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ORIGINAL ARTICLE – BREAST ONCOLOGY

Multifocal/Multicentric Ipsilateral Invasive Breast Carcinomas with Similar Histology: Is Multigene Testing of All Individual Foci Necessary?

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ABSTRACT

Background. Multiple synchronous ipsilateral invasive breast carcinomas (BCs) with similar histology usually have concordant receptor status. It is unknown whether individual foci with similar histology also share molecular and biological similarities or are heterogenous. This study examined the concordance of the 21-gene recurrence score (RS) in multiple synchronous morphologically similar ipsilateral BCs.

Patients and Methods. We identified patients with multiple ipsilateral BCs and available RS treated at our institution from 1/2014 to 6/2018. BCs were divided into three groups based on RS: (1) RS in same risk category, (2) RS in different risk categories but within 2-unit difference (e.g., RS 17 and RS 19), and (3) RS in different risk categories and a change of > 2 units. BCs in groups 1 and 2 were considered as concordant (no significant clinical impact) and BCs in group 3 as discordant (variation affects management).

Results. A total of 53 patients met the study criteria. RS was concordant in 46 (87%) cases. Seven (13%) cases were discordant (group 3). Of these, three (43%, 3/7) had biopsy cavity changes (BXC) adjacent to the BC with highest RS. In two cases the focus with higher RS had a lower percentage of progesterone receptor-positive tumor cells. In two cases, extensive ductal carcinoma in situ was associated with the BC focus with lower RS.

Conclusions. Morphologically similar multifocal ipsilateral BCs have concordant RS in 87% (46/53) of cases. Our results suggest that, in cases of morphologically similar multifocal BCs, testing of a single focus provides accurate prognostic and predictive information.

With the advancement of preoperative imaging, multiple ipsilateral foci of invasive breast carcinoma (BC), defined as multifocal or multicentric carcinoma, are identified more frequently;[1,2] the incidence of multifocal/multicentric BC ranges from 6 to 77%.[3] Multifocal/multicentric carcinoma can be due to intramammary spread of a single primary BC or to multiple synchronous independent primary BCs.[4–8] There are limited data on whether multiple synchronous tumors of similar histology have significant biological and molecular differences. The 21-gene recurrence score assay (Oncotype DX^{TM}, Genomic Health, Redwood City, CA) is a commercially available prognostic and predictive assay which measures the expression levels of 21 genes (16 cancer-related and 5 reference genes) by quantitative reverse-transcriptase polymerase chain reaction. The assay generates a numeric recurrence score (RS), on a scale from 0 to 100, to predict the 10-year risk of distant recurrence and magnitude of chemotherapy benefit in patients with early-stage, estrogen receptor-positive (ER +), human epidermal growth factor receptor 2-negative (HER2–) breast cancer. The RS stratifies patients into three risk categories: low (RS < 18), intermediate (RS 18–30), and high (RS ≥ 31).[9] Adjuvant chemotherapy is added to endocrine therapy for patients with high RS, while the

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benefit is estimated to be too low to outweigh the side effects in those with low RS.[10] The prospective randomized clinical trial TAILORx (Trial Assigning Individualized Options for Treatment) was designed to determine the benefits of adjuvant chemotherapy in patients with BC of intermediate RS.[11] In the TAILORx study, the risk categories are defined as low (RS < 11), intermediate (RS 11–25), and high (RS > 25). These categories differ slightly from the originally defined RS categories and are based on the threshold for recommending adjuvant chemotherapy, which is when the risk of local and distant recurrence is 5–10%, the level of risk associated with RS 11.[12] The TAILORx trial concluded that there was no chemotherapy benefit for RS < 25 in women over the age of 50 years and no benefit for RS ≤ 15 in women aged 50 years or younger.[13]

The National Comprehensive Cancer Network and American Society of Clinical Oncology recommend using RS to guide adjuvant systemic therapy in patients with ER +, HER2–, lymph node-negative invasive BC that are ≥ 0.5 cm.[14,15] The importance of genomic assays for risk stratification has been acknowledged in the 8th edition of the American Joint Commission on Cancer (AJCC) staging guidelines,[16] which incorporate RS into BC staging. It remains unclear whether the RS of morphologically similar multifocal/multicentric BCs differ significantly to warrant testing of multiple individual foci or if testing of a single representative tumor would provide adequate and accurate information. This study examines the concordance of the RS in multiple synchronous ipsilateral BCs with similar histology.

PATIENTS AND METHODS

At our center, all lymph node-negative, ER +, HER2– invasive BC measuring ≥ 0.5 cm in patients suitable for chemotherapy are routinely sent for 21-gene RS assay. The tissue block(s) for testing are selected by the pathologist at the time of initial histopathologic review. In cases with multiple synchronous foci, a sample of each individual focus is sent for testing if requested by the treating physician.

Through a retrospective search of our pathology database, we identified patients with multiple synchronous ipsilateral BCs who were treated at our institution between January 2014 and June 2018 and had available RS results. Multifocal tumors were defined as infiltrating carcinomas in the same breast, macroscopically distinct and measurable using available clinical and pathologic techniques.[17] Clinicopathologic features were obtained from pathology reports, including patient age at diagnosis, histologic subtype, size, grade, and ER and progesterone receptor (PR) expression level by immunohistochemistry. We also reviewed the original 21-gene assay reports and recorded the RS results. Cases reported as morphologically similar were included; if no comment regarding morphologic similarity was noted, hematoxylin and eosin (H&E)-stained slides of the tumor blocks submitted for analysis were reviewed and assessed for morphologic similarity.

Based on the RS of each tumor focus, BCs were divided into three groups: (1) BCs with RS in same risk category, (2) BCs with RS in different risk categories but within a 2-unit difference (e.g., RS 17 and RS 19), and (3) BCs with RS in different risk categories and a difference greater than 2 units. The selection of this 2-unit cutoff was based on the analytic validation studies of the Oncotype DX assay, showing a standard deviation of 2 RS units on a 100-unit scale.[9,18] BCs in groups 1 and 2 were considered concordant because there was no significant clinical impact due to the variation in RS result. BCs in group 3 were considered discordant because the RS variation was significant enough to impact therapeutic decisions. The H&E slides for all group 3 cases were reviewed by two pathologists (A.G., H.Y.W.). The institutional review board approved the study.

Protocol for Assessment of the 21-Gene Recurrence Score in Cases of Multifocal Invasive Carcinoma

According to verbal information obtained from a representative of the company that performs the 21-gene assay,[19] when multiple tumor foci are received, the reviewing pathologist determines which focus has the most worrisome histologic features, based on size and morphology, and selects that sample for testing first. In those carcinomas with similar morphology, the largest focus is tested first. If the RS is less than 25, the next sample is reflexively tested. When the assay result is greater than 25, the submitting clinician is contacted to advise whether testing of additional foci is needed.

RESULTS

Patient Selection and Tumor Characteristics

We identified a total of 108 patients with multiple ipsilateral BCs and available RS results. Fifty-three patients were excluded due to morphologic dissimilarity of the BCs, and two had tumors insufficient for testing. Fifty-three patients met the inclusion criteria. The patient median age was 52 years (range 32–73 years). The majority of patients (81%, 43/53) underwent mastectomy, while 19% (10/53) had breast conservation with wide local excision. BC types included ductal (n = 36), lobular (n = 14), and

mixed ductal and lobular (n = 3). The median tumor size was 1.2 cm (range 0.5–4.6 cm). Most BCs were Nottingham Grade 2/3 (79%, 42/53). In eight patients (15%), the BCs were grade 1/3, and in three patients (6%), the BCs were grade 3/3. Most (83%, 44/53) patients had two tumor foci tested, eight (15%) had three foci, and one patient (2%) had four separate invasive foci submitted for analysis.

Recurrence Score

A total of 116 BC samples from 53 patients were tested for RS. The median RS of the carcinomas was 14 (range 0–42). Most tumors (70%, 81/116) had low RS ($\leq$ 17). Thirty-three (28%) had intermediate RS (18–30), and two (2%) had high RS ($\geq$ 31).

Overall, the RS was concordant in 87% (46/53) of cases, including 44 (83%) in group 1 and 2 (4%) in group 2. The median difference in RS in group 1 was 2 (range 0–11). One patient in this group had two foci of BC with RS scores of 0 and 11. The median difference in group 2 was 1.5 (range 1–2). Seven (13%) cases were discordant (group 3, Table 1) with median RS difference of 9 (range 4–22).

Pathology Review of RS Discordant Cases

In three (43%) of the seven cases with BC foci having discordant RS, we found that the tissue sections of the BC that yielded higher RS contained biopsy cavity changes (BXC) (Fig. 1). In all three cases, the BC focus without BXC yielded low RS and the BC focus with BXC yielded intermediate RS. In four group 3 cases, no BXC were identified. In two cases, both with invasive ductal carcinoma (IDC), grade 2/3, immunohistochemical detection of PR expression showed varying results in each BC focus. One case showed nuclear positivity in 70% of cells in the focus with RS 13 and 40% in the focus with RS 23 (Fig. 2). A second case with foci having RS 1 and RS 23 showed nuclear positivity in 80% and 5% of cells, respectively. The remaining two discordant cases were IDC with associated intermediate nuclear grade ductal carcinoma in situ (DCIS). In both cases, the foci with lower RS had extensive DCIS admixed with the invasive carcinoma and no DCIS near the focus with higher RS.

TAILORx Criteria

Using TAILORx cutoffs, most BCs (70%, 81/116) had intermediate RS (11–25), with 25% (29/116) having low RS ($<$ 11) and 5% (6/116) high RS ($>$ 25). RS was concordant in 81% (43/53) of cases, all in group 1. The median difference in RS in group 1 was 2 (range 0–11). RS was discordant in 19% (10/53) of cases (Table 2). The median difference in RS in group 3 was 6.5 (range 5–22). Of the ten group 3 cases, 60% (6/10) were in women older than 50 years and all had RS $<$ 25 and 40% (4/10) were in women younger than 50 years and three had RS $\leq$ 15. One

TABLE 1 Summary of group 3 cases—oncotype DX criteria

Case	Tumor type	MBR grade	Tumor size (cm)	Recurrence score	Risk classification
1	Lobular with pleomorphic features	2	1.8	15	Low
			3.0	19[a]	Intermediate
2	Ductal, NOS	1	0.6	11	Low
			0.8	20[a]	Intermediate
3	Mammary with mixed ductal and lobular features	2	1.0	12	Low
			1.2	19[a]	Intermediate
			0.9	21[a]	Intermediate
4	Ductal with micropapillary features	2	1.0	13[b]	Low
			0.8	23	Intermediate
5	Ductal, NOS	2	1.4	1[b]	Low
			0.6	23	Intermediate
6	Ductal, NOS	1	0.8	14	Low
			0.6	19	Intermediate
7	Ductal, NOS	2	1.5	14	Low
			1.1	19	Intermediate
			1.9	11	Low

MBR modified Bloom–Richardson, *NOS* not otherwise specified

[a]Focus with adjacent BXC

[b]Focus with higher PR positivity

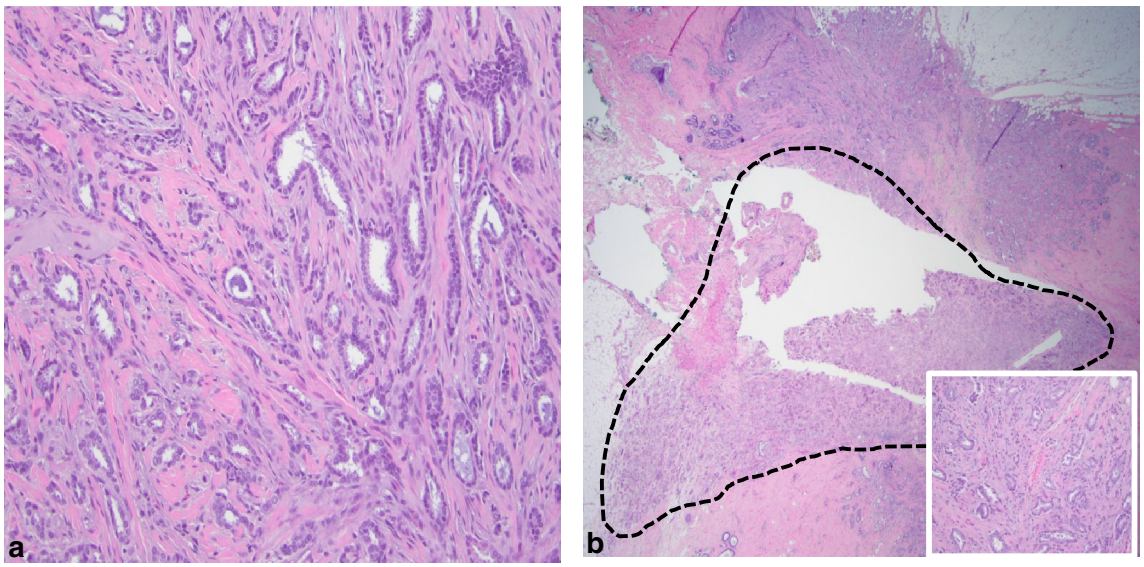

FIG. 1 Example of group 3 case with BXC associated with higher RS: **a** well-differentiated ductal carcinoma, 0.6 cm, with RS 11 (H&E, 200 ×), **b** well-differentiated ductal carcinoma with similar morphology but adjacent BXC (outlined), 0.8 cm, with RS 20 (H&E, 20 ×); inset: higher power showing similar morphology (H&E, 200 ×)

case in a 39-year-old woman had two BCs with high-risk RS 26 and 27 and one BC with intermediate RS 22. On morphologic review, all three BCs were cytologically similar [modified Bloom–Richardson (MBR) nuclear grade 3] and had similar mitotic activity (MBR mitotic count 2). The focus with RS 22 showed more tubule formation (MBR histologic grade 2) than the foci with RS 26 and 27 (MBR histologic grade 3).

DISCUSSION

According to College of American Pathologists recommendations, when there are multiple synchronous ipsilateral invasive carcinomas of the same histology, the largest invasive carcinoma is selected for classification and receptor assessment.[20] However, when multigene prognostic assays are utilized to guide decisions on adjuvant systemic therapy, there is no consensus on whether testing of all individual foci is necessary. At our institution, the treating physician decides whether one or multiple foci of BC are submitted for the 21-gene assay. Our data show that there is good correlation of RS with 87% concordance in patients with multifocal morphologically similar BCs.

Seven (13%) cases in our cohort showed discordant results; all had low RS in one BC focus and intermediate RS in the other. Upon rereview of H&E slides, biopsy cavity changes (BXC) were found adjacent to or admixed with the focus associated with higher RS in three of the seven discordant cases. Studies from Genomic Health[21] and others[22–24] have shown that BXC have a significant effect on the expression of individual genes in the 21-gene assay, which can lead to false elevation of the RS in low- to intermediate-grade tumors and a decrease in the RS in poorly differentiated tumors. Furthermore, Genomic Health showed that microdissected BXC had an average 13.4 ± 2.1 higher RS compared with microdissected invasive cancers.[21] However, it should be noted that, even with macro- or microdissection, there are intervening elements, such as inflammatory cells and reactive stromal fibroblasts, which cannot be adequately separated from tumor tissue and can potentially alter the RS. Acs et al. reported increased RS in low-grade invasive cancers associated with cellular stroma and/or inflammatory cells having an elevated proliferation index.[25] These findings emphasize the importance of avoiding or at least minimizing BXC and other mitotically active stromal components in tissue samples selected for multigene assays.

Together with the proliferation gene group, the ER-related gene group, which includes PR, is one of the most influential of the multiple gene groups assessed by the Oncotype DX assay.[26] PR expression is regulated by estrogen and is used to predict a functional ER pathway and therefore the likelihood of response to endocrine therapies.[27] Several studies have shown that PR– tumors have more aggressive profiles, benefit less from tamoxifen therapy, and have worse overall prognosis compared with PR + tumors.[27–29] Tang et al. found a similar correlation between PR expression and RS, reporting a significantly higher RS in PR– than PR + BCs.[30] Further validating these findings, an inverse relationship between PR expression level and RS independent of tumor grade has

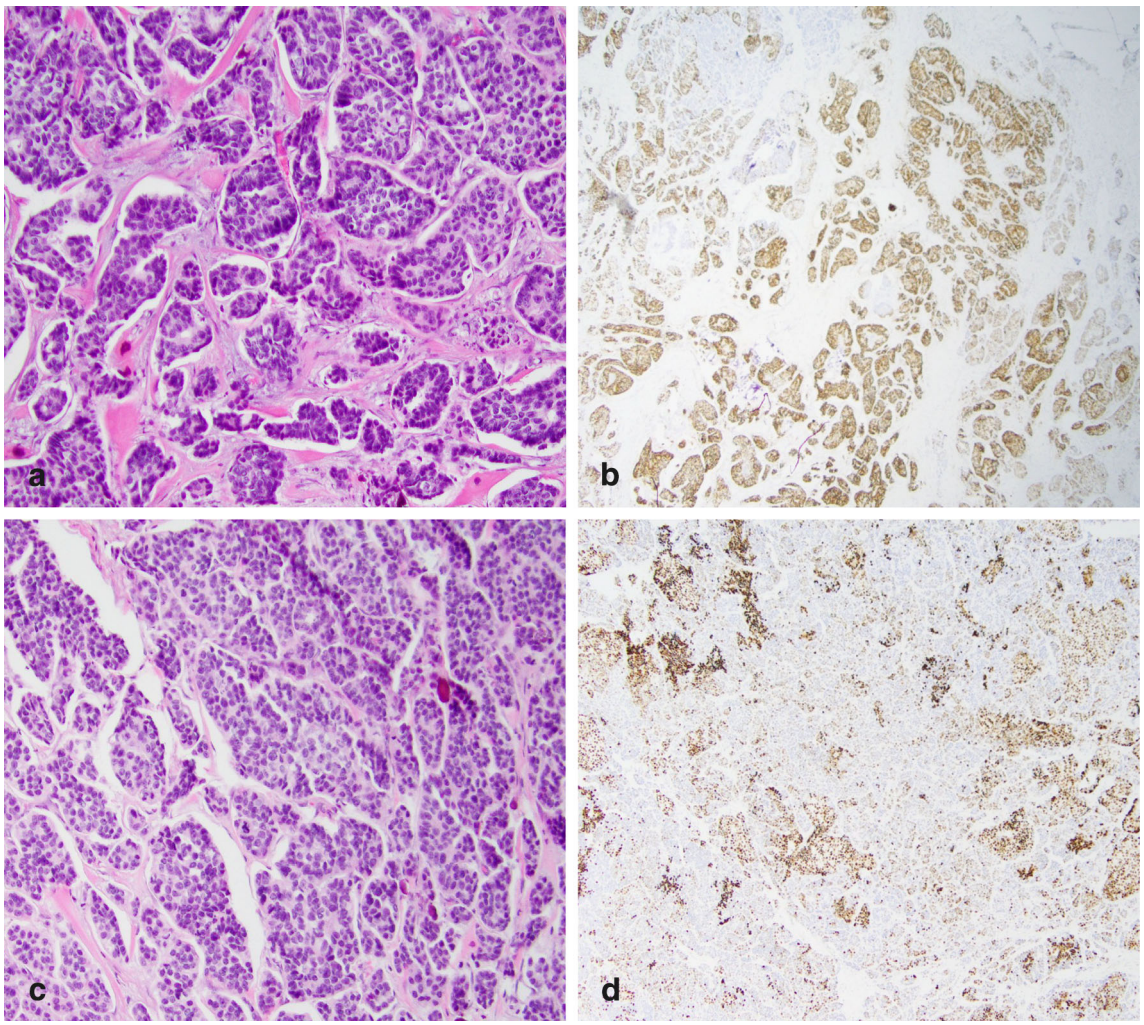

FIG. 2 Example of group 3 case with different PR expression: **a** ductal carcinoma with micropapillary features, 1.0 cm, with RS 13 (H&E, 100 ×), **b** PR immunohistochemistry (IHC) with 70% nuclear positivity (40 ×), **c** ductal carcinoma with micropapillary features, 0.8 cm, with RS 23 (H&E, 100 ×), and **d** PR IHC with 40% nuclear positivity (40 ×)

been identified.[22,28,31] Accordingly, two of the discordant cases in our study had different PR expression in each BC focus tested, all of which were IDC, grade 2/3. Despite having similar histology, each focus with low-risk RS had a greater percentage of nuclear staining for PR, while the focus with intermediate-risk RS showed less nuclear PR positivity. These differences in PR expression could be due to tumor heterogeneity or different clonality among tumor foci.

Two discordant cases showed a more predominant in situ component admixed with the BC focus having the lower RS. To the best of the authors' knowledge, there are no data examining how DCIS may affect the RS assay result. Whenever possible, it is advised to avoid tissue blocks containing extensive DCIS. Further investigation of the impact of DCIS on the RS assay is warranted.

Previous studies have evaluated the RS in multifocal breast cancers by evaluating bilateral tumors[32] or a mix of bilateral and ipsilateral BCs,[33] which may or may not have been histologically different. Karsten et al. examined synchronous bilateral tumors and found a 33% rate of discordance, ultimately recommending testing of all foci.[32] This is consistent with our practice of staging synchronous bilateral primary carcinomas separately, according to the AJCC staging guidelines. Toole et al. found that 50% of bilateral tumors and 22% of ipsilateral tumors had sufficiently different RS to warrant changes in therapeutic recommendations. This included bilateral BCs of different histologic subtype (75%) and ipsilateral BCs with different histologies (22%). However, they found a statistically significant difference in tumor histology and grade when comparing those patients who had discordant results versus those results that did not change management.[33]

TABLE 2 Summary of group 3 cases—TAILORx criteria

Case	Tumor type	MBR grade	Patient age (years)	Tumor size (cm)	Recurrence score
1	Ductal, NOS	1	43	1.1	5
				1.0	12
2	Ductal, NOS	3	39	2.4	26
				1.8	27
				1.9	22
3	Ductal, NOS	2	69	2.3	11
				0.6	6
				0.5	5
4	Classic lobular	2	44	1.7	12
				1.4	7
5	Ductal with focal micropapillary features	2	56	1.1	15
				0.6	7
6	Classic lobular	2	52	2.0	14
				1.0	8
7	Classic lobular	2	53	3.4	11
				1.1	0
8	Ductal, NOS	2	43	1.1	7
				1.0	15
9	Ductal, NOS	2	53	1.4	1
				0.6	23
10	Mammary with mixed ductal and lobular features	2	60	4.6	10
				1.0	13

NOS not otherwise specified

The TAILORx trial randomized patients with RS 11–25 to treatment with a combination of chemotherapy and endocrine therapy versus endocrine therapy alone. Analysis of the study results found that adjuvant chemotherapy is not beneficial in patients with RS < 25 and age over 50 years. However, women under age 50 years with BC having a mid-range RS score (RS 16–25) may still derive some benefit from chemotherapy.[13] Based on recent data from the TAILORx trial, 98% (52/53) of our cases were concordant in terms of adjuvant treatment recommendations. In the one discordant case, all three BC foci were in a woman under 50 years and had RS > 15 (RS 22, 26, and 27). The clinical management of this patient would be unchanged under the current guidelines.

Our study looked only at patients with ipsilateral morphologically similar tumor foci and is the largest series reported so far. Our data show a good correlation of RS between multiple tumors with 87% concordance. In the seven discordant cases, three had associated BXC, known to artifactually increase the RS. In two cases, the change in RS was due to varying expression of PR. In two cases, the presence of DCIS may have affected the RS assay. Our findings (1) emphasize the importance of avoiding the biopsy site when selecting tumor tissue blocks for testing, and (2) suggest that testing of a single focus from a multifocal BC is sufficient to provide accurate prognostic information and to direct treatment, provided the foci are morphologically similar and have similar ER and PR expression.

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DISCLOSURES MM and MD have consulted for Genomic Health.

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