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ORIGINAL REPORT

Breast Cancer Subtypes and the Risk of Local and Regional Relapse

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See accompanying editorials on pages 1625 and 1627 and articles on pages 1671 and 1677

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ABSTRACT

Purpose
The risk of local and regional relapse associated with each breast cancer molecular subtype was determined in a large cohort of patients with breast cancer. Subtype assignment was accomplished using a validated six-marker immunohistochemical panel applied to tissue microarrays.

Patients and Methods
Semiquantitative analysis of estrogen receptor (ER), progesterone receptor (PR), Ki-67, human epidermal growth factor receptor 2 (HER2), epidermal growth factor receptor (EGFR), and cytokeratin (CK) 5/6 was performed on tissue microarrays constructed from 2,985 patients with early invasive breast cancer. Patients were classified into the following categories: luminal A, luminal B, luminal-HER2, HER2 enriched, basal-like, or triple-negative phenotype–nonbasal. Multivariable Cox analysis was used to determine the risk of local or regional relapse associated the intrinsic subtypes, adjusting for standard clinicopathologic factors.

Results
The intrinsic molecular subtype was successfully determined in 2,985 tumors. The median follow-up time was 12 years, and there have been a total of 325 local recurrences and 227 regional lymph node recurrences. Luminal A tumors (ER or PR positive, HER2 negative, Ki-67 $< 14\%$) had the best prognosis and the lowest rate of local or regional relapse. For patients undergoing breast conservation, HER2-enriched and basal subtypes demonstrated an increased risk of regional recurrence, and this was statistically significant on multivariable analysis. After mastectomy, luminal B, luminal-HER2, HER2-enriched, and basal subtypes were all associated with an increased risk of local and regional relapse on multivariable analysis.

Conclusion
Luminal A tumors are associated with a low risk of local or regional recurrence. Molecular subtyping of breast tumors using a six-marker immunohistochemical panel can identify patients at increased risk of local and regional recurrence.

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INTRODUCTION

In the contemporary management of breast cancer, several possibilities exist for local and regional treatment. Patients and their oncologists must decide between various surgical options and the dose, volume, and technique of radiotherapy. These decisions may have a significant impact on treatment-related morbidity and survival from breast cancer.[1-4] A better understanding of the risk of local relapse (LR) and regional relapse (RR) would facilitate therapeutic decision making.

Gene expression profiling can be used to separate breast cancers into distinct molecular subtypes with prognostic significance.[5-8] Commercially available assays based on gene expression profiling, including Oncotype DX (Genomic Health, Redwood City, CA) and MammaPrint (Agendia, Amsterdam, the Netherlands), may provide useful prognostic information.[9,10] Other studies have found that using immunohistochemical surrogates for molecular subtyping can provide much of the prognostic information obtained by gene expression profiling.[11-13]

Although most studies of molecular subtypes in breast cancer report differences in survival, few have examined the differences in locoregional recurrence. The influence of breast cancer molecular subtypes on locoregional relapse and their relevance compared with established clinicopathologic

Table 1. Summary of Immunohistochemical Criteria for Defining Breast Cancer Intrinsic Subtypes

Criteria and Subtype	ER	PR	HER2	CK5/6	EGFR	Ki-67
Criteria for positive result	> 1% of tumor nuclei	> 1% of tumor nuclei	HercepTest* 3+ or 2+ and FISH amplification ratio > 2.0	Any cytoplasmic or membranous staining	Any cytoplasmic or membranous staining	≥ 14% of tumor nuclei
Subtype						
Luminal A	Either ER or PR positive		Negative	Any	Any	Negative
Luminal B	Either ER or PR positive		Negative	Any	Any	Positive
Luminal-HER2	Either ER or PR positive		Positive	Any	Any	Any
HER2 enriched	Negative	Negative	Positive	Any	Any	Any
Basal-like	Negative	Negative	Negative	CK5/6 or EGFR positive		Any
TNP-nonbasal	Negative	Negative	Negative	Negative	Negative	Any

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; CK, cytokeratin; EGFR, epidermal growth factor receptor; FISH, fluorescent in situ hybridization; TNP, triple-negative phenotype.
*Manufactured by Dako (Carpinteria, CA).

variables has not been defined. Biomarker studies can provide prognostic information that may facilitate treatment decisions. In this study, we describe the effect of breast cancer subtypes on LR and RR in a large cohort of patients with early breast cancer.

PATIENTS AND METHODS

Study Population

Between 1986 and 1992, 74% of all patients diagnosed with breast cancer in the province of British Columbia were referred for consultation at the British Columbia Cancer Agency (BCCA; Vancouver, British Columbia, Canada). All referred patients had tumor samples sent to a central laboratory for biochemical estrogen receptor (ER) testing. The cohort used for this study was derived from the archival paraffin-embedded breast tumor samples collected at the Vancouver General Hospital (Vancouver, British Columbia, Canada), one of the two institutions that performed centralized ER testing. Once a tumor sample was identified, patients were included in this cohort only if clinicopathologic data and updated outcomes were obtainable; all patients in this cohort have outcome data accurate to June 2004. Patients with in situ disease only or metastatic disease at presentation were excluded. The cohort includes 4,033 patients, accounting for approximately 41% of all patients referred to the BCCA during this time.

Table 2. Distribution of Clinical and Pathologic Characteristics Among the Various Breast Cancer Intrinsic Subtypes

	Luminal A (n = 1,304)		Luminal B (n = 713)		Luminal-HER2 (n = 185)		HER2 Enriched (n = 227)		Basal-Like (n = 295)		TNP-Nonbasal (n = 261)		
Characteristic	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	χ^2 P
Age at diagnosis, years													< .001
< 40	48	4	51	7	15	8	28	12	55	19	27	10	
40-55	371	29	230	32	65	35	68	30	111	38	92	35	
> 55	885	68	432	61	106	57	131	58	129	44	142	54	
Tumor size, cm													< .001
< 2	824	63	334	47	72	39	96	43	133	45	121	47	
2-5	445	34	345	49	102	55	112	50	138	47	124	48	
> 5	31	2	28	4	11	6	16	7	23	8	15	6	
Tumor grade													< .001
1/2	801	65	307	45	52	29	51	23	30	10	80	32.5	
3	430	35	382	55	127	71	169	77	259	90	166	67.5	
Lymph nodes													< .001
Negative	771	59	383	54	85	46	102	45	190	65	160	62	
Positive	532	41	329	46	99	54	124	55	104	35	100	39	
Lymphovascular invasion													< .001
Negative	761	61	329	48	75	41	102	46	171	60	142	57	
Positive	491	39	353	52	106	59	118	54	112	40	106	43	
Local treatment													< .001
Breast conservation and radiation	587	45	295	41	61	33	80	35	134	45	114	44	
Mastectomy	564	43	323	45	85	46	86	38	113	38	112	43	
Mastectomy and radiotherapy	153	12	95	13	39	21	61	27	48	16	35	13	

Abbreviations: HER2, human epidermal growth factor receptor 2; TNP, triple-negative phenotype.

For the local treatment of breast cancer, patients underwent either breast-conserving surgery (BCS) followed by adjuvant radiotherapy or mastectomy. Patients treated with BCS who did not receive adjuvant radiotherapy and patients with positive surgical margins were excluded from analysis. Patients typically had a level II axillary dissection, and the mean number of lymph nodes dissected was 11 nodes. Patients who underwent mastectomy may have received adjuvant radiotherapy at the discretion of the oncologist. In most cases, postmastectomy radiotherapy was limited to patients with high-risk disease (T3 tumors, > three involved lymph nodes, or any lymph node > 2 cm). Most patients were treated with adjuvant systemic therapy according to provincial management guidelines established by the BCCA.[14] No patients in this cohort were treated with neoadjuvant chemotherapy or adjuvant trastuzumab. This study was approved by the Clinical Research Ethics Board of the University of British Columbia and the BCCA.

Tissue Microarrays and Immunohistochemistry

Tissue cores were extracted from archival blocks of the primary breast tumor and used to construct a tissue microarray as previously described.[15] Immunohistochemical staining was performed for the biomarkers of ER, progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), Ki-67, epidermal growth factor receptor (EGFR), and cytokeratin (CK) 5/6 on each of the tissue microarray slides using the standard streptavidin-biotin complex method with 3′3′diaminobenzidine chromogen. Staining and interpretation of ER, PR, HER2, Ki-67, EGFR, and CK5/6 have been previously described.[12,13] Surgical pathologists scoring the tissue microarrays were blinded to the clinicopathologic characteristics and outcome of each patient. Samples with less than 50 tumor cells present in the tissue microarray cores were considered uninterpretable and were excluded from analysis. All of the stained tissue microarrays were digitally scanned, and primary image data are available for public access (http://www.gpecimage.ubc.ca/tma/web/viewer.php; username: localrecur; password: localrecur).

Breast cancer molecular subtypes according to immunohistochemical profile were categorized as follows: luminal A (ER positive or PR positive and Ki-67 < 14%; Table 1), luminal B (ER positive or PR positive and Ki-67 ≥ 14%), luminal-HER2 (ER positive or PR positive and HER2 positive), HER2 enriched (ER negative, PR negative, and HER2 positive), and basal-like (ER negative, PR negative, HER2 negative, and EFGR positive or CK5/6 positive). In addition triple-negative tumors (triple negative phenotype [TNP]) that were negative for both EGFR and CK5/6 were labeled TNP-nonbasal.

Statistical Analysis

All statistical analyses were carried out using SPSS Version 16.0 (SPSS, Chicago, IL) and R 2.8.1 (http://www.r-project.org). Differences in the clinicopathologic features between patients assigned to the six breast cancer molecular subtypes were examined using χ^2 tests. To avoid confounding factors related to locoregional therapy, survival analyses were conducted separately for patients treated with BCS versus mastectomy. Because the clinical implications and therapeutic options differ after LR or RR, we also chose to analyze these events separately. LR was defined as disease recurrence within the ipsilateral breast or chest wall. RR was defined as disease recurrence in the ipsilateral axillary nodes, internal mammary nodes, or supraclavicular nodes. Patients were censored at the time of last follow-up, at the date of distant relapse, or at the time of death.

For univariable survival analysis, LR-free survival (LRFS) and RR-free survival (RRFS) were estimated using Kaplan-Meier curves, and survival differences were assessed using log-rank tests. Cox proportional hazards models were used to calculate adjusted hazard ratios accounting for covariates. Clinicopathologic covariates included age at diagnosis, tumor size, tumor grade, presence of lymphovascular invasion, and lymph node status.[16,17] Treatment covariates included chest wall radiotherapy (for LR analysis after mastectomy only), nodal irradiation (for RR analysis only), boost irradiation (for LR after breast conservation only), chemotherapy, and hormonal therapy. Smoothed plots of weighted Schoenfeld residuals were used to test proportional hazard assumptions.[18] All statistical tests were two-sided, and $P < .05$ was considered statistically significant.

RESULTS

The Patient Cohort

Of 4,033 patients with nonmetastatic, newly diagnosed breast cancer, 59 were excluded because they had no primary surgery, and 133 patients were excluded because they had BCS without adjuvant breast radiotherapy. Four hundred six patients had positive surgical margins after BCS, and these patients were also excluded. Four hundred seventy-six patients were excluded because missing biomarker scores did not allow for assignment to a molecular subtype. In the final cohort of 2,985 tumors, the median age was 59 years, and median follow-up time for both LR and RR was 12 years. For patients treated with BCS, there were 130 LR events and 83 RR events. After mastectomy, there were 195 LR events and 144 RR events. The majority of these patients had luminal A tumors (44%, 1,305 of 2,985 patients), followed by luminal B (24%, 720 of 2,985 patients), basal-like (10%, 296 of 2,985 patients), HER2-enriched (8%, 227 of 2,985 patients), and luminal-HER2 tumors (6%, 185 of 2,985 patients). Nine percent of patients (261 of 2,985 patients) had TNP-nonbasal tumors. The clinicopathologic features are listed in Table 2, and there were significant differences in median age, tumor size, tumor grade, lymphovascular invasion, and node status between the subtype cohorts.

Forty-two percent of patients underwent BCS, and 58% underwent mastectomy; 25% of patients treated with mastectomy received

Table 3. Distribution of Clinical and Pathologic Characteristics Among Patients Treated With Breast Conservation Versus Mastectomy

Characteristic	Mastectomy and Radiation (n = 508)		Mastectomy Only (n = 1,492)		Breast Conservation and Radiation (n = 1,461)		
	No. of Patients	%	No. of Patients	%	No. of Patients	%	χ^2 P
Age at diagnosis, years							< .001
< 40	47	9	82	6	122	8	
40-55	170	34	379	25	513	35	
> 55	291	57	1,031	69	826	57	
Tumor size, cm							< .001
< 2	137	27	719	49	977	67	
2-5	265	53	714	48	465	32	
> 5	101	20	50	3	11	0.8	
Tumor grade							< .001
1/2	157	32	668	48	734	52	
3	327	68	727	52	688	48	
Lymph nodes							< .001
Negative	69	14	915	61	998	68	
Positive	438	86	575	39	460	32	
Lymphovascular invasion							< .001
Negative	125	26	808	57	915	64	
Positive	356	74	600	43	511	36	
Systemic chemotherapy treatment							< .001
No chemotherapy	263	52	1,212	81	1,113	76	
Chemotherapy	245	48	277	19	347	24	
Systemic hormonal treatment							< .001
No hormone therapy	204	40	909	61	1,008	69	
Hormone therapy	304	60	583	39	453	31	

postoperative radiotherapy. Fifty-seven percent of the total cohort of patients received adjuvant systemic therapy, consisting of chemotherapy (20%), hormonal therapy (31%), or both (7%). Patients undergoing mastectomy had larger and higher grade tumors and were more likely to have lymphovascular invasion and involved lymph nodes (Table 3).

LR and RR After BCS

Univariate survival analysis of patients treated with BCS and radiotherapy revealed statistically significant differences in LR (Fig 1A) and RR (Fig 1B) among the molecular breast cancer subtypes (Tables 4 and 5). For both LR and RR, patients with luminal A tumors had the most favorable prognosis, with LR and RR rates of only 8% and 3% at 10 years, respectively. Conversely, HER2-enriched and basal-like groups exhibited the highest rates of LR (21% and 14%, respectively) and RR (16% and 14%, respectively).

Multivariable Cox analysis revealed that young age at diagnosis and the HER2-enriched subtype were independent predictors of LR (Table 6) and that anthracycline-based chemotherapy was protective. Multivariable Cox analysis of RR demonstrated that age less than 40 years, more than three positive lymph nodes, and HER2-enriched and basal-like breast cancer subtypes were the strongest independent predictors of recurrence in the regional lymph nodes (Table 6).

Table 4. Ten-Year LRFS After Breast-Conserving Surgery by Subtype

Subtype	No. of Patients	No. of Events	10-Year LRFS (%)	95% CI (%)
Luminal A	587	55	92	90 to 95
Luminal B	295	27	90	86 to 94
Luminal-HER2	61	5	91	83 to 100
HER2 enriched	80	15	79	69 to 89
Basal-like	134	19	86	80 to 93
TNP-nonbasal	114	9	92	86 to 97

Abbreviations: LRFS, local relapse–free survival; HER2, human epidermal growth factor receptor 2; TNP, triple-negative phenotype.

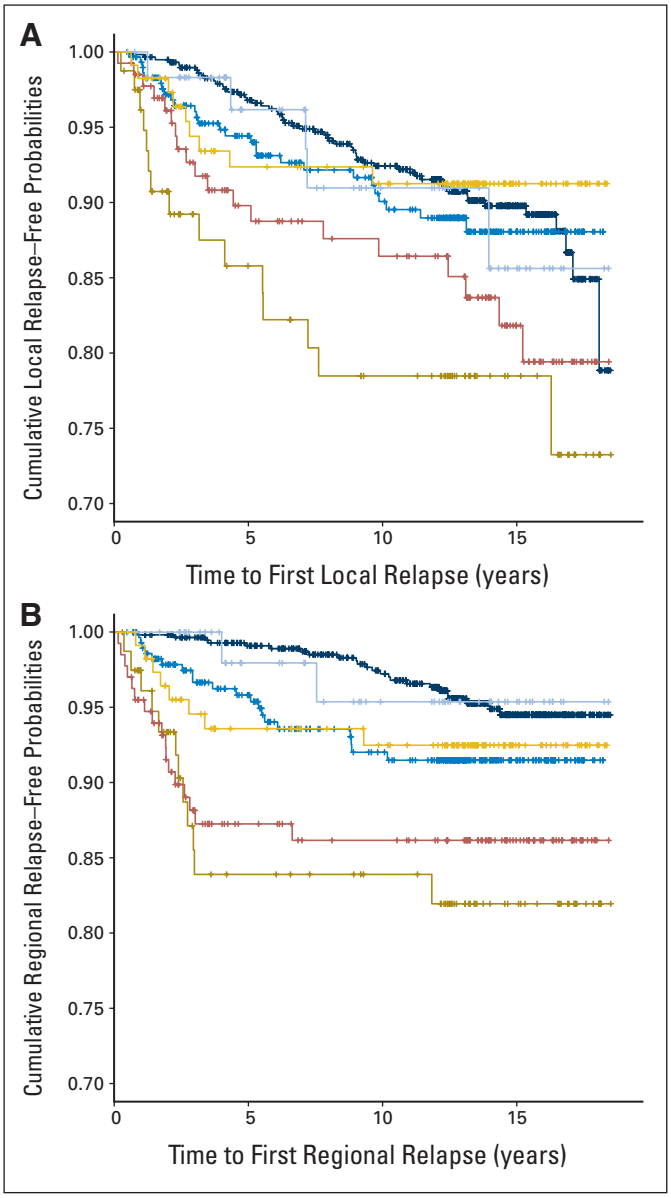

Fig 1. (A) Univariate analysis of local relapse–free survival in patients treated with breast-conserving therapy reveals significant differences among breast cancer intrinsic subtypes (log-rank test, $P = .00515$). (B) Univariate analysis of regional relapse–free survival among patients treated with breast-conserving therapy reveals statistically significant differences among breast cancer intrinsic subtypes (log-rank test, $P < .001$). Violet line, luminal A; light blue, luminal human epidermal growth factor receptor 2 (HER2); dark blue, luminal B; gold, five-marker negative phenotype; red, basal; beige, HER2 enriched.

LR and RR After Mastectomy

Locoregional relapse patterns observed among the various breast cancer subtypes were similar between BCS and mastectomy groups. In univariate analysis, statistically significant differences in LR and RR were observed (Fig 2; Tables 7 and 8). After treatment with mastectomy, patients with luminal A tumors again had the best prognosis, with relatively low rates of LR and RR (8% and 4%, respectively, at 10 years). All non–luminal A subtypes exhibited a greater risk of LR and RR.

Multivariable Cox analysis of LR in patients treated with mastectomy revealed that larger tumor size, high tumor grade, positive lymph nodes, and all non–luminal A subtypes (except TNP-nonbasal) were statistically significant independent predictors of a chest wall recurrence (Table 9). Chemotherapy and hormonal therapy were protective against LR. All of the non–luminal A subtypes (except for TNP-nonbasal) were also found to be independent predictors of a regional nodal recurrence after mastectomy (Table 9).

Table 5. Ten-Year RRFS After Breast-Conserving Surgery by Subtype

Subtype	No. of Patients	No. of Events	10-Year RRFS (%)	95% CI (%)
Luminal A	587	24	97	96 to 99
Luminal B	295	20	92	88 to 95
Luminal-HER2	61	2	95	83 to 99
HER2 enriched	80	12	84	73 to 91
Basal-like	134	17	86	79 to 91
TNP-nonbasal	114	8	93	86 to 96

Abbreviations: RRFS, regional relapse–free survival; HER2, human epidermal growth factor receptor 2; TNP, triple-negative phenotype.

Table 6. Multivariate Analysis of Local and Regional Relapse After Breast-Conserving Surgery and Adjuvant Radiotherapy

Variable	Local Relapse (n = 1,177) HR	95% CI	P	Regional Relapse (n = 1,177) HR	95% CI	P
Age, years						
> 55	1.0			1.0		
40-55	1.6	1.0 to 2.4	.050*	1.2	0.7 to 2.1	.57
< 40	1.7	0.9 to 3.3	.11	2.2	1.1 to 4.7	.035*
Tumor size, cm						
≤ 2	1.0			1.0		
> 2	1.0	0.7 to 1.5	.90	1.5	1.0 to 2.4	.072
Grade						
1/2	1.0			1.0		
3	1.4	0.9 to 2.1	.087	1.0	0.6 to 1.7	.94
Lymphovascular invasion						
Negative	1.0			1.0		
Positive	1.0	0.7 to 1.6	.86	1.4	0.8 to 2.4	.22
Lymph nodes						
Negative	1.0			1.0		
1-3 positive	1.3	0.8 to 2.2	.37	1.7	0.9 to 3.4	.10
≥ 4 positive	2.0	1.0 to 4.3	.058	3.2	1.2 to 9.0	.025*
Radiation boost						
No	1.0			NA		
Yes	1.0	0.6 to 1.9	.22			
Radiation to nodes						
No	NA			1.0		
Yes				0.5	0.2 to 1.1	.093
Chemotherapy						
No	1.0			1.0		
Anthracycline	0.4	0.2 to 0.9	.022*	0.5	0.2 to 1.2	.11
Nonanthracycline	1.0	0.6 to 1.9	.095	0.7	0.3 to 1.6	.46
Hormones						
No	1.0			1.0		
Yes	0.7	0.4 to 1.2	.17	0.6	0.3 to 1.3	.2
Subtype						
Luminal A	1.0			1.0		
Luminal B	1.0	0.6 to 1.7	.86	1.7	0.9 to 3.2	.12
Luminal-HER2	1.0	0.4 to 2.6	.99	0.9	0.2 to 3.8	.85
HER2 enriched	2.7	1.4 to 4.9	.0019*	4.7	2.2 to 10.2	< .001*
Basal-like	1.2	0.7 to 2.2	.48	2.7	1.3 to 5.8	.009*
TNP-nonbasal	0.9	0.4 to 1.8	.66	1.7	0.7 to 4.0	.23

Abbreviations: HR, hazard ratio; NA, not applicable; HER2, human epidermal growth factor receptor 2; TNP, triple-negative phenotype.
*Statistically significant.

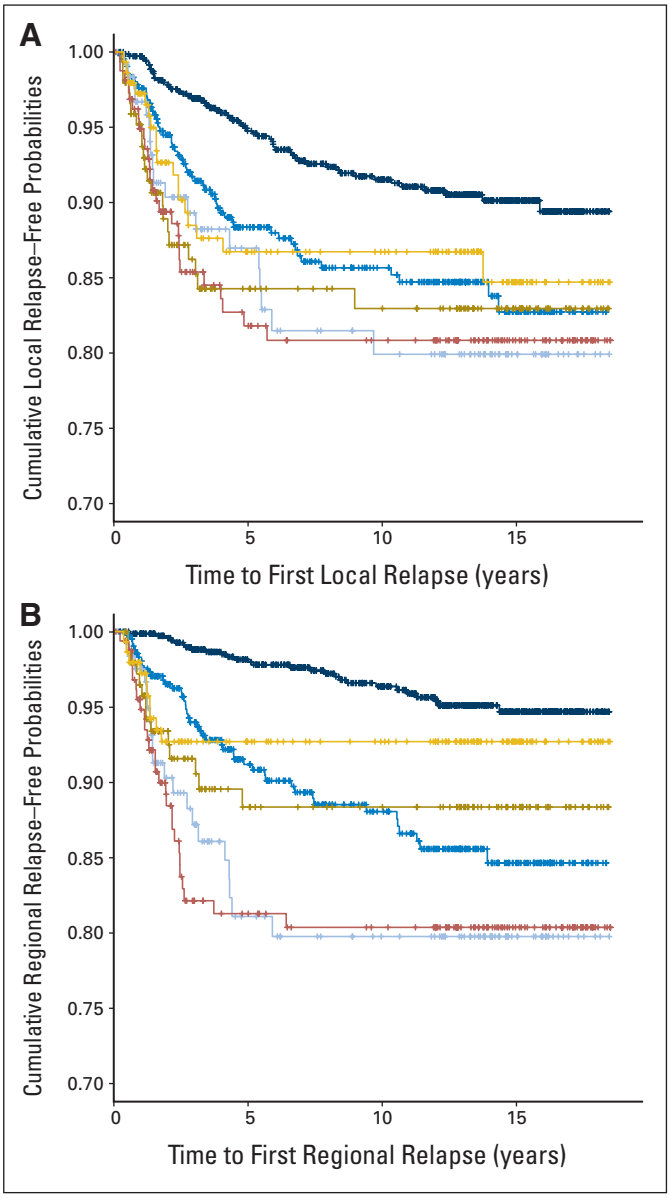

Fig 2. (A) Univariate analysis of local relapse–free survival after mastectomy by breast cancer subtypes reveals statistically significant differences (log-rank test, $P < .001$). (B) Univariate analysis of regional relapse–free survival after mastectomy reveals statistically significant differences (log-rank test, $P < .001$). Violet line, luminal A; light blue, luminal human epidermal growth factor receptor 2 (HER2); dark blue, luminal B; gold, five-marker negative phenotype; red, basal; beige, HER2 enriched.

Multivariable analysis for LR and RR in both treatment subgroups was repeated using a competing risks analysis (Appendix, online only). The breast cancer subtype hazard ratios obtained from competing risks analysis were consistent with those from the Cox model. Exploratory Kaplan-Meier analysis of patients who would generally not receive adjuvant radiation was performed to identify subgroups at high risk of locoregional relapse (Appendix).

DISCUSSION

In this study, the relevance of breast cancer subtypes as predictors of LR and RR was demonstrated. Multivariable analysis illustrates the independent prognostic value of tumor subtypes compared with established clinicopathologic variables. To date, relatively few studies have attempted to find an association between breast cancer molecular subtype and locoregional recurrence. Millar et al[19] used a similar five-marker immunopanel to subclassify 495 mostly low-risk breast cancers treated with BCS. Combining LR and RR (34 events), they found a 5-year locoregional recurrence rate of 15% for HER2-enriched tumors compared with 1% for luminal A tumors (statistically significant on univariable analysis).

Nguyen et al[20] examined a contemporary cohort of 793 patients with breast cancer treated with BCS. With 18 local events, the study found that HER2-enriched and TNP tumors were associated with an increased risk of local recurrence on multivariable analysis. Haffty et al[21] observed a higher overall incidence of local recurrence in a cohort

Table 7. Ten-Year LRFS After Mastectomy by Subtype

Subtype	No. of Patients	No. of Events	10-Year LRFS (%)	95% CI (%)
Luminal A	717	57	92	89 to 94
Luminal B	418	54	86	81 to 89
Luminal HER2	124	19	80	70 to 87
HER2 enriched	147	21	83	75 to 89
Basal-like	161	26	81	73 to 87
TNP-nonbasal	147	18	87	80 to 92

Abbreviations: LRFS, local relapse–free survival; HER2, human epidermal growth factor receptor 2; TNP, triple-negative phenotype.

of 482 patients treated with BCS. LR rate was 17% at 5 years, with no difference between TNP and non-TNP breast cancers. There was a small, but statistically significant, difference in nodal recurrence, with a higher risk observed in TNP cancers versus non-TNP cancers (5-year nodal recurrence rate of 6% *v* 1%, respectively). Dent et al[22] also did not find a difference in local recurrence rates for TNP breast cancer in 1,601 patients.

For patients treated with mastectomy, the Danish Breast Cancer Cooperative Group analyzed the prognostic and predictive value of ER, PR, and HER2 in 1,000 patients enrolled onto the Danish 82b and 82c postmastectomy radiation studies.[23-25] The Danish trials, in addition to a study from British Columbia,[26] were important studies that demonstrated an improvement in overall survival with postmastectomy radiotherapy. The Danish Breast Cancer Cooperative Group found that the TNP and HER2 (ER negative/PR negative/HER2 positive) subtypes were independent predictors of locoregional relapse. They also found that the survival benefit associated with postmastectomy radiation seemed to be isolated to ER- and PR-positive tumors, and there was no survival benefit for TNP and HER2-positive subtypes, suggesting that biomarkers may have a predictive role for radiotherapy response.

The current study represents the largest biomarker analysis of LR and RR in breast cancer reported to date. Luminal A tumors were associated with a low rate of LR of 8% at 10 years after either BCS or mastectomy. This result is concordant with repeated observations that luminal A tumors exhibit the best prognosis with respect to survival.[6,7] Given that only 46% of ER-positive patients were treated with adjuvant tamoxifen in this cohort, an even lower rate of relapse may be expected with more modern adjuvant hormonal therapy. Luminal A tumors also had infrequent RR (RR rate of 3% at 10 years for both BCS and mastectomy).

Table 9. Multivariable Cox Analysis of Local and Regional Relapse After Mastectomy With or Without Adjuvant Radiotherapy

Variable	Local Relapse (n = 1,512) HR	95% CI	*P*	Regional Relapse (n = 1,512) HR	95% CI	*P*
Age, years						
> 55	1.0			1.0		
40-55	1.43	0.9 to 2.2	.094	2.39	1.5 to 3.8	< .001*
< 40	1.77	1.0 to 3.3	.07	2.10	1.0 to 4.4	.049
Tumor size, cm						
< 2	1.0			1.0		
2-5	1.08	0.8 to 1.5	.66	1.19	0.8 to 1.8	.37
> 5	2.31	1.3 to 4.0	.0024*	1.0	0.4 to 2.2	.94
Grade						
1/2	1.0			1.0		
3	1.48	1.0 to 2.1	.027*	1.84	1.2 to 2.8	.0051*
Lymphovascular invasion						
Negative	1.0			1.0		
Positive	1.33	0.9 to 2.0	.15	1.73	1.1 to 2.7	.015*
Lymph nodes						
Negative	1.0			1.0		
1-3 positive	1.70	1.1 to 2.7	.022*	1.78	1.1 to 2.9	.024*
≥ 4 positive	2.87	1.7 to 5.0	< .001*	2.28	1.2 to 4.4	.013*
Radiation to chest wall						
No	1.0			NA		
Yes	0.67	0.4 to 1.0	.064			
Radiation to nodes						
No	NA			1.0		
Yes				0.51	0.3 to 0.9	.012*
Chemotherapy						
No	1.0			1.0		
Anthracycline	0.35	0.2 to 0.7	.0021*	0.43	0.2 to 0.9	.016*
Nonanthracycline	0.57	0.3 to 1.0	.048*	0.55	0.3 to 1.0	.059
Hormones						
No	1.0			1.0		
Yes	0.62	0.4 to 1.0	.03*	0.88	0.5 to 1.4	.61
Subtype						
Luminal A	1.0			1.0		
Luminal B	1.79	1.2 to 2.7	.0059*	2.89	1.7 to 4.8	< .001*
Luminal-HER2	2.05	1.2 to 3.6	.014*	2.75	1.4 to 5.5	.004*
HER2 enriched	1.77	1.0 to 3.1	.047*	2.81	1.4 to 5.6	.003*
Basal-like	1.90	1.1 to 3.2	.018*	4.22	2.3 to 7.8	< .001*
TNP-non basal	1.60	0.9 to 2.9	.12	1.45	0.6 to 3.4	.40

Abbreviations: HR, hazard ratio; NA, not applicable; HER2, human epidermal growth factor receptor 2; TNP, triple-negative phenotype.
*Statistically significant.

Table 8. Ten-Year RRFS After Mastectomy by Subtype

Subtype	No. of Patients	No. of Events	10-Year RRFS (%)	95% CI (%)
Luminal A	717	27	96	94 to 98
Luminal B	418	46	88	84 to 91
Luminal HER2	124	20	80	70 to 87
HER2 enriched	147	14	88	81 to 93
Basal-like	161	27	80	73 to 86
TNP-nonbasal	147	10	93	87 to 96

Abbreviations: RRFS, regional relapse–free survival; HER2, human epidermal growth factor receptor 2; TNP, triple-negative phenotype.

After BCS and adjuvant radiotherapy, there were statistically significant differences in LR observed in the other breast cancer subtypes. Of particular concern was the high rate of LR observed in the HER2-enriched subgroup (10-year LR rate, 21% for HER2 enriched *v* 8% for luminal A); this LR rate approaches the in-breast recurrence rate expected for patients treated with partial mastectomy alone, without adjuvant radiotherapy. Although this subgroup was small (n = 80), the HER2 subtype was an independent marker of LR after BCS (hazard ratio = 2.7, $P = .0019$). It is important to note that in the current treatment of breast cancer, adjuvant trastuzumab

would certainly reduce the risk of LR. There is insufficient evidence from this study to suggest that breast conservation is inappropriate for HER2-enriched tumors, but a radiation boost may be appropriate for some patients in this cohort, particularly if other high-risk local features exist.

Differences in LR among the molecular subtypes were more evident for patients treated with mastectomy. Although patients with luminal A tumors had a favorable prognosis (LR rate of 8% at 10 years), all other molecular subtypes displayed a higher rate of LR (13% to 20%). This was statistically significant on multivariable analysis for all non–luminal A tumors (except TNP-nonbasal). Currently, postmastectomy radiotherapy may be offered to patients with N+ or T3N0 disease and may also be beneficial in high-risk patients with T2N0 disease.[4,27] In the subgroup of patients with grade 3 and T1-2N0-1 breast cancer, LR rate after mastectomy only was 8% for luminal A tumors compared with 22% for luminal B tumors, suggesting that these patients may benefit from additional adjuvant treatment.

RR was rare for patients with luminal A tumors. Some studies have examined the omission of axillary node sampling for low-risk populations, and our study can provide additional criteria to aid in decision making if this option is being considered.[28,29] In our study, luminal B, luminal-HER2, HER2-enriched, and basal-like subtypes were associated with a higher risk of RR after BCS or mastectomy (as high as 20%). This was statistically significant on multivariate analysis for both treatment groups, with the exception of luminal B and luminal-HER2 tumors treated with BCS. In a subgroup of patients with grade 3, T2N0-1 breast cancer who did not receive radiotherapy to the regional lymph nodes, RR rate was 8% for luminal A tumors compared with 20% for basal tumors. Currently, the management of the axilla in breast cancer remains a challenging clinical decision; the impact of locoregional treatment on survival remains controversial, and there is the potential for significant morbidity, including arm lymphedema and neurologic injury. Although previous studies have suggested that basal breast cancers are associated with lymph node–negative disease at presentation,[30,31] we observed a high rate of regional nodal recurrence in this subtype.

An important finding of this study was the high risk of locoregional relapse observed in luminal B tumors, identified using Ki-67. Previous studies have applied HER2-positive status alone to identify higher risk tumors among the hormone receptor–positive breast cancers, and our results suggest that this is insufficient. Using a cutoff of 14% for Ki-67, we found that luminal B tumors were the second largest molecular subtype (35% of hormone receptor–positive and HER2-negative tumors), and they were associated with significantly higher rates of LR and RR. Consistent with our results, Colleoni et al[32] found that high Ki-67 predicted for recurrence in small ($<$ 1 cm), node-negative breast cancers. Furthermore, Mamounas et al[33] found that 25% of a cohort of ER-positive, node-negative breast cancers had a high-risk recurrence score (Oncotype DX assay), and this subgroup had a much higher risk of locoregional relapse compared with low-risk tumors (16% *v* 4%, respectively).

Studies looking at individual biomarkers also support the results of this study. p53 overexpression, a marker of basal-like breast tumors, is associated with local recurrence in both breast cancer[34-36] and ductal carcinoma in situ.[37,38] Elkhuizen et al[39] found that high expression of Ki-67, a luminal B marker, is also associated with an increased risk of local recurrence, whereas Jager et al[40] found that bcl-2 expression, a marker of the luminal A subtype, is associated with a lower risk of local recurrence.

A major limitation of this study is the underuse of systemic therapy, including the absence of adjuvant trastuzumab, in this cohort. Our results from multivariable analysis demonstrate the effectiveness of systemic therapy in reducing the risk of locoregional relapse. It follows that the differences in locoregional relapse between the molecular subtypes will likely be diminished in a population receiving modern systemic therapy. In addition, although the patient subgroup treated with mastectomy only provides the best insight into the locoregional behavior of the breast cancer subtypes, these patients are not directly comparable to the much higher risk subgroup treated with mastectomy and radiation therapy. Consequently, this study is not able to generate any substantial conclusions regarding the relative radiosensitivity and predictive value of breast cancer subtypes.

We have demonstrated that a small panel of immunohistochemical markers can identify patients at increased risk of LR and/or RR. However, the biology underlying these observations remains poorly understood. Nuyten et al[41] obtained gene expression data on 161 patients treated with BCS. Using a 380-gene list, they were able to isolate a subgroup of patients at high risk for local recurrence, and this classification was found to be prognostic on multivariable analysis. This work suggests that we will be able to identify the underlying biologic mechanisms associated with local tumor aggressiveness, nodal metastasis, and radiation response. Additional studies will be required to identify the most effective treatment modality to address a greater risk of locoregional relapse; these treatments could include more extensive surgery, systemic therapy, or radiotherapy. Because effective treatment modalities exist for the locoregional control of breast cancer, further investigation into breast cancer biomarkers, molecular subtypes, and the associated risk of locoregional relapse may profoundly affect the treatment of breast cancer.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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