BREAST CANCER AND TARGETED THERAPY

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Abstract

Breast cancer is one of the best examples of cancer diseases in which the molecular genetic alteration determination has changed its treatment options. Breast carcinoma is a *heterogeneous disease* (molecularly and clinically) with different natural courses of the disease and different response to therapy. Routine *clinical care* deals with *heterogeneity* for *decision making* depending on clinical prognostic factors, predictive, mixed prognostic/predictive biomarkers and guidelines. The assessment of prognostic factors, meanwhile, in order to provide a prediction of outcome, has become an essential part of the histopathologist's role in the handling and histological reporting of breast carcinomas. Following international guidelines and producing evidence by them has moved toward improvement the treatment of this disease. It is our duty as professionals to bring those guidelines in practice and influence the medicine policy makers in their decisions for better distribution of health resources which is is very important for developing countries like Albania

Introduction

Pathologic features of breast carcinoma allow patients to be placed into low-risk or high-risk categories in terms of recurrence, free or overall survival. Those features include lymph node status, tumor size, tumor type, histologic grade, lymphatic-vascular invasion, tumor proliferation rate, and hormone receptor status [1]. Sentinel or axillary lymph node status and tumor size are well established prognosticators in breast carcinoma patients and are part of the staging criteria outlined by the UICCAJCC scheme [2]. Although it is not possible to cover all the prognosticators in detail, hormone receptor statud and status of Her-2 (c-erb-B2) are discussed here.

Molecular pathology and specific oncogene therapy

For a long time one of the main goals of oncology has been the creation of therapies directed only against specific tumor cells, while sparing action in other cells of the body, in contrast to the toxicity caused by conventional chemotherapy and conventional radiotherapy. This kind of therapy is called *monoclonal therapy*, and consists in developing monoclonal antibodies against tumour cells, and more specifically against receptors or growth factors in the neoplastic cells inhibiting thus proliferation of them [3]. Malignant cells are characterized by self-sufficiency to growth signals, insensitivity to anti-growth signals, limitless of replicative potential, sustained of angiogenesis, evading apoptosis, tissue invasion and metastasis. All this steps of carcinogenesis are steps in developing novel prognostic and predictive markers and also of developing targeted therapies. There are also being developed other types of therapies the principle of action of which lies in the discovery and recognition of mutations in the genome of neoplastic cells [4]. This is not only a new area in the treatment of the patients but also in the relationship between oncologist – pathologist, for the choose of the right diagnosis and most appropriate therapy for the patient. Breast cacer is a *heterogeneous disease* (molecularly and clinically) with different natural courses of the disease (**prognosis**) and different response to therapy (**prediction**). Routine *clinical care* deals with *heterogeneity* for *decision making* depending on clinical prognostic factors (TNM, stage, comorbidity,.....); predictive, mixed prognostic/predictive biomarkers (ER, PR, HER2, grade....) and guidelines (NCI, NCCN, ...). The assessment of prognostic factors, meanwhile, in order to provide a prediction of outcome, has become an *essential part of the histopathologist's role* in the handling and histological reporting of breast carcinomas [5].

Proto-oncogene Expression

One of the most extensively studied proto-oncogenes in breast carcinoma is Her-2 (c-erb-B2) . The human epidermal receptor protein-2 (c-erbB-2; HER2) oncogene protein is a transmembrane

glycoprotein in the epidermal growth factor receptor family [6]. HER2 overexpression and/or gene amplification is an independent *prognostic marker* of clinical outcome, in both node-negative and node-positive patients. The major utility of HER2, however, is as a *predictive marker* [7]. Gene amplification and protein overexpression are reported to occur in 25% to 30% of breast carcinomas, especially those that are poorly differentiated, hormone receptor negative, lymph node positive, and flow aneuploid or show relatively high proliferation rates [8]. In our country Her2 testing in breast carcinoma has begun in 2004 with the precious contribution and strong determination of Prof Kadare. Breast cancer rate in Albania has increased over this period of time and also changed its mortality rate (fig 1). Our data also correspond with those of literature, and in a total number of 289 breast carcinoma patients for one year period the number of Her2 positive cases was 43 with a median value of 21% for a three year period (table 1).

Her-2 protein overexpression can be shown by immunohistochemical assays when there is complete or circumferential membrane staining and Her2 gene amplificatin by FSH/SISH. Her-2 status has been proposed as an eligibility criterion for new anti–Her-2 immunotherapies, such as trastuzumab (Herceptin), for patients with advanced breast cancer. Recent studies have shown a benefit in patients with Her-2–positive metastatic breast cancer who receive trastuzumab either alone or with adjuvant chemotherapy. Further studies have shown a benefit in Her-2 node-positive and high-risk patients receiving trastuzumab and adjuvant chemotherapy versus chemotherapy alone [9]. Nonetheless, as with hormone receptor assays, these studies suggest that Her-2 overexpression and gene amplification may become more of a predictive than a prognostic test. A prognostic test is used to predict the natural history of a disease regardless of therapy, whereas a predictive test predicts the response to a particular form of therapy, which
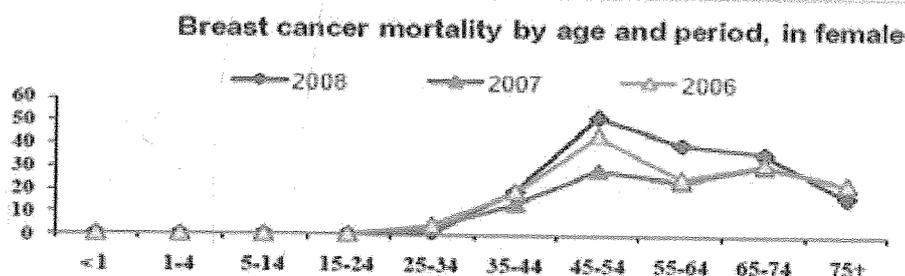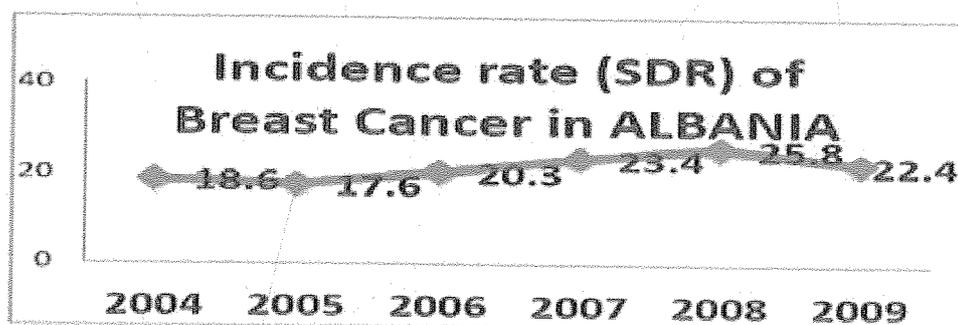

Figure 1: Source: *INSTAT*, Albania, Service of Oncology, UHC "Mother Theresa"

Table 1: Her 2 expression by IHC in a three year period

Year	0	1	2	3	Total
2009	76	86	33	58	253
2010	103	121	22	43	289
2011	75	128	35	63	301
2012 up to end of April	17	38	23	32	110
Total	271 (28%)	373 (39 %)	113 (12 %)	196 (21%)	953 (100%

would then have a positive impact on the natural history of the disease. The International Consensus Guidelines (2005) use Her-2 status to help stratify patients into low-, intermediate-, and high-risk groups with different recommendations for treatment plan [10]. HER2 status in breast cancer reflects, at least in part, the wide variation in methodology, instrumentation, and experience of the laboratories performing the testing [11]. Regarding to this, one of the main problems in HER 2 testing is *quality assurance* by optimizing laboratory equipment, choice of antibody, scoring system, validation assay and exclusion criteria. The problems encountered in our country while implementing and reporting HER 2 in breast cancer are given in figure 2.

15]. In a study of 257 primary breast carcinoma patients, Veronese and coworkers [15] found that tumor ER positivity was prognostically relevant for overall and relapse-free survival when the entire group was analyzed. The correlation of ER-PR status with outcome is, at least in part, closely linked to its ability to predict responsiveness to hormone manipulation therapy (about 77% of ER-positive and PR-positive tumors respond, 27% of ER-positive and PR-negative tumors respond, and 46% of ER-negative and PR-positive tumors respond). Yet approximately 11% of hormone receptor-negative patients respond to hormone therapy, and approximately 33% of hormone receptor-positive patients may not respond [16,17]. Some of these discrepancies may result from false negative
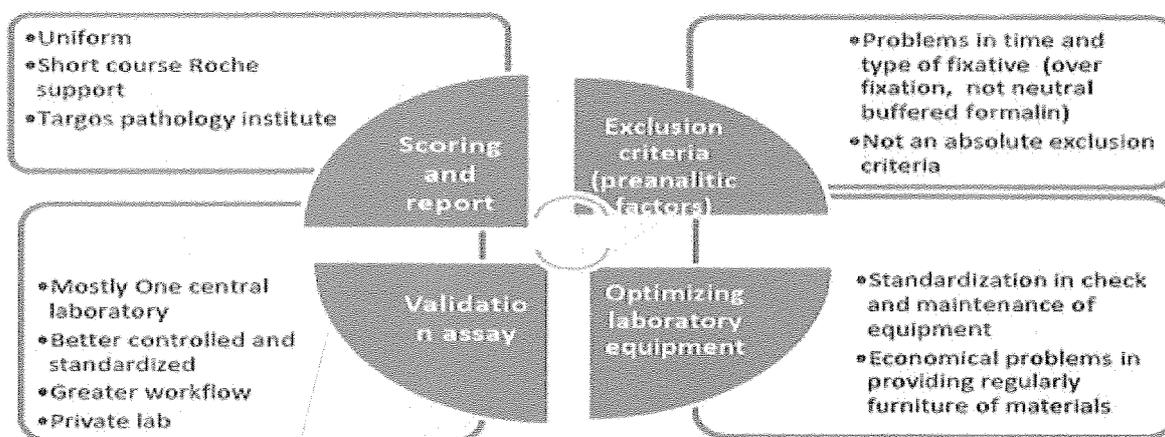

Figure 2 : Problems of quality assurance in HER2 testing in Abania

HER2 testing methodologies that are applied in our country are immunohistochemistry (IHC) and in situ hybridization (ISH). The IHC technique applied has been for several years manual IHC with HercepTest™ (Dako), c-erbB-2 Oncoprotein (Dako) used as antibodies and later (those two last years) Automated IHC BenchMark XT automated slide preparation system with pathway® anti-her-2/neu (4b5) Rabbit Monoclonal Primary Antibody.

Estrogen and Progesterone Receptors
Expression of ERs and PRs within tumors correlates well with low histologic grade and responsiveness to hormone manipulation, especially in postmenopausal patients [12] However, there is only an 8% to 10% absolute difference in disease-free survival between women with ER-positive node-negative invasive breast carcinoma and those with ER-negative disease, [13] and some studies have shown that any survival advantage of ER-PR positivity is lost after 5 years of follow-up [13, 14,

biochemical testing caused by the dilutional effect of abundant stroma or inadequate tumor sampling. Current recommendations from the International Consensus Guidelines use endocrine responsiveness (ER and PR status) as the most important selection factor for adjuvant chemotherapy and endocrine treatments in both node-negative and node-positive disease. Although the exact percentage of positive cells required for responsiveness has not been determined, some advocate 10% or more positive tumor cells as clearly indicative of endocrine responsiveness, 1% to 9% positive tumor cells as uncertain endocrine responsiveness, and 0% positive cells as endocrine nonresponsiveness [18].

## International Consensus Guidelines
The St. Gallen conferences play an important role on developing consensus opinions for the management of early breast cancer, and in Europe, the resulting guidelines are recognized as the leading treatment guidelines for the disease [19]. In the

United States, these recommendations are strongly supported by both the National Comprehensive Cancer Network (NCCN) Guidelines and the American Society of Clinical Oncology (ASCO) Technology Assessment of 2004 [20].

In January 2005, an international consensus panel of experts met during the Ninth Conference on Primary Therapy of Early Breast Cancer in St. Gallen, Switzerland, to develop a series of guidelines for the selection of treatments in specific patient populations. This panel sought to modify the previous guidelines based on new evidence that had emerged in the 2 years since the Eighth Conference [21]. For the first time, the 2005 guidelines moved away from risk assessment as the main criterion for treatment choice, introducing endocrine responsiveness as the most important factor in the selection of adjuvant chemotherapy and endocrine treatments in both node-negative and nodepositive disease. Three disease-responsiveness categories were identified: 1. Endocrine responsive. If tumor

treatment alone is doubtful, suggesting a need for adjuvant chemotherapy. 3. Endocrine nonresponsive. Cells have no detectable expression of steroid hormone receptors. The exact boundary between categories 1 and 2 is somewhat unclear and may be different in different clinical settings (e.g., according to the number of involved axillary lymph nodes or the patient's menopausal status). As noted earlier, some advocate 10% or more positive tumor cells as clearly indicative of endocrine responsiveness, 1% to 9% positive tumor cells as an uncertain endocrine response, and 0% positive cells as endocrine nonresponsive [25,26].

Those guidelines are being applied in our country also. In a group of 60 albanian breast cancer paients it has been the division of patients in risk categories using as one of the important parameers the ER, PR and HER 2 status. As we see in table 2 nearly half of the patients (56%) express ER, PR and do not express HER 2 being so part of the risk group which will respond to endocrine therapy.

Tab 2: Separation of patients according to ER, PR and HER 2 status
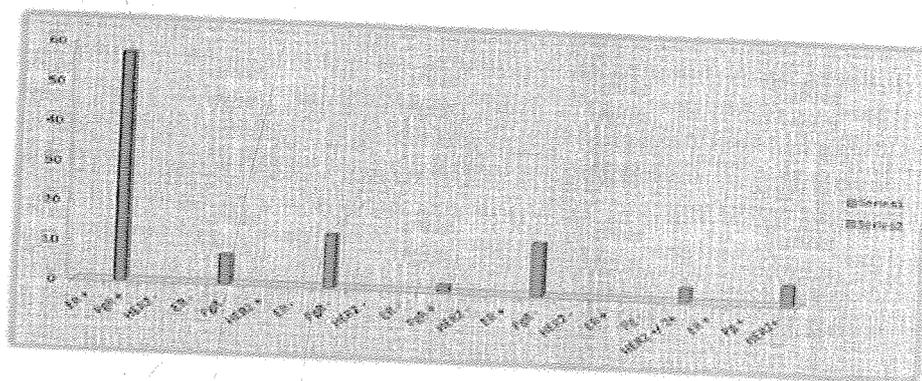
ER+/PR+/HER-	ER-/PR-/HER+	ER-+/PR-/HER2-	ER-/PR+/HER-	ER+/PR-/HER2-	ER+/PR-/HER-2+	ER+/PR+/HER+
34	4	8	1	8	2	3

cells express steroid hormone receptors it is probable that endocrine therapies will be effective in improving disease-free and overall survival. 2. Endocrine response uncertain. Features indicative of uncertainty include low levels of steroid hormone receptor immunoreactivity, lack of PRs (irrespective of the expression of ERs), Her-2/*neu* overexpression, high number of involved lymph nodes, high tumor levels of urokinase-type plasminogen activator–plasminogen activator inhibitor type 1, and increased proliferation markers [22,23,24]. Because any detectable steroid hormone receptor indicates some degree of endocrine responsiveness, such patients should receive endocrine therapy; however, the adequacy of such

Accordig to 2005 guidelines the nodal status remains an important feature for defining the risk category. However there is a subgroup of patients with node negative and low grade disease but other features conferring a worse prognosis These 3 markers were initially employed for prognostication but their role in treatment also rendered them of predictive value [27,28]. Newer molecular methods, especially high-throughput technologies, have shown that even morphologically similar subtypes of breast cancer can show molecular heterogeneity; moreover, infiltrating ductal carcinoma can be separated into at least 4 molecular subtypes designated *luminal* (ER+, PR+, and Her2/neu–), *Her2 overexpressing* (ER–, PR–, and Her2/neu+), *basal-like* (ER–, PR–

, Her2/neu–, and CK5/6+, EGFR+), and *normal breast-like* (ER–, PR–, and Her2/neu–), each with different clinical outcomes [29,30]. The importance of proliferative gene expression in these subtypes has been demonstrated and surrogate immunohistochemical markers include ER, PR, Her2/neu, and Ki67 for the more expensive molecular tests [31,32].

Conclusion: Breast cancer is one of the best examples of cancer diseases in which the molecular genetic alteration determination has changed its treatment options. Following international guidelines and producing evidence by them will improve the treatment of this disease even in our country. It is our duty as professionals to bring those guidelines in practice and influence the medicine policy makers in their decisions for better distribution of health resources which is is very important for developing countries like Albania.

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