BREAST CANCER CLASSIFICATION ACCORDING TO IMMUNOHISTOCHEMICAL MARKERS: CLINICOPATHOLOGIC FEATURES AND SHORT-TERM SURVIVAL ANALYSIS IN A POPULATION-BASED STUDY FROM THE SOUTH OF SWITZERLAND

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BACKGROUND

Systematic investigations of gene expression patterns and their correlation with specific features of phenotypic diversity are changing the way of classifying, at the molecular level, the phenotypes of breast cancers. In line with these reports, analysis of gene expression profiling and immunophenotypic characteristics suggests that breast cancer is not a single entity but a heterogeneous disease, composed of a growing number of recognized subtypes. The relationship between immunohistochemical (IHC) markers and responsiveness to therapeutic treatments has been extensively studied, whereas only a few population-based studies have investigated the relationship between molecular subtypes as defined by immunohistochemistry and clinical-pathological characteristics, particularly in European countries. Aim of the study was to investigate prevalence, clinical-pathological features and overall survival of breast cancer subtypes in a large population-based study.

MATERIALS AND METHODS

All invasive breast cancers, occurred between 2003 and 2007, were retrieved from the files of Ticino Cancer Registry. IHC studies for ER, PR and HER2 were performed prospectively on formalin-fixed paraffin-embedded tumour samples in the same pathology institute using an automated staining system (Ventana Medical Systems, Inc.). All cancers with ambiguous expression of HER2 (i.e. score 2+) were classified according to the results of FISH analysis (Vysis, Downer's Grove, IL). Four subtypes of breast cancers were identified: Luminal A (ER+ and/or PR+, HER2-); Luminal B (ER+ and/or PR+, HER2+); Basal-like (BCL) (ER-, PR-, HER2-); HER2+/neu (ER-, PR-, HER2+).

Differences among breast cancer subtypes were evaluated using 1-way analysis of variance for continuous variables; the Chi-square or Fisher's exact test for qualitative variables. Histotypes were classified as following: group A, including neuroendocrine carcinoma, apocrine adenocarcinoma, invasive ductal carcinoma, intraductal papillary adenocarcinoma with invasion, medullary carcinoma, inflammatory carcinoma, Paget's disease, cribiform, tubular or mucinous adenocarcinoma; group B: adenocarcinoma with spindle cell metaplasia and metaplastic carcinoma; group C: invasive lobular carcinomas; group D: mixed ductal and lobular carcinomas. Short-term OS analysis was carried out using the Kaplan-Meier method and the log-rank test was invoked to detect significant survival differences among molecular subtypes. Hazard ratios (HR) adjusted for patient age and AJCC stage were calculated through the multivariate Cox regression analysis. Statistical significance was determined at p-value<0.05. The statistical analysis was implemented in the SAS System version 9.1 (SAS Institute Inc, Cary, NC).

RESULTS

Of 1339 invasive breast cancers, 1214 (90.7%) had an IHC profile and were included in the study. Patient and tumour characteristics are summarized in Table 1. Mean patient age was equal to 62.7±14.0 years and mean tumuor diameter was 20.2±12.3 mm. As reported in Figure 1, most of the cases were classified as luminal A, whereas only 7.4% of tumours were BCL. The IHC subtypes differed significantly by age (p=0.0084), menopausal status (p=0.0044), tumour diameter (p=0.0001), AJCC stage group (<0.0001), tumour histotype (p<0.0001), histological grade (p<0.0001), ki-67 proliferation index (p<0.0001), synchronous *in situ* component with invasive lesion in the same breast (p<0.0001) (Table 1). BCL presented largely in pre-menopausal women (36.7%) and displayed aggressive features, such as the largest tumour size (26.0 mm), the highest prevalence of poorly differentiated cancers (75.9%), the highest proportion of cases with a high ki-67 proliferation index (75.3%). Luminal A included the highest percentage of patients over 70 years (35.4%), the highest proportion of stage I (47.4%), negative lymph nodes (62.2%), well/moderately differentiated (84.6%) and low ki-67 proliferation index tumours (33.9%). HER2+/neu subtype was more frequent in post-menopausal women (86.8%) and showed the highest prevalence of cases with stage IV (11.8%), positive lymph nodes (49.2%) and a synchronous *in situ* component (55.9%). As reported in Figure 2, the molecular subtypes significantly differed in survival (p=0.0446), BCL and HER2+/neu showing the lowest survival probability already at 2 years after the diagnosis (89.4% and 91.7%, respectively). After adjusting for patient age and AJCC stage, the hazard for death of patients with BCL was 4.1 times as great as that of luminal A cases (95%CI: 1.5; 11.6). Although not significant, also HER2+/neu showed a higher risk for death compared with luminal A (HR: 1.4; 95%CI: 0.3; 6.4).

Table 1
Association between IHC subtypes and main clinical-pathological characteristics

Variable	All cases N = 1214		BCL N = 90 7.4%		Her2/neu N = 68 5.6%		Luminal A N = 888 73.2%		Luminal B N = 168 13.8%		P-value
Age											
mean±sd (yrs)	62.7±14.0		58.5±14.6		62.3±12.5		63.4±13.7		61.4±15.0		0.0084
Age specific groups, n (%)											
<50	253	20.8%	32	35.5%	7	10.3%	173	19.5%	41	24.4%	0.0002
50-69	555	45.7%	34	37.8%	45	66.2%	401	45.1%	75	44.6%	
≥70	406	33.5%	24	26.7%	16	23.5%	314	35.4%	52	31%	
Pre-menopausal (ages51), n (%)	291	24.0%	33	36.7%	9	13.2%	205	23.1%	44	26.2%	0.0044
Post-menopausal (age>51), n (%)	923	76.0%	57	63.3%	59	86.8%	683	76.9%	124	73.8%	
Tumor size											
mean±sd (mm)	20.2±12.3		26.0±18.0		22.6±10.8		19.6±12.2		19.6±8.5		0.0001
Lymph node status, n (%)											
positive	436	39.6%	34	42.5%	30	49.2%	307	37.8%	65	44.5%	0.1546
negative	664	60.4%	46	57.5%	31	50.8%	506	62.2%	81	55.5%	
missing or set after therapy	114		10		7		75		22		
Clinical behaviour at diagnosis											
non-metastatic (M0)	1058	95.2%	83	96.5%	58	90.6%	780	95.6%	137	94.5%	0.2920
metastastatic (M1)	53	4.8%	3	3.5%	6	9.4%	36	4.4%	8	5.5%	
unknown	103		4		4		72		23		
AJCC stage group, n (%)											
stage I	436	42.9%	21	29.2%	10	19.6%	362	47.4%	43	33.1%	<0.0001
stage II	408	40.1%	39	54.2%	26	51.0%	283	37.0%	60	46.2%	
stage III	120	11.8%	9	12.5%	9	17.6%	83	10.9%	19	14.6%	
stage IV	53	5.2%	3	4.2%	6	11.8%	36	4.7%	8	6.1%	
unknown / unclassified	197		18		17		124		38		
Histological type, n (%)											
group A	992	83.7%	80	91.9%	67	100%	694	80.0%	151	92.6%	<0.0001
group B	3	0.3%	3	3.5%	0	0%	0	0%	0	0%	
group C	158	13.3%	3	3.5%	0	0%	147	16.9%	8	4.9%	
group D	32	2.7%	1	1.1%	0	0%	27	3.1%	4	2.5%	
unknown / unclassified	28		3		1		20		4		
Histologic grade (Elston/Ellis), n (%)											
well-/moderately differentiated	861	72.9%	21	24.1%	22	33.3%	733	84.6%	85	52.5%	<0.0001
poorly differentiated	320	27.1%	66	75.9%	44	66.7%	133	15.4%	77	47.5%	
unknown	33		3		2		22		6		
Ki67 proliferation index, n (%)											
≤5%	314	26.6%	6	6.7%	1	1.5%	292	33.9%	15	9.3%	<0.0001
5-20%	549	46.5%	16	18.0%	24	35.3%	434	50.3%	75	46.6%	
>20%	317	26.9%	67	75.3%	43	63.2%	136	15.8%	71	44.1%	
Multifocality/multicentricity, n (%)											
yes	222	18.3%	12	13.3%	18	26.5%	157	17.7%	35	20.8%	0.1402
no	992	81.7%	78	86.7%	50	73.5%	731	82.3%	133	79.2%	
Vascular invasion, n (%)											
yes	147	12.1%	10	11.1%	10	14.7%	100	11.3%	27	16.1%	0.3089
no	1067	87.9%	80	88.9%	58	85.3%	788	88.7%	141	83.9%	
Laterality, n (%)											
right	596	49.6%	37	41.1%	34	51.5%	447	50.9%	78	46.7%	0.2836
left	606	50.4%	53	58.9%	32	48.5%	432	49.1%	89	53.3%	
unknown	12		0		2		9		1		
Synchronous in-situ component, n (%)											
yes	421	34.7%	13	14.4%	38	55.9%	296	33.3%	74	44.1%	<0.0001
no	793	65.3%	77	85.6%	30	44.1%	592	66.7%	94	55.9%	

Figure 1
Distribution (%) of IHC subtypes

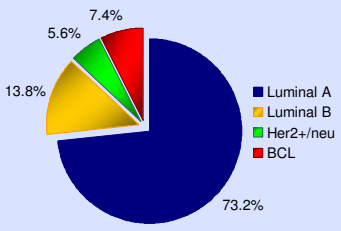
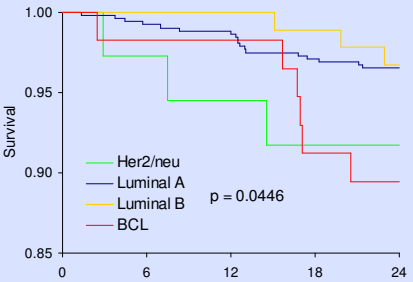


Figure 2
Short-term Overall Survival by IHC subtypes



Her2/neu	97.3%	94.5%	91.7%	91.7%
Luminal A	99.2%	98.6%	97.1%	96.5%
Luminal B	100.0%	100.0%	98.9%	96.7%
BCL	98.2%	98.2%	91.2%	89.4%

CONCLUSIONS

Since a consensus on the most appropriate IHC panel is currently lacking, we opted for a simple classification of molecular subtypes based on the expression of ER, PR and HER2. This definition has several advantages since the three markers are routinely carried out in pathology laboratories: staining and evaluation protocols are well established worldwide and quality controls are already available in several countries. This comprehensive European population-based study on breast cancer molecular subtypes (Spitale A *et al., Ann Oncol.* 2009;20(4): 628-35), as defined by the analysis of IHC markers, shows significant differences in subtypes distribution and clinical-pathological characteristics, also when compared with other population-based studies (Bauer KR et al., Cancer 2007;109(9):1721-8. Yang XR et al., *Cancer Epidemiol Biomarkers Prev* 2007; 16(3):439-43. Carey LA *et al., JAMA* 2006;295(21):2492-2502). In particular, our results provide strong evidence that BCL cancers, defined as triple-negative breast cancers, should be recognized as a distinct entity. We conclude that a molecular classification of breast cancers is useful for clinical management and has a superior value than the WHO classification, particularly in terms of short-term prognostic value.