Comparison of Oncotype DX and Mammostrat risk estimations and correlations with histologic tumor features in low-grade, estrogen receptor-positive invasive breast carcinomas

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Several molecular tests have been developed to estimate risk of distant recurrence and help clinical decision-making regarding adjuvant chemotherapy in patients with early stage breast carcinoma. Both Oncotype DX, a 21-gene expression profile, and Mammostrat, an immunohistochemistry-based assay, are validated to stratify patients into groups with low, intermediate and high risk of distant recurrence. However, they have not been compared head-to-head and little data are available regarding their correlation with clinicopathologic tumor features. In this study, we compared the clinicopathologic tumor features with risk estimations by Oncotype DX and Mammostrat in 106 low-grade estrogen receptor (ER)-positive breast carcinomas. Double immunohistochemical stain for pancytokeratin and Ki-67 was performed to assess cell proliferation in cancer *vs* stromal/inflammatory cells. Tumors showing intermediate/high risk by Oncotype DX, but not by Mammostrat, showed increased stromal cellularity, presence of inflammatory cells and increased proliferation in stromal/inflammatory cells. Discrepant cases showing intermediate/high risk by Oncotype DX but low risk by Mammostrat were associated with increased stromal cellularity, presence of inflammatory cells and increased proliferation in stromal/inflammatory cells, compared with concordant cases showing low risk by both assays. Our results suggest that low-grade ER-positive breast carcinomas with increased stromal/inflammatory cell proliferation may show an apparent increased risk of distant recurrence as assessed by Oncotype DX, which uses RNA extracted from a mixture of tumor and stromal/inflammatory cells in the assay. Mammostrat, which examines cancer cells only, may provide a better estimation of likely tumor behavior in a subgroup of low-grade breast carcinomas.

Breast cancer is a heterogeneous disease with a wide range of biological features and clinical behavior.[1,2] Besides patient characteristics (ie, age and comorbidities), oncologists have traditionally relied on clinicopathologic tumor features (tumor size, histologic grade, lymph node, hormone receptor and HER2 status) to assess risk of recurrence and guide recommendations for adjuvant chemotherapy. However, such treatment decisions remain challenging, especially with estrogen receptor (ER)-positive early stage tumors where the absolute benefit of chemotherapy is difficult to perceive.[3] Although the traditional clinicopathological prognostic/predictive factors show strong association with treatment response and patient outcome, concerns exist that they cannot adequately capture the diversity of clinical behaviors of breast cancer nor guide individualized treatment recommendations.

In recent years, based on gene expression profiling studies highlighting the marked heterogeneity of

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breast cancers and defining molecular subgroups of the disease,[4,5] multiple molecular assays to identify signatures associated with prognosis[6–8] and response to therapy[9] were developed to help clinical decision-making regarding adjuvant chemotherapy. Currently, the most widely used such assay in the United States is Oncotype DX (Genomic Health, Redwood City, CA, USA), an RT-PCR based assay analyzing 16 cancer-related and 5 reference genes to provide a Recurrence Score. The Recurrence Score is based on expression levels of genes related to hormone receptor/HER2 signaling and cell proliferation to predict a 10-year distant recurrence risk as a continuous variable, assuming that patients receive adjuvant hormonal manipulation. It is also subdivided into three risk categories: low (<18), intermediate (18–30) and high (>30) scores.

The Mammostrat immunohistochemical assay (Clarient, Aliso Viejo, CA, USA), based on the assumption that gene expression can be translated into protein expression data,[10] measures the expression of SLC7A5, involved in nutrient transport; p53, involved in cell cycle checkpoint control; HTF9C, a gene whose expression oscillates during the cell cycle; NDRG1, a stress- and hypoxia-inducible gene; and CEACAM5, a carcinoembryonic differentiation antigen.[10–12] Based on the expression of these proteins a Prognostic Index is calculated and patients are classified into three risk categories as well: low (≤0), moderate (>0 and ≤0.7) and high (>0.7) risk of recurrence.

Although both the Oncotype DX Recurrence Score[3,13,14] and the Mammostrat Prognostic Index[10–12] have been validated in multiple studies, and the Recurrence Score has been incorporated into clinical guidelines for management of breast cancer,[15,16] several questions remain unanswered. Although both were validated in the same cohorts of patients (NSABP B14 and B20), they have not been compared head-to-head and little data are available regarding their correlation with clinicopathologic tumor features. Although both tests were shown to predict outcome and response to therapy when analyzing large numbers of cases, their prognostic and predictive accuracy in a given individual case has not been adequately addressed and little has been done to study the possibility that some cases may have a falsely low- or high-risk prediction when compared with what would be expected based on clinicopathologic features and/or outcome.[17] Furthermore, the reproducibility of the Oncotype DX assay, performed with tissue extracts without microdissection of cancer cells from the stroma, is not well established and several studies suggest that stromal and inflammatory cells can have a significant effect on the Recurrence Score.[2,17–19] Indeed, we have recently shown that a proliferative, cellular stroma and inflammatory cells associated with tumor cells may account for unexpected intermediate-/high-risk estimations by Recurrence Score in low-grade breast carcinomas.[19] In contrast, the immunohistochemistry

Table 1 Summary of clinicopathologic features

	All carcinomas (n = 106)
Age (years, median, mean ± s.e.m.)	57.5 (58.3 ± 1.0)
Tumor size (cm, median, mean ± s.e.m.)	1.2 (1.4 ± 0.1)
Menopausal status (%)	
Premenopausal	37 (35)
Postmenopausal	69 (65)
Histologic type (%)	
Ductal (NST)	72 (67)
Lobular	4 (4)
Tubular	22 (21)
Cribriform	4 (4)
Mucinous	4 (4)
Tubule formation (%)	
1	30 (28)
2	71 (67)
3	5 (5)
Nuclear pleomorphism (%)	
1	20 (19)
2	85 (80)
3	1 (1)
Mitotic activity (%)	
1	106 (100)
2	0 (0)
3	0 (0)
Number of mitoses per 10 HPF (median, mean ± s.e.m.)	1 (1.8 ± 0.2)
Lymphatic invasion (%)	
Absent	104 (9)
Present	2 (2)
pN stage (%)	
0(i −)	93 (87)
0(i +)	5 (5)
1mi/1a	8 (8)
Percent ER reactivity (median, mean ± s.e.m.)	100 (95.1 ± 0.8)
ER H-score (median, mean ± s.e.m.)	295 (276.8 ± 3.3)
Percent PR reactivity (median, mean ± s.e.m.)	85 (75.6 ± 2.7)
PR H-score (median, mean ± s.e.m.)	222.5 (203.0 ± 8.7)
HER2 status (%)	
Negative	106 (100)
Positive	0 (0.0)
Cellular stroma (%)	
Absent	72 (68)
Present	34 (32)
Inflammatory cells (%)	
Absent	78 (74)
Present	28 (26)

Abbreviations: ER, estrogen receptor; HPF, high-power field; H-score, histologic score; NST, no special type; PR, progesterone receptor.

based Mammostrat assay examines only cancer cells and its results are not affected by the presence of inflammatory cells or the features of tumor stroma.

Table 2 Correlation of clinicopathologic tumor features with Oncotype DX Recurrence Score and Mammostrat Prognostic Index in low-grade, ER-positive invasive breast carcinomas

	Oncotype DX Recurrence Score			Mammostrat Prognostic Index		
	< 18 (n = 68)	RS ≥ 18 (n = 38)	P-value	<0 (n = 91)	> 0 (n = 15)	P-value
Age (years, median, mean ± s.e.m.)	57.5 (58.0 ± 1.1)	57.5 (59.0 ± 1.9)	0.6372*	57 (57.7 ± 1.0)	66 (62.5 ± 3.2)	0.0934*
Tumor size (cm, median, mean ± s.e.m.)	1.3 (1.4 ± 0.1)	1.2 (1.3 ± 0.1)	0.2721*	1.2 (1.3 ± 0.1)	1.5 (1.5 ± 0.2)	0.1597*
Menopausal status (%)						
Premenopausal	21 (31)	16 (42)	0.2905**	32(35)	5 (33)	1.000**
Postmenopausal	47 (69)	22 (58)		59 (65)	10 (67)	
Histologic type (%)						
Ductal (NST)	46 (67)	26 (68)	0.2107**	61 (67)	11 (7)	0.6869**
Lobular	1 (2)	3 (8)		4 (4)	0 (0)	
Other special type	21 (31)	9 (24)		26 (29)	4 (27)	
Tubule formation (%)						
1	24 (35)	6 (16)	0.0191**	27 (30)	3 (20)	0.4292**
2	43 (63)	28 (74)		59 (65)	12 (80)	
3	1 (2)	4 (10)		5 (5)	0 (0)	
Nuclear pleomorphism (%)						
1	12 (18)	8 (21)	0.6977**	19 (21)	1 (7)	0.0231**
2	55 (80)	30 (79)		72 (79)	13 (87)	
3	1 (2)	0 (0)		0 (0)	1 (7)	
Mitotic activity (%)						
1	68 (100)	38 (100)	NA	91 (100)	15 (100)	NA
2	0 (0)	0 (0)		0 (0)	0 (0)	
3	0 (0)	0 (0)		0 (0)	0 (0)	
Number of mitoses per 10 HPF (median, mean ± s.e.m.)	1 (1.6 ± 0.2)	1 (2.1 ± 0.4)	0.1444*	1 (1.7 ± 0.2)	1 (2.1 ± 0.5)	0.5180*
Lymphatic invasion (%)						
Absent	66 (97)	38 (100)	0.5357**	89 (98)	15 (100)	1.000**
Present	2 (3)	0 (0)		2 (2)	0 (0)	
pN stage (%)						
0(i −)	60 (88)	33 (87)	0.5358**	79 (87)	14 (93)	0.4672**
0(i +)	4 (6)	1 (3)		4 (4)	1 (7)	
1 mi/1a	4 (6)	4 (10)		8 (9)	0 (0)	
ER H-score (median, mean ± s.e.m.)	300 (278.8 ± 4.1)	285 (273.3 ± 5.6)	0.3267*	300 (277.9 ± 3.5)	290 (270.3 ± 10.3)	0.6763*
PR H-score (median, mean ± s.e.m.)	235 (218.1 ± 9.7)	200 (175.8 ± 16.3)	0.0345*	220 (201.1 ± 9.7)	240 (225.3 ± 17.4)	0.5634*
Cellular stroma (%)						
Absent	59 (87)	13 (34)	<0.0001**	61 (67)	11 (7)	0.7699**
Present	9 (13)	25 (66)		30 (33)	4 (27)	
Inflammatory cells (%)						
Absent	61 (6)	17 (45)	<0.0001**	67(74)	11 (73)	1.000**
Present	7 (33)	21 (55)		24 (26)	4 (27)	
Nottingham Prognostic Index						
Good prognosis (≤3.4)	67 (63)	37 (35)	1.000**	89 (84)	15 (14)	1.000**
Moderate prognosis (3.41–5.4)	1 (2)	1 (2)		2 (2)	0 (0)	

Abbreviations: ER, estrogen receptor; HPF, high-power field; H-score, histologic score; NST, no special type; PR, progesterone receptor.
*Student's *t*-test; **Kruskal–Wallis test.

In this study, we compared the clinicopathologic tumor features with risk estimations by Oncotype DX Recurrence Score and Mammostrat Prognostic Index in a series of low-grade ER-positive breast carcinomas.

Materials and methods

We selected 106 consecutive patients with early stage, ER-positive, low-grade invasive breast carcinoma who underwent Oncotype DX testing as part of their clinical care between 1 January 2006 and 31 March 2011. All hematoxylin and eosin-stained slides were reviewed to establish the diagnoses, including histologic type and grade.[20–22] All invasive carcinomas were graded according to the modified combined histologic grading system.[22] The number of mitoses was determined in 10 consecutive high-power (400 ×) fields in the mitotically most active areas of tumors. The tumor stroma was evaluated for increased cellularity (*vs* a more dense, fibrous stroma) and presence of associated inflammatory cells.

The clinicopathologic features of the tumors are summarized in Table 1. Surgical treatment consisted of lumpectomy in 72 (68%) patients, whereas 34 (32%) patients underwent mastectomy. Axillary lymph node staging was performed in all cases and consisted of sentinel lymph node biopsy in 101 (95%) and axillary lymph node dissection in 5 (5%) cases, respectively. The median number of lymph nodes per case examined was 2 (range 1–22). Axillary lymph node macro- (pN1a) and micrometastases (pN1mi) were present in four (4%) and four (4%) patients, respectively, whereas isolated tumor cells [pN0(i+)] were present in five (5%) patients. The median number of positive lymph nodes was 1 (range 1-–2). ER and progesterone receptor (PR) status were evaluated by immunohistochemistry as previously described.[19] In addition to categorical (positive *vs* negative) results, ER and PR expression were also assessed using a semi-quantitative immunohistochemical score (H-score)[23] taking in consideration the extent and intensity of nuclear

immunoreactivity with score ranges from 0 to 300. HER2/neu overexpression and/or gene amplification were determined by immunohistochemistry alone in 16 (15%) cases, fluorescence *in situ* hybridization (FISH) alone in 16 (15%) cases or by both methods in 74 (70%) cases, as previously described.[19] Twelve (11%) cases had equivocal HER2 immunohistochemical results, none of which showed gene amplification by FISH. The Nottingham Prognostic Index, taking in consideration tumor size, histologic grade and nodal status,[23] was used to estimate the risk of recurrence based on traditional clinicopathologic tumor features. Based on the Nottingham Prognostic Index, 91, 13 and 2 cases were considered to have an expected excellent (≤ 2.4), good (>2.4 but ≤ 3.4) and moderate prognosis (>3.4), respectively. As both patients in the excellent and good prognostic groups were reported to have excellent 10-year survival ($96 \pm 2\%$ and $93 \pm 2\%$, respectively),[24] these groups were combined for statistical analysis. At a mean
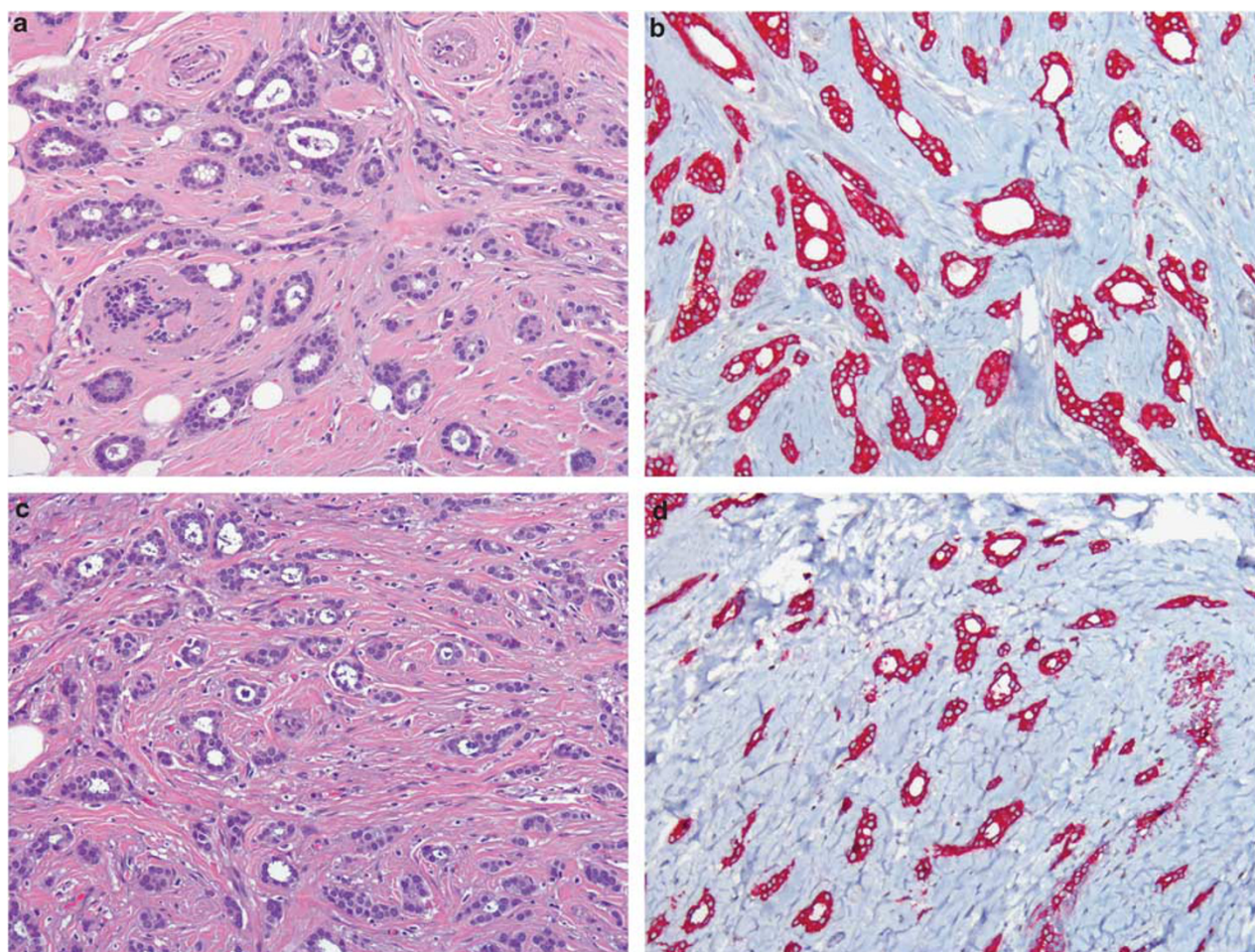

Figure 1 (**a–d**) Examples of low-grade invasive ductal carcinomas with low-risk estimations by both Oncotype DX Recurrence Score and Mammostrat. Note that the tumor stroma is fibrotic and lack significant proliferation as determined by Ki-67 immunohistochemistry (**b**, **d**). (**e–h**) Examples of low-grade invasive ductal carcinomas with intermediate-risk estimations by Oncotype DX Recurrence Score but low-risk estimations by Mammostrat. Note that the tumor stroma is cellular with spindled stromal and inflammatory cells. Ki-67 immunohistochemical stains show significant proliferative activity within the tumor stroma, but not in the cancer cells. (**a**, **c**, **e**, **g**) Hematoxylin and eosin stain; (**b**, **d**, **f**, **h**) double immunohistochemical stains for cytokeratin (red) and Ki-67 (brown).
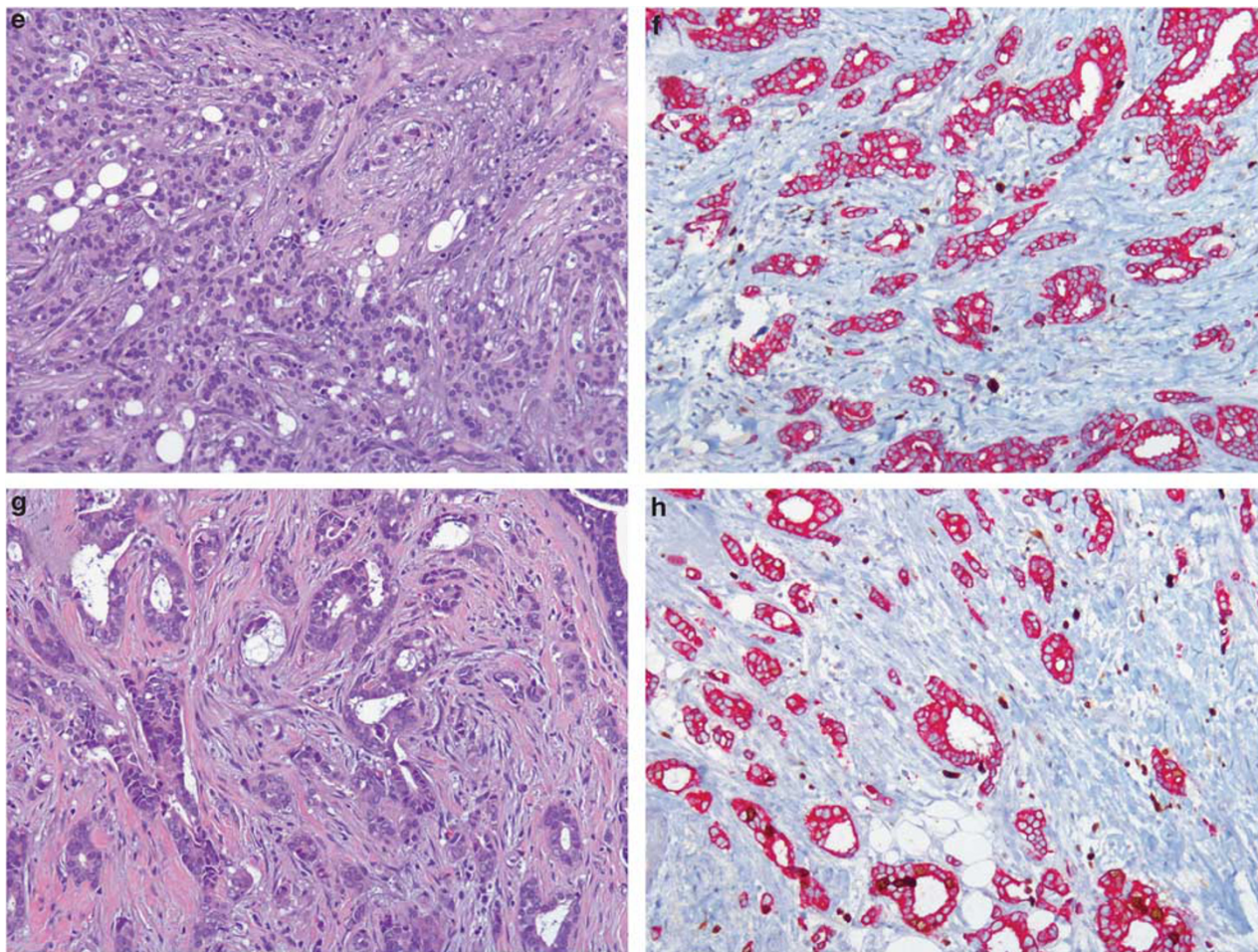
Figure 1 Continued.

follow-up time of 54.3 (range 20.1–83.2) months, no tumor recurrence was observed in any of the patients. Study protocols were approved by the University of South Florida Institutional Review Board.

The tumor tissue block used for Oncotype DX testing was used for immunohistochemical and Mammostrat assays in each case. The Mammostrat assays were performed and analyzed by Clarient as previously described.[10–12] A double immunohistochemical stain for pancytokeratin and Ki-67 was performed in each case to assess cell proliferation in cancer *vs* stromal/inflammatory cells, as previously described.[19] The areas of cancers showing the highest numbers of Ki-67-positive cancer and stromal/inflammatory cells were identified at low-power magnification. Three representative images of tumor and/or tumor stroma showing the highest numbers of Ki-67-positive cells were obtained using a digital camera. Each image taken at a magnification of $200 \times$ represented an area measuring $0.6554 \, mm^2$. Ki-67-positive stromal/inflammatory (pancytokeratin-negative) and cancer (pancytokeratin-positive) cells were counted manually and the mean values of the counts were obtained. As samples used for Oncotype DX testing do not undergo microdissection of tumor cells from stromal/inflammatory cells, results were expressed as the number of Ki-67-positive tumor and stromal/inflammatory cells per mm^2 of tissue.

The clinicopathologic features of carcinomas with Recurrence Score <18 (low risk) *vs* Recurrence Score ≥ 18 (intermediate/high risk), and Mammostrat Prognostic Index ≤ 0 (low risk) *vs* Prognostic Index >0 (moderate/high risk) were compared using the Mann–Whitney test, Student's *t*-test and χ^2 test, when appropriate. The rate of agreement between Oncotype DX and Mammostrat risk stratifications was assessed using kappa statistics. Statistical significance was determined if the two-sided *P*-value of a test was <0.05. Computations were performed using the Graphpad Prism (Version 5, GraphPad Software, San Diego, CA, USA) software.

Results

Among the 106 cases, based on Oncotype DX Recurrence Score and Mammostrat Prognostic Index 68, 38 and 0, and 91, 14 and 1 cases showed low-, intermediate- and high-risk estimates of distant
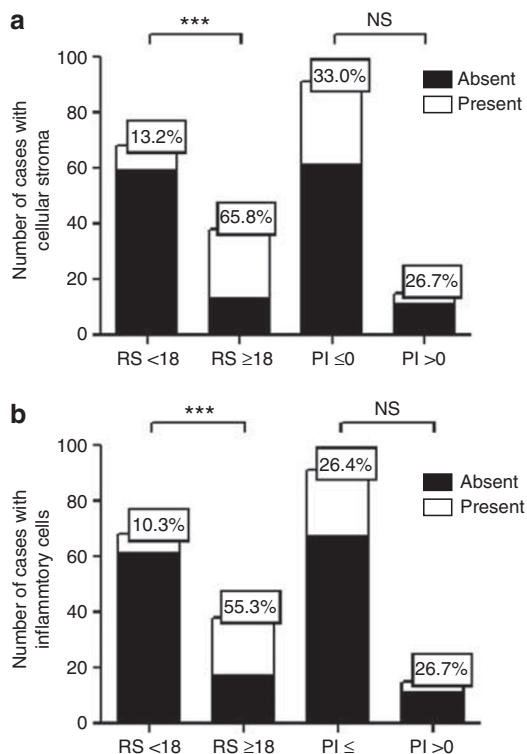
Figure 2 Comparison of the number of cases associated with cellular tumor stroma (**a**) and inflammatory cells (**b**) in breast carcinomas classified as low *vs* intermediate/high risk for distant recurrence by Oncotype DX Recurrence Score (RS) and Mammostrat Prognostic Index (PI), respectively. ***$P<0.0001$; NS, not significant, Chi-square test.

recurrence, respectively. The Recurrence Score and Prognostic Index agreed with the risk estimations based on clinicopathologic tumor features (Nottingham Prognostic Index) in 68 (65.2%) and 89 (84.0%) of 106 cases, respectively. The correlation of clinicopathologic tumor features with Recurrence Score and Prognostic Index is summarized in Table 2. Tumors showing intermediate-/high-risk estimation by the Recurrence Score showed significantly lower PR expression, increased stromal cellularity and presence of inflammatory cells intimately associated with the tumor cells (Figures 1 and 2). In contrast, we found no difference in PR expression, stromal cellularity or the presence of inflammatory cells between cases showing low *vs* intermediate/high recurrence risk estimation by the Prognostic Index.

Assessment of the concurrence between the Recurrence Score and the Prognostic Index to predict low *vs* intermediate/high risk of tumor recurrence showed a kappa value of 0.0541. When the comparison was made according to the three risk categories in both tests, the kappa value was less than that expected by chance. There was no statistically significant correlation between Recurrence Score and Prognostic Index values ($r = -0.0737$, $P = 0.4527$, Spearman test).

We found no statistically significant difference in the number of mitoses per 10 high-power fields present in cancer cells between tumors classified as low *vs* intermediate/high risk by either the Recurrence Score or the Prognostic Index (Table 2 and Figure 3a). Similarly, there was no difference in the number of Ki-67-positive cancer cells per mm^2 of tissue between tumors classified as low *vs* intermediate/high risk by either the Recurrence Score or the Prognostic Index (Figure 3b). In contrast, tumors showing intermediate/high Recurrence Score had significantly increased numbers of Ki-67-positive stromal/inflammatory cells per mm^2 of tumor tissue compared with tumors showing low Recurrence Score (Figure 3c); no such difference was seen between tumors classified as low *vs* intermediate/high risk by the Prognostic Index. The ratio of Ki-67-positive stromal/inflammatory *vs* tumor cells per mm^2 of tumor tissue was also significantly higher in cancers showing intermediate/high *vs* low Recurrence Score; no such difference was found between tumors showing intermediate/high *vs* low Prognostic Index (Figure 3d). The ratio of Ki-67-positive stromal/inflammatory *vs* tumor cells >1 had an area under the curve of 0.8929 ($P<0.0001$) and 0.5026 ($P=0.9823$) to predict intermediate/high recurrence risk based on the Recurrence Score and the Prognostic Index, respectively (Figures 3e and f).

Comparison of 'concordant' (low- *vs* intermediate-/high-risk estimations by both tests, $n = 64$) with 'discordant' (low by one but intermediate-/high-risk estimation by the other test, $n = 42$) cases showed no significant difference between 'concordant' and 'discordant' cases according to the number of tumor cell mitoses per 10 high-power fields or the number of Ki-67-positive tumor cells by mm^2 of tissue (Figures 4a and b). In contrast, the number of Ki-67-positive stromal/inflammatory cells per mm^2 of tissue and the ratio of Ki-67-positive stromal/inflammatory *vs* tumor cells was significantly higher in tumors classified as intermediate/high risk by the Recurrence Score but low risk by the Prognostic Index ($n = 33$) compared with cases classified as low risk by both assays ($n = 58$; Figures 4c and d).

Discussion

The main finding of this study is that in low-grade, ER-positive breast carcinomas, molecular prognostic/predictive assays examining tumor cells specifically to estimate likely biologic behavior appear to be more in line with what would be expected based on clinicopathologic tumor features and historical outcome data compared with assays also including molecular signatures from mitotically active tumor stroma and/or intimately associated inflammatory cells.

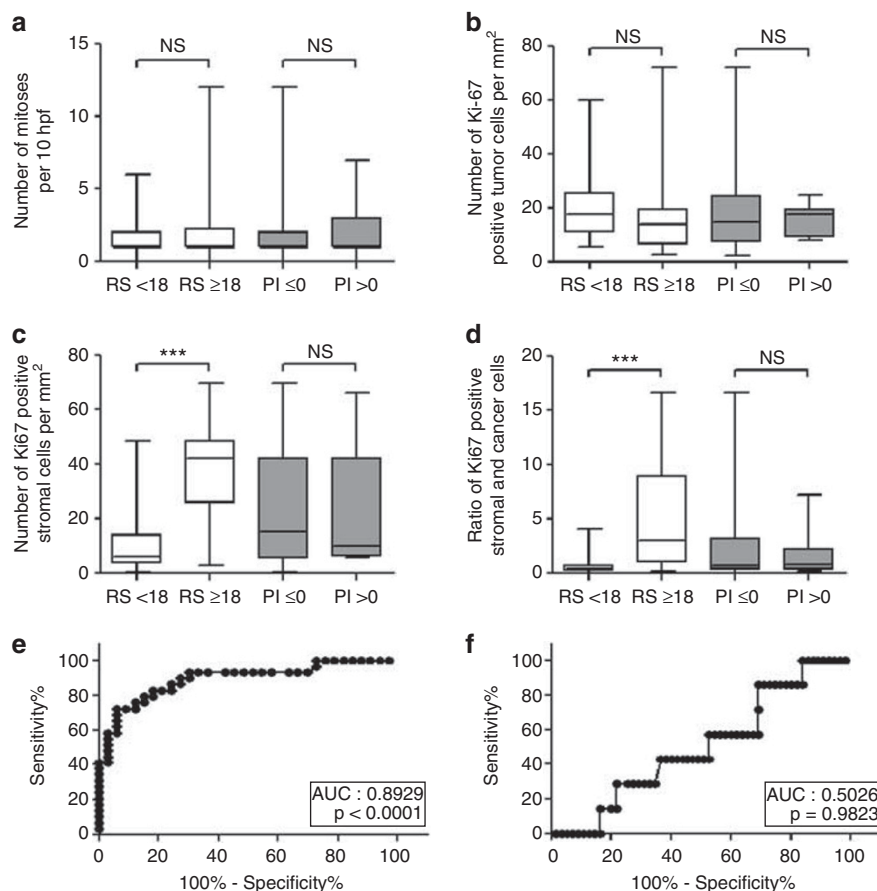Traditionally, treatment decisions for patients with breast cancer are based on clinicopathologic

Figure 3 Comparison of the number of mitoses in cancer cells per 10 high-power fields (**a**), the number of Ki-67-positive cancer cells (**b**), number of Ki-67-positive stromal/inflammatory cells (**c**) and the ratio of Ki-67-positive stromal and cancer cells (**d**) in low-grade invasive breast carcinomas with low- *vs* intermediate-/high-risk estimations by Oncotype DX Recurrence Score (RS) and Mammostrat Prognostic Index (PI). (**e**) Receiver operator curve (ROC) for the ratio of Ki-67-positive stromal and cancer cells to predict an intermediate/high Oncotype DX Recurrence Score. (**f**) Receiver operator curve (ROC) for the ratio of Ki67-positive stromal and cancer cells to predict an intermediate-/high-Mammostrat risk index. ***$P < 0.0001$; NS, not significant, Student's *t*-test.

tumor features and patient characteristics. One of the most established and validated histopathologic prognostic feature is tumor grade, as determined by the modified combined histologic (Nottingham) grading system.[22,23,25] The prognostic value of the Nottingham grade has been established and validated in multiple large independent studies[26–30] and incorporated into prognostic algorithms, such as the Nottingham Prognostic Index[23,24,31] and clinical guidelines.[32] Recent gene expression profiling studies have also shown that tumors of different histological grades show distinct molecular profiles suggesting that histologic grade is indeed an accurate morphologic reflection of the molecular makeup of breast cancers[7,26] and emphasized its relevance in breast cancer biology and behavior.[33,34] A compelling body of evidence suggests that histologic grade can accurately predict tumor behavior, particularly in early stage, small tumors, such as those included in this study.[25,29,35] Specifically, patients with breast cancers in the excellent and good Nottingham Prognostic Index groups (104 of 106 cases in this study) were reported

to have excellent (96 ± 2 and $93 \pm 2\%$, respectively) 10-year survival rates.[24] Several other large studies have also shown that patients with early stage, low-grade invasive breast carcinoma have an excellent outcome with over 95% survival.[35–37] Indeed, we did not observe any recurrence in our series of low-grade, early stage breast cancers, although the mean follow-up time was relatively short (54.3 months).

On the other hand, the gene expression profiling studies have also highlighted the perceived subjectivity associated with histologic grading[3,14] and some authors suggested that molecular tests perform better and may replace traditional histopathology as the 'gold standard' for prognostication and prediction of response to therapy.[38] However, in order to critically evaluate their role in breast cancer management, we must understand the limitations of the available molecular prognostic/predictive tests,[2] apart from the recognized subjectivity of the biostatistical approaches they employ.[26] Recent studies comparing various algorithms for molecular classification showed less than optimal agreement, comparing unfavorably with agreement

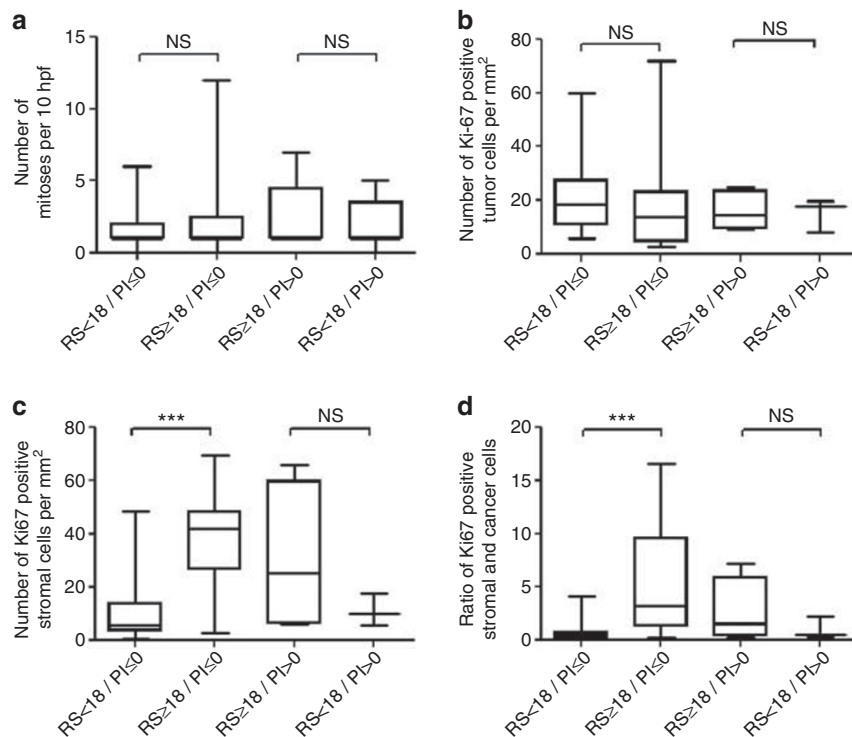**Figure 4** Comparison of the number of mitoses in cancer cells per 10 high-power fields (**a**), the number of Ki-67-positive cancer cells (**b**), number of Ki-67-positive stromal/inflammatory cells (**c**) and the ratio of Ki-67-positive stromal and cancer cells (**d**) in low-grade invasive breast carcinomas with concordant *vs* discrepant (low- *vs* intermediate-/high-) risk estimations by Oncotype DX Recurrence Score (RS) and Mammostrat Prognostic Index (PI). (RS < 18/PI ≤ 0, $n = 58$; RS ≥ 18/PI ≤ 0, $n = 33$; RS ≥ 18/PI > 0, $n = 5$ and RS < 18/PI > 0, $n = 10$). NS, not significant; ***$P < 0.0001$, Student's *t*-test.

rates for grading between experienced breast pathologists.[26,35,39] Based on our results in this highly selected group of low-grade, ER-positive, early stage breast cancers, the agreement between the Oncotype DX Recurrence Score and the Mammostrat Prognostic Index is poor, worse than agreement rates between pathologists regarding tumor grading,[26,35,39] and might be even poorer if cancers of all grades had been included.

Several reasons may exist accounting for the discordance between the assays examined. Of note, in contrast to Oncotype DX, Mammostrat does not incorporate hormone receptor and HER2 status, or measures of cell proliferation into its risk-stratification algorithm, resulting in an estimate based on tumors features different from those routinely assessed and reported by pathologists.[14] It was thus previously suggested that unlike Oncotype DX, Mammostrat appears to identify biological drivers of disease relapse that actually complement conventional pathological and biological markers (ie, ER, PR, HER2, Ki-67) and its results could be interpreted in conjunction with the conventional clinicopathologic features of breast cancers.[14]

It is also important to note that, apart from other methodological differences (RT-PCR *vs* IHC), Mammostrat specifically examines tumors cells, whereas cDNA from both tumor and associated stromal/inflammatory cells is included in the Oncotype DX assay and not controlled for when the Recurrence

Score is calculated. As such, Oncotype DX suffers from the inherent limitations present when a portion of tissue is used that contains both invasive cancer and associated stromal/inflammatory cells. Regarding the possible effect of inflammation on the Recurrence Score, one previous study suggested that in some cases a high Recurrence Score may be more related to peritumoral inflammatory response rather than the tumor cells themselves.[40] Our recent data indicate that in low-grade ER-positive breast cancers, a mitotically active tumor stroma and/or inflammatory cells can indeed contribute to increased risk estimations by the Recurrence Score compared with those expected based on clinicopathologic features and reported outcome data.[19] Recent meta-analyses of microarray-based expression profiling studies have also demonstrated that the apparent prognostic impact of the molecular signatures investigated, including the Recurrence Score, mainly stems from the proliferation-related genes,[41,42] either expressed by tumor or associated stromal/inflammatory cells.

Our current results suggest that in contrast to the Recurrence Score, risk estimations by Mammostrat are independent of stromal/inflammatory cell proliferation and appear to be more in line with what would be expected based on clinicopathologic data and the historically excellent outcome in early stage, low-grade, ER-positive breast cancers. This is further highlighted by the finding that the only

difference between 'concordant' and 'discordant' cases as estimated by the two assays was increased stromal/inflammatory cell proliferation, but not features of the cancer cells themselves, in tumors with intermediate/high Recurrence Score. Indeed, this is not surprising given the dependence of the Recurrence Score primarily on cell proliferation, markers for which are not included in Mammostrat.

It is well known that tumor–stroma interactions have a significant role in tumor development and progression, and alterations in the stromal microenvironment, such as enhanced vasculature, modified extracellular matrix composition, inflammatory cells and unbalanced protease activity, are essential regulatory factors of tumor growth and invasion.[43] However, these effects are mainly thought to be mediated by the expression of cytokines and growth factors by the cancer associated fibroblasts/inflammatory cells and it is unlikely that the proliferative activity of these cells per se (as measured by the Oncotype DX assay) has a significant role in these processes.

Although it is clear that the best method to determine which assay is more accurate in predicting recurrence would be actual long-term outcome data, our relatively short-term follow-up (despite the lack of tumor recurrence in any of the cases so far), does not allow us to address this issue at the present time. Nevertheless, based on reported outcome data in small, low-grade, node-negative, ER-positive breast cancers,[35,36] we expect an excellent outcome in the cases included in this study.

In summary, our study suggests that the presence of a mitotically active, cellular tumor stroma and/or inflammatory cells associated with the tumor in low-grade invasive breast carcinomas may contribute to Oncotype DX Recurrence Score results suggesting an apparently increased risk of recurrence. As the Mammostrat assay examines only tumor cells, this potential confounder/bias does not seem to have a role in risk estimations by this latter test. Given the inherent characteristics of the assay, clinicians and pathologists should be alert to the possibility that the Oncotype DX Recurrence Score may not be an accurate representation of the true cancer biology in a subgroup of low-grade breast cancers associated with a cellular tumor stroma and/or admixed inflammatory cells. Careful pathologic assessment, correlation with histopathologic features and utilization of alternative molecular tests examining tumor cells specifically in such cases may help in determining appropriate patient management.

Acknowledgements

Disclosure/conflict of interest

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