Clinicopathologic Significance of Molecular Classification of Breast Cancer: Relation to Nottingham Prognosis Index

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ABSTRACT

Purpose: The aim of this work is to determine the possible relationship between the different profiles of molecular expression of hormone receptors and Her-2/neu receptors to clinical and histopathological known prognostic variables for breast cancer.

Material and Methods: A total of 110 breast carcinoma tumor samples were included. In this study 4 groups or immunohistochemical profiles were defined, based on expression of hormone receptors (estrogen and/or progesterone) and/or Her2/neu (Luminal A, Luminal B, HER2 overexpressing profile, and triple-negative profile). We studied whether there were differences between them regarding clinical and histopathological variables with a known prognostic significance in addition to Nottingham Prognosis Index (NPI).

Results: In this series, 65 cases corresponded to Luminal A (59.1%), 18 cases (16.4%) were Luminal B, in 14 cases (12.7%) HER2 was over-expressed, while 13 cases (11.8%) were of the triple negative subtype. It is worth noting the relationship between the triple negative and HER2 over-expressing immunophenotypes and the high NPI (>3.4) in comparison with the Luminal A and Luminal B immunophenotypes (p=0.029). The association of the former two types with higher tumor grade was also observed, but such association did not reach statistical significance.

Conclusion: The subgroups without hormone receptor expression, with Her2/neu overexpression or without (triple-negative group), have characteristics associated with variables of a poorer prognosis.

Key Words: Breast cancer – Hormone receptors – Her2/neu – Classification – Nottingham prognosis index.

INTRODUCTION

Over the last few decades, there have been outstanding advances in breast cancer manage-
Recent attention has been directed singularly at molecular classifications of breast cancer. While molecular and genetic testing is very elegant, prognostic and predictive, it is expensive and not yet widely available. Also, despite the prognostic information provided by the molecular test, current reports of assay results impart little specific guidance of response to targeted and proven therapy; for example, endocrine and trastuzumab therapy for tumors expressing estrogen receptor/progesterone receptor (ER/PR) or human epidermal growth factor receptor 2 (HER2) proteins, respectively [5,6].

Immunohistochemical (IHC) classification provides both therapeutic and prognostic information. Studies using immunohistochemical techniques are more accessible to clinical practice and, since they reveal the expression of certain proteins in tumor cells, could be considered a valid reflection of applicable molecular biology studies. In fact, it has proved useful to classify breast cancer patients according to the expression and overexpression of certain molecules, mainly hormone receptors and receptor HER2 [7].

The aim of this work is to determine whether molecular classification using immunohistochemistry, based on the expression of hormone receptors and overexpression of Her2/neu, coincide with clinical, and histopathological variables of known prognostic value in addition to the NPI.

**MATERIAL AND METHODS**

One hundred and ten female patients aged between 32 and 75 years, diagnosed as invasive breast cancer and treated by modified radical mastectomy or with wide local excision and axillary clearance, were included in this study. The cases included only the invasive ductal and lobular carcinomas that were not otherwise specified, with 94 cases (85.5%) for the former and 16 cases (14.5%) for the latter. Data were retrieved from the Pathology Department at Misr University for Science and Technology Hospital and Ain Shams University Hospitals, in addition to the consultation files of the third author. All the cases received during a three-year period, between January 2007 and December 2009, and had their routine immunohistochemical profile for ER, PR, and Her2/neu available were selected.

Nottingham prognostic index (NPI) was calculated for each case as follows [3]:

\[
\text{NPI} = (\text{Tumor size} \times 0.2) + \text{grade} + \text{lymph node score}
\]

Lymph node score was calculated as:

- 0 involved lymph node was given score 1.
- 1-3 involved lymph nodes was given score 2.
- More than 3 involved lymph nodes was given score 3.

Patients were then grouped into two groups according to their NPI as:

- NPI 2-3.4, excellent to good prognosis.
- NPI >3.4, moderate to poor prognosis.

Estrogen receptor (ER), progesterone receptor (PR) and Human epidermal growth factor 2 receptor (Her2/neu) status was determined using IHC and/or chromogen in situ hybridization (CISH) for cases with Her2/neu +2 score by IHC on the initial diagnostic material. The results were available from the original pathology reports. In brief, the IHC for ER, PR, and HER2 was performed on the formalin-fixed, paraffin-embedded tissue slides. The established protocols, the ready-to-use ER, PR antibodies and EGFR pharm Dx kit for HER2 staining (Dako Corporation, Carpentaria, CA) were used. In our institutions, ER and PR results are reported as positive when more than 10% of neoplastic cells are positive (Fig. 1A,B). HER2 slides were reviewed and HER2 was considered positive with either 3+ immunoreactivity [diffuse strong reactivity in >30% of the tumor cells] (Fig. 1C) or amplification by CISH (defined as 10 dots, or large clusters, or mixture of multiple dots and large clusters of the HER2 gene present per nucleus in 50% tumor using SPOT-Light HER2 CISH kit- Invitrogen/Zymed-CA-USA). All 2+ cases by immunohistochemistry were followed by CISH. This is in accordance with the recently published College of American Pathologists/American Society for Clinical Oncology guidelines for HER2 testing [8]. Equivocal CISH result was considered as negative for HER2 in this study.

Tumors were grouped according to their ER, PR and HER2 status into four subtypes according to the previously published studies [9-11]:

- Luminal A subtype (ER+ and/or PR+, and HER2–).
- Luminal B subtype (ER+ and/or PR+, and HER2+).
HER2+ over expressing subtype (HER2+/ ER– PR–).

• Triple negative phenotype "TNP" (ER– PR– HER2–).

Each of these subtypes was evaluated according to patient age, tumor size, tumor grade, number of involved lymph nodes and lastly according to NPI.

Statistical analysis: All statistical analyses were carried out using the SPSS statistical software package (V. 18, PASW IBM Corp., USA, 2010). Differences between these breast cancer molecular subtypes with regard to clinicopathologic characteristics and NPI were examined using either the Chi-square tests or Fisher's exact test as appropriate. \( p \)-value <0.05 was considered significant.

RESULTS

Among the studied 110 cases, 65 cases were Luminal A, 18 cases Luminal B, 14 cases HER2 over expressing and 13 cases of triple negative subtype, representing 59.1%, 16.4%, 12.7%, and 11.8%, respectively.

Upon relating the four subtypes to the tumor size, a significant difference was revealed (Table 1, Fig. 2). Luminal B and HER2 over expressing tumors presented with larger tumor size relative to Luminal A tumors (72.2%, 78.6 Vs. 44.6%).

The relationship of the IHC molecular subtypes to the tumor grade was explored. Although the difference between the four subtypes did not reach statistical significance, yet both the triple negative and HER2 over expressing subtypes were represented only among grade II and III tumors, while Luminal A and Luminal B groups were seen among grade I tumors as well (Table 2).

The association between the four IHC subtypes and the number of involved lymph nodes was investigated. It was noted that the percentage of the tumors with involvement of more than three lymph nodes was highest among the triple negative tumors (61.5%) compared to 46.2%, 38.9%, and 35.7% among Luminal A, Luminal B, and HER2 overexpressing tumors, respectively (Table 3).

The association between the four IHC subtypes and the Nottingham prognostic index NPI was explored. The difference among the four subtypes regarding NPI was not statistically significant (Table 4). Yet, on gathering the two Luminal subtypes (Luminal A and Luminal B) and comparing them to the two remaining subtypes also in congregate, a statistical significant difference was noted with the HER2 overexpressing and TNP subtypes having higher NPI (Table 5, Fig. 3).

Lastly, when the four IHC subtypes were related to the patient age, no significant difference as regards the predilection towards any age group was demonstrated (\( p=0.764 \)) (Table 6).

Fig. (1): Microscopic images corresponding to positive results with immunohistochemical stains in breast carcinoma cases. A: Estrogen receptors (X200). B: Progesterone receptors (X200). C: HER2/neu receptors (X400).
Table (1): Relationship between the four IHC subtypes of breast cancer and tumor size.

<table>
<thead>
<tr>
<th>IHC subtype</th>
<th>N (%)</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>(n=65)</td>
<td>1-3 cm: 36 (55.4%)</td>
</tr>
<tr>
<td>Luminal B</td>
<td>(n=18)</td>
<td>5 (27.8%)</td>
</tr>
<tr>
<td>HER2 overexpressing</td>
<td>(n=14)</td>
<td>3 (21.4%)</td>
</tr>
<tr>
<td>TNP</td>
<td>(n=13)</td>
<td>5 (38.5%)</td>
</tr>
</tbody>
</table>

$X^2 = 8.364, \quad p = 0.039.$

Table (2): Relationship between the four IHC subtypes of breast cancer and tumor grade.

<table>
<thead>
<tr>
<th>IHC subtype</th>
<th>N (%)</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>(n=65)</td>
<td>I: 5 (7.7%)</td>
</tr>
<tr>
<td>Luminal B</td>
<td>(n=18)</td>
<td>1 (5.6%)</td>
</tr>
<tr>
<td>HER2 overexpressing</td>
<td>(n=14)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>TNP</td>
<td>(n=13)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Fisher’s exact test, $p=0.238$.

Table (3): Relationship between the four IHC subtypes of breast cancer and the number of the involved lymph nodes.

<table>
<thead>
<tr>
<th>No of involved LN</th>
<th>IHC subtype</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>(n=65)</td>
<td>0-3: 35 (53.8%)</td>
</tr>
<tr>
<td>Luminal B</td>
<td>(n=18)</td>
<td>11 (61.1%)</td>
</tr>
<tr>
<td>HER2 overexpressing</td>
<td>(n=14)</td>
<td>9 (64.3%)</td>
</tr>
<tr>
<td>TNP</td>
<td>(n=13)</td>
<td>5 (38.5%)</td>
</tr>
</tbody>
</table>

$X^2 = 2.218, \quad p = 0.528.$
DISCUSSION

Breast cancer is a heterogeneous disease whose traditional prognosis assessment has been based on clinical and histopathological parameters. Staging of cases has mainly been based on the size of the tumor and lymph node affection. However, although this traditional assessment facilitates the comparison of series and different treatments, morphological classifications are not able to evaluate the biological behavior of the different tumors with sufficient preciseness. TNM classification enables patients to be grouped together in different stages representing differing probabilities of recurrence. It is therefore possible for TNM classification to establish a prognosis and help indicate adjuvant therapies after surgery [12]. But this is especially true in very early stages (0 to I), with recurrence in less than 5% of cases, and in advanced stages (III), with recurrence in approximately 80% of cases within 5 years, and in those cases in which the indication or non-indication of chemotherapy and/or radiotherapy is clearer. Unfortunately, in the intermediate stages, the lack of more specific prognostic indicators can lead to under-or overtreatment, with associated morbidity in the latter case [11].

Gene expression analysis has demonstrated distinct classes of breast carcinomas based on the degree of expression of a selected number of genes. However, cost and complexity issues have rendered gene expression profiling impractical as a routine hospital diagnostic tool [13].

The purpose of the present study was to discover whether immunohistochemical techniques, which are much more simple to perform and interpret in daily practice than molecular biology techniques, can define immunophenotypes with different variables of prognostic interest, which may serve as a guide to design studies to find differences in overall and disease-free survival, and which may even define a predictive value for response to treatment. The first step, which this work hoped to present, consisted of determining whether there were differences among the different immunophenotypes defined in comparison with clinical and histopathological variables with a known prognosis significance, so that these immunophenotypes could be classified in relation to this prognosis. The 110 Egyptian cases were classified according to the determination of 3 immunohistochemical tests of which the prognosis and predictive importance were known, namely hormone receptors (estrogen and progesterone) and Her2/neu [14,15]. Cases were classified into defined groups based on their combination. The proportion of cases in each group basically corresponded to that found in the literature using microarray techniques, with a greater frequency of Luminal A immunophenotype, which in the Carolina Breast Cancer Study series was 67% [16] and was 59.1% in the current series. The percentage of triple negative cases (defined by the lack of immunohistochemical expression of estrogen, progesterone and Her2/neu receptors, without considering cytokeratins) also coincided with that expressed in other series (16.3%) [17].

When we investigated the four subtypes with regard to patients age, no age predilection among any subtype was noted, which is consistent with the findings of Nguyen and his colleagues [18]. Although Carey et al. [19] reported the triple negative subtype to be more common in the young age group, they collected their data from Afro-American patients only. As regard the tumor size, we noted a discrepancy between the Luminal A and either Luminal B or HER2 overexpressing, with the latter two subtypes presenting with larger tumor size than the former. This may be explained by the design of the groups which defined a mixed group (Luminal B) as cases in which there were as many hormone receptors as Her2/neu, which did not statistically “contaminate” the pure Luminal A group and devalue the differences which may be found due to the expression of the Her2/neu receptor. Not taking this mixed group into account, and defining a tumor as Luminal by the simple fact that it expresses hormone receptors, regardless of whether it expresses Her2/neu, is erroneous. Brenton et al. reported no difference among different immunohistochemical phenotypes as regard tumor size [13].

More interestingly, and more importantly, when assessing the use of these IHC criteria for classifying breast cancer from a prognostic point of view, was the previously reported relationship between the triple negative and HER2 over-expressing groups, with the histologically more poorly differentiated forms [17,20]. When
we considered the four subtypes as regard tumor grade, the difference did not reach a statistical significance. Yet, we noted that the triple negative and HER2 over expressing subtypes tended to be of higher grade than the Luminal A and Luminal B subtypes, as 42.8%, 30.7% of the formers were grade III while only 15.3%, and 11.1% of the latter subtypes were grade III tumors. Another study, as well, reported higher tumor grades in triple negative and HER2 over-expressing subtypes [21].

No statistical difference was found among the groups in term of the number of involved lymph nodes. Yet, the percentage of tumors with involvement of more than three lymph nodes in this work was found utmost in the triple negative phenotype (61.5%), compared to the other three subtypes with a percentage ranging from 35.7 to 46.2%. Although these results are in accordance with the general concept of bad prognostic impact of the triple negative subtype [11], and also with Kim et al. [20] results who reported an association between triple negative breast cancer and lymph node involvement, other researchers have correlated the triple negative subtype with node-negative patients [9].

Several reports proved a relation between different bad prognostic parameters and the groups of HER2 over-expression and TNP [6,11]. The HER2 over-expressing group was reported to have the worst cumulative breast cancer specific survival, both after five and ten years of follow-up, in a large series of four thousand forty six invasive breast cancers [9]. This correlates with our findings that proved a statistically significant difference when comparing the HER2 overexpressing and TNP subtypes on one hand and the Luminal A and Luminal B subtypes on the other hand with respect to NPI.

In general, it is to be noted that the predictive value of this type of classification is just as or more important than, its prognosis use. This would mean that different immunophenotypes would respond differently to different therapies. Luminal groups with receptor expression would therefore adopt hormone treatment, while patients from the Her2/neu group would benefit from adding biological therapy using trastuzumab to chemotherapy. The triple negative immunophenotype would require chemotherapy [22].

With respect to the adjuvant treatment of breast cancer, the influence of immunohistochemical expression on response to primary or neoadjuvant therapies has also been confirmed [23].

Within this context, it is worth mentioning that this study design was planned to be simple yet reproductive. It overlooked the importance of further dividing the seemingly heterogeneous TNP [9] to segregate the basal-like tumors with additional work up. In terms of its clinical application, it is also important to point out that the pattern depending on the expression or non-expression of progesterone receptors is also an important factor when considering specific hormone therapy regimes [24-28] and therefore further dividing the Luminal groups with respect to the progestrone receptor status, whether positive or negative, seems justified.

In light of the above, and given the growing number of new drugs, in particular hormonal ones, and biological therapies, this type of classification must be investigated and taken into account when assessing response to these treatments. This will open up this line of work with immunohistochemicals, which will probably facilitate the definition of specific groups with a better or poorer response to these therapies.

In short, the results obtained seem to confirm that there are differences among the immunophenotypes considered, so that classification using these criteria can distinguish forms with a different biology, although follow-up studies are required to confirm this point. Among these differences, the subgroups without expression of hormone receptors, with expression of Her2/neu (HER2 over-expressing group) or without (triple negative group) present characteristics associated with poorer prognosis.

REFERENCES


