PRECLINICAL STUDY

Correlation of circulating or disseminated tumor cells with the Oncotype DX Recurrence Score

Puneet Singh[1] · Sarah E. Tevis[1,2] · Carolyn S. Hall[1] · Salyna Meas[1] · Rosa F. Hwang[1] · Anthony Lucci[1]

Abstract

Purpose New biomarkers are emerging to predict recurrence risk in women with early-stage breast cancer. High Oncotype DX Recurrence Score® (RS) is associated with worse disease-free and overall survival. Similarly, circulating tumor cells (CTCs, blood) and disseminated tumor cells (DTCs, bone marrow) have prognostic value in breast cancer. We investigated the association between high RS and CTCs or DTCs.

Methods Using a prospective database, we evaluated patients with hormone receptor-positive/HER2-negative, node-negative invasive breast cancer from 1/2005 to 1/2017. RS was classified using TAILORx study cutoff points: low (< 11), intermediate ($11–25$), and high (> 25). CTCs were assessed using CellSearch® and DTCs using cytospin specimens of bone marrow aspirates. Positive result was defined as one or more CTCs or DTCs identified. Chi-square analyses were utilized to evaluate the relationship between RS and CTCs or DTCs.

Results 233 patients were identified from a prospective database, of which 96 had RS results. Of these patients, 88 had CTC results and 58 had DTC results. CTCs were detected in 17/88 (19%) patients, while DTCs were detected in 20/58 (34%). Patients with high RS were not more likely to have CTCs (18%) compared to patients with low/intermediate RS (20%; $p = 0.919$). Similarly, high RS was not associated with DTC detection, with DTCs present in 40% of patients with high RS versus 33% with low/intermediate RS ($p = 0.687$). In the subgroup of patients ≤ 50 years, no associations were found between high RS and CTCs ($p = 0.383$) or DTCs ($p = 0.234$).

Conclusions High Oncotype DX RS did not correlate with CTCs in blood or DTCs in bone marrow in our study.

Keywords Circulating tumor cells · Disseminated tumor cells · Oncotype DX · 21-Gene recurrence score · Biomarker · Breast cancer

Background

There has been increasing investigation of new and novel biomarkers that can predict the risk of recurrence in women with early-stage breast cancer. Oncotype DX RS is a 21-gene breast cancer assay that is commercially available and utilized in hormone receptor-positive breast cancer as a prognostic and predictive tool [1]. A high RS (≥ 31) has been found to be associated with worse disease-free and survival overall in patients with early-stage breast cancer [1, 2]. Similarly, CTCs in the peripheral blood and DTCs in the bone marrow have prognostic value in patients with operable breast cancer, though these are not routinely used in clinical practice due to unproven predictive value at this time. Studies have shown that CTCs are present in 25% of non-metastatic breast cancer [3, 4]. Lucci et al. demonstrated that detection of one or more CTCs in chemonaïve patients was associated with decreased progression-free survival and overall survival (OS) [4]. A large pooled analysis with 3173 patients from five institutions with non-metastatic breast cancer also demonstrated that the presence of CTCs was an independent prognostic factor for disease-free survival, distant disease-free survival, breast cancer-specific survival, and OS [5]. A published study of DTCs in non-metastatic breast cancer has been reported by Braun et al., who performed a pooled analysis of nine studies with 4703 patients

✉ Anthony Lucci
alucci@mdanderson.org

1 Department of Breast Surgical Oncology, MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, USA

2 Present Address: Department of Surgery, University of Colorado, Aurora, CO, USA

with stages I–III breast cancer [6]. DTCs were found to be present in 30.6% of patients. In a subset of 1036 pathologic T1, node-negative tumors who received no adjuvant systemic therapy (chemotherapy or endocrine therapy), there was an increased breast cancer-specific mortality and risk of distant metastases in patients with DTCs [6]. The initial results from the PADDY study, the largest pooled analysis of 10,320 patients with early breast cancer, demonstrated that 27.4% had DTCs present, and this was an independent predictor for disease-free survival, distant disease-free survival, breast cancer-specific survival, and OS [7]. Multiple other studies have also validated the detection of DTCs as a prognostic factor [8–11]. Thus, RS, CTCs, and DTCs have been shown to have prognostic impact in early-stage breast cancer.

Two studies have examined whether there is an association between RS and CTCs and/or DTCs [12, 13]. Aktas et al. evaluated 68 patients with pathologic T1-3, N0-1 breast cancer of which 13% had a high RS (≥ 31). CTCs and DTCs were detected in 19% and 28% of patients, respectively. There was no correlation between RS ≥ 31 and CTCs or DTCs [13]. Conversely, in a cohort of 114 patients with early-stage breast cancer, Hartkopf and colleagues demonstrated that DTCs were present in 11%, and there was an association between intermediate/high RS (≥ 18) and DTCs [12]. Therefore, the data on correlation between high RS and CTCs or DTCs currently remain unclear.

We sought to evaluate the association between high RS (≥ 26) and CTCs and DTCs in patients with early-stage breast cancer and hypothesized that there would be a positive association between identification of CTCs and/or DTCs and high RS. To our knowledge, this is the largest study to evaluate the relationship between RS and both CTCs and DTCs.

Methods

Patients

Patients were retrieved from a retrospective review of a prospectively maintained database from January 2005 to January 2017. Patients who underwent primary surgery for hormone receptor-positive, HER2-negative, invasive breast cancer were included in the study. Other inclusion criteria included Oncotype DX RS testing, and testing for DTCs or CTCs at baseline (prior to surgery or systemic adjuvant therapy). Patients were excluded if they had clinically or pathologically positive lymph nodes since at the time of enrollment for this study; node-positive patients were not routinely tested with Oncotype DX.

Determination of Oncotype DX RS, CTCs, and DTCs

The Oncotype DX RS is a genomic assay that evaluates 21 genes, including 16 cancer-related genes and five reference genes. We categorized RS based on the TAILORx study cutoff points [14], as these are now considered the standard cut-points for clinical decision-making, following the publication of the study. In TAILORx, Arm A, with RS < 11, had the lowest event (invasive disease-free survival events) rate. The randomization within TAILORx was for the group with RS cut-points from 11–25, formerly known as the intermediate risk group. TAILORx used RS of ≥ 26 as the cutoff for the high-risk group.

DTCs are disseminated tumor cells in the bone marrow, while CTCs are circulating cells in the blood. CTCs were assessed using the Circulating Tumor Cell Kit and Cell-Search® technology (Menarini Silicon Biosystems). For DTCs, cytospin specimens of bone marrow aspirates were collected from bilateral iliac crests, enriched for epithelial cells by Ficoll density gradient separation, cytospun onto microscope slides, immunostained using a pancytokeratin cocktail of antibodies, including AE1/AE3, CAM5.2, MNF116, cytokeratin 8 (CK8), and CK18. CTCs and DTCs were considered to be positive if one or more CTCs or DTCs were identified, respectively.

Statistical analysis

Descriptive statistics were utilized to characterize the study population demographic and clinicopathologic features. Chi-square analyses were utilized to evaluate the relationship between CTCs and Oncotype RS low (0–10), intermediate (11–25), and high risk (≥ 26). Analyses combining low and intermediate RS groups had a score of 0–25. The association between DTCs and Oncotype RS was similarly evaluated. In addition, we analyzed CTCs and DTCs in relation to Oncotype RS in the subset of women under the age of 50. All statistics were performed in IBM SPSS Statistics, version 24 (IBM Corp, Armonk, NY, USA) and p values < 0.05 were considered significant.

Results

There were 96 patients included in the study, and characteristics of the patient population are shown in Table 1. All patients had an Oncotype DX RS and either CTC or DTC testing. Mean age was 54 years (32–75) and mean BMI was 28.1 (18.2–47.9). The majority of women were post-menopausal (67%), compared to pre-menopausal (33%). Tumor histology was 77% ductal, 13% lobular, 7% mixed ductal

Table 1 Characteristics of the cohort

	$N=96$ n (%)	CTC status		DTC status	
		Positive n (%)	p-value[a]	Positive n (%)	p-value[a]
Age (years)					
Mean	54.4				
Range	32–75				
BMI					
Mean	28.1				
Range	18.2–47.9				
Menopausal status					
Pre-menopausal	32 (33)	7 (23)		8 (40)	
Post-menopausal	64 (67)	10 (17)	0.493	12 (32)	0.521
Histology[b]					
Ductal	74 (77)	13 (19)		16 (36)	
Lobular	12 (13)	4 (22)	0.726	4 (31)	0.749
Mixed	7 (7)				
Other	3 (3)				
T-stage					
T1	67 (70)	14 (24)		17 (40)	
T2	29 (30)	3 (10)	0.135	3 (20)	0.171
Nuclear grade					
I/II	87 (91)	16 (20)		16 (31)	
III	9 (9)	1 (17)	0.865	4 (57.1)	0.179
Cancer stage					
I	68 (71)	14 (23)		17 (39)	
II	28 (29)	3 (11)	0.163	3 (21)	0.238
Circulating tumor cells (CTCs)					
Absent	71 (74)				
Present	17 (18)				
Unknown	8 (8)				
Disseminated tumor cells (DTCs)					
Absent	38 (40)				
Present	20 (20)				
Unknown	38 (40)				
Oncotype DX RS					
Low/intermediate risk (0–25)	83 (86)				
High risk (≥ 26)	13 (14)				

[a]Chi-square analysis

[b]Mixed and other histologies were combined with lobular histology, and compared to ductal histology, for Chi-square analysis

and lobular, and 3% other. Most tumors were small in size, with 70% being T1 stage and 30% T2 with overall stage I in 70% of cases. Nearly all tumors were grade I (18%) or grade II (73%). Oncotype DX RS was low/intermediate risk in 83 (86%) and high risk in 13 (14%). CTC testing was performed in 88 patients of which 17/88 (19%) had CTCs present in blood versus absent in 71/88 (81%). Median RS was 18 (range 3–47) for patients with CTCs present, and 16 (3–36) for those without CTCs. DTC testing was performed in 58 patients, and DTCs were present in bone marrow in 20/58 (34%) and absent in 38/58 (66%). There was no difference in median RS in patients with DTCs present (16, range 3–47), versus absent (16, range 4–35). There were no associations with clinicopathologic factors and CTC or DTC status. Mean survival was 1953 days (range 71–3775).

Table 2 demonstrates the presence or absence of CTCs stratified by RS. CTCs were present in 20% of patients with low/intermediate RS and 18% with high RS. Patients with high RS were not more likely to have CTCs as compared with patients who had low/intermediate RS ($p = 0.919$).

Table 2 Presence of CTCs stratified by Oncotype DX RS

Oncotype DX RS	CTCs absent	CTCs present	p-value
Low/Intermediate risk (<26)	62 (80.5)	15 (19.5)	
High risk (≥26)	9 (81.8)	2 (18.2)	0.919

CTC circulating tumor cells

Table 3 Presence of DTCs stratified by Oncotype DX RS

Oncotype DX RS	DTCs absent	DTCs present	p-value
Low/intermediate risk (<26)	32 (66.7)	16 (33.3)	
High risk (≥26)	6 (60.0)	4 (40.0)	0.687

DTC disseminated tumor cells

Table 4 Association of CTCs and Oncotype DX RS® in subjects with age ≤50

Oncotype DX RS	CTCs absent	CTCs present	p-value
Low risk (<16)	10 (76.9)	3 (23.1)	
Intermediate risk (16–25)	12 (75.0)	4 (25.0)	
High risk (≥26)	5 (83.3)	1 (16.7)	0.917

CTC circulating tumor cells

Table 5 Association of DTCs and OncotypeDX RS in subjects with age ≤50

Oncotype DX RS	DTCs absent	DTCs present	p-value
Low risk (<16)	4 (44.4)	5 (55.6)	
Intermediate risk (16–25)	8 (66.7)	4 (33.3)	
High risk (≥26)	3 (100.0)	0 (0.0)	0.208

DTC disseminated tumor cells

DTCs were present in 33% of patients with low/intermediate RS and 40% with high RS (Table 3). There was no association between high RS and presence of DTCs in the bone marrow ($p = 0.687$) in our study group. Using the RS risk categories defined prior to the TAILORx study13 with low RS < 18, intermediate 18–30, and high ≥ 31, there was still no association between high RS and CTCs or DTCs (data not shown).

For the subgroup of women age 50 years or younger ($n = 37$, 39%), there was no correlation between CTCs in the high RS (≥ 26) group (17%) versus low (< 16; 23%) or intermediate (16–25; 25%) RS groups ($p = 0.917$; Table 4). Similarly, there was no correlation between DTCs in the high RS group (0%) versus low (56%) or intermediate (33%) RS group ($p = 0.208$; Table 5).

Discussion

This study included 96 patients with stage I or II, node-negative breast cancer and found no association between high RS and CTCs or DTCs. Overall, CTCs were detected in 18% and DTCs were detected in 20% of patients. Furthermore, in a subgroup analysis of younger women, there was no association between high RS and the presence of CTCs or DTCs. Despite previous literature demonstrating the prognostic value of the RS, CTCs, and DTCs independently, we did not find correlations between RS and the studied biomarkers in the overall patient cohort, or in the younger age subgroup.

Aktas et al. evaluated both CTCs and DTCs in addition to other biomarkers including stemness-like tumor cells (slCTCs), urokinase-type plasminogen activator, plasminogen activator inhibitor type I, and Ki-67 in a smaller cohort of 68 patients [13]. Similar to our study, there was no association between RS and CTCs ($p = 0.100$) and DTCs ($p = 0.883$). The only biomarker that correlated with RS was Ki-67 ($p < 0.001$). Unlike our study, patients with T3 or N1 tumors were included, yet, the same rate of CTC detection was demonstrated in the Aktas et al. study (19%), compared to our study (19%). DTC detection rates were also similar (28% versus 34% in our study), and they did not find an association with RS. Thus with a larger cohort, our results confirm the results of this previously published report.

Hartkopf et al. included 114 patients with primarily T1, N0 breast cancers, though 31% were staged as T2–T4, and 28% were node-positive breast cancers [12]. They found a significant association between RS and DTCs when comparing intermediate/high RS to low RS ($p = 0.03$). One of the key differences was the low overall rate of 11% DTC detection compared to 34% in our study, and 31% in the literature [6]. Furthermore, the patients included in our study were all node-negative. Additionally, we utilized the RS cutoffs based on the TAILORx study [14] compared to Aktas et al. and Hartkopf et al., who utilized RS < 18 to define the low-risk group.

The Oncotype DX RS is increasingly utilized to aid in clinical decisions regarding chemotherapy benefit. The prognostic and predictive significance of the RS has facilitated its widespread clinical use. There are other genomic signatures that are available for clinical use; however, we were not able to locate any other published data evaluating the association of these genomic signatures with CTCs or DTCs.

Other prognostic biomarkers, such as detection of CTCs and DTCs,, have not been incorporated into routine patient care and are largely utilized for research. CTCs and DTCs are biomarkers that represent minimal residual disease,

which in theory could be a direct precursor to recurrence. In a prospective study by our group of 302 patients with stage I–III breast cancer, of which 61% were node-negative, the presence of CTCs independently predicted decreased progression-free and overall survival [4]. However, in this study, our results do not show a correlation between high RS and these biomarkers of minimal residual disease. Perhaps the mechanisms by which the RS and CTCs and DTCs are prognostic differ and are independent of each other. A limitation of this study, however, is the small sample size, particularly for patients who had DTC testing, though a strength of our study is that we analyzed both CTCs and DTCs. Also, another possible limitation is that this was a retrospective review of a prospectively maintained database at a single institution.

In conclusion, this study is the first to evaluate the correlation between RS and CTCs and DTCs in early-stage, node-negative breast cancer. We found no association between high RS and either CTCs or DTCs. Future studies should focus on minimal disease biomarkers such as CTCs and circulating tumor DNA, to determine whether these markers can be used to assess response to therapy, and possibly identify novel targets against microscopic disease. We anticipate that in the future, assessment of the primary tumor, as well as the blood-based biomarkers, will provide a more complete assessment of risk for relapse, and possibly guide optimal clinical management.

Compliance with ethical standards

Conflict of interest Anthony Lucci is on the speaker's bureau for Exact Sciences. The other authors have no conflicts of interest to report.

References

1. Kwa M, Makris A, Esteva FJ (2017) Clinical utility of gene-expression signatures in early stage breast cancer. Nat Rev Clin Oncol 14(10):595–610

2. Paik S, Shak S, Tang G et al (2004) A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 351:2817–2826

3. Hall CS, Karhade MG, Bowman Bauldry JB et al (2016) Prognostic value of circulating tumor cells identified before surgical resection in nonmetastatic breast cancer patients. J Am Coll Surg 223(1):20–29

4. Lucci A, Hall CS, Lodhi AK et al (2012) Circulating tumour cells in non-metastatic breast cancer: a prospective study. Lancet Oncol 13:688–695

5. Janni WJ, Rack B, Terstappen LW et al (2016) Pooled analysis of the prognostic relevance of circulating tumor cells in primary breast cancer. Clin Cancer Res 22(10):2583–2593

6. Foroni C, Broggini M, Generali D, Damia G (2012) Epithelial-mesenchymal transition and breast cancer: role, molecular mechanisms and clinical impact. Cancer Treat Rev. https://doi.org/10.1016/j.ctrv.2011.11.001

7. Hartkopf ADBS, Taran F et al (2019) Abstract nr GS5–07 International pooled analysis of the prognostic impact of disseminated tumor cells from the bone marrow in early breast cancer: results from the PADDY study (abstract). Cancer Res. https://doi.org/10.1158/1538-7445.SABCS18-GS5-07

8. Gerber B, Krause A, Muller H et al (2001) Simultaneous immunohistochemical detection of tumor cells in lymph nodes and bone marrow aspirates in breast cancer and its correlation with other prognostic factors. J Clin Oncol 19(4):960–971

9. Chiang AC, Massague J (2008) Molecular basis of metastasis. N Engl J Med 359(26):2814–2823

10. Diel IJ, Kaufmann M, Costa SD et al (1996) Micrometastatic breast cancer cells in bone marrow at primary surgery: prognostic value in comparison with nodal status. J Natl Cancer Inst 88(22):1652–1658

11. Wiedswang G, Borgen E, Karesen R et al (2003) Detection of isolated tumor cells in bone marrow is an independent prognostic factor in breast cancer. J Clin Oncol 21(18):3469–3478

12. Hartkopf AD, Wallwiener M, Kommoss S, Taran FA, Brucker SY (2016) Detection of disseminated tumor cells from the bone marrow of patients with early breast cancer is associated with high 21-gene recurrence score. Breast Cancer Res Treat 156(1):91–95

13. Aktas B, Bankfalvi A, Heubner M, Kimmig R, Kasimir-Bauer S (2013) Evaluation and correlation of risk recurrence in early breast cancer assessed by Oncotype DX((R)), clinicopathological markers and tumor cell dissemination in the blood and bone marrow. Mol Clin Oncol 1(6):1049–1054

14. Sparano JA, Gray RJ, Makower DF et al (2018) Adjuvant chemotherapy guided by a 21-Gene expression assay in breast cancer. N Engl J Med 379(2):111–121

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.