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THE PREDICTIVE ROLE OF ANATOMOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL MARKERS IN BREAST CANCER

PhD THESSIS ABSTRACT

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Introduction

Breast cancer is the most common type of neoplasia in women globally, regardless of age or ethnicity. In our country, it is estimated that there are 50 new cases per 100,000 people annually, accounting for more than a third of all cancers and resulting in over 3,200 deaths each year. The genetic polymorphism of breast cancers, reflected in each phenotype, makes this pathology a constant challenge for the medical world, necessitating the discovery of new prognostic and predictive factors to guide the most effective therapeutic approaches. [1]

Most breast cancers are indicated for neoadjuvant therapy (PST) before surgery, and the response to therapy will guide the surgeon towards a conservative or radical technique. The quantification of the tumor response to various neoadjuvant chemotherapy regimens is performed using clinical, imaging, and anatomopathological methods, with data obtained from the resection piece. In reality, the anatomopathological analysis of the surgical specimen is considered the optimal method for evaluating the response to PST.

This open, retrospective, and descriptive observational study aimed to identify predictive factors of response to neoadjuvant treatment in patients with breast cancer to increase the number of cases addressed through conservative surgery at the mammary gland level.

The RCB score, the tumor-infiltrating lymphocyte (TIL) score, and the Chevallier score are validated scoring systems based on anatomopathological information from the surgical resection specimen, each demonstrating predictive and prognostic value in breast cancers. However, no comparison and consequently no correlation between these scores has existed until now [2,3].

The first stage of the research, in addition to studies conducted so far where the RCB score was reported only to immunohistochemical subtypes, aimed to select individual variables (age, menarche, menopause, family history), clinical/paraclinical characteristics, anatomopathological markers, and immunohistochemical markers capable of predicting the response to neoadjuvant chemotherapy and validating them using the RCB score.

The second stage of the research compares the evaluation of the response to PST using the RCB score with the evaluation through the Chevallier System and the TIL Score. It also creates a new research direction - predicting the response to adjuvant therapy by calculating the TIL Score on both the biopsy and surgical resection specimen.

I. General Part

1. Breast Cancer

1.1 Epidemiology

Globally, in 2018, there were approximately 21 million newly diagnosed cases of breast cancer, representing nearly 1 in 4 cases of malignant tumors among women. It is the most common neoplasm in women, accounting for between 20% and 32% of all cancers, with percentages below 1% in men [1].

1.2 Etiopathogenesis

Multiple risk factors have been associated over time with an increased risk of developing breast cancer, but most lead to a small or moderate increase in this risk. At least half of the women who develop breast cancer have no identifiable risk factors other than gender and advanced age [4].

1.3 Clinical and Paraclinical Investigations

It can take between 2 and 8 years for the tumor to become accessible to clinical examination. Early detection in subclinical stages remains fundamental for improving the quality of life and prognosis of this disease [5]. Various national or regional population screening programs have been implemented at the European Union level to detect breast cancers early. Depending on the patient's age, mammography and breast ultrasound remain the investigations of choice for screening, sometimes complemented by MRI [6].

1.4 Anatomopathological Diagnosis

Following suspicion raised by clinical and paraclinical investigations, a definitive diagnosis of neoplasia is made through an anatomopathological examination, with the tissue most commonly obtained from a biopsy. TRU-CUT biopsy represents the method of choice in diagnosing breast lesions[7].

1.5 Breast Cancer Staging

This paper will use the TNM (Tumor size, Lymph Nodes affected, Metastases) staging system of the AJCC (American Joint Committee on Cancer) [8]. The classic anatomopathological staging has incorporated other characteristics, such as ER, PR, and HER2 biomarkers, tumor grading, and in N-/ER+/HER2- neoplasms, the Oncotype DX genomic analysis [9].

2. Breast Cancer Treatment

2.1 Neoadjuvant Treatment

The decision regarding systemic neoadjuvant treatment should be based on evaluating sensitivity to particular therapy types, considering short- and long-term toxicity, the patient's biological age, general health, and comorbidities. Neoadjuvant chemotherapy should be used in most cases, with exceptions only for Luminal A subtype cancers, where chemotherapy will be considered for T3/T4 N+ clinically or >4LNs+ in SLNB [10]. The most common regimens used in clinical practice are anthracycline and/or taxane-based regimens, anti-HER2+ therapy, and platinum derivatives [11–13].

2.2 Surgical Treatment

The Madden technique, used in all patients indicated for radical surgery, is the surgical technique of choice currently in cases where conservative surgery is not feasible. Proposed by Madden in 1965, it involves complete removal of the mammary gland with preservation of both pectoral muscles [14].

Axillary lymphadenectomy is an obligatory component of modified radical mastectomy. Guidelines recommend the SLNB technique in evaluating the status of axillary lymph nodes in T1-T2 cases with clinically/imagistically negative axillary nodes at diagnosis. In all other cases with large tumors (T3/T4), clinically/imaging-positive axillary nodes at diagnosis, or after PST, ALND will be performed as the first intention [15–17].

3. Prognostic and Predictive Factors in Breast Cancer

3.1 Immunohistochemical Subtypes

Molecular studies based on different patterns of gene expression have divided breast cancers into four subtypes [8]. The differences between these molecular subtypes are reflected in the biology of the malignant cell and manifest through individualized evolutions. These subtypes are:

- Luminal A (ER and/or PR+ with low-grade characteristics)
- Luminal B (ER and/or PR+ with high-grade characteristics or HER2+)
- HER2 overexpression (ER-/PR-/HER2+ HER2-enriched)
- Basal type (ER-/PR-/HER2- triple-negative/basal-like)

3.2 Anatomopathological Scores

Several systems have been developed to evaluate the pathological response to PST in breast cancer. The pathological response and the Chevallier system are related but not exactly the same. The pathological response broadly refers to the evaluation of how a tumor has responded to PST based on tissue sample examination.

The Chevallier system is a specific method of assessing the pathological response in breast cancer. However, in the context of subjective variability related to the pathologist's examiner classification in grade 2 and 3 of the Chevallier system, as well as the overlap of these two categories with pPR, a classification that encompasses both systems was used in this study [2,18].

The morphological evaluation of the tumor-infiltrating lymphocyte (TIL) in breast cancer has captured the medical world's interest due to its prognostic and potential predictive value as an immunological marker. This score has demonstrated prognostic value in triple-negative and HER2-positive breast cancers [19–21].

The RCB Score represents the latest validated method of evaluating the response to neoadjuvant therapy and is an important and independent prognostic factor. It mathematically analyzes certain characteristics of the anatomopathological specimen, such as the size of the

primary tumor, the percentage of invasive versus in situ tumor, and adds the analysis of the number of positive lymph nodes and the size of the largest positive node [3,22].

II. Personal Contributions

4. Working Hypothesis and General Objectives

Study Design:

This is an open, retrospective, and descriptive observational study developed in a hospital in Romania, specifically in the General and Esophageal Surgery Clinic at "Sfanta Maria" Clinical Hospital in Bucharest. The study was conducted in accordance with the Declaration of Helsinki and the Good Clinical Practice Guidelines. The study protocol was approved by the Ethics Committee of the Hospital.

Purpose:

To identify predictive factors of response to neoadjuvant treatment in breast cancer patients to increase the number of cases addressed through conservative surgery at the mammary gland level.

Objectives:

- Selecting individual variables (age, menarche, menopause), clinical/imaging characteristics, anatomopathological and immunohistochemical markers capable of predicting the response to neoadjuvant chemotherapy.
- Reporting these factors to different anatomopathological methods of evaluating the chemotherapy response to validate them as predictive factors.

5. General Research Methodology

Between July 2018 and December 2022, data were collected on the 240 breast cancer cases surgically treated in the General and Esophageal Surgery Clinic at "Sfanta Maria" Clinical Hospital in Bucharest, ultimately selecting a lot of 88 cases, all female, aged between 37 and 77 years.

In the first stage of the research (July 2018 - May 2020), data were collected on 175 breast cancer cases, from which 53 cases were selected.

Inclusion Criteria:

- Cases indicated for neoadjuvant treatment (except for Luminal A cancers/clinically or imaging primary tumors under 20mm cN0 with low proliferation marker values indicated for BCS+RT+ET - none of the 240 cases).
- Cases where complete anamnestic data (menarche, menopause, APP, concomitant diseases), clinical (clinical characteristics of the primary tumor and status of axillary lymph nodes), imaging (US/mammography/MRI performed pre-/post-PST), diagnostic (complete anatomopathological and immunohistochemical data, supplemented with CISH/FISH if necessary) could be collected.

Exclusion Criteria:

- Cases with distant secondary determinations (metastatic breast cancers have a different approach, both medicinal and surgical - ESMO 2020/ABC Guidelines).
- Patients with associated diseases (cardiac, hepatic, renal pathologies, etc.) that represented contraindications for ChT and could not benefit from PST.
- Cases where the anatomopathological slides with the surgical resection specimen could no longer be accessed (slides analyzed in other centers for CISH/FISH).

The ***database*** created with information about each patient (after obtaining their informed consent) includes:

- Name, age, menopausal status
- Personal oncological pathological history
- Other significant personal pathological history
- Location of the primary tumor (right/left breast) and quadrant SE (C50.4)/SI (C50.2)/IE (C50.5)/II (C50.3)/C(C50.1), multicentric/multifocal tumors (Overlapping lesion of breast - OL) (C50.8)
- Histopathological type: NST (8500/3), lobular (8520/3), mixed (8522/3, 8523/3, 8524/3)
- Grading: G1/G2/G3 - Nottingham Score
- Immunohistochemical markers: ER/PR/HER2 with percentage values
- Proliferation marker Ki67: values and reference intervals ≤10%/10-30%/≥30%
- Immunohistochemical phenotype: LuminalA/Luminal B/HER2+/Basal (Triple negative)

- In situ hybridization techniques results (CISH/FISH) in HER2 equivocal cases (2+)
- Initial tumor size (clinical/imaging) and tumor size on the surgical resection specimen + percentage of residual tumor + residual tissue mass
- Number of positive nodes from the total resected and the diameter of the largest positive node
- Presence of vascular and/or perineural invasion
- Anderson Score (RCB-0/I/II/III)
- TIL (tumor-infiltrating lymphocytes) score - percentage
- Adapted Chevallier System - pR (pCR, pPR, pNR)
- TNM staging pre/postoperative respectively c/p/yp
- Neoadjuvant therapy - basic therapy +/- Paclitaxel +/- Anti-HER2 number of cures
- Type of surgical intervention (BCS with axillary lymph node clearance or modified radical mastectomy Madden type).

Paraclinical Investigations:

A thoracic CT scan, an ultrasound (US), abdominal CT or MRI, and bone scintigraphy were performed on all patients with clinically positive axillary nodes, tumors over 5 cm, aggressive tumor biology, and the presence of signs/symptoms or constant biological values suggestive of distant metastases (45 cases).

In those receiving anthracyclines and/or trastuzumab, a complete cardiac evaluation, including echocardiography, was performed [23].

As mentioned earlier, all patients were indicated for ChT, including Luminal A/T2 cases (no T1 cases), with clinically/imaging positive axillary nodes (no N0 cases), followed by RT and/or individualized adjuvant ET (post-surgical intervention).

Surgical Techniques:

The two surgical techniques used in both the initial (53 cases) and extended (88 cases) lots were the modified radical mastectomy Madden type and BCS with ALND [24,25]. For various reasons explained during each research phase, SLNB was not performed in any cases, with ALND practiced across the entire lot. All interventions were conducted without major peri-procedural complications, with optimal final results.

Statistical Analysis:

The likelihood ratio test was used for univariate analysis. When p values were compared, they were adjusted using the Bonferroni method. The Chi-square test was used for analyzing categorical data (to evaluate associations or differences between variable categories) and to estimate the necessary sample size and statistical power for the study. Statistical analysis was performed using SPSS software version 23.0 (IBM Corp. Armonk, NY), with $p < 0.05$ considered statistically significant.

6. Predictive Factors of Response to Neoadjuvant Chemotherapy in Breast Cancer Validated by Residual Cancer Burden Score

6.1. Working Hypothesis and Specific Objectives

In this first stage of research, the RCB score was chosen as the method for quantifying this response using anatomopathological data obtained from the surgical resection specimen. The RCB score estimates the area still containing residual malignant cells, marks this area on each slide analyzed microscopically, and ultimately reconstructs an area represented by the actual tumor.

The originality of this study consists in reporting the RCB index not only to age, T, N, and grading but also to other variables such as menopausal status, histopathological and immunohistochemical type of neoplasm, clinical stage of the disease, and the type of neoadjuvant chemotherapy administered.

Purpose:

To identify predictive factors of response to neoadjuvant treatment in breast cancer patients using the RCB Score.

6.2. Material and Method

Between July 2018 and May 2020, data were collected on 175 breast cancer cases surgically treated in the General and Esophageal Surgery Clinic at "Sfanta Maria" Clinical Hospital in Bucharest, between January 2013 and December 2019. Ultimately, 53 cases were selected based on inclusion/exclusion criteria.

The online RCB calculator, developed by the MD Anderson Cancer Center of the University of Texas, USA, calculates a response index, with 0 being equivalent to pCR, RCB-1 (minimal), RCB-II (moderate), and RCB-III (extensive).

Neoadjuvant Therapy in the First Stage of Research

ChT was administered every 21 days/4 cycles for the AC regimen (doxorubicin 60 mg/m² plus cyclophosphamide 600 mg/m²). For the TAC regimen, ChT was administered every 21 days/4 cycles (docetaxel 75 mg/m² plus doxorubicin 50 mg/m² plus cyclophosphamide 500 mg/m²). The CMF regimen (cyclophosphamide 100 mg/m² days 1 - 14, methotrexate 40 mg/m² days 1 and 8, plus 5-fluorouracil 600 mg/m² days 1 and 8) was applied as 6 cycles every 28 days [26,27].

Paclitaxel (PTX) 80 mg/m² was administered after the AC or CMF regimens weekly for 12 weeks. Trastuzumab was applied in HER2+ cases in different doses based on body weight [28–30].

6.3. Results

Within the lot, only 3 cases had tumors ≤20mm (T1) after neoadjuvant treatment, including a case with multiple tumors (OL-Overlapping Lesion/C50.8) that could not benefit from BCS. Thus, 51 cases were surgically treated with modified radical mastectomy Madden type, and only 2 through BCS and ALND.

Following the anatomopathological analysis of the 53 cases, only 6 patients fell into the RCB=0 category, having a complete pathological response to neoadjuvant chemotherapy; 8 had a moderate response (RCB-II), and a significant 73.6% (39 patients) had a minimal response with RCB=III. No patients fell into the RCB-I category (minimal response).

All 6 pCR cases were invasive NST carcinomas (ICD-O-8500/3), with 5 receiving 4 AC cures and one receiving 6 TAC cures, followed by Paclitaxel in all 6 patients. Favorable response cases did not have a stage higher than IIIA with N0 or N1, all being single tumors (no multiple tumors) of up to 5 cm (no T4 cases).

The first parameter analyzed in relation to the RCB score was the age of the patients, most falling into the 50-59 age groups. Of the total of 6 patients with RCB=0, 66.7% were under 49 years old, suggesting a better response to PST in this category compared to older ages.

The predominant histopathological type was invasive NST carcinoma, with 43 cases (81%). All RCB=0 patients belonged to the NST subgroup, without statistically demonstrating the association between the NST histological type and favorable response to ChT (p 0.45).

Of the 53 cases, 16 (30.2%) had multiple tumors (OL), and 87.5% (14 cases) had a minimal or no response to neoadjuvant ChT. The low response rate to PST in OL (at the statistical significance limit with p 0.08) suggests that multifocality/multicentricity, along with the imaging estimation of a total tumor volume, could be important negative predictive factors for the response to ChT [31].

The primary tumor size was another analyzed parameter, with most falling between 2-5 cm (40 cases), 11 being over 5 cm, and only 2 cases with tumors ≤2 cm (both Luminal B/Her2- cases indicated for neoadjuvant ChT). The small number of breast cancers that could benefit from BCS (51 out of 53 cases having tumors over 2 cm) indicates the late diagnosis of this cancer type, limiting treatment options and benefits. Analysis showed that 70% of large tumors (over 5 cm) and 75% of those between 2-5 cm had a minimal or no response to ChT, making conservative surgical techniques impracticable even after PST.

In the analyzed lot, 45 patients had clinically positive axillary nodes at diagnosis (the other 8 cases indicated for neoadjuvant ChT due to the primary tumor size - T2/T3/T4 or the immunohistochemical phenotype - Luminal B/HER2+/triple-negative), and 92.3% (36 cases) had a minimal or no response to neoadjuvant treatment.

According to the TNM classification, the lot of patients in this study was in stages II (A-6 cases and B-29 cases) and III (A-8 cases and B-10 cases), with 54.7% of cases in stage IIB. Anatomic staging (Anatomic Stage Group - T/N/M) did not statistically correlate with the chemotherapy response rate (p 0.69).

Following immunohistochemical analysis, 40 cases had positive estrogen receptors (ER+), and 37 cases had positive progesterone receptors (PR+). A significant 87.2% of ER+ cancers fell into RCB-III, having significant residual disease compared to the RCB-II category, where ER was positive in only 37.5% of cases (p <0.01). Similarly, for PR, differences of 82.1% in RCB-III compared to 37.5% in RCB-II (p 0.01) were evident. In this study, RCB-0 was achieved in 7.5% of ER+ cases and only 5.4% of PR+ cases, still debating whether neoadjuvant chemotherapy brings benefits to this patient category [32,33].

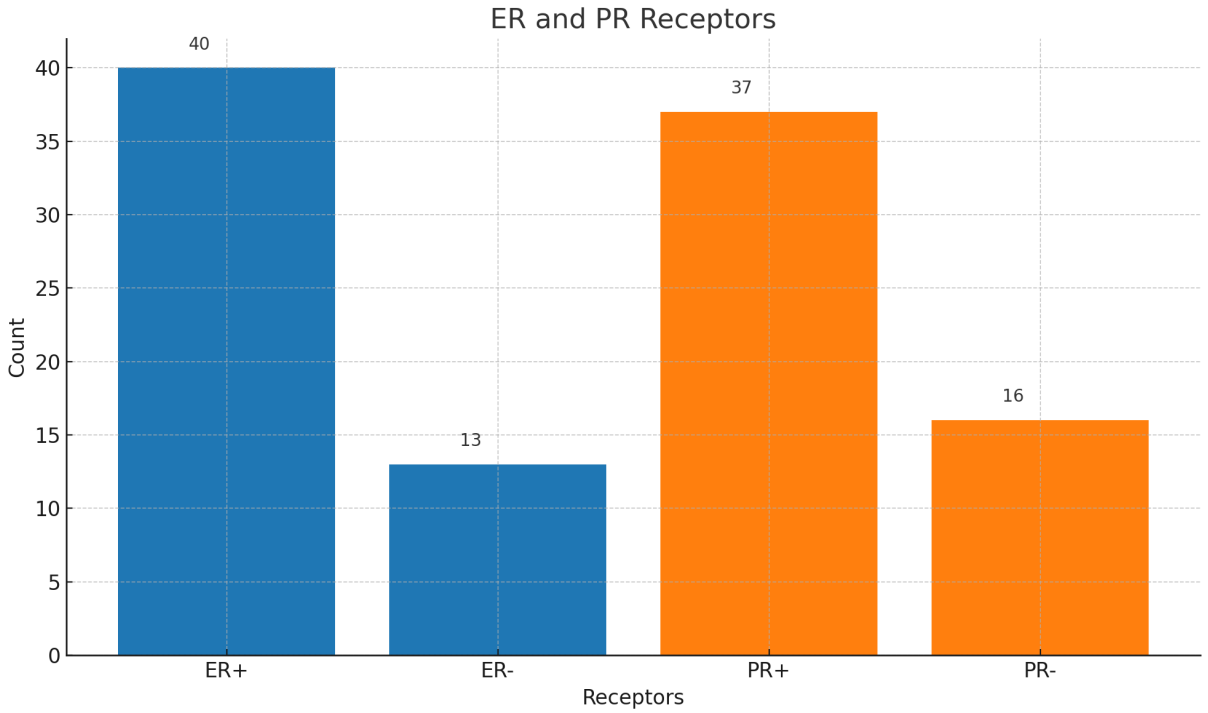

Figure 6.3.1 Distribution of Patients Based on the Presence of Hormone Receptors

The cell cycle and cell proliferation marker Ki67 was analyzed in biopsy specimens in 37 out of 53 cases, with 7 cases having values below 10%, 9 cases between 10-30%, and the majority (21 cases) with values over 30%. 81% had a minimal or no response to neoadjuvant chemotherapy. However, all RCB-0 and RCB-II cases had Ki67 values over 30% (p 0.057). This can be explained by the association of high Ki67 values with highly responsive tumor subtypes to targeted chemotherapy, such as Luminal B HER2+ or triple-negative.

HER2+ status was found in 11 cases (6 HER2+ subtype and 5 Luminal B/HER2+) of the 53 analyzed cases, all receiving targeted anti-HER2 therapy (trastuzumab). Most patients fell into the Luminal B subtype (HER2- 21 cases, HER2+ 5 cases), followed by Luminal A - 14 cases, Basal - 7 cases, and HER2+ 6 cases.

In the Luminal A category, no patient had a complete response to ChT, with 85.7% in the RCB-III category. In the Luminal B/HER2- subgroup, 85.7% of cases were RCB-III. It was observed that 50% of total cases falling into RCB-II (moderate response) belonged to the Basal immunohistochemical subtype, with only 5.1% of this subtype in the RCB-III category. This statistically significant association (p 0.02) of triple-negative cancers with partial response to PST highlights the importance of ChT in this aggressive breast cancer subtype.

Statistical analysis of all neoadjuvant chemotherapy regimens showed a statistically significant association only with Paclitaxel administration. It was observed that using this chemotherapeutic agent placed 50% of patients in the RCB-II category, with a significantly higher percentage of 89.7% in the RCB-III category, suffering from minimal or no response to Paclitaxel administration. Although taxanes represent the first line in breast cancer chemotherapy treatment alongside anthracyclines, a decrease in efficacy was observed due to the development of malignant cell resistance to this therapy [11,29].

6.4. Discussions and Conclusions:

In this study, the possibility of performing BCS increased by only 11% after PST. Most cases had a minimal or no response to neoadjuvant ChT, with pCR achieved in less than 15%. The unfavorable response to PST can be explained by the high number of ER+/PR+ cases, with the association between HR+BC and lack of response to ChT regimens being statistically demonstrated.

Although it was observed that all cases achieving pCR benefited from anthracycline-based therapies and none receiving CMF had a complete response, none of these associations could be proven. Trastuzumab therapy contributed to achieving pCR in less than 30% of HER2+ cases receiving this agent. Sequential Paclitaxel administration after anthracycline-based ChT regimens did not increase the response rate to PST, with the association between Paclitaxel and significant residual disease on the surgical resection specimen being statistically demonstrated.

Additionally, clinically/imaging positive axillary nodes at diagnosis and multicentricity/multifocality appear to be negative predictive factors for the response to PST.

Conversely, age under 49, high Ki67 values at diagnosis, and the TNBC subtype are predictive factors for a favorable response to neoadjuvant ChT, but require validation in prospective studies on large patient cohorts [34].

7. Residual Cancer Burden Score, Chevallier System, and Tumor-Infiltrating Lymphocytes Score in Evaluating Response to Neoadjuvant Chemotherapy in Breast Cancer

7.1. Working Hypothesis and Specific Objectives

The response to PST is a major factor in deciding the selection of patients who might benefit from breast-conserving surgery (BCS) and those with a clear indication for radical surgery, simultaneously serving as an important and independent prognostic factor for recurrence and survival. The RCB score, the tumor-infiltrating lymphocyte (TIL) score, and the Chevallier score are validated scoring systems based on anatomopathological information from the surgical resection specimen, each demonstrating predictive and prognostic value in breast cancers. However, no comparison and consequently no correlation between these scores has existed until now [22,35,36]

According to the mentioned inclusion/exclusion criteria, a lot of 88 cases, all female (no male breast cancer cases from 2013 to 2022), aged between 37 and 77 years, was selected.

Purpose:

To identify predictive factors of response to neoadjuvant treatment in breast cancer patients to increase the number of cases addressed through conservative surgery at the mammary gland level and validate them through three anatomopathological scores.

7.2. Material and Method

Chevallier/pCR System

The pathological response and the Chevallier system are interconnected but not exactly the same. The pathological response broadly refers to the evaluation of how a tumor has responded to PST based on tissue sample examination. The Chevallier system is a specific method of assessing the pathological response in breast cancer. However, due to subjective variability related to the pathologist's examiner classification in grade 2 and 3 of the Chevallier system, and the overlap of these two categories with pPR, a classification encompassing both systems was used in this study [18,37]:

- Grade 1 (pCR): Complete response - Absence of any residual invasive tumor cells in the breast and regional lymph nodes.
- Grade 2 (pPR): Residual micrometastases - Presence of carcinoma in situ in the breast but no invasive tumor and no tumor invasion in regional lymph nodes.
- Grade 3 (pPR): Partial response - Significant reduction in tumor volume but with the presence of invasive tumor cell foci in the breast and/or regional lymph nodes.
- Grade 4 (pNR): Minimal or absent response - The primary tumor and/or regional lymph nodes still have a significant amount of invasive tumor cells, indicating a minimal or absent response to neoadjuvant treatment.

TIL Score

The morphological evaluation of tumor-infiltrating lymphocytes (TIL) in breast cancer has captured the medical world's interest due to its prognostic and potential predictive value as an immunological marker. The TIL score can be evaluated using tissue obtained from biopsies before neoadjuvant treatment or from specimens obtained after surgical resection before adjuvant treatment. Recommendations for calculating the TIL score in breast cancer include reporting TILs as a percentage of the specific stromal compartment, the percentage of mononuclear inflammatory cells in the entire stromal area respecting the tumor margins, and excluding normal tissue or DCIS as well as necrotic or hyalinized areas [38,39].

In this study, the TIL score could only be estimated on surgical resection specimens and not on diagnostic biopsy specimens at diagnosis (diagnostic biopsies performed in multiple centers whose slides could not be subsequently obtained).

7.3. Results

7.3.1. Chevallier System

Out of the lot of 88 patients, 16 had pCR post-neoadjuvant chemotherapy, and the remaining 72 had pPR, with no patient falling into the pNR category. As mentioned earlier, the pPR category includes both grade 2 and grade 3 of the classic Chevallier system.

Patients with pCR were from all age groups, but with statistically significant differences in the 40-49 age subgroup, with 43.8% pCR compared to 15.3% pPR, and in the 60-69 age

subgroup, with 12.5% pCR compared to 38.9% pPR (p=0.01). Closely related to age, significant statistical differences were also obtained regarding menopausal status, with half of the patients who achieved pCR being postmenopausal (50%) compared to 88.9% of patients with partial response to neoadjuvant chemotherapy (p<0.01).

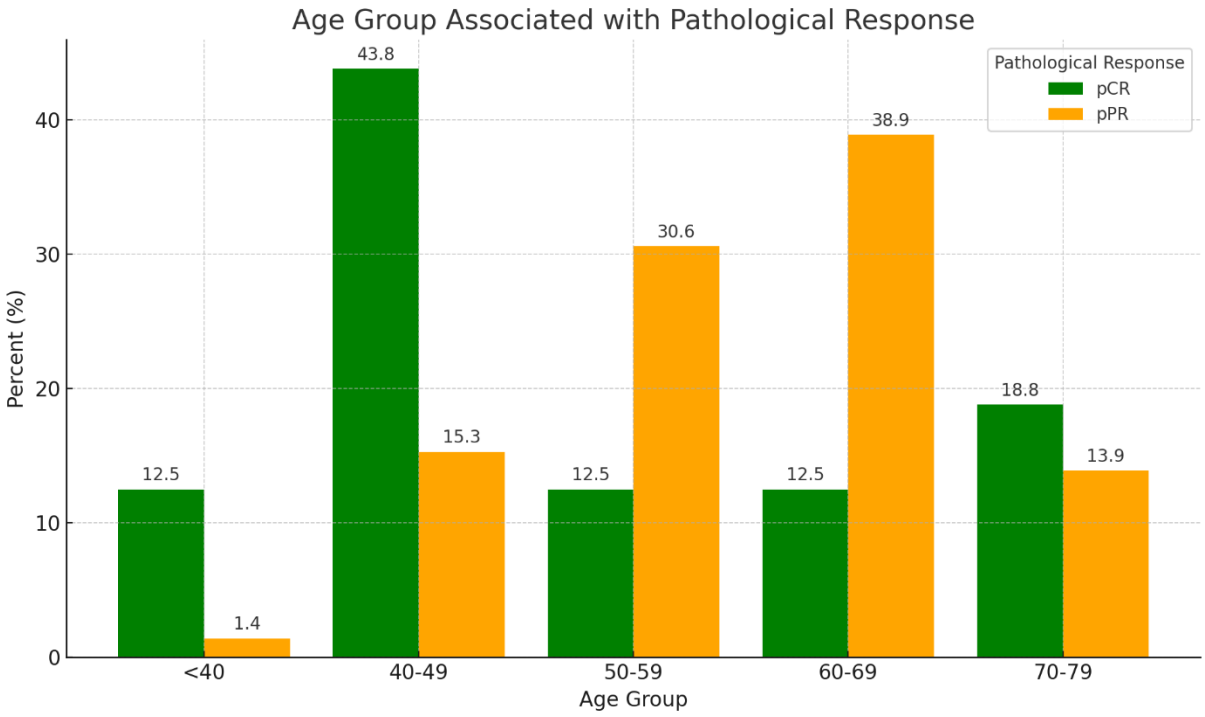

Figure 7.3.1.1 Distribution by Age Groups in Relation to pCR

Of the 16 patients, 9 cases were Luminal B, 5 Basal, and 2 HER2(-), with no Luminal A cases. It can be seen that 80.6% of pPR patients belonged to the Luminal A and Luminal B subgroups. Statistically significant results were obtained in the Luminal A subgroup, with differences between 0% pCR compared to 26.4% pPR (p 0.01).

Regarding Ki67 status, significant results were obtained in the ≤10% subgroup, with 0% pCR compared to 27.8% pPR (p<0.01), suggesting a weaker response to neoadjuvant chemotherapy in tumors with low Ki67 percentages, which could be considered a negative predictive factor for PST response.

Differences were observed in the G1 (well-differentiated) subgroup, with 18.8% pCR cases compared to 2.8% pPR, and in the G2 (moderately differentiated) subgroup, with 37.5% pCR compared to 72.2% pPR (p 0.01).

Regarding histopathological type, all 16 pCR cases were ductal type, with percentage differences in the ductal subgroup of 100% pCR compared to 75% pPR and in the lobular subgroup with 0% pCR compared to 20.8% pPR (p=0.04).

7.3.2. RCB Score

Following the anatomopathological analysis of the 88 cases, only 14 patients fell into the RCB=0 category, having a complete pathological response to neoadjuvant chemotherapy; 4 patients had a very good response with minimal residual disease, 23 had a moderate response (RCB-I), and a significant 53.4% (47 patients) had a minimal or no response to therapy with significant residual disease and RCB=III.

All 14 pCR cases were invasive NST carcinomas (ICD-O-8500/3), with 50% falling into the 40-49 age group, 57.1% Luminal B, with only one case of multiple tumors, and no cN2 cases (only cN0 or cN1).

Significant statistical results ($p<0.01$) were obtained between the RCB-0 and RCB-III groups, with only 6 out of 14 patients (42.9%) with a complete chemotherapy response being postmenopausal compared to 42 out of 47 (89.4%) postmenopausal in the RCB-III group.

The number of patients with tumor sizes ≤2cm increased from 2 to 18 (3.7% to 20.4%) after finalizing the patient lot (from 53 to 88), indicating earlier breast cancer diagnosis over time and easier access to screening and diagnostic methods for this pathology.

Regarding the primary tumor size, 56 cases (63.6%) fell into the 2-5cm range, 18 cases had sizes ≤2cm, and 14 cases were over 5cm. Comparing the PST response of these three size subgroups, significant statistical differences ($p<0.01$) were observed, with RCB-0 and RCB-I predominating in the ≤2cm and 2-5cm ranges, and RCB-II and RCB-III predominating in the 2-5cm and 5cm ranges.

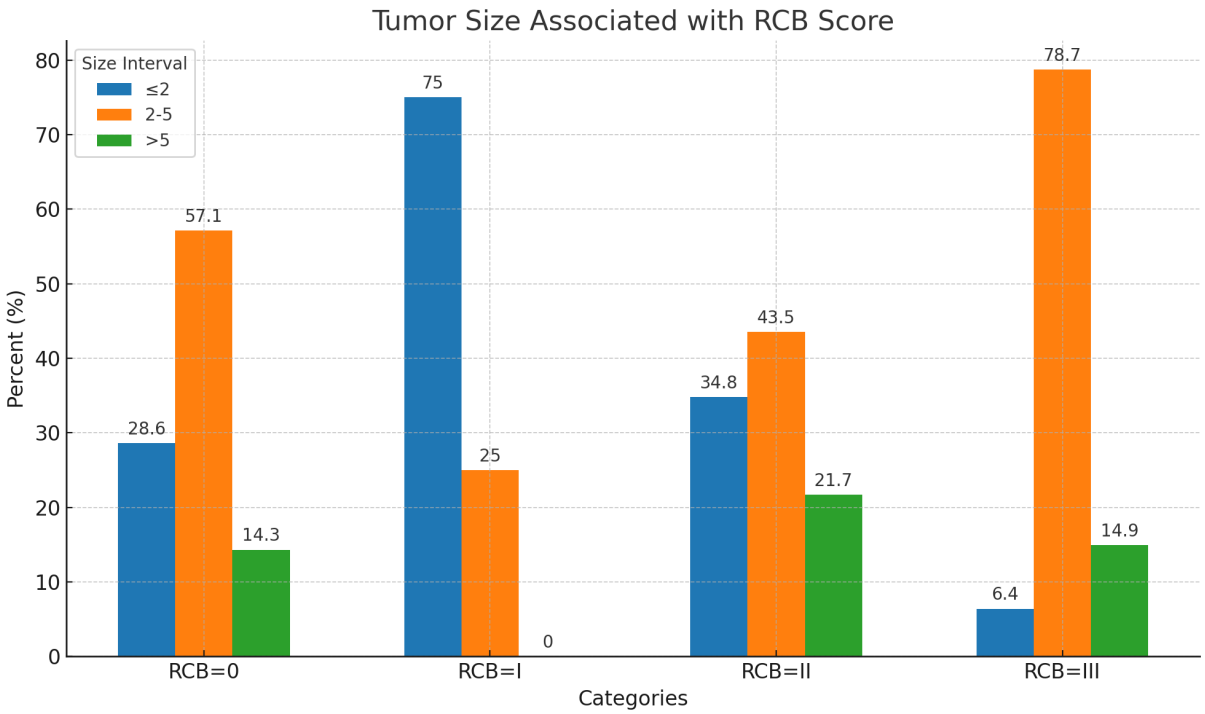

Figure 7.3.2.1 Distribution of the Lot Based on Tumor Size in Relation to RCB

Statistical analysis demonstrated significant differences ($p<0.01$) of 87.2% in RCB-III (41 out of 47 patients being +cN) compared to 43.5% (only 10 out of 23 patients with +cN) in the RCB-II subgroup, suggesting an association between poor chemotherapy response and the clinical status of axillary nodes. After completing the clinical examination with necessary imaging methods for precise staging, the number of N0 cases decreased from 23 to 17.

In the patient lot, axillary lymphadenectomy (ALND) was performed on all patients (of the 23 -cN patients, 10 had large tumors T3/T4, 7 either did not have access to SLNB at diagnosis or refused post-surgical tangential RT, opting for total node clearance, and 6 were excluded from the cN0 category after imaging examination, subsequently falling into the +cN category).

Classifying cases into immunohistochemical subtypes, most patients fell into the Luminal B subtype - 48 cases (HER2- 37 cases, HER2+ 11 cases), followed by Luminal A - 19 cases, Basal - 15 cases, and HER2+ 6 cases. In the Luminal A category, no cases reached RCB-0, with 73.6% falling into RCB-III. In the Luminal B category, 56.2% of cases fell into RCB-III, similar to the previous smaller lot of this study, indicating the need to develop new selection criteria for cases that would benefit from ChT and those that should receive only ET.

Significant statistical differences ($p<0.01$) were observed in the G1 subgroup, with 21.4% of patients in RCB-0 compared to 0% (no patients) in RCB-III, and in the G2 subgroup, with 28.6% of patients in RCB-II compared to 80.9% in RCB-III, suggesting a decrease in response rate to neoadjuvant ChT with increased grading and decreased differentiation.

7.3.3. TIL Score

The TIL score was analyzed in 87 out of 88 surgical resection specimens in our lot. Most cases fell into the TIL 1 subgroup (46 cases, 52.8%), followed by TIL 0 (19 cases), TIL 2 (17 cases), and TIL 3 with only 5 cases.

Significant statistical differences were observed between TIL 0 (63.2% postmenopausal patients) compared to 100% in the TIL 2 subgroup ($p<0.01$), suggesting an association between menopause and high TIL scores.

An association ($p<0.01$) was demonstrated between low Ki67 values (<10%) and higher TIL scores, with no patients in the Ki67 <10% subgroup falling into TIL 0, but 36.6% in TIL 1.

In the TIL 0 subgroup, all patients had high or intermediate Ki67 values, suggesting a clear association between low TIL scores even after neoadjuvant chemotherapy in aggressive cancers with high Ki67 values.

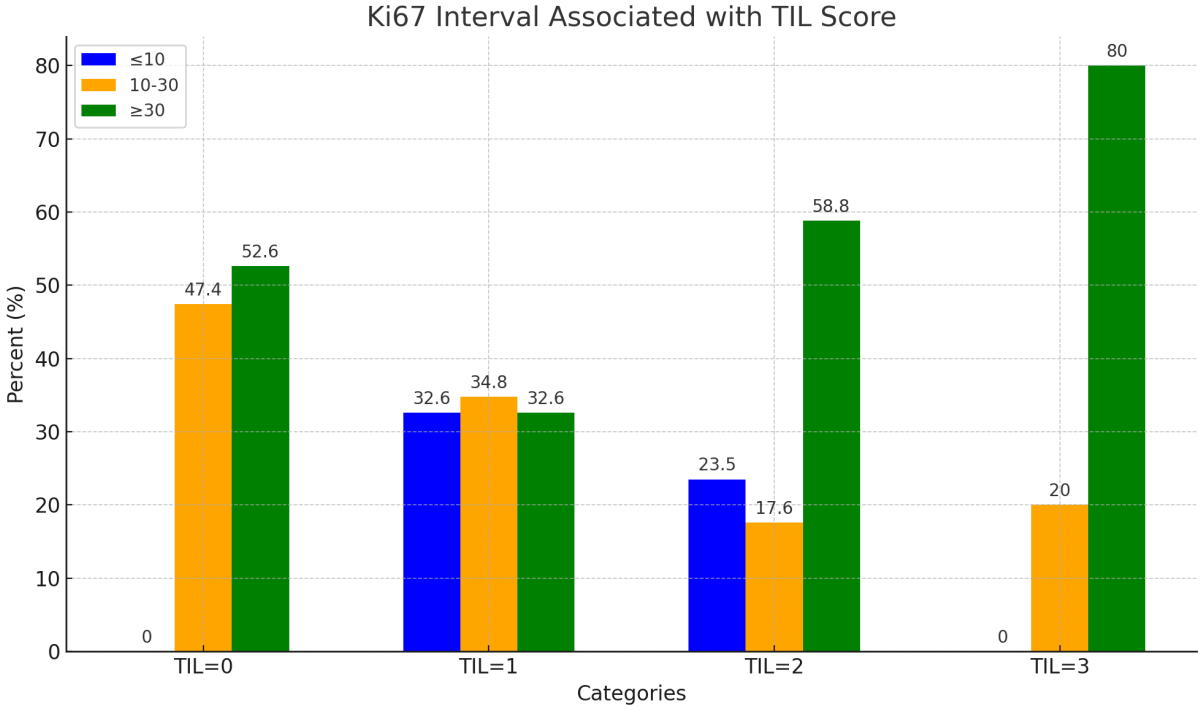

Figure 7.3.3.1 Distribution of the Lot Based on Ki67 in Relation to the TIL Score

In the AC (Doxorubicin + Cyclophosphamide) subgroup, significant differences were found between 26.3% of cases in TIL 0 compared to 70.6% in TIL 2, but also between this last percentage and 0% in TIL 3, suggesting the potential of this chemotherapeutic regimen to place neoplasms post-PST in intermediate TIL groups (TIL 1 and TIL 2), with no TIL 3 cases but relatively low TIL 0 percentages.

Regarding differentiation grade (Nottingham Score), significant statistical differences ($p=0.03$) were observed in the G2 subgroup, with 36.8% of cases in TIL 0 compared to 78.3% in TIL 1, noting the association of this intermediate differentiation grade with low TIL scores (75% of G2 cases falling into TIL 0 and TIL 1). Practically, cancers with intermediate and high grading are associated with low TIL scores, both characteristics indicating aggressive forms, poor chemotherapy response, and poor prognosis.

7.4. Discussions and Conclusions:

There is a close correlation between the two anatomopathological methods of quantifying the response to neoadjuvant ChT (RCB Score and Chevallier System - pCR), with predictive factors confirmed by statistical analysis corresponding to both methods.

Age under 49 and premenopausal status are predictive factors for a favorable response to neoadjuvant ChT but are also associated with low TIL scores and thus a poor response to post-operative adjuvant therapy.

Multifocality/multicentricity, clinically/imaging-positive axillary nodes, and low differentiation grade are negative predictive factors for the response to PST. ER+/PR+ cancers respond poorly or not at all to PST, while TNBC represents an aggressive breast cancer subtype with promising results regarding PST response.

Intermediate and high-grading BC is associated with low TIL scores, both characteristics indicating aggressive forms, poor chemotherapy response, and poor prognosis. Additionally, TNBC and HER2+ cancers with intermediate TIL scores post-PST based on anthracyclines or CMF will have a favorable prognosis.

8. Conclusions and Personal Contributions

This open, retrospective, and descriptive observational study developed in a hospital in Romania aimed to identify predictive factors of response to neoadjuvant treatment in breast cancer patients to increase the number of cases addressed through conservative surgery at the mammary gland level, largely achieving its objectives.

We succeeded in selecting individual clinical, paraclinical, anatomopathological, and immunohistochemical variables capable of predicting the response to neoadjuvant chemotherapy and validating them through two anatomopathological methods of evaluating the chemotherapy response to validate them as predictive factors.

From a technical-economic standpoint, the study followed diagnostic and treatment steps according to current guidelines within a state hospital without additional costs for patients or medical staff.

According to guidelines, almost all breast cancer cases are indicated for neoadjuvant ChT, especially Luminal B/HER2+, HER2+, and TNBC cases. The chosen surgical method, whether total or partial mastectomy - BCS, largely depends on the PST response. The percentage of cases that could benefit from BCS increased from 6% at diagnosis to 20% post-PST, but for reasons discussed earlier, only 11.3% were treated with BCS.

Evaluation of the neoadjuvant therapy response is preferably done through anatomopathological methods on the surgical resection specimen; currently, there is no clear correlation between different anatomopathological scores. The main objective of the study was to identify predictive factors of the neoadjuvant ChT response and validate them concurrently through the three most important anatomopathological scores at present: RCB, TIL, and the Chevallier system (overlapping with pCR).

Most predictors of the neoadjuvant ChT response in breast cancer were confirmed by both the RCB score and the Chevallier/pCR system with similar statistical results. On the other hand, the TIL score was only correlated with certain factors. A limitation of this study is the lack of information regarding the TIL score calculated on the biopsy specimen at diagnosis, so its evolution post-ChT could not be quantified.

Invasive/mixed lobular HR+ cancers, clinically positive nodules at diagnosis, and low differentiation grade are negative predictive factors for PST response, confirmed by at least two anatomopathological scores. TNBC, high Ki67 value, age under 49, and premenopausal status

are predictive factors for a favorable PST response. Additionally, TNBC and HER2+ cancers with intermediate TIL scores post-PST based on anthracyclines or CMF will have a favorable prognosis. Conversely, a high Ki67 value after PST and young age are associated with low TIL scores, a poor response to adjuvant therapy, and a poor survival prognosis.

Future research directions will consider following patients for predetermined periods (1, 2, 5, and 10 years) to quantify the prognostic value of individual clinical, paraclinical, anatomopathological, and immunohistochemical variables, as well as the three mentioned anatomopathological scores. Additionally, data on the TIL score obtained by puncture-biopsy at diagnosis and on the surgical resection specimen post-PST will be collected to make clearer associations between it and various predictive factors of the PST response.

Furthermore, the current study can be viewed as a pilot study for a much larger, possibly multicentric study that uses more than three scores to validate predictive factors and allows for a complex multivariate statistical analysis comparing existing scores.

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