TRIPLE-NEGATIVE BREAST CANCER DOES NOT FULLY OVERLAP WITH “BASAL-LIKE” MOLECULAR PROFILE – A MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

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Summary

Breast cancer comprises a diverse group of diseases in terms of clinical presentation, morphology, molecular profile, and response to therapy. Recent microarray studies indicated that breast cancer is a heterogeneous disease and may be divided into several distinct subtypes. It is not clear if the “triple-negative breast cancer” (TNBC) category corresponds completely to the basal-like group. The aim of the study was to identify the basal-like subtype of breast cancers among the group of TNBCs by examining their morphology and immunohistochemical characteristics. The majority of triple-negative cancers were high-grade invasive ductal carcinomas of no special type (NST)-72%, 22% were medullary breast carcinomas and 6% were others. Morphologically, TNBC were highly cellular tumors characterized by solid architecture with little or no tubule formation. Among 77 TNBCs using the five-marker method criteria for identification of basal-like breast cancers 62/77 (80%) were found positive for basal marker panel while 15/77 (20%) were negative for ER, PgR, Her2 and Cytokeratin 5/6 and EGFR as well. All 77 TNBCs are highly proliferative tumors showed high nuclear Ki-67 immunostaining. Although a significant overlap with basal-like carcinoma was observed, it seemed clear that ‘triple negativity’ should not be used as a surrogate marker for basal-like cancers.

Key words: breast cancer, triple negative breast cancer, immunohistochemistry

Introduction

Breast cancer comprises a diverse group of diseases in terms of clinical presentation, morphology and histology, molecular profile, and response to therapy [1, 2].

Recent microarray studies have indicated that breast cancer is a heterogeneous disease and may be divided into several distinct and reproducible subtypes associated with different outcomes and prognosis.

Expression profiles have categorized invasive breast carcinomas into the following groups: luminal A and B subtypes (ER+/HER2-/+), HER2 + subgroup (ER-/HER2+), the basal-like subgroup (ER- and HER2-) characteristics of breast myoepithelial cells [3, 4].
Among the ones that have attracted the attention of investigators in recent years, is basal-like cancer. Tumors from this group have been found to be positive for expression of basal cytokeratins, and negative for oestrogen receptor (ER) and HER 2.

The so-called “triple-negative breast cancer” TNBC is a term recently introduced in literature that refers to cancers not expressing ER, PgR, and HER 2 receptors [1, 5]. TNBCs are aggressive cancers that affect young women of low social status. [6].

According to J. A. Sparano et al there exist significant differences in gene expression between the TNBC and hormone receptor positive groups [7].

To date, it is not clear if the TNBC category corresponds completely to the basal-like group. However, patients with TNBC are clinically relevant because the tumors are more aggressive. Chemotherapy in these cases is the only treatment available because there are no specific molecular targets. TNBCs are also associated with later diagnosis and shorter survival. The normal female breast is a gland composed of branching ducts which originate from lobules and end in ducts at the nipple area. Normal breast ducts contain at least three types of epithelial cells: luminal cells, basal/myoepithelial cells, and progenitor cells [8]. The basal cells are confined in a basal membrane which separates the luminal epithelial component from the specialized breast connective tissue and adipose tissue.

Myoepithelial and luminal epithelia can be distinguished by their different CK expression patterns. Myoepithelial cells typically express high-molecular weight cytokeratins such as CK 5/6, CK 14 and CK 17, while luminal cells typically express CK 8 and 18. Immunohistochemical studies for basal/myoepithelial and luminal CKs appear to be helpful in subtyping invasive breast carcinomas into distinct biological subtypes.

Basal/myoepithelial cytokeratins and other markers have been used to identify a subset of ER- and HER2-negative breast carcinomas that are associated with a poor prognosis, further supporting the idea that a basal-like phenotype exists [5-9, 10, 11, 12, 13].

However, there is currently no internationally recognized immunohistochemical panel to define basal-like breast cancer and several combinations of basal cytokeratins and myoepithelial markers have been proposed.

The aim of the investigation was to identify the basal-like subtype of breast cancers among the group of TNBCs by examining their morphology and immunohistochemical characteristics.

**Patients and Methods**

Archival, histological, formalin-fixed and paraffin-embedded materials from 77 female II and III stage breast cancer patients, verified in previous pathology reports as “triple-negative”, were obtained in a retrospective study of the archives of the Department of Clinical Pathology, University Hospital-Pleven. Our study group was selected from a regional population database.

For that purpose a total of 513 clinical cases of breast cancer, treated surgically at the Department of Surgical Oncology, University Hospital - Pleven, during the period 2006-2009 were studied retrospectively.

The mean age of the studied group was 58 years-ranging from 36 to 78 years.

All H&E stained sections of these tumors were reviewed to confirm conventional morphological parameters according to the WHO classification 2003 by two pathologist (S.P and I.I) [2].

Histological grades were assigned using modified Elston & Ellis criteria [14].

All sections were immunohistochemically stained for ER, PgR, HER-2, epidermal growth factor receptor (EGFR), CK5/6 and Ki-67 according to protocols provided by the manufacturer (Table 1.).

CK5/6, and EGFR were chosen as they are established markers of basal/myoepithelial cells [15-17].

Cytokeratin 5/6 was scored positive if more than 5% (weak or strong) cytoplasmic and/or membranous invasive carcinoma cell staining was observed.

The Ki-67 positivity was quantified as percentage of positive nuclear staining of the tumor cells, 10% being a cut-off for active proliferation.

EGFR immunostaining was evaluated according to the FDA approved Dako EGFR PharmDX kit instruction. According to that, membrane reactivity above the background in more than 1% of tumor cells is considered a positive result.
Table 1. Antibody, Clone, Dilution and Manufacturer

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Dilution</th>
<th>Manufacturer</th>
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<tbody>
<tr>
<td>ER</td>
<td>Mouse AntiHuman 1D 5</td>
<td>1:1</td>
<td>DakoCytomation</td>
</tr>
<tr>
<td>PgR</td>
<td>PR-PgR 636 Mouse AntiHuman</td>
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<tr>
<td>HER-2</td>
<td>Polyclonal AntiHuman c-erb-B-2 Oncoprotein 1100</td>
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<td>Ki-67</td>
<td>Anti-human Ki-67 Antigen Clone MIB-1</td>
<td>1:1</td>
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<tr>
<td>Cytokeratin 5/6</td>
<td>Anti-human Cytokeratin 5/6-clone D5/16 B4</td>
<td>1:50</td>
<td>Dako Cytomation</td>
</tr>
<tr>
<td>EGFR</td>
<td>Mouse monoclonal -clone 18C9 NOVO</td>
<td>1:100</td>
<td>DakoCytomation</td>
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Results

TNBCs were found to be rare – 77 cases (15% of breast cancers diagnosed and treated during the period 2006-2009). The majority of triple-negative cancers were high-grade invasive ductal carcinomas of no special type (NST) 72%, 22% were medullary breast carcinomas, and 6% were other types of carcinomas (Fig. 1).

Morphologically, TNBC were highly cellular tumors characterized by solid architecture with little or no tubule formation. We found geographic necrosis in 40 cases (52%) of the TNBC, pushing margins in 44 cases (57%), and lymphocytic infiltrate in 33 cases (43%) (Fig. 2).

Fig. 1. Distribution of TNBT according their morphology

Fig. 2. A Ribbon -like architecture of invasive ductal carcinoma, areas of haemorrhages. Hematoxylin-eosin stain; original magnification, x100
B Medullar carcinoma of the breast –the tumor is composed of solid sheets of tumor cells, lack of tubule formation and lymphoplasmacytic infiltrate. Hematoxylin-eosin staining; original magnification, x100
C Grade III invasive ductal carcinoma of no special type IDC-NST or poorly differentiated invasive ductal carcinoma with high degree of pleomorphism and a moderate amount of mitoses. (original magnification x100
D Medullar carcinoma of the breast- synticial architecture and prominent nuclear pleomorphism. Hematoxylin-eosin staining; original magnification, x10.)
The nuclear chromatin pattern ranged from coarse to vesicular. Nucleoli were ranged from inconspicuous to prominent high histological grade and high mitotic index.

Of the 77 TNBCs, using the five-marker method criteria by Nielsen et al. [5] for identification of basal-like breast cancers, 62 of 77 (80%) were found positive for basal marker panel. The remainder 15 of 77 (20%) were negative for ER, PgR, Her2, as well as for Cytokeratin 5/6 and EGFR (Fig. 3).

Of the 77 TNBCs, 58 (75%) of the cases were found with 90% or more tumor cell nuclei intensively stained, when tested with Ki-67. Seventeen (22%) cases showed Ki-67 nuclear immunostaining between 50-60%. In only two cases (2.6%) the stained nuclei were under 30% of all nuclei examined.

Discussion

In our study, the group of triple-negative cancers was heterogeneous. Most of the tumors were found to be high grade IDC NST, or medullary carcinoma.

Despite the great interest in basal-like cancers, there is still no internationally accepted definition of these tumours.

From a scientific point of view, microarray-based expression profiling analysis remains the “golden standard” for identification of basal-like breast cancers.

This technology is unlikely to be introduced in daily routine diagnostic practice in the foreseeable future, and results of microarray-based expression profiling using RNA extracted from formalin-fixed archival samples are suboptimal.

Several attempts to define an immunohistochemical surrogate for basal-like cancers have been described. The best example to date is the panel proposed by Nielsen et al., where basal-like cancers are defined as those lacking both ER and HER2 expression and expressing CK5/6 and/or EGFR. This panel has a specificity of 100% and a sensitivity of 76% for the identification of basal-like cancers [5].

Using the triple negative phenotype (TNP) method, basal-like carcinomas were negative for all routinely tested biomarkers: ER, PR, and HER2 (ER–PR–HER2–), and this surrogate definition of basal-like was referred to as TNP.

Using the five-biomarker method, TNP were divided into two groups: triple-negative cases (ER–, PR–, HER2–), which were also positively expressed for either EGFR or CK5/6. These cases were referred to as Core Basal TNBCs.

There was a five-marker negative phenotype (5NP), which was triple negative, and furthermore expressed neither EGFR nor CK5/6. EGFR expression has been investigated in groups of triple-negative tumours, and was found to vary between 56% and 84% [18-19].

Patients with triple-negative cancers expressing a basal phenotype have been reported to have a significantly shorter disease-free survival, as compared with those with triple-negative cancers lacking the expression of basal markers. This is the reason why it is important to stratify patients with TNBCs [20].

EGFR activating gene mutations are remarkably rare. EGFR gene amplification has been shown in up to 25% of cases of metaplastic breast cancers - a subgroup of tumours that consistently show a triple-negative/basal-like phenotype [21].

A meta-analysis of more than 5000 breast cancer patients from 40 different series [22] has reported EGFR expression in 48% of the cases, using different techniques (range 14-91%).

In our study, positive staining for EGFR was
found in 57 of the 77 tumors (74%). Our results provide strong evidence that the use of five bi-marker surrogate (ER, PgR, HER 2 EGRF and Ck 5/6) to define the basal-like subtype of breast cancer is significant for prognostication and proper clinical trial design.

Conclusion

The term triple-negative breast cancer encompasses a heterogeneous group of tumours that possess distinctive yet rather heterogeneous pathological and clinical features.

Although a significant overlap with basal-like carcinoma was observed, it seems that 'triple negativity' should not be used as a surrogate marker for basal-like cancers.

The development of new drugs and targeted therapies for triple-negative cancers is of paramount importance and requires better understanding of the complexity of this heterogeneous group of tumours.

By adding EGFR and CK5/6 as positive markers to TNP, a significantly worse outcome group can be identified among triple-negative cases. The Core Basal definition is associated with even poorer breast cancer survival in the whole population-based group and this high risk group may benefit from a more aggressive chemotherapy.

Acknowledgment

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References

2. Tavassoli FA, Devilee P. WHO Classification of Tumours: Pathology and Genetics of Tumours of the Breast and Female Genital Organs, Lyon: IARC Press; 2003. p.9-110
10. Kesse-Adu R, Shousha S. Myoepithelial markers are expressed in at least 29% of oestrogen receptor negative invasive breast carcinoma. Mod Pathol. 2004;17:646-652
17. Santini D, Ceccarelli C, Tardio ML, Taffurelli M,


