Protein Biomarker Expression in Invasive Breast Carcinoma, NST Classified According to Age, TNM and Original Nottingham Prognostic Index – A Study in Kosovo

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Abstract
Prognosis and management of patients with invasive breast carcinoma, NST is dependant on a few established parameters such as: TNM stage, Nottingham Prognostic Index, age, Estrogen and Progesterone Receptor expression, Her-2/neu amplification, proliferation index as measured by Ki-67 and molecular profiling.

In our study we analyzed immunohistochemical expression of Estrogen Receptor, Progesterone Receptor, Her-2/neu, Ki-67 and p53 in in patients classified according to Nottingham Prognostic Index, TNM stage and age.

In patients classified according to the original Nottingham Prognostic Index there was significant difference in biomarker expression. In contrast, this difference was not observed when patients were classified according to TNM. In analysis of marker expression in different age groups, significant difference was observed only with p53, expression of which was seen more frequently in younger age group.

In conclusion, prognostic stratification of patients by the original Nottingham Prognostic Index is related to expression of well-established biomarkers of breast cancer. p53 showed to be the only biomarker with significantly higher expression in younger patients.

Keywords: Protein Biomarker, Invasive Breast Carcinoma, NST, Age, TNM, Nottingham Prognostic Index, Prognosis.

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Introduction
Prognosis and management of patients with invasive breast carcinoma, NST is dependant on a few established parameters such as: TNM stage, Nottingham Prognostic Index, age, Estrogen and Progesterone Receptor expression, Her-2/neu amplification, proliferation index as measured by Ki-67 and molecular profiling.1\textsuperscript{9}

Histologic grading by Nottingham is a rigorous modification of Elston and Ellis criteria, which in turn, are a modification of Scarff-Bloom-Richardson system. This system is based on three important histological parameters such as a). level of tubule formation, scored as 1-3 (1=more than 75% tubule formation, 2=10-75% tubule formation and 3=less than 10% tubule formation by the tumor cells), b). nuclear atypia/polymorphism scored as 1 to 3 and c). mitotic activity scored 1 to 3 (1=less than 8/10HPF, 2=8 to 15/10HPF and 3=more than15/10HPF). Histological grade is subsequently expressed as a score of 1 to 3 which is determined by adding the values of the individual parameter. Values 3 to 5 are scored as grade 1, values 6 to 7 are scored as grade 2 and values 8 to 9 are scored as grade 3.2\textsuperscript{9}

Nottingham Prognostic Index (NPI) is a numerical value calculated by adding the values of a).tumor size expressed in cm and multiplied by a coefficient of 0.2, b). number of positive lymph nodes (1=negative lymph nodes, 2=one-to-three positive lymph nodes and 3=more than three positive lymph nodes), and c). histological grade (1,2 or 3). According to this system the patients are stratified in three prognostic groups, namely a). good prognostic group, with a value of
≤3.4, b). moderate prognostic group, with a value of 3.4-5.4 and, c). poor prognostic group, with a value above 5.4.9

Besides NPI, it is generally accepted that young age is also an independent prognostic indicator. Less than 10% of women with breast cancer are diagnosed before the age of 40 years. Survival rates are worse when compared to those in older women, and multivariate analysis has shown younger age to be an independent predictor of adverse outcome.10

Material and methods

Paraffin blocks from patients diagnosed with invasive breast carcinoma, NST were retrieved from our archive. The cases included in our study were female patients who were treated with radical mastectomy and axillary dissection. The cases had been previously staged according to the pathologic tumor-node-metastasis (pTNM) system and appointed histological grade according to Nottingham criteria (Table 1).

<table>
<thead>
<tr>
<th>NPI</th>
<th>Value</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤3.4</td>
<td>good</td>
<td></td>
</tr>
<tr>
<td>3.41-5.4</td>
<td>moderate</td>
<td></td>
</tr>
<tr>
<td>≥5.4</td>
<td>poor</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. The original Nottingham Prognostic Index classification.

Table 2. Immunohistochemical stains.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Source</th>
<th>Pretreatment</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>125</td>
<td>DAKO</td>
<td>pH9.0</td>
<td>1:35</td>
</tr>
<tr>
<td>PR</td>
<td>636</td>
<td>DAKO</td>
<td>pH9.0</td>
<td>1:50</td>
</tr>
<tr>
<td>Her-2/neu</td>
<td>HercepTest</td>
<td>DAKO</td>
<td>pH9.0</td>
<td>RTU</td>
</tr>
<tr>
<td>p53</td>
<td>DO-7</td>
<td>DAKO</td>
<td>pH9.0</td>
<td>1:1000</td>
</tr>
</tbody>
</table>

Immunohistochemistry

All biopsy samples were previously evaluated by two independent pathologists. The biopsy samples had been fixed in 10% neutral buffered formalin and cut in 3-4 micron thick sections. Antigen was retrieved by incubating the slides in 95 degrees Celzius for 45 minutes in steamer in target retrieval solution, pH6.0 (DAKO, K534011) or pH 9.0, respectively (Table 2). The slides were incubated with primary antibody (Table 2) for 30 min. The visualization was carried out with dextran polymer conjugated with peroxidase and secondary antibody (EnVision+, DAKO, K534011) for 30 min.

Results

Positive expression of ER and PR was inversely related to the NPI numerical value. This difference was statistically significant between the groups (p<0.001). Also, the incidence of ER positive, PR negative cases increased with increasing NPI numerical value. This difference was statistically significant between the groups (p=0.002).
Amplification of Her-2/neu was in direct correlation with NPI numerical value. This difference was statistically significant between the groups (p=0.001). Similarly, high rate of Ki-67 increased with increasing NPI value. This difference was statistically significant between the groups (p<0.001).

In contrast, as shown in Table 4, analysis of expression of protein biomarkers in different T
stages showed no significant difference between the groups.

Additionally, as shown in Table 5 and Figure 4, analysis of biomarker expression in different age groups showed significant difference only for p53.

Discussion

In our study we observed that expression of routinely applied immunohistochemical antibodies namely, ER, PR, Her-2/neu and Ki-67 showed significant differences among the NPI groups when the original, three-tiered NPI system is used (Table 3). Hence, according to these basic observations we decided to use the original, three-tiered NPI classification instead of newer, modified, five-to-six tiered NPI classifications. The threshold of Low versus High Ki-67 proliferation rate was set according to the Sg.Gallen criteria. Additionally, there was a particular observation of PR loss in cases with high numerical NPI value, emphasizing its role as adverse prognostic marker. This was also described in other studies.

In a study by the Nottingham group, immunohistochemical analysis of tissue microarrays was carried out for ER, PR, Her-2/neu, EGFR, Her-3, Her-4, p53, CK5/6, CK7/8 and Mucin 1. According to this study, breast carcinoma cases were classified in a similar way as studies employing gene expression profiling.

Benefits of this methodology are lower cost and wider applicability in Institutions with limited resources. Luminal A and Luminal B phenotypes are characterized by Her-3, Her-4 positivity in contrast to Luminal N phenotype which is Her-3 and Her-4 negative. Despite inability to prove any significant difference of p53 expression among NPI groups, we observed significant increase of expression of this protein in younger patients. Many data based on molecular and pathological studies support the important role of p53 in breast carcinogenesis. Nevertheless, despite these observations regarding loss of function of p53 in breast carcinoma, mutations of this gene are found in lower frequency compared to other solid tumors. During the last years, new knowledge related to the regulation of p53 protein have described new transcription products of p53. These have described alternative molecular mechanisms, besides mutations, through which p53 is deactivated in breast cancer. The molecular analysis of different stages of activity of p53 protein may have a diagnostic, prognostic and therapeutic implications in the future.

In our study, we observed significant difference of prevalence of Her-2+/ER- phenotype between three NPI groups whereas Her-2+/ER+ phenotype was not significantly different. This observation also corresponds to the results of the study by Green et al.

Conclusions

The results of our study prove that stratification of patients according to the original, three-tiered, Nottingham Prognostic Index is in correlation with “good” and “bad” prognostic biomarkers. Cases with “favourable immunophenotype” are more prevalent in “favourable NPI groups” whereas the opposite is true for cases with “adverse immunophenotype”. p53 expression is seen more in patients less than forty years of age, correlating with general view in terms of correlation between young age and adverse prognosis.

Declaration of Interest

Authors declare no competing interests.

Acknowledgements

Nora Shabani designed the study and wrote the manuscript. Fisnik Kurshumliu analyzed data and contributed in writing the manuscript. Ljube Ivkovski analyzed data and contributed in writing the manuscript. Suzana Manxhuka Kërliu provided important contribution in study design and data interpretation.

References


