

# Triple-negative breast cancer: clinicopathological characteristics and relationship with basal-like breast cancer

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**Triple-negative breast cancer, defined as that with negative expression of estrogen and progesterone receptors and *cerbB2*, accounted for 11% of invasive breast cancers in our study, drawn from an original cohort of 7048 women diagnosed with breast cancer from the files of the Department of Pathology, Singapore General Hospital, over 14 years. Women with triple-negative breast cancer were generally postmenopausal, with adverse pathological characteristics of high histological grade and frequent nodal metastases. Using a set of 61 invasive breast cancers earlier profiled into molecular subtypes with expression arrays, we defined specificity and sensitivity values for different immunohistochemical panels of basal keratins (CK5/6, CK14, CK17, 34 $\beta$ E12), CD117, EGFR, p63 and SMA in defining basal-like breast cancer. Subsequent application of a tri-panel of CK14, EGFR and 34 $\beta$ E12 (specificity 100% and sensitivity 78%) to our group of 653 triple-negative breast cancers delineated 84% to be basal-like. Immunohistochemical expression of individual biological markers correlated with unfavorable pathological parameters. We conclude that triple-negative breast cancers in an Asian population harbor adverse pathobiological features, and an immunohistochemical surrogate panel can be reliably used to define basal-like cancers among them.**

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Breast cancer is a heterogeneous disease, which can be characterized into clinically, morphologically and biologically meaningful groups. There has been recent intense interest in the subset of breast cancer referred to as triple-negative breast cancer that lacks the expression of hormone receptors and HER2 (*cerbB2*). Triple-negative breast cancer has more aggressive clinical behavior,<sup>1–4</sup> distinctive metastatic patterns<sup>5,6</sup> and poor prognosis.<sup>7,8</sup> Attention on triple-negative breast cancer also relates to the lack of tailored therapies for this group of breast cancer patients, and its overlap with descriptions of basal-type breast cancer, as many triple-negative breast

cancers manifest basal characteristics in their molecular profiles. Triple-negative breast cancer accounts for 10–17% of all breast carcinomas depending on thresholds used to define estrogen receptor (ER) and progesterone receptor (PR) positivity, as well as methods and criteria for HER2 assessment.<sup>8–15</sup> Both triple-negative and basal-like breast cancers tend to be of high histological grade with limited therapeutic options, and a significant overlap in their biological and clinical characteristics is repeatedly shown by molecular techniques.<sup>2,7,16–18</sup>

The advent of tissue microarrays and high-throughput pathology has placed demands on the quality, reproducibility and accuracy of immunohistochemical assays, many of which have been used to address and develop immunohistochemical surrogates for tumors interrogated through molecular profiling.<sup>19</sup> It is understandable that immunohistochemistry may be a more appropriate and

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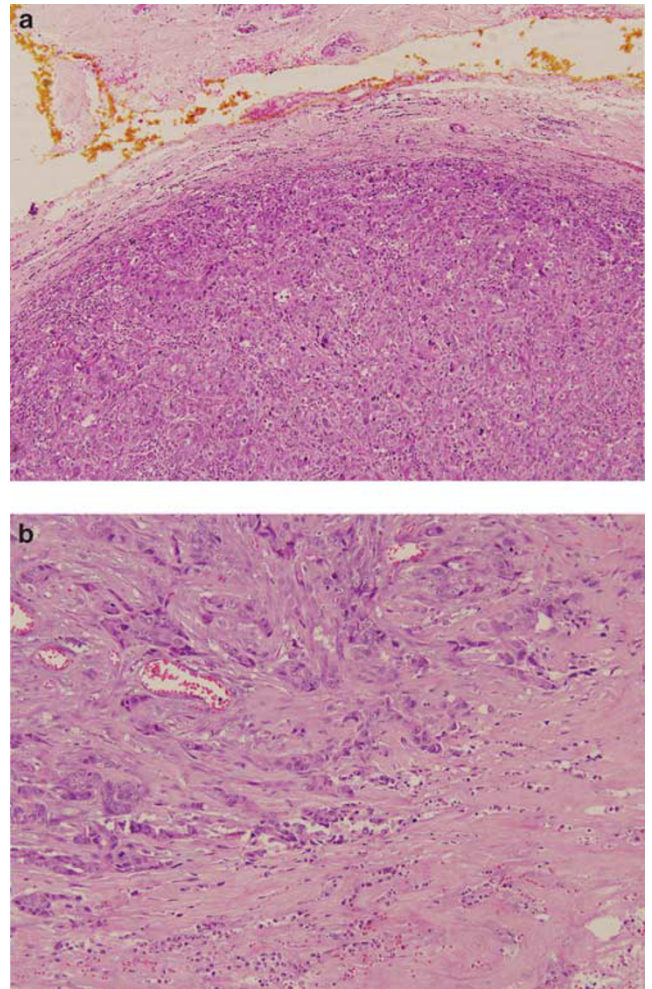
convenient technique in the clinical setting than gene expression-based platforms, in view of cost and equipment availability issues of the latter in routine service laboratories. To date, the true relationship between triple-negative breast cancer and those showing basal-like expression profiles or basal-type proteins (basal-like cancers) is not completely enunciated. There have been reports lumping triple-negative breast cancer with basal-like breast cancers. Yet, there are studies that reveal that not all triple-negative breast cancers express basal characteristics, and hence these two groups of breast cancers are not synonymous.<sup>20</sup>

Our study aimed to pathologically illustrate triple-negative breast cancer in our population, and subject these cancers to a broad panel of biomarkers, including those of a basal nature, to better comprehend the relationship between triple-negative and basal-like breast cancers in our women. In addition, we compared basal protein expression in a subset of molecularly characterized basaloid breast cancers on the basis of expression profiling.

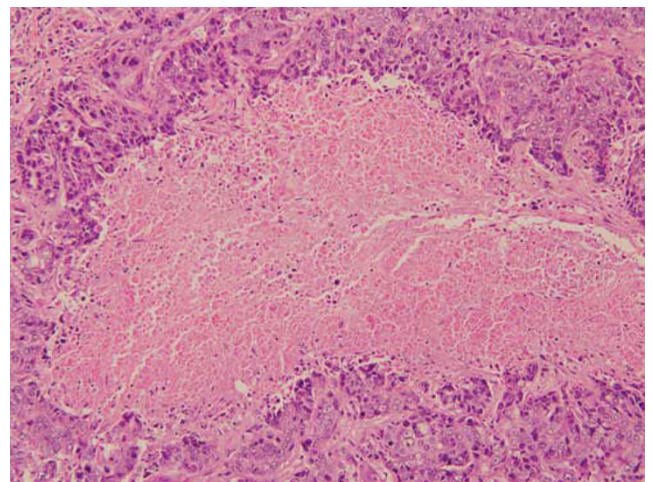
## Materials and methods

### Patients and Tumors

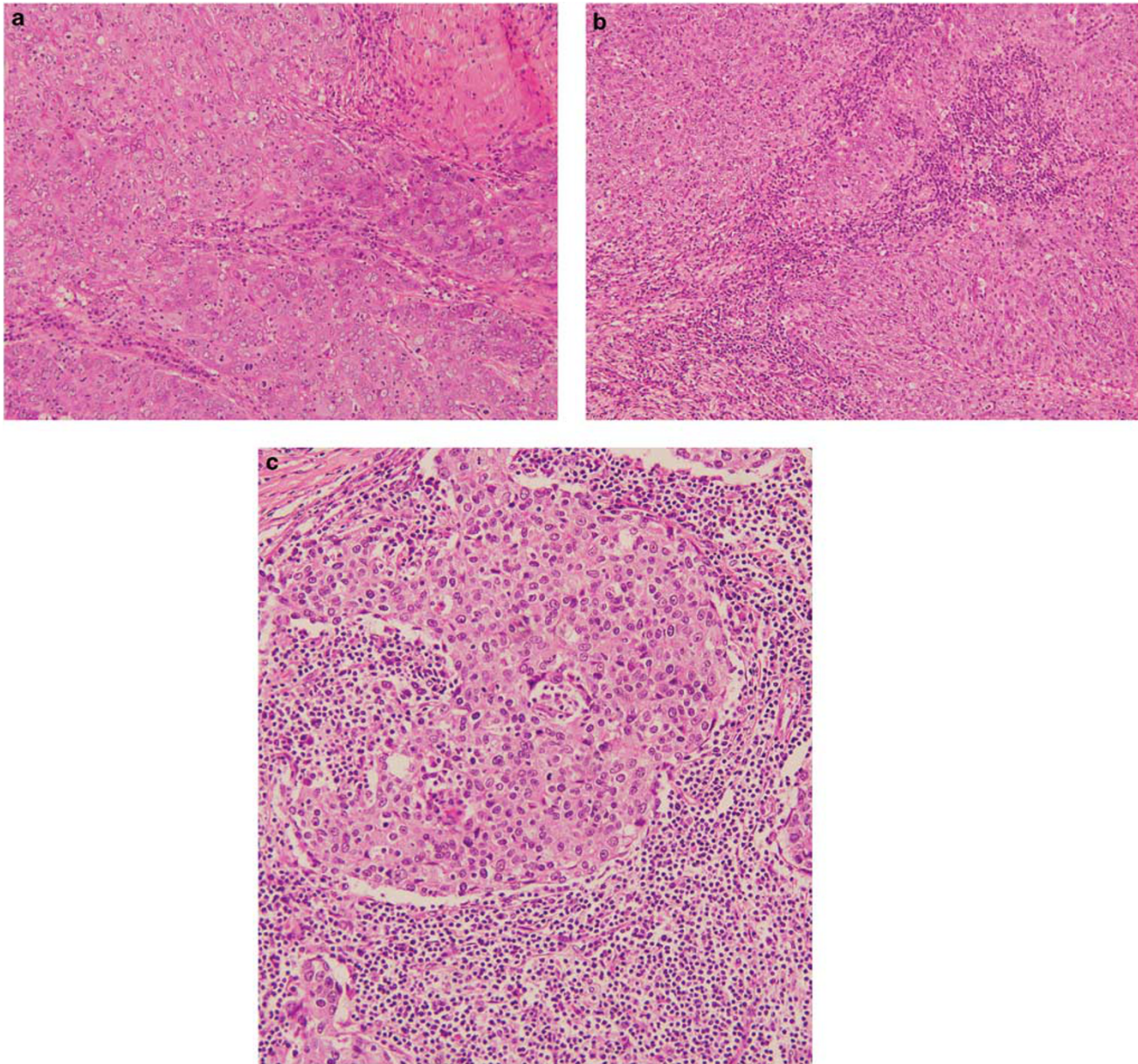
Of the 7048 women diagnosed with primary breast cancer between 1994 and 2007 at the Department of Pathology, Singapore General Hospital, 767 invasive tumors were triple negative according to archival reports that documented ER, PR and *cerbB2* negativity. Clinicopathological parameters including age, ethnicity, tumor size, histological grade, nuclear pleomorphism, mitotic score, tubule formation/score, histological subtype, associated ductal carcinoma *in situ*, lymphovascular invasion and nodal status were evaluated. Histological grade, nuclear pleomorphism, mitotic score, tubule formation and histological subtype were assessed in accordance with standard guidelines.<sup>21</sup> Ductal carcinoma *in situ* was classified according to nuclear grade. Tumor margin, necrosis, degree of lymphocytic infiltrates and growth pattern were reviewed. Tumor margin was considered infiltrating when there were tongue-like protrusions of cancer cells into the surrounding stroma, beyond the general contours of the tumor. A pushing margin was one with generally rounded peripheries (Figure 1). Necrosis was assessed as present or absent (Figure 2). Lymphocytic infiltrates were stratified into mild, moderate and marked, and assessed on the basis of the quantum of lymphocytes present in the tumor (Figure 3). When less than a third of the tumor incorporated lymphocytic infiltrates, a third to two-thirds of the tumor included lymphocytes and more than two-thirds of the tumor harbored lymphocytic accompaniment, they corresponded to mild, moderate and marked lymphocytic infiltrates, respectively. Trabecular growth inferred groups of tumor cells arranged in bars and beams, whereas syncytial growth was defined by tumor



**Figure 1** (a) Pushing or rounded margin at which the tumor edge showed a circumscribed boundary. (b) Infiltrative margin at which there were permeative tumor tongues at the advancing tumor front.



**Figure 2** Necrosis observed within the tumor.



**Figure 3** (a) Mild lymphocytic infiltrates with  $<1/3$  of the tumor area revealing lymphoplasmacytic collections. (b) Moderate lymphocytic infiltrates showing just over  $1/3$  of the tumor harboring lymphocytes and plasma cells. (c) Marked lymphocytic infiltrates with  $>2/3$  of the tumor area obscured by lymphocytes and plasma cells.

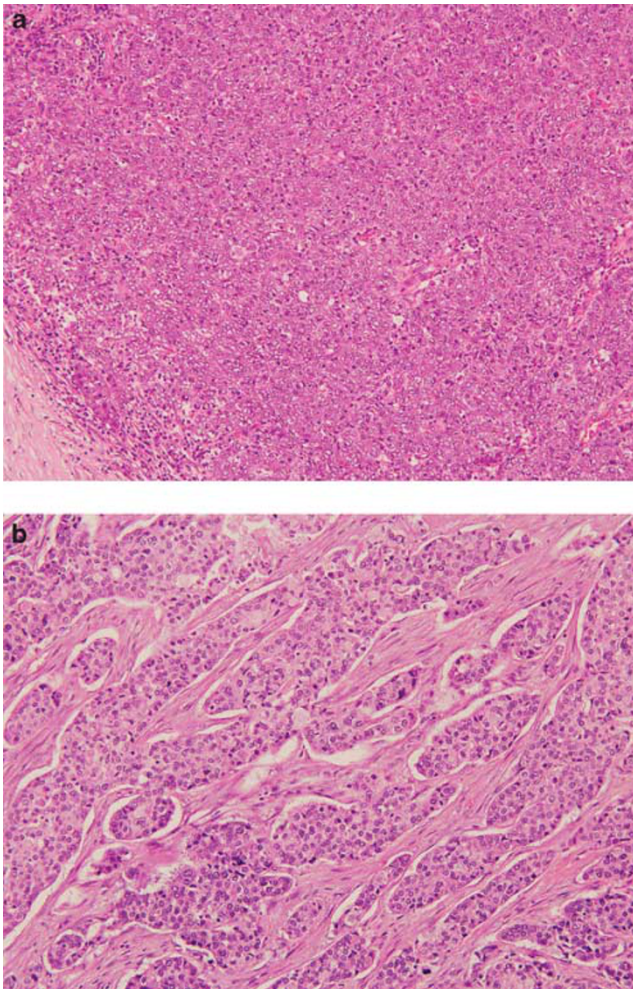
cells intimately apposed and nestled against one another without distinct cytoplasmic membranes, forming a large syncytium (Figure 4).

#### Tissue Microarrays and Immunohistochemistry

Histological slides were retrieved and reviewed, and representative areas were selected for tissue microarray construction, using the Beecher microarrayer with 1 mm cores and two cores per case. Of the 767 triple-negative breast cancers from our archival files, 40 tumors were diagnosed on core biopsies and in 48 cases, suitable archival paraffin blocks were not

available for tissue microarray construction. Finally, a total of 679 triple-negative tumors were subjected to tissue microarray construction.

For immunohistochemistry,  $4\mu\text{m}$  sections were cut from tissue microarray blocks and fished onto coated slides (POLYSINE, Menzel-glaser) in a similar orientation to facilitate evaluation. Sections were restained with anti-ER, anti-PR and anti-*cerbB2* antibodies using routine protocols. Briefly, paraffin sections of formalin-fixed tissue were stained for ER using Neomarker RM9101-S with 1:50 dilution, PR using Neomarker RM9102-S with 1:200 dilution and *cerbB2* using Neomarker RM9103-S with 1:200 dilution. The sections were pretreated using a



**Figure 4** (a) Syncytial growth pattern with sheets of cells intimately apposed without discernible cell membranes. (b) Trabecular growth pattern with broad but discrete tumor beams.

microwave at 98°C for 12 min in Ventana CCI solution (Ventana Medical Systems, Arizona, USA). The detection system used was Linked Streptavidin Biotin. The tissue microarray cores were reviewed and triple negativity was reconfirmed. For ER and PR, a result was considered positive if at least 10% of tumor cells displayed a minimum of 2+ nuclear staining. For *cerbB2*, a test was considered positive if at least 30% of tumor cells exhibited 3+ cell membrane staining and a borderline/equivocal result was given when at least 10% of tumor cells showed 2+ cytoplasmic membrane staining. The results that failed to fulfill the above criteria were considered negative. Tumors in which any one of the above three markers showed immunoreactivity were excluded from the analysis. Benign breast epithelium served as internal positive controls for ER and PR, whereas a tumor with known *cerbB2* overexpression was used as the positive control for *cerbB2*.

After repeat ER, PR and *cerbB2* immunostaining, 10 tumors showed ER positivity, 2 revealed PR

**Table 1** Details of antibodies and dilutions

Antibody	Clone	Source	Dilution
CK5/6	D5/16 B4	Dako M7237	1:20
CK14	LL002	NovocastraNCL-LL002	1:20
CK17	E3	Dako M7046	1:20
CD117	Ra	Dako A4502	1:200
EGFR	E30	Dako M7239	1:50
p63	7JUL	Novocastra NCL-p63	1:100
SMA	1A4	Dako 0851	1:500
Cytokeratin HMW	34βE12	Dako M0630	1:200

Microwave pretreatment in Tris-EDTA pH 8.7.

positivity, 3 showed both ER and PR positivity, and 11 tumors disclosed *cerbB2*-positive immunore-expression. All these 26 cases were excluded, with a final 653 triple-negative breast cancers forming the basis of this study.

These 653 triple-negative breast cancers were subjected to antibodies to CK5/6, CK14, CK17, EGFR, CD117, p63, SMA and 34βE12, which were applied to sections cut from tissue microarray blocks using routine immunohistochemical protocols. Details of antibodies are shown in Table 1. Cytoplasmic staining for CK5/6, CK14, CK17, SMA, CD117, 34βE12, nuclear reactivity for p63 and cytoplasmic membrane positivity for EGFR were considered positive. Appropriate controls were used. Staining intensity was scored as 0, 1+, 2+ and 3+ (no staining, weak, moderate and strong staining, respectively). The percentage of positively stained tumor cells was assessed as a proportion of the total number of tumor cells present in the section. Immunoreactive score and intensity percentage score were documented. Immunoreactive score was calculated as follows: (3 × % strong staining) + (2 × % moderate staining) + (1 × % weak staining). Intensity percentage score was defined as the product of the maximum immunostaining intensity and the percentage of tumor cells stained.

Cases of invasive lobular carcinoma were confirmed with negative staining for E-cadherin (Clone NCH-38, Dako M3612, dilution 1:30, with microwave antigen retrieval).

### Molecular Subtypes of Breast Cancer

On the basis of an earlier published study on clinical validation of a customized multiple signature microarray for breast cancer molecular subtypes, including the basaloid subtype,<sup>22</sup> we performed immunohistochemical protein validation in 61 cases of invasive ductal breast carcinomas which had been classified on expression profiling. These included 28 cases designated as basaloid, 13 *cerbB2* and 20 luminal phenotypes on expression arrays. Corresponding tumor paraffin blocks of these cases were subjected to tissue microarray construction and immunohistochemistry as described above.

Antibodies to ER, PR, cerbB2, basal markers (CK5/6, CK14, CK17, 34 $\beta$ E12), CD117, EGFR, p63 and SMA were applied to 4  $\mu$ m sections cut from the constructed tissue microarray block and immunohistochemical detection was performed using the Linked Streptavidin Biotin method. Immunoscoring was applied as described.

### Statistical Analysis

Findings were analyzed using statistical software SPSS for Windows, Version 16. The relationship between clinicopathological parameters, immunohistochemical staining intensity, percentage of positively stained tumor cells, immunoreactive score and intensity percentage score of markers was tested using the  $\chi^2$  and Fisher's exact tests. A *P*-value < 0.05 defined statistical significance.

Immunohistochemical reactivity of basal and other biological markers in 61 cases of invasive ductal carcinoma earlier characterized on expression profiling was assessed to determine the distribution of cases into true and false-positive, true and false-negative categories. We calculated percent sensitivity as  $100 \times [\text{true positive}/(\text{true positive} + \text{false negative})]$  and specificity as  $100 \times [\text{true negative}/(\text{true negative} + \text{false positive})]$ . To identify criteria providing optimal discrimination, we plotted receiver-operating characteristic curves. We also estimated areas under the receiver-operating characteristic curves using the method of successive parallelograms.<sup>23</sup> Estimates were considered statistically significant for two-tailed values of *P* < 0.05.

## Results

### Clinicopathological Features

Of 7048 women diagnosed with primary breast cancer between 1994 and 2007 at the Department of Pathology, Singapore General Hospital, 1145 (16%) were ductal carcinoma *in situ*, making the total number of invasive breast cancers 5903 (84%). On the basis of archival pathology reports, 741 of 5903 (13%) cases were triple negative for ER, PR and cerbB2. However, on repeat immunohistochemical evaluation of 679 archivally documented triple-negative breast cancers, 26 cases showed positivity of one or more of these markers. After exclusion of cases that were not retested from the denominator of invasive breast cancers, the proportion of triple-negative breast cancers among our women is finalized as 11%.

Clinicopathological characteristics of 653 triple-negative breast cancers are shown in Table 2. Ninety-eight (15%) patients were 40 years of age or younger. The majority (538/653, 82%) were Chinese. Triple-negative breast cancers in our study cohort were mostly high grade/grade 3 (503 out of 653,

**Table 2** Clinicopathological features of triple-negative breast cancer (*N* = 653)

Clinicopathologic parameters	Number of cases (%)
<i>Age (years) (mean 53, median 52, range 25–89)</i>	
< 40	98 (15%)
40 to 50	193 (30%)
> 50	355 (54%)
Not available	7 (1%)
<i>Ethnicity</i>	
Chinese	538 (82%)
Malay	55 (8%)
Indian	35 (5%)
Others	23 (4%)
Not mentioned	2 (1%)
<i>Tumor size (mm) (mean 29, median 25, range 0.9–200)</i>	
≤ 20	169 (26%)
> 20	460 (70%)
Unknown	24 (4%)
<i>Histologic grade</i>	
1	17 (2%)
2	130 (20%)
3	503 (77%)
Not assessable	3 (1%)
<i>Histologic subtype</i>	
IDC (NOS)	606 (92%)
ILC	15 (2%)
Mixed IDC and ILC	2 (1%)
Papillary carcinoma	2 (1%)
Medullary carcinoma	18 (2%)
Metaplastic carcinoma	9 (1%)
Mucinous carcinoma	1 (1%)
<i>Associated DCIS</i>	
Absent	196 (30%)
Low nuclear grade	9 (1%)
Intermediate nuclear grade	66 (10%)
High nuclear grade	220 (34%)
Not mentioned	162 (25%)
<i>Lymphovascular invasion</i>	
Absent	464 (71%)
Present	189 (29%)
<i>Lymph node status</i>	
No nodal metastasis	320 (49%)
Metastasis in 1 to 3 lymph nodes	129 (20%)
Metastasis in 4 or more lymph nodes	130 (20%)
Not available	74 (11%)
<i>Nuclear pleomorphism</i>	
Mild	3 (1%)
Moderate	227 (35%)
Marked	423 (64%)
<i>Mitotic score (field diameter 0.5 mm)<sup>a</sup></i>	
1	60 (9%)
2	137 (21%)
3	453 (69%)
Not assessable	3 (1%)
<i>Tubule formation/score</i>	
> 75% tubule formation (score 1)	15 (2%)
10% to 75% tubule formation (score 2)	104 (16%)
< 10% tubule formation (score 3)	534 (82%)
<i>Margins</i>	
Infiltrating margin	548 (84%)
Pushing margin	105 (16%)
<i>Necrosis</i>	
Absent	10 (2%)
Present	643 (98%)
<i>Lymphocytic infiltrates</i>	
Mild (< 1/3 tumor area)	296 (45%)
Moderate (1/3 to 2/3 tumor area)	350 (53%)
Marked (> 2/3 tumor area)	7 (2%)
<i>Growth pattern</i>	
Trabecular	290 (44%)
Syncytial	363 (56%)

<sup>a</sup>Among 295 tumors associated with DCIS, 75% (220/295) DCIS were high nuclear grade.

IDC, infiltrative ductal carcinoma; ILC, infiltrative lobular carcinoma; DCIS, ductal carcinoma *in situ*; Mitotic activity 0–6/10HPFs (score 1), 7–13/10HPFs (score 2), ≥ 14/10HPFs (score 3).

77%) and T2 tumors (460 out of 653, 70%). Infiltrative ductal carcinoma was the commonest subtype (606 out of 653, 93%). A total of 295 tumors were associated with ductal carcinoma *in situ*, and of these, 220 (75%) were of high nuclear grade. All 15 cases of infiltrative lobular carcinoma were confirmed with E-cadherin-negative immunohistochemical staining.

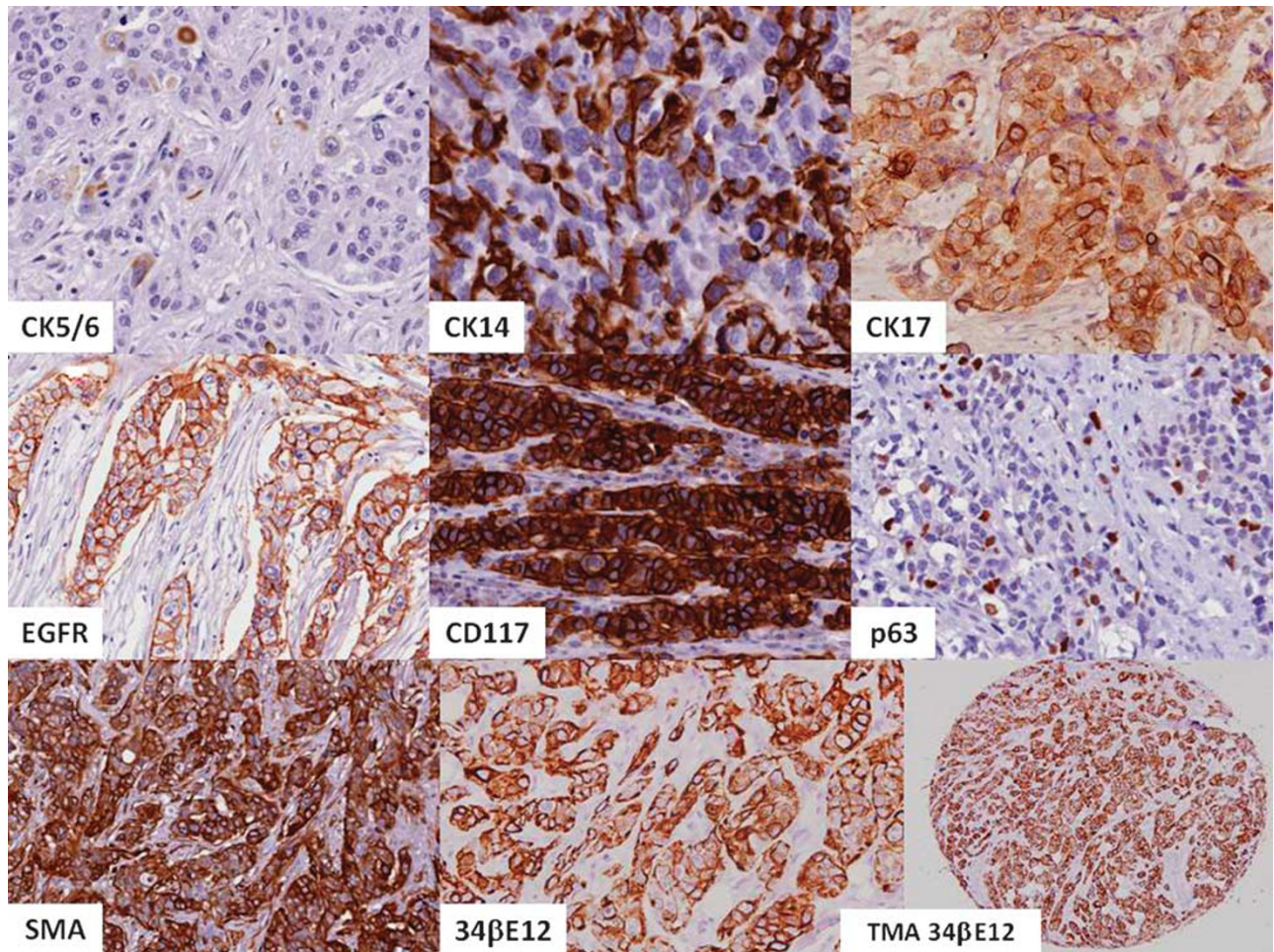
Tumor size of triple-negative breast cancer was significantly associated with ductal carcinoma *in situ*, lymphovascular invasion and nodal status ( $P=0.000$ , 0.021, 0.001). Histological grade was correlated with the subtype ( $P=0.001$ ), whereas lymphovascular invasion was associated with nodal status ( $P=0.000$ ). There were no statistically significant correlations among other clinicopathological parameters.

### Immunohistochemical Findings

Among the basal antibodies, CK5/6 stained the least (38 out of 653, 6%) and 34 $\beta$ E12 the most (460 out of

653, 70%) cases in our series of 653 triple-negative breast cancers (Figure 5). The results are detailed in Table 3.

CK5/6 staining intensity correlated with larger tumors ( $P=0.033$ ) and absence of lymphovascular invasion ( $P=0.034$ ). Percentage of CK14-positive tumor cells was associated with high-grade tumors ( $P=0.05$ ), marked nuclear pleomorphism ( $P=0.044$ ) and a high mitotic score ( $P=0.025$ ). Both CK17 staining intensity and percentage of positive tumor cells correlated with larger tumors ( $P=0.002$ , 0.001), high mitotic scores ( $P=0.034$ , 0.01) and infiltrative margins ( $P=0.009$ , 0.001). The total percentage of CK17-positive tumor cells correlated with high-grade tumors ( $P=0.006$ ), marked nuclear pleomorphism ( $P=0.000$ ) and mild lymphocytic infiltrates ( $P=0.002$ ). EGFR staining intensity correlated with high histological grade ( $P=0.016$ ) and marked nuclear pleomorphism ( $P=0.013$ ) and this also correlated with the total percentage of positive EGFR tumor cells ( $P=0.039$ ). p63 staining intensity correlated with syncytial growth pattern ( $P=0.017$ ) and total percentage of p63-positive cells was



**Figure 5** Immunohistochemical staining for CK5/6 with focal patchy reactivity of tumor cells; CK14, CK17, SMA and 34 $\beta$ E12 showed strong cytoplasmic staining of tumor cells; cytoplasmic and membrane positivity for CD117; cytoplasmic membrane reactivity with EGFR; focal nuclear staining with p63.

**Table 3** Immunohistochemical reactivity of basal and other biological markers in 653 cases of TNBC

Basal marker	Positive expression				Negative expression
	1+	2+	3+	Total	
CK5/6	33 (5%)	5 (1%)	—	38 (6%)	615 (94%)
CK14	55 (8%)	69 (11)	187 (29%)	311 (48%)	342 (52%)
CK17	276 (42%)	30 (5%)	23 (3%)	329 (50%)	324 (50%)
EGFR	159 (24%)	24 (4%)	15 (2%)	198 (30%)	455 (70%)
CD117	238 (36%)	35 (6%)	19 (3%)	293 (45%)	361 (55%)
p63	101 (16%)	27 (4%)	13 (2%)	141 (22%)	512 (78%)
SMA	44 (7%)	24 (4%)	96 (14%)	164 (25%)	489 (75%)
34βE12	236 (36%)	112 (17%)	112 (17%)	460 (70%)	193 (30%)

related to marked nuclear pleomorphism ( $P=0.028$ ). CD117 staining intensity was associated with lymphovascular invasion ( $P=0.014$ ), infiltrative margin ( $P=0.001$ ), presence of necrosis ( $P=0.014$ ) and syncytial growth pattern ( $P=0.029$ ), whereas the total percentage of CD117-positive tumor cells was related to larger tumors ( $P=0.003$ ), lymphovascular invasion ( $P=0.002$ ), high mitotic score ( $P=0.001$ ) and infiltrative margin ( $P=0.03$ ). The proportion of SMA-positive tumor cells correlated with high mitotic score ( $P=0.014$ ). The total percentage of 34βE12-positive tumor cells correlated with marked nuclear pleomorphism ( $P=0.022$ ) and syncytial growth pattern ( $P=0.025$ ), whereas its staining intensity was related to the presence of necrosis ( $P=0.03$ ). No other significant correlations were found.

#### Immunohistochemical Expression Compared against Molecular Subtypes of 61 Invasive Ductal Carcinomas categorized through Expression Profiling

In the group of 61 invasive ductal carcinomas classified through expression profiling, 34βE12 is the most sensitive (sensitivity 70%) in detecting the basal phenotype; however, it showed less specificity (specificity 55%). There was a trend for single markers with high specificity to be accompanied by low sensitivity as shown in Table 4. When a combination of markers was evaluated immunohistochemically, there was an improved diagnostic discrimination between the basal versus *cerbB2* and luminal subtypes categorized on expression arrays. A panel of two protein markers confirmed basal-type breast cancer with higher sensitivity and specificity than did a single marker (Table 5). A high sensitivity and specificity could be shown using a combination of three markers (a triple panel) to confirm basal protein immunophenotypic expression among cases categorized as such on expression profiling (Table 6). When we examined the receiver-operating curves to search for criteria providing optimal discrimination, we found that there was a left shift, as combinations of two or three immuno-

**Table 4** Sensitivity and specificity of immunohistochemistry for individual markers in determining a basal phenotype in 61 infiltrative ductal carcinomas subtyped on expression profiling

Marker	Sensitivity (%)	Specificity (%)	AUC <sup>a</sup>	P-value
CK14	46	88	0.672	0.022
CK17	46	88	0.672	0.022
CD117	61	88	0.771	0.000
EGFR	57	94	0.541	0.582
P63	14	94	0.743	0.001
SMA	29	91	0.597	0.193
34βE12	86	55	0.701	0.007

<sup>a</sup>AUC, area under the ROC curve.

**Table 5** Sensitivity and specificity of immunohistochemistry for a combination of two markers in determining a basal phenotype in 61 infiltrative ductal carcinomas subtyped on expression profiling

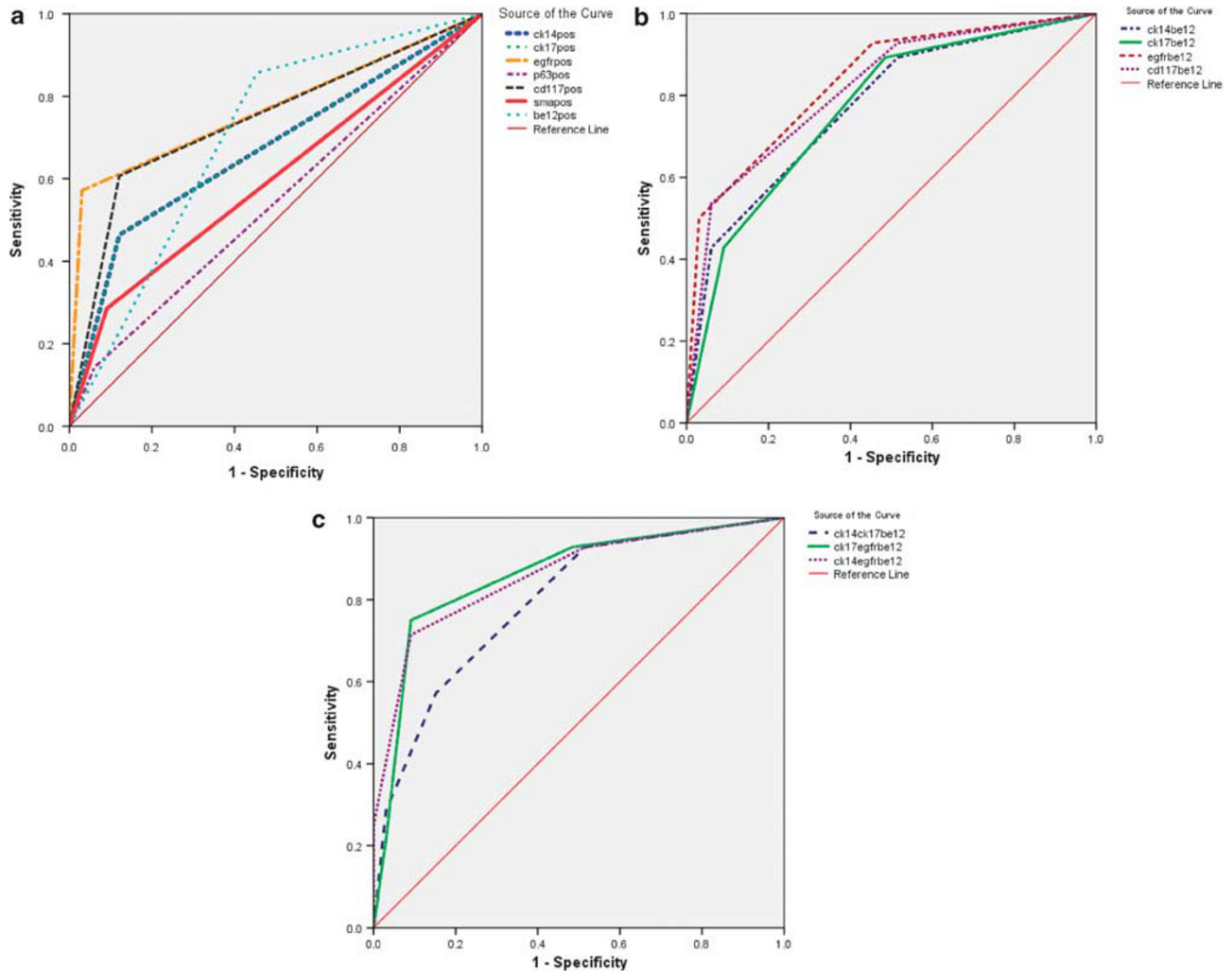
Two marker combination	Sensitivity (%)	Specificity (%)	AUC <sup>a</sup>	P-value
CK14, 34βE12	80	89	0.772	0.000
CK17, 34βE12	80	85	0.767	0.000
EGFR, 34βE12	88	95	0.837	0.000
CD117, 34βE12	88	89	0.817	0.000

<sup>a</sup>AUC, area under the ROC curve.

**Table 6** Sensitivity and specificity of immunohistochemistry for a combination of three markers in determining a basal phenotype in 61 infiltrative ductal carcinomas subtyped on expression profiling

Three marker panel	Sensitivity (%)	Specificity (%)	AUC <sup>a</sup>	P-value
CK14, CK17, EGFR	44	100	0.838	0.000
CK14, CK17, 34βE12	80	94	0.797	0.000
CK14, EGFR, 34βE12	78	100	0.860	0.000
CK17, EGFR, 34βE12	75	94	0.860	0.000

<sup>a</sup>AUC, area under the ROC curve.



**Figure 6** (a) ROC curves for individual immunohistochemical markers. Although they were aligned to the left of the reference line, they remained relatively close to the diagonal reference line. (b) ROC curves for a combination of two immunohistochemical markers (double panel) shifted to the left compared with the curves of the single markers. (c) ROC curves for a combination of three immunohistochemical markers shifted to the left compared with the curves of the single and double immunomarker panels.

**Table 7** Sensitivity and specificity of immunohistochemistry for a combination of four markers in determining a basal phenotype in 61 infiltrative ductal carcinomas subtyped on expression profiling

Four markers panel	Sensitivity (%)	Specificity (%)	AUC <sup>a</sup>	P-value
CK14, CK17, EGFR, p63	86%	73%	0.863	0.000
CK14, CK17, EGFR, CD117	86%	73%	0.863	0.000
CK17, EGFR, p63, CD117	86%	76%	0.859	0.000
CK17, EGFR, p63, SMA	79%	79%	0.813	0.000

<sup>a</sup>AUC, area under the ROC curve.

histochemical markers were used to detect basal phenotype breast cancers as shown in Figures 6a–c, indicating high sensitivity and specificity. This documentation was supported by the area under the receiver-operating characteristic curve values and by significant ‘P-values (Tables 5 and 6). When

four markers were studied in combination, specificity was decreased (Table 7). On the basis of the findings, it would seem that a triple panel of CK14, EGFR and 34βE12 provided the best specificity (100%) with reasonable sensitivity (78%), and applying this panel to our 653 cases, revealed 549 (84%) expressing the basal phenotype.

## Discussion

The incidence of triple-negative breast cancer in our population stands at 11%, which is within the range of 10%–16% generally reported in the literature.<sup>20</sup> There are scant data on triple-negative breast cancer in Asian women. In Japan, the triple-negative subtype accounted for 15.5% of breast cancers derived from surveillance data of the Registration Committee of the Japanese Breast Cancer Society, with a predominance of postmenopausal women



whose cancers were diagnosed at more advanced stages with more frequent nodal metastases.<sup>24</sup> In another Asian cohort of 683 Korean women with breast cancers, triple-negative breast cancer formed 19.9% (136 cases) of these tumors, which seems higher than that reported in other studies.<sup>25</sup> A similar result was obtained with a smaller group of 116 Chinese cases, in which 22 (19%) were triple negative.<sup>26</sup> With regard to the relationship with basal-like breast cancers, an investigation of 793 Japanese patients with breast cancer reported that only 8% were basal like with aggressive biological characteristics and a significantly poorer prognosis.<sup>27</sup> This could be potentially extrapolated to imply that basal-like breast cancers constitute about half of the proportion of triple-negative breast cancers in Japanese women, supporting the premise that these tumors are not synonymous, although the definition and methodology used to delineate the basaloid phenotype will obviously affect the rates. In our study, using a combination of three markers (CK14, EGFR and 34 $\beta$ E12), 84% of our triple-negative breast cancers comprised basal-like tumors, which is at the higher end of the range of 56–84% reported in the literature of triple-negative breast cancers expressing basal cytokeratins and EGFR.<sup>20</sup> The ethnic distribution of women in our study did not differ significantly from that observed in our country, in which breast cancers are diagnosed in 83.7% Chinese, 9.6% Malay and 5% Indian women.<sup>28</sup>

Among our cases, the mean and median age of women harboring triple-negative breast cancer was in the early postmenopausal phase of 52 years, and the incidence of women who were 40 years or less among this cohort was not different from the overall rate (15%) of breast cancer in this age group documented in our population, suggesting that younger women did not represent a significantly higher proportion of those with triple-negative breast cancer.<sup>28</sup> Although this is in keeping with Japanese data that indicated a predominance of postmenopausal women among their triple-negative cases,<sup>24</sup> it differs from that generally reported in which triple negativity affects younger patients under 50 years of age,<sup>20</sup> as well as from data from the Korean study.<sup>25</sup> A recent study that compared triple-negative breast cancers in women from Vietnam and the United States found that Vietnamese cases were significantly smaller tumors, predominantly ductal in phenotype, less often grade 3, with fewer metaplastic and no medullary subtypes, as compared with those diagnosed in the United States.<sup>29</sup>

The pathological characteristics in our series confirmed a morphologically aggressive phenotype, with the majority of cases being grade 3 infiltrative ductal carcinomas that measured >20 mm in size, with frequent histological identification of necrosis. When ductal carcinoma *in situ* was present, it was predominantly of high nuclear grade. Although

many triple-negative breast cancers have been described to have rounded pushing margins, we found that our cases with such an appearance formed a minority of only 16%. Similarly, marked lymphocytic infiltrates were rare (2%), and although a syncytial growth pattern was seen in slightly over half of the cases (56%), a significant number showed trabecular appearances (44%) observed in conventional infiltrating ductal carcinomas. Unusually, a few tumors of special subtypes were encountered among the triple-negative breast cancers, suggesting that triple negativity can occur in all histological subtypes of breast cancer with possible implications on their pathogenesis, progression and prognosis. Unlike Dent *et al*,<sup>9</sup> who found no relationship between the tumor size of triple-negative breast cancer and nodal metastasis, our cases showed an increasing incidence of axillary lymph node metastases with enlarging tumor size.

When comparisons were made between immunohistochemical detection of individual biological markers and pathological parameters, there was in general an association of protein expression with aggressive histological features. This is in keeping with our earlier study in which we found that expression of basal keratins in breast cancers of young women correlated with adverse pathological parameters.<sup>30</sup> Hence, apart from inherent challenges to effective therapies for triple-negative breast cancers that are devoid of currently known therapeutic targets, the expression of basal keratins and other biological markers evaluated in this study may confer added aggressive qualities. The finding of SMA positivity in 25% of our cases is not surprising. SMA, similar to p63, are myoepithelial markers, both of which have been reported to be expressed in some basal-like breast cancers. Their relatively infrequent documentation, however, has argued against a myoepithelial origin of these tumors.<sup>31</sup>

The relationship between triple-negative and basal-like breast cancer has been discussed and debated at length, and there now seems to be some clarification of the earlier confusion that enveloped these two closely related yet different entities.<sup>20,32,33</sup> It is recognized that both triple-negative and basal-like breast cancers share many similar characteristics. Using microarray-based expression analysis, basal-like breast cancers are mostly triple negative, and vice versa. However, it has been found that 15–45% of basal-like breast cancers defined on expression profiling express either ER, PR or *cerbB2*, and only 56–84% of triple-negative breast cancers manifest basal cytokeratins and EGFR.<sup>20</sup> It has been reported that patients with triple-negative breast cancer expressing a basal phenotype had significantly shortened disease-free survival than those devoid of basal expression.<sup>11,12</sup> Interestingly, Fulford *et al*<sup>5</sup> documented a better prognosis in patients whose basal-like cancers possessed a diffuse expression of cytokeratin 14. More recently, Liu *et al*<sup>34</sup> discovered worse disease-free and overall survivals

in a series of 112 triple-negative breast cancers that disclosed positive staining for CK5/6 or CK17.

Although expression profiling represents the gold standard in molecular subclassification of breast cancer, it is not a tool that would be readily available for routine use in many laboratories, taking into consideration its prohibitive cost and technical difficulties in achieving optimal results with paraffinized material. It is thus useful to determine immunohistochemical surrogates that can be applied to standard paraffin sections that could accurately indicate the presence of a basal phenotype among triple-negative breast cancers.

Analyzed against a group of earlier profiled breast cancers using expression microarrays, we obtained a specificity of 100% and a sensitivity of 78% in detecting basal-like cancer, for a triple protein immunohistochemical combination of CK14, EGFR and 34 $\beta$ E12, which exceeds the specificity and sensitivity that could be achieved with dual or four marker panels. Using this immunohistochemical selection, 84% of our 653 triple-negative breast cancers would be considered basal-like. Nielsen *et al*<sup>3</sup> proposed using negative ER and *cerbB2*, with a positive expression of CK5/6 and EGFR, to define basal-like cancers, and reported 100% specificity and 76% sensitivity. Our results with CK5/6, however, were disappointing, as it decorated only 6% of triple-negative breast cancers when evaluated individually. We believe that our recommended panel from this study will be efficacious in both cost and technique, as these antibodies are familiar and routinely used in the majority of laboratories. 34 $\beta$ E12 has the advantage of being a basal cell cocktail of high molecular weight cytokeratins 1, 4, 10 and 14, thereby incorporating several keratin forms into one antibody cocktail. The inclusion of EGFR may potentially allow the exploration of the possibility of being a therapeutic target if overexpressed, and CK14 has already been described as defining a better prognosis when exhibiting a diffuse pattern of staining in basal-like breast cancers.<sup>32</sup>

In summary, our study documents clinicopathological features of 653 triple-negative breast cancers derived from a large cohort of 5903 invasive breast carcinomas diagnosed in our department over 14 years from 1994 to 2007, affirming their generally more adverse histological characteristics. Immunohistochemical expression of basal keratins (CK5/6, CK14, CK17, 34 $\beta$ E12), EGFR, CD117, p63 and SMA was evaluated, and a triple panel of CK14, EGFR and 34 $\beta$ E12 seems to be the most specific and sensitive in defining basal-like breast cancers among triple-negative breast cancers. Although progress continues to be sought and made in the management of these therapeutically challenging breast tumors, understanding their link with basal-like cancers may provide not only clues to their biology but also offer potential targets that can enhance and improve their treatment options.

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## Disclosure/conflict of interest

The authors declare no conflict of interest.

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