Application of a New Classification to a Breast Tumor Series from a Population-Based Cancer Registry

Demographic, Clinical, and Prognostic Features of Incident Cases, Palermo Province, 2002–2004

Maurizio Zarcone,[a] Rosalba Amodio,[b] Ildegarda Campisi,[a] Rosanna Cusimano,[b,c] Cecilia Dolcemascolo,[a] Vitale Miceli,[a] Adele Traina,[a] and Maurizio Macaluso[d]

[a]*Palermo Breast Cancer Registry and Experimental Oncology Unit, Department of Oncology, ARNAS-Civico, Palermo, Italy*

[b]*Palermo Province Cancer Registry, Department of Hygiene, University of Palermo, Italy*

[c]*Department of Epidemiology of ASL6, Palermo, Italy*

[d]*Residency Program in Hygiene and Preventive Medicine, Department of Hygiene, University of Palermo, Italy*

A new classification based on gene expression profiling or immunohistochemical (IHC) characteristics may replace current histopathological classifications and predict better clinical outcomes. We used IHC markers to classify incident cases ascertained by the Palermo Breast Cancer Registry (2002–2004) into four subtypes: luminal-A (ER+ or PgR+ and HER2/neu−); luminal-B (ER+ or PgR+, HER2/neu+); basal-like (ER−, PgR−, HER2/neu−); and HER2+/ER− (HER2/neu+, ER−, PgR−). We evaluated HER2/neu, ER and PgR in 1300/1985 (65%) cases. The most common IHC-subtype was luminal-A (68%), whereas luminal-B, basal-like, and HER2+/ER− accounted for 14%, 13%, and 5%, respectively. IHC-subtypes were not associated with tumor size, geographic location within the province, or menopause, but differed by NPI ($P < 0.0001$), grading ($P < 0.0001$), lymph-node involvement ($P = 0.04$), metastases ($P = 0.04$), and TNM stage ($P = 0.04$). Endocrine therapy was administered to 81% of 519 postmenopausal, luminal-A, and luminal-B cases and to 32% of 114 postmenopausal, basal-like, and HER2+/ER− cases.

Key words: **breast cancer; luminal classification; basal-like; endocrine treatment, immunohistochemical (IHC) characteristics**

Introduction

Breast cancer is a heterogeneous malignancy comprising categories with distinct molecular characteristics often correlated with clinical outcome. This disease has become a model for the development of therapies that target tumor-specific biologic pathways used by cancer cells to escape homeostatic control of growth and differentiation.

As the employment of targeted therapy increases, it becomes more important to classify cases according to the particular biomarker profiles for which specific treatment protocols are available.[1–3] Breast cancer classification based on gene expression profiling or immunohistochemical (IHC) characteristics may soon replace the traditional histopathological classification because it may provide a more

Address for correspondence: Adele Traina, M.D., Experimental Oncology Unit, Department of Oncology, M. Ascoli, ARNAS-Civico, Via Carmelo Lazzaro 2, 90127 Palermo, Italy. Voice: +39 091 666 4345 or 4347; fax: +39 091 666 4352. registrotumori@ospedalecivicopa.org

Steroid Enzymes and Cancer: Ann. N.Y. Acad. Sci. 1155: 222–226 (2009).
doi: 10.1111/j.1749-6632.2008.03693.x

accurate tool to match a specific disease type with the most appropriate treatment protocol. IHC markers allow classifying cases into four subtypes, which differ markedly in prognosis and in the repertoire of therapeutic targets they express.[4–6] The incidence and distribution of these molecular subtypes in the population have not been systematically evaluated. Adjuvant hormonal therapy substantially decreases a woman's risk of developing recurrent breast cancer and reduces overall mortality. For almost two decades, a 5-year course of tamoxifen was the standard treatment offered to almost all women with hormone-receptor positive disease.[7,8] Over the past decade, aromatase inhibitors have emerged as highly effective agents in the treatment of hormone-receptor positive breast cancer.[9] IHC subtypes incorporate hormone receptor status and better describe patients for whom adjuvant endocrine treatment is indicated.

We applied the new classification system to incident cases identified by the Palermo Breast Cancer Registry during 2002–2004.[10] In this article, we evaluated the demographic, clinical, and pathological features of incident cases classified into IHC subtypes and assessed whether endocrine therapy was administered.

Materials and Methods

Study Population

The Palermo Breast Cancer Registry (BCR-PA) BCR-PA is a population-based registry covering a population of about 1.2 million residents of the Palermo province in Italy. Palermo (2003 population: 680,000) is the largest city in Sicily and the seat of its regional government. Whereas about half of the population resides in the city of Palermo, the province is a diverse territory including smaller cities and rural villages. The BCR-PA is a "high resolution" specialized registry whose information system couples active case ascertainment with extensive collection of clinical–pathological data as well as follow-up information and survival on each incident case. For the purpose of this analysis we selected invasive breast cancer cases ascertained by the BCR-PA information system between January 2002 and December 2004.

Definition of Breast Cancer IHC Subtypes

Gene expression analysis using DNA microarrays has helped classify breast cancer into four newly defined subtypes: luminal A (with estrogen or progesterone receptor positive [ER+ or PgR+], and human epithelial growth factor receptor-2 negative [HER2/neu−]); luminal B (ER+ or PgR+, HER2/neu+); basal-like (ER−, PgR−, HER2/neu−); and HER2+/ER− (HER2/neu+, ER−, PgR−).[1,2] Large-scale typing using gene expression profiling is not currently feasible with formalin-fixed, paraffin-embedded samples.[11] Immunohistochemistry, however, can provide a valid surrogate methodology for classifying cases according to the four categories described above. IHC subtypes have been validated against gene expression profiles.

Immunohistochemistry of HER2/neu Expression

The procedure to assess HER2/neu has been described in detail elsewhere.[12] Briefly, 2–4-μm thick tissue sections were cleared in xylene, rehydrated in ethanol, and rinsed in distilled water. The slides were then incubated with primary antibody (rat polyclonal antibody antihuman HER2/neu protein) for 30 min at room temperature, the reaction was revealed through incubation with the visualization reactant (EN Vision/HRP Plus, rabbit) and a chromogen agent (DAB) for 10 min, and counterstained with hematoxylin for 30 s. The slides were then dehydrated and mounted using a natural resin. At the end of this procedure, the membranes of HER2/neu+ cells were stained in red-brown, while the cells not

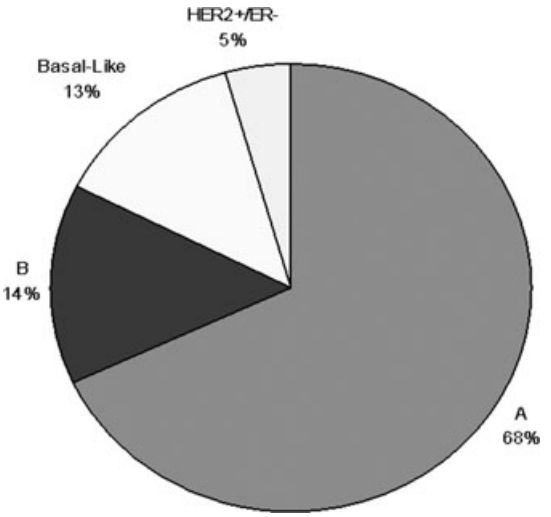

Figure 1. Distribution of new classification of IHC markers in breast cancer patients.

TABLE 1. Incident Breast Cancer Cases According to IHC Subtype and Calendar Year[a]

	2002	2003	2004	Total
Luminal A	285	264	336	885
Luminal B	36	93	57	186
Basal-like	68	53	49	170
HER2+/ER−	16	21	22	59
Insufficient data	237	227	221	685
Total	642	658	685	1985

[a]Palermo province, 2002–2004

displaying the receptors were stained in blue-violet. HER2/neu stain intensity was scored as 0 (no staining), 1+ (fewer than 10% cells stained), 2+ (10% or more cells stained, weak to moderate stain intensity); or 3+ (10% or more cells stained, strong intensity).

FISH Analysis of HER2/neu Amplification

As reported elsewhere,[12] 2–4-μm paraffin-embedded tissue sections were cleared and hydrated, enzymatically digested, denatured, and finally incubated for 12 h with the DNA HER2/neu gene probe (INFORM HER2/neu probe for automation, Ventana), labeled with biotin.

After hybridization, samples were washed in buffer and incubated with a detection system

TABLE 2. Significant Correlations between IHC Subtypes and Clinical Features

Luminal type subtypes	NPI				Grading				Lymph node involvement					TNM stage				
	Good	Moderate	Poor	NA	G1	G2	G3	NA	N0	N1	N2	N3	NA	I	II	III	IV	NA
Luminal A	34.7	18.4	28.0	18.9	11.5	49.7	29.6	9.2	50.6	36.7	2.3	1.7	8.7	35.7	42.0	11.4	4.3	6.6
Luminal B	21.5	20.4	46.3	11.8	4.3	42.5	49.5	3.7	35.5	48.4	4.3	2.1	9.7	21.5	53.8	12.4	5.9	6.4
Basal-like	24.7	17.6	36.5	21.2	2.4	35.9	47.6	14.1	52.3	38.2	1.8	1.2	6.5	31.2	41.2	12.3	10.0	5.3
HER2+/ER-	13.6	20.3	47.5	18.6	0.0	32.2	66.1	1.7	37.3	49.1	1.7	3.4	8.5	23.7	47.5	13.5	8.5	6.8
Chi-square (DF)		44.22 (9)				90.55 (9)					22.07 (12)					33.24 (12)		
P-value		<0.0001				<0.0001					0.037					0.044		

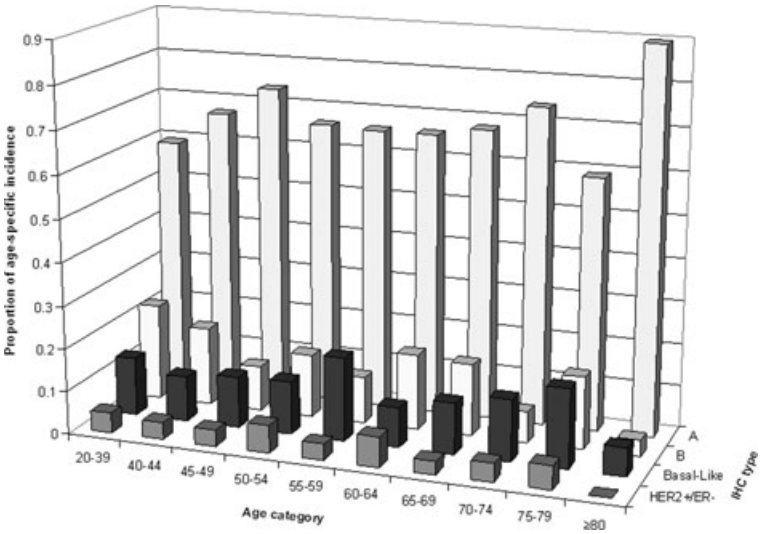

Figure 2. Estimated proportional incidence (age-specific) of breast cancer by IHC type. Palermo province, 2002–2004.

consisting of a first antibiotinrat monoclonal antibody labeled with fluorescein isothiocyanate (FITC) and a second FITC-labeled antirat IgG antibody to further amplify the signal. The slides were finally counterstained using the VECTASHIELD system (Vector Laboratories), examined in a fluorescence microscope, and then scored according to the number of fluorescein signals counted in 20 nuclei of invasive tumor cells for each field. The sample was classified as positive if the mean number of fluorescence signals was greater than 4.

Statistical Methods

The goal of the present analysis was to estimate the incidence of IHC breast cancer subtypes in a population-based sample of breast cancer cases and to examine correlations with clinicopathologic variables. We employed the BCR-PA database to ascertain the availability of IHC marker data on each incident case, and classified the cases with sufficient information into one of the four IHC subtypes. To compute incidence rates, we grouped cases according to IHC subtype and standard age and calendar time groups to obtain the numerator of the rate, then divided each numerator by the appropriate estimate (Istituto Nazionale di Statistica) of the female resident population ($\times 100{,}000$). We also employed stratified and regression analyses to evaluate the association of IHC subtypes with age, year, geographic location (Palermo city limits vs. rest of the province), menopause, TNM stage, Nottingham Prognostic Index (NPI), grading, and endocrine therapy administration (by menopausal and hormone receptor status).

Results and Discussion

IHC marker data on HER2/neu, ER, and PgR were available for 1300/1985 (65%) invasive cases of breast cancer incident during 2002–2004. These cases could be assigned to a category of the luminal-type classification. The most common IHC subtype was luminal A (68%), whereas luminal B, basal-like, and HER2+/ER− accounted for 14%, 13%, and 5%, respectively (Fig. 1). There was significant heterogeneity of IHC subtypes by year and age, with basal-like cases increasing and

HER2+/ER− cases decreasing ($P < 0.001$) with time (Table 1); and luminal-A cases being older at diagnosis (mean: 59.5 years) than others (mean: 57.7 years, $P = 0.03$) (see Fig. 2).

IHC subtypes were not associated with geographic location or menopausal status. Each group differed systematically by NPI ($P < 0.0001$), grading ($P < 0.0001$), lymph node involvement ($P = 0.04$), metastases at diagnosis ($P = 0.04$), and TNM stage ($P = 0.04$), but not tumor size (see Table 2). This was consistent with a better prognostic profile at diagnosis for luminal A type and, to a lesser extent, luminal B cases, whereas the basal-like cases and the HER2+/ER− cases tended to have a more aggressive profile at diagnosis. Overall, 82.5% of all breast cancer were hormone-receptor positive, with the majority presenting in postmenopausal women (70.2%). Endocrine therapy was administered to 420 (81%) of 519 postmenopausal luminal A and B cases (for whom adjuvant therapy is indicated), and to 36 (32%) of 114 postmenopausal basal-like and HER2+/ER− cases (for whom adjuvant therapy may not be necessary).

Recently developed high-throughput genomic analysis techniques have offered the opportunity to challenge the molecular complexity of breast cancer and provided evidence for classifying breast cancer into biologically and clinically distinct groups based on gene expression patterns.[2–4] These novel breast cancer categories are also distinct with respect to response to therapy and outcomes. Our results suggest that we can use "high resolution" cancer registry data to monitor different therapeutic strategies for breast cancer cases' subgroups defined according to their biology. It is possible that in the future new hormonal therapy strategies will be targeted to specific histotypes, also considering differences in genetic makeup, environmental exposures, and dietary or other lifestyle factors.

Conflicts of Interest

The authors declare no conflicts of interest.

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