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 primary breast cancer with special attention to triple negative tumors: a review

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ABSTRACT

Purpose

There is a strong need to improve the prognostication of breast cancer patients in order to prevent over- and undertreatment, especially when considering adjuvant chemotherapy. Tumor stroma characteristics might be valuable in predicting disease progression.

Methods

Studies regarding the prognostic value of the tumor-stroma ratio (TSR) in breast cancer were evaluated.

Results

A high stromal content was related to a relatively poor prognosis. The most pronounced prognostic effect of this parameter seemed to be observed in the triple-negative breast cancer subtype.

Conclusions

TSR assessment might represent a simple, fast and reproducible prognostic factor at no extra costs, and could be incorporated into routine pathological diagnostics. Despite these advantages, robust clinical validation of this parameter has yet to be established in prospective studies.

INTRODUCTION

According to the European cancer statistics for 2018, the estimated number of new breast cancer cases is 522.500 and the estimated number of breast cancer related-deaths is 137.700 (1). Breast tumors are classified into four molecular subtypes, namely luminal A, luminal B, human epidermal growth factor receptor 2 (HER2)-enriched and basal-like (2, 3). The triple-negative breast cancer (TNBC) belongs to the basal-like phenotype in the vast majority, which is an aggressive form of breast cancer with a shorter relapse-free period (RFP) and relative survival compared to luminal A and B (4, 5). However, gene-expression analyses have shown that this group is notoriously heterogeneous, with some molecular subtypes even associated with a relatively favorable prognosis (5). Approximately 16% of all breast cancer cases are represented by TNBC (6).

In recent years, extensive research has been performed to discover new prognostic biomarkers and determine optimal prognostication schemes for breast cancer patients. Molecular tests, such as the 70-gene signature (MammaPrint, Agendia BV, The Netherlands) and the 21-gene assay (Oncotype DX, Genomic Health, United States) have shown to improve clinical decision making in early-stage breast cancer of certain molecular and clinical subtypes, such as estrogen receptor (ER)-positive or HER2-negative breast cancer (7, 8). These molecular markers are now endorsed into routine clinical practice, according to the American Society of Clinical Oncology Clinical Practice guideline, to reduce the administration of adjuvant chemotherapy and prevent overtreatment (9).

Despite the fact that alterations in the tumor microenvironment have been recognized as important drivers of tumor progression, the tumor environment has not been integrated in routine clinical decision making yet. A parameter which translates the amount of tumor-associated stroma is the tumor-stroma ratio (TSR), which has been extensively described as a rich source of prognostic information for various solid cancer types (10-38). The TSR was first described as a prognostic factor in breast cancer in 2011 by De Kruijf et al. and has been validated in numerous studies (12-15, 17).

For TSR assessment, the amount of tumor-associated stroma is determined on routine hematoxylin and eosin (H&E) stained slides of the primary tumor tissue.

Each tumor is assigned to either the stroma-high or stroma-low category based on a set cut-off value (10).
In this review, literature investigating the effect of the TSR as a prognostic factor in female breast cancer is discussed with a special interest in the prognostic effect in TNBC patients.

RATIONALE

The influence of the tumor-associated stroma on epithelial tumor progression is mostly derived from functional in vitro studies. Similarly, those in vitro studies have demonstrated events in the stromal compartment that occur during carcinogenesis and could contribute to tumor progression. The production of growth factors and proteases by cancer cells initiate changes in the stromal environment (39). Those alterations lie within remodeling of the matrix, recruitment of fibroblasts, the migration of immune cells and angiogenesis, all contributing to tumor progression (40). Cancer-associated fibroblasts (CAFs) contribute to carcinogenesis through the development of unique functions, including an amplified extracellular matrix (ECM) production, higher proliferation rate and the secretion of several cytokines, like vascular endothelial growth factor (VEGF), stromal cell-derived factor 1 (SDF1) and platelet-derived growth factor (PDGF), leading to angiogenesis (40). Transforming growth factor-β (TGF-β) is another factor that is thought to be strongly involved in the tumor-promoting effects of CAFs as described in colon cancer by Hawinkels et al. (41). Those behavioral modifications lead to an elevated expression of enzymes, like matrix metalloproteinases (MMPs), resulting in remodeling and deposition of the ECM, with concurrently the release of pro-angiogenic factors (42).
The ECM is frequently disorganized in tumors. One of the most important mechanisms in the ECM contributing to tumor progression is collagen crosslinking. Due to crosslinking collagen by lysyl oxidase (LOX), the ECM of the tumor becomes more stiff, leading to increased focal adhesions and enhanced PI3K signaling, thereby indirectly ensuring tumor progression (43). Besides the fact that alterations in the tumor niche lead to progression directly, the tumorigenesis can also be strengthened indirectly due to the aforementioned production of

pro-angiogenic factors by CAFs and immune cells. Thus, during the process of tumorigenesis, changes occur in the organization of stromal cells, contributing both directly and indirectly to tumor growth and progression.

Previous studies investigating gene-expression profiles in stromal cells have demonstrated gene signatures related to clinical outcome and response to treatment in breast cancer (44, 45). Clinical application of these signatures was impractical and a definitive indication was never discovered. However, these studies did provide a strong indication that valuable clinical information was ignored by solely focusing on the epithelial compartment. As the stromal processes that are reflected by these assays likely have a quantitative relationship with the amounts of stromal tissue within the tumor, quantitative stromal parameters might equally express prognostic information just by morphology alone (45).

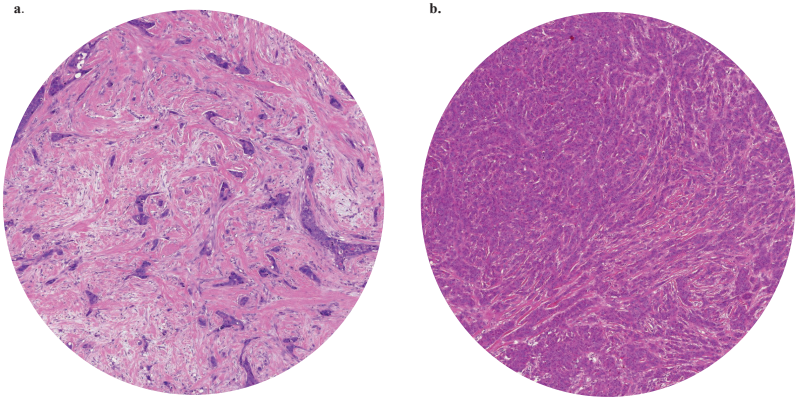
METHODS USED FOR TSR ASSESSMENT

In literature, two methods are described for TSR assessment in breast cancer. The visual scoring method utilized by Mesker et al. and the automated point counting method, a semi-automated approach, utilized by West et al. (10, 18).

Visual eyeballing

Mesker et al. and others determined the TSR by visual eyeballing (10, 12-17). The microscopic determination of the amount of stroma in the primary tumor is performed on routine H&E stained slides. A 2.5x or 5x objective is used to determine the most stroma-abundant area on the slide. In this area, image-fields with tumor cells at all borders of the image are used to determine the amount of stroma, using a 10x objective. The stroma percentage is estimated in increments of 10% per image-field, considering the highest scored stroma percentage as decisive. A stroma percentage ≤50% is categorized as stroma-low and a stroma percentage >50% is categorized as stroma-high, based on the statistical determination, initially performed on colon cancer and subsequently verified for breast cancer (figure 1) (10, 18). Considerable segments of necrosis or *in situ* tumors were excluded in the evaluation of the TSR by neglecting them in the analysis (12, 14).

FIGURE 1. Microscopic evaluation of the tumor-stroma ratio on hematoxylin and eosin stained sections of breast tumors with a 10x objective categorized in stroma-high tumors (>50% stroma) and stroma-low tumors (≤50% stroma) by visual eyeballing. **a.** Stroma-high **b.** Stroma-low.



Semi-automated point counting

West and colleagues have objectified the measurement by evaluating the tumor tissue slides in colon carcinoma using 300 random measurement points validated for breast cancer by Downey et al. (18, 46, 47). Four-micrometer-thick H&E stained sections are scanned using a 20x objective and subsequently two areas without large segments of necrosis are selected with a digital slide viewer. In this method, the two sampled 9 mm^2 areas are in the tumor-leading edge, as well as in the non-leading edge. The group utilizes a grid with a sample of 300 random points, superimposed on the selected area. Under each of the 300 points, the histopathology is categorized in 'tumor', 'stroma' or 'unclassified' (necrosis, blood vessels, inflammation, etc.). The ultimate TSR is the proportion of 'stroma' under the 300 points, compared with all points per section. In other words, the TSR is the number of points, categorized as 'stroma' divided by the total number of points, categorized as 'tumor' and 'unclassified' (18, 46, 47). Downey et al. used 0.49 (i.e. 49%) as a cut-off value in their study in 2014, with ^{3}0.49 being stroma-high and <0.49 stroma-low, based on

statistical analysis (46). However, in another study, cut-off values of 0.31 for OS and 0.46 for DFS are used for categorizing the TSR (47).
The inter-observer variation of these two methods, determined by the Cohen's kappa coefficient (K) or intraclass correlation coefficient (ICC), lies in the range of 0.68-0.85, indicating substantial to good agreement between observers in both methods (table 1).

THE TUMOR-STROMA RATIO IN BREAST CANCER PATIENTS

The first study on the TSR in breast cancer was published by De Kruijf et al. (12). The TSR was estimated by visual eyeballing according to the method described by Mesker et al. (10). The authors showed that the TSR was an independent prognostic parameter in 574 breast cancer patients with invasive breast tumors without distant metastasis (pT1-4, pN0-3, M0). Stroma-high tumors were associated with a worse RFP (HR 1.97, 95% CI 1.47-2.64, $p < 0.001$) and overall survival (OS) (HR 1.50, 95% CI 1.18-1.91, $p = 0.001$) analyzed with multivariate Cox regression analysis (table 1) (12). Vangangelt et al. analyzed the prognostic value of the TSR in a subset of the cohort of De Kruijf et al. in combination with the immune status of tumors. Determination of classical human leukocyte antigen (HLA) class I, HLA-E, HLA-G, natural killer cells and/or regulatory T cells in addition to the TSR showed to have an even stronger prognostic effect (16).
Dekker et al. investigated the prognostic value of the amount of stroma determined by visual eyeballing in 403 premenopausal node-negative breast cancer patients (cT1-3) (14). These patients were selected from the perioperative chemotherapy trial (POP trial, 10854) (48). This study supported the earlier finding of the TSR as an independent prognostic parameter for disease-free survival (DFS) (HR 1.85, 95% CI 1.33-2.59, $p < 0.001$) in favor of stroma-low tumors and borderline statistical significance for OS (HR 1.60, 95% CI 1.00-2.57, $p = 0.050$) (14).
Gujam et al. assessed the TSR on the H&E slides of 361 patients with invasive carcinoma of no special type (NST) (T1-3, N0->3, grade I-III) and subsequently found a correlation between stroma-high tumors and a poor 15-year cancer-specific survival (CSS) (HR 2.12, 95% CI 1.37-3.29, $p = 0.001$) in the multivariate survival analysis (15). Downey et al. dispute this finding in their work by analyzing the

TABLE 1. Detailed overview of studies on the prognostic value of the tumor-stroma ratio in the main study population and triple-negative breast cancer patients.

	Author, year	Sample Size	Percentage of stroma-high tumors	Population	Method	Inter-observer variation	Outcome	Most favorable prognosis
Results of the main study population (except triple-negative breast cancer as main cohort or subgroup)								
General BC	De Kruijf et al., 2011 (12)	574	68%	pT1-4, pN negative-positive, grade I-III	VS	K = 0.85	OS: HR 1.50, 95% CI 1.18-1.91, $p = 0.001$ RFP: HR 1.97, 95% CI 1.47-2.64, $p < 0.001$	Stroma-low
General BC	Dekker et al., 2013 (14)	403	40%	cT1-3, N0, grade I-III	VS	K = 0.804	OS: HR 1.60, 95% CI 1.00-2.57, $p = 0.050$ DFS: HR 1.85, 95% CI 1.33-2.59, $p < 0.001$	Stroma-low
General BC	Roeke et al., 2017 (17)	737	38%	T1-3, N negative–positive, grade I-III	VS	K = 0.68	OS: HR 1.56, 95% CI 1.18-2.05, $p = 0.002$ RFS:HR 1.35, 95% CI 1.01-1.81, $p = 0.046$ DMFS: HR 1.52, 95% CI 1.12-2.06, $p = 0.008$	Stroma-low
Invasive carcinoma of NST	Gujam et al., 2014 (15)	361	30%	T1-3, N0->3, grade I-III	VS	ICC = 0.83	CSS: HR 2.12, 95% CI 1.37-3.29, $p = 0.001$	Stroma-low
Estrogen receptor positive BC	Downey et al., 2014 (46)	118		N0-3, grade I-III	APC	K = 0.70	OS: HR 0.2-0.7, $p = 0.008$ RFS: HR 0.1-0.6, $p = 0.006$	Stroma-high
Inflammatory BC	Downey et al., 2015 (47)	45		N0-3, grade I-III	APC		OS: $p = 0.53$ DFS: $p = 0.66$	No difference

TABLE 1. Continued.

	Author, year	Sample Size	Percentage of stroma-high tumors	Population	Method	Inter-observer variation	Outcome	Most favorable prognosis
Results of triple-negative breast cancer as main study population or subgroup								
TNBC	Moorman et al., 2012 (13)	124	40%	pT1-4, pN0-3, grade I-III	VS	K = 0.74	OS: HR 3.00, 95% CI 1.08-8.32, p = 0.034 RFP: HR 2.39, 95% CI 1.07-5.29, p = 0.033	Stroma-low
TNBC	De Kruijf et al., 2011 (12)	82	56%	pT1-4, pN negative-positive, grade I-III	VS		OS: HR 1.87, 95% CI 1.07-3.26, p = 0.028 RFP: HR 2.92, 95% CI 1.36-6.32, p = 0.006	Stroma-low
TNBC	Dekker et al., 2013 (14)	69			VS		DFS: HR 2.71, 95% CI 1.11-6.61, p = 0.028	Stroma-low
TNBC	Roeke et al., 2017 (17)	77	26%		VS		OS: p = 0.221	No difference
Invasive carcinoma of NST TNBC	Gujam et al., 2014 (15)	151	24%	T1-3, N0->3, grade I-III	VS		CSS: p = 0.151	No difference

Abbreviations: APC = automated point counting, BC = breast cancer, CSS = cancer-specific survival, DFS = disease-free survival, DM = distant metastasis, DMFS = distant metastasis-free survival, ICC = intraclass correlation coefficient, K = Cohen's kappa value, NST = no special type, OS = overall survival, RFP = relapse-free period, RFS = recurrence-free survival, TNBC = triple-negative breast cancer, VS = visual scoring

stromal content with semi-automated point counting (46). They showed that a high tumor-stroma content in 118 women with ER-positive invasive breast tumors (grade I-III) was independently associated with a better OS and relapse-free survival (RFS) (95% CI 0.2-0.7, $p = 0.008$ and 95% CI 0.1-0.6, $p = 0.006$, respectively) (46). After their first study, Downey and colleagues investigated the stromal content in 45 patients with inflammatory breast cancer, a rare and aggressive form of breast cancer, using the semi-automated point counting method (47, 49). However, no statistically significant difference was observed for this series (OS $p = 0.53$, DFS $p = 0.66$) (47).

Roeke et al. (T1-3, N0-2, grade I-III) validated by visual TSR assessment that a high stromal content was a prognostic factor for worse OS (HR 1.56, 95% CI 1.18-2.05, $p = 0.002$), distant-metastasis-free survival (DMFS) (HR 1.52, 95% CI 1.12-2.06, $p = 0.008$) and RFS (HR 1.35, 95% CI 1.01-1.81, $p = 0.046$) in their study of 737 patients with primary operable invasive breast cancer (17). Unlike the work of Downey et al., patients with ER-positive stroma-high tumors were associated with a worse OS (HR 1.43, 95% CI 1.04-1.99, $p = 0.030$) (17).

THE TUMOR-STROMA RATIO IN TRIPLE-NEGATIVE BREAST CANCER

For the applicability of the TSR as a prognostic parameter in TNBC patients, a study has been performed by Moorman et al. in 2012. They analyzed the TSR in a retrospective cohort study consisting of TNBC patients (pT1-4, pN0-3, grade I-III) (n = 124) (13). The amount of stroma was evaluated by visual eyeballing. Multivariate Cox regression analysis showed that the TSR was an independent prognostic factor for both RFP (HR 2.39, 95% CI 1.07-5.29, $p = 0.033$) and OS (HR 3.00, 95% CI 1.08-8.32, $p = 0.034$), in favor of stroma-low tumors. The 5-year RFP and OS for patients with stroma-low tumors compared to stroma-high tumors were 85% and 89% versus 45% and 65%, respectively (13).

Subgroup analysis of 82 TNBC in the cohort of De Kruijf et al. supported the results of Moorman and colleagues that patients with stroma-high tumors had a significant shorter RFP (HR 2.92, 95% CI 1.36-6.32, $p = 0.006$) and OS (HR 1.87, 95% CI 1.07-3.26, $p = 0.028$) (12). After 5 years of follow-up, 81% of the TNBC

patients with stroma-low tumors were relapse-free compared to 56% of patients with stroma-high tumors (12).
Among the 403 patients in the cohort of Dekker and colleagues, 69 patients were diagnosed with TNBC. A separate analysis of patients with stroma-high TNBC validated a 2.71 greater risk of developing a recurrence compared to patients with stroma-low TNBC (DFS: HR 2.71, 95% CI 1.11-6.61, $p = 0.028$) (14).
However, in the study of Gujam et al., the percentage of tumor stroma was not found to be an independent prognostic factor for cancer-specific survival in 151 TNBC patients ($p = 0.151$) (15). Likewise, Roeke et al. were not able to prove this correlation either ($p = 0.221$) (table 1) (17).

THE TUMOR-STROMA RATIO IN OTHER SUB-GROUPS

De Kruijf et al., Gujam et al. and Roeke et al. described the role of the TSR in other subgroups. The results of De Kruijf et al. showed an independent prognostic value of the TSR in patients who only received local therapy ($p < 0.001$), adjuvant chemotherapy ($p = 0.038$) or adjuvant endocrine therapy ($p = 0.024$) (12). The latter was confirmed by Roeke et al. ($p = 0.001$) (17). The same results were seen in patients with TNBC who received only local therapy ($p = 0.006$).
In non-TNBC patients ($p = 0.013$), ER-positive patients ($p = 0.030$) and HER2-negative tumors the TSR was also of independent prognostic value (12, 17). This was not the case for ER-negative and PR-negative breast tumors (17). In node-negative tumors the TSR was also proved to be statistically significant for CSS and OS ($p = 0.002$ and $p = 0.003$, respectively) in two different studies (15, 17). Table 2 presents a summary of these results.

DISCUSSION OF CURRENT LITERATURE

Extensive research has been performed to determine prognostic biomarkers for patient prognosis. Molecular tests, as the MammaPrint and Oncotype DX, have seemed to be valuable for the improvement of clinical decision making in early-stage breast cancer (7, 8). These tests will possibly be endorsed into routine

clinical practice to reduce the administration of adjuvant chemotherapy and prevent overtreatment (9). However, the disadvantages of the aforementioned molecular testing are the relatively high cost and the far more unknown influence of tumor heterogeneity. More specifically, intermingled non-tumor tissue may have a profound influence on the test results (50).

The TSR has shown to be of prognostic value in addition to the traditional prognostic markers which are implemented in standard clinical care, for example, TNM stage, receptor status and HER2 expression, in breast cancer with a robust inter-observer variability. In supplementary table 1 and supplementary table 2 the effect of the TSR in addition to the most important traditional prognostic markers is shown for the entire study population and triple-negative tumors, respectively.

So far, seven studies regarding the TSR have been performed in the field of breast cancer, of which five have shown a significant association between high tumor stroma content and a poor prognosis (12-15, 17). However, the results of both studies of Downey and colleagues were not in line with the other five (46, 47). As Downey et al. have determined the TSR with semi-automated point counting instead of visual eyeballing and have utilized different cut-off values in both studies, it may be concluded that a standardized estimation of the TSR is essential for a robust method, which can be applicable for patient management. The method of determining the TSR differed considerably, resulting in underestimating the heterogeneity (51). In contrast with previous studies, where the ultimate TSR category is based on the highest stroma rate in the sample, Downey and colleagues only scored an area of 9 mm^2 at the edge of the tumor (10, 46, 51, 52).

Although the difference in results can be attributed to this inconsistency, the different breast cancer subgroups regarding basic characteristics must be taken into consideration as well. The applicability in the subtypes, namely TNBC, ER-positive and inflammatory breast cancer, may differ and subsequently the individual relevance of the TSR has to be determined in breast cancer subgroups, as is previously performed by Roeke and colleagues (17). For example, in lobular carcinomas, the question is raised on how to determine which part is tumor induced stroma or tumor supportive stroma. This should be further determined in larger cohorts. Concerning TNBC, five studies have investigated this subgroup, of which three studies have shown significant results (12-15, 17). The results of these three

TABLE 2. The results of the multivariate Cox regression analysis on the prognostic value of the tumor-stroma ratio in different subgroups of breast tumors described in literature (data on the main cohort of publication and triple-negative tumors are presented in table 1). Stroma-low is used as a reference value.

Subgroups	**De Kruijf et al., 2011**				**Gujam et al., 2014**				**Roeke et al. 2017**			
	Recurrence-free period				Cancer-specific survival				Overall survival			
	n (% stroma-high)	HR	95% CI	*p*-value	n (% stroma-high)	HR	95% CI	*p*-value	n (% stroma-high)	HR	95% CI	*p*-value
Treatment												
Only local therapy (no systemic therapy)	244 (66)	2.06	1.42-2.97	<0.001								
Only adjuvant chemotherapy	88 (68)	1.83	1.04-3.25	0.038								
Only adjuvant endocrine therapy	27 (29)	2.59	1.13-5.91	0.024						2.02	1.34-3.07	0.001
Only local therapy in TNBC		4.12	1.49-11.39	0.006								
Hormone receptor and/or HER2 status												
Non-TNBC		1.50	1.09-2.07	0.013								
ER-positive										1.43	1.04-1.99	0.030
ER-negative and PR-negative												No statistically significant difference (data not shown)
HER2-negative												Results comparable with results of ER-positive tumors (data not shown)
Tumor stage												
Lymph node-negative					54 (26)	3.11	1.53-6.33	0.002		1.90	1.24-2.90	0.003

Abbreviations: ER = estrogen receptor, HER2 = human epidermal growth factor receptor 2, PR = progesterone receptor, TNBC = triple-negative breast cancer

studies are rather promising regarding the prognostic effect of the TSR (12-14). However, two other studies have not validated this prognostic effect despite the favorable results showed earlier. As mentioned by Roeke et al., this discordance could be contributed to the relatively low amount of stroma-high tumors in the TNBC subgroup (17). The similar reason could be the cause for the effect of the TSR in TNBC patients in the study of Gujam et al. (15). Another explanation could be that the histological type of TNBC plays a role.

Although different studies researched the prognostic value of TSR, little is known about the composition of the stroma. Even when using conventional light microscopy, vast differences in stromal morphology can be appreciated, which are surely reflective of enormous differences in stromal functionality. Molecular analyses have identified multiple molecular markers that are associated with varying degrees of stromal activation (53-55). These findings might allow us to distinguish activated, highly tumor-promoting stromal tissues from non-activated or only mildly active stromal tissues. Future studies investigating stromal activation might therefore solely focus on specific highly active subsets of stromal tissues as opposed to counting all stromal tissues equally, thereby further refining this parameter. For instance, as shown in a previous publication by the identification of PA28 as a marker of stromal activation (53).

Similarly, Ahn et al. investigated the stromal composition of breast cancer tissue. Besides the TSR, the dominant histological stroma type (collagen, fibroblast or lymphocytes) offers additional prognostic information. Five- and 10-year RFS rates were most favorable in the lymphocytic stroma type, followed by the fibroblast and collagen type. The latter was associated with the most aggressive tumor and consequently poorest prognosis (56). Interestingly, Ahn et al. observed a trend between TNBC and a predominantly lymphocytic stroma type, with 56.1% of the samples classified as ‘lymphocytic’. Considering TNBC has a relatively poor prognosis, the observed trend between TNBC and a predominantly lymphocytic stroma type, with a favorable prognosis, is striking. Leon-Ferre and colleagues showed similar results in early-stage TNBC in which the presence of low tumor-infiltrating lymphocytes (TILs) contributes to a poor prognosis (57).

Considering the aforementioned generally promising prognostic effect in TNBC, this subgroup is the most obvious candidate for further exploration of the TSR.

Currently, adjuvant systemic chemotherapy is advocated for all patients that present with operable TNBC due to the aggressive nature of this tumor subgroup. Regarding TNBC, unlike other molecular subtypes, there is no Food and Drug Administration (FDA) approved targeted therapy yet. Forasmuch as both the aggressive nature of the subtype as the devoid of therapeutic options, supplementary research is necessary. For the development of curative therapeutics in TNBC, stromal targets have to be determined. Given the fact that TNBC predominantly consists of lymphocytic stroma, according to Ahn and colleagues, the possible target might lie within this stroma. The quantity of programmed death-ligand 1 (PD-L1), expressed on tumor cells, could be prognostic as well. Tomioka et al. have shown that low TILs, in combination with high PD-L1 expression, predicts an unfavorable prognosis. Within the abundant lymphocytic stroma in TNBC, PD-L1 could operate as a target for therapeutic options (58). Thus, in further research, in addition to a standardized estimation of the TSR, the biology or quality of the stroma should be taken into account as well, in both general breast cancer and especially in TNBC patients to clarify the paradox and subsequently to lay a foundation regarding targeted therapy. Lastly, it should be noted that although previous studies demonstrated prognostic value in the past, these studies have always been performed as part of retrospective studies by researchers and pathologists with a specific interest in stromal tissues. Breast cancer is a heterogeneous disease, and for this reason, additional larger retrospective studies could add valuable information about the prognostic value of TSR in specific subgroups as well. Moreover, no prospective feasibility studies have been performed, and as such, it remains to be seen whether the broad application of this parameter would lead to reproducible test results. Current research efforts in this direction are, however, ongoing.

CONCLUSIONS

The current breast cancer prognostication schemes do not adequately predict patient prognosis. This leads to both over- and undertreatment with adjuvant chemotherapy. To better predict tumor biology and prevent unwarranted chemotherapy, additional prognostic parameters are necessary. The TSR can be a valuable biomarker for determining patient prognosis. The scoring can easily be performed by the

pathologist during routine pathological examination of H&E stained slides in less than a minute and without additional costs, as it is a quick, simple method with a high reproducibility. The field of tumor stroma provides promising perspectives, although standardization of the methodology is desired. There is a trend toward high stromal content and a poor prognosis, being most applicable in TNBC. The TSR, in this case, could be used to predict both disease progression and patient prognosis.

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SUPPLEMENTARY DATA

SUPPLEMENTARY TABLE 1. Prognostic value of the tumor-stroma ratio in addition to traditional prognostic tumor characteristics in the main study cohort discussed in the literature calculated by Cox regression analysis.

Tumor characteristics	**De Kruijf et al., 2011**						**Dekker et al., 2013**						**Gujam et al., 2014**					
	General breast cancer cohort						General breast cancer cohort						Invasive carcinoma of no special type					
	Recurrence-free period						Disease-free survival						Cancer-specific survival					
	Univariate analysis			*Multivariate analysis*			*Univariate analysis*			*Multivariate analysis*			*Univariate analysis*			*Multivariate analysis*		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Tumor size	2.49	1.71-3.64	<0.001	1.86	1.24-2.79	0.009	3.17	1.37-7.36	0.024	2.72	0.99-7.47	0.150	2.17	1.54-3.07	<0.001			0.142
Lymph node involvement	3.06	2.38-3.95	<0.001	2.66	2.03-3.49	<0.001							1.97	1.51-2.56	<0.001	1.97	1.46-2.66	<0.001
Tumor grade	2.02	1.33-3.08	0.001	1.71	1.09-2.70	0.022	1.85	1.26-2.72	0.006	1.32	0.82-2.13	0.440	1.85	1.30-2.60	<0.001	1.72	1.18-2.51	0.005
Histological type	1.24	0.83-1.85	0.291															
ER status	1.05	0.81-1.36	0.725				0.87	0.60-1.26	0.454				0.52	0.34-0.79	0.002			0.240
PR status	0.96	0.74-1.24	0.744				0.83	0.60-1.16	0.275				0.44	0.32-0.82	0.006			0.184
HER2 status	1.21	0.78-1.88	0.401				1.27	0.83-1.97	0.275				1.44	0.88-2.35	0.145			
Ki67 expression	1.00	0.71-1.42	0.994				2.06	1.30-3.27	0.002	1.73	1.02-2.92	0.042						
Lymphovascular invasion													2.07	1.39-3.09	<0.001			0.864
Angiogenesis							1.21	0.81-1.80	0.349									
Tumor-stroma ratio	1.62	1.23-2.13	0.001	1.97	1.47-2.64	<0.001	1.69	1.23-2.31	0.001	1.85	1.33-2.59	<0.001	1.89	1.26-2.82	<0.001	2.12	1.37-3.29	0.001

SUPPLEMENTARY TABLE 1. Continued.

Tumor characteristics	Downey et al., 2014						Downey et al., 2015						Roeke et al., 2017					
	Estrogen receptor-positive breast cancer						Only inflammatory breast cancer						General breast cancer					
	Relapse-free survival						Disease-free survival						Recurrence-free survival					
	Univariate analysis			*Multivariate analysis*			*Univariate analysis*			*Multivariate analysis*			*Univariate analysis*			*Multivariate analysis*		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Tumor size													1.61	0.40-6.52	0.670	1.33	0.30-5.84	0.709
Lymph node involvement													4.41	2.68-7.26	<0.001	4.58	2.51-8.36	<0.001
Tumor grade													2.73	1.74-4.30	<0.001	1.78	1.06-2.99	0.028
Histological type													0.97	0.61-1.55	0.910			
ER status													0.53	0.38-0.74	<0.001	0.82	0.52-1.28	0.375
PR status													0.59	0.44-0.79	<0.001	0.78	0.54-1.12	0.183
HER2 status													1.09	0.77-1.54	0.633	1.04	0.73-1.48	0.819
Ki67 expression																		
Lymphovascular invasion																		
Angiogenesis																		
Tumor-stroma ratio		0.1-0.6	0.006						0.66				1.26	0.95-1.67	0.113	1.35	1.01-1.81	0.046

Abbreviations: ER = estrogen receptor, HER2 = human epidermal growth factor receptor 2, PR = progesterone receptor

The reference group used in the univariate and multivariate Cox regression analyses was different between the included studies. In this table, only the traditional prognostic markers are shown. In the original papers, more parameters were included in de multivariate Cox regression analysis. Tumor size; pT1 versus pT3/4 (De Kruijf et al.), cT1 versus cT3 (Dekker et al.), ≤20 mm versus >50 mm (Gujam et al.) and ≤20 mm versus >50 mm (Roeke et al.). Lymph node involvement; pN-negative versus pN-positive (De Kruijf et al.), 0 versus >3 involved lymph nodes (Gujam et al.) and pN0 versus pN3 (Roeke et al.). Tumor grade; grade I versus grade III. Histological type; invasive carcinoma of no special type versus others (De Kruijf et al.) and invasive carcinoma of no special type versus lobular carcinoma (Roeke et al.). ER status; negative versus positive. PR status; negative versus positive. HER2 status; no overexpression versus overexpression (De Kruijf et al.) and negative versus positive (Dekker et al. and Roeke et al.). Ki67 expression; negative versus positive (De Kruijf et al.) and low versus high (Dekker et al.). Angiogenesis; low microvessel density versus high microvessel density. Lymphovascular invasion; no versus yes. Tumor-stroma ratio; stroma-low versus stroma-high.

SUPPLEMENTARY TABLE 2. Prognostic value of TSR in addition to traditional prognostic tumor characteristics in the triple-negative breast cancer population described in the discussed literature calculated by Cox regression analysis.

Tumor characteristics	De Kruijf et al., 2011						Moorman et al., 2012						Dekker et al., 2013					
	Recurrence-free period						Relapse-free period						Disease-free survival					
	Univariate analysis			*Multivariate analysis*			*Univariate analysis*			*Multivariate analysis*			*Univariate analysis*			*Multivariate analysis*		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Tumor size	3.53	1.03-12.08	0.131										10.17	2.29-45.11	0.009	6.19	1.04-36.96	0.075
Lymph node involvement	2.30	1.61-4.57	0.017	1.88	0.89-3.96	0.096	3.38	1.27-9.00	0.010									
Tumor grade	1.72	0.80-3.69	0.163										0.84	0.12-6.22	0.478			
Histological type																		
Ki67 expression	0.70	0.31-1.61	0.403										2.44	0.33-17.91	0.382			
Lymphovascular invasion							2.46	1.19-5.07	0.012									
Angiogenesis													1.53	0.61-3.84	0.364			
Tumor-stroma ratio	3.19	1.49-6.83	0.003	2.92	1.36-6.32	0.006	2.93	1.37-6.26	0.004	2.39	1.07-5.29	0.033	2.21	1.004-4.84	0.049	2.71	1.11-6.61	0.028

The reference group used in the univariate and multivariate Cox regression analysis was different between the included studies. In this table, only traditional prognostic markers are shown. In the original papers, more parameters were included in de multivariate Cox regression analysis. Tumor size; pT1 versus pT3/4 (De Kruijf et al.), T1 versus T3 (Dekker et al.) and ≤20 mm versus >50 mm (Gujam et al.). Lymph node involvement; pN-negative versus pN-positive (De Kruijf et al.), pN0 versus pN2/3 (Moorman et al.) and 0 versus >3 involved lymph nodes (Gujam et al.). Tumor grade; grade I/II versus grade III (De Kruijf et al.), grade I versus grade III (Gujam et al. and Dekker et al.). Ki67 expression; negative versus positive (De Kruijf et al.) and low versus high (Dekker et al.). Lymphovascular invasion; no versus yes. Angiogenesis; low microvessel density versus high microvessel density. Tumor-stroma ratio; stroma-low versus stroma-high.

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