The Importance of Immunohistochemistry Techniques in Assessing the Prognosis of Multimodally Treated Breast Cancer

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The identification of prognostic factors in the invasive breast cancer is particularly important for the therapeutic approach appropriate to the aggression of neoplasia. Determining the impact of multiple prognostic factors and correlating clinical and immunohistochemical factors allow the treatment to be adjusted so as to avoid the over-treatment of less aggressive neoplasms and to select the optimal multimodal neoadjuvant and adjuvant treatment in cancers with increased aggressiveness. Tissue samples obtained by breast tumor biopsy were studied by classical histopathology methods and by immunohistochemistry techniques, followed by the determination of the histological type, degree of tumor differentiation, status of estrogenic and progesterone receptors, Her2 status. Subsequently, the analysis of the particularities of the prognostic factors associated with the cases with aggressive, metastatic evolution was performed.

Key words: breast cancer, immunohistochemistry, chemotherapy, radiotherapy

Prognostic and predictive factors are a present subject, intensively studied in breast cancer in an attempt to identify subgroups of patients with particular development who would receive individualized treatment [1-3].

The usual anatomo-pathological exam offers important impact elements for predicting prognosis in breast cancer: lymph nodes status, primary tumor size, histological type, histological grade and vascular and lymphatic invasion[4, 5].

Immunohistochemistry techniques provide additional information on prognosis by establishing the status of estrogen receptor (RE), progesterone receptor (RP), HER2neu receptor status, quantification of cell proliferation markers (Ki67).

The normal mammary gland cells and some mammary tumor cells have protein receptors to which estrogen and progesterone hormones are attached form the hormone-receptor complex, which upon activation mediates gene transcription and promotes tumor growth and dissemination. Progesterone receptor activation requires the presence of estrogens. Breast tumor cells may have one, both or none of these receptors.

Breast tumors that have estrogen receptors are called ER-positive (ER+), those with progesterone receptors are called PR-positive (PR+) and those lacking these receptors are called ER-negative (ER-), respectively PR-negative (PR-).

Hormone therapy consists of administering drugs to reduce estrogen levels or block estrogen-receptor interactions with beneficial effects for patients with hormone receptor positive (predictive role) but no benefit in tumors with negative hormone receptors [6, 7].

HER2 oncogene amplification is a negative prognostic factor, the HER2 protein being involved in cell signaling and cycling. HER2 amplification is present in 10-30% of invasive breast tumors and also has a predictive role in response to Herceptin / Trastuzumab-targeted therapy, antiHER2 monoclonal antibody used in adjuvant or metastatic disease.

HER2 positive status corresponds to the immunohistochemistry score 3+, HER2 negative status is associated with 0/1 + score, and FISH or CISH (in situ fluorescence / in situ chromogenic hybridization) is performed for 2+ (uncertain) [8].

The significance of HER2 analyzed alone as a prognostic factor is controversial, but HER2 hyperexpression in the primary tumor in patients with positive axillary lymph nodes indicates a negative prognosis [9, 10].

Interpretation of Ki67 proliferation marker values in breast cancer by immunohistochemistry is difficult because of the lack of standardization of reagents, procedures and scores.

Experimental part
Material and method

In this study we used the IRO Radiotherapy Clinic Iasi database from May 2012 to December 2013. Of the total of 223 treated patients, for 96 patients who were monitored for an average of 48 months, we compared the prognostic factors of the group of patients with invasive breast cancer with rapid metastatic evolution (group A, 31 patients) and the group of patients lacked metastasis in evolution (group B, 65 patients). The groups were similar in age to diagnosis, diagnostic methods, staging and treatment, mean follow-up period.

The mean age of patients was 55 years (32-78 years) for group A and 57 years (32-76 years) for group B. All cases were clinically diagnosed, imaging (mammography and mammary echography) and by corebiopsy, which allows the pre-therapeutic setting of the histological type, but also of the molecular subtype of mammary carcinoma [11,12]. Hormonal receptor status and HER2 status were determined for all patients and the Ki67 value for 20 patients (64.5%) in group A and 46 patients (70.8%) in group B. The staging of patients in the two groups were conducted according to the TNM 7th AJCC 2010 edition.

The surgical intervention was conservative in 4 patients (12.9%) of group A and 28 patients (43.08%) in group B and the radical type in 27 patients (87.1%) in group A, respectively in 37 patients (56.92%) in group B.

The chemotherapy was neoadjuvant or adjuvant type, 4-8 cycles, based on anthracyclines or taxanes, according to guidelines.

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Radiotherapy was adjuvant for all patients, with standard total doses and fractional doses. The treatment plan was done by simulation using a CT scanner, followed by target and risk volume outlines, dosimetry, and treatment parameters establishment. The treatment was administered at the linear particle accelerator with 6MV / 10MV photon energy for all patients in both groups.

24 patients (77.42%) of group A and 56 patients (86.15%) of group B received adjuvant hormone therapy.

Biological therapy with Trastuzumab was administered to 8 patients (25.81%) of group A and 3 (4.62%) of group B respectively.

Results and discussions

Stage distribution was for group A: stage I - 0 patients (0%), IIA - 1 patient (3.22%), IIB - 3 patients (9.68%), IIIA - 4 patients (12.9%), IIIB - 8 patients (25.8%), IIIC - 15 patients (48.39%), and for group B: stage I - 13 patients (20%), IIA - 10 patients (15.37% IIB - 13 patients (20%), IIIA - 18 patients (27.7%), IIIB - 2 patients (3.08%), IIIC - 9 patients (13.85%) (fig. 1).
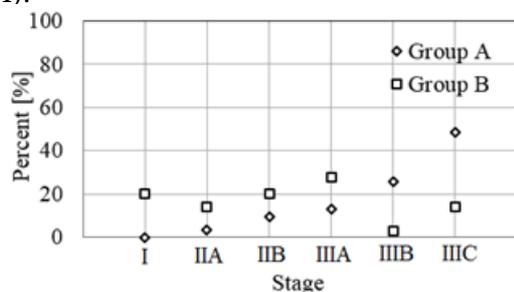

Fig.1. Stage distribution of groups

The results support the important prognostic role of the stage of metastatic evolution, the proportion of patients with advanced stages (especially IIIB and IIIC) being much higher in group A compared to group B, the results are statistically significant (p = 0.05).

The primary tumors of the two groups were: T1 - 2 patients (6.45%), T2 - 15 patients (48.39%), T3 - 4 patients (12.9%), T4 - 10 patients (32, 26%) in group A and T1-22 patients (33.85%), T2 - 29 patients (44.62%), T3-11 patients (16.92%), T4 - 3 patients (4.61%) in group B (fig. 2).
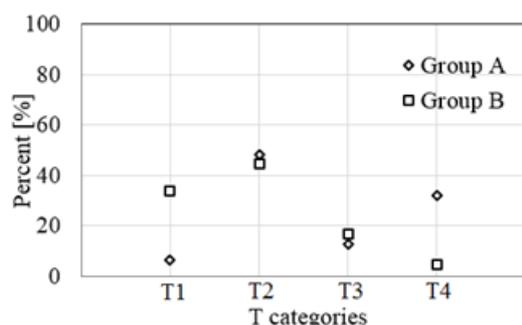

Fig.2. Distribution of T categories

Intermediate categories T2 and T3 were in similar proportions in the groups, but small T1 tumors were much more common in group B, while advanced T4 tumors predominated in group A (p = 0.48).

The status of the lymph nodes identified was: N0 - 1 patient (3.22%), N1 - 6 patients (19.35%), N2 - 8 patients (25.81%), N3 - 16 patients (51.61%) in group A and N0 - 25 patients (38.46%), N1 - 18 patients (27.69%), N2 - 13 patients (20%), N3 - 9 patients (13.85%) in group B fig. 3).

Similar to the T4 distribution, but with higher gradient, the weight of the N3 category is higher in group A, statistically significant results (p = 0.02).
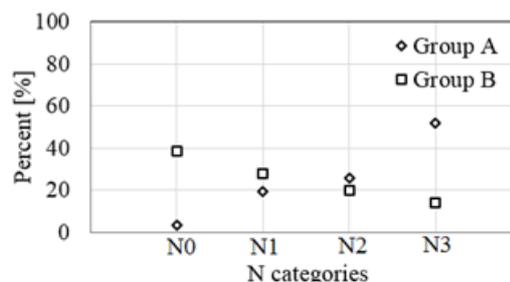

Fig.3. Distribution of N categories

The histological types identified for group A were: invasive ductal carcinoma (CDI) - 24 patients (77.42%), invasive lobular carcinoma (CLI) - 3 patients (9.68%), mixed carcinoma - %), cribriform - 1 patient (3.22%), and for group B: CDI - 56 patients (86.15%), CLI -2 patients (3.08%), mixed carcinoma - 69%), mucositis - 2 patients (3.08%), statistically insignificant differences (p = 0.21). Multicentric carcinomas represented 19.35% (6 cases) in group A, respectively 7.69% (5 cases) in group B (p = 0.39).

The degree of tumor differentiation was determined for 18 patients in group A (58.06%), of which 11.11% (2 cases) G1, 38.89% (7 cases) G2 and 50% (9 cases) G3, respectively for 52 patients in group B (80%), of which 26.92% (14 cases) G1, 51.92% (27 cases) G2 and 21.16% (11 cases) G3. In group A, the proportion of poorly differentiated forms, G3, was higher than in group B, but statistically insignificant (p = 0.6).

The molecular subtypes identified in lots A and B were luminal A (ER + and / or PR +, HER2-, Ki67 <14%) - 2 cases (6.45%) and 16 cases (24.61%) respectively, luminal B (ER + and / or PR +, HER + or ER + and / or PR +, HER2- and Ki67>14%) - 13 cases (41.93%) and 26 cases (39.99%), respectively, subtype HER2 (ER-, PR-, HER2 +) - 2 cases (6.45%), respectively 2 cases (3.07%), the basaloid subtype (triple negative) - 6 cases (19.35%) and 7 cases (10.77%) (fig. 4). Due to lack of Ki67 values in some patients, 8 cases (25.8%) of group A and 14 cases (21.54%) of group B with ER +, PR +, HER2- were not included in the classical molecular subtypes (X). The luminal A (LA) subtype, considered favorably prognostic, was more common in group B, and the triple negative (B), poor prognosis subtype was more frequent in group A, with statistical significance close to staging (p =0.06).
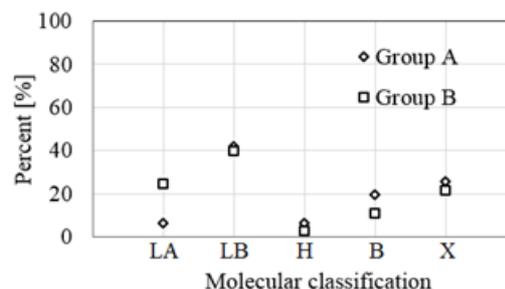

Fig.4. Distribution of molecular subtypes

The location of metastasis for patients in group A was the following: single site - bone 12 patients (38.71%), brain 7 patients (22.58%), lung 2 patients (6.45%), pleural 2 patients (6.45% %), liver 1 patient (3.22%), cervical lymph node 1 patient (3.22%), ovary 1 patient (3.22%), multiple site - 5 patients (16.13%).

Conclusions

From the prognostic factors for the multimodally treated invasive breast cancer, which rapidly evolves metastatically, the most important remains the lymph nodes status, followed by the clinical stage and the molecular subtype.

The identification and quantification of molecular subtypes, associated with clinical prognostic factors of aggressiveness, allow an individual therapeutic approach to each patient.

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