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Leiden
The Netherlands

The tumor-stroma ratio in epithelial cancer types: towards implementation in diagnostic pathology

Smit, M.A.

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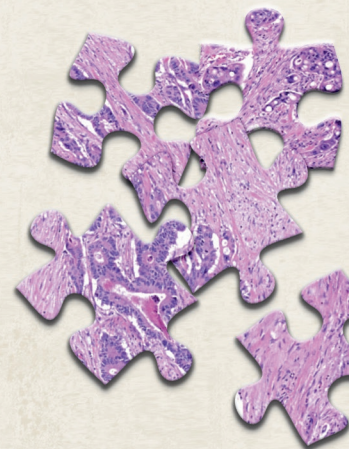
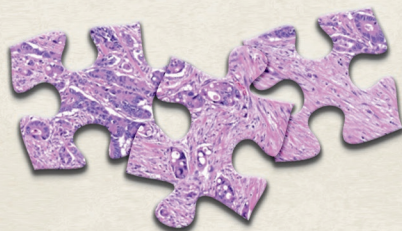


CHAPTER 8

70-Gene signature and tumor-stroma ratio select different groups of patients with breast cancer at risk for recurrence

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Marloes A Smit | Julia EC van Steenhoven | Rob AEM Tollenaar
Thijs van Dalen | Paul J van Diest | Wilma E Mesker



Abstract

Background: Strategies for breast cancer care are improving. However, there is a clinical need for a better selection of patients who are at risk for recurrence and benefit from treatment with chemotherapy. The 70-gene signature (70-GS) guides chemotherapy decision making based on the mRNA microarray assessment but is expensive. The tumor-stroma ratio (TSR) is a morphological and cheap biomarker based on the amount of stroma in the primary tumor. In the present study the association between the 70-GS and TSR was investigated.

Materials and methods: Frozen tumor tissue sections of 102 ER+/Her2- patients were sent to Agendia for 70-GS analysis in an earlier study. The results of the 70-GS were available, and hematoxylin and eosin stained sections of the 102 tumors were scored for TSR. Concordance between the TSR and the 70-GS was analyzed. No data of patient outcome is available.

Results: Of the patients in the 70-GS high risk category (n=46), 23 patients (50%) were classified into the stroma-high group according to previously established thresholds. 55% (n=31) of the low-risk 70-GS patients, had a stroma-low profile. The overall concordance between the 70-GS and TSR was 53% and no association was observed (Chi-square: $p=0.917$). Patients with a 70-GS high-risk test result had a significantly higher tumor grade and stroma-high patients were significantly older and had larger tumors.

Conclusion: In conclusion, no association between the 70-GS and TSR was found. This suggests that both methods select different groups of patients at risk for recurrence, and may have additional prognostic value.

Introduction

Breast cancer is the most common cancer affecting women. Over the past decades, mortality rates have declined significantly mainly due to improved systemic treatment strategies (1, 2). Breast cancer treatment not only exists of surgery, but is often in combination with radiotherapy. It also encompasses treatment with adjuvant chemotherapy. Systemic treatment decisions are guided by clinical and pathological characteristics to estimate the risk for recurrence and the benefit of adjuvant chemotherapy. However, due to the heterogeneity of the disease, patients with comparable clinicopathological features may have different outcomes (3). Therefore, optimization in breast cancer risk stratification is of great importance to reduce overtreatment with adjuvant systemic therapy, but also to prevent under treatment.

Prognostic and predictive tests can be molecularly or morphologically based. In recent years, several gene expression profiles have been developed to assess the risk of recurrence based on the expression of, predominantly tumor proliferation related, genes (4). One such test is the 70-gene signature (70-GS (MammaPrint®)) that assigns patients to high- or low-risk recurrence group (3, 5-8). The guidelines suggest the use of a validated gene-expression profile in patients with estrogen-receptor positive (ER+) and Her2 receptor negative (HER2-) disease in whom treatment with adjuvant chemotherapy is debated on the basis of clinicopathological characteristics alone (9, 10). However, as gene-expression profiles are relatively costly, attempts have been made to search for alternative tests that predict prognosis accurately.

A promising and less expensive morphological biomarker is the tumor-stroma ratio (TSR). TSR is based on the microenvironment of the primary tumor and showed to be a good independent prognostic parameter for (disease free and overall) survival in multiple epithelial cancer types, including breast cancer (11-14). Tumors with high stromal content are characterized by invasive behavior and worse survival. Stroma-high tumors have been associated with enriched extracellular matrix and activated fibroblasts which likely contribute to the metastatic capacity of cancer cells (15). The TSR can be scored during diagnostic pathology in the hospital without extra laboratory costs. It is often asked if the TSR and the 70-GS are related to each other.

In the present study, we aimed to determine whether the 70-GS and TSR select similar groups of patients at risk for recurrence. Therefore, the association between the 70-GS and the TSR was investigated.

Materials and Methods

Patient cohort

The study cohort of 102 patients was selected from “the Symphony Triple A Study” (a prospective multicenter observational study regarding the influence of the 70-GS on adjuvant chemotherapy decision) (16). The distribution between the 70-GS high-risk profile and low-risk profile was equal in the selected population, further patient selection was random. All patients had been diagnosed with early stage invasive ductal ER+ breast cancer, with an uncertain benefit of adjuvant therapy (16). Clinicopathological information regarding age, tumor size, invasive grade, lymph-node involvement, ER, PR, Her2 status and the genomic test result was obtained from the Symphony Triple A database.

70-Gene signature

A fresh frozen or paraffin embedded tissue sample of the tumor was sent to the Agendia Laboratory (Amsterdam, the Netherlands) for analysis of the 70-GS as part of routine care in the aforementioned study. The 70-GS (MammaPrint®) assigned patients to an either high or low risk of dissemination group based on the expression of 70 genes. The analyses were performed blinded to clinical and pathological data (16).

Tumor-stroma ratio

The TSR was scored at the Leiden University Medical Center (Leiden, the Netherlands) on hematoxylin and eosin stained sections of the resection specimen, used for routine pathology. The percentage of stroma in the primary tumor was scored in increments of 10. A tumor was classified as stroma-low when $\leq 50\%$ stroma was present and stroma-high if a tumor had $>50\%$ stroma. For detailed description of the scoring method see references (14, 17, 18). Two well-trained observers (WM, MS) scored the sections for TSR, both blinded to clinicopathological data and each other’s score. Interobserver agreement was calculated.

Statistical analysis

Baseline characteristics were presented in percentages. Non-normally distributed continuous parameters were visualized with a median and range was calculated. Differences between non-normal variables and groups were analyzed using the Mann-Whitney U test. Contingency tables were used to visualize categorical variables. Percentage concordance between TSR and 70-GS was calculated, and Chi-square test was used for measuring the statistical coherence between TSR and 70-GS. The interobserver agreement was calculated using Cohen’s kappa. P-values <0.05 were considered significant. Statistical analyses were conducted in SPSS statistical package version 25.0 (SPSS, Inc. an IBM Company Chicago, IL, USA).

Table 1. Baseline characteristics of 102 invasive ER+/HER2- breast cancer patients studied for tumor-stroma ratio (TSR) and 70-gene signature (70-GS).

	70-GS			TSR		
	Low-risk	High-risk	<i>p-value</i>	Stroma-low	Stroma-high	<i>p-value</i>
All						
N=102 (100%)	56 (55%)	46 (45%)		54 (53%)	48 (47%)	
Age, years, median (range)	53 (33-73)	59 (35-70)	0.151	53 (33-73)	60 (42-70)	0.009*
Tumor size, mm, median (range)	16 (7-130)	15 (7-32)	0.457	15 (7-32)	17 (9-130)	0.041*
pN status			0.862			0.104
Negative	88 (86%)	39 (85%)		44 (81%)	44 (92%)	
Positive	12 (12%)	5 (11%)		9 (17%)	3 (6%)	
Missing	2 (2%)	2 (4%)		1 (2%)	1 (2%)	
Tumor grade			0.020*			0.115
1	19 (19%)	5 (11%)		7 (13%)	12 (25%)	
2	63 (62%)	27 (59%)		33 (61%)	30 (63%)	
3	20 (20%)	14 (30%)		14 (26%)	6 (13%)	
PR status			0.106			0.313
Negative	12 (12%)	8 (17%)		8 (15%)	4 (8%)	
Positive	86 (84%)	36 (78%)		44 (81%)	42 (88%)	
Missing	4 (4%)	2 (4%)		2 (4%)	2 (4%)	

* Significant results.

70-GS 70-Gene Signature, N Lymph nodes, p Pathological, PR Progesterone, TSR tumor-stroma ratio

Results

Baseline

In total, tissue samples of 102 women diagnosed with ER+/Her2- disease, were analyzed. The median age of the patients was 56 years (range 33-73) and the majority of patients had intermediate grade tumors, without axillary lymph node involvement (**table 1**).

Forty-five percent (n=46) of patients were classified as 70-GS high-risk and 47% (n=48) of patients were classified stroma-high. Patients in the stroma-high group were significantly older (median 60 vs. 53 years) and had larger tumors (median 17mm vs. 15mm) compared to patients in the stroma-low group, whereas no differences were observed regarding age and tumor size between 70-GS low- and high-risk patients. Patients with a 70-GS high-risk test result had a significantly higher tumor grade compared to 70-GS low-risk patients. No difference was observed for TSR and tumor grade. Lymph node involvement and PR status were comparable in the 70-GS and TSR groups (**table 1**). A good interobserver agreement for TSR ($\kappa=0.78$) was obtained.

Comparison of the 70-GS versus TSR

Both prognostic variables classified similar proportions of patients into the risk group associated with poor prognosis. Of the 70-GS high-risk patients (n=46), 50% (n=23) were classified as stroma-high (**table 2**). The overall concordance between the 70-GS and TSR was 53% and no association was found (Chi-square: $p=0.917$).

Table 2. Comparison of 70-gene signature and tumor-stroma ratio in 102 invasive ER+/HER2- breast cancer patients ($p=0.917$, Chi-square test) ER+/HER2- breast cancer patients ($p=0.917$, Chi-square test).

		70-GS		Total
		Low-risk	High-risk	
TSR	Stroma-low	31 (55%)	23 (50%)	54
	Stroma-high	25 (45%)	23 (50%)	48
Total		56	46	102

70-GS 70-Gene Signature, TSR tumor-stroma ratio

Discussion

The current study did not show an association between the 70-GS and the TSR. This suggests that both markers select different groups of breast cancer patients at risk for developing recurrence. Both tests have shown to select patients with a high-risk or worse disease free survival compared to the low-risk group (6-8, 11-14). In the panel of the 70-GS, mostly tumor

related genes are included, but also some stromal genes. Since the 70-GS result is binary, no data is available whether individual stromal genes are up- or down regulated. It would be interesting to see whether the stromal genes of the 70-GS in the stroma-high group are up regulated compared to the stroma-low group.

TSR can be scored on all available tissue sections of a primary tumor, the 70-GS can only be performed when the tumor tissue contains 30% or more tumor cells. Because of this restriction a selection bias was created. However, no differences in distribution for TSR was seen in this cohort, compared to previous TSR publications due to a variation in the distribution of TSR in different cohorts (11-14, 18). Stroma-high patients were significantly older and had larger tumors than stroma-low patients, this is consistent with a recent publication of Vangangelt et al. who showed that intratumoral stroma increases with age (18). A larger size of stroma-high tumors is in line with earlier research, the clinical relevance can be debated because of the small difference (2mm) (13, 18).

Currently, no follow-up data is available for this cohort and will not be expected before the end of 2020. Both tests have shown their prognostic value. For TSR as described in a review by Kramer et al. who provided an overview of the prognostic value of the TSR validated in several independent international studies (11). The 70-GS has proven to be valuable because it is included in the international guidelines (9). In our opinion there is no reason to believe that the prognostic value of the tests will be different in this cohort. So, the assumption is that low/low-risk patients (low 70-GS and stroma-low) have a better prognosis than intermediate-risk patients (high 70-GS and stroma-low or vice versa), while high/high-risk patients (high 70-GS and stroma-high) have the worst prognosis. This is interesting and should be further investigated. Clinical conclusions can be drawn when outcome of our patient cohort is known.

Conclusion

The present study did not find an association between the 70-GS (MammaPrint®) and the TSR. This indicates that 70-GS and TSR, both may have additional prognostic value.

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