Diploma Thesis

Outcome of breast cancer patients treated with neoadjuvant therapy considering the Residual Cancer Burden

submitted by

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Statutory declaration

‘‘I hereby declare that this dissertation is my own original work and that I have fully acknowledged by name all of those individuals and organisations that have contributed to the re-search for this dissertation. Due acknowledgement has been made in the text to all other material used. Throughout this dissertation and in all related publications I followed the guidelines of “Good Scientific Practice”

Linz, 25.06.2017 *Gumpoldsberger Melanie eh.*

You're quite right, Mr. Hatter. I do live in a topsy-turvy world.
It seems like I have to do something wrong first, in order to learn from what not to do.
And then, by not doing what I'm not supposed to do, perhaps I'll be right.
But I'd rather be right the first time, wouldn't you?

unknown, Alice in wonderland

Zusammenfassung

Brustkrebs ist Ursache für die meisten tumorbezogenen Tode österreichischer Frauen. Die Abschätzung von Risikofaktoren und das Stellen von langfristigen Überlebensprognosen stellt hier eine Herausforderung für die behandelnden Ärzte dar. Der RCB-Score wurde eingeführt um den Erfolg von einer neoadjuvanten Chemotherapie zu messen und somit die Überlebenschancen besser beurteilen zu können. Das Ziel dieser Arbeit war es die Prognosefähigkeit des RCB-Scores über das krankheitsfreie Überleben (DFS) und Gesamtüberleben (OS) in einer unabhängigen Studie zu überprüfen.

212 BrustkrebspatientInnen, die eine neoadjuvante Therapie mit anschließender Operation im Landeskrankenhaus Graz erhalten hatten, wurden retrospektiv analysiert. Sowohl Chemotherapie, als auch Hormontherapie und HER2-gerichtete Therapien wurden inkludiert. Der RCB-score und die RCB-Gruppe (0, I, II, III) wurden nach Symmans et al. berechnet. Informationen über Alter, Histologie, Östrogen- und Progesteronrezeptordichte, HER2-Status, erhaltene Therapie und Nachsorge wurden den Patientenakten der Onkologie entnommen. Zur statistischen Analyse wurde Microsoft Excel® und IBM SPSS® verwendet. Kaplan-Mayer Kurven wurden zur Berechnung der Korrelation zwischen RCB-Score und DFS bzw. OS verwendet.

Mit diesen Berechnungen konnte die allgemeine Prognosefähigkeit des RCB-Scores für das DFS (p=0,000006) und OS (p=0,000306) statistisch bestätigt werden. Außerdem bestand ein Zusammenhang zwischen dem pathologischen T-Status und beiden Überlebensparametern (DFS p=0,0003 und OS p=0,005). Menopausenstatus, Hormonrezeptordichte, HER2-Status und histologischer Subtypen korrelierten hingegen weder mit DFS, noch mit OS.

Abstract

As breast cancer causes most tumor related deaths in Austrian women, the evaluation of risk and prognosis remains a challenge for physicians. Therefore, the RCB-score was introduced to measure response to neoadjuvant treatment and enable physicians to make more accurate prognoses on long-term survival. It was the goal of our research to independently test the prognostic value of the RCB-score on disease-free survival (DFS) and overall survival (OS).

A retrospective analysis was performed on 212 breast cancer patients who received neoadjuvant therapy and surgery at the Medical University of Graz. We included patients with neoadjuvant chemotherapy, endocrine therapy and HER2-directed therapy. The RCB-score and group (0, I, II, III) were calculated according to Symmans et al. Information about age, histological subtypes, receptor density, received regimen and follow-up was collected from paper-based files. Microsoft Excel® and IBM SPSS® were used for statistical examination. Kaplan-Mayer curves were used to calculate the correlation of the RCB-score with DFS and OS.

We were able to confirm the general prognostic value of the RCB-score to DFS ($p=0.000006$) and OS ($p=0.000306$). Furthermore, the pathological T-status correlated with both DFS ($p=0.0003$) and OS ($p=0.005$). In contrast, menopause status, endocrine-receptor density, HER2-status and histological subtype did not correlate with the survival factors.

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Abbreviations

A	doxorubicin
AI	aromatase inhibitors
C	cyclophosphamide
DCIS	ductal carcinoma in situ
DNA	deoxyribonucleic acid
DFS	disease free survival
E	epirubicin
EFS	event free survival
ER	estrogen receptor
F	fluorouracil
FISH	fluorescence in-situ hybridization
H	trastuzumab
HER2	human epidermal growth factor receptor 2
IDC	invasive ductal carcinoma
ILC	invasive lobular carcinoma
LCIS	lobular carcinoma in situ
OS	overall survival
PR	progesterone receptor
pCR	pathological complete response
RFS	relapse free survival
T	taxan

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1 Introduction

1.1 Epidemiology and Etiology

With 5.521 newly identified cases of breast cancer in 2012, breast cancer has become the prevalent tumor for Austrian women. In addition, breast cancer has been counted responsible for 1.528 deaths in 2012, making the cancer entity liable for the most tumor associated deaths in Austria.

The age-adjusted incidence accounted for more than 75 per 100.000 and has been consistent for 10 years. In addition, the mortality declined during the same period, now resting at 17 per 100.000, as can be seen in figure 1 (1, 2).

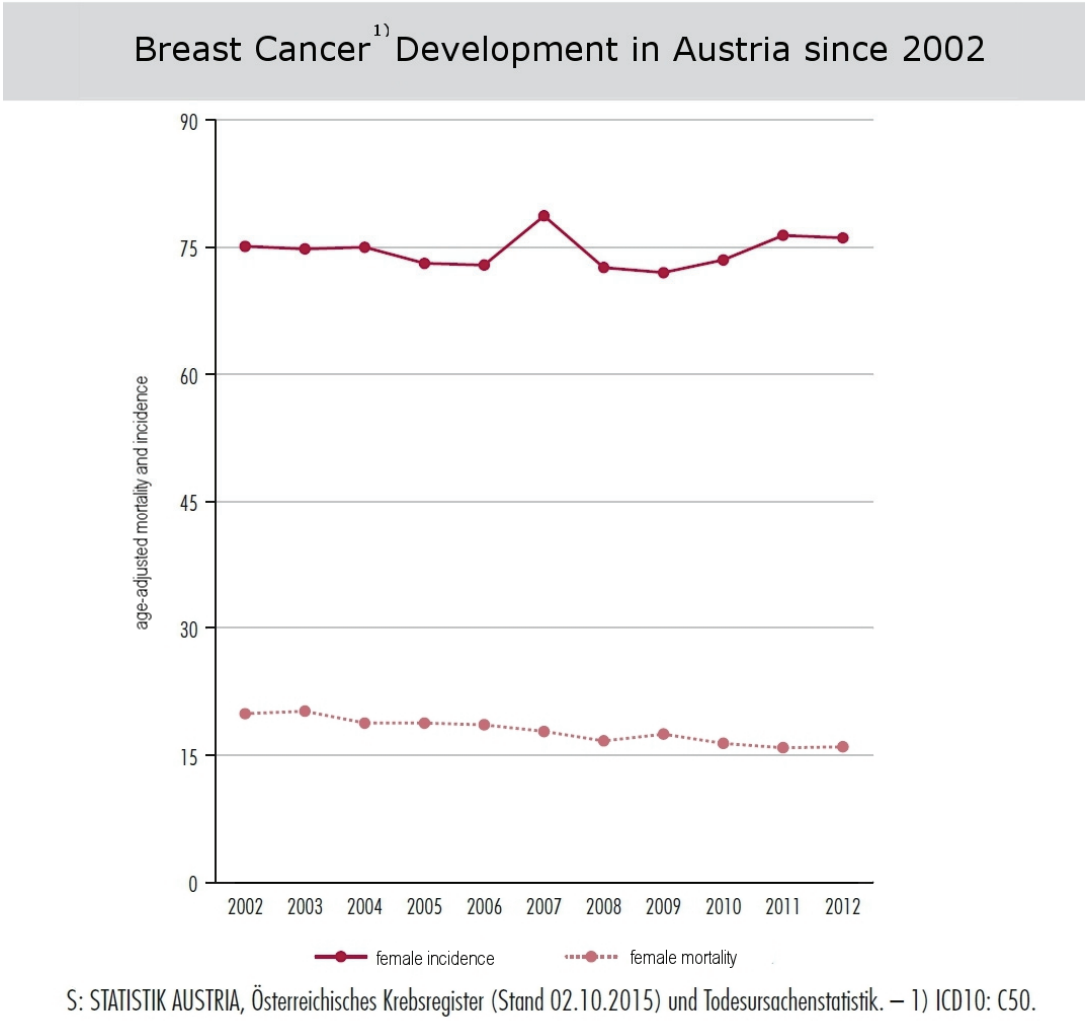

Figure 1: Statistik Austria - Incidence and mortality of malign neoformation of the female breast since 2002, modified according to Hackl, M. et al. (2).

The decrease in death rate is mainly awarded to mammography screening offered to women over 45 for early detection (3). Nevertheless, due to the persistent high prevalence of breast cancer, a good risk evaluation remains a prerequisite for a high quality of diagnosis and therapy, which can be pertained through better histological classification of residual cancer cells. The following paragraphs mean to describe the general factors needed to identify the risk evaluation in breast cancer.

Although the etiology of breast cancer has not fully been explored, the following factors have been verified to raise the risk of occurrence

- positive family history: breast- or ovary carcinoma in first degree relatives
- early menarche / late menopause: before the age of 12 and after 55
- nulliparous and late primiparous women, short phase of breastfeeding
- hormonal imbalance: especially higher estrogen levels
- obesity
- age, as frequency rises with age
- carcinoma of contra lateral side
- precursor lesions: ductal carcinoma in situ (DCIS)
- mutation of the BRCA1-, BRCA2- or p53-gene

(4–6).

1.2 Classification

Breast cancer is most likely to develop in the upper outer quadrant of the breast because there is more tissue located than in the other quadrants. One study described the location of around 60% of mamma carcinomas in the upper outer quadrant, 18 % in the lower inner quadrant, 15% in the upper inner quadrant and 7% in the lower outer quadrant (7).

1.2.1 Precursor Lesions

The WHO describes the carcinoma in situ (CIS) as the direct pre-stage of the invasive mamma cancer. Histologically CIS is defined as a neoplastic proliferation of malign epithelial cells without perforating the basal membrane. The CIS lies dissociated, independent of surrounding fat and conjunctive tissue, and only seldom infiltrates blood- or lymph vessels. Therefore, it does not metastasize (1, 8).

The **ductal carcinoma in situ** (DCIS) represents 95% of in situ lesions. It spreads segmental in the milk ducts and is usually found accidentally through microcalcifications in mammography. If the diagnosis is confirmed, surgical therapy is recommended, since about half of DCIS evolve into an invasive carcinoma (8–10).

The other 5% of in situ lesions show a proliferation of monomorphic cells in the lobules of the mamma, called **lobular carcinoma in situ** (LCIS).

1.2.2 Invasive Mamma Carcinoma

Invasive carcinoma of the breast is a malign epithelial tumor, defined by its ability to break through the basal membrane and infiltrate the surrounding tissue. It can metastasize through haematogenous and lymphatic vessels and leads to death in 30-40% of cases (1, 11).

The invasive ductal carcinoma (IDC) accounts for 80% of invasive growing breast cancers (12, 13). It develops in the epithelia of milk ducts and does not show a special histological growth pattern in 70%, thus it is not otherwise specified (NST). The following patterns of mamma carcinoma can be counted as invasive breast cancer and only make up a few percentages of all breast tumors:

- medullary carcinoma: often shows necrosis
- mucinous carcinoma: produces mucus
- tubular carcinoma: usually low-grade with tubular glands
- papillary carcinoma
- Paget's disease of the nipple
- inflammatory breast cancer

(1, 14, 15).

The **invasive lobular carcinoma** (ILC) is the infiltrative growing tumor of the glandular lobules, and with only 15% far rarer than the IDC. It is characterized by its dissociative growth and isolated cancer cells (12, 16).

1.2.3 Staging

Because stage of expansion is important for prognosis and further procedure, TNM-classification is performed on every kind of cancer. It shall be mentioned in advance, that both the pT and pN-status are used to calculate the RCB-score. For further information see chapter 1.4

pT	Primary tumor
pTX	tumor cannot be evaluated
pT0	no signs of tumor
pTis	carcinoma in situ
pT1	tumor ≤ 2cm in greatest dimension
pT2	tumor > 2cm but < 5cm
pT3	tumor > 5cm in greatest dimension
pT4	tumor of any size that invades skin or thoracic wall
pN	Regional lymph nodes, clinical
pNX	regional lymph nodes cannot be evaluated
pN0	no regional lymph node metastasis
pN1	metastasis to moveable ipsilateral level I and II axillary lymph nodes
pN2	Metastases in ipsilateral level I, II axillary lymph nodes that are clinically fixed or matted; or in clinically detected ipsilateral internal mammary nodes in the absence of clinically evident axillary lymph node metastases
pN3	Metastases in ipsilateral infraclavicular (level III axillary) lymph node(s) with or without level I, II axillary lymph node involvement; or in clinically detected ipsilateral internal mammary lymph node(s) with clinically evident level I, II axillary lymph node metastases; or metastases in ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymph node involvement
pM	Distant metastasis
MX	distant metastasis cannot be evaluated
M0	No clinical or radiographic evidence of distant metastases
M1	Distant detectable metastases as determined by classic clinical and radiographic means and/or histologically proven >0.2 mm

Table 1: Table 1: pTNM-classification, modified according to S3-guidelines 2012 (1, 17)

Before the start of breast cancer treatment medical contraindications against surgery are taken into consideration, such as pregnancy at diagnosis. Neoadjuvant therapy might be an option for those patients (18).
In addition neoadjuvant therapy should be taken into consideration if the tumor is primarily inoperable or if mastectomy would be required and the patient wishes to have breast-conserving surgery or in the case of a tumor with unfavourable biology.
Before administering systemic therapy, the gathering of receptor status is essential. In up to 20% of breast carcinomas human epidermal growth factor receptor 2 (HER2) is over expressed due to gene amplification. HER2 induces proliferation and suppresses apoptosis of carcinoma cells (19, 20).
Fluorescence in-situ hybridization (FISH) is a cytogenetic test that localizes DNA sequences. It is used to detect HER2-overexpression (21).
Furthermore, steroid hormones estrogen and progesterone support tumor growth, so it is reasonable to determine receptor density of estrogen (ER) and progesterone (PR) prior to therapy (1).

1.3 Neoadjuvant Therapy Options

Goal of every therapy is to reduce the chance of distant metastasis and consequently better overall survival (OS). Therefore, surgery used to be the means of choice. Systemic therapy before an operation can, however, oftentimes reduce the size of primary inoperable cancer to make extraction possible. Also, cosmetic outcome is positively influenced, since smaller operations are needed for resection.
Studies show that HER2 positive and triple negative (HER2 negative, ER negative, PR negative) breast-cancers are associated with a high likelihood of response to neoadjuvant therapy. Then again, HER2 negative, HR positive cancers less frequently show a significant reaction to neoadjuvant therapy and are, therefore, often initially treated with surgery (22–24).

1.3.1 Chemotherapy

Chemotherapy prevents growth of cells with high proliferation rate. Because cancer usually multiplies quickly, it is a well-aimed target. This systemic therapy can greatly minimize the risk of distant metastasis, however, because of the described mechanism, side-effects like alopecia, bone marrow suppression and mucosal damage are expectable (25, 26). Also, nausea and vomiting occur regularly.

Antineoplastic substances target tumor cells through one of three mechanisms:

- Damaging DNA

  Firstly, DNA is damaged by attaching an alkyl-group to the guanine of the DNA, which inhibits further proliferation of the cancer cell. Cyclophosphamide (C) is one of these alkylating agents and is regularly used for breast cancer treatment.

  Secondly, platinum-based agents cause crosslinking in the DNA or between DNA-strands. As a result, DNA-metabolism is damaged, leading to toxic effects for the whole cell. Carboplatin is a typical substance of this group used against cancer of the mamma, particularly recently in the treatment of triple negative disease (27).

  Thirdly, anthracycline antibiotics are among the most effective chemotherapeutics available and are regularly used to treat breast cancer. They prevent cell proliferation through integration into the DNA-strand, thus causing breakage in the DNA. They also inhibit the topoisomerase II enzyme, which is of great importance for proliferation overall.

  Most commonly used agents are doxorubicin (A) and epirubicin (E). However, both chemotherapeutics are cardiotoxic and can lead to neutropenia (28).

- Interference with DNA synthesis

  It is both possible to inhibit synthesis of DNA- building blocks and to incorporate wrong blocks into the newly formed DNA. 5-fluorouracil (F) is a very common pyrimidine antimetabolite. When built into the DNA instead of thymine or cytosine it results in inhibited proliferation (29).

- Interference with microtubules

  Microtubules are the main components of the mitotic spindles and, therefore, of great importance for effective cell proliferation. Paclitaxel and docetaxel both belong to the group of taxanes (T). Through binding onto the microtubule-beta-subunit they inhibit depolymerisation and thereby deformed microtubules are constructed. (30)

Chemotherapy can be administered both neoadjuvant, before surgery, and adjuvant, after surgery. Against the initial assumption that neoadjuvant chemotherapy would positively influence survival rates, there could be no difference detected in comparison to adjuvant therapy (31). However, neoadjuvant therapy does broaden the surgical options and often makes breast-conserving operations possible (32, 33).

It also enables the physician to evaluate the effectiveness of the treatment during an earlier stage and allows a sooner modification if necessary.

Usually, in neoadjuvant chemotherapy the same regimens are used as in an adjuvant setting. In both cases the choice should rest upon the tumor biology and the receptor status (33). If not contraindicated, anthracyclines are incorporated in common regimens such as FEC, FAC, EC and AC. These polychemotherapies were shown to improve OS-rates in comparison to non-anthracycline regimens (34, 35). However, an increased dose of anthracyclines exceeding recommendations did not result in higher survival rates and is therefore not administered (36).

Instead, anthracycline-based regimens can be combined with paclitaxel or docetaxel. Studies show that a simultaneous or sequential application of a taxane results in higher rates of pCR (also meaning higher rates of RCB-0) in comparison to anthracycline-based regimens without taxanes. These patients also had better DFS- and OS-rates (31).

Non-anthracycline regimens are commonly used if there is a contraindication against anthracyclines. These therapy options are, usually, well tolerated, resulting in less neutropenic and neurotoxic side effects (37, 38). Unfortunately, there is still no data about OS in the neoadjuvant setting available.

All regimens above can be and are regularly applied to women and men alike. However, studies suggest that men might profit even more from the use of endocrine therapy. (39)

1.3.2 Endocrine Therapy

Because some tumors overexpress receptors for estrogen and progesterone, there are treatment options which specifically target these tumor cells. For hormonal treatment there are two different mechanisms:

- Selective Estrogen Receptor Modulators

  Substances of this group react tissue-specifically, either antagonistic or agonistic. They attach to the DNA, thus changing conformation of the receptor-protein. Tamoxifen, a substance with antagonistic effects on the breast is most commonly used. In neoadjuvant therapy it can be used on ER positive tumors to reduce tumor size. In adjuvant therapy it is used for at least 5 years to reduce risk of recurrence. It is most often administered on premenopausal women and men with hormonal positive breast cancer. During application, estrogen-like side effects such as thrombotic embolisms or proliferation of the endometrium regularly occur. Also, it can accumulate in the lysosomes of cells, resulting in vision defects (40, 41).

- Aromatase Inhibitors (AI)

  Aromatase is an essential enzyme for estrogen synthesis. It catalyzes the aromatization of androgens into estrogens. Aromatase inhibitors are used in neoadjuvant and adjuvant treatment of ER positive breast cancers, especially in post-menopausal women. Compared to tamoxifen, these inhibitors don't show estrogen-like side effects. However, climacteric side-effects like hot flushes, sweating or dizziness have been described. Exemestan, letrozol and anastrozol are the most common substances; all three can be applied orally (42, 43).

The following treatment options are generally used on patients with positive hormone receptor-, but negative HER2 status. For HER2 directed therapy see chapter below.

Neoadjuvant hormonal therapy in post-menopausal HER2 negative women is a well-researched topic. Studies show, that neoadjuvant AI are as well or better tolerated than tamoxifen (44), resulting in less side-effects and a higher probability for breast-conserving surgery (45, 46). In comparison to neoadjuvant chemotherapy, neoadjuvant endocrine therapy reaches similar OS-rates with less toxic side-effects in post-menopausal women (47). It was, however, hypothesized that PR-status might influence survival-outcomes. This question remains for further investigation.
Unfortunately, there is only little data about the neoadjuvant usage and none about OS of endocrine therapy in premenopausal HER2 negative women.

Against the initial assumption that endocrine positive cancer in men might be similar to the equivalent in post-menopausal women, research shows fundamental differences. Positive hormonal status is more frequent in male than in female patients (48). However, since male breast cancer is generally less common, endocrine treatment options were long considered off-label-use.
Studies show that endocrine therapy in men resulted in better OS than chemotherapy (39). Also, men treated with tamoxifen reached better OS-rates than those treated with an AI (49). Therefore, treatment with tamoxifen represents the first-line option for adjuvant systemic therapy for this group.
AI may remain an option if there are any contraindications against tamoxifen (50).

1.3.3 HER2-directed Therapy

Up to 20% of breast carcinomas show an over-expression of HER2, providing a potent therapeutic target for anti Her2 therapies.
Trastuzumab (H) and pertuzumab are monoclonal antibodies, which bind onto the HER2 receptor, thus inducing its depletion (19, 20, 51). The apoptosis of tumor cells is initiated and angiogenesis is stopped.
However, during initial application patients can suffer from anaphylactic reactions. Also, as HER2 receptors are also located on the heart, the application can lead to toxic cardiac side-effects, especially if patients are also treated with anthracyclines (52, 53). Neutropenia is a seldom but potentially dangerous side-effect of pertuzumab (54).

Studies suggest that the additional use of trastuzumab in neoadjuvant therapy leads to higher pCR- and EFS-rates than chemotherapy-use only (55, 56).
HER2 positive, hormone receptor negative women seem to get the greatest benefit from being treated with trastuzumab. These women generally have a better OS and surgery-outcome than females with positive ER/PR-status (57).
Unfortunately, these promising results can often not be reached because of trastuzumab-resistance. Up to 60% of HER2 positive women are either initially resistant or develop resistance during treatment (58). This issue can partially be overcome by adding pertuzumab. Again, this incorporation results in higher pCR-rates, without adding major toxicity (54). If this, however, also leads to higher OS-rates is yet to be determined.
In a recent annual meeting of American Society for Clinical Oncology (ASCO) data from the adjuvant trial of adding pertuzumab to trastuzumab were shown, with a significant, but clinically not very meaningful impact on disease free survival (DFS).

1.4 Residual Cancer Burden

As neoadjuvant systemic therapy in breast cancer is still a topic of ongoing research, standardized methods to determine individual outcome have yet to be further established. In 2007 the MD Anderson Cancer Center in Houston TX, developed the RCB-score. The RCB was designed as prognostic factor on OS for patients who had received neoadjuvant chemotherapy.
For pathological evaluation of the success of a neoadjuvant treatment the tumor-tissue obtained at surgery had to be histologically examined. It was then compared to the biopsy, which was isolated at time of diagnosis. The pathologist then evaluated the following parameters in order to calculate the RCB-score (59):

- two dimensions of the primary tumor bed (mm)
- the cancer cellularity fraction (%) of invasive cancer and CIS
- the number of metastasis positive axillary lymph nodes
- diameter of the largest lymph node metastasis (mm)

The resulting score then divided the patient-population to one of four groups, with RCB-0 corresponding to a pathological complete response (pCR), RCB-I to

minimal residual disease, RCB-II to moderate residual disease and RCB-III to extensive residual disease.

Symmans et al were able to prove the prognostic significance of the RCB-score on OS. In their first trial 382 patients with invasive breast cancer were reviewed. All had received neoadjuvant chemotherapy with anthracyclines and/or taxanes. Study outcomes demonstrated a reverse correlation between survival-rates and the RCB-score. Participants who were rated as RCB-0 or RCB-I showed a significantly higher survival rate compared to patients in groups RCB-II and –III. (59,60). For patients who had reached pCR (RCB-0) the probability of relapse within the first five years was estimated at 5.4% and 2.4% for those with RCB-I. It was, in contrast, 53.6% for the RCB-III group (59).

Further studies confirmed that the usage of residual cancer measurements of the breast combined with those of the lymph nodes led to a better defined prognosis than either alone (61–63).

A second trial published in 2017 reviewed five patient cohorts with collectively 1158 patients. Three cohorts received T/FAC as neoadjuvant systemic therapy, one received FAC and one cohort received H+T/FEC. Also, all patients were classified by their phenotype in one of the subgroups HER2+, triple negative or HR+/HER2-.

Symmans et al were again able to show that the RCB-score is prognostic for OS after neoadjuvant systemic therapy for all three phenotype subsets.

For triple negative patients the estimated 10-year relapse-free survival (RFS) rates were 86% for the pCR-group, 81% for the RCB-I class, 55% for the RCB-II class and 23% for the RCB-III class. These results were similar in the H+T/FEC cohort, with 95% for the pCR-group and 77%, 47% and 21% for the RCB-classes I, II and III. For HR-positive / HER2-negative patients in the combined T/FAC cohorts the 10-year RFS was 83% for pCR, 97% for RCB-I, 74% for RCB-II and 52% for RCB-III. (64)

The researchers did, however, also express the need of further external validation of their findings.

1.5 Reason for this study

Although treatment options and outcomes for breast cancer have rapidly developed over the last years, it is still a challenge for modern medicine to timely diagnose and treat invasive breast cancer. Consistent therapy options are still being developed. It remains difficult for physicians to make a detailed prognosis about survival-rates. However, prognosis after a neoadjuvant systemic treatment is of great importance for the patient and the physician in charge.

The RCB-score is a relatively new tool developed in 2007 by Symmans et al to help physicians set a more accurate prognosis based on clinical and histopathological findings.

In the initial trial the RCB-score was only tested on patients who had undergone chemotherapy.

In this study the RCB-score will be tested on patients who have received any kind of neoadjuvant treatment, including chemo-, endocrine- and HER2-directed-therapy. The prognostic value on survival-rates regardless of the neoadjuvant approach will be researched.

Although this study is smaller than the original one, the patient population, all doctors and the writer are fully unattached to the study in 2007. This research shall independently test the prognostic ability of the RCB-score.

1.6 Research Questions

1. Do the various patients in the four RCB-groups show a difference in their progression-free- and overall-survival?
2. Can the menopausal-status, hormone- or HER2-receptor status, histological subtype or the TNM-classification influence survival outcomes?

1.7 Hypothesis

1. It is assumed that the RCB-score shows differences in prognosis for both DFS and OS in all four groups, although the patient population was extended to all neoadjuvant regimens compared to the original study from Symmans et al.
2. It is hypothesized that in addition to the RCB-score other factors are prognostic for the DFS- and OS.

2 Materials and Methods

2.1 Ethical review

Not all patients were still in the follow-up at Medical University of Graz during this retrospective analysis. The study was approved by the ethical review committee of Graz (EK-number: 29-075 ex 16/17) in November 2016.

2.2 Sample

This retrospective analysis included patients who were treated with neoadjuvant therapy at the Internal Medicine – Division of Oncology at Medical University of Graz. Both men and women were included in this trial. All participants were diagnosed with invasive breast cancer between 2011 and 2016, had received any sort of neoadjuvant treatment and were over 18 years old. All histological- and receptor-subtypes were included.
Patients who were diagnosed with another form of breast cancer (i.e. CIS only) were excluded from this trial. Furthermore, patients who had only undergone surgery and adjuvant therapy were excluded.

2.3 Medical patient data

All relevant patient-data was extracted from paper-based patient files at Oncology Medical University of Graz or found within the electronical hospital-in-house information system MEDOCS®. The following parameters were collected:

- Residual cancer burden-score during surgery
- date of birth of the patient, used for calculating the age at diagnosis
- menopausal status
- whether the tumor was a first occurrence
- localisation (left, right, both breasts)
- the pathological review date, used as date of diagnosis
- initial histological subtype before treatment

- estrogen- and progesterone- status at diagnosis
- HER2/neu status at diagnosis
- pTNM-classification
- mode of neoadjuvant treatment (chemotherapy, endocrine therapy, HER2-directed) and exact regimen used
- date of radiological or pathological confirmation of metastasis or relapse, used to calculate DFS
- last known date alive or date of death, used for OS
- last known survival status (alive or dead) and last known tumor status

Before further analysis patient names and birthdays were anonymised. All participants were adjusted with a numerical 8-digit code and birthdays were reduced to birth years.

2.4 Statistical analysis

Microsoft Excel® and IBM SPSS® were used for statistical examination.
All participants were categorized for population description according to their age, menopausal status, histological- and receptor subtype, treatment and RCB-score. Corresponding figures were then generated with SPSS.
For survival analysis, the pathological verification was used as starting date. The last date of follow-up marked the endpoint for DFS and for OS of all event-free participants. For patients who had suffered from relapse or metastasis the date of radiological or pathological confirmation was used as endpoint for DFS. The date of death was used as endpoint for DFS for metastasis-free patients who had passed away and for OS for all patients who had died during follow-up.
Kaplan-Meier procedure was then applied for the analysis of the survival of all subgroups. The Log-rank test confirmed statistical significance and $p<0.05$ was used as significance level.

3 Results

3.1 General Data

Initially 213 women were included in this study. Unfortunately, no male patient who satisfied all criteria could be identified. All women included in the study were diagnosed with invasive breast cancer between the years 2011 and 2016, received neoadjuvant systemic therapy, were at least 18 years old and had a RCB-Score evaluated after surgery. Not included were patients with metastasis in other organs at the time of diagnosis or any patient under the age of 18. Also, patients with an inconclusive RCB-Score had to be excluded.

During follow-up ten women changed clinic, therefore, their health status was unknown at the end of study. Nevertheless, the family physicians of six of those women were able to provide the necessary information. The health status of the other four women remains unknown.

Also, one woman did undergo sentinel-lymph node biopsy before neoadjuvant therapy, thus leaving the RCB-Score indeterminable. She was included in general descriptive-, but excluded from the survival-statistics.

3.1.1 Data about the Participants

The median age at diagnosis was 56; the youngest patient was 28, the oldest 92. The age distribution of all participating women can be seen in figure 2.

Of all women 58 were found to be premenopausal (27.2%) and 128 were postmenopausal (60.1%). The menopausal-status remains unknown or unclear for 27 women (12.7%).

190 tumors (89.2%) were a first occurrence; the other 23 tumors (10.8%) either recurrence or a secondary new histological subtype. 97 women (45.5%) developed cancer in their right breast, 114 women in their left (53.5%). Two women were primarily diagnosed with cancer on both sides (0.9%).

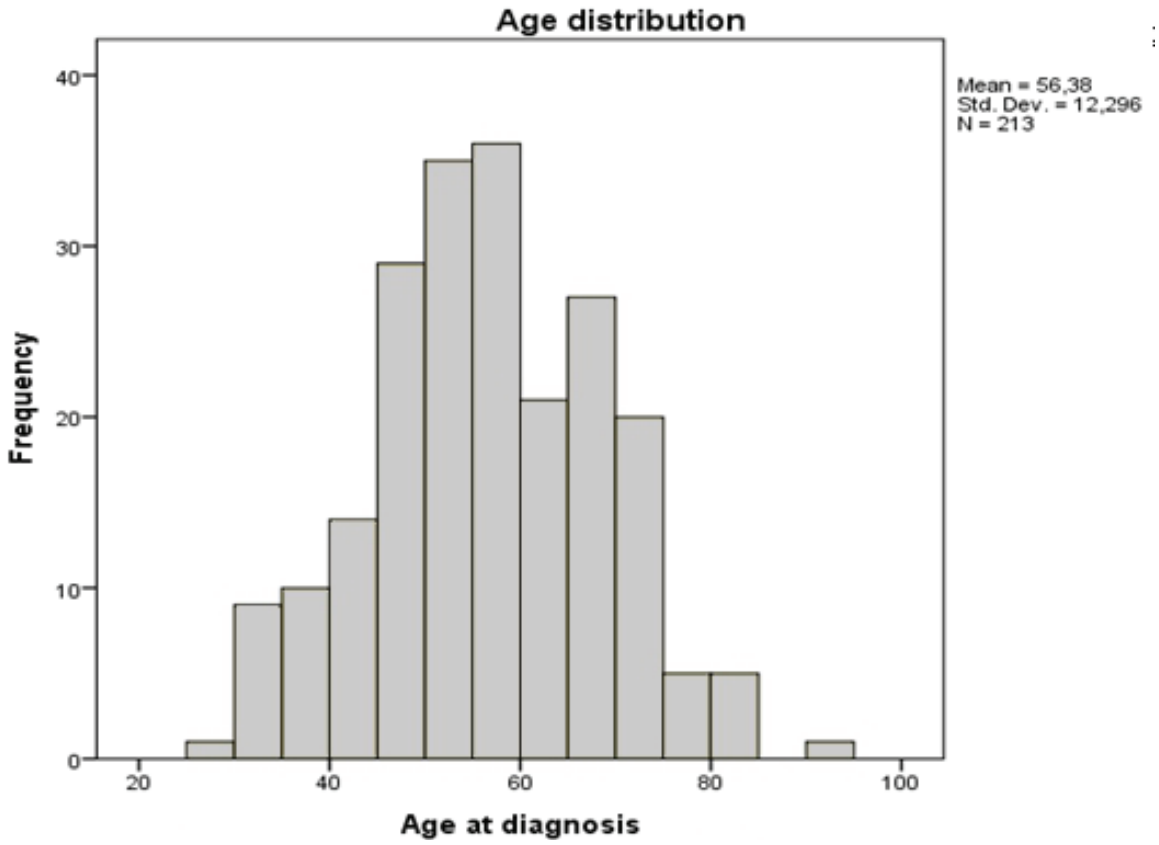

Figure 2: Age distribution of the 213 participants

3.1.2 Data about receptor distributions

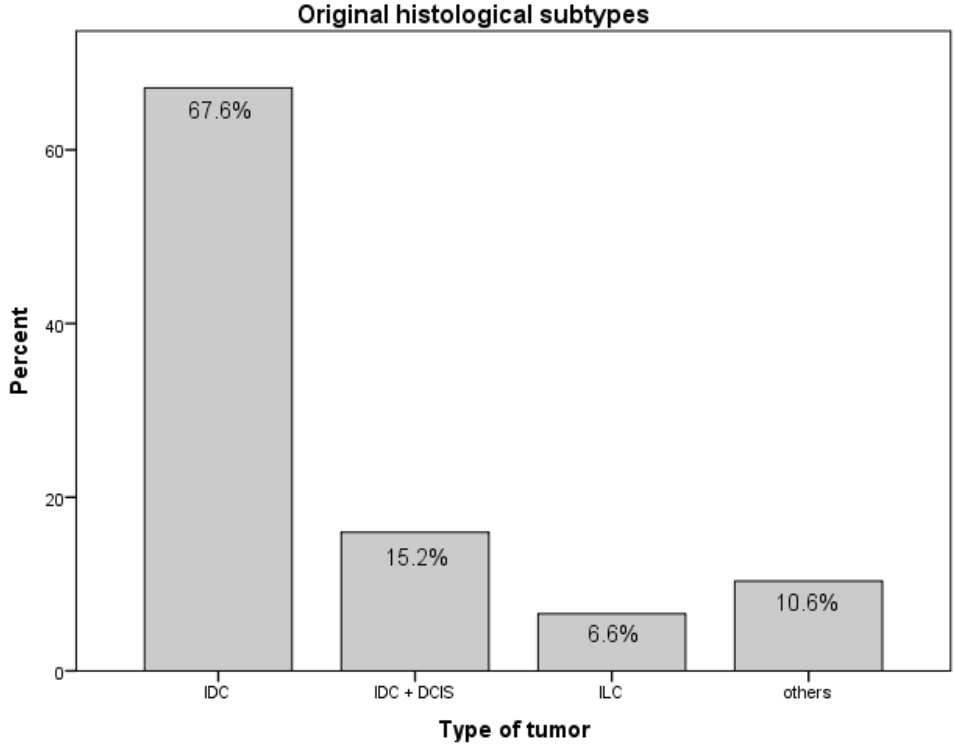

Figure 3: Original histological subtype found at biopsy before neoadjuvant treatment was introduced

Before undergoing neoadjuvant treatment, all women underwent biopsy extraction to confirm malignancy and to identify histological- and receptor-subtypes.
As seen in figure 3 above, 144 tumors (67.6%) were diagnosed as IDC (according to the new classification NST), another 34 (15.2%) were identified as a combination of IDC (NST) and DCIS. 14 women (6.6%) were diagnosed with ILC. All other tumor-identities were summarized (10.6%). Latter category included five aprocrine, four medullar, four mucinous, three papillar, three IDC with ILC, one inflammatory and one clear cell carcinoma.

As seen in figure 4 below, of all women included in this study 94 (44.1%) were negative for ER, 6 patients (2.8%) were low-grade positive, 7 patients (3.3%) were moderately positive and 106 women (49.8%) were high-grade positive.
Concerning PR, 96 women (45.1%) were negative, 30 (14.1%) were low-grade positive, 26 women (12.2%) were moderately positive and 53 (24.9%) were high-grade positive. The PR-status remained unknown for 8 women (3.8%).
Since the ER is usually the main determinant for the use of endocrine therapy, it was more closely evaluated than the progesterone-receptor status (PR). Women were again categorized as either hormone-receptor (HR) positive or negative according to their ER-status and according to their endocrine-treatment-status.

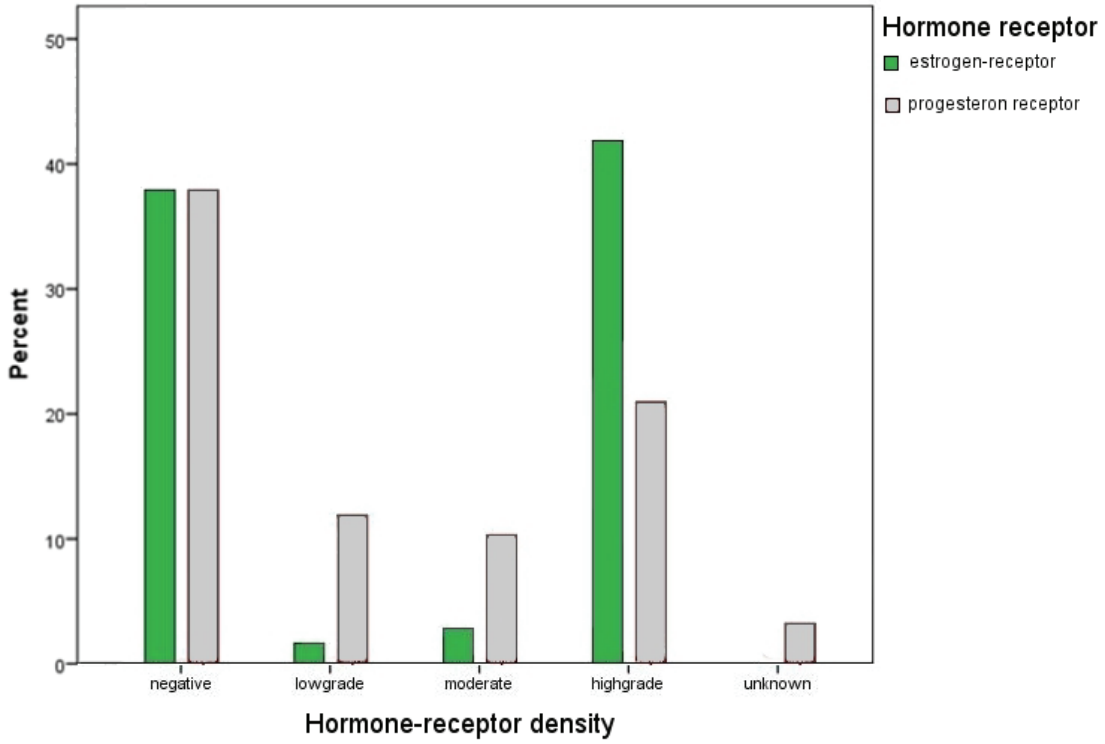

Figure 4: Distribution of hormone receptor density

ER high-grade (>51% of cells positive) and moderate (>11% positive) women were included in the HR positive group, while all ER negative (0%) women were included in HR negative. It remains a difficult question whether low-grade (<10% positive) women should be counted as positive or negative (65–67). As this is a retrospective analysis of prior treatments, the decision where to categorize them was left to the physicians in charge. Only three low-grade women were treated with anti-endocrine substances after surgery and, therefore, rated as HR positive. The other four low-grade women had not received an endocrine treatment and were, therefore, categorized as HR negative. Consequently, this left 97 women (45.5%) rated as HR negative and 116 (54.5%) as HR positive.

Also, all women were categorized as either HER2 positive or negative according to their immune-histo-chemistry and FISH-status. Consequently, 150 women (70.4%) were rated as HER2 negative and 63 (29.6%) as HER2 positive.

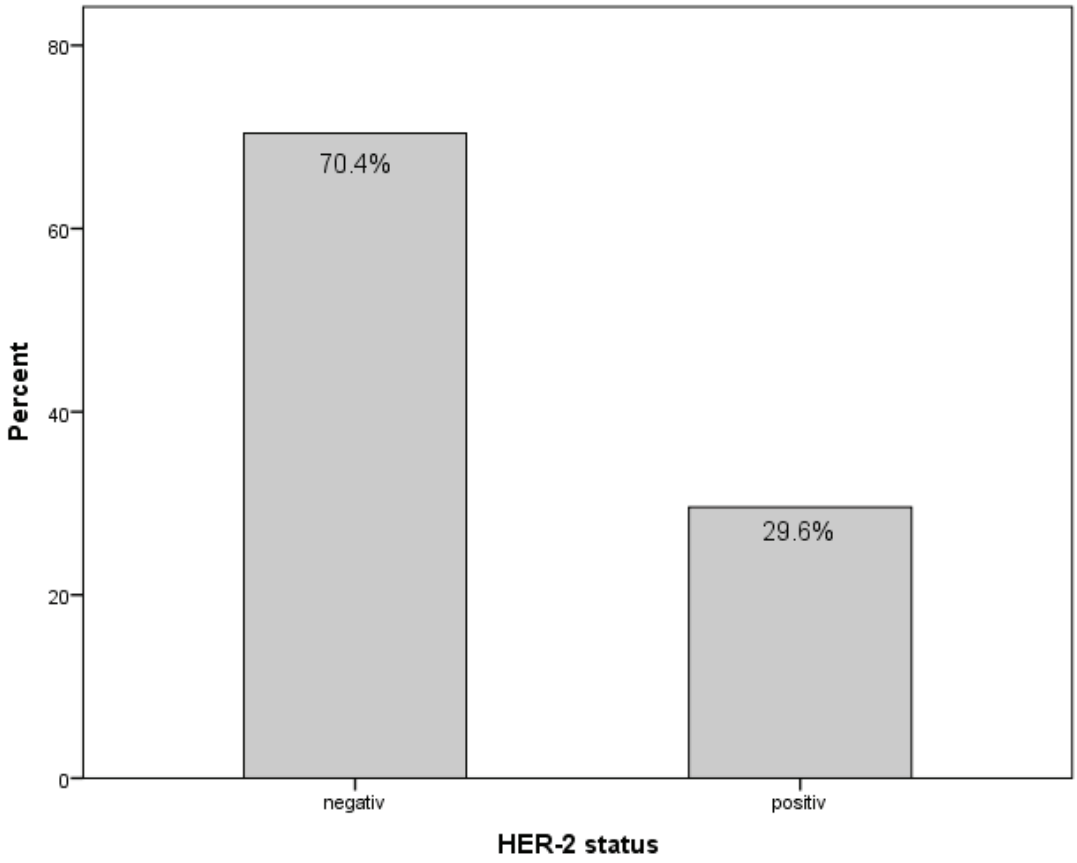

Figure 5: Distribution of HER2 status of the 213 patients

As seen in table 2 and figure 6, four subgroups within all receptor statuses were identified.

Firstly, one group contained 65 women (30.5%), who suffered from TNBC (HR- and HER2-status both being negative).

Secondly, 85 women (39.9%) were positive for HR, but negative for HER2.

Thirdly, 30 patients (14.1%) were grouped according to their positive HER2- and HR-receptors status (“triple positive”).

Fourthly, 33 women (15.5%) were positive for HER2 and negative for HR.

	subtype	frequency	percent
HER2 positive		63	29.6%
	HER2+, HR-	33	15.5%
	HER2+, HR+	30	14.1%
HER2 negative		150	70.4%
	HER2-, HR+	85	39.9%
	TNBC	65	30.5%

Table 2: HER2 and HR subtype distribution of the 213 participants

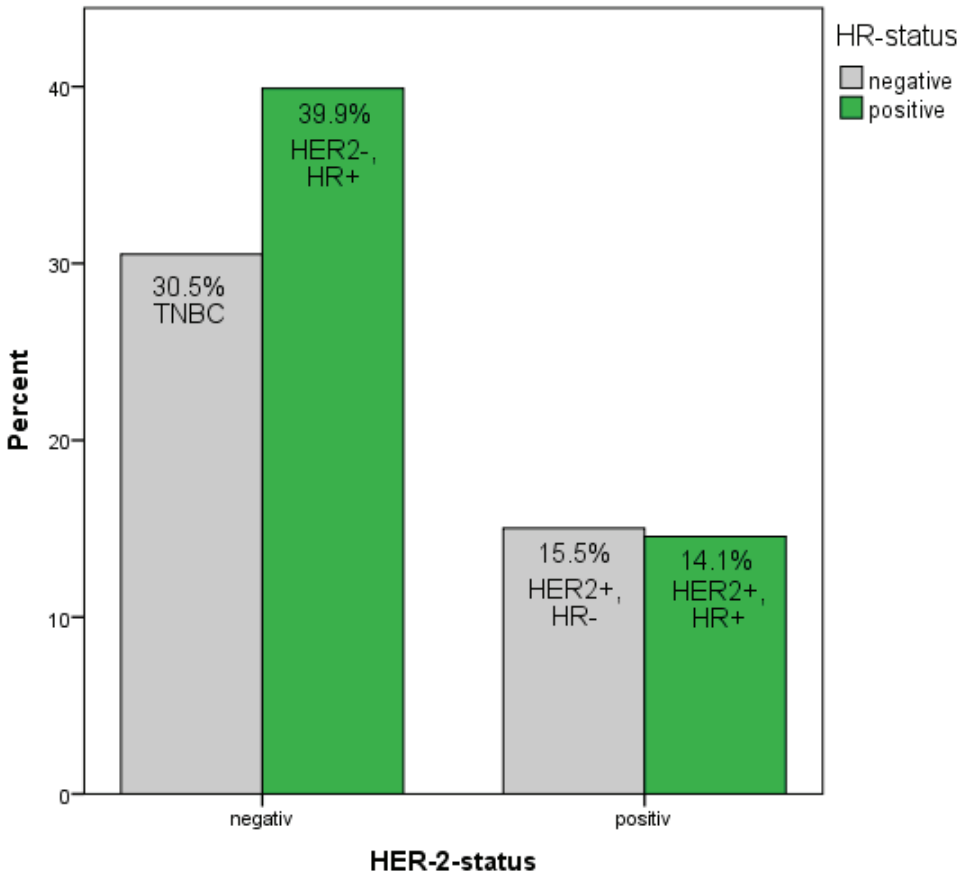

Figure 6: Distribution of receptor subtypes

3.1.3 Data about treatment

Four women (1.9%) received monochemotherapy, of those three with paclitaxel and one with 5-fluorouracil.

The majority of women, with 185 in number, (86.9%) were treated with at least two cytostatic substances, thus being categorized as polychemotherapy. Most common regimens were FEC/T, EC/T and AT, as can be seen in figure **7**. Common and reverse sequences were summarized.

24 women (11.3%) did not receive any neoadjuvant chemotherapy, but instead an endocrine or HER2-targeted therapy.

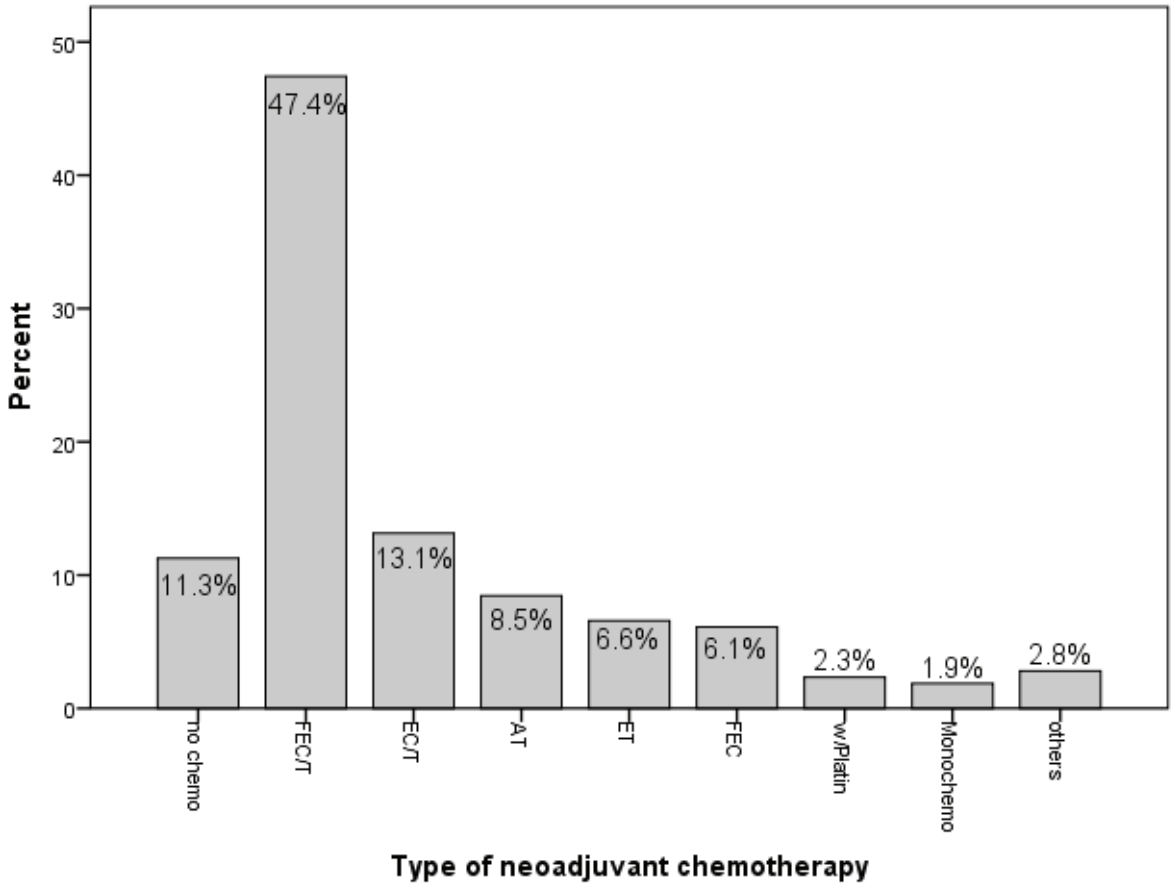

Figure 7: Frequency of chemotherapy regimens

Concerning HER2-directed therapy, 37 women (17.4%) underwent neoadjuvant treatment with trastuzumab. 23 women (10.8%) received both trastuzumab and pertuzumab. Furthermore, a single patient received bevacizumab as neoadjuvant therapy, counting for 0.5 %. 152 women (71.4%) did not receive any HER2-targeted treatment as neoadjuvant therapy.

Regarding endocrine-therapy, 25 women (11.7%) received neoadjuvant hormonal treatment. Of this group, 19 women were treated with letrozole, four with exemestane, one was treated with anastrozol and one with tamoxifen.
Thus, 188 patients (88.3%) did not undergo neoadjuvant hormonal therapy.
Figure 8 shows the summarized distribution of all neoadjuvant regimens used during this trial. As seen below, almost 40% of women received a neoadjuvant approach including either HER2 directed or endocrine therapy.

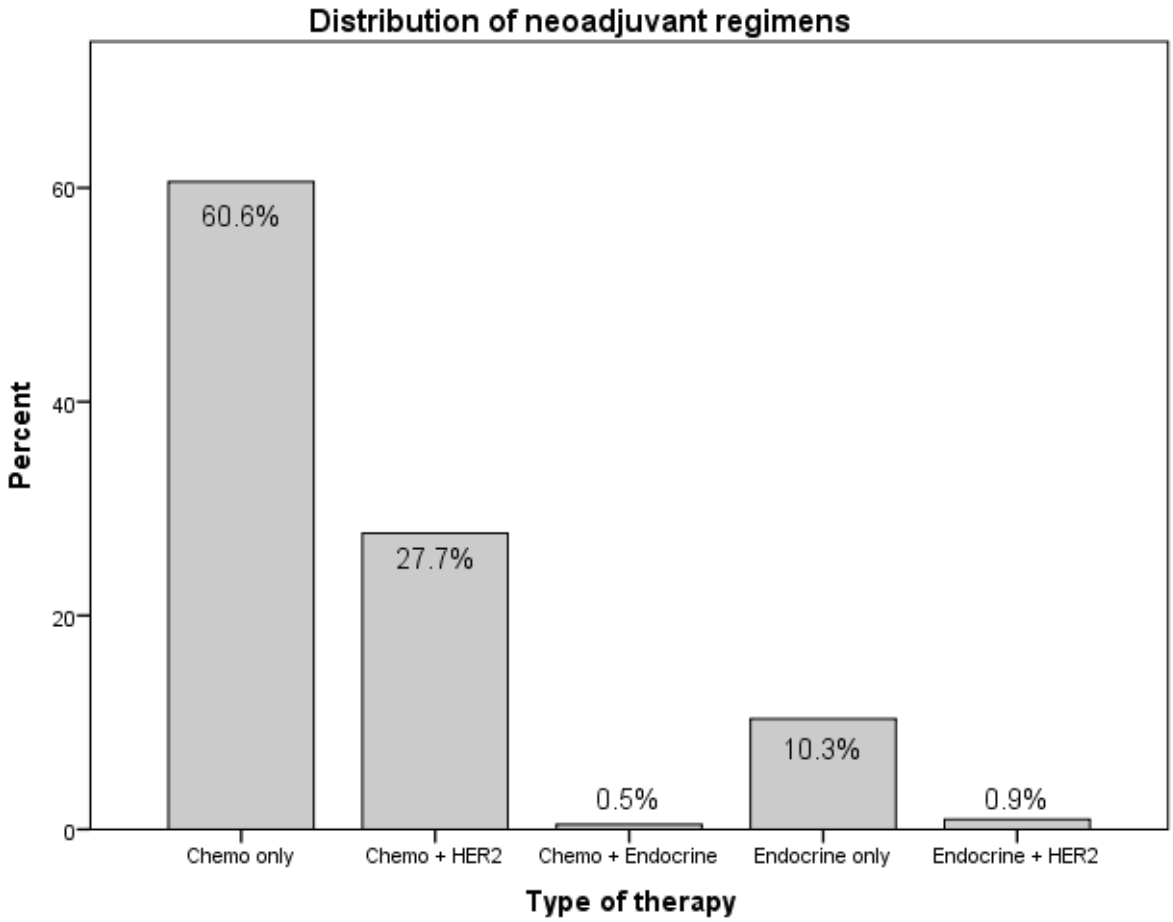

Figure 8: Form of neoadjuvant therapy of the 213 participants

3.1.4 Data about the Residual cancer Burden

As mentioned above, one patient was excluded after undergoing neoadjuvant sentinel-lymph node biopsy, thus leaving 212 participants in the study. Among these patients were 164 (77.4%) who only had to be operated once. Another 33 women (15.5%) had to undergo surgery twice, leaving 15 patients (7.1%) with three surgeries or more.

During surgery 54 women (25.5%) were found to have a RCB-Score of 0.
31 women (14.6%) did receive a RCB-Score of 1,
87 patients (41.0%) were diagnosed with a RCB-Score of 2,
and 40 women (18.9%) were found to have a RCB-Score of 3.

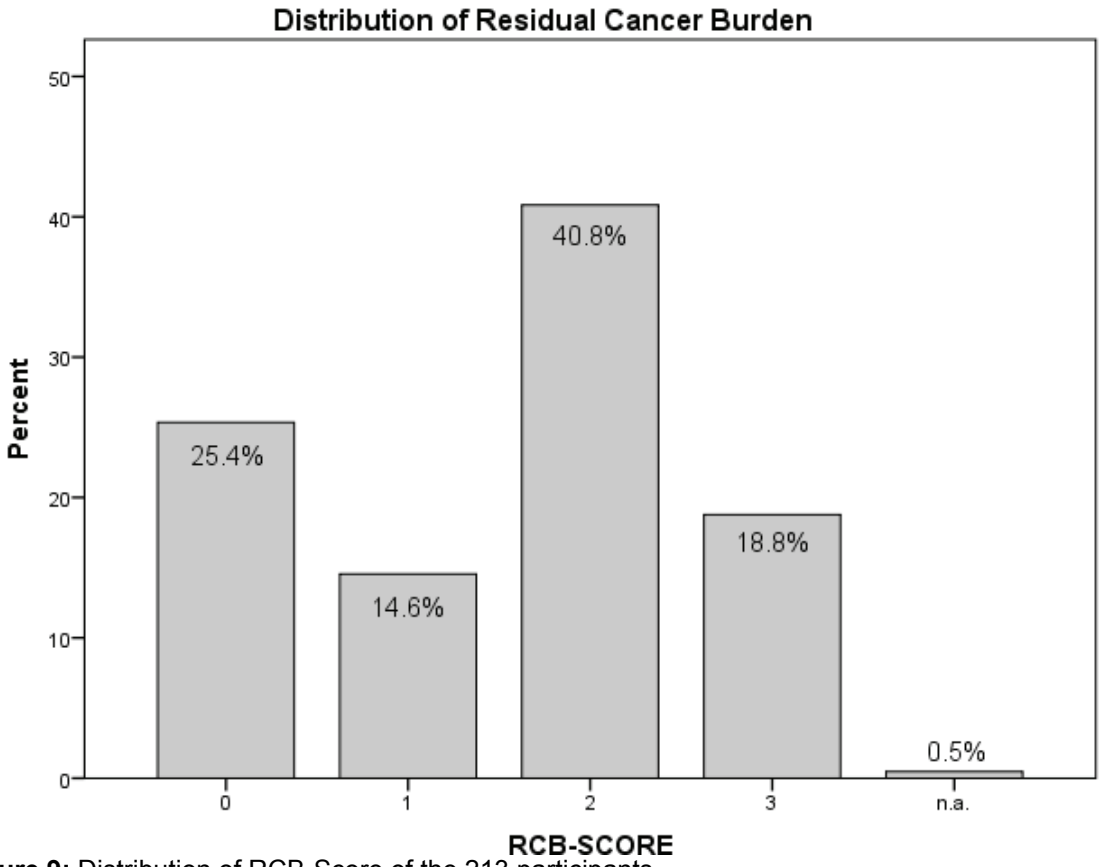

Figure 9: Distribution of RCB-Score of the 213 participants

Among the 54 women who reached pCR, there were 25 who had undergone only chemotherapy as neoadjuvant treatment and 29 who had received a combination of chemotherapy and HER2-directed therapy.

	Chemo only	Chemo + HER2	Chemo + Endocrine	Endocrine only	Endocrine + HER2	Total
RCB-0	25	29	0	0	0	54
RCB-I	18	12	0	1	0	31
RCB-II	60	12	1	13	1	87
RCB-III	26	5	0	8	1	40
Total	129	58	1	22	2	212

Table 3: Distribution of RCB-score among choice of neoadjuvant treatment

3.1.5 Survival- and Tumor Status at End of the Study

During ongoing study 38 women (17.8%) suffered from an event. An event was defined as metastasis in a peripheral organ or death of the patient.

Overall 22 deaths occurred during the time of ongoing study. One patient died tumor-independently. Three patients suffered from tumor-related primary death without known metastasis. 18 deaths occurred after the women had suffered from metastasis first.

Figure 10 shows the status distribution at end of the trial. The survival status was unclear for only one woman (0.5%), all other women were categorized as alive or dead. Also, figure 10 shows the tumor status of all women.

Altogether 34 women (16.0%) were diagnosed with metastasis during follow-up. Among these women ten were diagnosed with cerebral metastasis, nine with lymph node metastasis (axillar not included), nine patients with liver metastasis, six with bone metastasis, five with lung metastasis, two with skin metastasis, two with metastasis in the thoracic wall and one patient was diagnosed with colon metastasis. Moreover, five women developed a secondary tumor during the study and two women suffered from local relapse.

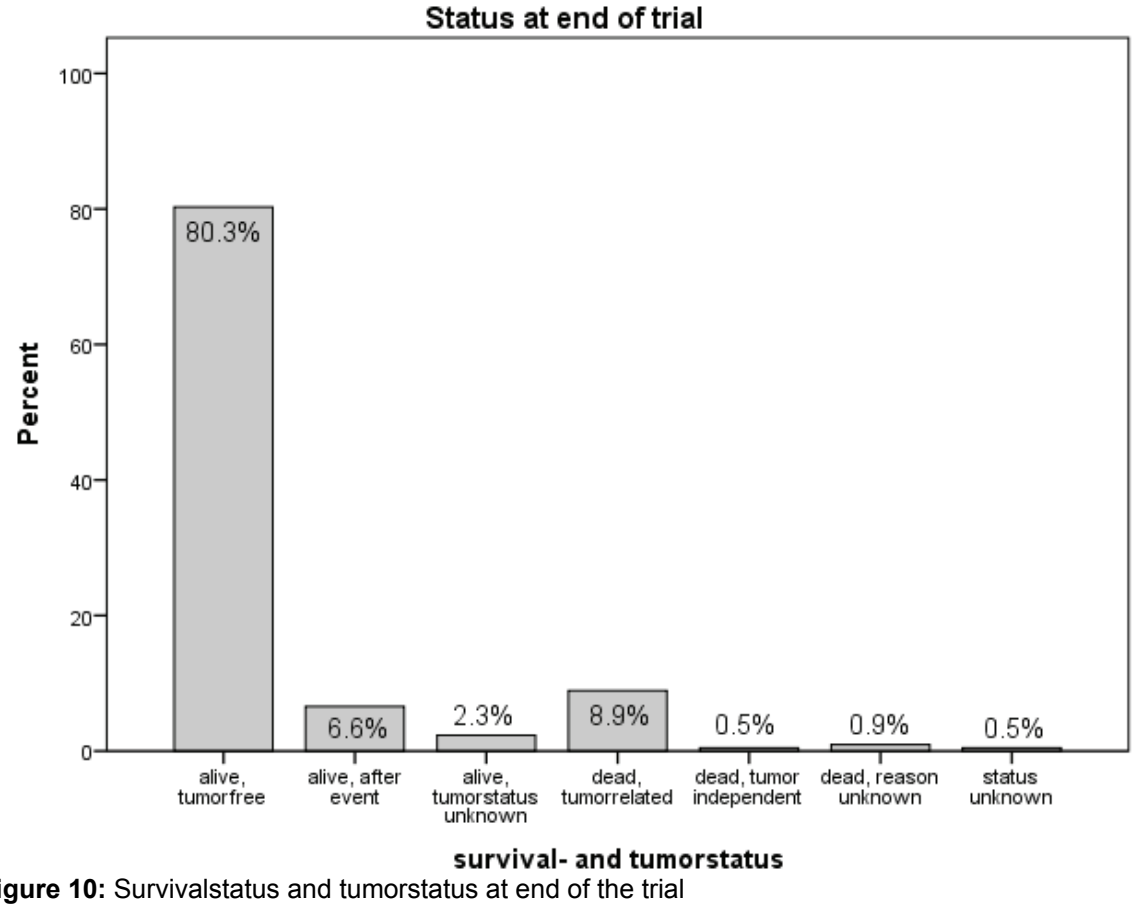

Figure 10: Survivalstatus and tumorstatus at end of the trial

3.2 Survival Data

3.2.1 Primary Endpoint – RCB-Score

The primary endpoint of this trial was to evaluate the Residual Cancer Burden as a prognostic factor for OS and DFS. Data about the RCB-Score was not re-categorized, but left just as defined by the pathologist according to Symmans et al. As seen in figures 11 and 12 there was a correlation between the RCB-Score found at surgery and the survival rates. There was a statistical significance in both the DFS (p=0.000006) and OS (p=0.000306). The mean OS-rates were 56.8 months (+/- 1.1) for RCB-0, 57.4 months (+/- 1.6) for RCB-I, 56.1 months (+/- 1.8) for RCB-II and 46.2 months (+/- 3.4) for RCB-III.

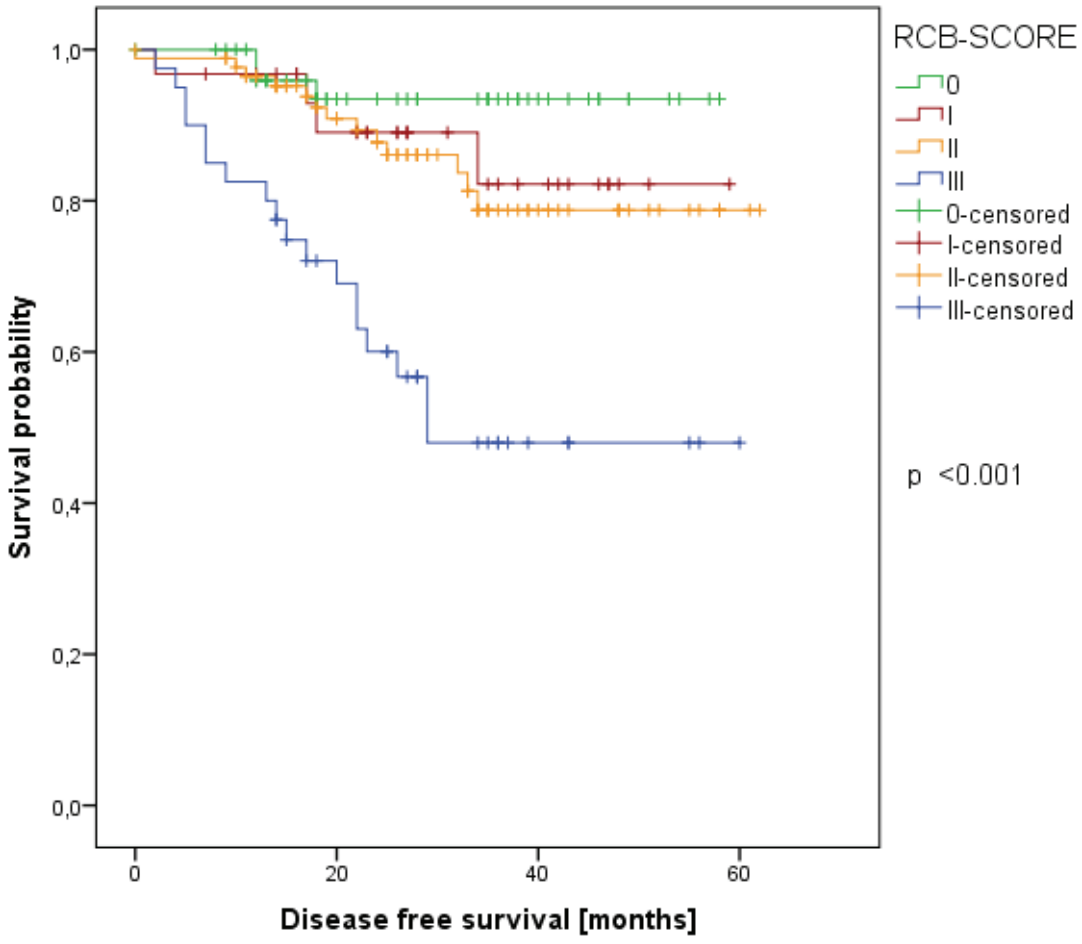

Figure 11: Disease free survival associated with RCB-Score

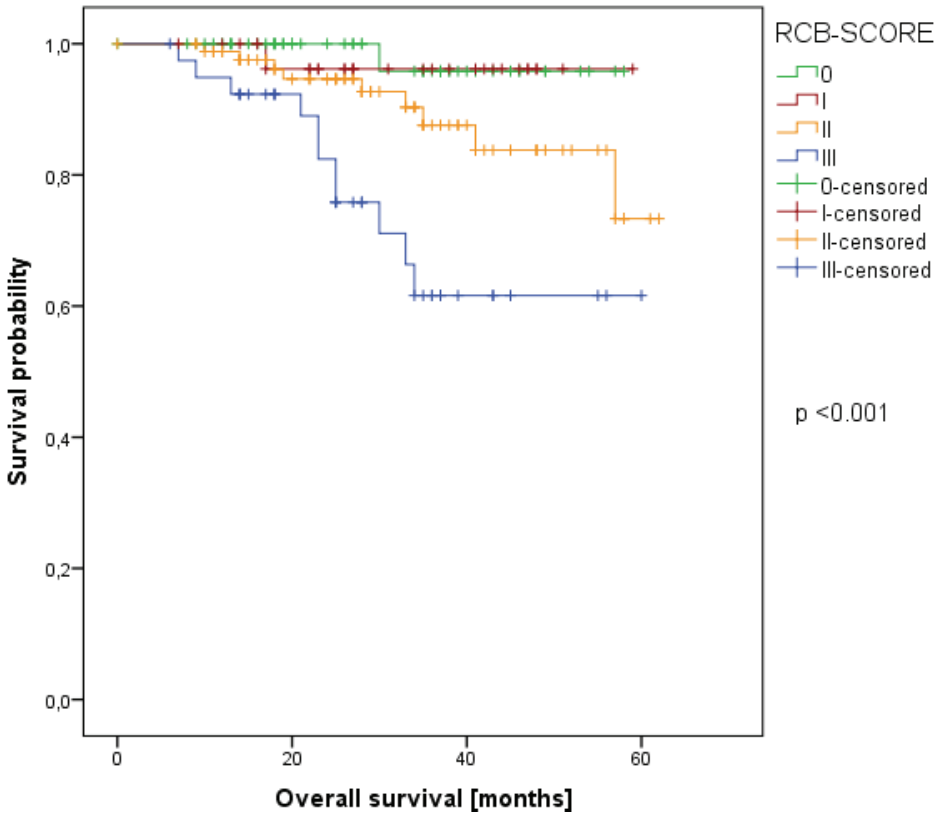

Figure 12: Overall survival associated with RCB-Score

3.2.2 Secondary endpoints

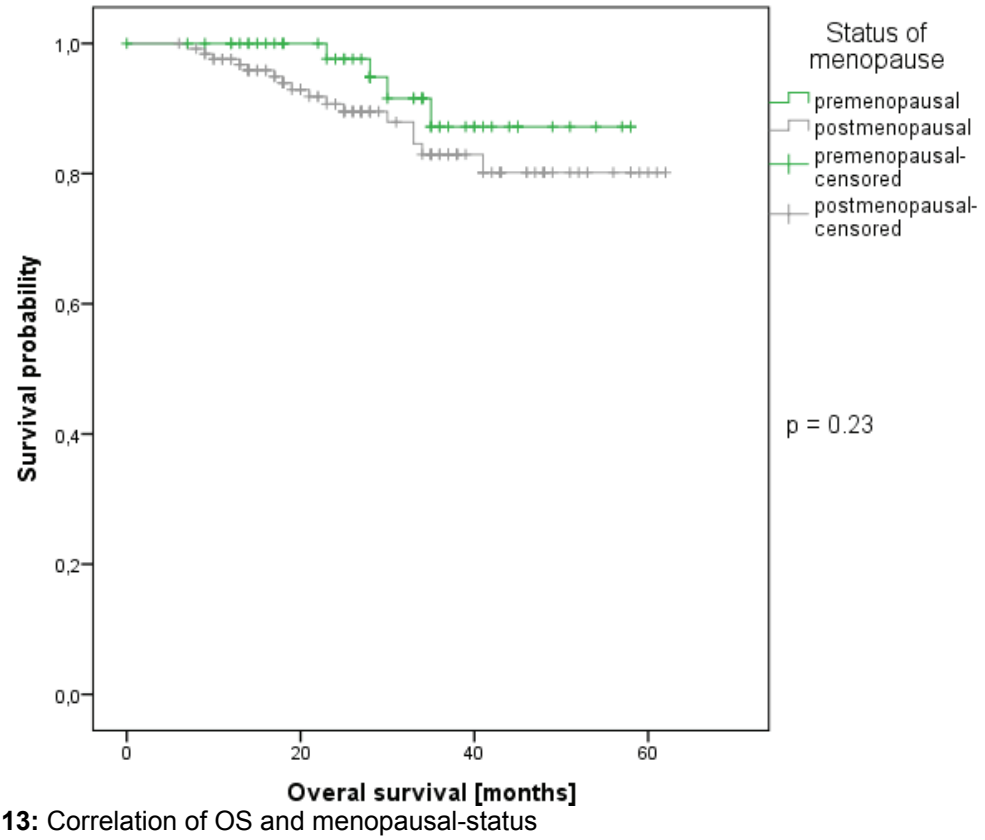

Figure 13: Correlation of OS and menopausal-status

One of the secondary endpoints was to find out whether the menopause–status correlated with the survival-rates. In 185 women the menopausal-status was defined, for the others it remains unknown. 58 patients were premenopausal (27.2%) and 128 were postmenopausal (60.1%).
However, there was no statistical significance determined neither for DFS (p=0.737), nor for OS (p=0.23) as can be seen in figure 13.

Also, the correlation of the histological subtype found at first biopsy and their association with survival-rates was evaluated. There was no statistically significant difference between subtypes concerning DFS (p=0.722) and OS (p= 0.864). Histological subtypes were categorized as IDC (NST), IDC (NST) + DCIS, ILC and others, as can be seen in figure 14.

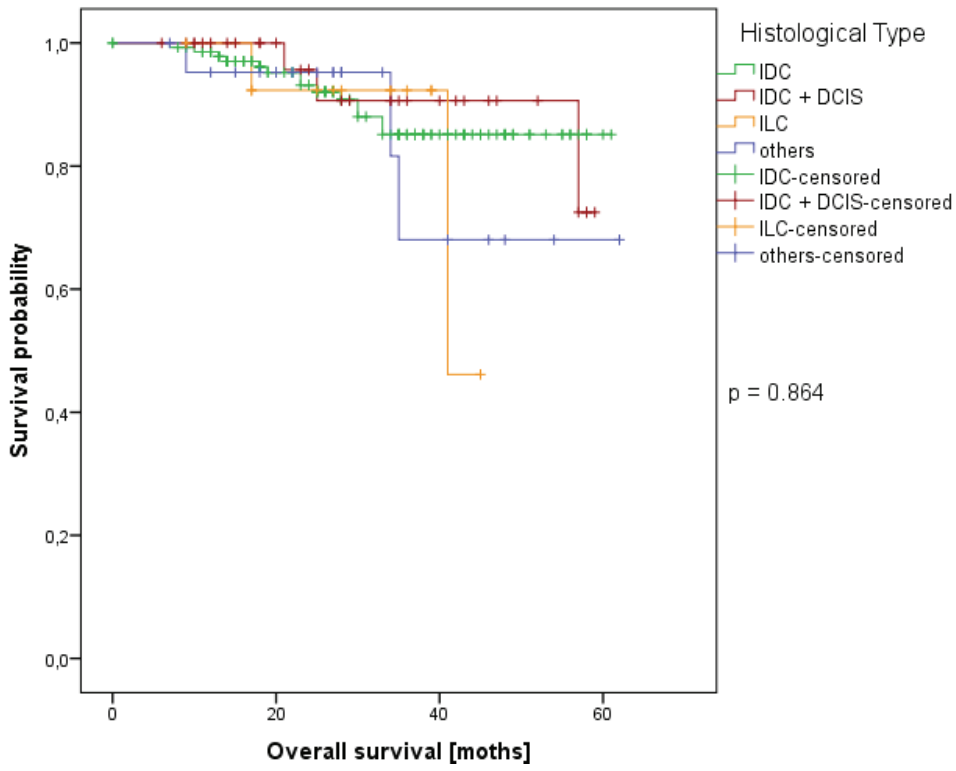

Figure 14: Correlation of OS and histological tumor type

The endocrine-receptor status HR was, as mentioned above, only classified as positive or negative. All women were included in the survival-rate measurements of this factor; 97 as HR negative and 116 as HR positive, according to their ER- and endocrine-treatment-status. There was, however, no difference in DFS (p=0.615) or OS (p=0.437) detected, as seen in Figure 15.

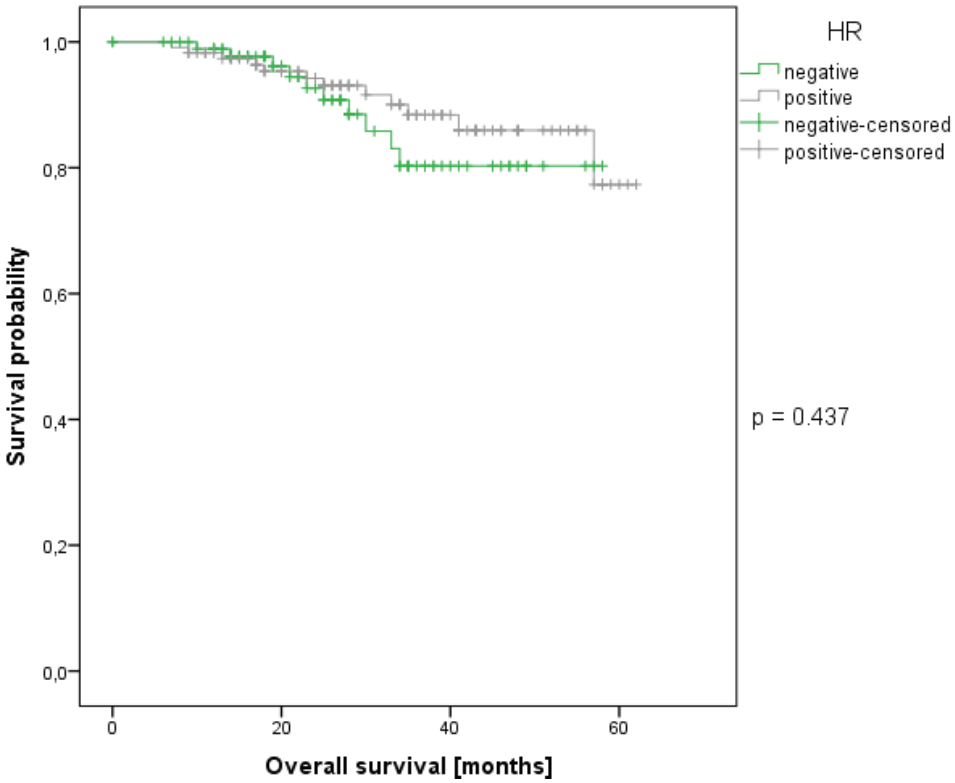

Figure 15: Correlation of OS and endocrine-receptor-status

Furthermore, 62 women were found to show an overexpression of HER2, while 150 were negative for the receptor. Concerning survival-rates, the status of the HER2 receptor did not reveal a statistical significance in DFS (p=0.794) or OS (p=0.182), as seen in figure 16.

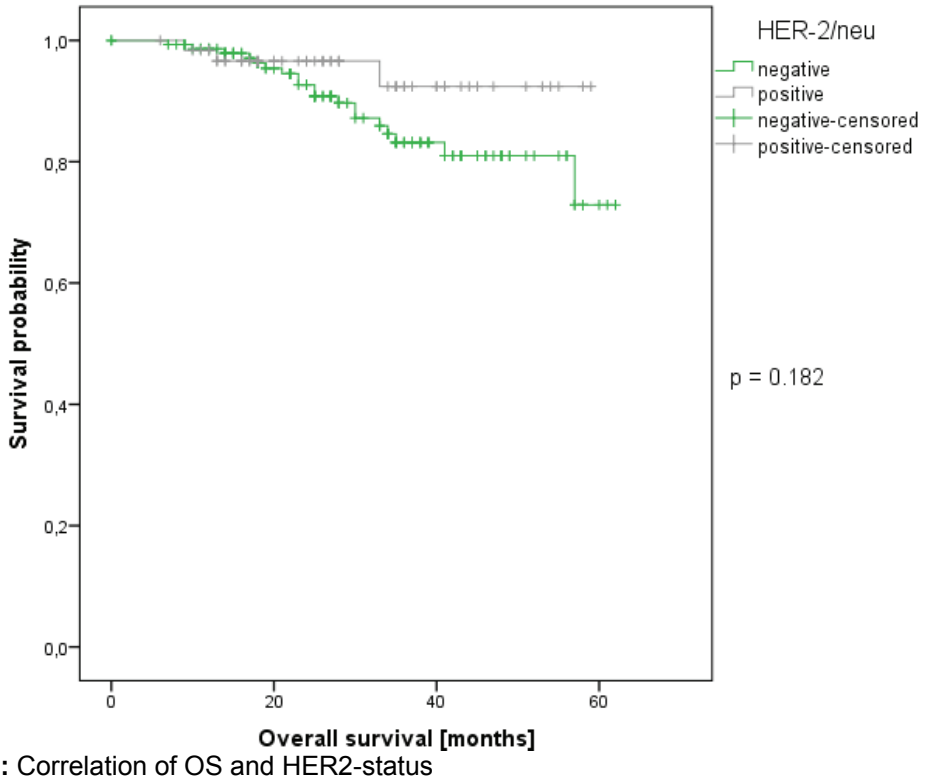

Figure 16: Correlation of OS and HER2-status

As seen in figure 8, of all 212 women participating in survival-study, 129 received only chemotherapy as a neoadjuvant treatment. 58 received a combination of chemotherapy and HER2-directed neoadjuvant therapy. 22 women were neoadjuvantly treated with endocrine therapy only. Only two women were treated with a combination of chemotherapy and endocrine therapy and only one woman with a combination of HER2-directed- and endocrine therapy. These last three women were not included in the following analysis. The choice of neoadjuvant treatment was shown to influence survival rates. Though there was no statistical significance detected for DFS (p=0.414), as seen in figure 17, the difference in OS was significant (p=0.025).

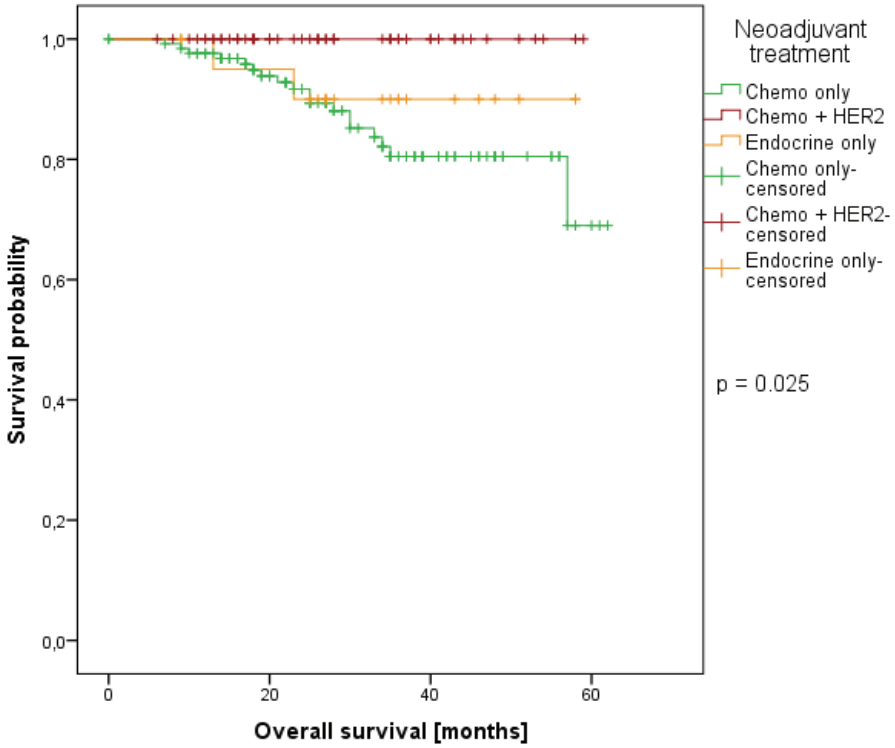

Figure 17: Correlation of OS and the choice of a neoadjuvant regimen

Finally, pathological T-status of the 212 women during surgery was examined. Of those, 42 (19.8%) were labelled as ypT0. 17 women (8.0%) were found to have a remaining carcinoma-in-situ (ypT0is). 99 patients (46.7%) were identified as pT1. 33 (15.6%) women were staged as pT2. 16 women (7.5%) were distinguished as pT3, leaving 5 patients (2.4%) pT4.

Statistical research found a difference in both DFS (p=0.0003) and OS (p=0.005), as seen in figures 18 and 19 below.

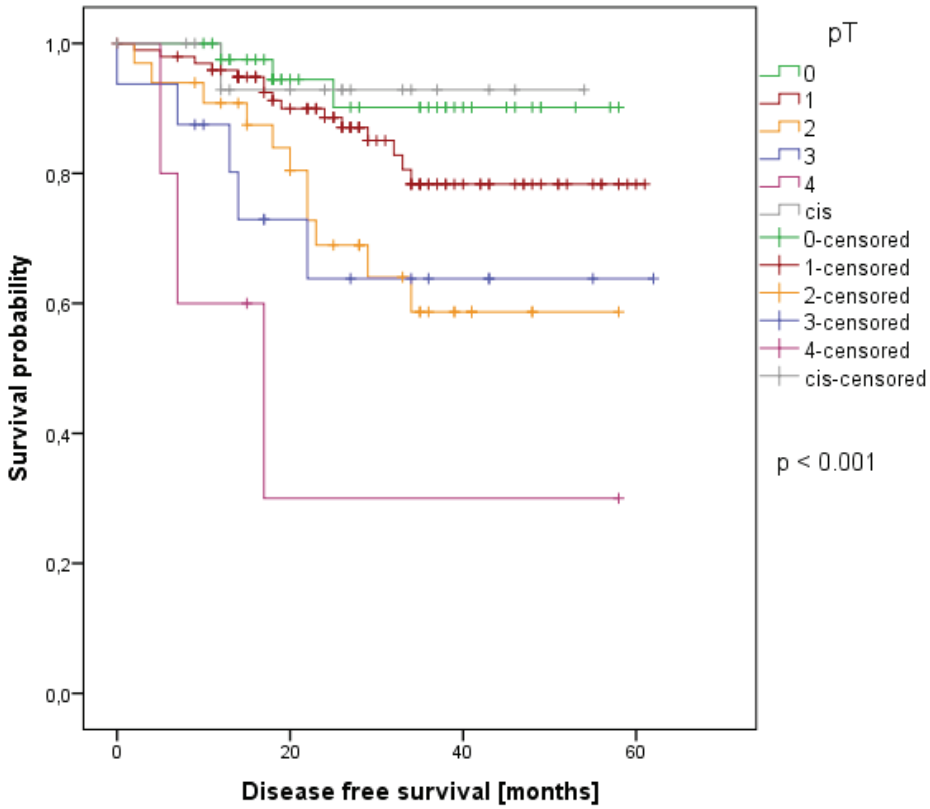

Figure 18: Correlation of DFS and pT-stadium

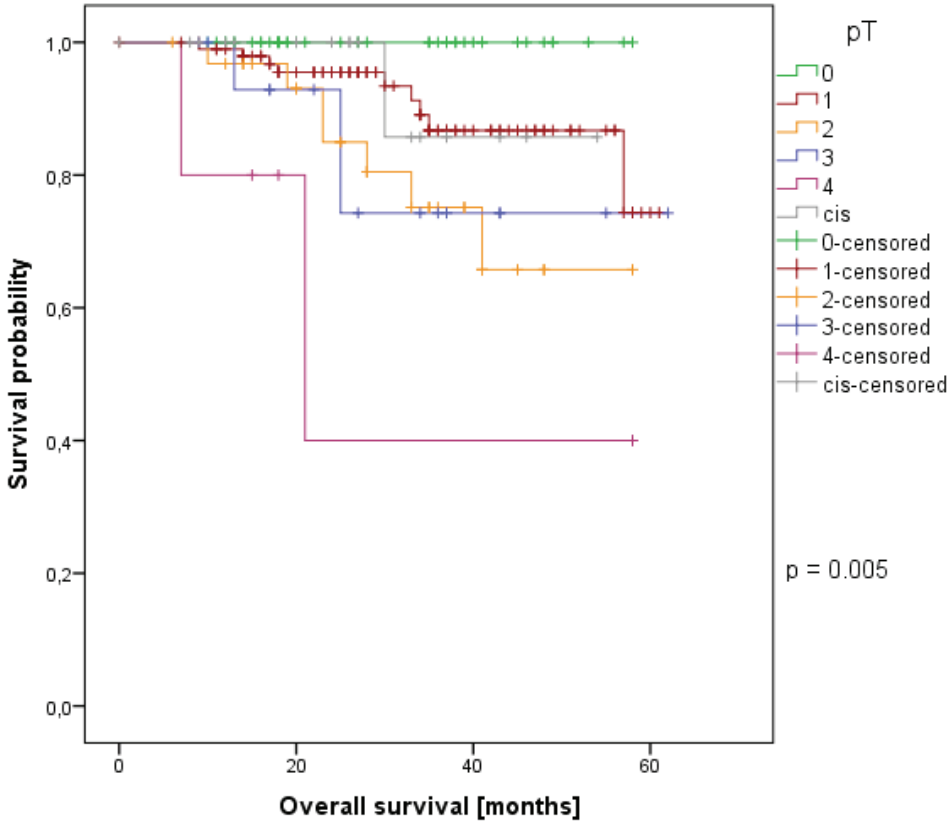

Figure 19: Correlation of OS and pT-stadium

4 Discussion

4.1 Evaluation of primary endpoint

The main endpoint of this study was to validate the findings of Symmans et al. from 2007 and 2017 concerning the prognostic value of the RCB-score. In both studies their group was able to show a correlation between OS and the RCB-score after neoadjuvant treatment (59, 64).

Concerning the patient collective in our study, as shown in figure 11 and 12, there was a significant reverse correlation between RCB-score and both survival parameters. Patients among the RCB-0 and RCB-I group had better DFS- and OS-rates than patients who were categorized in the RCB-II and RCB-III group. These results seem obvious, as patients from groups II and III have bigger residual tumors with higher cellularity, in combination with more and larger lymph node metastasis, than their fellow participants.

This is in agreement with our findings concerning the pathological T-status. Because the pathological T-status (tumor size) is a part of the RCB-score and since the RCB-score proved to be related to survival rates, it only seemed logical that the pathological T-status by itself should also correlate to the survival of patients. As seen in figure 18 and 19 the size of residual cancer at the time of surgery was related to DFS and OS.

These findings were already expected before the beginning of the study and confirmed both our hypothesis and the results published by Symmans et al.

It should be considered that in their first trial in 2007 all patients received neoadjuvant chemotherapy with anthracyclines and/or taxanes, while in their second trial in 2017 all patients were treated with T/FAC, FAC or H+T/FEC.

In contrast to the two trials of Symmans et al., our study incorporated all types of neoadjuvant treatments, including HER2-directed and endocrine therapy. This change did, however, not influence the prognostic value of the RCB-score. This might be due to the fact that the majority of our patients were treated with the most effective treatments, and the results achieved (RCB 0 and I) were correlated to the high response rates in the chemotherapy and chemotherapy along with Her2

directed therapy. However, it can be concluded that the RCB-score correlates with the overall and the DFS independently of the choice of neoadjuvant treatment.
Although at the current stage we did not analyse the impact of the RCB-score in different biological groups, but only overall, our results suggest that this should be analysed further. Additional evaluation should focus on the chemotherapy group, contrasting hormone receptor positive versus triple negative subgroups. In our study the subgroups at the end of the observation period were too small to calculate for significance. However, with a larger population, as well as a longer follow up, these analyses will become increasingly important.

4.2 Evaluation of secondary endpoints

The age analysis described in figure 2 shows a correlation of higher age with likelihood of breast cancer occurrence. This is consistent with already known risk factors for breast cancer (4–6). The mean age of women at the time of diagnosis in our study was 56. However, findings from Statistik Austria and S3-guidelines, both from 2012, show the median age at diagnosis to be over 60 (1, 2). The age difference might be due to the difference in included tumor types. While Statistik Austria and the S3-guidelines also included benign forms of breast tumors and less aggressive breast cancer, with lower stage, our study only researched women with invasive breast cancer selected for neoadjuvant treatment. Typically, patients with worse biology of the tumors, such as triple negative or Her2 positive are selected for the neoadjuvant treatment. In the group of hormone receptor positive patients, only patients with larger tumors, worse grading, lymph node metastases are selected for the neoadjuvant treatment, and these patients have worse prognosis in the group of hormone receptor positives. It can be further hypothesized that the most aggressive form of breast cancer is generally diagnosed at an earlier age compared to all types of breast tumors. This could be connected to a higher rate of genetically determined breast cancer; however, data about gene analysis of our patients is unavailable.
One could assume that the aggressive cancer forms among younger women would influence survival rates, however, there was no difference found between younger- premenopausal and older- postmenopausal women. Only 58 women were known to be premenopausal at diagnosis, while 128 were definitely

postmenopausal. Results from other studies about whether younger age at diagnosis would lead to better or poorer prognosis are controversial. This might be, however, due to the choice of different cut-off points for age groups among existing studies (68). Moreover, this is a consequence of generally poorer prognosis of all patients selected (including the ER positive group) for the neoadjuvant treatment, as suggested above.

Concerning the histological subtype, women were more than ten times more likely to suffer from IDC than ILC. This is concurrent to other findings about the contribution of histological subtypes of breast carcinomas (12, 13, 16). Again, there was no significant difference between the histological subtypes in DFS or OS. In their study from 2004 Arpino G. et al. (16) did not find a difference in 5-year OS between ILC and IDC either. Nonetheless, in 2012 Lips EH. et al. (69) published a study that suggested better responses from IDC to neoadjuvant therapy compared to ILC. They did, however, explain these results with differences in HR- and HER2- characteristics and not with the histological subtype. Current neoadjuvant treatments do not focus on histology, but instead on receptor status for HR and HER2. If and how the histological subtype influences OS remains a topic for future studies.

Of all women, 54.5% were rated as positive for endocrine receptors, while 45.5% were considered negative. This distribution was very similar to the original study from Symmans et al. from 2007 (59), who found 56% positive and 39% negative. In their study the HR-status did not correlate with long-term survival. Neither was there a correlation with DFS or OS in our study.
Interestingly, the 22 women only treated with endocrine therapy before surgery in our study, did not show a disadvantage in DFS or OS when compared to the 129 women only treated with chemotherapy. This confirms the results of Marcus DM et al. (47) from 2013, who found neoadjuvant endocrine therapy to be as effective as neoadjuvant chemotherapy among their 99 patients. Although in our study only 22 out of 116 HR positive women received endocrine neoadjuvant therapy, it should be considered a reasonable choice for selected patients. This selection is mostly based on the biology of tumors and age of patients.

Moreover, 58 women received a combination of chemo- and HER2-directed neoadjuvant therapy. Initially 63 women had been rated as HER2 positive, making up 29.6%. While women with a positive HER2 receptor did not show a benefit in DFS and OS, the combination of treatment led to better OS rates than chemotherapy alone. These findings were similar to the two following studies. In 2008 Peintinger et al. (56) suggested that patients treated with additional trastuzumab reached better survival rates than patients treated with chemotherapy only. Gianni et al. showed clearly in 2014 (55) that the use of additional trastuzumab leads to better pCR- and survival rates. Again, our results showed that HER2 positive women benefit from the treatment combination by reaching better OS. According to the biology of the Her2 disease, most Her2 positive breast cancer patients are selected for the neoadjuvant treatment, and receive either trastuzumab alone, or in combination with pertuzumab. Also, this combination was a suitable possibility as a neoadjuvant regimen.

During the follow-up 34 women were diagnosed with metastasis. Most women suffered from multiple metastases; among those cerebral, lymph node, liver, bone and lung metastasis were the most common ones. This contribution is similar to the findings of H.Kennecke et al. (70) and C.Savci-Heijink et al. (71). Kennecke included 3,726 patients and Savci-Heijink 256 patients. Both found the brain, liver, lung, bones and distant nodi to be the organs with most cases of distant metastasis. Also, both described multiple sites of metastasis in their patient population.

4.3 Conclusion

Breast cancer remains the prevalent cancer among Austrian women.

This single centre study with 212 patients confirmed the prognostic value of the RCB-score on survival rates. We were able to verify a correlation with both the DFS and OS. Further, we were able to show that the prognostic value of the RCB-score is independent of the choice of neoadjuvant treatment.

In addition, our study demonstrated pre-existing results concerning neoadjuvant therapy regimens. For HER2-positive patients the combination of neoadjuvant chemotherapy with additional trastuzumab-use continues to be the recommended regimen. Neoadjuvant endocrine therapy remains a suitable choice for selected patients with positive hormone receptor disease. Further follow up of our study on the correlation of molecular subtypes and RCB Score on survival with increasing number of patients and longer follow up will be performed.

5 References

1. Deutsche Krebsgesellschaft, Deutsche Gesellschaft für Gynäkologie und Geburtshilfe. Interdisziplinäre S3-Leitlinie für die Diagnostik, Therapie und Nachsorge des Mammakarzinoms [Langversion 3.0, Aktualisierung 2012]

[cited 2017 May 7].

2. Hackl M, Karim-Kos HE. Krebserkrankungen in Österreich 2016: STATISTIK AUSTRIA. Wien; 2016.

3. WHO Regionalbüro für Europa. Brustkrebs; 2016. Available from:

URL: http://www.euro.who.int/de/health-topics/noncommunicable-diseases/cancer/news/news/2012/2/early-detection-of-common-cancers/breast-cancer.

4. Barnard ME, Boeke CE, Tamimi RM. Established breast cancer risk factors and risk of intrinsic tumor subtypes. Biochim Biophys Acta 2015; 1856(1):73–85.

5. O'Brien KM, Sun J, Sandler DP, DeRoo LA, Weinberg CR. Risk factors for young-onset invasive and in situ breast cancer. Cancer Causes Control 2015; 26(12):1771–8.

6. McTiernan A. Behavioral Risk Factors in Breast Cancer: Can Risk Be Modified? Oncologist 2003; 8(4):326–34.

7. Chan S, Chen J-H, Li S, Chang R, Yeh D-C, Chang R-F et al. Evaluation of the association between quantitative mammographic density and breast cancer occurred in different quadrants. BMC Cancer 2017; 17(1):274.

8. Sanders ME, Schuyler PA, Dupont WD, Page DL. The natural history of low-grade ductal carcinoma in situ of the breast in women treated by biopsy only revealed over 30 years of long-term follow-up. Cancer 2005; 103(12):2481–4.

9. Erbas B, Provenzano E, Armes J, Gertig D. The natural history of ductal carcinoma in situ of the breast: a review. Breast Cancer Res Treat 2006; 97(2):135–44.

10. Betsill WL. Intraductal Carcinoma. JAMA 1978; 239(18):1863.

11. Klevesath MB, Pantel K, Agbaje O, Provenzano E, Wishart GC, Gough P et al. Patterns of metastatic spread in early breast cancer. Breast 2013; 22(4):449–54.

12. Borst MJ IJA. Metastatic patterns of invasive lobular versus invasive ductal carcinoma of the breast. Surgery 1993; 114(4):637–42.

13. Arps DP, Healy P, Zhao L, Kleer CG, Pang JC. Invasive ductal carcinoma with lobular features: a comparison study to invasive ductal and invasive lobular carcinomas of the breast. Breast Cancer Res Treat 2013; 138(3):719–26.

14. Park I, Kim J, Kim M, Bae SY, Lee SK, Kil WH et al. Comparison of the characteristics of medullary breast carcinoma and invasive ductal carcinoma. J Breast Cancer 2013; 16(4):417–25.

15. Cao A-Y, He M, Liu Z-B, Di G-H, Wu J, Lu J-S et al. Outcome of pure mucinous breast carcinoma compared to infiltrating ductal carcinoma: a population-based study from China. Ann Surg Oncol 2012; 19(9):3019–27.

16. Arpino G, Bardou VJ, Clark GM, Elledge RM. Infiltrating lobular carcinoma of the breast: tumor characteristics and clinical outcome. Breast Cancer Res 2004; 6(3):R149-56.

17. Senkus E, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rutgers E et al. Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2015 [cited 2017 May 17]; 26 Suppl 5:v8-30.

18. Loibl S, Han SN, Minckwitz G von, Bontenbal M, Ring A, Giermek J et al. Treatment of breast cancer during pregnancy: An observational study. The Lancet Oncology 2012; 13(9):887–96.

19. Baselga J, Cortes J, Kim S-B, Im S-A, Hegg R, Im Y-H et al. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. N Engl J Med 2012; 366(2):109–19.

20. Sadeghi S, Olevsky O, Hurvitz SA. Profiling and targeting HER2-positive breast cancer using trastuzumab emtansine. Pharmgenomics Pers Med 2014; 7:329–38.

21. Pauletti G, Dandekar S, Rong H, Ramos L, Peng H, Seshadri R et al. Assessment of methods for tissue-based detection of the HER-2/neu alteration in human breast cancer: a direct comparison of fluorescence in situ hybridization and immunohistochemistry. J Clin Oncol 2000; 18(21):3651–64.

22. Minckwitz G von, Untch M, Blohmer J-U, Costa SD, Eidtmann H, Fasching PA et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. J Clin Oncol 2012; 30(15):1796–804.

23. Sikov WM, Dizon DS, Strenger R, Legare RD, Theall KP, Graves TA et al. Frequent pathologic complete responses in aggressive stages II to III breast cancers with every-4-week carboplatin and weekly paclitaxel with or without trastuzumab: a Brown University Oncology Group Study. J Clin Oncol 2009

[cited 2017 Jan 6]; 27(28):4693–700. Available from:

URL: http://ascopubs.org/doi/pdf/10.1200/jco.2008.21.4163.

24. Carey LA, Dees EC, Sawyer L, Gatti L, Moore DT, Collichio F et al. The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. Clin Cancer Res 2007; 13(8):2329–34.

25. Natale JJ. Reviewing current and emerging antiemetics for chemotherapy-induced nausea and vomiting prophylaxis. Hosp Pract (1995) 2015; 43(4):226–34.

26. Shin H, Jo SJ, Kim DH, Kwon O, Myung S-K. Efficacy of interventions for prevention of chemotherapy-induced alopecia: a systematic review and meta-analysis. Int J Cancer 2015; 136(5):E442-54.

27. Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol 2014; 740:364–78.

28. Rabbani A, Finn RM, Ausio J. The anthracycline antibiotics: antitumor drugs that alter chromatin structure. Bioessays 2005; 27(1):50–6.

29. Mojardin L, Botet J, Quintales L, Moreno S, Salas M. New insights into the RNA-based mechanism of action of the anticancer drug 5'-fluorouracil in eukaryotic cells. PLoS One 2013; 8(11):e78172.

30. Churchill CDM, Klobukowski M, Tuszynski JA. Elucidating the mechanism of action of the clinically approved taxanes: a comprehensive comparison of local and allosteric effects. Chem Biol Drug Des 2015; 86(5):1253–66.

31. Rastogi P, Anderson SJ, Bear HD, Geyer CE, Kahlenberg MS, Robidoux A et al. Preoperative chemotherapy: updates of National Surgical Adjuvant Breast and Bowel Project Protocols B-18 and B-27. J Clin Oncol 2008; 26(5):778–85.

32. Fisher B, Bryant J, Wolmark N, Mamounas E, Brown A, Fisher ER et al. Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. J Clin Oncol 1998; 16(8):2672–85.

33. Gralow JR, Burstein HJ, Wood W, Hortobagyi GN, Gianni L, Minckwitz G von et al. Preoperative therapy in invasive breast cancer: pathologic assessment and systemic therapy issues in operable disease. J Clin Oncol 2008; 26(5):814–9.

34. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: An overview of the randomised trials. The Lancet 2005; 365(9472):1687–717.

35. Levine MN, Pritchard KI, Bramwell VHC, Shepherd LE, Tu D, Paul N. Randomized trial comparing cyclophosphamide, epirubicin, and fluorouracil with cyclophosphamide, methotrexate, and fluorouracil in premenopausal women with node-positive breast cancer: update of National Cancer Institute of Canada Clinical Trials Group Trial MA5. J Clin Oncol 2005; 23(22):5166–70.

36. Henderson IC, Berry DA, Demetri GD, Cirrincione CT, Goldstein LJ, Martino S et al. Improved outcomes from adding sequential Paclitaxel but not from escalating Doxorubicin dose in an adjuvant chemotherapy regimen for patients with node-positive primary breast cancer. J Clin Oncol 2003; 21(6):976–83.

37. Kesler SR, Blayney DW. Neurotoxic Effects of Anthracycline- vs Nonanthracycline-Based Chemotherapy on Cognition in Breast Cancer Survivors. JAMA Oncol 2016; 2(2):185–92.

38. Chen XS, Nie XQ, Chen CM, Wu JY, Wu J, Lu JS et al. Weekly paclitaxel plus carboplatin is an effective nonanthracycline-containing regimen as neoadjuvant chemotherapy for breast cancer. Ann Oncol 2010; 21(5):961–7.

39. Giordano SH, Perkins GH, Broglio K, Garcia SG, Middleton LP, Buzdar AU et al. Adjuvant systemic therapy for male breast carcinoma. Cancer 2005; 104(11):2359–64.

40. Lee W-L, Cheng M-H, Chao H-T, Wang P-H. The Role of Selective Estrogen Receptor Modulators on Breast Cancer: From Tamoxifen to Raloxifene. Taiwanese Journal of Obstetrics and Gynecology 2008; 47(1):24–31.

41. Lewis JS, Jordan VC. Selective estrogen receptor modulators (SERMs): mechanisms of anticarcinogenesis and drug resistance. Mutat Res 2005; 591(1-2):247–63.

42. Brodie AM, Njar VC. Aromatase inhibitors in advanced breast cancer: Mechanism of action and clinical implications. The Journal of Steroid Biochemistry and Molecular Biology 1998; 66(1-2):1–10.

43. Ghorab MM, Alsaid MS, Samir N, Abdel-Latif GA, Soliman AM, Ragab FA et al. Aromatase inhibitors and apoptotic inducers: Design, synthesis, anticancer activity and molecular modeling studies of novel phenothiazine derivatives carrying sulfonamide moiety as hybrid molecules. Eur J Med Chem 2017; 134:304–15.

44. Chlebowski RT, Haque R, Hedlin H, Col N, Paskett E, Manson JE et al. Benefit/risk for adjuvant breast cancer therapy with tamoxifen or aromatase inhibitor use by age, and race/ethnicity. Breast Cancer Res Treat 2015; 154(3):609–16.

45. Ellis MJ, Ma C. Letrozole in the neoadjuvant setting: the P024 trial. Breast Cancer Res Treat 2007; 105 Suppl 1:33–43.

46. Smith IE, Dowsett M, Ebbs SR, Dixon JM, Skene A, Blohmer J-U et al. Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: the Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen (IMPACT) multicenter double-blind randomized trial. J Clin Oncol 2005; 23(22):5108–16.

47. Marcus DM, Switchenko JM, Prabhu R, O'Regan R, Zelnak A, Fasola C et al. Neoadjuvant Hormonal Therapy is Associated with Comparable Outcomes to Neoadjuvant Chemotherapy in Post-Menopausal Women with Estrogen Receptor-Positive Breast Cancer. Front Oncol 2013; 3:317.

48. Wang-Rodriguez J, Cross K, Gallagher S, Djahanban M, Armstrong JM, Wiedner N et al. Male breast carcinoma: correlation of ER, PR, Ki-67, Her2-Neu, and p53 with treatment and survival, a study of 65 cases. Mod Pathol 2002; 15(8):853–61.

49. Eggemann H, Ignatov A, Smith BJ, Altmann U, Minckwitz G von, Rohl FW et al. Adjuvant therapy with tamoxifen compared to aromatase inhibitors for 257 male breast cancer patients. Breast Cancer Res Treat 2013; 137(2):465–70.

50. Gnant M, Harbeck N, Thomssen C. St. Gallen 2011: Summary of the Consensus Discussion. Breast Care (Basel) 2011; 6(2):136–41.

51. Valabrega G, Montemurro F, Aglietta M. Trastuzumab: mechanism of action, resistance and future perspectives in HER2-overexpressing breast cancer. Ann Oncol 2007; 18(6):977–84.

52. Gunaldi M, Duman BB, Afsar CU, Paydas S, Erkisi M, Kara IO et al. Risk factors for developing cardiotoxicity of trastuzumab in breast cancer patients: An observational single-centre study. J Oncol Pharm Pract 2016; 22(2):242–7.

53. Mantarro S, Rossi M, Bonifazi M, D'Amico R, Blandizzi C, La Vecchia C et al. Risk of severe cardiotoxicity following treatment with trastuzumab: a meta-analysis of randomized and cohort studies of 29,000 women with breast cancer. Intern Emerg Med 2016; 11(1):123–40.

54. Gianni L, Pienkowski T, Im Y-H, Roman L, Tseng L-M, Liu M-C et al. 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. The Lancet Oncology 2012; 13(1):25–32.

55. Gianni L, Eiermann W, Semiglazov V, Lluch A, Tjulandin S, Zambetti M et al. Neoadjuvant and adjuvant trastuzumab in patients with HER2-positive locally advanced breast cancer (NOAH): Follow-up of a randomised controlled superiority trial with a parallel HER2-negative cohort. The Lancet Oncology 2014; 15(6):640–7.

56. Peintinger F, Buzdar AU, Kuerer HM, Mejia JA, Hatzis C, Gonzalez-Angulo AM et al. Hormone receptor status and pathologic response of HER2-positive breast cancer treated with neoadjuvant chemotherapy and trastuzumab. Ann Oncol 2008; 19(12):2020–5.

57. Wang Y, Sun T, Wan D, Sheng L, Li W, Zhu H et al. Hormone receptor status predicts the clinical outcome of human epidermal growth factor 2-positive metastatic breast cancer patients receiving trastuzumab therapy: a multicenter retrospective study. Onco Targets Ther 2015; 8:3337–48.

58. Nahta R, Esteva FJ. Trastuzumab: triumphs and tribulations. Oncogene 2007; 26(25):3637–43.

59. Symmans WF, Peintinger F, Hatzis C, Rajan R, Kuerer H, Valero V et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol 2007; 25(28):4414–22.

60. Peintinger F, Sinn B, Hatzis C, Albarracin C, Downs-Kelly E, Morkowski J et al. Reproducibility of residual cancer burden for prognostic assessment of breast cancer after neoadjuvant chemotherapy. Mod Pathol 2015; 28(7):913–20.

61. Choi M, Park YH, Ahn JS, Im Y-H, Nam SJ, Cho SY et al. Assessment of pathologic response and long-term outcome in locally advanced breast cancers after neoadjuvant chemotherapy: comparison of pathologic classification systems. Breast Cancer Res Treat 2016; 160(3):475–89.

62. Bossuyt V, Symmans WF. Standardizing of Pathology in Patients Receiving Neoadjuvant Chemotherapy. Ann Surg Oncol 2016; 23(10):3153–61.

63. Provenzano E, Bossuyt V, Viale G, Cameron D, Badve S, Denkert C et al. Standardization of pathologic evaluation and reporting of postneoadjuvant specimens in clinical trials of breast cancer: recommendations from an international working group. Mod Pathol 2015; 28(9):1185–201.

64. Symmans WF, Wei C, Gould R, Yu X, Zhang Y, Liu M et al. Long-Term Prognostic Risk After Neoadjuvant Chemotherapy Associated With Residual Cancer Burden and Breast Cancer Subtype. J Clin Oncol 2017; 35(10):1049–60.

65. Prabhu JS, Korlimarla A, Desai K, Alexander A, Raghavan R, Anupama C et al. A Majority of Low (1-10%) ER Positive Breast Cancers Behave Like Hormone Receptor Negative Tumors. J Cancer 2014; 5(2):156–65.

66. Yi M, Huo L, Koenig KB, Mittendorf EA, Meric-Bernstam F, Kuerer HM et al. Which threshold for ER positivity? a retrospective study based on 9639 patients. Ann Oncol 2014; 25(5):1004–11.

67. D. Craig Allred MD. ASCO/CAP Guideline Recommendations for IHC: Testing of ER and PgR in Breast Cancer. Journal of Clinical Oncology 28:2784-2795, 2010 [cited 2017 May 14]. Available from:

URL: http://uscapknowledgehub.org/site~/100th/pdf/companion11h01.pdf.

68. Brandt J, Garne JP, Tengrup I, Manjer J. Age at diagnosis in relation to survival following breast cancer: a cohort study. World J Surg Oncol 2015; 13:33.

69. Lips EH, Mukhtar RA, Yau C, Ronde JJ de, Livasy C, Carey LA et al. Lobular histology and response to neoadjuvant chemotherapy in invasive breast cancer. Breast Cancer Res Treat 2012; 136(1):35–43.

70. Kennecke H, Yerushalmi R, Woods R, Cheang MCU, Voduc D, Speers CH et al. Metastatic behavior of breast cancer subtypes. J Clin Oncol 2010; 28(20):3271–7.

71. Savci-Heijink CD, Halfwerk H, Hooijer GKJ, Horlings HM, Wesseling J, van de Vijver MJ. Retrospective analysis of metastatic behaviour of breast cancer subtypes. Breast Cancer Res Treat 2015; 150(3):547–57.