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ORIGINAL ARTICLE

BREAST CANCER RESPONSE TO NEOADJUVANT CHEMOTHERAPY QUANTIFIED BY RESIDUAL CANCER BURDEN (RCB) SCORE

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Abstract

Primary systemic therapy (PST) is the first weapon employed in fighting against breast cancer (BC) and the response to the numerous chemotherapy (ChT) regimens currently used is the key to the conservative surgical approach. The objective of this study was to analyse the efficiency of ChT by assessing the anatomopathological response on the surgical resection piece. 53 patients with BC have been investigated pre/post-PST within this retrospective study and the quantification of the tumour response was made using the Residual Cancer Burden Score (RCB). 6 patients achieved pathological complete response (pCR), and 13 patients presented a tumour < 2 cm. Except for the 4 patients with multiple tumours, the percentage of cases that could have benefited from post-PST conservative surgery (BCS) increased to 16.9% (9/53). 87.2% of the cases classified as RCB-III were ER+ (p = 0.005) and 82.1% PR+ (p = 0.014), while the utility of ChT in case of these BC subtypes is still in debate. The Paclitaxel (PTX) sequential administration following anthracycline-based regimens did not increase the PST response rate and was associated with significant residual disease (p = 0.027). The anthracycline-based regimens or the anti-HER2 therapy (trastuzumab) (p = 0.986) did not influence the pCR reach in a statistically significant manner. The BC low response rate to PST is associated with hormone receptor+ (HR+) subtypes with a weak response to ChT, but might also be due to malignant cells' innate resistance to taxane therapy, presently considered as one of the cornerstones in BC treatment.

Rezumat

Terapia neoadjuvantă (PST) reprezintă prima armă utilizată în lupta contra cancerului mamar (BC) iar răspunsul la numeroasele scheme de chimioterapie (ChT) utilizate în prezent este cheia abordării chirurgicale conservatoare. Obiectivul studiului a fost de a analiza eficiența regimurilor ChT prin evaluarea răspunsului anatomopatologic pe piesa de rezecție chirurgicală. 53 de paciente cu BC au fost investigate pre/post-PST în cadrul acestui studiu retrospectiv, iar cuantificarea răspunsului tumoral s-a realizat cu ajutorul Scorului RCB (*Residual Cancer Burden*). 6 paciente au atins pCR (*pathological complete response*), iar 13 paciente au prezentat o tumoră < 2 cm. Exceptând cele 4 paciente cu tumori multiple, procentul cazurilor ce ar fi putut beneficia de o intervenție chirurgicală conservatoare (BCS) post-PST a crescut la 16,9% (9/53). 87,2% dintre cazurile încadrate RCB-III au fost ER+ (p = 0,005) și 82,1% PR+ (p = 0,014), rămânând încă în dezbatere utilitatea ChT în cazul acestor subtipuri de BC. Administrarea secvențială de paclitaxel (PTX) după scheme bazate pe antracicline, nu a crescut rata de răspuns la PST, asociindu-se cu existența bolii reziduale semnificative (p = 0,027). Regimurile bazate pe antracicline sau terapia anti-HER2 (trantuzumab) (p = 0,986) nu au influențat statistic semnificativ atingerea pCR. Rata scăzută de răspuns a BC la PST se asociază subtipurilor hormon receptor+ (HR+) slab responsive la ChT, dar se poate datora și rezistenței înnăscute a celulei maligne la terapia cu taxani.

Keywords: breast cancer, neoadjuvant chemotherapy, pathologic response, Residual Cancer Burden Score

Introduction

BC is the most common type of female cancer world-wide, irrespective of age or ethnicity, with annual incidences of up to 2.1 million newly diagnosed cases, accounting for nearly 1 in 4 cases of female malignant tumours among women [1-3]. Over recent years, the medical world's attention focused on the pre-surgical approach of breast cancers, particularly on PST, which proved to be a powerful weapon against this type of cancer [4-6]. The quantification of the tumour response to the numerous neoadjuvant ChT regimens currently available is made by clinical and imaging methods before the surgical intervention, but also by pathological anatomy methods after surgery, the latter being considered the optimal evaluation method for the PST response. The pathological complete response (pCR) is highly correlated with overall survival, disease-free survival, and recurrence-free survival, being defined as the absence of residual invasive cancer in the mammary gland or regional lymph nodes [7]. The RCB Score is a pCR quantification method, but also an independent

prognostic factor for all breast cancer subtypes, calculated based on the data from the surgical resection specimen. Given that PST optimization has become primordial for the conservative surgical approach of BC, as well as for patients' survival, we chose to analyse the efficiency of the major neoadjuvant ChT regimens currently used by assessing the anatomopathological response as an objective of this study.

Materials and Methods

Study design

This is an open, retrospective, observational study developed in a Romanian hospital. The study was conducted by the Declaration of Helsinki and the Good Clinical Practice guidelines. The study protocol was approved by the Hospital Ethics Committee. The purpose of the research was to analyse the efficiency of the major neoadjuvant ChT regimens currently used by assessing the anatomopathological response.

Population

From July 2018 to May 2020, data have been collected on 175 BC cases surgically treated in the Department of General Surgery I, "Sf. Maria" Clinical Hospital, Bucharest, Romania, during the period January 2013 - December 2019. Were included only cases with complete clinical data (clinical characteristics of the primary tumour and status of the axillary lymph nodes), imaging data (ultrasonography/mammography/MRI (magnetic resonance imaging) performed pre- and post-PST), diagnostic data (anatomopathological and immunohisto-chemistry (IHC)) and those with neoadjuvant ChT indication. The selected cases were those with tumours exceeding 2 cm, with positive axillary nodes by imaging or clinical means, particularly those of aggressive subtypes, such as human epidermal growth factor 2+ (HER2+), triple-negative (TNBC), and also Luminal B/HER2+.The exclusion was decided for cases with small tumours, without a regional lymphatic extension, of Luminal A subtype, with low values of tumour proliferation markers, as well as for those with distant metastases or associated pathologies which accounted for contraindications to ChT.

Methods

The BC diagnosis was made on the tumour tissue obtained by core needle biopsy/TRU-CUT [8, 9], using of a histological analysis of the tumour type and tumour grade. For the grading type classification, Nottingham Histological Score System was used.

Through the IHC methods, analysis was made on the status of the hormonal receptors (ER and PR) and HER2, as well as on the Ki67 cell proliferation marker [10].

For the IHC detection of tissue antigens, the method used is two-stage, based on a polymeric network visualization system (DAKO EnVision). The technique is applied to fragments of tumour tissue embedded in paraffin, sectioned to 3 mm thick. The antibodies used are Clone SP1 (DAKO, Carpinteria CA, USA) in dilution 1:200, for ER alpha with specificity for mammary epithelial cell nuclei; Clone PPG5/10 (DAKO, Carpinteria CA, USA) in dilution1:10, for ER beta with specificity for mammary epithelial cell nuclei; Clone 16 (Novocastra Leica- microsystems.com) in dilution1:100, for PR with specificity for mammary epithelial cell nuclei; Clone MIB1 (VENTANA) Ready-made solution for Ki67 specific for mammalian epithelial cell nuclei and Polyclonal Serum (Dako, Carpinteria, CA USA) in dilution 1:800 for CERBb2 with specificity for the tumour cell membrane.

For ambiguous results concerning the HER2 (2+) status, CISH (chromogenic in situ hybridization) or FISH (fluorescence *in situ* hybridization) confirmation tests were performed [11].

Clinical findings were integrated with imaging (mammography or US) to determine the size of the primary tumour (T) and the presence or absence of regional lymph-nodes metastases (N) [12, 13].

A thoracic computed tomography or abdominal MRI and bone scintigraphy were made on all the patients with positive axillary lymph nodes by clinical/imaging means, tumours exceeding 5 cm, and signs/symptoms or values of the biological constants suggestive of distant metastases.

ChT was given every 21 days/4 cycles for AC regimen (doxorubicin 60 mg/m^2 plus cyclophosphamide 600 mg/m^2). For TAC regimen ChT was given every 21 days/4 cycles (docetaxel 75 mg/m^2 plus doxorubicin 50 mg/m^2 plus cyclophosphamide 500 mg/m^2). CMF regimen (cyclophosphamide 100 mg/m^2 days 1 - 14, methotrexate 40 mg/m^2 days 1 and 8 plus 5-fluoro-uracil 600 mg/m^2 days 1 and 8), was applied as 6 cycles every 28 days. Paclitaxel (PTX) 80 mg/m^2 was administered after AC or CMF regimens, weekly, for 12 weeks. Trastuzumab was applied in HER2+ cases, in different doses depending on body weight [6, 14, 15].

After 6 weeks from the end of PST, patients were re-evaluated and given surgical treatment: BCS - partial axillary lymphadenectomy and lumpectomy – removal of lymph node stations I and II (Berg) [16-18] or Madden modified radical mastectomy.

The pCR quantification by RCB score was performed based on the anatomopathological data from the surgical resection specimen. The score was mathematically calculated by analysing characteristics such as primary tumour area and percentage of the invasive tumour cells, as well as the number of positive lymph nodes and size of the largest positive lymph node metastasis. The RCB online calculator made by the University of Texas MD Anderson Cancer Center, USA, determines a response index, 0 being equivalent to pCR, RCB-1 (minimal residual disease), RCB-II (moderate residual disease), and RCB-III (extensive) [19-21].

Statistical analysis

To evaluate the statistical associations between the ChT regimens and the PST pathological response, the

Likelihood-Ratio test was used [22, 23], in univariate analysis. For the case where values were compared [24, 25], these were adjusted using the Bonferroni method [26, 27]. The statistical analysis was performed by using the SPSS software (version 23.0), and $p < 0.05$ was considered statistically significant.

Results and Discussion

In line with the inclusion/exclusion criteria, this retrospective study analysed a group of 53 cases, all female, with ages between 37 and 77 years.

Regarding the size of the primary tumour, most of them fall between 2 and 5 cm (40 cases), 11 have dimensions over 5 cm, and only 2 cases with tumours ≤ 2 cm. 45 patients had clinically positive axillary lymph nodes at the time of diagnosis. According to TNM (Tumour size, Lymph Nodes affected, Metastases) classification cases were in stages II (A – 6 cases and B – 29 cases) and III (A – 8 cases and B – 10 cases). The histopathological classification was made following World Health Organization recommendations [28], the predominant histopathological type was invasive carcinoma NST (no special type) in 43 cases (81%), while the lobular type in 8 cases (15%) and the mixed type were much less common, being found in 2 cases (4%).

40 cases presented Grade II and 13 cases Grade III.

The analysed group had 40/53 ER+ cases and 37/53 PR+ cases. HER2 protein expression (overexpression/ gene amplification) was confirmed for 11/53 cases (6 within the HER2+ subtype and 5 in Luminal B/HER2+). As a marker for cell cycle and cell proliferation, the Ki67+ cell percentage was examined for every specimen obtained by biopsy in 37 of the 53 group cases, of which 7 cases had values under 10%, 9 cases between 10 - 30%, and the majority of them, respectively 21 showed values exceeding 30%. According to the ER/ PR/HER2/Ki67 status, 26 cases fell within the Luminal B subtype (HER2-negative – 21 cases, HER2-positive – 5 cases), followed by Luminal A – 14 cases, TNBC – 7 cases and HER2+ – 6 cases.

AC regimen was used in most of the cases – 43 patients (81.1%) TAC regimen was administrated in 7 patients (13.2%) and CMF regimen was given to 3 patients. Paclitaxel was administered in 45 cases after AC or CMF regimens. All 11 HER2+ cases received different doses of trastuzumab depending on body weight.

After PST, clinical and imaging evaluation highlighted that only 13 cases had tumours ≤ 20 mm, potentially suitable for BCS, among which 4 cases with multiple tumours unfit for BCS, 7 cases in which the patients opted for a total mastectomy, and 2 cases in which we performed BCS.

Within the analysed group, 10 patients had negative axillary lymph nodes in the post-PST clinical/imaging examinations, 6 of the cases without indication for Sentinel Lymph Node Biopsy (SLNB) due to the size of the primary tumour (T3/T4) and the other 4 not having access to this method at the time of diagnosis (given the lack of the required technical equipment in the medical centres at that time: 2013 - 2015) [29-31]. Therefore, 51 cases were surgically treated by Madden modified radical mastectomy and 2 cases by.

The number of patients who might benefit from BCS increased from 0/53 at the time of diagnosis to 9/53 after PST, accounting for merely 16.9%.

Furthermore, the reluctance of the patients aged over 60 toward conservative surgery has lowered this percentage to 3.77%. At the moment, it is considered that approximately 60% - 80% of the incipient BCs might benefit from BCS and radiotherapy, before or after PST, with similar or even superior results compared to the radical techniques [31-35]. The reported percentages have increased from 3.5% in 1992 [36] to approximately 40% - 60% over the recent years, with variations that depend on the stage at the time of diagnosis, on the tumour subtype, but mainly on the PST response [37, 38].

On the surgical resection specimen, analysis was conducted for the primary tumour size (T), vascular and perineural invasion, residual tumour mass, percentage of residual tumour and carcinoma in situ (CIS), the number of invaded axillary lymph nodes and size of the largest lymph node metastases removed. The data obtained were used for calculating the RCB score. 6 (11.3%) patients fell within the RCB-0 category, showing a complete pathological response to neoadjuvant ChT, 8 (15.1%) of them had a moderate residual disease (RCB-II), while most of them, 73.6% (39 patients), showed a minimal or even absent response to therapy, with a significant residual disease and RCB-III. There were no patients under RCB-I class (minimal residual disease). All 6 pCR cases were invasive carcinomas NST, they did not have a stage higher than IIIA, with N0 or N1, all being single tumours of 5 cm maximum (no T4 cases) and none of the cases of Luminal A subtype.

Table I

Pathologic response depending on oestrogen/progesterone receptors

	RCB = 0 (n = 6)	**RCB = II (n = 8)**	**RCB = III (n = 39)**	**p value (test)**
ER+	3/6 (50.0%)	3/8 (37.5%)	34/39 (87.2%)	0.005863 (Likelihood Ratio)
PR+	2/5 (40.0%)	3/8 (37.5%)	32/39 (82.1%)	0.014776 (Likelihood Ratio)

ER: oestrogen receptor; PR: progesterone receptor; RCB: Residual Cancer Burden score

One can notice that 87.2% of the cases classified as RCB-III were ER+ ($p < 0.01$) and 82.1% PR+ ($p < 0.02$), the utility of ChT remaining in debate for those HR+ BC subtypes. The obtained results join those of the literature mentioning percentages of merely 11% [19] for reaching pCR post-PST, the endocrine therapy being the first-line therapy in approaching these cases [39]. ChT should be taken into consideration in cases of T3/T4 tumours, aggressive by grading or proliferation markers. In case of uncertain indication for ChT initiation, the use of genetic testing is recommended, for determining the individual recurrence risk and the ChT general potential benefit in HR+ BC [40, 41].

Following the analysis of the neoadjuvant ChT regimens in relation to RCB score, it was noticed that all the patients classified as RCB-0 have benefited from ChT based on anthracyclines, more specifically on doxorubicin (83.3% treated with AC and 16.7% with TAC), however without being able to statistically demonstrate the association of anthracyclines with pCR. Although the sequential administration of anthracyclines and taxanes is currently the cornerstone in BC treatment, the percentages of reaching a pCR have been only 11.3%, comparable with data reported by other studies in the field, with 10 - 35% percentages [42-44].

Maintaining low PST response rates over time illustrates the need of conducting further studies on complementing and optimizing the currently used ChT regimens, but also continuing studies based on reversal of taxane/ anthracycline sequence [45].

Table II

The impact of various ChT regimens on the pathological response

	RCB = 0	RCB = II	RCB = III	p value (test)
ChT regimens				0.471617 (Likelihood Ratio)
AC	5/6 (83.3%)	7/8 (87.5%)	31/39 (79.5%)	
TAC	1/6 (16.7%)	0/8 (0.0%)	6/39 (15.4%)	
CMF	0/6 (0.0%)	1/8 (12.5%)	2/39 (5.1%)	

AC: doxorubicin + cyclophosphamide; TAC: docetaxel + doxorubicin + cyclophosphamide;
CMF: cyclophosphamide + methotrexate + 5-fluorouracil; RCB: Residual Cancer Burden score

None of the 3 cases treated with CMF have reached pCR. Furthermore, the efficiency of the first-generation therapies such as CMF, useful in TNBC and in patients with other comorbidities due to less severe adverse reactions, seems surpassed by the new therapies with ADCs (antibody-drug conjugates), PARP inhibitors (poly ADP-ribose polymerase) or AR antagonists (androgen receptor), which are promising surprising effects within selected subgroups of patients [46, 47]. Given the involvement of AR in various hormonal processes, its blockage plays a critical role in BC, but also in the management of other hormone-dependent cancers [48].

Table III

Pathologic response depending on anti-HER2 and paclitaxel therapy

	RCB = 0	RCB = II	RCB = III	p value (test)
Trastuzumab	3/6 (50.0%)	1/8 (12.5%)	6/39 (15.4%)	0.173535 (Likelihood Ratio)
Paclitaxel	6/6 (100%)	4/8 (50.0%)	35/39 (89.7%)	0.017450(Likelihood Ratio)

RCB: Residual Cancer Burden score

Half of the cases reaching pCR have also received anti-HER2 targeted therapy (2 cases Her2+ and 1 case Luminal B Her2+), suggesting the need of supplementing the ChT regimens with trastuzumab in aggressive BC subtypes. On the other hand, the existence of a 72.7% percentage of the HER2 cases with a moderate/minimal response to trastuzumab denotes the importance of the genetic polymorphism of this cancer subtype within the targeted therapy resistance, as well as the need to associate other anti-HER2 molecules, such as pertuzumab, lapatinib, neratinib, trastuzumab emtansine (TDM-1) [49-52]. Although inexistent as a current guideline recommendation, there are studies that mention the superiority of associating dual HER2 blockade (trastuzumab/lapatinib or trastuzumab/ pertuzumab) to standard ChT, compared to associating a single agent like trastuzumab [53, 54].

Significant percentage differences in the taxane treatment response can be noticed, namely 50% of RCB-II cases which received PTX, compared to 89.7% classified as RCB-III, suggesting the weak response of BC to this ChT agent ($p < 0.02$). Although it was proven that taxanes (PTX, cabazitaxel and docetaxel) improve the ChT effectiveness regardless of tumour size, grading, HR, or axillary node status [55-57] or in other cancers [58], a decrease was however noticed in their efficacy due to the existence of an innate resistance of the malignant cells to this therapy. The mechanisms of this resistance, still unclear, might be explained however by hypotheses such as overexpression of the proteins responsible for drug efflux in the malignant cell, such as P- glycoprotein (ABCB1) and MDR-1 [59, 60], both pertaining to the ABC superfamily (ATP-binding cassette). Also, SAC proteins (spindle assembly checkpoint) like Mad2, BubR1, or Aurora A (Aur-A) may constitute important markers of PTX resistance [61, 62]. The overexpression of Aur-A kinase, noticed particularly in TNBC cases, proved to be responsible for protecting the tumour cell against PTX [63]. The expression alterations of the proteins

associated with microtubules (MAPs) such as Tau or MAP4 are considered useful markers in selecting the patients for PTX therapy. Their decreased expression is making microtubules much more vulnerable and the malignant cells more sensitive to these agents [64]. Good knowledge of the ways accountable for malignant cell resistance to PTX is the key to selecting those cases which could have clear benefits from following this therapy, as well as discovering new target molecules capable of mitigating or even cancelling this resistance [65].

Conclusions

Within this study, the possibility of performing BCS increased by only 11% after PST. Most of the cases had a minimal or even absent response to neoadjuvant ChT, pCR being reached in percentages below 15%. The PST unfavourable response may be explained by a large number of ER+/PR+ cases, the association between HR+BC and lack of response to ChT regimens being statistically demonstrated. Although, it was noticed that all the cases reaching pCR have benefited from anthracycline-based therapies and none of those which received CMF had a complete response, none of these associations could be proven. Trastuzumab therapy has contributed to pCR reach for less than 30% of the HER2+ cases which have benefited from treatment with this ChT agent. The PTX sequential administration after anthracycline based ChT regimens did not increase the PST response rate, the association between PTX and significant residual disease on the surgical resection specimen being statistically proven.

Conflict of interest

The authors declare no conflict of interest.

References

1. Bray F, Ren JS, Masuyer E, Ferlay J, Global estimates of cancer prevalence for 27 sites in the adult population in 2008. *Int J Cancer*, 2013; 132(5): 1133-1145.
2. Howlader N, Noone AM, Krapcho M, Miller D, Brest A, SEER Cancer Statistics Review, 1975-2016, National Cancer Institute. Bethesda, based on November 2018 SEER data submission, posted to the SEER web site, April 2019.
3. Bray F, Ferlay J, Soerjomataram I, Siegel R, Torre L, Jemal A, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. 12 September 2018.
4. Spring LM, Fell G, Arfe A, Sharma C, Greenup R, Reynolds KL, Smith BL, Alexander B, Moy B, Isakoff SJ, Parmigiani G, Trippa L, Bardia A, Pathologic Complete Response after Neoadjuvant Chemotherapy and Impact on Breast Cancer Recurrence and Survival: A Comprehensive Meta-analysis. *Clin Cancer Res.*, 2020; 26(12): 2838-2848.
5. Sutton TL, Schlitt A, Gardiner SK, Johnson N, Garreau JR, Time to surgery following neoadjuvant chemotherapy for breast cancer impacts residual cancer burden, recurrence, and survival. *J Surl Oncol*., 2020; 122(8): 1761-1769.
6. Cardoso F, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rubio IT, Zackrisson S, Senkus E, ESMO Guidelines Committee, Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*., 2019; 30(8): 1194-1220.
7. Spring L, Greenup R, Niemierko A, Schapira L, Haddad S, Jimenez R, Coopey S, Taghian A, Hughes KS, Isakoff SJ, Ellisen LW, Smith BL, Specht M, Moy B, Bardia A, Pathologic Complete Response After Neoadjuvant Chemotherapy and Long-Term Outcomes Among Young Women With Breast Cancer. *J Natl Compr Canc Netw.*, 2017; 15(10): 1216-1223.
8. ali Bdour M, Hourani S, Mefleh W, Shabatat A, Karadsheh S, Nawaiseh O, Al Ebous AD, Comparison between fine needle aspiration cytology and tru-cut biopsy in the diagnosis of breast cancer. *J Surg Pak.*, 2008; 13(1): 19-21.
9. Yong WS, Chia KH, Poh WT, Wong CY, A comparison of trucut biopsy with fine needle aspiration cytology in the diagnosis of breast cancer. *Singapore Med J*., 1999; 40(9): 587-589.
10. Penault-Llorca F, Radosevic-Robin N, Ki67 assessment in breast cancer: an update. *Pathology*, 2017; 49(2): 166-171.
11. Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, Bilous M, Ellis IO, P Fitzgibbons, Hanna W, Jenkins RB, Press MF, Spears PA, Vance GH, Viale G, McShane LM, Dowsett M, Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/ College of American Pathologists Clinical Practice Guideline Focused Update. *J Clin Oncol.*, 2018; 36(20): 2105-2122.
12. Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, Meyer L, Gress DM, Byrd DR, Winchester DP, American Joint Committee on Cancer Staging Manual - 8th edition, 2017, published by Springer International Publishing AG, pp 570-591.
13. Sinna HP, Kreipeb H, A Brief Overview of the WHO Classification of Breast Tumors, 4th Edition, Focusing on Issues and Updates from the 3rd Edition. *Breast Care (Basel)*, 2013; 8(2): 149-154.
14. Early Breast Cancer Trialists' Collaborative Group (EBCTCG), Peto R, Davies C, Godwin J, Gray R, Pan HC, Clarke M, Cutter D, Darby S, McGale P, Taylor C, Wang YC, Bergh J, Di Leo A, Albain K, Swain S, Piccart M, Pritchard K, Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. *Lancet*, 2012; 379(9814): 432-444.
15. Samuel JA, Wilson JW, Bandos H, Bandos H, Elledge R, Robidoux A, Fehrenbacher L, Ward P, Polikoff J, Brufsky A, Provencher L, Paterson A, Hamm J, Carolla R, Baez-Diaz L, Rastogi P, Julian T, Wickerham D, Swain S, Geyer C, Mamounas E, Wolmark N, A randomized phase III trial comparing six cycles of 5-fluorouracil (5-FU), epirubicin, and cyclophosphamide (FEC) to four cycles of adriamycin and cyclophosphamide

(AC) in patients (pts) with node-negative breast cancer. *Cancer Res.*, 2015; 75(9_Suppl.): S3-02.
16. Association of Breast Surgery at Baso 2009, Surgical guidelines for the management of breast cancer. *Eur J Surg Oncol.*, 2009; 35(Suppl. 1): 1-22.
17. de Glas NA, Kiderlen M, Liefers GJ, Survival after lumpectomy and mastectomy for early stage invasive breast cancer: the effect of age and hormone receptor status. *Cancer*, 2013; 19(7): 3253-3254.
18. van Maaren MC, de Munck L, de Bock GH, Jobsen JJ, van Dalen T, Linn SC, Poortmans P, Strobbe LJA, Siesling S, 10 year survival after conserving surgery plus radiotherapy compared with mastectomy in early breast cancer in the Netherlands: a population-based study. *Lancet Oncol.*, 2016; 17(8): 1158-1170.
19. Yau C, Osdoit M, van der Noordaa M, Shad S, Wei J, de Croze D, Hamy A-S, Laé M, Reyal F, Sonke GS, Steenbruggen TG, van Seijen M, Wesseling J, Martín M, del Monte-Millán M, López-Tarruella S, I-SPY 2 Trial Consortium, Boughey JC, Goetz MP, Hoskin T, Gould R, Valero V, Edge SB, Abraham JE, Bartlett JMS, Caldas C, Dunn J, Earl H, Hayward L, Hiller L, Provenzano E, Sammut SJ, Thomas JS, Cameron D, Graham A, Hall P, Mackintosh L, Fan F, Godwin AK, Schwensen K, Sharma P, DeMichele AM, Cole K, Pusztai L, Kim M-O, van 't Veer LJ, Esserman LJ, Symmans WF, Residual cancer burden after neoadjuvant therapy and long-term survival outcomes in breast cancer: A multi-center pooled analysis. *Lancet Oncol.*, 2022; 23(1): 149-160.
20. Hamy AS, Darrigues L, Laas E, De Croze D, Topciu L, Lam GT, Evrevin C, Rozette S, Laot L, Lerebours F, Pierga JY, Osdoit M, Faron M, Feron JG, Laé M, Reyal F, Prognostic value of the Residual Cancer Burden index according to breast cancer subtype: Validation on a cohort of BC patients treated by neoadjuvant chemotherapy. *PLoS One*. 2020; 15(6): e0234191: 1-17.
21. Miglietta F, Dieci M, Tsvetkova V, Griguolo G, Vernaci G, Menichetti A, Faggioni G, Giarratano T, Mioranza E, Genovesi E, Cumerlato E, Bottosso M, Saibene T, Michieletto S, Mele ML, Conte P, Guarneri V, Validation of Residual Proliferative Cancer Burden as a Predictor of Long-Term Outcome Following Neoadjuvant Chemotherapy in Patients with Hormone Receptor-Positive/Human Epidermal Growth Receptor 2-Negative Breast Cancer. *Oncologist*, 2020; 25(9): e1355-e1362.
22. King G, Unifying Political Methodology: The Likelihood Theory of Statistical Inference. New York: Cambridge University Press, 1998; 84.
23. Li B, Babu GJ, A Graduate Course on Statistical Inference. Springer. 2019: 331.
24. Aschwanden C, Not Even Scientists Can Easily Explain P-values. Five Thirty Eight, 2015, Archived from the original on 25 September 2019. Retrieved 11 October 2019.
25. Wasserstein RL, Lazar NA, The ASA's Statement on *p*-Values: Context, Process, and Purpose. *American Statistician*, 2016; 70(2): 129-133.
26. Dunnett CW, A multiple comparisons procedure for comparing several treatments with a control. *J Am Statistic Assoc.*, 1955; 50(272): 1096-1121.
27. Shaffer JP, Multiple Hypothesis Testing. *Ann Rev Psychol.*, 1995; 46: 561-584.
28. Lakhani SR, Ellis IO, Schnitt SJ, Tan PH, van de Vijver MJ, Classification of Breast Tumors, 4th Edition, Ed. WHO, 2012; volume 4.
29. Gebruers N, Verbelen H, De Vrieze T, Coeck D, Tjalma W, Incidence and time path of lymphedema in sentinel node negative breast cancer patients: a systematic review. Arch Phys Med Rehabil., 2015; 96(6): 1131-1139.
30. Krag DN, Anderson SJ, Julian TB, Brown AM, Harlow SP, Costantino JP, Ashikaga T, Weaver DL, Mamounas EP, Jalovec LM, Frazier TG, Noyes RD, Robidoux A, Mc Scarth H, Wolmark N, Sentinel-lymph-node resection compared with conventional axillary-lymph-node dissection in clinically node-negative patients with breast cancer: overall survival findings from the NSABP B-32 randomised phase 3 trial. *Lancet Oncol.*, 2010; 11(10): 927-933.
31. Giuliano AE, Hawes D, Ballman KV, Whitworth PW, Blumencranz PW, Reintgen DS, Morrow M, Leitch AM, Hunt KK, McCall LM, Abati A, Cote R, Association of occult metastases in sentinel lymph nodes and bone marrow with survival among women with early-stage invasive breast cancer. *JAMA*, 2011; 306(4): 385-393.
32. Hwang ES, Lichtensztajn DY, Gomez SL Fowble B, Clarke CA, Survival after lumpectomy and mastectomy for early stage invasive breast cancer: the effect of age and hormone receptor status. *Cancer*, 2013; 119(7): 1402-1411.
33. van Maaren MC, de Munck L, de Bock GH, Jobsen JJ, van Dalen T, Linn SC, Poortmans P, Strobbe LJA, Siesling S, 10 year survival after breast-conserving surgery plus radiotherapy compared with mastectomy in early breast cancer in the Netherlands: a population-based study. *Lancet Oncol.*, 2016; 17(8): 1158-1170.
34. Lagendijk M, van Maaren MC, Saadatmand S, Strobbe LJA, Poortmans PMP, Koppert LB, Tilanus-Linthorst MMA, Siesling S, Breast conserving therapy and mastectomy revisited: breast cancer-specific survival and the influence of prognostic factors in 129,692 patients. *Int J Cancer*, 2018; 142(1): 165-175.
35. Gentilini OD, Cardoso MJ, Poortmans P, Less is more. Breast conservation might be even better than mastectomy in early breast cancer patients. *Breast*, 2017; 35: 32-33.
36. Nattinger AB, Gottlieb MS, Veum J, Yahnke D, Goodwin JS, Geographic Variation in the Use of Breast-Conserving Treatment for Breast Cancer. *N Engl J Med.*, 1992; 326: 1102-1107.
37. Shaziya Hassan Ali, Somashekhar, Arun Kumar, Rate of Breast-Conserving Surgery *vs* Mastectomy in Breast Cancer: a Tertiary Care Centre Experience from South India. *Indian J Surg Oncol.*, 2019; 10(1): 72-76.
38. Fisher S, Yasui Y, Dabbs K, Winget M, Re-excision and survival following breast conserving surgery in early stage breast cancer patients: a population-based study. *BMC Health Serv Res.*, 2018; 18(1): 94: 1-10.
39. Albain KS, Barlow WE, Ravdin PM, Farrar WB, Burton GV, Ketchel SJ, Cobau CD, Levine EG, Ingle JN, Pritchard KI, Lichter AS, Schneider DJ, Abeloff MD, Henderson IC, Muss HB, Green SJ, Lew D,

Livingston RB, Martino S, Osborne CK, Breast Cancer Intergroup of North America, Adjuvant chemotherapy and timing of tamoxifen in postmenopausal patients with endocrine-responsive, node-positive breast cancer: a phase 3, open-label, randomised controlled trial. *Lancet*, 2009; 374(9707): 2055-2063.

40. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, Baehner FL, Walker MG, Watson D, Park T, Hiller W, Fisher ER, Wickerham DL, Bryant J, Wolmark N, A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med.*, 2004; 351(27): 2817-2826.
41. Sparano JA, Gray RJ, Makower DF, Pritchard KI, Albain KS, Hayes DF, Geyer CE Jr, Dees EC, Perez EA, Olson JA Jr, Zujewski JA, Lively T, Badve SS, Saphner TJ, Wagner LI, Whelan TJ, Ellis MJ, Paik S, Wood WC, Ravdin P, Keane MM, Gomez Moreno HL, Reddy PS, Goggins TF, Mayer IA, Brufsky AM, Toppmeyer DL, Kaklamani VG, Atkins JN, Berenberg JL, Sledge GW, Prospective Validation of a 21-Gene Expression Assay in Breast Cancer. *N Engl J Med.*, 2015; 373: 2005-2014.
42. Malhotra V, Dorr VJ, Lyss AP, Anderson CM, Westgate S, Reynolds M, Barrett B, Perry MC, Neoadjuvant and adjuvant chemotherapy with doxorubicin and docetaxel in locally advanced breast cancer. *Clin Breast Cancer*, 2004; 5(5): 377-384.
43. Lee KS, Ro J, Nam BH, Lee ES, Kwon Y, HS Kwon, Chung KW, Kang HS, Kim EA, Kim SW, Shin KH, Kim SK, A randomized phase-III trial of docetaxel/ capecitabine *versus* doxorubicin/cyclophosphamide as primary chemotherapy for patients with stage II/III breast cancer. *Breast Cancer Res Treat.*, 2008; 109(3): 481-489.
44. Ayers M, Symmans WF, Stec J, Damokosh AI, Clark E, Hess K, Lecocke M, Metivier J, Booser D, Ibrahim N, Valero V, Royce M, Arun B, Whitman G, Ross J, Sneige N, Hortobagyi GN, Pusztai L, Gene Expression Profiles Predict Complete Pathologic Response to Neoadjuvant Paclitaxel and Fluorouracil, Doxorubicin, and Cyclophosphamide Chemotherapy in Breast Cancer. *J Clin Oncol.*, 2004; 22(12): 2284-2293.
45. Earl HM, Vallier AL, Hiller L, Fenwick N, Young J, Iddawela M, Abraham J, Hughes-Davies L, Gounaris I, McAdam K, Houston S, Hickish T , Skene A, Chan S, Dean S, Ritchie D, Laing R, Harries M, Gallagher C, Wishart G, Dunn J, Provenzano E, Caldas C, Neo-tAnGo Investigators, Effects of the addition of gemcitabine, and paclitaxel-first sequencing, in neoadjuvant sequential epirubicin, cyclophosphamide, and paclitaxel for women with high-risk early breast cancer (Neo-tAnGo): an open-label, 2x2 factorial randomised phase 3 trial. *Lancet Oncol.*, 2014; 15(2): 201-212.
46. Gadi VK, Davidson NE, Practical Approach to Triple-Negative Breast Cancer. *J Oncol Pract.*, 2017; 13(5): 293-300.
47. Aysola K, Desai A, Welch C, Xu J, Qin Y, Reddy V, Matthews R, Owens C, Okoli J, Beech DJ, Piyathilake CJ, Reddy SP, Rao VN, Triple Negative Breast Cancer – An Overview. *Hereditary Genet.*, 2013; 2013(Suppl 2): 001: 1-7.
48. Prutianu I, Căruntu ID, Manole MB, Giuşcă SE, Profire B, Timofte AD, Profire l, Gafton B, Developments in prostate cancer therapy: from the androgen receptor to antiandrogens and beyond. *Farmacia*, 2021; 69(3): 389-395.
49. Gonzalez-Angulo AM, Litton JK, Broglio KR, Meric-Bernstam F, Rakkhit R, Cardoso F, Peintinger F, Hanrahan EO, Sahin A, Guray M, Larsimont D, Feoli F, Stranzl H, Buchholz TA, Valero V, Theriault R, Piccart-Gebhart M, Ravdin PM, Berry DA, Hortobagyi GN, High risk of recurrence for patients with breast cancer who have human epidermal growth factor receptor 2-positive, node-negative tumors 1 cm or smaller. *J Clin Oncol.*, 2009; 27(34): 5700-5706.
50. Gianni L, Pienkowski T, Im YH, Roman L, Tseng LM, Liu MC, Lluch A, Staroslawska E, de la Haba-Rodriguez J, Im SA, Pedrini JL, Poirier B, Morandi P, Semiglazov V, Srimuninnimit V, Bianchi G, Szado T, Ratnayake J, Ross G, Valagussa P, Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. *Lancet Oncol.*, 2012; 13(1): 25-32.
51. Martin M, Holmes FA, Ejlertsen B, Delaloge S, Moy B, Iwata H, von Minckwitz G , Chia S, Mansi J, Barrios CH, Gnant M, Tomašević Z, Denduluri N, Šeparović R, Gokmen E, Bashford A, Ruiz Borrego M, Kim SB, Hugger Jakobsen E, Ciceniene A, Inoue K, Overkamp F, Heijns JB, Armstrong AC, Link JS, Abraham A, Bryce R, Wong A, Moran S, Yao B, Xu F, Auerbach A, Buyse M, Chan A, ExteNET Study Group, Neratinib after trastuzumab-based adjuvant therapy in HER2-positive breast cancer (ExteNET): 5-year analysis of a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol.*, 2017; 18(12): 1688-1700.
52. Cameron D, Piccart-Gebhart MJ, Gelber RD, Procter M, Goldhirsch A, de Azambuja E, Castro G Jr, Untch M, Smith I, Gianni L, Baselga J, Al-Sakaff N, Lauer S, McFadden E, Leyland-Jones B, Bell R, Dowsett M, Jackisch C, Herceptin Adjuvant (HERA) Trial Study Team, 11 years' follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive early breast cancer: final analysis of the HERceptin Adjuvant (HERA) trial. *Lancet*, 2017; 389(10075): 1195-1205.
53. Baselga J, Bradbury I, Eidtmann H, Di Cosimo S, de Azambuja E, Aura C, Gómez H, Dinh P, Fauria K, Van Dooren V, Aktan G, Goldhirsch A, Chang TW, Horváth Z, Coccia-Portugal M, Domont J, Tseng LM, Kunz G, Sohn JH, Semiglazov V, Lerzo G, Palacova M, Probachai V, Pusztai L, Untch M, Gelber RD, Piccart-Gebhart M, NeoALTTO Study Team, Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. *Lancet*, 2012; 379(9816): 633-640.
54. Guarneri V, Frassoldati A, Bottini A, Cagossi K, Bisagni G, Sarti S, Ravaioli A, Cavanna L, Giardina G, Musolino A, Untch M, Orlando L, Artioli F, Boni C, Generali DG, Serra P, Bagnalasta M, Marini L, Piacentini F, D'Amico R, Conte P, Preoperative chemotherapy plus trastuzumab, lapatinib, or both in human epidermal growth factor receptor 2-positive operable breast cancer: results of the randomized phase II CHER-LOB study. *J Clin Oncol.*, 2012; 30(16): 1989-1995.

55. Vici P, Viola G, Rossi S, Botti C, Vitucci C, Sergi D, Ferranti FR, Saracca E, Di Lauro L, Corsetti S, Foggi P, Fattoruso SIS, Lopez M, Optimal sequence of anthracyclines and taxanes as adjuvant breast cancer treatment. *Clin Ter.*, 2008; 159(6): 453-456.
56. Kellokumpu-Lehtinen P, Tuunanen T, Asola R, Elomaa L, Heikkinen M, Kokko R, Järvenpää R, Lehtinen I, Maiche A, Kaleva-Kerola J, Huusko M, Möykkynen K, Ala-Luhtala T, Weekly paclitaxel—An effective treatment for advanced breast cancer. *Anticancer Res.*, 2013; 33(6): 2623-2627.
57. Van Vuuren RJ, Visagie MH, Theron AE, Joubert AM, Antimitotic drugs in the treatment of cancer. *Cancer Chemother Pharmacol.*, 2015; 76(6): 1101-1112.
58. Merino Almazán M, Duarte Pérez JM, Marín Pozo JF, Evaluation of docetaxel use in non-small cell lung cancer: real-world health outcomes. *Farmacia*, 2021; 69(3): 419-425.
59. Wang H, Vo T, Hajar A, Li S, Chen X, Parissenti AM, Brindley DN, Wang Z, Multiple mechanisms underlying acquired resistance to taxanes in selected docetaxel-resistant MCF-7 breast cancer. *BMC Cancer*, 2014; 14: 37: 1-15.
60. Childs S, Ling V, The MDR superfamily of genes and its biological implications. *Important Adv Oncol.*, 1994: 21-36.
61. McGrogan BT, Gilmartin B, Carney DN, McCann A, Taxanes, microtubules and chemoresistant breast cancer. *Biochim Biophys Acta*, 2008; 1785(2): 96-132.
62. Sudo T, Nitta M, Saya H, Ueno NT, Dependence of paclitaxel sensitivity on a functional spindle assembly checkpoint. *Cancer Res.*, 2004; 64(7): 2502-2508.
63. Yang N, Wang C, Wang J, Wang Z, Huang D, Yan M, Kamran M, Liu Q, Xu BL, Aurora kinase A stabilizes FOXM1 to enhance paclitaxel resistance in triple-negative breast cancer. *J Cell Mol Med.*, 2019; 23(9): 6442-6453.
64. Rouzier R, Rajan R, Wagner P, Hess KR, Gold DL, Stec J, Ayers M, Ross JS, Zhang P, Buchholz TA, Kuerer H, Green M, Arun B, Hortobagyi GN, Symmans WF, Pusztai L, Microtubule-associated protein tau: A marker of paclitaxel sensitivity in breast cancer. *Proc Natl Acad Sci USA.*, 2005; 102(23): 8315-8320.
65. Abu Samaan T, Samec M, Liskova A, Kubatka P, Büsselberg D, Paclitaxel's Mechanistic and Clinical Effects on Breast Cancer. *Biomolecules*, 2019; 9(12): 789: 1-22.