Thesis

Residual Cancer Burden as a prognostic factor in breast cancer patients considering the outcome, different subtypes and dose-density of the neoadjuvant therapy

submitted by

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Graz, 25.07.2023

Declaration of Academic Integrity

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Zusammenfassung

Hintergrund: Der RCB-Score (residual cancer burden) ist ein vielversprechender prognostischer Marker mit klinischer Relevanz für Brustkrebs-PatientInnen, die eine neoadjuvant Chemotherapie erhalten haben. In dieser Studie wurde der RCB-Score unabhängig als prognostischer Marker in einer erweiterten Kohorte evaluiert. Zusätzlich wurde ein möglicher Zusammenhang zwischen dem RCB-Score und der Dosisreduktion der neoadjuvanten Chemotherapie als ein explorativer Endpunkt analysiert.

Material und Methoden: In dieser retrospektiven Studie wurden die Follow-up Daten zum Rezidiv-freien Überleben (HR), distantem krankheitsfreien Überleben (DDSF) und Gesamtüberleben (OS) von 367 Brustkrebs PatientInnen, die an der Klinischen Abteilung für Onkologie am Universitätsklinikum Graz therapiert worden sind, analysiert. Um eine mögliche Interaktion zwischen Dosisreduktion von der neoadjuvanten Chemotherapie und dem RCB-Score festzustellen, wurden standardisierte kumulative Dosen von Anthrazyklinen und Taxanen berechnet.

Ergebnisse: Unabhängig von den Subtypen von Brustkrebs war ein höherer RCB-Score mit einem schlechteren klinischen Outcome assoziiert (HR für RFS = 1.60, 95% CI 1.33-1.93, $p < 0.0001$; HR für DDFS = 1.70, 95% CI 1.39–2.05, $p < 0.0001$; HR für OS = 1.67, 95% CI 1.34–2.08, $p < 0.0001$). In dem Beobachtungszeitraum von 5 Jahren gab es keinen Unterschied des klinischen Outcomes zwischen RCB-Score 0 und 1.
49.1% aller PatientInnen (n=180) erhielten eine Dosisreduktion. In dieser Studie konnte ein signifikanter Zusammenhang zwischen einem höheren RCB-Score und einer Reduktion der Chemotherapie Dosis festgestellt werden (Interaktion p-Wert 0.042).

Fazit: Diese retrospektive Studie hat die Validität des RCB-Scores als unabhängigen, prognostisch relevanten Marker bestätigt. Weiters konnte gezeigt werden, dass eine Dosisreduktion der neoadjuvanten Chemotherapie mit einem höheren RCB-Score assoziiert ist. Diese Ergebnisse haben wichtige Implikationen für den klinischen Alltag.

Abstract

Background: The residual cancer burden (RCB) score demonstrates promising prognostic value with clinical significance in breast cancer patients treated with neoadjuvant chemotherapy. In this study, the RCB score was independently evaluated as a prognostic marker in an expanded cohort of patients. As a secondary endpoint, a potential association between the RCB score and the decrease in dosage of neoadjuvant chemotherapy was also analyzed.

Material and Methods: In this retrospective study, follow-up data on overall survival (OS), distant disease-free survival (DDFS), and recurrence-free survival (RFS) of 367 breast cancer patients treated at the Department of Oncology, University Hospital of Graz, Austria, were evaluated. In order to determine a possible interaction between dose reduction of neoadjuvant chemotherapy and the RCB score, standardized cumulative doses of anthracyclines and taxanes were calculated.

Results: Irrespective of the breast cancer subtype, a higher RCB score demonstrated a correlation with unfavorable clinical outcomes. The hazard ratios (HR) for RFS, DDFS, and OS were 1.60 (95% CI 1.33-1.93, $p < 0.0001$), 1.70 (95% CI 1.39-2.05, $p < 0.0001$), and 1.67 (95% CI 1.34-2.08, $p < 0.0001$) respectively. However, over the 5-year observation period, there was no detectable disparity in clinical outcomes between RCB scores 0 and 1. Additionally, 49.1% of all patients (n=180) experienced a dose reduction. This study found a notable correlation between a higher RCB score and a reduction in chemotherapy dose (interaction p-value 0.042).

Conclusion: The validity of the RCB score as an unrelated prognostic marker was confirmed in this retrospective study. Furthermore, reducing the dose of neoadjuvant chemotherapy was linked to higher RCB scores, a finding with important implications for clinical work.

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List of Abbreviations

AIs ..... aromatase inhibitors
AR ..... androgen receptor
A-T, A/T ..... anthrazycline + taxane
DDFS ..... distant disease-free survival
DSF ..... disease free survival
EFS ..... event free survival
ER ..... estrogen receptor
HR ..... hormone receptor, hazard rate
ICI ..... immune checkpoint inhibitors
IHC ..... immunohistochemical
NAC/NAT ..... neoadjuvant chemotherapy
OR ..... objective response
OS ..... overall survival
pCR ..... pathological complete remission
PD-L1 ..... programmed cell death ligand
PR ..... progesterone receptor
RCB ..... residual cancer burden
RFS ..... recurrence/residual free survival
TNBC ..... triple negative breast cancer

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1 Introduction

1.1. Entity of Breast Cancer

1.1.1. Histopathological classification

The histopathological classification depends on the growth pattern of breast cancer. Invasive ductal carcinoma has the highest prevalence with up to 75% of breast cancers being this subtype. The remaining 25% of all breast cancer are of a special type, which consists of at least 17 subtypes. Invasive lobular breast cancer is the most commonly encountered special type, with a prevalence ranging from 5% to 15%. (1)

1.1.2. Markers

The most important biomarkers in breast cancer are estrogen receptors (ER), progesterone receptors (PR), and HER2 (human epidermal receptor 2).
ER and PR status are determined by immunohistochemical (IHC) methods alone. While the PR Status does not confer from endocrine therapy, the ER status is clinically more relevant and helps to identify tumors that benefit from endocrine therapy. HER2 is detected by the combination of IHC and FISH assays. Detecting HER2 positive patients is important because these patients benefit from targeted therapies. (2)

1.1.3. Clinicopathological classification

The clinicopathological classification divides breast cancer into 5 subtypes depending on their expression of estrogen receptors, progesterone receptors, Ki-67, and HER2:

Subtypes	ER and PR	HER2	Ki-67
Luminal A	ER and/or PR positive	negative	low
Luminal B	ER and/or PR positive	negative	high
HER2 pos. Luminal B	ER and/or PR positive	positive	any
non luminal HER2+	absent	negative	/
triple negative	negative	negative	/

Table 1 Molecular Classification of breast cancer (2)

The most prevalent subtype is Luminal A, which is associated with a lower histopathological grading and better prognosis because of a low Ki-67 expression. Ki-67 is a nuclear marker produced in actively proliferating cells. A higher Ki-67 expression reflects a faster cell proliferation rate of malignant neoplasms and therefore a higher malignancy. However, Luminal A-type tumors show less sensitivity to chemotherapy. Generally speaking, luminal subtypes exhibit a more favorable prognosis due to their responsiveness to hormone receptors.
Since the breakthrough of targeted therapies for HER2-positive tumors, patients with this subtype have had a better overall outcome than before. (2, 3)
Triple-negative breast cancer occurs in 13% of all breast cancer and is determined by the deficiency of ER, PR, and HER2 expression and shows the worst prognosis of all subtypes. (2, 4)

1.2.Neoadjuvant systemic therapy

In the past neoadjuvant therapy was just used for patients who were expected to receive adjuvant systemic therapy and needed tumor shrinkage to improve surgical approaches, i.e., patients with regionally advanced breast cancer. However, in recent years, studies have revealed that neoadjuvant therapy has not only a positive impact on the extent and morbidity of curative surgery, increasing the probability of breast-conserving surgery but even more importantly could minimize the chance of metastatic disease. (5, 6)
Besides patients with inflammatory, unresectable, or locally advanced breast cancer neoadjuvant therapy should also be used in patients with triple-negative or high-risk HER2-positive breast cancer, where a residual tumor would lead to the recommendation of adjuvant therapy and escalation of treatment. Benefits from neoadjuvant therapy are downstaging the tumor, better resectability, better cosmetic outcome, and reduced in postoperative complications. (6)

1.2.1. Chemotherapy

The standard neoadjuvant treatment regimen is based on the combination of an anthracycline plus cyclophosphamide followed by a taxane. (7)

In patients with TNBC carboplatin can additionally be offered as a part of the neoadjuvant treatment, the decision should be based upon potential benefits and harms and should not be used routinely. (6, 8) Even though the GEPARSixto Study showed that the incorporation of carboplatin to chemotherapy led to a significant elevation in pCR rates and a significantly better DFS in TNBC, the effect on long-term outcome on OS was not significant. (9) The pCR rates improved up to 20% due to the addition of platinum but it is also important to note, that patients who were administered carboplatin had a higher probability to endure grade 3 and 4 hematological side effects (e.g. thrombocytopenia and neutropenia), which increased the number of patients that required a dose modification or treatment discontinuation in comparison to patients who did not receive carboplatin. (6) However especially in breast cancer dose-dense regimens and receiving full-dose chemotherapeutic agents seem to have a major influence on a positive long-term outcome. (10) Regimens that were based on anthracycline in combination with taxane or had a higher cumulative dose of anthracycline showed to reduce cancer mortality by about one-third. (11)

Patients with HER2-positive tumors benefit from combining standard neoadjuvant chemotherapy (i.e., anthracycline and taxane-based or non-anthracycline based) with the monoclonal antibodies trastuzumab and pertuzumab. The pCR rates are approximately twice as high when trastuzumab is added to the neoadjuvant regimen as compared to the standard chemotherapy regimen. However, the pCR rates increased further with the combination of trastuzumab with pertuzumab without increasing severe side effects. (6, 7, 12) While patients with TNBC certainly experienced advantages from the incorporation of carboplatin in the neoadjuvant setting and, more recently, from pembrolizumab, the data for the Her2-positive subtype are not clear. Nevertheless, it is reasonable to assume that neoadjuvant chemotherapy for Her2-positive breast cancer should include carboplatin. (9, 13, 14)

1.2.2. Endocrine Therapy

Endocrine therapy represents an additional treatment option for patients with HR-positive and HER2-negative breast cancer.

The administration of endocrine therapy is contingent on the menopausal status of the patient. (6) Premenopausal estrogen is produced primarily in the ovaries, whereas

postmenopausal estrogen is produced by the enzyme aromatase, which converts androgens to estrogen, primarily in peripheral muscle and adipose tissue. Endocrine therapy either works by obstructing the effect of estrogen at the receptor or by suppressing estrogen synthesis. (15)

There are different classes of endocrine therapy that use different mechanisms and therefore have different indications.
Tamoxifen, classified as a selective estrogen receptor modulator (SERM), exhibits an anti-estrogenic impact in mammary tissue and the vaginal mucosa, while concurrently demonstrating an estrogenic effect in other tissues, such as bones and the endometrium. Tamoxifen is indicated in pre-and postmenopausal women, because of its tissue-specific behavior.
Another important class are aromatase inhibitors (AIs). Those agents block the enzyme aromatase and consequently the synthesis of estrogen. This group can be divided into two subgroups: steroidal (exemestane) and nonsteroidal (anastrozole and letrozole) aromatase inhibitors. The difference between these two subgroups is that steroidal AIs are irreversible inhibitors of the enzyme, whereas non-steroidal AIs are reversible inhibitors of the enzyme.
AIs are indicated only in postmenopausal women because premenopausal inhibition of peripheral estrogen causes increased ovarian estrogen production. Other classes of endocrine therapy are tyrosine kinase inhibitors (TKIs), selective estrogen receptor down regulators (SERD), and luteinizing hormone-releasing hormone agonists (LHRH Agonists). (15)

While premenopausal patients should not generally be offered endocrine therapy as a neoadjuvant treatment option, postmenopausal patients may benefit from an aromatase inhibitor as a locoregional treatment choice. (6) One reason why neoadjuvant endocrine therapy is mainly used in postmenopausal women is that the majority of research on the use of neoadjuvant endocrine therapy was conducted on postmenopausal women.(16)
The IMPACT study compared the effect of preoperative endocrine therapy with anastrozole, tamoxifen, or a combination of these two therapeutic agents. The main focus of this study was the clinical objective response (OR) as the key endpoint. While there

were no significant differences within the groups, anastrozole still showed a significant downstaging of breast cancer and minimized the necessity of mastectomy. (17)
A Meta-Analysis of 20 trials conducted by Spring et al confirmed that aromatase inhibitors were more effective than tamoxifen and showed a significantly higher clinical and radiological response rate. (18)
Even though small clinical trials showed that HR-positive breast cancer exhibited a similar outcome after endocrine therapy as to chemotherapy. However, none of the trials reached pCR rates over 10%. (8) The role of endocrine therapy in the neoadjuvant setting is not fully understood as there are still open questions about the rates of pCR, the application in premenopausal women, and the optimal length of treatment. (15, 16)

1.2.3. Immunotherapy

Especially for patients with TNBC immunotherapy is expected to play a significant role in future treatment possibilities. Currently, the treatment approach for TNBC consists of surgery, chemotherapy, and radiation. In comparison to other types of breast cancer this treatment lacks the targeted precision, which is also noticeable in reduced OS, higher recurrence rate, higher risk of metastasis, and a worse outcome. (19)
Currently, immune checkpoint inhibitor (ICI) treatment is limited to advanced PD-L1 (programmed cell death ligand 1)-positive TNBC and more recently to early-stage neoadjuvant triple-negative breast cancer. There are multiple trials that review the future role of ICIs in neoadjuvant therapy in combination with chemotherapy. However, the results are not very conclusive. For example, the KEYNOTE-522 study shows that PD-L1 status in localized breast cancer occurs to be a general marker of therapy response because PD-L1 positive tumors exhibit a better response to neoadjuvant therapy irrespective of the use of immunotherapy. (20) KEYNOTE-522 also demonstrated that with the use of pembrolizumab patients experienced improvement irrespective of PD-L1 Status. The study demonstrated that incorporating pembrolizumab significantly enhances pCR rates in TNBC. However, it was also observed that the rate of treatment-related adverse effects was higher as a result. To conclude, immune checkpoint inhibitors have progressively become part of routine therapeutic choices for patients with early-stage TNBC. (6) However, this occurred after the conduction of the current study, and the findings of our study are not impacted by the addition of pembrolizumab.

1.3. Outcome

1.3.1. Remission rates for HER2-positive and triple-negative breast cancer

Aggressive breast cancer subtypes showed the highest correlation between pathological complete remission and long-term outcomes. Pathological complete remission is defined as no remnant invasive tumor in the operative preparation of the primary breast or lymph node, which would translate to ypT0/is and ypN0 according to the TNM classification. Achieving pCR is important because it is associated with an improved DFS. (4, 21)

HER2-positive/HR-negative breast cancer showed a significant association with pCR and achieved the highest pCR rates after NAC in combination with trastuzumab (50.3%), without the addition of trastuzumab the pCR rate is around 30.2%. HER2-positive/HR-positive breast cancer showed lower rates of pCR and no statistically significant association with pCR. The NeoSphere trial showed that pCR rates improved further with dual HER-2 blockade consisting of trastuzumab in combination with pertuzumab. (4, 21, 22)

In TNBC, high rates of pCR were also observed (33.6%) after standard neoadjuvant chemotherapy (NAC). However, the inclusion of carboplatin or bevacizumab in the NAC regimen significantly enhances the likelihood of achieving pCR. (4, 23) In comparison, less aggressive breast cancer subtypes (HR-positive subtypes) showed the lowest pCR rates (around 7.5%). (4)

1.3.2. Relevance of complete remission

At present pathologic complete remission is used as a clinical guideline for the decision-making of adjuvant treatment options. Patients who attain complete remission following neoadjuvant therapy demonstrate a notably improved prognosis in comparison to those with residual disease. The achievement of pCR with neoadjuvant chemotherapy is a good prognostic factor. Especially patients with HER2-positive breast cancer, who were administered trastuzumab in the neoadjuvant setting, or with TNBC showed the most beneficial outcomes after pCR. (6, 21)

The CTNeoBC analysis compared 12 international trials of neoadjuvant breast cancer treatment for the association between pCR. In all studies, there was consistent evidence indicating that patients who achieved pCR exhibited longer overall survival (OS) and event-free survival (EFS) in contrast to patients with residual disease. Patients with high-grade carcinomas had a stronger association between pCR and the outcome.
Regardless of HR status, there was a correlation between pCR and long-term outcome in HER2-positive patients. It is noteworthy that there was no correlation between a greater prevalence of pCR and the effect of treatment on OS and EFS in this trial-level analysis. Pathological remission is not considered a prognostic factor in HER2-positive breast cancer, as well as luminal A and luminal B breast cancer. (21)
Patients with TNBC have significantly higher pCR rates than other subgroups. (24). However, in the long-term outcome there is still a great potential for improvement, and the hope to establish novel targeted therapy options and improve knowledge on the heterogeneity and aggressive mechanisms of this subtype. (4) While there is a significant decrease in OS in TNBC patients with residual disease compared to other subtypes with residual disease, the results do not indicate any significant difference in patients who achieved pCR. (24) The heterogeneous response of TNBC to NAC implies the presence of distinct subtypes within TNBC. Currently, 6 subgroups are classified by gene expression. For example, Androgen-receptor-positive (AR) While the AR-positive subtype exhibited a significantly worse response to neoadjuvant chemotherapy (NAC), TNBC still showed a more favorable prognosis and survival compared to AR-negative TNBC (4)
Achieving pCR is very important in patients with TNBC, as demonstrated by the fact that in the first three years after neoadjuvant therapy the survival of patients who were unable to reach pCR is significantly worse compared to those who did. (24)

However, another systematic review concluded that pCR is not a suitable substitute endpoint in NAT trials. It was suggested to distinguish between prognosis- and biology-related pCR. Biology-related pCR does not influence on survival rates, it is dependent on NAT and only indicates pathological disease. Whereas prognosis-related pCRs seem to be independent of NAT, are associated with an improved of OS and DSF but are limited to a subgroup of patients with initially good prognosis. With this distinction, it is possible to explain, why patients with pCR had improved survival at the individual level, while there

was no link between pCR and overall survival at trial level. (25) Recent studies have shown a substantial link between pCR and long-term outcome parameters in TNBC and Her2-positive breast cancer. Patients with these subtypes who achieve pCR have the best outcome. (21, 26)

Achieving pCR after the use of NAC also increases the possibility of breast-conserving therapy. (27) Furthermore, extended follow-up examinations demonstrated that preoperative chemotherapy elevated the likelihood of breast-conserving surgery (BCS) while maintaining comparable rates of locoregional recurrence. (28, 29)

1.4. Residual Cancer Burden

As described above pathological complete remission is an excellent marker to validate the efficacy of neoadjuvant chemotherapy but cannot be used as a surrogate endpoint for enhanced DFS and OS in the individual studies. (30) For the evaluation of neoadjuvant trials and the decision about adjuvant treatment it is important to have a standardized method to measure residual disease or pCR because throughout different studies there is no common method whether patients with in situ carcinoma, minimal residual disease or lymph node metastasis are classified as pCR.(30, 31)
In 2007, Symmans et al., therefore, developed the RCB-Score. This tool measures the residual cancer burden (RCB) based on the size of the primary tumor, the number of metastatic axillary lymph nodes, the sizes of the largest metastasis, and the cellularity of the residual tumor bed. (31, 32) The classification system categorizes patients as follows: RCB-0 for no residual disease, RCB-I for minimal residual disease, RCB-II for moderate residual disease, and RCB-III for extensive residual disease. (31) In comparison to other pathological classification systems, RCB was the only one that was a significant predictive factor for OS and distant disease-free survival for all four subtypes. (33)

1.4.1. Outcome

In the primary investigation conducted by Symmans et al., the RCB-Score was assessed in 382 patients who underwent neoadjuvant treatment for invasive breast cancer.

The study revealed a significant connection between the RCB score and the recurrence risk. The subgroups RCB-0 to RCB-III had a progressively worse prognosis, with each one unit increase the RCB score almost doubling the risk of recurrence.
Higher RCB values were overall associated with a higher risk of 5-year distant relapse. The increase was disproportionate in high RCB values after T/FAC chemotherapy. Patients who received adjuvant hormonal treatment had an overall lower risk of recurrence but also showed a gradual increase. (31)
In 2022 Yau et. al. published a multicenter pooled analysis involving 5161 patients to assess the link between RCB and the long-term outcome. The findings showed that across the entire population and in all cohorts, a higher RCB score demonstrated a significant correlation with a poorer long-term outcome, including reduced event-free survival and distant relapse-free survival. It is worth mentioning that RCB delivers prognostic information even if pCR has not been reached after NAT. (26)

1.4.2. Dependence on neoadjuvant therapy

Since relapse-free survival also depends on adjuvant treatment, Symmans et al. compared the relapse risk within groups with or without adjuvant treatment. Regardless of whether patients with RCB-0 and RCB-I after NACT received adjuvant hormone treatment the 5-year relapse-free prognosis was excellent. Patients with RCB-III had the worst prognosis: 13% of patients with hormone receptor-positive tumors had RCB-III after NACT and 40% of those relapsed within 5 years regardless of adjuvant hormonal treatment. However, within 27 months 9 out of 9 hormone-receptor-negative breast cancer patients with RCB-III all relapsed after NACT. (31)

1.4.3. Subtypes

In 2017, Symmans et al. conducted another study to identify the long-term prospect in each phenotypic subtype after NACT alone or with HER2-targeted therapy linked to the residual cancer burden. (32)
Not only were patients with triple-negative breast cancer more probable to achieve RCB-0 or RCB-I (45.7%), but they also had a good prognosis when achieving pCR with an estimated residual free survival (RFS) of 94% after 5 years and 86% after 10 years. (32,

34). TNBC who only achieved RCB-II or RCB-III after NACT had an inferior prognosis. The estimated RSF with RCB-II was 62% after 5 years and 55% after 10 years, while RCB-III had an estimated RFS of 26% after 5 years and 23% after 10 years. In TNBC, the RCB score was the sole independent prognostic factor for RFS. (32)

In this study, the prognosis of HER2-positive breast cancer was compared between NACT alone with NACT administered together with trastuzumab. Overall, the incorporation of HER2-targeted therapy was associated with a superior RCB class compared to NACT alone. (32) HER2-positive breast cancer patients were the most likely to achieve RCB-0 or RCB-I and those who achieved pCR with NACT + trastuzumab had a significantly better long-term prognosis in comparison to the other RCB classes with an estimated RFS of 95% at 5 and at 10 years. (32, 34) Although fewer patients had RCB-III (7% compared to 15% with NACT alone), they experienced a significantly elevated risk of early recurrence. NACT + trastuzumab with RCB-III had an estimated RSF of 21% after 5 years, while NACT alone had an estimated RSF of 47% after 5 years. The analysis showed that for patients who were treated with NACT + trastuzumab, the RCB score was the only independent prognostic factor. (32)
Patients with HR-positive/HER2-negative breast cancer showed the best prognosis while achieving pCR or RCB-I with an estimated RFS after 5 years of 88% (pCR) or 100% (RCB-I). (32) However, HR-positive breast cancer patients had a higher likelihood of achieving RCB-II or RCB-III and patients with RCB-III had a significantly worse estimated RFS of 70% after 5 years and 52% after 10 years. (32, 34) Surprisingly, pCR, RCB index, and pre-treatment clinical stage III cancer were found to be independent prognostic factors within the cohort of HR-positive/HER2-negative breast cancer patients. (32)

1.5. Previous study at the Medical University of Graz

In 2019, the Division of Oncology at the Medical University of Graz published a retrospective study to validate the residual cancer burden as a prognostic factor after neoadjuvant treatment. Over the course of 5 years (during the years 2011 and 2016) the data of 184 breast cancer patients were compiled and analyzed retrospectively.

Breast cancer subtypes and Ki-67 were discovered to be unrelated predictive markers of RCB. Low RCB scores were associated with TNBC, HER2-positive breast cancer, and high Ki-67. During the mean follow-up of 4 years, 43 patients had an event during RFS. Those 43 patients had on average a significantly higher RCB Score than the 141 event-free patients (2.43 vs. 1.39).

The analysis also confirmed that higher RCB scores are associated with worse RFS throughout all subgroups. The analysis of OS as a secondary endpoint also showed that patients with higher RCB scores (class III) had a prognostically worse outcome.

This study confirmed the RCB score as a valid prognostic marker. (35)

Therefore, we decided to perform further analyses in larger cohort of patients and evaluate in addition whether the administered chemotherapy doses correlated with the RCB.

2. Material and Methods

2.1. Trial Design

This study is an extension of a prior research validating the RCB-score as a prognostic tool that was conducted and published at the Division of Oncology, Department of Internal Medicine at the Medical University of Graz. (35) Ethical approval for this study was granted by the ethics committee of the Medical University of Graz (ethical approval number 31-212 ex 18/19). Data was collected from 410 patients (final cohort after exclusion n=367) who were treated with neoadjuvant chemotherapy at the Division of Oncology, Department of Internal Medicine at the Medical University of Graz between the years 2011 - 2020. Patient data were collected with MEDOCS®, the database used within this hospital, and physical medical charts, and subsequently were recorded with the web application REDCap®. (36, 37) The following data were collected:

- patient data
- tumor characteristics
- RCB score
- treatment details
- medical outcome (OS, DDSF and RFS)
- last known survival status (via Austrian Social Security Providers Association)

The Department of Pathology, Hospital Graz II conducted the pathological procedure and diagnosis as well as the calculation of the RCB score. Pretherapeutic histological characteristics were determined by core-cut biopsies and the RCB scores and classification after treatment were conducted on the operative sample. (35) The RCB score and RCB class were evaluated in a standardized manner using the RCB calculator available on the MD Anderson website. (31, 32, 38) After surgery, all patients underwent regular clinical visits and specific medical imaging taking place quarterly the first 3 years, biannually the following 2 years, and subsequently annually for a maximum follow-up duration of 10 years. (39)

2.2. Statistical Methods and Analysis

The focus of this study is to validate RCB as a predictive marker in a larger cohort with a prolonged follow-up time and the influence of neoadjuvant dose density on the RCB score. The Steep criteria were used to define the endpoints in a standardized way. (40) The primary endpoint was the five-year recurrence-free survival (RFS). RFS was characterized as the period between the time of definite surgery until local relapse or distant metastasis or mortality from any reason within the first five years after surgery. Five-year overall survival (OS) and five-year distant disease-free survival (DDFS) were among the secondary endpoints. The definition of DDFS was the date of definitive surgery to the first appearance of distant metastasis or mortality from any reason. On the other hand, OS was characterized as the period between definitive surgery and mortality from any reason or until survival was counted. (39)

To allow for comparison between the dose density of neoadjuvant chemotherapy, standardized cumulative doses were calculated using the multiplication factors of the three anthracyclines used (doxorubicin with an anthracycline multiplication factor aMF =1), Epirubicin (aMF = 0.67), and Myocet (aMF =1) and the three taxanes used (paclitaxel tMF = 1), nab-paclitaxel tMF = 0.64, and docetaxel tMF = 3.2). The doses of taxanes and anthracyclines were multiplied by their corresponding tMF and aMF. The cumulative doses of taxanes and anthracyclines were calculated individually for each patient and then adjusted based on the patient's body surface area, which was determined using the Dubois formula. Out of 124 HER2+ patients, 63.7% underwent neoadjuvant combined inhibition with pertuzumab and trastuzumab, while the remaining 36.3% received trastuzumab alone, which was the standard treatment at the time this study was conducted. (39)

Statistical analyses were conducted using Stata 16.1 (Stata Corp., Houston, TX, USA). Continuous variables were presented as medians [25th-75th percentile], and count data were expressed as absolute numbers (%). To compare the distribution of continuous variables between two groups, rank sum tests were used. The investigation into the association between two categorical variables involved the utilization of Pearson Chi-square and Fisher's exact tests (39) Simple and multiple linear regression models were examined to establish the relationship between the continuous RCB score and covariates including the dose density of anthracyclines and taxanes and the switching effect between them. Median follow-up time was calculated with the reverse Kaplan-Meier method. (41)

To make a comparison between the clinical outcome parameters OS and RFS Kaplan-Meier estimators were used and their functions among two or more groups were compared by utilizing log-rank tests. The relationship between prognostic factors (i.e., RCB scores and classes and the likelihood of metastasis or death) were computed with uni- and multivariable Cox proportional hazard models. With Roxston-Parmar models under proportional hazards time-dependent recurrence rate graphics based on RCB class were created. (39, 41, 42)

3. Results

3.1. General Data

3.1.1. Baseline Characteristics of Patients

Between 2011 and 2020 410 breast cancer patients received with preoperative systemic treatment at the Division of Oncology in Graz. Of these, 43 patients were excluded in total. 30 patients were excluded because they received monotherapy or a different therapeutic regimen, 7 were missing chemotherapy doses, 4 were missing follow-up data and 2 were missing breast cancer data (1 HER2 status and 1 ypN status). As seen in Figure 1, 367 patients were finally analyzed. All 367 participants were female; no male breast cancer patients were enrolled in this investigation.
At the initiation of neoadjuvant therapy, the median age of female patients was 54.6 years. (39) (**Figure 1**)

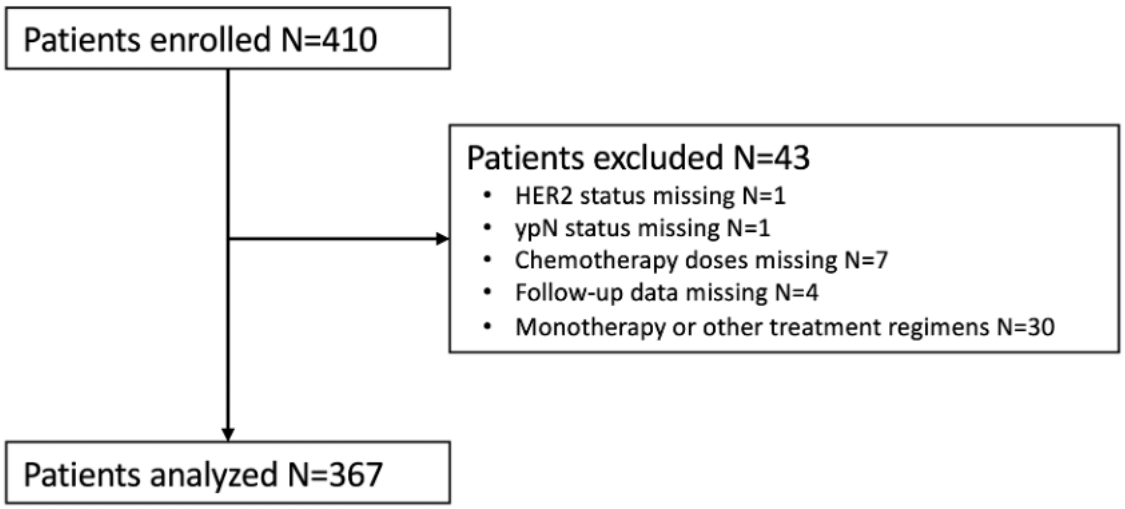

Figure 1 *Consort diagram (39)*

3.1.2. Primary Tumor Data

The most common breast cancer subtype was HR-positive/Her2-negative with 36.0% (n=132), followed closely by the HER2+ subtype with 34.6% (n=127). The rarest subtype was triple-negative breast cancer with a proportion of 29.4% (n=108). The histologic grading of the primary tumor was known for 358 patients; the most common grading was

G3 in 234 (65,4%) cases, followed by G2 in 121(33.8%) and just 3 (0.8%) cases with G1 grading.
The median Ki67 index of 366 patients was at 40% (27.5 – 70). (39)

3.1.3. Surgical Outcome and Adjuvant Therapy

The majority of patients (n=255, 69.5%) received breast conservation therapy, while only 112 (30.5%) underwent a mastectomy. In 241 cases (65.7%) axillary lymph node dissection was performed as definitive care of the axilla, while in 126 cases (34.4%) only the sentinel lymph node was removed. After surgery, tumor category and lymph node status were reassessed.
The most common post-neoadjuvant tumor category was ypT1 in 157 cases (42.8%), closely followed by ypTis-ypT0 in 140 cases (38.1%). In third place, there were 51 ypT2 cases (13.9%) and the lowest was ypT3-ypT4 in 19 cases (5.2%). In terms of post-neoadjuvant lymph node status, 265 patients (72.2%) had negative lymph node status after neoadjuvant chemotherapy (ypN0), 64 (17.4%) cases had ypN1 stage, 32 cases (8.7%) had ypN2 stage and only 6 cases (1.6%) had ypN3 stage.
The median RCB score was 1.52 (0.00-2.43). 123 patients (33.5%) achieved a pCR and therefore an RCB class 0, 47 patients (12.8%) achieved RCB class 1, 143 patients (39.0%) attained RCB class 2, and 54 patients (14.7%) were diagnosed with an RCB class 3.
Slightly over half of the patients (n=191, 52.0%) received adjuvant endocrine therapy, while 145 patients (39.5%) also received adjuvant chemotherapy ± anti-HER2 therapy. (39)

3.2. Connotation between Clinical Outcome and RCB Score

Over the median follow-up time of 4.1 years (five-year cut-off), 60 RFS, 56 DDFS, and 43 OS events were observed. Overall, 11 patients had local recurrence, 48 patients experienced distant metastases, and 48 patients died during the follow-up period. For the total trial population, the approximate five-year RFS rate was 73% (95%, CI: 66-79), the DDFS rate was 76% (95%, CI: 68-81), and the OS rate was 83% (95%, CI: 77-87).
When comparing cases who had a RFS event in the surveillance period to those who did not, it is apparent that those with a RFS event not only had a significantly increased

occurrence of mastectomy and axillary lymph node dissection but also significantly larger tumor sizes (ypT) and worse lymph node status (ypN) post-neoadjuvant. Similarly, within these cases, the median RCB score appeared to be significantly higher (2.21 vs. 1.33), along with a significantly higher RCB class on average. (39)

A univariable Cox regression model was employed to ascertain the hazard rates (HR) of RFS, DDSF, and OS. Higher RCB scores (per one-point increase) demonstrated significantly worse outcomes in RFS (HR = 1.60, $p < 0.0001$), DDFS (HR = 1.70, $p < 0.0001$), and OS (HR = 1.67, $p < 0.0001$). Other univariable predictors of significantly worse outcome in RFS, DDFS, and OS were high ypT stage (large tumor after neoadjuvant chemotherapy) and positive post-neoadjuvant lymph node status (high ypN stage), both of which are used in the calculation of RCB scores. As can be seen in Figure 2, a higher RCB class also exhibited a significantly worse outcome in all three outcome parameters. However, there was no considerable distinction in the clinical outcome between RCB class 0 and 1 after the five-year follow-up period. (39) (**Figure 2**)

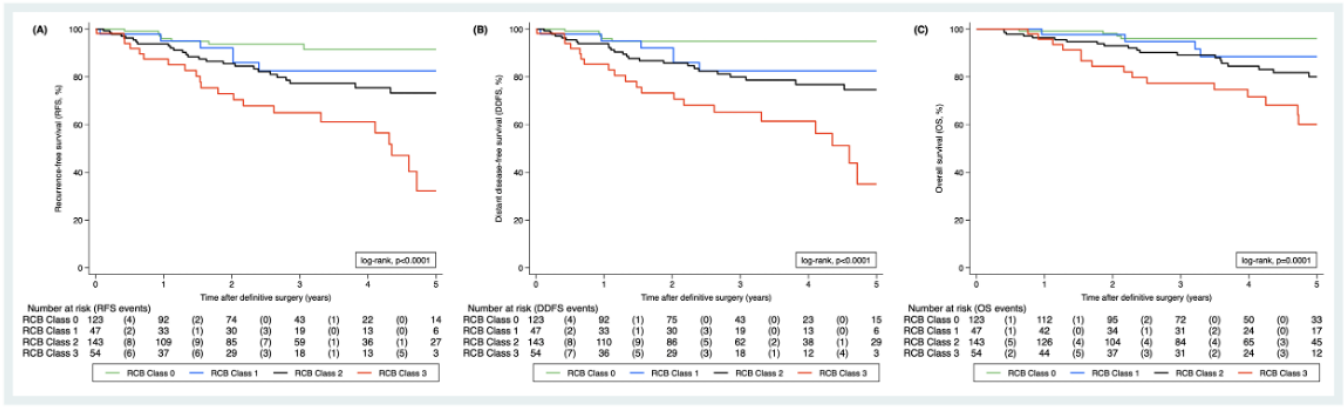

Figure 2 *Survival curves for (A) RFS, (B) DDSF, and (C)OS based on RCB class (39)*

Estimated recurrence rates within the five-year observation period were calculated using flexible parametric survival modeling. As can be seen in Figure 3(A), RCB class 0 tumors consistently showed a low risk for recurrence, as also shown above in the Kaplan-Meier curve. Tumors of RCB class 1 and 2 showed a slight peak after 1-2 years. RCB class 3 tumors showed a clear peak of recurrence after 1-2 years and the estimated recurrence rate was significantly increased during the entire follow-up period in comparison to the other RCB classes. (39) (**Figure 3**)

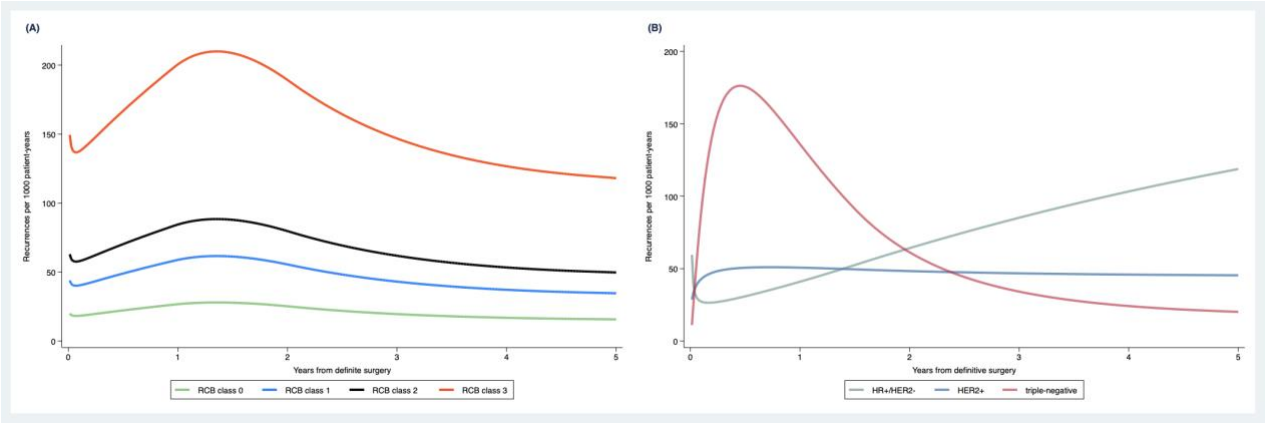

Figure 3 *projected relapse rates in relation to (A) RCB class and (B) subtypes 5 years after definitive surgery (39)*

3.3. Association of Tumor Subtypes and RCB Score

The univariable Cox regression model showed no significant difference in five-year RFS, DDFS, and OS among different tumor subtypes. In parametric survival analysis, patients with HR+/HER2- tumors showed a constant increase in the recurrence rate over the five-year surveillance period, while patients diagnosed with HER+ breast cancer demonstrated a relatively lower yet steadily increased recurrence rate. Patients with triple-negative tumors showed a peak in recurrence after one year, but their recurrence rate overall decreased over the remainder of the observation period, as seen in chart 3(B). As seen in Figure S1, the distribution of RCB scores varies between subtypes. An interaction test found no significant association between molecular subtype and RCB score. While HER2+ (HR=1.71, $p = 0.003$) and triple negative (HR = 2.14, $p < 0.001$) subtypes were found to have a significantly worse RFS with an increase in RCB score, the HR+/HER2-(HR = 1.44, $p = 0.056$) subtype does not have this significant impact. According to linear regression analysis, the most significant indicators of a favorable RCB score are triple-negative breast cancer, HER2-positive breast cancer, a high Ki67 index, and high tumor grading G3. Ki67 and subtypes appeared to be the only independent predictors according to a multivariable regression model. (39) (**Figure S1**)

3.4. Association of Neoadjuvant Chemotherapy Dose Modification and RCB Score

An exploratory analysis was conducted to investigate whether reducing the dose of neoadjuvant A/T chemotherapy was linked to a higher RCB score. In the entire study population, dose reduction of neoadjuvant chemotherapy was recorded in 180 patients (49.1%). In a multiple linear regression model, a significant p-value of 0.047 was observed in an interaction term for dose modification of A/T chemotherapy. This suggests that when the amount of dose reduction is small, the potential negative effects on neoadjuvant treatment are small, and vice versa. (**Figure 4**) In another multivariable regression analysis where the Ki67 index and breast cancer molecular subtype were included, the interaction term of cumulative A/T dose reduction stayed statistically meaningful with a p-value of 0.042. (39)

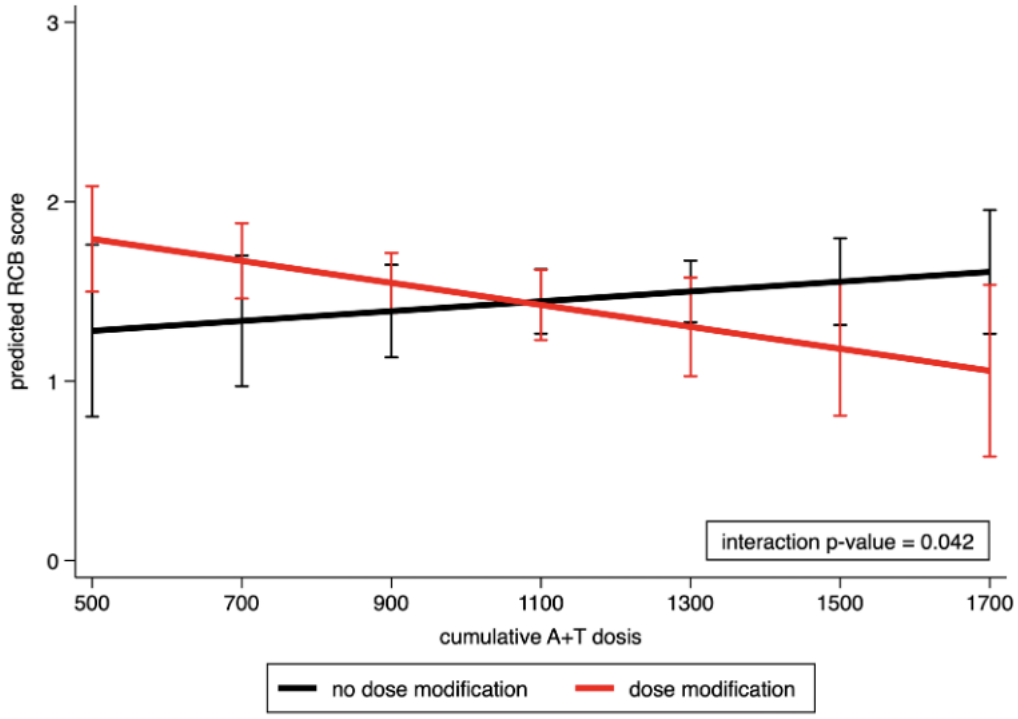

Figure 4 *RCB Score and dose density of neoadjuvant A+T therapy (39)*

4. Discussion

In this retrospective study, the RCB scores of 367 patients were evaluated. After neoadjuvant chemotherapy, 33.5% achieved an RCB score of 0 or pCR. 12.8% achieved an RCB score of 1, 39% an RCB score of 2, and 14.7% an RCB score of 3. The median RCB score was 1.54. 60% of the total patients had G3 differentiated tumor and the median Ki67 index was 40%. (39) These results were confirmatory of the findings of the first study by Müller et al. conducted at the Department of Oncology, Medical University of Graz, in 2019. In this study, data from 184 patients were analyzed. The RCB score had a median value of 1.57, with 28% of patients achieving an RCB score of 0, 16% an RCB score of 1, 39% an RCB score of 2, and 17% an RCB score of 3. 35%. 66% of the entire group had G3 differentiation and the median Ki67 was 35%. (35)

The primary outcome variable of our study was the clinical outcome. RFS, DDSF, and OS were observed over a 5-year follow-up period (median 4.1 years). The 5-year OS, DDFS, and RS rates for the complete research cohort were estimated to be 83%, 76%, and 73%. Patients who experienced an RFS event displayed a notably higher mean RCB score compared to patients who did not encounter an RFS event. (RCB score 2.22 vs. 1.81). In addition, patients with a higher RCB score (HR by a one-point increase) were associated with a significantly worse outcome in OS (1.67), DDSF (1.70), and RSF (1.60). (39) These results align with the findings from a multicenter pooled analysis of 5161 patients conducted by Yau et al. and published in 2022, where higher RCB scores were also significantly correlated with unfavorable outcomes, with HR per unit increase in RCB of 1.86 for DDSF and 1.82 for RFS. (26) In both studies, a ypT3 or ypT4 tumor stage was significantly correlated with an elevated hazard of an RSF event or a DDSF event, respectively. While histologic grade 3 was also significantly associated with an elevated chance of relapse in the study by Yau et al, we were unable to prove this in our study. (26, 39) The fact that our study had a much smaller population may explain this difference.

In our study population, breast cancer subtypes were distributed as 36% HR-positive/HER2 negative, 34.6% HER2 positive, and 29.4% triple negative.
Regarding the different subtypes, no statistically significant relationship was established between their specific outcome and the RCB score. However, the negative predictive

impact of increasing RCB score on recurrence-free survival (RFS) was found to be significant only in the triple-negative and HER2+ positive subtypes, while it was not observed in the HR+/HER- subtype. (39) These findings differ from the analysis of the I-SPY2 Randomized Clinical Trial by Symmans et al. published in November 2021. In their study, every subtype of breast cancer showed a significant worsening of EFS with an increase in the RCB score. (43) However, these findings are consistent with the analysis by Yau et al., which also found a significant association in all subtypes. (26) The lack of significance regarding the HR+/HER2- subtype may be, in part, due to the considerably smaller study size population, which may have limited the ability to detect such differences and the dynamics of relapse which in case of HR-positive disease tend to accumulate over time, and great proportion occurs after 5 years. (26, 39, 43)

The short follow-up of 5 years (mean 4.1 years) is another limitation of our study. Our analysis showed no significance in all three clinical outcome parameters during the follow-up period between RCB class 0 and class 1. However, there was a significant increase in the recurrence rate observed in the RCB class 3 group. (39) While Yau et al. confirmed the significantly increased risk of recurrence for RCB class 3 in all subtypes, they also demonstrated a significant distinction in the outcome between RCB class 0 and 1 for triple-negative and HR-positive/HER2-positive subtypes. This is in line with the expectations that each response correlates with the outcome. Notably, the HR-positive/HER2-positive subtype with targeted HER2 therapy did not show any difference between RCB class 0 and 1 after a 5-year follow-up, but only after a follow-up of 10 years. (26) This observation is particularly relevant for patients with HR-positive breast cancer, as this subtype has a higher incidence of late recurrence. (44, 45) Although this limitation of the duration of the follow-up is relevant for the HR-positive subtype, triple-negative and HER2-positive tumors tend to recur mainly in the first 5 years, as confirmed in our study. (39, 45-47) For these subtypes, the larger size of the study would allow for distinguishment of groups even with much smaller differences. This finding is also congruent with the view that ER-negative tumors benefit most from chemotherapy in the first three years. (47) On the other hand, ER-positive tumors are most likely to benefit from prolonged endocrine therapy. (48, 49)

An exploratory analysis aimed to assess the impact of dose reduction of neoadjuvant chemotherapy on the RCB score. (39) In breast cancer therapy, anthracyclines and taxanes

are still the most potent cytotoxic agents. (50-52) However, the optimal dose and timing of administering these drugs are still unknown. Our study revealed an association between the decrease in anthracycline- and taxane-based chemotherapy doses and an unfavorable RCB score. A significant interaction term suggests that the possible unfavorable effects of reducing dose on RCB score are likely to depend on the quantity of dose reduction.
During their neoadjuvant chemotherapy regimen, 49.1% of our patients underwent a dose reduction. (39) This result is similar to the observations of the retrospective study by Lyman et al, who analyzed data from more than 20,000 patients. In this study, 55% of all patients received a dose reduction during treatment. (53) The most common reasons for dose reductions are related to hematologic toxicity (e.g., neutropenia, thrombocytopenia, anemia). However, dose reductions without an objective medical reason or at the request of the patient occurred in many cases. (54, 55) A reduction in the dose intensity of A/T chemotherapy is associated with a worse survival outcome. (56, 57)
Theoretically, increasing dose intensity leads to decreased tumor cell growth and increased tumor cell killing, potentially resulting in a better outcome. (58-60) Dose intensity can be increased in several ways. The first option would be to escalate chemotherapy and administer a higher total amount of cytotoxic agents. However, escalation of anthracyclines beyond the standard dose did not show any apparent benefit, according to Henderson et al. (61) Other options for increasing dose intensity without increasing the total dose would be sequential scheduling or dose-dense administration (shorter intervals between treatment cycles) of chemotherapy. A meta-analysis comprising data from over 37,000 patients demonstrated that these two variants of drug administration resulted in improved outcomes (lower risk of 10-year recurrence and mortality) without an increase in mortality from other causes. (62)
However, it is crucial to highlight that the analysis of this exploratory endpoint in our study was conducted in a heterogeneous cohort of patients with a variety of neoadjuvant chemotherapy regimens, patient ages, and dose density and intensity. (39) Nevertheless, the trend demonstrating the decremental impact of dose reduction of systemic treatment on the response of patients, thereby potentially impacting the outcome of our patients strongly suggests that we should be very cautious when deciding to reduce treatment and rather evaluate other options of supportive therapy in order to continue the scheduled treatment and potentially improve their outcome.

5. Conclusion

The primary focus of this study was to validate the RCB score as a prognostic indicator for the identification of high-risk patients. This study demonstrated that the RCB score can be used as a predictor in clinical practice to select patients susceptible to recurrence who may profit from intensified adjuvant therapy and follow-up.
In addition, this study provided initial findings regarding the impact of dose modification or interruption of neoadjuvant chemotherapy on RCB score and prognosis.
In addition, initial data are collected in this study on the impact of dose density of neoadjuvant chemotherapy regarding the RCB score. In conclusion, at this point, changes in dose should be used with caution in clinical practice. However, a follow-up study is needed before the exact association with the RCB score can be determined.

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Appendix

Supplemental Figure

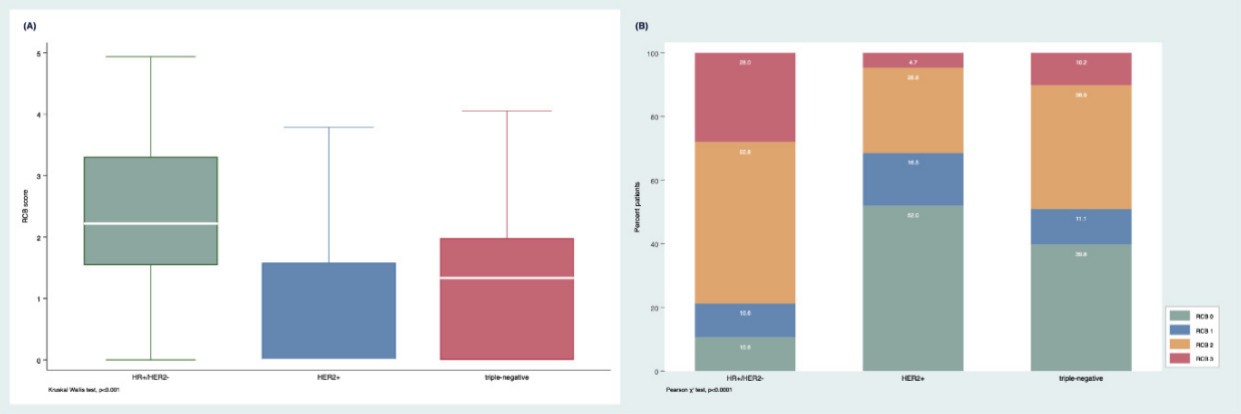

Figure S1 *Connection between breast cancer subtypes and (A) RCB-Score and (B) RCB Class (39)*