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Basal Subtype, as Approximated by Triple-negative Phenotype, is Associated with Locoregional Recurrence in a Case-control Study of Women with 0-3 Positive Lymph Nodes after Mastectomy

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Abstract

Purpose/Objectives—Recent literature appears to support the importance of breast cancer biologic subtype as a discriminator of locoregional recurrence risk (LRR). More specifically, the basal subtype, as approximated by the triple-negative phenotype (ER-PR-Her2-), has correlated with higher LRR in several studies of patients undergoing breast-conserving therapy. Indications for post-mastectomy RT (PMRT) in women with 0-3 positive lymph nodes remain unclear. We evaluated the importance of biologic subtype in a cohort of women with LRR after mastectomy.

Materials/Methods—We retrospectively identified 22 women with 0-3 postive nodes who suffered LRRs after mastectomy and had available paraffin-embedded tissue blocks from the primary mastectomy specimen. None of these women received PMRT. We case-control matched these to 28 women with 0-3 positive nodes who had mastectomy (no PMRT) and remained without evidence of disease at last follow-up and had available primary specimens for processing. We matched controls for age (+/−3 years) and follow-up duration (<5y vs more). Paraffin-embedded specimens were used to construct a triple-redundant tissue microarray. Biomarker expression was correlated with clinicopathological factors (grade, LVSI, ECE, use of systemic therapy) and outcomes. We used conditional logistic regressions between each predictor and LRR, output was based on odds ratio (OR). We conducted multivariate analysis for each biomarker and all clinical variables with stepwise selection and significance level at entry = 0.15 and at exit = 0.25 to define the best model.

Results—On univariate analysis, ER+, PR+ or the combination was strongly associated with lower odds of LRR. Basal subtype, as approximated by ER-PR-Her2- (TN) was associated with higher LRR (OR 8.5, p=0.048). Use of chemotherapy was also associated with lower LRR (OR

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0.119, p=0.0073). On multivariate analysis, ER or PR+, triple-negative status, and use of chemotherapy continued to remain significant.

Conclusions—Basal subtype as approximated by triple-negative status is associated with higher LRR in women with 0-3 positive nodes on mastectomy specimens. This data is concordant with reports from others demonstrating that TN phenotype is associated with higher LRR and can be considered along with other predictors of LRR when selecting women for PMRT.

Keywords

breast cancer; basal subtype; triple-negative

Introduction

Post-mastectomy radiation therapy (PMRT) is associated with unequivocal improvements in local-regional recurrence (LRR), disease-free survival and overall survival[1–4]. Several professional societies including ASTRO have issued guidelines on the appropriate indications for PMRT, and there is general consensus that women with advanced presentations are very likely to benefit from PMRT. It has been less clear that PMRT is routinely indicated in women with intermediate-risk presentations, such as women with N1a disease. Several large series of patients with 1-3 positive nodes treated in the United States and elsewhere have reported local-regional recurrence rates that hover in the range of 6-13%[5–8]. Several groups have attempted to identify clinicopathological variables that can reliably identify women at higher risk of LRR in un-irradiated cohorts within this intermediate-risk group. For example, Wallgren et al identified high-grade disease and vascular invasion as risk factors of LRR in women with N1a disease. Others have identified multicentric diease, tumor size and gross extracapsular extension as independent predictors of LRR. Indeed even selected women with lower risk, *node-negative* disease may benefit from PMRT. For example, Jagsi et al examined a cohort of node-negative women treated at Massachusetts General Hospital and identified margin status (<2 mm), premenopausal status, size (>2 cm), and LVI as independent predictors of LRR. In summary it appears that while the overall LRR risks for node-negative and stage IIA patients may be low, the simultaneous presence of several secondary risk factors may indicate a higher than average recurrence risk.

A clinicopathological constellation of adverse feature may be a surrogate for adverse underlying disease biology. Thus biologic determinants of LRR may contribute to risk assessment. Recent work has identified biologically distinct subtypes of breast cancer (based on gene expression patterns) that are associated strongly with clinical outcome. These subtypes can be approximated by assessing expression levels of a handful of markers; prognostic information on metastasis and death is conserved even with these phenotypic consructs[9]. Several groups have examined LRR rates as a function of biologic subtype, but the results have been mixed. We attempted to identify whether biologic subtype correlated with LRR in a cohort of mastectomy patients with low-intemediate risk disease burden.

Methods and Materials

Patients

We retrospectively identified 22 women with 0-3 postive nodes who suffered LRRs as either first or only sites of failure after mastectomy and had available paraffin-embedded tissue blocks from the primary mastectomy specimen. None of these women received PMRT. We case-control matched these to 29 women with 0-3 positive nodes who had mastectomy (no PMRT) and remained without evidence of disease at last follow-up and had available primary specimens for processing. We matched controls for age (+/−3 years) and follow-up duration (<5y vs more). Information about each patient's clinical history was retrospectively abstracted from patient charts and assembled into a database. The size of the primary tumor was defined as the largest tumor diameter reported by the pathologist after surgery. Lymph node status was determined by histological evidence of lymph node metastases. The study was conducted after obtaining approval from the participating institutions' Human Investigations Committees.

All patients in this study were treated with mastectomy with or without axillary lymph node dissection, as clinically indicated and based on the standard practice patterns of treating surgeons throughout the interval. Adjuvant chemotherapy was administered to patients as clinically indicated in accordance to the standard practice of medical oncologists during this interval. Adjuvant hormone therapy was routinely given to ER-positive patients.

Construction of Tissue Microarray

A pathologist (NB) examined hematoxylin- and eosin-stained slides of the archived paraffin blocks of breast cancer tissue and circled representative tumor sections. Areas of tumor that were distinct from normal epithelium were identified and marked for subsequent analysis. From these tumor sections, two 0.6-mm cores were extracted using a tissue microarray device (Beecher Instruments, Silver Spring, MD). Three cores were retrieved from each block (triple-redundancy). Microarrays were cut into 5-μm-thick sections with a tape-based tissue transfer system (Intrumedics, Hackensack, NJ) and processed onto slides.

Staining

Immunohistochemical analysis was performed on 5-μm thick tissue sections prepared from formalin-fixed, paraffin-embedded archival tissue from the tissue microarray block constructed. Tissue sections were deparaffinized and then quenched in 2% hydrogen peroxide–methanol solution. Samples were then pretreated to promote antigen retrieval with the DAKO Target Retrieval Solution (DAKO, Carpinteria, CA). A 3% hydrogen peroxide solution was then used for endogenous peroxidase blocking. Slides were then incubated with rabbit polyclonal antibody DDX1 (1:250; Bethyl Laboratories, Montgomery, TX). Slides were additionally incubated overnight at 4°C with the following antibodies: 1) ER, mouse monoclonal antihuman ER (DAKO); 2) PR, mouse monoclonal antihuman PR (DAKO); 3) HER2neu: rabbit polyclonal anti-HER-2/neu oncoprotein (DAKO). Slides were then incubated with secondary antibody, labeled with avidin-biotin complex streptavidin-peroxidase (Elite; Vector Laboratories, Burlingame, CA), and incubated with the chromogen diaminobenzidine tetrahydrochloride as a chromogenic substrate. Finally, slides were

counterstained with hematoxylin, dehydrated with ethanol, and mounted. A known positive case was included as a positive control. For the negative control, the primary antibody was replaced with nonimmune mouse serum.

Quantitative and qualitative assessment of all biomarkers stained was performed by a single experienced pathologist (H.W.) who was blinded to patient outcomes. For cores that were uninterpretable because of tissue loss or lack of tumor cells, a score of “not applicable” was given. For each core, the region of predominant staining intensity was scored. ER and PR were assessed by the number of positive-stained nuclei. For HER2neu, only membrane staining was scored positive. A numeric score ranging from 0 to 3 that reflected the staining intensity and patterns in 10% or more tumor was used. For HER2 status, numeric scores of 2 or 3 were considered positive.

Statistical analysis

Biomarker expression was correlated with clinicopathological factors (grade, LVSI, ECE, use of systemic therapy) and outcomes. We used conditional logistic regressions between each predictor and LRR, output was based on odds ratio (OR). We conducted multivariate analysis for each biomarker and all clinical variables with stepwise selection and significance level at entry = 0.15 and at exit = 0.25 to define the best model.

Results

A description of study cohort variables is presented in Table 1. There was one missing/unevaluable patient in each cohort due to loss of tissue on the TMA. Table 2 presents a univariate analysis of standard clinicopathological variables and odds of LRR. Only use of chemotherapy was associated with lower LRR (OR 0.119, p=0.0073). On univariate analysis of biomarkers and phenotypic subtype constructs, ER+, PR+ or the combination was strongly associated with lower odds of LRR (Table 3). Basal subtype, as approximated by ER-PR-Her2- (TN) was associated with higher LRR (OR 8.5, p=0.048). On multivariate analysis, ER or PR+, triple-negative status, and use of chemotherapy continued to remain significant (Table 4).

Discussion

Breast cancer has recently been classified into biologically distinct subtypes (based on gene expression patterns) with varying clinical potential[10]. These subtypes can be approximated by assessing expression levels of just a handful of markers; prognostic information appears to be conserved even with these subtype consructs[9]. Since the recognition of these subtypes, several groups have studied the implications of subtype on local-regional recurrence.

Kyndi et al retrieved paraffin-embedded tumor blocks for 1078 patients enrolled on the Danish post-mastectomy trials who had a minimum of 8 lymph nodes examined[11]. Tissue microarrays were constructed from 1000 of these patients and then interrogated for expression analysis with standard immunohistochemical methods. With a median follow-up of 17 years, they found that triple-negative status and receptor-negative/Her-2 positive (Her-2 driven) were prognostic for LRR and overall mortality. Of all variables, only nodal

status correlated more strongly with all endpoints (LRR, DM and mortality) compared to Her-2 driven tumors. Of patients randomized to observation after mastectomy (n=510), triple-negative tumors were associated with inferior overall mortality, DM rate, and LRR probability. Her-2 tumors were associated with mortality and DM but not LRR. In patients treated with PMRT (n=486), triple-negative status was associated with worse LRR, but did not correlate with survival or metastasis rate. Notably, it this analysis of patients receiving PMRT, triple-negative status and Her-2 enriched status were the strongest associations with LRR, exceeding even nodal status and tumor size. Furthermore, PMRT only appeared to benefit patients with favorable biologic subtypes (constructed luminal A) with no statistical improvement in mortality for patients with luminal B, triple-negative, and Her-2 subtypes.

Voduc et al also analyzed biologic subtype as a predictor of local-regional recurrence in a cohort of over 4000 women treated in the British Columbia Cancer Agency (BCCA) system[12]. Approximately 1500 women were post-mastectomy patients. Basal and Her-2 positive subtypes predicted for higher rates of local and regional failure in both post-lumpectomy and post-mastectomy cohorts. Non-luminal A subtypes were found to be independent variables for risk of chest wall and regional nodal failure on Cox multivariate analysis. The 10-year local relapse-free survival for luminal A patients was 92% while the regional relapse-free survival was 96% while the corresponding rates were 81% and 80% for basal subtype.

Dominici et al reported on a cohort of 819 patients who underwent mastectomy at the MD Anderson Cancer Center[13]. Most of these patients received systemic therapy at the discretion of their treating oncologist but notably none received PMRT. The majority of patients were either T1 (75%) or N0 (72%). Approximately 27% of patients (219/819) had 1-3 positive lymph nodes. With a median follow-up of 58 months, the overall 5-year risk of LRR was only 2.5%. However, patients with triple-negative tumors had a 10.9% incidence of LRR, which was higher than other phenotype constructs ($p<0.01$). On multivariate analysis, having 4 or more positive lymph nodes and triple-negative status were significant predictors of LRR.

Using a three-marker classification, Billar retrospectively analyzed recurrence rates by constructed subtype in a cohort of 1061 patients of whom 35% were mastectomy patients[14]. Local or regional recurrence developed more frequently in patients with triple negative phenotype (5.7%) compared to Her-2+ (2.9%) and ER+ (1%), $p=0.001$.

Wang et al recently reported a multicenter randomized trial evaluating the benefit of PMRT in triple-negative breast cancer patients[15]. Six-hundred and eighty-one women were randomly assigned to receive either no further treatment or 50 Gy in 25 fractions to the chest wall +/− regional lymph nodes after a mastectomy and systemic chemotherapy. Notably, inclusion was restricted to Stage I and II patients. All patients had tumors that were no larger than 5 cm, and over 60 % were node negative. With a median follow-up of 86.5 months, patients who received PMRT demonstrated improvements in both 5-year relapse-free (74.6% vs 88.3%) and overall-survival rates (78.7% vs 90.4%) compared to patients treated with chemotherapy alone.

Similar to the studies cited, our report also demonstrates higher odds for local-regional recurrence in hormone-receptor negative and triple-negative phenotypes compared to hormone-receptor positive tumors. Our study is unique in that we began with a cohort of patients with Stage I and II tumors who had chest wall or nodal failures and matched these to similar controls without recurrence. Our findings are in keeping with the results of others and, taken together, we believe these data strongly suggest that PMRT should at least be considered for most women with triple-negative or basal subtype cancers.

Acknowledgments

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Table 1

Descriptive analysis of study cohorts

	Cases	Controls	Total
Primary Size (>2cm vs. <= 2cm)	13/22 (59.09%)	16/29 (55.17%)	51
Multicentricity	5/22 (22.73%)	8/29 (27.59%)	51
Extracapsular_extension	5/22 (22/73%)	3/28 (10/71%)	50
Use of Chemo	11/22 (50%)	26/29 (89.66%)	51
Use of Hormonal therapy	10/22 (45.45%)	18/29 (62.08%)	51
Histologic grade (grade 3 vs. others)	7/22 (31.81%)	16/29 (55.17%)	51
LVSI	6/22 (27.27%)	13/29 (44.83%)	51
ER positive	10/21 (47.62%)	25/28 (89.26%)	49
PR positive	10/21 (47.62%)	21/28 (75%)	49
HER2 positive	3/21 (14.29%)	5/28 (17.86%)	49
ER+ or PR +	12/21 (57.14%)	26/28 (92.86%)	49
TNP	7/21 (33.33%)	1/28 (3.57%)	49
Luminal A	11/21 (52.38%)	22/28 (78.57%)	49
Luminal B	1/21 (4.76%)	4/28 (14.29%)	49
COX (3+)	5/22 (22.73%)	11/29 (37.93%)	51
P53 (3+)	1/22 (4.55%)	7/29 (24.14%)	51

Table 2

Univariate analysis of clinicopathological variables and odds of recurrence

Variable	Estimate (S.E.)	Odds Ratio (95% CI)	p-value
Primary Size (>2cm vs. <= 2cm)	0.3079 (0.51)	1.361 [0.5, 3.703]	0.5467
Multicentricity (1 vs. 0)	−0.1031 (0.61)	0.902 [0.276, 2.953]	0.8647
Extracapsular_extension (1 vs. 0)	1.8071 (1.14)	6.093 [0.653, 56.831]	0.1127
Use of Chemo (1 vs. 0)	−2.1303 (0.79)	0.119 [0.025, 0.563]	0.0073
Use of Hormonal therapy (1 vs. 0)	−0.7025 (0.57)	0.495 [0.162, 1.516]	0.2183
Histologic grade (grade 3 vs. others)	−0.678 (0.64)	0.508 [0.146, 1.767]	0.2846
LVSI (1 vs. 0)	−0.6696 (0.63)	0.512 [0.149, 1.754]	0.2866

Table 3

Univariate analysis of biomarker phenotype and odds of recurrence

Biomarker	Estimate (S.E)	Odds Ratio (95% CI)	p-value
ER (+ vs. −)	−1.9293 (0.79)	0.145 [0.031, 0.684]	0.0147
PR (+ vs. −)	−1.4718 (0.81)	0.230 [0.047, 1.123]	0.0692
HER2 (+ vs. −)	−0.2515 (0.83)	0.778 [0.152, 3.969]	0.7624
ER+ or PR+ vs. others	−2.3090 (1.07)	0.099 [0.012, 0.803]	0.0303
TNP (ER-PR-HER2-) vs. others	2.1374 (1.08)	8.477 [1.016, 70.711]	0.0483
Luminal A (ER+ or PR+ and HER2−) vs. others	−0.9864 (0.62)	0.373 [0.111, 1.257]	0.1117
Luminal B (ER+ or PR+ and HER2+) vs. others	−1.3926 (1.21)	0.248 [0.023, 2.666]	0.2501
COX (3+ vs. others)	−0.6519 (0.63)	0.521 [0.153, 1.776]	0.2975
P53 (3+ vs. others)	−1.6967 (1.08)	0.183 [0.022, 1.512]	0.1151

Table 4

Multivariate analysis of clinicopathological variables and biomarker phenotype and odds of recurrence

Phenotype	**Estimate (S.E)**	**Odds Ratio (95% CI)**	**p-value**
ER (+ vs. −)	−2.4150 (1.09)	0.089 [0.011, 0.758]	0.0268
Use of Chemo (1 vs. 0)	−2.6167 (1.17)	0.073 [0.007, 0.723]	0.0252
Phenotype	**Estimate (S.E)**	**Odds Ratio (95% CI)**	**p-value**
PR (+ vs. −)	−2.6667 (1.36)	0.069 [0.005, 0.983]	0.0485
Use of Chemo (1 vs. 0)	−2.7116 (1.10)	0.066 [0.008, 0.575]	0.0138
Use of Chemo (1 vs. 0)	−1.8726 (0.91)	0.154 [0.026, 0.913]	0.0394
Phenotype	**Estimate (S.E)**	**Odds Ratio (95% CI)**	**p-value**
ER+ or PR+ vs. others	−3.4936 (1.66)	0.073 [0.001, 0.781]	0.0349
Use of Chemo (1 vs. 0)	−1.8731 (0.90)	0.154 [0.026, 0.895]	0.0372
Phenotype	**Estimate (S.E)**	**Odds Ratio (95% CI)**	**p-value**
TNP vs. others	3.5468 (1.73)	34.702 [1.179, >>]	0.0398