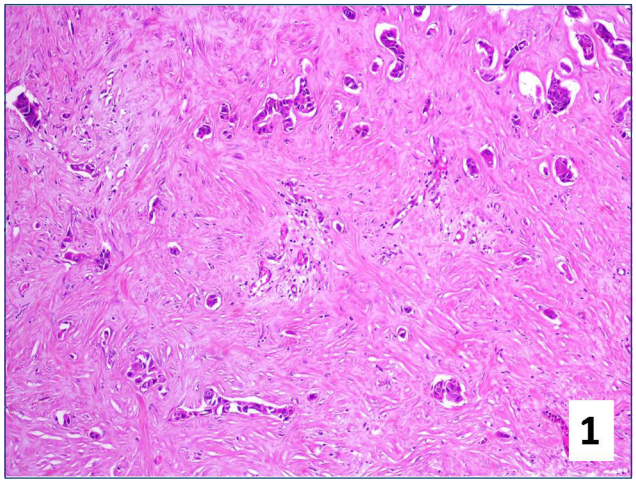

Figure 1. Example of stroma-rich (stroma ratio ≥50%). Hematoxylin and eosin-stained 4 µm sections of primary breast tumors (original magnification ×100).

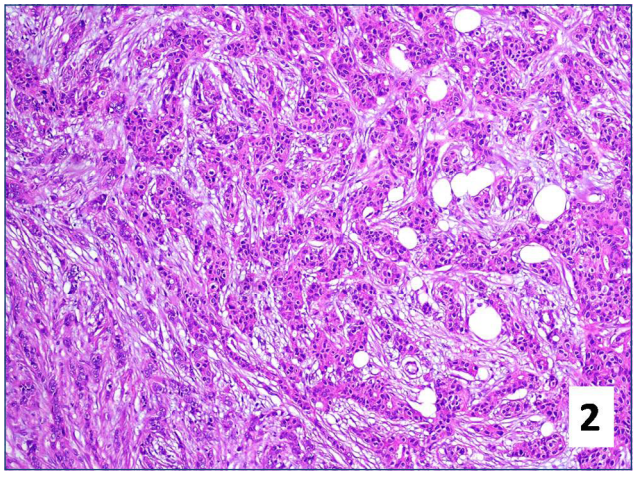

Figure 2. Example of stroma-poor (stroma ratio <50%). Hematoxylin and eosin-stained 4 µm sections of primary breast tumors (original magnification ×100).

Clinical and demographic data and tissues

We selected the patients with invasive breast cancer between 2010 and 2020 from the database of our hospital. Patients who received neoadjuvant chemoradiotherapy, who had distant organ metastasis at the time of diagnosis, who died due to post-op complications in the first month after surgery, whose clinical data could not be reached, who were not followed up after the operation in our hospital, whose hormone profile was not interpreted, who were out of follow-up for any reason, and whose HE slides and formalin-fixed paraffin-embedded blocks could not be found in our archive were excluded from the study.

The clinical information was obtained from retrieving the medical records, including gender, age, histological tumor type, grade, tumor size, lymph node status, type of surgery, and patient follow-up information. All cases were divided into molecular subtypes based on the ER, PR, HER2, and Ki-67 immunohistochemical staining patterns and histological types, according to the classification of breast cancer by World Health Organization[2,12].

As a result, 363 patients were included in the study, and the relationship between TSR, and clinicopathological parameters, disease-free (DFS), and overall survival (OS) were investigated.

Statistical analysis

Statistical analysis was performed using SPSS version 21 software. Kolmogorov–Smirnov test, histogram graphics, Mann–Whitney U test, chi-square, Fisher's exact test, Hosmer–Lemeshow test, log-rank test, Cox regression, and Kaplan–Meier survival analysis were performed. For statistical significance, the p-value was accepted as <0.05.

RESULTS

Tumor–stroma ratio and clinical and pathological parameters

Tumor–stroma ratio could be evaluated in a total of 363 patients, 2 of whom were men. The median age of patients was 55 (25–100) years, and the median follow-up time was 46 (2–132) months. A total of 324 of the cases had invasive ductal carcinoma (IDC), of whom 20 had invasive lobular carcinoma (ILC) and 19 patients had other histological types. When the cases were evaluated according to the T stages, 146 cases were found in T1, 196 cases were in T2, and 21 cases were found in the T3 stage. According to the molecular subtypes, 135 cases were Luminal A, 160 cases Luminal B, 30 cases Her2, and 32 cases were in the triple-negative group.

There was no statistical relationship between TSR and clinicopathological parameters such as age, hormone status, molecular

and histological type, and angiolymphatic invasion. Among the clinicopathological parameters, there was a statistically significant difference between axillary lymph node metastasis and TSR (p=0.004). Accordingly, as the TSR increased, the incidence of axillary lymph node metastasis increased. The main characteristics of the included studies are listed in Table 1.

Table 1. Correlations between tumor–stroma ratio and well-established prognostic factors.

		TSR low		TSR high		p
		N	%	N	Column N %	
Age	<57.2	104	61.50	101	52.10	0.069
	>57.2	65	38.50	93	47.90	
Histological types	IDC	149	88.20	175	90.20	0.594
	ILC	9	5.30	11	5.70	
	Others	11	6.50	8	4.10	
ER expression	ER negative	35	21.00	33	17.20	0.363
	ER positive	132	79.00	159	82.80	
PR expression	PR negative	54	32.30	52	27.10	0.277
	PR positive	113	67.70	140	72.90	
Ki67 expression	Low	54	37.00	70	44.00	0.211
	High	92	63.00	89	56.00	
Her2 expression	Her2 negative	110	65.90	122	63.20	0.361
	Her2 positive	32	19.20	48	24.90	
	Unknown	25	15.00	23	11.90	
Molecular subtypes	Luminal A	57	34.30	78	40.80	0.488
	Luminal B	76	45.80	84	44.00	
	HER2	15	9.00	15	7.90	
	Triple negative	18	10.80	14	7.30	
Nuclear grade	1	5	4.30	7	5.60	0.059
	2	84	73.00	104	83.20	
	3	26	22.60	14	11.20	
Histological grade	1	13	8.30	14	7.50	0.116
	2	109	69.40	146	78.50	
	3	35	22.30	26	14.00	
T stage	PT1	70	41.40	76	39.20	0.698
	PT2	91	53.80	105	54.10	
	PT3	8	4.70	13	6.70	
Perineural invasion	Negative	123	74.10	138	71.50	0.582
	Positive	43	25.90	55	28.50	
Anjiolymphatic invasion	Negative	96	56,80	91	46.90	0.06
	Positive	73	43.20	103	53.10	
Lymph node metastasis	Negative	104	61.50	90	46.40	0.004
	Positive	65	38.50	104	53.60	
Breast cancer-related death	Alive	159	94.10	160	82.50	0.001
	Dead	10	5.90	34	17.50	
Distant metastasis	Negative	152	89.90	161	83.90	0.089
	Positive	17	10.10	31	16.10	

Tumor–stroma ratio and prognostic associations with outcome

A total of 194 (54%) patients were classified as TSR high and 169 (46%) patients as TSR low. OS was found for patients with TSR-high tumors as compared to patients with TSR-low tumors in univariate analysis (p=0.003). In multivariate analysis, the TSR was an independent prognostic variable for OS (Table 2).

In univariate analyses (p=0.86), the TSR was not an independent prognostic variable for DFS. After correction for the factors also used in multivariate analyses for DFS, no significant difference was obtained.

Molecular subtypes and axillar lymph node metastasis were independent prognostic variables for DFS in multivariate analysis. Even performing Kaplan–Meier curve for OS showed a significant difference between TSR-high and TSR-low patients (p=0.002) (see Figure 3). A trend was seen toward a worse DFS for patients with TSR-high tumors compared to patients with TSR-low tumors in the Kaplan–Meier curve (p=0.082) (see Figure 4).

A total of 104 (61.5%) node-positive Luminal A and B breast carcinoma patients were classified as TSR high and 65 (38.5%) patients as TSR low. OS was found for these patients with TSR-high tumors as compared to patients with TSR-low tumors in univariate analysis (p:0.003). In multivariate analysis, the TSR was an independent prognostic variable for OS [Hazard ratio (HR) 5.33; 95%CI 1.224–23.203; p=0.026]. Patients with TSR-high node-positive Luminal A and B tumors show a significantly worse overall survival compared to patients with TSR-low tumors in the Kaplan–Meier curve(p<0.001) (see Figure 5). A trend was seen toward a worse DFS for patients with TSR-high tumors compared to patients with TSR-low tumors in the Kaplan–Meier curve (p=0.066) (see Figure 6).

Table 2. Cox univariate and multivariate analysis for overall survival of all patients.

Variables	Univariate	Multivariate	HR (95%CI)
	p	p	
Tumor–stroma ratio	0.003	0.042	2.381 (1.033–5.485)
Metastasis	<0.001	<0.001	7.038 (2.917–16.978)
ER expression	0.012		
PR expression	0.02		
Her2 expression	0.031		
Ki67 proliferation index	0.015		
Molecular subtypes	<0.001	0.001	10.382 (2.731–39.467)
Nuclear grade	0.223		
Histological grade	0.079		
Tumor size	0.013		
Age	<0.001	0.008	1.035 (1.009–1.062)
Lymph node metastasis	<0.001	0.025	3.945 (1.187–13.110)
Angiolymphatic invasion	<0.001		
Perineural invasion	0.375		

DISCUSSION

The relation of TSR with survival status was first investigated by Mesker et al. They found that patients with TSR more than 50% showed significantly worse OS and DFS. They suggested that TSR could serve as an independent parameter for predicting clinical outcomes in early-stage colon cancer[11].

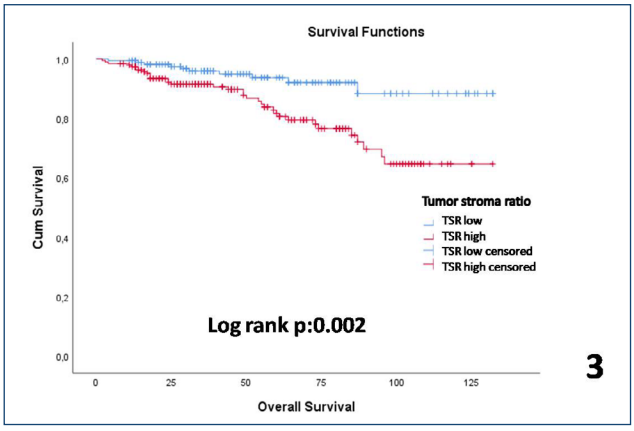

Figure 3. Kaplan–Meier curves for tumor–stroma ratio for the total patient population. Patients with TSR-high tumors show a significant overall survival compared to patients with TSR-low tumors.

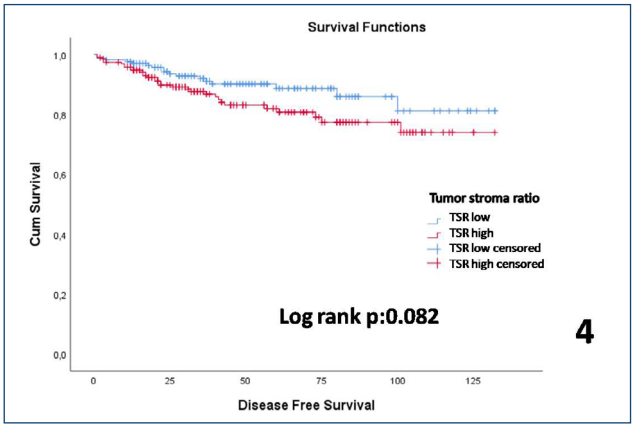

Figure 4. A trend was seen toward a worse disease free survival for patients with TSR-high tumors compared to patients with TSR-low tumors.

The tumor stroma consists of fibroblasts, pericytes, bone marrow-associated mesenchymal stem cells, adipocytes, macrophages, and immune cells[13]. These components play a role in neoangiogenesis, metastasis, and tumor progression[14]. It is not always morphologically possible to evaluate these components separately. Additional studies may be needed to evaluate these components; however, TSR can only be evaluated by light microscopy.

Routine pathology reports include the following parameters that affect patient prognosis, such as nuclear and histological grade, molecular subtype, lymph node metastasis, and lymphovascular invasion[15]. In addition to these parameters, the TSR, an independent prognostic indicator with many studies, is a convenient and valuable parameter for patient prognosis.

Studies in the literature evaluate stroma in BC using digital methods such as machine learning algorithms and automated analyses[9]. The advantage of digital methods over light microscopy is to evaluate whole tumor tissue with digital methods, while a more limited area is evaluated in light microscopy. While evaluating the stroma, the area of necrosis, the previous biopsy area, mucinous areas, and peripheral sides of tumors should not be evaluated. Therefore, the pathologist has a role in the selection of the appropriate tissue in digital analyzes. Digital methods are also not readily available, and extra costs are required to use them. For this reason, our study is based on visual eyeballing evaluation with light microscopy, which is very practical and does not require extra cost. Also, in our study, high intra-observer agreement kappa values for TSR prove strongly that TSR is a highly reproducible method.

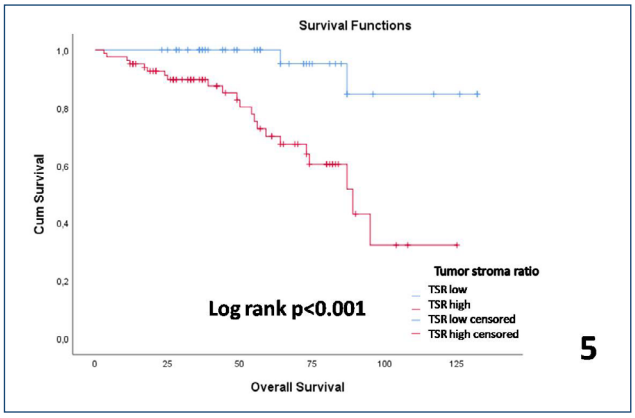

Figure 5. Kaplan–Meier curves for tumor–stroma ratio for node-positive Luminal A and B tumors. Patients with TSR-high tumors show a significant overall survival compared to patients with TSR-low tumors.

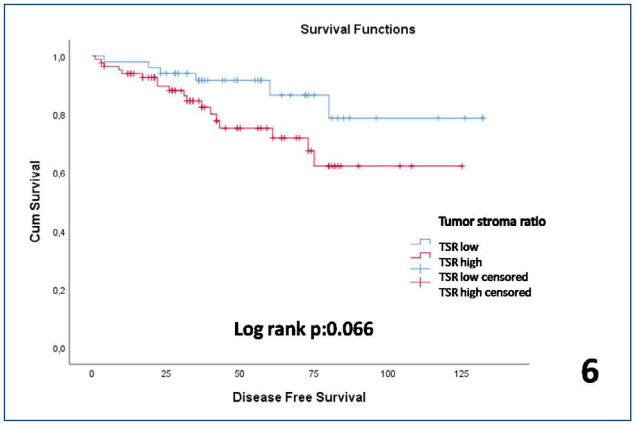

Figure 6. A trend was seen toward a worse disease free survival for patients with TSR-high tumors compared to patients with TSR-low tumors.

Recent studies have mainly worked on TSR of triple-negative breast cancers, which are negative for ER, PR, and HER2. Vangangelt et al. reported that a high amount of stroma predicts poor survival in TN BC. Kruijf et al. showed TSR to be an independent prognostic factor for DFS in breast cancer patients, especially in those with TN BC. Also, Dekker et al. confirmed this finding by a validation study in the EORTC peri-operative chemotherapy trial[18]. The relationship between molecular subtypes and TSR was evaluated in our study, but no statistically significant relationship was found (p>0.05). This result supports the idea that the molecular properties of tumor cells are independent of the molecular properties of the tumor stroma.

In this study, TSR was found to be associated with lymph node metastasis, which is a prognostic factor independent of clinicopathological parameters. The incidence of lymph node metastasis increases in patients with TSR high (p=0.004) compared to patients with TSR low. When the overall survival was evaluated according to TSR high and TSR low among 363 patients whose survival information was available, the OS of the group with TSR high was significantly shorter than TSR low (p=0.002) patients. Also, in univariate (p=0.003) and multivariate cox regression models, TSR was an independent prognostic variable for OS.

Tumor–stroma ratio was also examined in node-positive Luminal A and B groups to evaluate the relationship between TSR and survival in a more homogeneous group. The overall survival of this group with TSR high was significantly shorter than TSR low (p<0.001). Also, in univariate (p=0.003) and multivariate cox regression models, the TSR was an independent prognostic variable for OS. TSR is an independent risk factor in this group of patients whose survival may differ. It is a parameter that can be used to determine prognosis. A potential limitation of our study was that patients with a short follow-up period were also included in this study in order to evaluate more patients. This may be the reason why TSR and DFS are not associated.

Adjuvant chemotherapy is controversial in early-stage TN and ER-positive BC. 17th St. Gallen International Breast Cancer Conference suggested genomic assays in addition to

clinicopathological parameters in patient selection for treatment in PT1/PT2 N0 ER-positive patients[10]. However, in the same panel, it was reported that these genomic assays are not easily accessible universally and cost much more than routine pathology procedures. In our study, we showed that TSR is a decisive, independent prognostic factor. Therefore, TSR may be a parameter in the treatment decision, especially in this group of patients who have difficulties making treatment decisions.

There are publications in the literature reporting that clinical features are also effective in prognosis in BCs, regardless of the histological type[20-22]. One of the limitations of this study is that clinical features were not evaluated. Another limitation is that the molecular and histological types of the cases are not homogeneously distributed.

CONCLUSION

Tumor–stroma ratio is an independent prognostic parameter that can be evaluated quite easily in all molecular subtypes of all BCs and does not require extra cost and time to evaluate. Therefore, TSR is a candidate practical parameter that can be included in routine pathological reports.

ETHICS

This study was conducted at Recep Tayyip Erdogan University Research and Training Hospital, Rize, Turkey, and conducted in accordance with the Declaration of Helsinki. The Ethics Committee approved the study protocol of Recep Tayyip Erdogan University.

AUTHORS' CONTRIBUTIONS

ÇÖ, OO: Conceptualization, Investigation, Visualization, Project administration. **ÇÖ, OO, BŞ:** Data curation, Writing – orginal draft. **ÇÖ, BŞ:** Formal Analysis, Methodology. **RB:** Funding acquisition, Validation. **ÇÖ, RB:** Resources. **BŞ:** Software. **OO, RB:** Supervision. **OO, BŞ, RB:** Writing – review & editing.

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