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Tumour biomarker expression relative to age and molecular subtypes of invasive breast cancer

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BACKGROUND: Quantitative differences in biomarker expression relative to age and molecular subtypes have not been well documented in invasive breast cancer (IBCA).

METHODS: Oestrogen receptor (ER), progesterone receptor (PR), HER2, ki67, p53 and DNA ploidy was performed by image analysis in 162 consecutive IBCAs in women ($\leqslant$40 years) and compared with women $\geqslant$50 years (100 cases). Molecular subtypes were defined by immunohistochemistry (IHC).

RESULTS: Among young women, tumours were frequently ER negative ($P = 0.01$) with lower ER ($P < 0.00$), PR ($P = 0.03$), higher ki67 index (KI) ($P = 0.01$) and p53 ($P = 0.00$) compared with older women. Triple negative was more frequent among young women with frequent lymph node involvement compared with older women. Luminal B among young *vs* old women showed lower ER (67% *vs* 88%), PR (32% *vs* 52%), higher KI (48% *vs* 34%) and p53 (19% *vs* 7%). Linear regression model showed increasing KI ($P < 0.0001$) and p53 ($P = 0.0003$) according to the molecular subtypes. Survival difference among subtypes was demonstrated by multivariate analysis ($P = 0.0092$) after adjusting for age, race, tumour size, grade and stage.

CONCLUSION: We demonstrated significant differences in biomarker expression relative to age and molecular subtypes. Molecular subtype defined by IHC was an independent prognostic factor.

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Breast cancer among young women (<40 years) is a rare disease associated with poor prognosis (Anders *et al*, 2009). Factors such as race, advanced stage, large tumour size and hormone receptor (HR)-negative status have been shown to contribute to poor prognosis in this age group (Bonnier *et al*, 1995; Walker *et al*, 1996; Shavers *et al*, 2003; Carey *et al*, 2006; Anders *et al*, 2009). Recently, gene expression analysis has identified molecular subtypes of invasive breast cancer (IBCA) with distinct biological behaviour and prognosis (Sorlie *et al*, 2001; Sotriou *et al*, 2003). Routine immunohistochemistry (IHC) has been used as a substitute for gene expression with fairly good correlation (Bhargava *et al*, 2009; Cheang *et al*, 2009; Voduc *et al*, 2010). It has been suggested that molecular subtypes of IBCA among young women may be different from those among older women (Carey *et al*, 2006; ihemelangu *et al*, 2007; Lin *et al*, 2009). Tumour proliferation and p53 overexpression have important roles in IBCA prognosis (Chae *et al*, 2009; Yerushalmi *et al*, 2010), however, their relationship to age and molecular subtypes has not been well elucidated. The aims of this study were to determine if there are differences in biomarker expression (oestrogen receptor (ER), progesterone receptor (PR), human epidermal receptor 2 (HER2), Ki67 and p53) and DNA ploidy relative to age ($\leqslant$40 *vs* $\geqslant$50 years) and molecular subtypes of IBCA using digital image analysis. We also evaluated the relationship of the subtypes to disease outcome.

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MATERIALS AND METHODS

We identified 162 consecutive cases of IBCAs among women $\leqslant$40 years. In all, 158 cases had information on tumour biomarkers and DNA ploidy performed between 1999 and 2009 at the UT Southwestern Medical Centre, Dallas. In four cases biomarker were performed at other institutions and not quantified by image analysis and were excluded. A comparison was made with IBCA occurring among women $\geqslant$50 years during the same period. There were 100 cases in this group, of which one case was excluded due to lack of biomarker information. Clinical information, including race, age at diagnosis, clinical stage, pre-surgical chemotherapy, type of surgery, post-surgical therapy and follow-up information, was obtained from the electronic medical records after Institutional Review Board approval. Tumour size, nodal stage, histological subtypes, tumour grade and results of biomarkers (ER, PR, HER2, Ki67 and P53) and DNA ploidy were extracted from the pathology database. These were performed prospectively on excision specimens at the time of diagnosis, as part of the clinical work-up. Histological classification was done according to the World Health Organisation. Tumour was graded according to the modified Nottingham grading system.

Immunohistochemistry and quantitation of biomarker expression

Slides were stained on an automated immunostainer (Dako autostainer, Carpintaria, CA, USA) using ER (clone1D5, 1 : 800), PR (PgR 636, 1 : 1000) and Ki67 (MIB-1, 1 : 300), HER2/neu (1 : 600) and p53 (DO-7, 1 : 220) (Dako autostainer). Scoring and quantification was performed using the computerised Automated Cellular Imaging System (ACIS, Clarient, Inc., San Juan Capistrano, CA, USA). This consists of an automated robotic bright-field microscope module, a computer and a Windows NT-based software interface. Sub-regions were selected from the digital images of the IHC-stained slides for analysis. Positive staining in $\geqslant 5\%$ of the tumour cells for ER and PR in 10 selected sub-regions of the tumour was the cut-off for positive result. The results were reported as percentage of positive staining nuclei and intensity was graded from 1+ to 3+.

Computer-generated results were reviewed by pathologists with experience in image analysis to confirm that the area of invasive tumour was analysed. The computer scores were taken as the final scores. To assess HER2 overexpression, ACIS provided an average score for five selected sub-regions of the tumour with the highest cytoplasmic membrane-staining intensity for HER2. Tumours with $>10\%$ cells with an average score $\geqslant 2.0$ (3+) were reported as positive overexpression, scores between 1.4 and 1.9 as borderline (2+) and <1.4 as negative (0 and 1+). All the positive and borderline results were confirmed by fluorescent *in situ* hybridisation (FISH) using the FDA-approved PathVysion kit (Abbott-Vysis Lab, Abbott Park, IL, USA) according to the manufacturer's protocol. A ratio of the number of HER2 gene signals to chromosome 17 >2.0 was reported as amplified using the FDA criteria. The P53 expression in $\geqslant 10\%$ of the tumour cells was considered as overexpression.

DNA ploidy analysis

DNA analysis was performed by image analysis using Feulgen-stained paraffin sections from the same tumour block. DNA ploidy was analysed using the Autocyte Pathology Workstation (Tripath, Burlington, NC, USA). Briefly, a total of 200–300 nuclei were collected and DNA index was obtained by measuring the optical density of tumour cells in comparison with those of stromal cells in the section, using the latter as the diploid reference (value of 1.0). Tumours were classified as diploid or aneuploid/multiploid.

Molecular classification of IBCA by IHC

The Ki67 index (KI) was used to define luminal A and B tumours according to Cheang *et al* (2009). Tumours were classified as luminal A (ER+/PR±/HER2−, Ki67 $\leqslant 14\%$); luminal B (ER+/PR±/HER2−, Ki67 $>14\%$), luminal-Her2 (ER+/PR±/ HER2+++); HER2+ (ER−/PR−/HER2+++) and triple negative (ER−/PR−/HER2−).

Statistical methods

The Fisher's exact test was used to analyse categorical variables and the two-sample *t*-test for continuous variables. Multivariate analysis was performed using logistic regression model and the general linear model to analyse categorical and continuous variables, respectively, after adjusting for age, race, tumour grade, ploidy, tumour size and T and N stage. Univariate analyses were performed for each of the IBCA subtypes to compare tumour size, grade and lymph node status and biomarker expression in the two groups. Linear regression models and F-tests were used to determine the relationship of ki67 and p53 expression and molecular subtypes.

Disease-free survival (DFS) was defined as time from diagnosis to recurrence or death, whichever occurred first, and was censored at the date of last follow-up for those alive without recurrence. Survival curves were produced using the Kaplan–Meier method and the log-rank test was used to compare survival curves between the two age groups and molecular subtypes. Cox regression analysis was performed to determine if molecular subtypes retained significance after adjusting for T and N stages, race, tumour size, grade and ploidy.

Two-sided *P*-values <0.05 were considered significant. All analyses were conducted using SAS 9.2 software package (SAS Institute Inc., Cary, NC, USA).

RESULTS

A comparison of clinico-pathological variables is shown in Table 1. The mean age at diagnosis was 34.6 years in young and 61.8 years in old women. Young women frequently belonged to minority race. Tumours among young women were significantly larger, of higher grade and with advanced T and N stages compared with older women. There was no significant difference in DNA ploidy in the two groups. Young women were more likely to receive pre-surgical chemotherapy, modified radical and bilateral mastectomies and frequent post-surgical chemotherapy and radiation than older women.

A comparison of biomarkers expression is shown in Table 2. Among young women, tumours were frequently ER negative with lower ER levels compared with older women. There was a trend towards PR negativity and lower PR levels among young women. There was no difference in rates of HER2 overexpression between the two groups. The KI, frequency of p53 overexpression and levels of p53 was significantly higher among young *vs* older women. Of the molecular subtypes, triple negative and HER2 subtypes were more frequent among young compared with older women ($P=0.000$ and 0.015, respectively).

By multivariate analysis, the mean ER ($P=0.040$) and PR ($P=0.031$) levels, tumour grade ($P<0.0001$) and DNA ploidy ($P=0.0021$) were independently associated with age, after controlling for race, T and N stages, tumour size and grade, whereas the KI was not significant ($P=0.469$) and p53 was mildly significant ($P=0.064$). After adjusting for molecular subtypes, the levels of ER ($P=0.009$) and p53 ($P=0.027$) were independently associated with age but PR (0.872) and KI ($P=0.246$) were not. Tumour size ($P=0.048$) and molecular subtypes ($P=0.049$) were independently associated with age.

Table 3 shows the comparison of tumour size, grade and lymph node status and biomarker expression for each of the molecular subtypes according to age groups. In luminal A tumours, the ER level was lower among young compared with older women. Among young women with luminal B subtypes, the ER and PR values were significantly lower with higher KI and p53 compared with those among older women.

Luminal B subtype among young women was predominantly high grade, 28 out of 43 (65.1%) *vs* 9 out of 29 (31.0%) among older women ($P=0.007$). Similarly, triple-negative subtype among young women was predominantly high grade, 54 out of 57 (94.7%) *vs* 17 out of 25 (68%) in older women ($P=0.002$), and was frequently associated with positive lymph nodes, 40 out of 54 (74.1%) *vs* 13 out of 25 (52.0%) compared with the older group ($P=0.072$).

Linear regression model showed a positive trend toward increasing KI with molecular subtypes in both age groups ($P<0.0001$), Figure 1. Similar trend was noted for p53 levels in young ($P<0.0001$) and old women ($P=0.0003$), Figure 2.

Survival analysis

The median DFS was 84 months (95% CI: 84, NA) for old and 107 months (95% CI: 60, 144) for young women. Among young

Table 1 Comparison of clinico-pathological characteristics in young *vs* old women

Variables	⩽40 years	⩾50 years	*P*-value[a]
Age (Mean)	34.6	61.8	<0.00
Race	162 (%)	100 (%)	<0.00
Caucasian	32 (19.8)	46 (46)	
African American	54 (33.3)	36 (36)	
Hispanic	69 (42.6)	16 (16)	
Other	7 (4.32)	2 (2)	
Grade	153	96	<0.00
1	9 (5.9)	11 (11.4)	
2	31 (20.2)	47 (49.0)	
3	113 (73.9)	38 (39.6)	
DNA ploidy	150	98	0.11
Aneuploid	112 (74.7)	64 (65.3)	
Diploid	38 (25.3)	34 (34.7)	
Tumour size (cm)	4.33 ± 3.27	3.30 ± 3.18	0.01
Tumour stage	155	97	0.02
1	37 (23.9)	38 (39.2)	
2	63 (40.7)	34 (35.0)	
3	32 (20.6)	19 (19.6)	
4	23 (14.8)	6 (6.2)	
Nodal stage	147	97	0.00
0	43 (29.2)	48 (49.5)	
1	77 (52.4)	33 (34.0)	
2	10 (6.8)	9 (9.3)	
3	17 (11.6)	7 (7.2)	
Clinical stage	161	100	0.00
I	22 (13)	29 (29)	
II	61 (38)	40 (40)	
III	62 (39)	28 (28)	
IV	16 (10)	3 (3)	
Treatment			
Pre-surgical therapy	151	100	0.03
Yes	56 (37.1)	24 (24)	
No	95 (62.9)	76 (76)	
Surgery	141	100	0.00
Partial mastectomy	53 (37.6)	56 (56)	
Radical mastectomy	57 (40.4)	19 (19)	
Total mastectomy	21 (14.9)	24 (24)	
Bilateral total mastectomy	10 (7.1)	1 (1)	
Post-surgical therapy	134	71	
Chemotherapy	36 (26.9)	31 (43.7)	0.00
Radiation	35 (26.1)	6 (8.4)	
Chemotherapy and radiation	63 (47.0)	34 (47.9)	
Hormonal therapy	134	71	0.00
Yes	70 (52.2)	41 (57.7)	
No	64 (47.8)	30 (42.3)	

[a]*P*-value <0.05 is significant.

Table 2 Comparison of biomarker expression and molecular subtypes among young *vs* old women

Variables	Women ⩽40 (%)	Women ⩾50 (%)	*P*-value
ER status	*n* = 158	*n* = 99	0.01
Positive	79 (50.0)	65 (65.7)	
Negative	79 (50.0)	34 (34.3)	
Level (%)	33.04 ± 41.67	57.87 ± 44.65	<0.00
PR status	*n* = 158	*n* = 99	0.07
Positive	60 (38.0)	49 (49.5)	
Negative	98 (62.0)	50 (50.5)	
Level (%)	18.43 ± 30.49	27.9 ± 37.12	0.03
Her2 status	*n* = 157	*n* = 99	1.00
Positive	35 (22.3)	23 (23.2)	
Negative	122 (77.7)	76 (76.8)	
Amplification level (FISH)	4.89 ± 2.66	4.65 ± 2.91	
KI67	*n* = 149	*n* = 98	0.01
High (>14%)	126 (82.9)	75 (76.5)	
Low (⩽14%)	23 (17.1)	23 (23.5)	
Level (%)	49.58 ± 30.96	40.11 ± 29.78	0.01
P53	*n* = 143	*n* = 98	0.00
Normal (<10%)	79 (55.2)	72 (73.5)	
High (⩾10%)	64 (44.8)	26 (26.5)	
Level (%)	31.34 ± 38.76	17.90 ± 32.16	0.00
Molecular subtypes	*n* = 157	*n* = 99	0.05
Luminal A	18 (11.4)	20 (20.2)	
Luminal B	45 (28.7)	30 (30.3)	
Her2 subtype	21 (13.4)	8 (8.1)	
Luminal-Her2	14 (8.9)	15 (15.2)	
Triple negative	59 (37.6)	26 (26.2)	

Abbreviation: FISH = fluorescent *in situ* hybridisation.

women, there were 54 (74.0%) adverse events *vs* 19 (26.0%) among old women. Young women with triple-negative tumours had 26 out of 32 (81.3%) adverse events *vs* 6 out of 32 (18.7%) among old women ($P = 0.1469$).

Kaplan–Meier survival curves for DFS in young *vs* old women was significant ($P = 0.032$) by log-rank test (Figure 3). The DFS differed significantly by molecular subtypes and univariate log-rank test ($P = 0.0094$) (Figure 4). The median DFS was 144 months (95% CI: 84, 144) for luminal B, 60 months (95% CI: 35, 60) for Lum-Her2 and 63 months (95% CI: 25, NA) for triple negative. The median DFS was not estimable for luminal A and HER2 due to lack of DFS events. The difference in survival among the subtypes was significant ($P = 0.0092$) by multivariate Cox proportional hazard model after adjusting for race, age, tumour size, grade and T and N stage. Tumour size ($P < 0.001$), T ($P = 0.047$) and N stage ($P < 0.001$) were significant predictors of DFS.

DISCUSSION

In this study, young women with IBCA had significantly higher clinical stage at presentation, larger tumour size, poorly differentiated histology and lower frequency of HR-positive status when compared with their older counterpart, similar to previous studies (Walker *et al*, 1996; Colleoni *et al*, 2002; Anders *et al*, 2009). We have demonstrated the utility of digital image analysis for semi-quantification of biomarkers expression applied to routine clinical specimens. The advantage of this technique is the consistency of results with less intra- and inter-observer variability compared with manual estimation (Gokhale *et al*, 2007; Faratian *et al*, 2009; Bolton *et al*, 2010). By semi-quantitative analysis, we have demonstrated for the first time that young women had significantly lower ER and PR levels, higher KI and p53 overexpression compared with older women. On multivariate analyses, tumour size, grade, ER and PR were independently associated with age, but KI was not.

Molecular characterisation of IBCA by gene expression is highly complex and can be performed only in reference laboratories. Routine IHC is simple, economical and several of the biomarkers can be performed simultaneously using the standardised automated techniques. We used IHC as a surrogate tool for gene

Table 3 Biomarker expression values (%) according to the molecular subtypes and age groups

	LUM-A ($n=38$)		LUM-B ($n=75$)		LUM-HER2 ($n=29$)		HER2 ($n=29$)		TRIPLE-NEG ($n=85$)	
Variables	**Mean**	**P^a**	**Mean**	**P^a**	**Mean**	**P^a**	**Mean**	**P^a**	**Mean**	**P^a**
Ki67 (%)										
⩾50 years	7.1 ± 3.36	0.07	33.8 ± 13.9	0.00	41.7 ± 25.2	0.43	58.0 ± 26.8	0.56	67.5 ± 30.2	0.05
⩽40 years	9.1 ± 3.66		48.4 ± 24.2		50.8 ± 33.4		51.1 ± 29.1		62.7 ± 30.3	
P53 (%)										
⩾50 years	2.2 ± 3.63	0.02	6.9 ± 11.6	0.03	36.3 ± 39.4	0.96	23.1 ± 42.9	0.35	31.0 ± 42.5	0.22
⩽40 years	7.3 ± 16.9		18.5 ± 29.8		37.0 ± 44.5		39.5 ± 41.3		43.5 ± 42.0	
ER (%)										
⩾50 years	92.5 ± 9.32	0.02	87.5 ± 17.9	0.00	83.5 ± 28.1	0.97	NA		NA	
⩽40 years	76.6 ± 26.2		66.5 ± 35.6		83.2 ± 19.3		NA		NA	
PR (%)										
⩾50 years	40.4 ± 36.8	0.49	52.3 ± 40.3	0.01	25.8 ± 32.7	0.38	NA		NA	
⩽40 years	48.9 ± 38.7		31.7 ± 32.3		37.5 ± 34.6		NA		NA	

NA = not applicable. [a]*P*-value of the two-sample *t*-test.

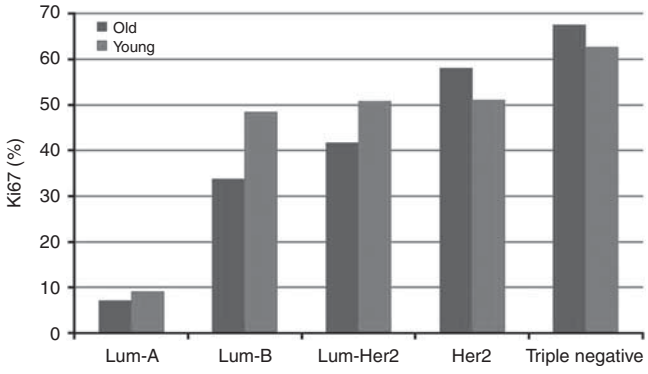

Figure 1 Relationship between KI and molecular subtypes of IBCA in young *vs* old women ($P<0.0001$).

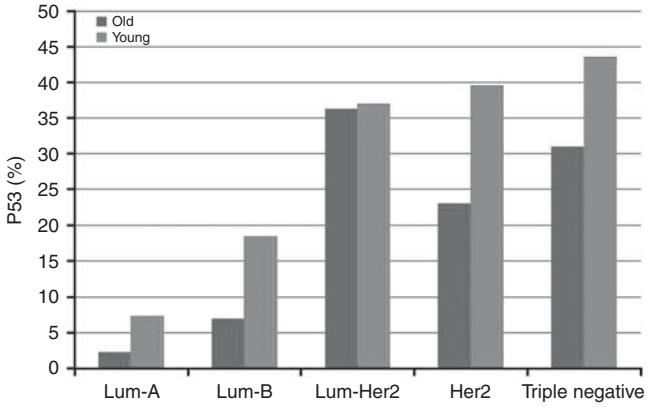

Figure 2 Relationship between p53 expression and molecular subtypes of IBCA in young and old women ($P<0.0001$ and <0.0003, respectively).

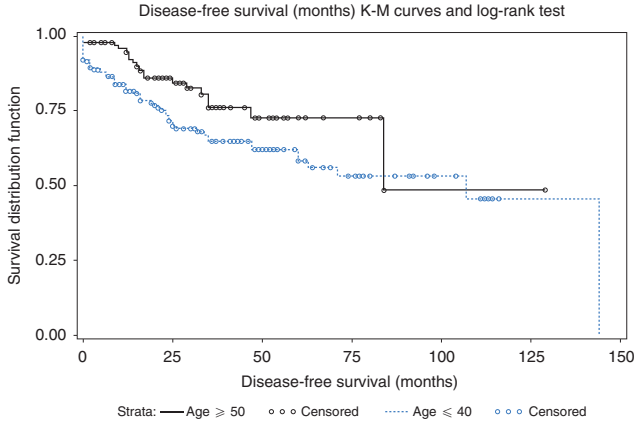

Figure 3 Kaplan–Meier survival curves for DFS in young *vs* old women ($P=0.032$) by log-rank test.

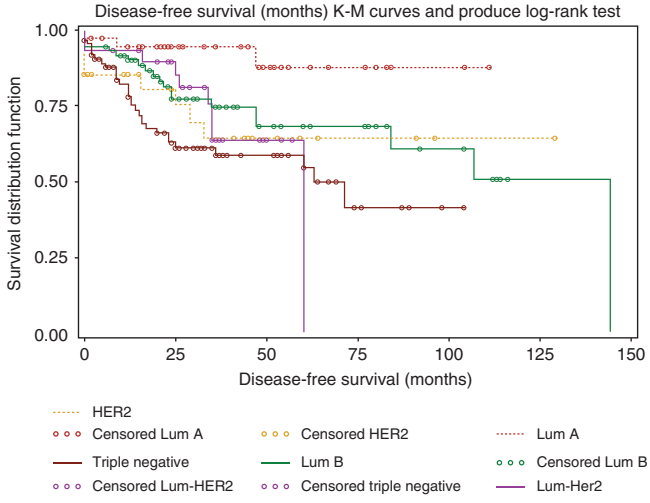

Figure 4 Kaplan–Meier survival curves for DFS in the molecular subtypes of IBCA; univariate log-rank test ($P=0.0094$) and multivariate Cox proportional hazard model ($P=0.0092$).

expression. We observed that molecular subtypes were independently associated with age by multivariate analysis. Triple-negative tumours comprised 38% among young women *vs* 26% in old group. The higher prevalence of triple-negative tumours is most likely due to the predominance of Hispanics and African American women in our population. Triple-negative tumours occur more frequently in young Black and Hispanic women than in young women of other racial groups (Carey *et al*, 2006; Foulkes *et al*, 2010). In the Carolina Breast Cancer study, the prevalence of basal phenotype in premenopausal African American women was 39% compared with only 16% in other racial groups (Carey *et al*, 2006). It has been suggested that the interaction of societal and genetic

factors may put Black and Hispanic women at increased risk for triple-negative breast cancer (Foulkes *et al*, 2010). It may be argued that triple negative is not synonymous with basal phenotype. However, the majority of triple-negative tumours express basal phenotypes and both have similar clinical behaviour (Foulkes *et al*, 2010), although their molecular heterogeneity has been highlighted (Rakha *et al*, 2009). In our study, triple-negative tumours were poorly differentiated with high KI and p53 and frequently associated with positive lymph nodes compared with other subtypes. Previous observations reported lower incidence of positive lymph node, suggesting that this subtype has a higher propensity to spread by hematogenous route (Fulford *et al*, 2007; Crabb *et al*, 2008; Foulkes *et al*, 2010). Furthermore, triple-negative tumours among young women, when compared with those of older women, were predominantly poorly differentiated with higher frequency of positive lymph nodes. Genetic factors, such as BRCA mutation, have important role in young women with IBCA (Robson *et al*, 1998; Greenblatt *et al*, 2001). Triple-negative phenotype, loss of p53 function and high ki67 are the hallmark of BRCA-associated IBCA (Greenblatt *et al*, 2001). In our study, genetic testing was not consistently performed in all young patients and BRCA mutation was positive in 10 out of the 19 young women that were tested (data not shown).

In our study, luminal B subtype was more prevalent (66%) than luminal A (34%). The mean KI for both age groups was $<10\%$ in luminal A and was 48% and 34% among young and old women in luminal B, respectively. Previous studies have shown luminal A to be more common than luminal B, however, proliferation was not evaluated and it is possible that luminal tumours with high proliferation may have been included in the luminal A category (Carey *et al*, 2006; Ihemeladanu *et al*, 2007). A very recent study showed luminal B to be more prevalent (77%) than luminal A (23%), using proliferation criteria (Cancello *et al*, 2010). Studies have shown that only 30% of luminal B tumours overexpress HER2 (Carey *et al*, 2006; Cheang *et al*, 2009), therefore in the past, the majority of luminal B tumours were inappropriately classified as luminal A. In our study, HER2 overexpression was seen in 28% of all the luminal B tumours. Luminal B tumours with HER2 overexpression are distinct from those without HER2 overexpression (Cheang *et al*, 2009).

The importance of proliferation in luminal tumours has been shown recently. The Oncotype Dx assay provides prognostic information in patients with HR-positive breast cancer (Paik *et al*, 2004). The recurrence score is heavily weighted on proliferation besides ER and PR expression (Paik *et al*, 2004). The MK167 gene that encodes the ki67 protein is highly expressed in luminal B tumours (Perou *et al*, 1999; Paik *et al*, 2004; Cheang *et al*, 2009). The KI in luminal tumours correlated with survival, and the 10-year survival for luminal A, B and luminal-her2 was 92%, 79% and 78%, respectively (Cheang *et al*, 2009). In our study, women with luminal B subtype had inferior survival compared with luminal A. It has been shown that women with luminal B tumours were less responsive to anti-hormonal therapy with significantly higher rates of recurrence and regional relapse compared with women with luminal A tumours (Cheang *et al*, 2009; Voduc *et al*, 2010).

An interesting observation not documented previously is that luminal B tumours among young women, when compared with the older group, demonstrated more aggressive features, such as significantly higher histological grade, KI, p53 and significantly lower levels of ER and PR expression. Recently, it was shown that very young women with luminal B breast cancer had worse outcome compared with older women with similar subtype (Cancello *et al*, 2010) and were more likely to have endocrine unresponsive disease with inferior prognosis compared with older women (Aebi *et al*, 2000; Colleoni *et al*, 2002; Ahn *et al*, 2007; Haffty and Buchholz, 2010). The high proliferation and p53 level, coupled with low ER and PR expression, suggests that these tumours may originate from less-differentiated luminal cells.

There are conflicting reports regarding incidence of Her2 overexpression in young women. Some reported higher incidence in young women (Agrup *et al*, 2002; Hartley *et al*, 2006; Anders *et al*, 2008), whereas others showed no difference in the two age groups (Colleoni *et al*, 2002; Maru *et al*, 2005). This reflects the different methodologies and criteria used to determine HER2 status. In this study, the overall rate of HER2 overexpression by FISH assay was similar in the two age groups, although the HER2 subtype was more common among young women.

We have demonstrated for the first time the significant relationship between KI and p53 and molecular subtypes of IBCA in both age groups. Differences in proliferation and p53 among the subtypes have been shown by gene expression (Perou *et al*, 1999; Sorlie *et al*, 2001). Others have reported only a modest association between proliferation and molecular subtypes using tissue microarray (Bhargava *et al*, 2009). This may be due to underestimation of proliferation because of sampling error. Assessment of KI by automated analysis on whole-tumour section provides a more accurate estimation of the proliferation index.

We found significant differences in DFS among the subtypes by univariate and multivariate analysis and this was independent of N and T stage, tumour size and age, similar to previous other studies (Carey *et al*, 2006; Sorlie *et al*, 2001).

This retrospective study has several limitations. Breast cancer in young women has strong genetic influence and risk factors such as family history, parity and genetics were not consistently evaluated in this study.

The classification of IBCA using IHC does not reflect all the molecular subtypes defined by gene expression. However, this study demonstrated that a basic panel of ER, PR, HER2 and ki67 could identify distinct subtypes with different survival outcomes. More importantly, these were performed prospectively by image analysis in a real world fashion and cases were not pre-selected based on tumour size and availability of tissue samples.

CONCLUSION

We demonstrated significant quantitative differences in biomarker expression relative to age and molecular subtypes. The KI served as a useful parameter for identifying luminal tumours with high proliferation that showed a worse outcome compared with those with low KI. Triple-negative and luminal B subtypes among young women appear to show more aggressive features than those among older women. Molecular subtype defined by IHC was an independent prognostic factor.

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