The effects of neoadjuvant chemotherapy on grade and proliferation of breast cancer

Ph. D. Thesis

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LIST OF PAPERS THAT SERVED AS THE BASIS OF THE PH.D. THESIS

I. **Ádám Ferenczi**, Gábor Cserni. Changes in breast cancer grade from biopsy to excision following surgery or primary chemotherapy. Pathologica 2024; 116:22-31, doi: 10.32074/1591-951x-958

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II. Tamás Zombori, **Ádám Ferenczi**, Anita Sejben, Szintia Almási, Veronika Szelestei, Renáta Koszó, Tamás Lantos, Zsuzsanna Kahán, Gábor Cserni. The prognostic value of histological grade determined after neoadjuvant chemotherapy of breast cancer. Pathol Res Pract 2025; 265:155732, doi: 10.1016/j.prp.2024.155732

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III. **Ádám Ferenczi**, Tamás Lantos, Gábor Cserni. Temporality of change in Ki67 labelling following neoadjuvant systemic chemotherapy of breast cancer – The common drop often subsides with time. Virchovs Arch 2025; Nov 13, doi: 10.1007/s00428-025-04263-7

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IV. **Ádám Ferenczi**, Levente Kuthi, István Sejben, Anita Sejben. Colonic tubular adenoma with clear cell change – case report with whole exome sequencing and updated review of the literature. Pathobiology 2024; 91:375-381, doi: 10.1159/000538705

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V. **Ferenczi Ádám**, Sejben Anita. Cysticus lymphangioma az ileumban. Orv Hetil 2024; 165(29):1135-1139, doi: 1556/650.2024.33089

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VIII. **Ádám Ferenczi**, Sofia Germano, Joana Afonso, Levente Kuthi, Anita Sejben. Duodenal foveolar adenoma – case presentation with whole exome sequencing and review of the literature. Pathobiology 2025; doi: 10.1159/000546139

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IX. **Ferenczi Ádám**, Sejben Anita. Hyperplasticus polyp-szerű átalakulás első gyulladásos bélbetegség diagnózis mellett. Orv Hetil 2025; 166(31): 1230-1233, doi: 10.1556/650.2025.33363

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X. **Ádám Ferenczi**, Karim Rashid, Yaffa Alkawasmi, El Samad Rayan, Sawako Yoshida, Ahmed Friji, Tran Anh Phuong, Tamás Lantos, Anita Sejben. Hepatic and splenic hyaloserositis. Diagnostics 2025; 15(15):1949, doi: 10.3390/diagnostics15151949

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XI. Sejben Anita, **Ferenczi Ádám**, Parsa Abbasi, Pósfai Boglárka, Zombori Tamás, Lantos Tamás. Májáttétek hisztopatológiai növekedési mintázatai. Orv Hetil 2025; 166(34):1323-1332, doi: 10.1556/650.2025.33358

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XII. Anita Sejben, Bence Baráth, Szintia Almási, Boglárka Pósfai, **Ádám Ferenczi**, Parsa Abbasi, Tamás Zombori, Tamás Lantos. Reproducibility examination of histopathological growth patterns of liver metastases in a retrospective, consecutive, single center cohort with literature review. Med Sci 2025; 13(4):220, doi: 10.3390/medsci13040220

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XIII. Berkenye Csonka, **Ádám Ferenczi**, Anita Sejben. Beyond the inflamed appendix: Low-grade appendiceal mucinous neoplasm – emphasis on macroscopic findings. Diagnostics 2026; 16(2):315, doi: 10.3390/diagnostics16020315

IF (2024/25): 3,3 (Scimagojr: Q1)

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LIST OF ABBREVIATIONS:

BxG – histological grade from biposy specimen

CHIR – the group treated with primary surgery

CISH – chromogenic in situ hybridization

CNB – core needle biopsy specimen

ER – oestrogen receptor

EWGBSP – European Working Group for Breast Screening Pathology

EXC – surgical excision specimen

FISH – fluorescent in situ hybridization

G – Nottingham histological grade

HER2 – human epidermal growth factor receptor 2, c-ErbB2

HR – hormone receptor

ICCR – International Collaboration on Cancer Reporting

IHC – immunohistochemitry

ISH – in situ hybridization

Ki67 – antigen Kiel 67

Ki67 LI – Ki67 labelling index

M – histological grade area adjusted mitotic rate subscore

NACT – neoadjuvant chemotherapy

P – histological grade nuclear pleomorphism subscore

pCR – pathological complete regression

PR – progesterone receptor

PST – the group treated with primary systemic therapy

T – histological grade tubule formation subscore

TIL – tumour-infiltrating lymphocytes

TN – triple negative (breast cancer)

WHO – World Health Organization

yG – postneoadjuvant histological grade

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1. INTRODUCTION

Breast cancer is a highly prevalent disease with the 2nd highest incidence among cancers ranked by site of origin, while its mortality rate has been showing a decreasing trend over the years, largely due to advances in prevention, early diagnosis and modern, personalised treatment [1-3].

The treatment of neoplastic disease is more and more shifted to personalization. For this, many parameters need to be assessed. As concerns the tumours, prognosticators (variables with proven effect on outcome) and predictive markers (variables predicting the effectiveness of a given treatment) have to be considered in treatment allocation.

The pathology report contains crucial prognostic and predictive factors that play a role in the selection of the most appropriate treatment plan. Along with many important prognostic factors, such as the age of the patient, histological type of the tumour, presence of vascular or lymphovascular invasion, primary tumour size (pT category), lymph node status (pN category), tumour-infiltrating lymphocytes (TIL), oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (c-erbB-2 or HER2) statuses, the report must also contain the histological grade (G) according to the Nottingham grading system [4-7].

The first three-tiered histological grading system for breast cancer was published by Greenhough in 1925. This was followed by the Bloom and Richardson grading system and finally, the currently used Nottingham or Elston and Ellis grading scheme [5, 8-10]. Histological grade reflects the differentiation and biological behaviour of the tumour, and in the case of breast cancer, it is determined by summarizing the subscores for tubule formation (T), nuclear pleomorphism (P) and area adjusted mitotic rate (M), each being scored on a three-tiered scale. According to the sum of these subscores, the tumour can be well differentiated (Grade 1, 3-5 points), moderately differentiated (Grade 2, 6-7 points) or poorly differentiated (Grade 3, 8-9 points). The evaluation of grade contains subjective factors and may be influenced by the experience of the evaluator, thus its reproducibility is considered moderate [11, 12]; nonetheless the prognostic significance of grade remains clear even in the era of modern molecular testing and gene expression profiling [13, 14].

The report must also contain the ER-, PR- and HER2-statuses. When assessing hormone-receptor (HR) status by immunohistochemistry (IHC), the threshold for positivity is 1%; expression of <1% being considered as negative and ≥1% as positive. However, according to recent guidelines,

tumours displaying 1-10% positive staining by IHC should be categorised as “weakly positive”, as they are less sensitive to endocrine therapy compared to tumours with stronger expression [15]. For HR assessment, the Allred quick score is advised and, if possible, only blocks containing internal control should be used.

The assessment protocol of HER2-status has undergone major changes, and according to the updated guidelines, 4 categories should be used instead of the previous positive-negative dichotomic classification. As a first step, IHC should be performed. Tumours displaying a strong, circular membranous staining in >10% of tumours cells are classified as 3+, positive. In case of complete, but only weak to moderate staining in >10% of tumour cells, in situ hybridization (ISH) reflex test should be performed (chromogenic – CISH, fluorescent – FISH or silver-labelled - SISH) to determine the HER2 gene amplification status and, if amplified, the HER2 status is 2+, positive (amplified). Tumours displaying 2+ IHC results and a lack of amplification by ISH, as well as cases with incomplete, weak staining in >10% of tumour cells (1+), formally negative, are now classified as “HER2-low”, and the same criteria (i.e. incomplete, weak staining on IHC) being met in ≤10% of tumour cells form the “HER2-ultra low” (0+) category. Only in the case of a complete absence of staining can a tumour be classified as completely negative (0) [15].

The treatment of breast cancer includes loco-regional modalities like surgery and radiotherapy, and systemic options also acting at distant sites, but also locally and regionally, like endocrine therapy, chemotherapy, targeted treatments and immunotherapies. These latter will be listed in more details, as they are connected to the topic of the present thesis.

HR-positive tumours may benefit from endocrine therapy, which includes aromatase inhibitors (e.g. letrozole or anastrozole), selective oestrogen receptor modulators (e.g. tamoxifen), selective oestrogen receptor degraders (e.g. fulvestrant), and endocrine therapy of advanced, metastastic or high-risk early breast cancers may be combined with cyclin dependent kinase 4/6 (CDK4/6) inhibitors (e.g. palbociclib, abemaciclib, ribociclib) as well. More recent advances in the treatment of metastatic HR-positive breast cancers include datopotamab-deruxtecan, which targets the tumour cells using trophoblast cell surface antigen 2 (TROP2) protein.

Currently, HER2-targeting therapy may be composed of trastuzumab or trastuzumab-pertuzumab combination therapy (classical anti-HER2 treatments), as well as the more recent group of antibody-drug-conjugates, such as trastuzumab-emtansine (T-DM1) and trastuzumab-deruxtecan

(T-DXd). Some patients also derive benefit from small molecule HER2/EGFR tyrosine-kinase inhibitor (lapatinib).

Other novel therapeutic agents used in the treatment of breast cancer include phosphoinositide 3-kinase (PI3K) inhibitors (e.g. alpelisib, inavolisib) and protein kinase B (AKT) inhibitors (e.g. capivasertib) in the case of PI3K or AKT mutations being present. Furthermore, mammalian target of rapamycin (mTOR) inhibitors (e.g. everolimus) may be used mainly in case of resistance to CDK4/6 or PI3K inhibitors. In addition, poly (ADP-ribose) polymerase (PARP) inhibitors (e.g. olaparib) may be valuable in BRCA-mutated cancers.

In the era of molecular diagnostics, breast cancer has become an even more heterogenous disease with multiple molecular subtypes being described. The main molecular subtypes are luminal A, luminal B, basal-like and HER2-enriched. In everyday clinical practice, the use of genomic tests is not as widespread, thus, according to the 2015 St. Gallen consensus guidelines, surrogate molecular subtypes may be used. Luminal A-like tumours display a strong HR expression and low Ki67 proliferation index, the tumour mass is usually smaller and the tumours are most often of pT1-pT2 and pN0-pN1 category. Triple negative (TN) tumours lack ER, PR and HER2 expression. HER2-positive tumours show overexpression of the HER2 protein and variable HR expression. The group of luminal B-like tumours is quite heterogenous and not well defined: it is composed of tumours with lesser HR expression, higher Ki67 labelling index, larger tumour mass, ≥pN2, ≥pT3, grade 3, extensive lymphovascular invasion and possible simultaneous HER2 expression [15-17].

Systemic chemotherapy for non-metastatic breast cancer may be administered before and/or after surgery and thus we can define it as neoadjuvant or adjuvant chemotherapy. According to the International Collaboration on Cancer Reporting (ICCR) guidelines, histological grade must be assessed from both preoperative core needle biopsy (CNB) specimens and surgical excision (EXC) specimens, as well as following neoadjuvant chemotherapy (NACT), although its prognostic value has less evidence in this latter case [15]. The use of NACT is becoming more and more widespread, due to its ability to downstage locally advanced tumours, thus allowing for breast-conserving surgery and also serving as a way to monitor tumour response to the treatment, as well as eliminating micrometastases or even greater metastatic deposits in high-risk early or locally advanced breast cancers [18]. Reporting the response of the tumour to NACT is also a core element of the pathology report according to the ICCR recommendations [19]. For this purpose, several

regression grading systems have been implemented and according to the most recent Hungarian guidelines, the Residual Cancer Burden (RCB) and European Working Group for Breast Screening Pathology (EWGBSP) Tumour Response (TR) and Nodal Response (NR) categories should be used while reporting [15]. The RCB system takes into account the primary invasive tumour bed area (the two greatest perpendicular dimensions), overall cancer cellularity, percentage of in situ disease, number of positive lymph nodes and diameter of the largest metastasis, resulting in RCB-I, -II and -III categories. The EWGBSP TR system contains 3 major categories with further subdivision: 1a (pCR, no residual carcinoma), 1b (no residual invasive disease but residual DCIS is present), 2a (<10% residual disease), 2b (10-50% residual invasive disease), 2c (>50% residual invasive tumour) and 3 (no signs of regression). The condition for pathological complete response (pCR) is the complete processing of the tumour bed and all resected lymph nodes and their lack of any tumour cells. Pathological complete response has been found to be a good predictor of survival [20].

Most contemporary systemic chemotherapy regimens for breast cancer contain taxanes (eg. docetaxel or paclitaxel) combined with anthracyclines (eg. doxorubicin or epirubicin) or platinum-based chemotherapeutic agents in case of cardiac comorbidities. The general effect of chemotherapy can be described as a reduction in the proliferating fraction of the tumour by inhibiting different components of the mitotic cycle, such as inhibition of the topoisomerase II enzyme or microtubule formation, or causing apoptosis and inhibition of DNA repair via crosslinking. Tumour proliferation can be assessed, among many other ways, by Ki67 IHC and the labelling index (Ki67 LI) which describes the percentage of staining cells. Ki67 is a nuclear protein expressed during the G1, S, G2 and M phases of the cell cycle, but not in the resting state, G0 [21]. As it describes tumour proliferation, Ki67 LI has been found to be an independent prognosticator for both overall survival (OS) and recurrence-free survival (RFS) when assessed from core biopsies (CNB-Ki67), postneoadjuvant surgical specimens (yKi67), as well as the change of these values from biopsy to excision specimen (ΔKi67) [22]. Prior research has also shown that Ki67 LI is a strong predictor of pCR and higher values forecast a better response to chemotherapy, while higher yKi67 values indicate worse prognosis and a higher risk of early recurrence [18, 23-31]. Despite profound literature concerning Ki67 and its prognostic value, the dynamics and temporality of change in Ki67 LI following NACT are not well documented.

2. AIMS

The aims of the thesis and the articles serving as its basis are as follows:

1. To describe the changes observed in histological grade of breast cancer and its subscores for tubule formation, nuclear pleomorphism and mitotic rate following NACT and compare these with cases operated on without NACT.

2. To assess the prognostic significance of yG seen following NACT of breast cancers.

3. To analyse tumour proliferation as reflected by Ki67 IHC before and after NACT and evaluate its dynamics.

3. MATERIALS AND METHODS

3.1. ASSESSMENT OF CHANGES IN HISTOLOGICAL GRADE AND ITS COMPONENTS FOLLOWING NEOADJUVANT CHEMOTHERAPY

Data of patients with invasive breast cancer diagnosis from the time period of 2010 to 2022 were collected from the digital medical records of the Bács-Kiskun County Teaching Hospital. The patients were required to have both CNB and EXC specimens available.

Of all the cases seen at the Department of Pathology, the following were excluded from the study:

- multifocal tumours in which the histological grade of the foci was different, thus it was unclear which focus was sampled preoperatively by the CNB (this exclusion was not applied to cases in which the multiple foci possessed identical histological grade);
- the CNB specimen was crushed or had limited diagnostic value, leading to the assessment of grade being inadequate (e.g., tumour dimension of less than 10 high power fields corresponding to $2mm^2$ or crush artefacts leading to uncertain assessment of nuclear pleomorphism or lumen formation);
- tumours with the diagnosis of in situ carcinoma from CNB;
- cases receiving neoadjuvant endocrine therapy;
- cases displaying pathological complete response or the residual disease being unsuitable for grading.

In all included cases, histological grade and its subscores were extracted from the original reports, and all data were recorded in Microsoft Excel spreadsheets. The cases were divided into groups according to the type of therapy received, thus the group receiving NACT was named PST, while the group treated with surgery and no neoadjuvant therapy was named CHIR.

Histological grade was assessed according to the Nottingham grading system by a pathologist expert in breast pathology. This method of grading is also part of the most recent recommendation by the World Health Organization (WHO) Classification of breast tumours. For each case and its pair of samples before and after treatment, the three-tiered values of histological grade (G), T, P and M were recorded. The corresponding variables were matched for the CNB and EXC specimen of each case, thus representing the first and second measurement of that variable.

The distributions for each value of every variable (i.e. G, T, P, M) in the CHIR and PST groups, as well as the concordance rates in both groups were assessed by Chi-square test for both CNB and EXC specimens.

Assessment of change in T, P, M and G from CNB to EXC in both groups was carried out by nonparametric Wilcoxon signed rank test, as it accounts for negative and positive changes in the variables and analyses whether the change seen is random or carries some sort of tendency. The statistical calculations were carried out in Microsoft Excel's Real Statistics Resource Pack add-in, using the corrections recommended by Charles Zaiontz, the creator and author of the add-in. Namely, for calculations where the case number (n) is greater than 25, the T statistics shows near-normal distribution, and a second correction is also recommended for better variance estimation if a large number of cases shows concordance between the first and second measurement values. Lastly, the author recommends the continuity correction which adds a new factor to the usual calculation of the z-score, because we estimate the change of a discrete variable with a continuous one. The final, resulting z-score in a two-tailed test with 95% confidence intervals was significant above 1.96. Level of significance was $p < 0.05$.

3.2. EXAMINATION OF THE PROGNOSTIC VALUE OF POSTNEOADJUVANT GRADE

The retrospective consecutive study included breast cancer patients who received NACT followed by surgery at the Departments of Oncotherapy and Surgery of the Albert Szent-Györgyi Clinical Centre of the University of Szeged between 1999 and 2018, as well as patients treated at the Bács-Kiskun County Teaching Hospital, Kecskemét, between 2000 and 2018.

Exclusion criteria in this study were the lack of sufficient residual tumour or undeterminable yG due to poor tissue fixation, as well as detection of distant metastasis (M1, stage IV) within 6 months from initial diagnosis, and lacking follow-up data from the digital charts.

For all included cases, multiple clinicopathological parameters, such as gender, age, histological diagnosis, histological grade from the biopsy specimen (BxG) of the tumour, type of NACT, type of surgery, completeness of resection, biopsy based ER, PR and HER2 status, presence or absence of lymphovascular invasion, yG, ypT, ypN categories, and lastly overall survival (OS) and recurrence free survival (RFS) were collected and recorded in Microsoft Excel spreadsheets. For survival assessment, time from initiation of systemic treatment to last follow-up or recurrence (RFS) / death (OS) was used; patients lost to follow-up were censored at the last follow-up date.

The change in histological grade has been assessed with contingency tables and Chi-square test in this cohort of cases, too.

The prognostic value of yG was assessed using Kaplan-Meier analyses and log-rank test, and to further solidify our findings, univariate Cox proportional hazards model was applied as well. Each variable proving significant in the univariate model has also been tried in a multivariate model. The statistical calculations and creation of graphs were carried out using SPSS Statistics V 23.0 software (Armonk, USA).

3.3. DESCRIPTION OF THE CHANGE IN KI67 LABELLING INDEX FOLLOWING NEOADJUVANT TREATMENT AND ITS TEMPORALITY

Invasive breast cancer cases treated with NACT with both CNB and EXC specimens available were collected at the Bács-Kiskun County Teaching Hospital between 2009 and 2024. Inclusion required Ki67 immunohistochemistry (IHC) stained slides available for both samples, and cases with technically suboptimal staining, cut out material, and tumours only available within megaslides were excluded.

For each of the included cases, data such as patient age, tumour type, grade, ER, PR and HER2 status, Ki67 IHC results via eye-balling based subjective estimation of the percentage of stained tumour cells by an expert pathologist (EB-EST), T, P and M scores according to the Nottingham grading system, mitotic index (MI: number of mitoses per 10 high power fields, corresponding to $2mm^2$), exact dates of surgery and last cycle of NACT prior to surgery, type of NACT and response

to treatment were collected in Microsoft Excel spreadsheets. The tumour response was characterized according to Residual Cancer Burden (RCB) I, II and III categories and the European Working Group for Breast Screening Pathology Tumour Regression (TR) categories 2a, 2b, 2c and 3, where the RCB-I and TR2a categories represent an excellent response to treatment (minimal residual disease), while RCB-III and TR3 categories represent no response at all. Apart from the relatively subjective EB-EST method, the proportion of Ki67 positive cells was also determined using 2 more objective methods.

The relevant Ki67 stained slides were collected from the archives of the Department and scanned for each case using a Pannoramic 250 scanner (3Dhistech, Budapest, Hungary). Three images were taken from each slide with the SlideViewer software (3DHistech, Budapest, Hungary) at 30X magnification with the aim of uniformly representing the tumours as much as possible; in tumours displaying homogenous staining, 3 random areas were photographed, while in heterogeneous tumours we aimed to take 2 images reflecting the dominant density and one representing the minority, to allow some weighting for the average. Specific attention was paid not to include pictures of areas with in situ (DCIS) component. These pictures served as the basis of our further analyses.

One method was the ImmunoRatio (IR) 1.0c plugin [32] with the Fiji imagej.net image processing package. This software is able to determine positive and negative nuclei by analysing the image according to brown and blue colour thresholds. The source image scale (4.0 pixel(s) / μm) and correction equation ($y = ax^3 + bx^2 + cx + d$; $a = 6.442$, $b = 0$, $c = 0.611$, $d = 0.43$) values were kept as default. The brown and blue colour threshold values were modified at each image to best represent the unique nuclear staining observable on each slide. During analysis of slides containing large foci of connective tissue or inflammatory cell infiltrate, the image was masked so that it only contained tumour cells for the recognition analysis.

The third method of assessing Ki67 staining proportion was a grid counting (GRID) according to the description by Vörös et al [33], thus all images were exported to Microsoft PowerPoint, and a grid of horizontal lines 1.5 cm apart, starting from the middle of the slide in both directions, were spread over each image. The nuclei being intersected by these grid lines were then counted manually and the ratio of positive to all cells (i.e. the Ki67 labelling index [LI]) was calculated.

For the EB-EST method, the whole slide was the basis for the estimation of the average labelling, done by the same pathologist (GC) to limit interobserver variability; for the IR and GRID methods, the average of the quantification of the 3 representative images were used, thus, by the end, for each tumour, there were three pairs of CNB and EXC Ki67 LI values to be correlated with time elapsed after the last cycle of NACT using linear regression models. The change in Ki67 LI (i.e. delta Ki67 [ΔKi67]) was defined as the preoperative biopsy specimen value being subtracted from the surgical excision specimen value, thus, positive ΔKi67 values correspond to an increase in proliferation, while negative values represent a decrease. Concordance between the observation methods was evaluated using Bland-Altman plots.

The studies reported were granted ethical approval by the Regional and Institutional Review Board of Human Investigations in University of Szeged (91/2021 SZTE-RKEB).

4. RESULTS

4.1. ASSESSMENT OF CHANGES IN HISTOLOGICAL GRADE AND ITS COMPONENTS FOLLOWING NEOADJUVANT CHEMOTHERAPY

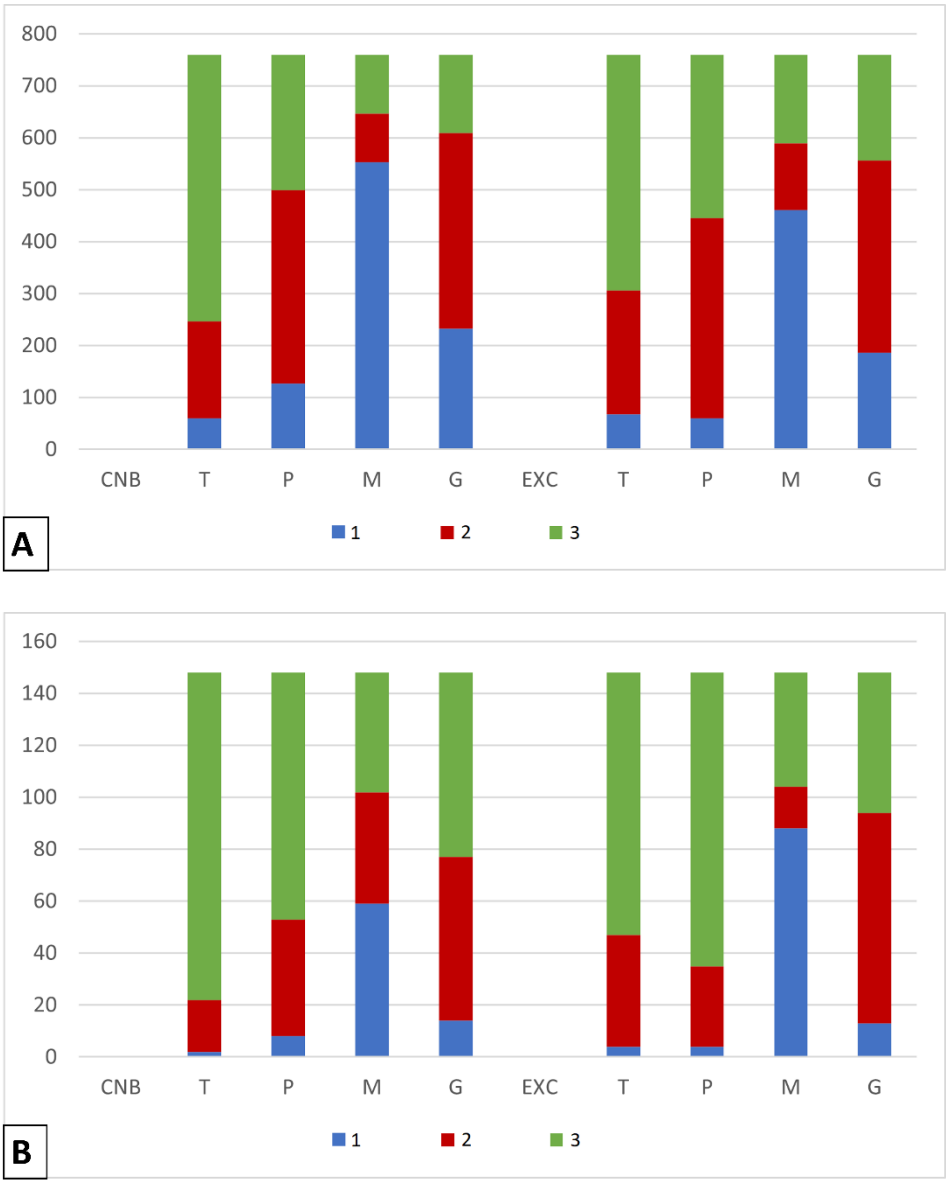
In total, 1257 pairs of CNB and EXC breast cancer specimens could be identified, from which 908 were left after all exclusions. The CHIR group receiving primary surgery consisted of 760 pairs of samples (n=760), while the PST group receiving primary systemic chemotherapy had 148 pairs (n=148). Taxane chemotherapy was used in the majority of cases in the latter group (132/148, 89.2%) and was generally paired with anthracyclines, mostly epirubicin, or platinum-based agents. In HER2 positive tumours (i.e. HER2-overexpressing or *ERBB2*-amplified), anti-HER2 treatment was also generally administered. The distribution of T, P and M subscores and histological grade values in the CHIR and PST groups are shown in Table 1 and Figure 1.

Table 1. – Case numbers, (percentages and [95% confidence interval limits]) of tubule formation, nuclear pleomorphism and mitotic activity subscores and histological grades in the two types of specimens in the two groups investigated.

CHIR (n = 760)				PST (n = 148)			
CNB	1	2	3	CNB	1	2	3
T	60 (7.9% [6.2- 10.0])	187 (24.6% [21.7- 27.8])	513 (67.5% [64.1- 70.7])	T	2 (1.4% [0.4- 4.8])	20 (13.5% [8.9- 20.0])	126 (85.1% [78.5- 90.0])
P	127 (16.7% [14.2- 19.5])	372 (49.0% [45.4- 52.5])	261 (34.3% [32.0- 37.8])	P	8 (5.4% [2.8- 10.3])	45 (30.4% [23.6- 38.2])	95 (64.2% [56.2- 71.5])
M	553 (72.8% [69.5- 75.8])	94 (12.4% [10.2- 14.9])	113 (14.9% [12.5- 17.6])	M	59 (39.9% [32.3- 47.9])	43 (29.1% [22.3- 36.8])	46 (31.1% [24.2- 38.9])
G	233 (30.7% [27.5- 34.0])	376 (49.5% [45.9- 53.0])	151 (19.9% [17.2- 22.9])	G	14 (9.5% [5.7- 15.3])	63 (42.6% [34.9- 50.6])	71 (48.0% [40.1- 56.0])
EXC				EXC			
T	68 (9.0% [7.1- 11.2])	238 (31.3% [28.1- 34.7]	454 (59.7% [56.2- 63.2])	T	4 (2.7% [1.1- 6.7])	43 (29.1% [22.3- 36.8])	101 (68.2% [60.4- 75.2])
P	60 (7.9% [6.2- 10.0])	386 (50.8% [47.2- 54.3])	314 (41.3% [37.9- 44.9])	P	4 (2.7% [1.1- 6.7])	31 (21.0% [15.2- 28.2])	113 (76.4% [68.9- 82.5])
M	461 (60.7% [57.1- 64.1])	129 (17.0% [14.5- 20.0])	170 (22.4% [19.6- 25.5])	M	88 (59.5% [51.4- 67.0])	16 (10.8% [6.8- 16.8])	44 (29.7% [23.0- 37.5])
G	186 (24.5% [21.6- 27.7])	371 (48.8% [45.3- 52.4])	203 (26.7% [23.7- 30.0])	G	13 (8.8% [5.2- 14.5])	81 (54.7% [46.7, 62.5])	54 (36.5% [29.2- 44.5])

CHIR: the group treated by primary surgery, CNB: core needle biopsy, EXC: excision specimens, G: histological grade, M: mitotic activity, P: nuclear pleomorphism, PST: the group treated with primary chemotherapy before surgery, T: tubule (gland) formation.

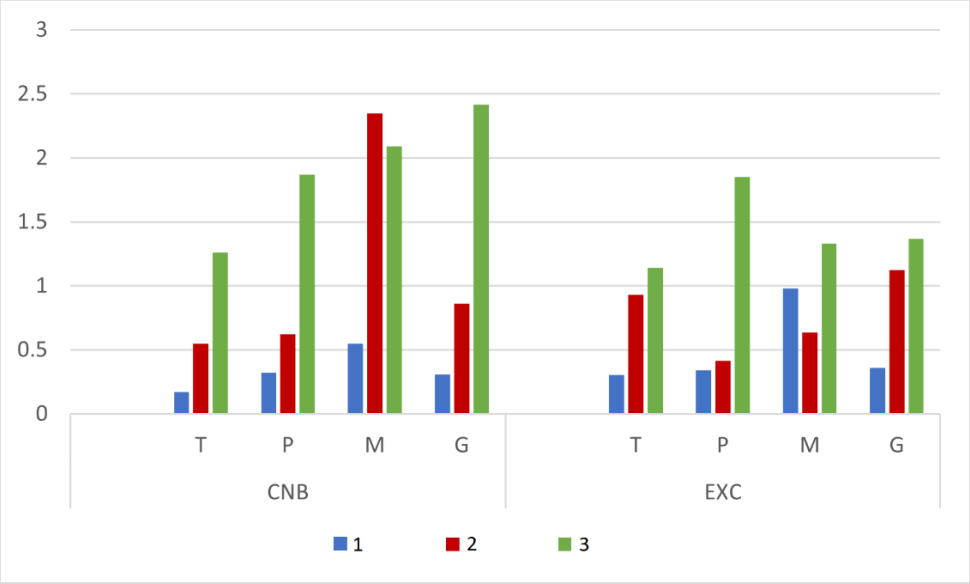
Figure 1. Distributions of case numbers for each grading subscore (T, P and M) and grade (G) in the group treated with A: primary surgery (CHIR) or B: primary chemotherapy (PST).



CNB: core needle biopsy, EXC: excision specimen. CHIR: the group treated with primary surgery, G: histological grade, M: mitotic activity, P: nuclear pleomorphism, PST: the group treated with primary chemotherapy before surgery, T: tubule (gland) formation.

To better illustrate the differences in distributions of the subscores and grade between the groups, the relative frequencies of each variable are shown on Figure 2. It is remarkable that at the time of treatment planning, based on CNB specimens, a greater proportion of poorly differentiated, high-grade (G3) and highly proliferative (i.e. higher M score) tumours can be observed in the PST group, since all of these characteristics serve as the basis for eligibility for NACT. The unchanged parameters from core biopsy to excision are summarized in Table 2.

Figure 2. PST/CHIR ratio of relative frequencies per grading subscores (T, P and M) and grade (G).



Values above 1 (especially >1.5) reflect greater incidence of a variable in the PST group. CHIR: the group treated with primary surgery, CNB: core needle biopsy, EXC: excision specimens, G: histological grade, M: mitotic activity, P: nuclear pleomorphism, PST: the group treated with primary chemotherapy before surgery, T: tubule (gland) formation.

Table 2 - Unchanged parameters from CNB to EXC in the two groups investigated.

CNB to EXC:	1-1	2-2	3-3	All
T (CHIR)	46 (7.7%)	134 (22.3%)	420 (70%)	600
P (CHIR)	43 (8.2%)	265 (50.6%)	216 (41.2%)	524
M (CHIR)	435 (76.7%)	38 (6.7%)	94 (16.6%)	567
G (CHIR)	148 (27.4%)	267 (49.4%)	126 (23.3%)	541
T (PST)	0 (0%)	16 (14%)	98 (86%)	114
P (PST)	2 (1.9%)	17 (16.3%)	85 (81.7%)	104
M (PST)	46 (62.2%)	4 (5.4%)	24 (32.4%)	74
G (PST)	7 (7.7%)	45 (49.5%)	40 (44%)	91

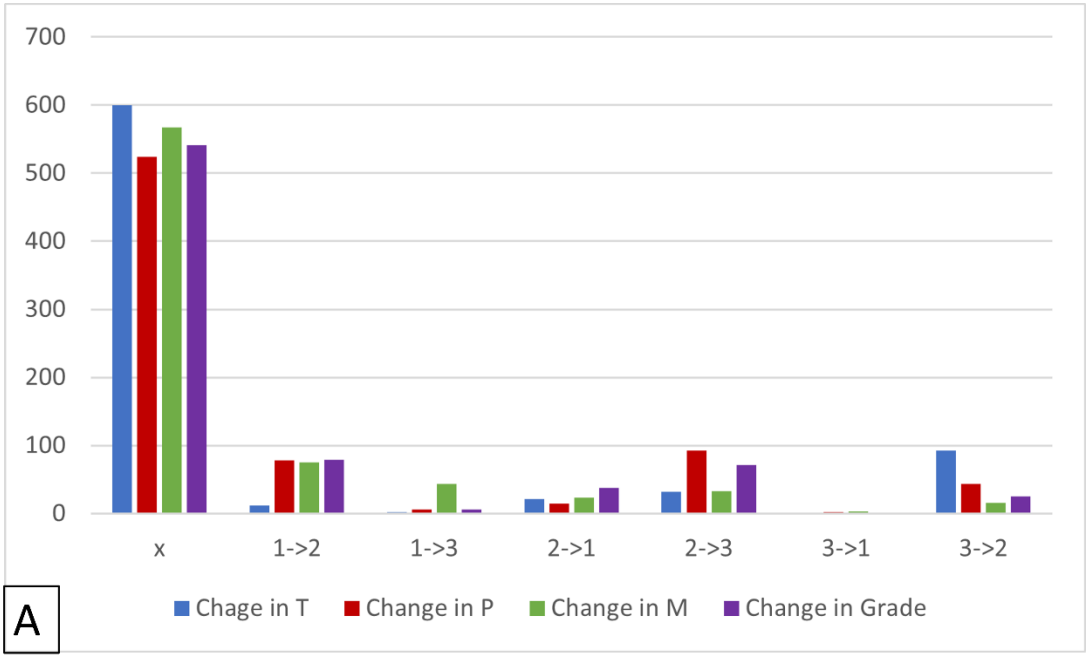
CHIR: the group treated by primary surgery, CNB: core needle biopsy, EXC: excision specimens, G: histological grade, M: mitotic activity, P: nuclear pleomorphism, PST: the group treated with primary chemotherapy before surgery, T: tubule (gland) formation.

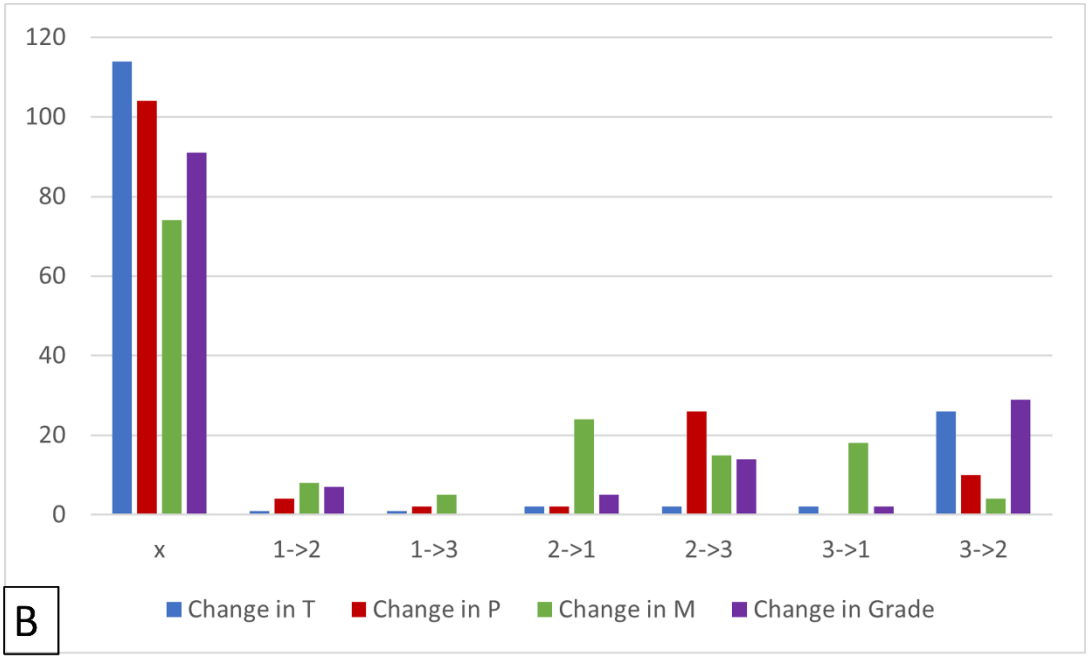
When comparing the distributions of the subscores and grade values of the CHIR and PST groups, in the case of CNBs significant differences could be observed for all parameters (T, P, M and G, all $p < 0.001$), whereas in EXC specimens T ($p < 0.05$), P ($p < 0.001$) and G ($p < 0.001$) were significantly different, but not M ($p = 0.544$).

Regarding the change of subscores and grade from CNB to EXC, a high concordance rate was observed in both the CHIR (T: 78.9%, P: 68.9%, M: 74.6%, G: 71.2%) and PST (T: 77%, P: 70.3%, M: 50%, G: 61.5%) groups. In the discordant cases, the most common change was that of a difference of one score. The concordance rates were significantly different between the CHIR and PST groups in G ($p = 0.024$) and the M subscore ($p < 0.0001$), but not in the T ($p = 0.68$) and P ($p = 0.82$) subscores. For discordant cases, the following trends could be identified. Changes in the subscore of T were predominantly from a low tubule formation to a higher tubule formation tendency (i.e. from high to a lower subscore) in both groups. In the CHIR group, the most commonly observed changes in P and M were that of a single point increase (1→2 or 2→3), however in the case of M, 2-point increases were also present. In the PST group, the P subscore tended to increase (2→3), however the dominant change in M subscore was a single- or 2-point-

reduction. As a result of the changes described above, the grade more commonly increased in the CHIR (71.2%, 95% CI: 64.7%-77.0%) and decreased in the PST (63.2%, 95% CI: 50.2%-74.5%) groups. The results of the Wilcoxon signed-rank test indicate a statistically significant change across all evaluated parameters in both groups (CHIR group: T, P, M and G, all $p < 0.001$; PST group: T, $p < 0.001$, P, M and G, $p < 0.05$). Changes in the values of T, P, M subscores and G are illustrated on Figure 3.

Figure 3. Changes in the values of T, P, M subscores and grade (G) from CNB to EXC in the two patient groups





(A: CHIR and B: PST groups). CHIR: the group treated with primary surgery, CNB: core needle biopsy, EXC: excision specimens, G: histological grade, M: mitotic activity, P: nuclear pleomorphism, PST: the group treated with primary chemotherapy before surgery, T: tubule (gland) formation, x: no change in the values.

4.2. EXAMINATION OF THE PROGNOSTIC VALUE OF POSTNEOADJUVANT GRADE

Altogether 355 patients were included in this study, all of whom were diagnosed with breast cancer, received NACT and residual invasive carcinoma was present in their excision specimens. The basic clinicopathological characteristics of the cohort are summarised in Table 3. The most common tumour type was breast carcinoma of no special type (NST), ER-positive, BxG 3. Most common type of treatment was taxane-based NACT and mastectomy. Approximately 40% of patients were diagnosed with ypT1 and 70% of patients with ypN1-3 categories, yG1 was barely identified, while the frequency of yG2 and yG3 categories was similar. The median time of follow-up was 73 months (range: 3.5-236 months), 86 patients died and 155 experienced disease recurrence.

Table 3. Basic clinicopathological characteristics of included patients.

Histological type	**n**	**%**
NST (IDC)	298	84
ILC	36	10
others	21	6
BxG		
G1	19	5
G2	109	31
G3	192	54
No data	35	10
ER		
Positive	225	63
Negative	127	36
No data	3	1
PR		
Positive	193	54
Negative	158	45
No data	4	1
HER2		
Positive	52	15
Negative	280	79
Unknown	23	6

Surrogate molecular subtypes		
Triple (ER/PR/HER2) negative	88	25
HER2 positive (ER and PR negative)	26	7
HER2 positive (ER or PR positive)	26	7
"Luminal-like" HER2-negative (ER and/or PR positive)	192	54
Unclassifiable (missing data)	23	6
Neoadjuvant chemotherapy		
Taxane-based	285	80
Anthracycline-based	57	16
Other	8	2
Not specified	5	1
Anti-HER2 treatment (mostly trastuzumab alone, or with pertuzumab)(added)	35	10
Surgery		
Excision	105	30
Mastectomy	250	70
yG		
yG1	31	9
yG2	169	48

yG3	155	43
Lymphovascular invasion		
Positive	100	28
Negative	250	71
No data	5	1
ypT	n	%
ypT1a-b	59	16
ypT1c	81	23
ypT2	127	36
ypT3	50	14
ypT4	10	3
ypTx	28	8
ypN		
ypN0	109	31
ypN0(i+)/1mi	14	4
ypN1	113	32
ypN2	73	21
ypN3	37	10
ypNx	9	2

NST: invasive breast carcinoma of no special type, IDC: invasive ductal carcinoma, ILC: invasive lobular carcinoma, BxG: grade in the core needle biopsy, ER: oestrogen receptor, PR: progesterone receptor, HER2: human epidermal growth factor receptor-2, NACT: neoadjuvant chemotherapy, yG: grade in the resection specimen after neoadjuvant therapy)

Changes in grade according to surrogate molecular subtypes and the nature of change (i.e. decrease or increase) are summarized in Table 4. The highest rate of change was observed in HR-positive, HER2-positive tumours (chi-square: 22.26, degrees of freedom: 3, $p < 0.0001$), followed by HR-positive, HER2-negative cases. When all luminal-like tumours were combined, their proportion of grade change remained significantly higher than that of triple-negative or HER2-positive, HR-negative breast cancers (chi-square: 11.32, degrees of freedom: 2, $p = 0.004$). Results of the Kaplan-Meier analyses are presented in Figure 4. Several of the differences in both OS and RFS across the different comparisons of BxG and yG categories were statistically significant. In general, higher grade categories of both BxG and yG were correlated to unfavourable prognoses regarding RFS and OS estimates, respectively. Subgroup analysis was carried out for cases with different BxG and yG (Figure 4. E-F), which indicated an adverse impact of higher yG categories on both RFS and OS estimates. Out of all cases, both BxG and yG data were available for 320 tumours; there was no change in grade in 221 (69%) cases, while an increase could be observed in 21 (7%) and a decrease in 78 (24%) cases. The results of univariable and multivariable Cox proportional hazard models are summarised in Table 5.

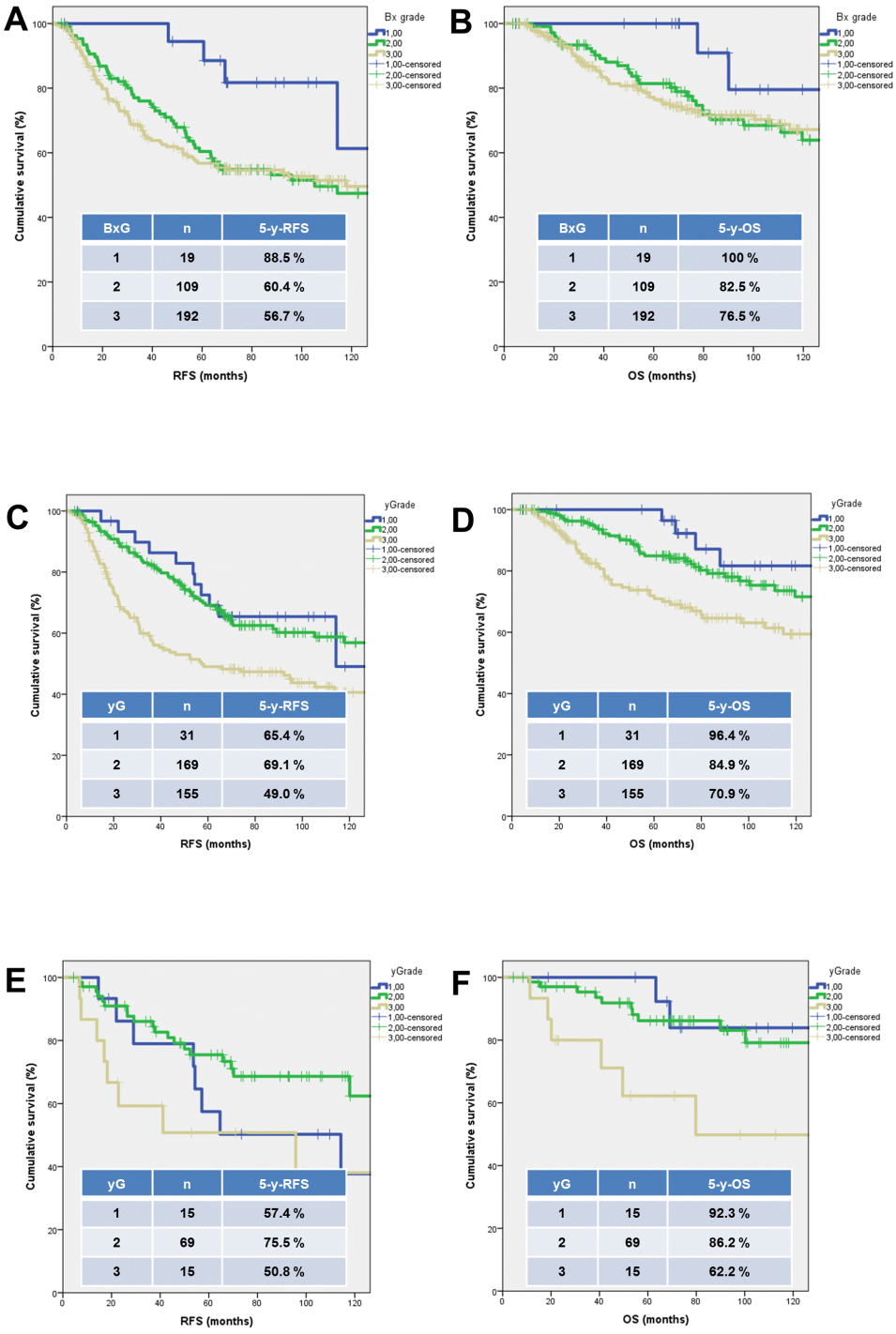
Table 4. Grade changes per surrogate molecular subtypes.

Type	All/assessable*	Grade changes (%)**	Grade decrease/increase ***
Triple-negative	88/78	15 (19%)	9 (0.6) / 6 (0.4)
HER2+ (HR-)	26/23	5 (22%)	4 (0.8)/1(0.2)
HER2+ (HR+)	26/25	17 (68%)	17 (1.0)/0
HR+ (HER2-)	192/175	61 (35%)	47 (0.77)/14(0.23)
Unclassified	23/19	1 (5%)	1 (1.0)/0

HR: hormone receptor, i.e. oestrogen receptor and/or progesterone receptor, HER2: human epidermal growth factor receptor-2

*assessable: having both BxG and yG values; ** percent of assessable cases; *** proportion of grade changes in parentheses

Figure 4. The results of the Kaplan-Meier analyses.



A and B: Regarding BxG, significant differences were identified between the RFS estimates of BxG 1 vs. 2 (p_{RFS}=0.022) and BxG 1 vs. 3 (p_{RFS}=0.019) but not those of BxG 2 vs. 3 (p_{RFS}=0.356). Furthermore, significant differences were identified between the OS estimates of BxG 1 vs. 3 (p_{OS}=0.05) but not between BxG 1 vs. 2 (p_{OS}=0.075) and BxG 2 vs. 3 (p_{OS}=0.518), respectively.

C and D: Concerning yG, significant differences were seen between RFS estimates of yG 1 vs. 3 (p_{RFS}=0.010) and yG 2 vs. 3 (p_{RFS}<0.001) but not those of yG 1 vs. 2 (p_{RFS}=0.788). Significant differences were demonstrated between OS estimates of yG 1 vs. 3 (p_{OS}=0.008) and yG 2 vs. 3 (p_{OS}<0.001) but not between yG 1 vs. 2 (p_{OS}=0.185), respectively.

E and F: Subgroup analysis of the cases where BxG and yG were different (n=99). Significant differences were found between RFS estimates of yG 2 vs. 3 (p_{RFS}=0.022), but not between yG 1 vs. 2 (p_{RFS}=0.213) and yG 1 vs. 3 (p_{RFS}=0.463). Furthermore, significant differences were found between OS estimates of yG 1 vs. 3 (p_{OS}=0.048), and yG 2 vs. 3 (p_{OS}=0.008) but not between yG 1 vs. 2 (p_{OS}=0.555), respectively.

Table 5. Results of univariable and multivariable Cox proportional hazards models regarding OS and RFS estimates.

Variable	OS						RFS					
	univariable			**multivariable**			**univariable**			**multivariable**		
	HR	**95% CI**	***p***	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Age (years)												
<53	Ref.						Ref.					
≥53	**1.59**	**1.034 -2.45**	**0.0 34**				1.23	0.90-1.69	0.1 88			
BxG												
1	Ref.						Ref.					

2	2.90	0.69-12.17	0.144				**2.75**	**1.01-7.61**	**0.050**			
3	3.06	0.74-12.60	0.121				**2.86**	**1.05-7.82**	**0.040**			
ER												
positive	Ref.			Ref.			Ref.			Ref.		
negative	**1.90**	**1.24-2.90**	**0.003**	**2.71**	**1.74-4.20**	**<0.001**	**1.70**	**1.24-2.34**	**0.001**	**1.72**	**1.14-2.59**	**0.009**
PR												
positive	Ref.						Ref.					
negative	**1.71**	**1.12-2.61**	**0.013**				**1.57**	**1.14-2.15**	**0.005**			
HER2												
negative	Ref.						Ref.					
positive	1.189	0.90-1.55	0.208				0.97	0.77-1.22	0.81			
Neoadjuvant therapy												
Taxane	Ref.						Ref.					
Anthracyclin	1.05	0.61-1.81	0.864				1.04	0.69-1.57	0.833			
Others	1.61	0.51-5.11	0.421				1.16	0.42-3.14	0.772			
Surgery												

Breast-conserving surgery	Ref.						Ref.					
Mastectomy	1.33	0.82-2.11	0.251				**1.63**	**1.13-2.35**	**0.009**			
yG												
1	Ref.						Ref.					
2	1.87	0.66-5.27	0.235				1.07	0.57-1.99	0.822			
3	**3.39**	**1.22-9.41**	**0.019**				**1.88**	**1.02-3.45**	**0.041**			
Complete resection												
R0	Ref.						Ref.					
R1	**1.94**	**1.13-3.30**	**0.014**				1.34	0.86-2.09	0.192			
Lymphovascular invasion												
Absent	Ref.			Ref.			Ref.			Ref.		
Present	**2.94**	**1.90-4.51**	**<0.001**	**2.26**	**1.42-3.58**	**0.001**	**2.18**	**1.57-3.02**	**<0.001**	**1.84**	**1.27-2.69**	**0.001**
ypT												
ypT1a	Ref.						Ref.					
ypT1b	0.42	0.11-1.59	0.205				**0.27**	**0.07-0.93**	**0.039**			

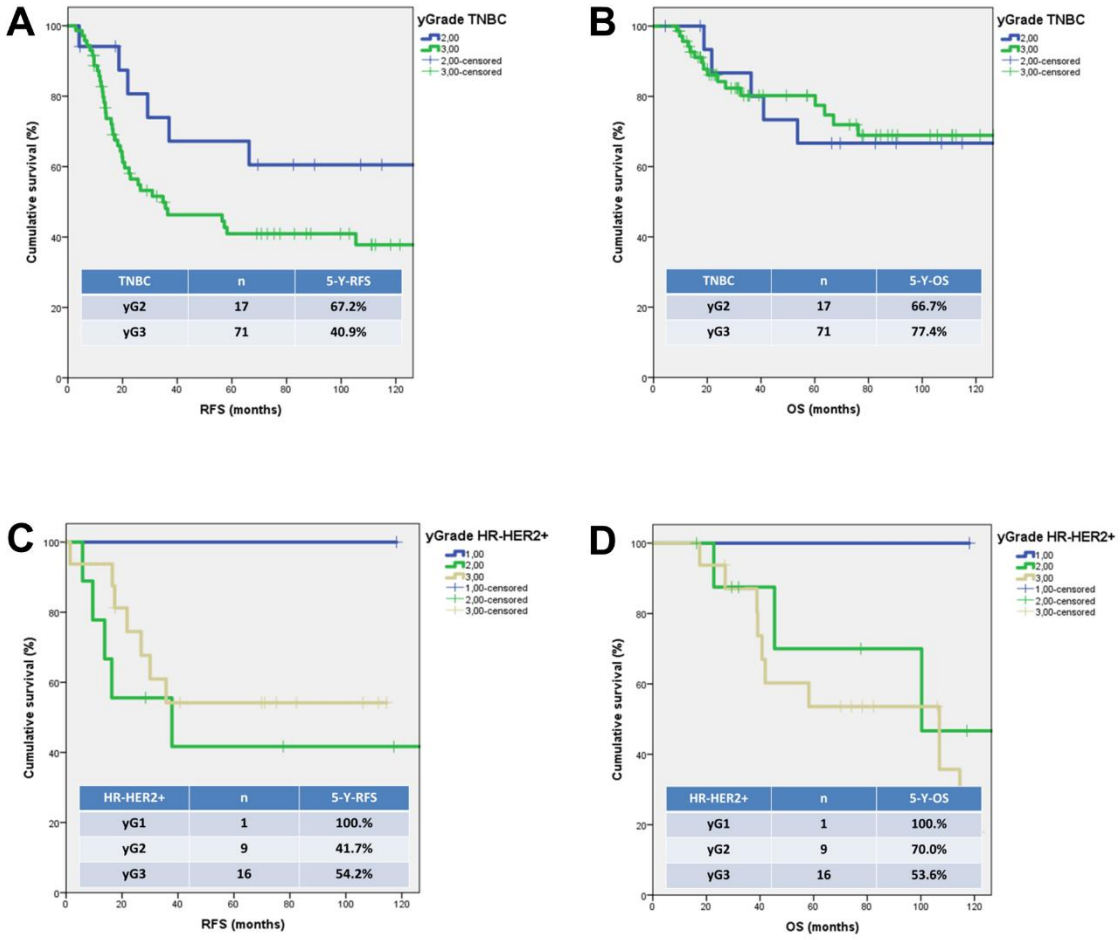
ypT1c	1.19	0.54-2.59	0.658				1.42	0.77-2.61	0.262			
ypT2	1.17	0.55-2.46	0.676				1.33	0.73-2.38	0.344			
ypT3	1.34	0.58-3.08	0.482				1.61	0.84-3.09	0.150			
ypT4	**4.27**	**1.42-12.83**	**0.009**				**4.2**	**1.59-11.05**	**0.004**			
ypN												
ypN0	Ref.			Ref.			Ref.			Ref.		
ypNmi	1.11	0.14-8.50	0.919	1.75	0.23-13.39	0.591	0.98	0.23-4.12	0.983	2.26	0.52-9.74	0.274
ypN1a	**3.32**	**1.75-6.28**	**<0.001**	**2.95**	**1.52-5.73**	**0.001**	**2.46**	**1.58-3.84**	**<0.001**	**2.36**	**1.42-3.92**	**0.001**
ypN2	**3.50**	**1.76-6.97**	**<0.001**	**3.03**	**1.46-6.24**	**0.003**	**3.06**	**1.90-4.94**	**<0.001**	**3.33**	**1.93-5.75**	**<0.001**
ypN3	**5.11**	**2.39-10.92**	**<0.001**	**4.29**	**1.88-9.79**	**0.001**	**3.16**	**1.78-5.57**	**<0.001**	**3.10**	**1.61-5.97**	**0.001**

HR: hazard ratio, CI: confidence interval, Ref.: reference, BxG: grade in the core needle biopsy, ER: oestrogen receptor, PR: progesterone receptor, HER2: human epidermal growth factor receptor-2, NACT: neoadjuvant chemotherapy, yG: grade in the resection specimen after neoadjuvant therapy

In univariate analysis older age, lack of ER and PR expression, presence of lymphovascular invasion, incomplete resection and higher ypT, ypN and yG categories were significant adverse prognostic parameters regarding OS, while lack of ER and PR expression, mastectomy, presence of lymphovascular invasion and higher ypT, ypN, BxG and yG categories proved to be significant prognosticators of unfavourable outcome regarding RFS. In multivariate analysis, concerning OS

and RFS estimates, significant independent prognosticators were ER status (HR_{OS}: 2.71, 95% CI: 1.74–4.20, $p_{OS}<0.001$; HR_{RFS}: 1.72, 95% CI: 1.14–2.59, $p_{RFS}=0.009$), lymphovascular invasion (HR_{OS}: 2.26, 95% CI: 1.42–3.58, $p_{OS}=0.001$; HR_{RFS}: 1.84, 95% CI: 1.27–2.69, $p_{RFS}<0.001$) and the ypN category (Table 5.), respectively. Subgroup analysis was carried out for cases with different BxG and yG categories and univariate analysis indicated older age (HR_{OS}: 5.83, 95% CI: 1.69–20.06, $p_{OS}=0.005$) and higher yG (HR_{OS}: 5.13 95% CI: 1.03–25.88 $p_{OS}=0.045$) category had an adverse impact on prognosis. None of them proved to be independent in multivariable analysis. The results of the Kaplan Meier subgroup analyses focusing on the prognostic impact of yG and molecular subtypes are displayed in Figure 5. and 6. Among HR-positive, HER2-negative breast cancer cases, significant differences were found between the RFS and OS estimates of yG1 vs. yG3 and yG2 vs. yG3 categories (p_{RFS} yG1 vs. yG3 = 0.021; p_{RFS} yG2 vs. yG3 = 0.002; p_{OS} yG1 vs. yG3 = 0.005; p_{OS} yG2 vs. yG3 = 0.001).

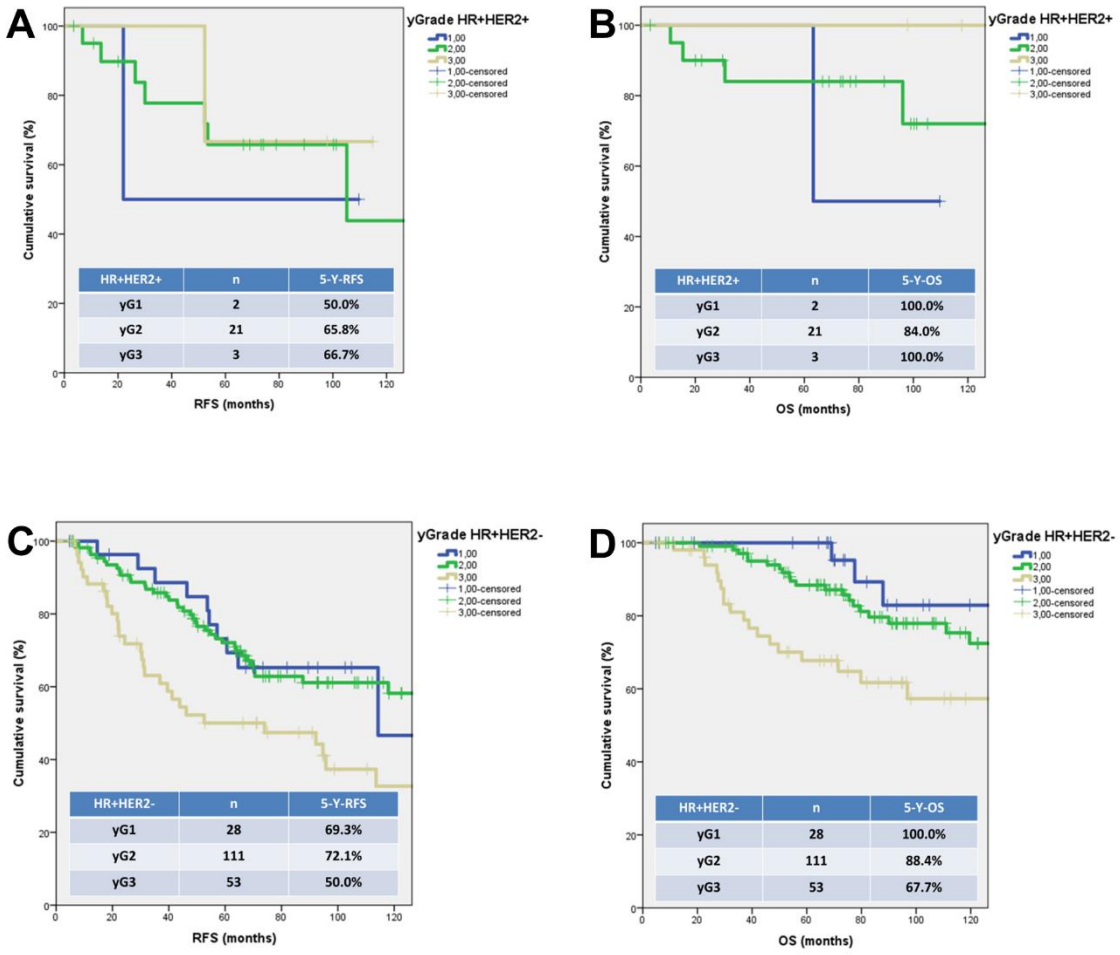
Figure 5. The results of the Kaplan-Meier subgroup analyses focusing on the prognostic impact of yG and the molecular subtypes of breast cancer (1).



A and B: There were no significant differences between yG2 and yG3 among triple-negative breast cancers (TN) (p_{RFS} yG2 vs. yG3=0.087; p_{OS} yG2 vs. yG3=0.937)

C and D: Regarding HR-HER2+ cases, yG did not have a significant impact on RFS and OS estimates (p_{RFS} yG1 vs. yG3=0.444; p_{RFS} yG1 vs. yG2=0.372; p_{RFS} yG2 vs. yG3=0.428; p_{OS} yG1 vs. yG3=0.237; p_{OS} yG1 vs. yG2=0.418; p_{OS} yG2 vs. yG3=0.455)

Figure 6. The results of the Kaplan-Meier subgroup analyses focusing on the prognostic impact of yG and the molecular subtypes of breast cancer (2).



A and B: Concerning HR+HER2+ cases, there were no significant differences between the RFS and OS estimates of the different grade categories (p_{RFS} yG1 vs. yG3=0.592; p_{RFS} yG1 vs. yG2=0.840; p_{RFS} yG2 vs. yG3=0.716; p_{OS} yG1 vs. yG3=0.221; p_{OS} yG1 vs. yG2=0.558; p_{OS} yG2 vs. yG3=0.336)

C and D: Regarding HR+HER2- cases, significant differences were found between the RFS and OS estimates of yG1 vs. yG3 and yG2 vs. yG3, but not between that of yG1 vs. yG2 (p_{RFS} yG1 vs. yG3=0.021; p_{RFS} yG1 vs. yG2=0.799; p_{RFS} yG2 vs. yG3=0.002; p_{OS} yG1 vs. yG3=0.005; p_{OS} yG1 vs. yG2=0.201; p_{OS} yG2 vs. yG3=0.001)

4.3. DESCRIPTION OF THE CHANGE IN KI67 LABELLING INDEX FOLLOWING NEOADJUVANT TREATMENT AND ITS TEMPORALITY

Following exclusions, a total of 54 paired cases were included in the study. Clinical data and tumour characteristics of the cohort are summarized in Table 6. The most common surrogate molecular subtype observed was luminal B-like (n=22), followed by TN (n=16), HER-positive (n=12) and lastly luminal A-like (n=4). The most common regression grades were RCB-II (n=39) and TR2b (n=20). The average time between the last cycle of NACT and surgical operation was 40.3 days (range: 8-82). Taxane-containing NACT regimen was used in 52/54 cases and it most often involved the sequential administration of epirubicin and docetaxel, however, some patients received a taxane and platinum combination regimen or other taxane-containing regimens. As for the two remaining cases, one consisted of 3 cycles of epirubicin (as the patient declined further preoperative chemotherapy), and the other consisted of capecitabine. Anti-HER2 treatment in the form of trastuzumab (5 ER+ and 1 ER- tumours) or a dual blockade with trastuzumab and pertuzumab (4 ER- tumours) was administered in 10/12 patients with HER2-positive tumours.

Table 6. Clinical data and tumour characteristics with corresponding number of cases

Age mean, (median, range)	57.85	58, 32-78
ER status	Positive	31
	Negative	23
PR status	Positive	29
	Negative	25
HER2 status	Positive	12
	Negative	32
Grade		
	I	2
	II	18
	III	34
yGrade		
	I	6
	II	33
	III	15
ypTNM		
ypT	ypT1a	3
	ypT1b	13

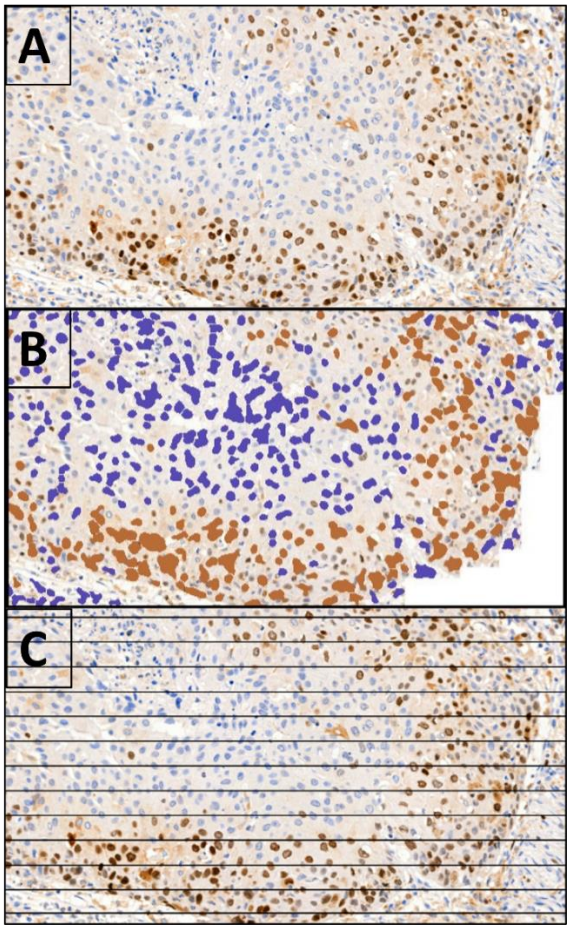
	ypT1c	25
	ypT2	10
	ypT3	1
	ypT4	2
ypN	ypN0	30 [16 sn]
	ypN1	18 [5 sn; 6mi]
	ypN2	5
	ypN3	1
yM	yM0	18 (+36 not assessed/available)
cTNM		
cT	cT1a	0
	cT1b	1
	cT1c	15
	cT2	32
	cT3	6
	cT4	0
NACT regimens		
LumA-like	EC+Taxane	3
	Capecitabine	1
LumB-like	EC+Taxane	22
TN	EC+Taxane	15
	EC	1
HER2-positive	EC+Taxane	2
	EC+Taxane+Trastuzumab	3
	EC+Taxane+Trastuzumab+Pertuzumab	3
	EC+Taxane+Carboplatin+Trastuzumab	2
	EC+Taxane+Carboplatin+Trastuzumab+Pertuzumab	1
	EC+Taxane+5-FU+Trastuzumab	1

EC: epirubicin-cyclophosphamide, ER: oestrogen receptor, HER2: human epidermal growth factor receptor-2, NACT: neoadjuvant chemotherapy, PR: progesterone receptor, TN: triple-negative breast cancer, 5-FU: 5-fluorouracil

All cases were reported by the same pathologist and the slides reported by others were reviewed by the same pathologist (GCs) to limit interobserver variability. The same microscope was used for

scoring mitoses during the period of data collection. A representation of the results created by the IR method, as well as the gridlines used for the GRID method are illustrated in Figure 7.

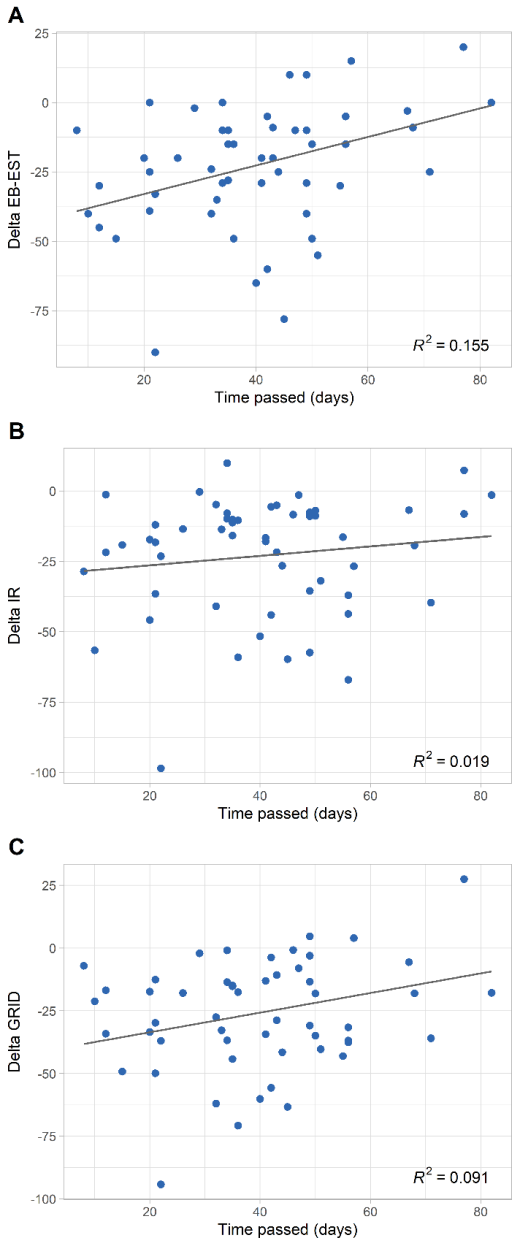
Figure 7. Examples of the methods of Ki67 evaluation in the study.



A: Digital image of a scanned Ki67 immunostained slide; B: Result example of the ImmunoRatio software; C: Example of the gridlines used for counting the positive cells for the GRID method.

In the linear regression model, before subgroup analysis, the best goodness-of-fit was achieved by the EB-EST method, indicating an upward trend and thus a decreasing change in Ki67 LI over time ($p=0.003$, $R^2=0.1548$). Delta Ki67 (ΔKi67) represented the difference achieved by subtracting the preoperative biopsy specimen value from the surgical excision specimen value. The linear regression results and R2 values for each quantification method when all cases were grouped together are shown in Figure 8.

Figure 8. Linear regression results and R^2 values for all cases, according to quantification method.

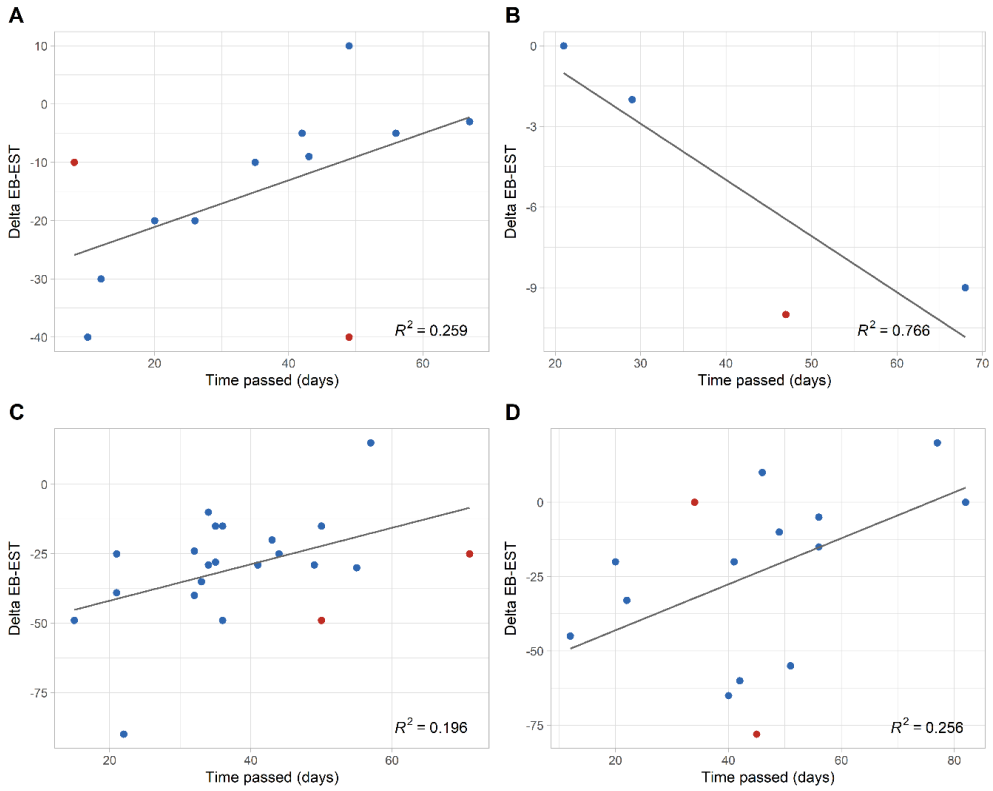


Delta: difference between post- and pretreatment Ki67 LI values; EB-EST: eyeballing-based estimation method; IR: ImmunoRatio software results; GRID: gridline-based counting and proportion of positive cells

In the subgroup analysis based on surrogate molecular subtypes, illustrated in Figure 9, Luminal A-like tumours exhibited the best fit with the linear regression model (p=0.125, R2=0.766), indicating a decreasing trendline and thus an increase in the proliferation blocking effect as time

passes following last cycle of NACT, albeit this subgroup contained the fewest number of cases (n=4), and therefore no conclusions can be drawn for this subgroup. HER2-positive (p=0.091, R2=0.2589), TN (p=0.045, R2=0.2563) and Luminal B-like (p=0.039, R2=0.1961) tumours all demonstrated an upward trendline, suggesting that the proliferation blocking effect of NACT in these tumours decreases over time.

Figure 9. Linear regression models and R^2 values of the subgroup analysis based on surrogate molecular subtypes.

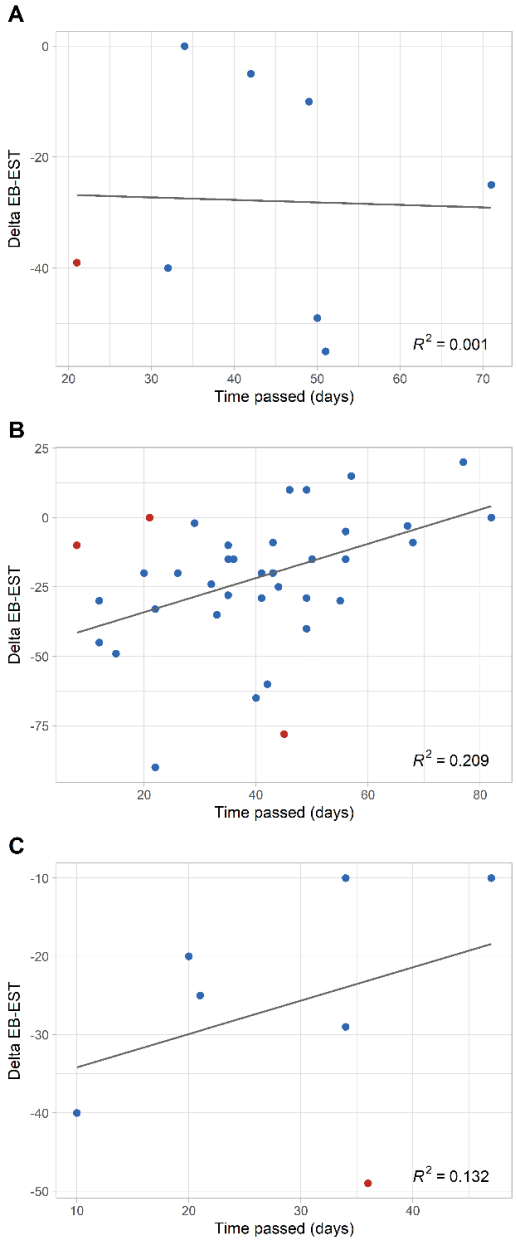


A: HER2+; B: Luminal A-like; C: Luminal B-like and D: TN tumours; delta: difference between post- and pretreatment Ki67 LI values;; statistical outliers are indicated in red

Subgroup analysis based on RCB regression grade scores demonstrated discordant trendlines between RCB-I versus RCB-II and RCB-III categories, with the former showing a downward trend and the latter two indicating the inverse; the upward trend, meaning a decrease in proliferation reduction following NACT. The subgroups RCB-I and RCB-III had a suboptimal number of cases

(n=8 and n=7, respectively), a known limitation of our study. The results of this subgroup analysis are shown on Figure 10.

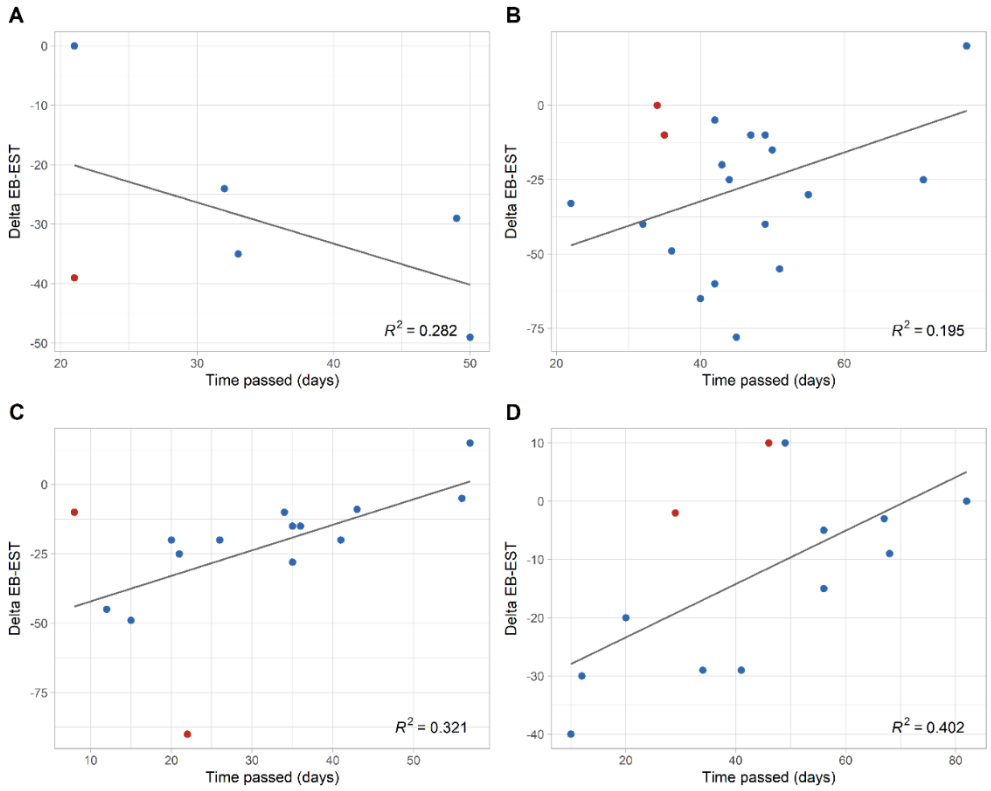
Figure 10. Linear regression models and R^2 values of the subgroup analysis based on RCB regression grades.



A: RCB-I; B: RCB-II; C: RCB-III; delta: difference between post- and pretreatment Ki67 LI values; statistical outliers are indicated in red

Lastly, subgroup analysis results based on the EWGBSP TR regression grade categories are illustrated on Figure 11. The linear regression trendline for TR2a tumours showed a downward trend and thus a sustained effect of chemotherapy (p=0.276, R^2=0.2819), while TR2b (p=0.051, R^2=0.1951), TR2c (p=0.028, R^2=0.3209) and TR3 (p=0.02, R^2=0.4019) groups all demonstrated an opposite, upward trend.

Figure 11. Linear regression models and R**2** values of the subgroup analysis based on EWGBSP TR regression grade categories.

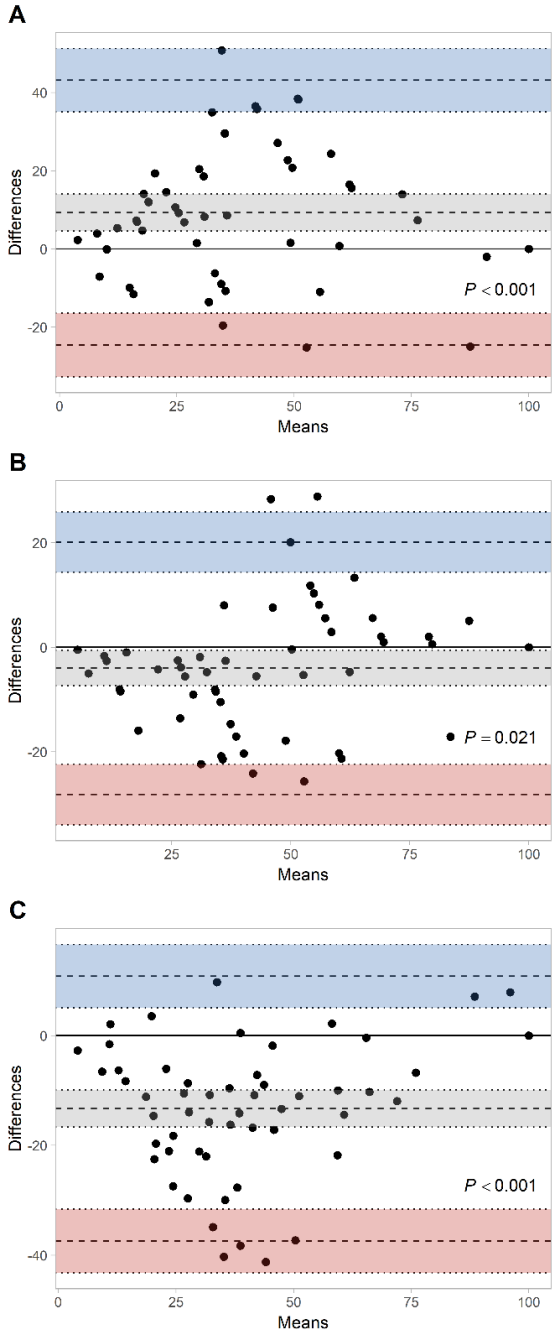


A: TR2a; B: TR2b; C: TR2c and D: TR3; delta: difference between post- and pretreatment Ki67 LI values; statistical outliers are indicated in red

In core biopsies, the Bland-Altman analysis assessing the agreement between the EB-EST and IR, and the EB-EST and GRID (in this order, pairwise) estimation methods showed a mean difference (bias) of 9.3 (95% CI: 4.6 to 14.0; p<0.001) and -4.0 (95% CI: -7.3 to -0.6; p=0.02), with limits of agreement ranging from -24.6 to +43.2 and -28.1 to +20.2, respectively.

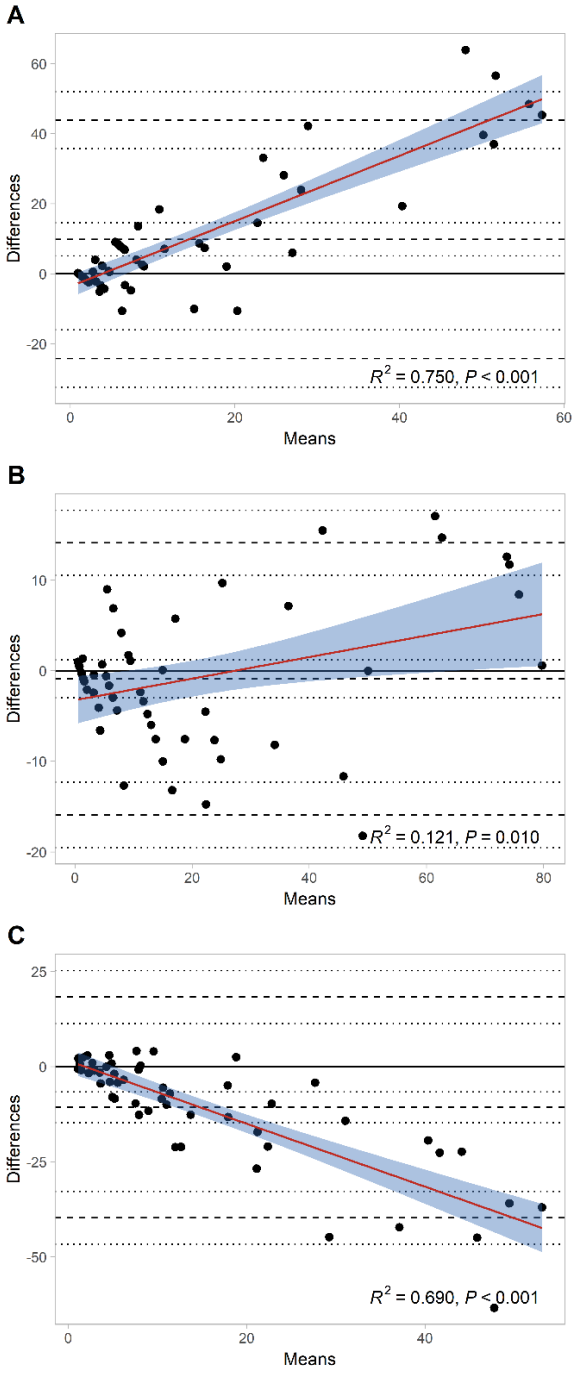
As for the values in excision specimens, the Bland-Altman analysis demonstrated an average difference of 9.8 (95% CI: 5.1 to 14.6; $p<0.001$) and -0.9 (95% CI: -3.0 to +1.2; $p=0.4$) between EB-EST and IR, and EB-EST and GRID, respectively. The ranges for limits of agreement were -24.2 to +43.9 and -15.9 to +14.1, respectively. Additionally, significant increasing trends in the differences were detected across the range of methods ($p<0.001$ and $p=0.01$, respectively), which were not present in the core biopsy results ($p=0.874$ and $p=0.112$, respectively). Specifically, the difference between the estimation methods increases with the magnitude of the Ki67 LI value – that is, EB-EST tends to produce higher values than IR or GRID as values increase. Results of the Bland-Altman analyses based on core biopsies and excision specimens are presented on Figure 12 and Figure 13, respectively.

Figure 12. Bland-Altman plots (the difference against the mean of two measurements) demonstrating the bias and limits of agreement (with 95% confidence bands) and p-values in core biopsies.



A: EB-EST and IR, B: EB-EST and GRID, C: IR and GRID; the grey band illustrates the mean difference and its 95% confidence interval, the blue and red bands represent the ±1.96 SD values and their 95% confidence intervals

Figure 13. Bland-Altman plots (the difference against the mean of two measurements) demonstrating the bias and limits of agreement (with 95% confidence bands) in excision specimens, as well as the linear regression trendline indicating a significant bias with p- and R^2-values.



A: EB-EST and IR, B: EB-EST and GRID, C: IR and GRID

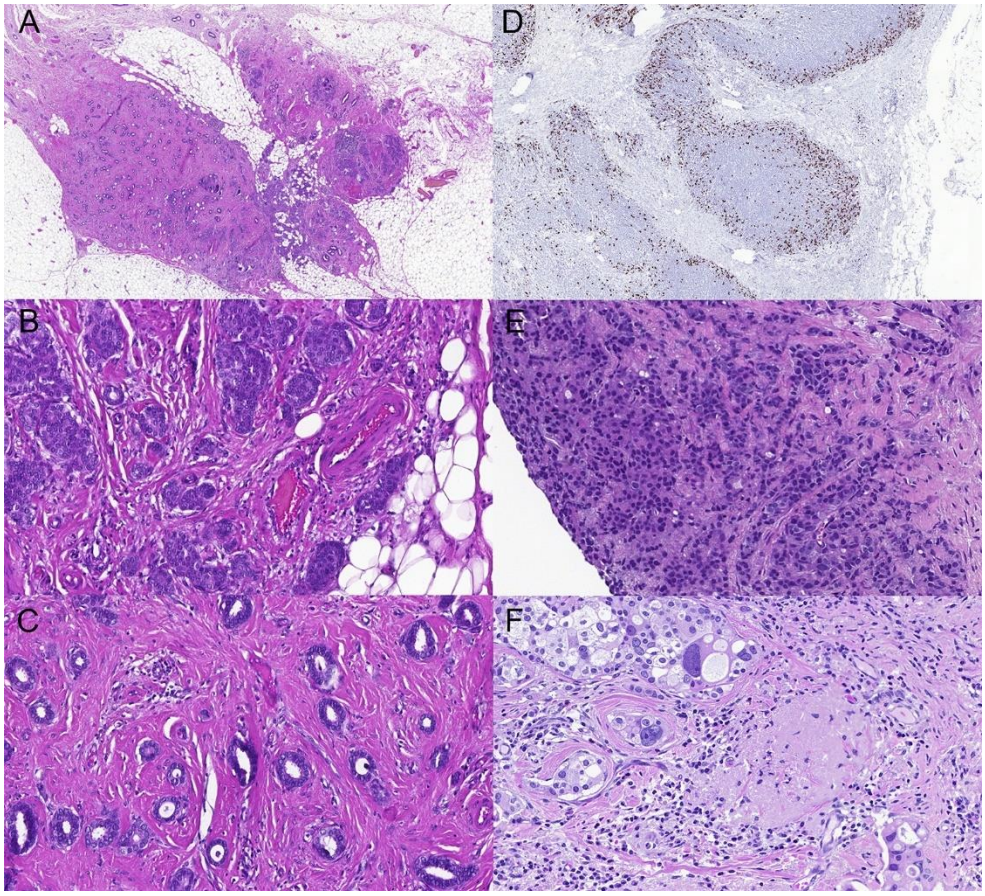
5. DISCUSSION

The traditional prognostic factors of breast cancer, such as histological type and grade, remain relevant even in the era of molecular and genomic testing [6, 34], and histological grade is considered a good prognosticator despite interobserver variability. A higher BxG (i.e. G3) is associated with pCR following NACT, which is an independent prognosticator based on multivariate analysis [35]. Previous studies demonstrated that in spite of concordance in grade in preoperative and operative samples in most of the cases, discordant cases show some trends. A meta-analysis based on 33 studies (4980 patients) suggested concordance in grade in the majority of cases (range: 59% to 94%, pooled estimate: 71%), however, in discordant CNB and EXC grades, it indicated an underestimation twice as commonly (19%) as overestimation (9%) [36]. Some of the included studies also reported on the subscores for grade calculation, and in discordant cases, specific trends could be observed in these as well: 1. Concordance was predominant, but when faced with discordance, 2. Tubule formation (T) was more commonly overscored than underscored (13% vs 9% based on pooled percentages of 12 studies), 3. Nuclear pleomorphism (P) was more likely to be underestimated than overestimated (17% vs 10% based on 14 studies), 4. Lastly, mitotic activity (M) was more often underestimated than overestimated (30% vs 8% on the basis of 13 studies) [36]. Our results based on the analysis of change in grade and its subscores are in keeping with the pooled analyses presented. Concordance rate for grade between CNB and EXC specimens was 71%, not only nearly identical to the pooled results, but also a reliable data, as the study with the greatest weight included in the meta-analysis by Knuttel et al. included 300 cases, less than half of the number of cases included in the CHIR group of our study. Our data also reinforce the previously described tendency to overestimate T and underestimate P, M and G, with G being nearly twice as often underestimated than overestimated. The highest rate of concordance for the subscores was observed for T in our study as well (79% vs 78%), however, there was a minor discrepancy between our results and that of the meta-analysis: our second highest concordance was seen in connection with M (75% vs 62%), followed by P (69% vs 73%).

Discrepancies between grades determined from CNB or EXC specimens have generally been explained by underrepresentation by CNB. This effect is even more pronounced in breast cancers that show heterogeneity within the tumour mass, such as mixed tubular carcinomas. These tumours often contain one part, generally the central area, which demonstrates plenty of tubule formation, whereas the peripheral area may lack tubule formation completely (Figure 14) [37]. Another

recognised phenomenon is the zonation of proliferation and the stemming recommendation to count mitotic figures at the periphery of the tumour, as these areas present a more optimal environment for proliferation (i.e. lack of hypoxia).

Figure 14. Histological variability that may account for differences in subscores and grades between core needle biopsies and excisions.



A-C: Mixed tubular carcinoma: A: x2; right side with less than 10% gland formation (B: x20) and left side with 100% tubule formation characteristic of tubular carcinoma (C: x20). D: A Ki-67 immunostain highlighting inhomogeneity and zonal distribution of the cells in the cell cycle, a zonation that may also characterise the distribution of mitotic figures (Ki67, x5). E-F: The same tumour before chemotherapy in the core needle biopsy and after chemotherapy in the excision, with P scores 2 and 3, respectively (x30).

Both heterogenous tubule formation and zonal distribution of proliferative cells can lead to discrepancies on the basis of tangential versus radial sampling by the needle and these variabilities

play a lesser role in EXC specimens where we may observe the complete cut surface of the lesion. As the size and quality of the CNB sample (at least 2 mm^2 tumour area, 10 high power fields, no crush artefacts) are also important to be able to adequately determine grade, these samples may end up being unassessable. Differences in cold ischaemia time and fixation are also mentioned as a possible cause for discrepancies. Lehr and colleagues have rather confidently explained why larger specimen size, slower permeation by formalin, delayed fixation and the development of more easily identifiable mitotic figures may be the principal cause for the consistent underestimation of M in CNB samples, or more precisely, an overestimation in EXC specimens [38]. Less than perfect interobserver variation has also been mentioned as a possible cause of discrepancies, since the reproducibility of histological grading of breast cancers has been found to be only moderate [11, 12], however, interobserver variability did not influence the results of our study, as all cases have been reported by the same pathologist, and intraobserver agreement of grading has always been reported as better than interobserver agreement [39]. All aforementioned phenomena should play a role in discrepancies seen in the PST group and dissonant trends may be attributed to the differences of the populations or as effects of NACT. In the PST group, while the majority of cases still demonstrated concordance, the rate was significantly lower than in the CHIR group (71% vs 62%). In discordant cases, the most notable change was that of a decrease in G (88%). Regarding the subscores, T and P indicated a substantial concordance between CNB and EXC samples and the rates were nearly identical to those observed in the CHIR group (PST vs CHIR: T (77% vs 79%) and P (70% vs 69%)). By contrast, the M subscore demonstrated discordance in half of the cases, significantly more often than in the CHIR group. For discordant cases, T and M scores more often decreased (in 88% and 62%, respectively), whereas P more commonly increased (73%). Taxanes may cause the formation of pleomorphic tumour giant cells and bizarre, large nuclei by disrupting microtubule function and mitotic spindle formation, ultimately increasing pleomorphism subscore [40, 41], however, this might not play a significant role, as most of the cases were of P score 3 originally. Changes in the M subscore may be attributed as the desired and expected effect of NACT. As a combined result of the above-mentioned effects and especially of the drastic decrease in M subscores in the PST group, G tended to decrease rather than increase in the EXC specimens, an effect attributable to NACT.

As most breast cancers treated with NACT are of high grade, and reflect subscores 3 for most assessed parameters, aggravation cannot occur in most cases, but a reduction in proliferation can

lead to decreased grade in the residual tumour. Whether this change is associated with improved prognosis is not well known, nonetheless, reporting of histological grade following NACT is part of several recommendations [19, 42, 43]. Despite of this, a survey done in the US among 23 academic pathologists with experience in breast pathology revealed that postneoadjuvant grade was not reported by 6 (26%), furthermore, their added comments included the lack of evidence on correlation of yG and prognosis, as well as the drop of mitotic scores with potential senseless downgrading. Comments were also added by those who included grade in their reports, mentioning that the majority of cases had identical grades with some cases displaying a change with no known evidence of prognostic significance to the responder [44]. The extent of residual tumour following NACT has been proven to be of prognostic relevance as reflected by the residual cancer burden (RCB) [45-48], but the addition of Ki67 proliferation index, ER status and yG further improves prognostication [49], suggesting that yG has prognostic importance, too. It should be noted, that the cases analysed in our study have not reached pCR, and therefore represent a cohort with worse prognosis. In our first, single-centre study [50], we noted a decrease in grade in 24% of tumours following NACT, while in our second study, grade decreased comparably in 24%. An earlier French study documented a reduced yG in 34/171 of the assessable cases (20%, 95% CI: 14-27%), seen mostly in responsive tumours and was thus interpreted as a benefit of NACT, though survival analysis was not performed in that study [51]. Another previous study based on the results of 485 patients treated with anthracycline or anthracycline + taxane based NACT indicated the prognostic impact of both BxG and yG, with the impact being greater in the yG setting. The cohort included 8% G1, 41.5% G2 and 50.5% G3 tumours, pCR was achieved in 115 cases (23.7%). The remaining 370 cases with residual disease showed a split of 12% yG1, 55% yG2 and 33% yG3 and the Cox proportional hazard model showed a significant overall and distant disease-free survival disadvantage for yG3 (but not yG1 and yG2) compared to pCR, though yG was not assessed separately for cases with residual tumours only [46].

As our previous results demonstrate [50], the leading factor in grade reduction following NACT is the marked reduction in the M subscore, that is, proliferation of the tumour. The reaction of the tumour to NACT can be described as heterogeneous, as its highly dependent on the biological behaviour of breast cancer, supported by the fact that it is more often given to patients with poorly differentiated, highly proliferative tumours, as these patients may benefit more from the proliferation blocking effects of NACT. Tumour regression can be graded according to multiple

systems, such as the RCB or EWGBSP TR and NR categories. Pathological complete response following NACT is a good predictor of survival, however, it is only achieved in approximately 15-20% of cases [20, 52]. Discovering further predictive factors for pCR and prognosticators, as well as monitoring tumour response to NACT may assist in identifying the most optimal patient group according to cost-vs-benefit from the therapy.

Dynamically adjusted therapy regimens based on biomarkers may offer a solution to personalized therapeutic pathways for well-responders and non-responders, and help avoid over- or undertreatment. The WSG-ADAPT-HR+/HER2- sub-trial assessed this question based on Ki67 response in HR-positive, HER2-negative intermediate risk tumours. A second, interim biopsy was taken after 3 weeks of induction endocrine therapy and according to the Ki67 LI value further treatment could be modified. Patients with ≤10% Ki67 LI on biopsy continued to receive endocrine therapy only, while those with >10% Ki67 LI also received chemotherapy. The trial reached the conclusion that interim biopsies proved to be a helpful tool in evaluating tumour response to therapy. The POETIC trial reached a similar conclusion; following surgery performed 2 weeks after randomization, patients with yKI67 LI ≤10% in the EXC specimens were considered responders, and endocrine therapy alone proved to be sufficient, whereas non-responders with higher yKi67 LI values were considered as potentially deriving benefit from additional chemotherapy [53-56].

Interim biopsies have also been used for NACT regimen adjustments as reported by Mukai et al., who assessed Ki67 in HER2-positive tumours from these samples and divided the patients into groups of responders versus non-responders. Worse pCR rates were observed when the original weekly paclitaxel and trastuzumab therapy regimen was switched to epirubicin-cyclophosphamide and trastuzumab in non-responders compared to when the patients were maintained on the original NACT [57].

Another importance of Ki67 arises during subdivision of breast cancers into surrogate molecular subtypes. According to the 2015 St. Gallen consensus conference, the main IHC surrogate subtypes are TN, HR+HER2+ (a subset of luminal B-like tumours), HR-HER2+ and HR+HER2- groups, the latter consisting of luminal A-like and luminal B-like (HER2-negative) tumours, among which Ki67 is an important differentiating factor [16], although there is no agreed upon cut-off value between high and low proliferation. During literature review, the most commonly found values were between 15% and 30%, the latter also being the threshold for high proliferation currently

suggested by both the International Ki67 in Breast Cancer Working Group and the latest St. Gallen Consensus Conference dealing with this matter. The cut-off value separating luminal A-like and luminal B-like tumours was 20% [18, 20, 21, 28, 29, 58-62].

Following NACT, Chen et al. reported a 37.5% discordance rate in Ki67 LI between CNB and EXC specimens using a >20% cut-off value to dichotomise tumours of high or low proliferation. Patients with CNB-Ki67 and yKi67 both ≤20% had a significantly better survival than any of the concordantly high or discordant low to high or high to low converted cases. Our results based on mitotic counts indicate and underestimation of proliferation in discordant cases following surgery without preoperative systemic therapy, while in NACT cases, proliferation tended to be higher in core biopsies than in surgical specimens, a result that is in keeping with the results of Chen et al [20].

While ΔKi67 is also recognised as prognostic, yKi67 remains the main factor predicting early local and distant metastasis risk and survival, with higher yKi67 values corresponding to poorer prognosis, regardless of the extent of the change [22, 27, 29-31]. Many authors seem to agree on the prognostic value of yKi67 LI [22, 29, 49, 63]. A comprehensive meta-analysis was done by Li et al., examining preoperative and postoperative Ki67 as well as ΔKi67 from biopsy to excision in terms of OS and DFS. Their study concluded that all three of these markers are independent prognostic factors of both OS and DFS, this finding being backed by other studies since then [22, 28, 52, 64]. Furthermore, yKi67 proved to be a stronger predictor of DFS than of OS [22, 59, 60].

We may conclude from previous studies that Ki67 LI assessed both from CNB and EXC specimens carries important information and has an impact on prognosis. Not only the starting and end values (i.e., CNB and EXC specimen values) but also the degree of change between the two can alter the expected prognosis, and yet, the temporality and trend of this change over time between the last cycle of chemotherapy and surgery remain to be less investigated. Our study aimed to fill this gap in knowledge. Our cumulative data may suggest that the drop in proliferation after NACT may gradually be lost, and therefore, time from the last chemotherapy administration may also influence the prognostic value of yKi67 and ΔKi67. This suggestion would be more straightforward if all tumours behaved similarly to NACT, which is not the case. When we tried to analyse subsets of tumours according to their molecular subtype using the surrogate molecular classification or the degree of response to NACT, we saw a similar tendency for a drop in yKi67 in nearly all subsets,

except for the good prognosis luminal A-like tumours and the tumours with the best response to treatment (RCB class I and EWGBSP TR2a), all underrepresented in the cohort. The data are purely descriptive and seem to reflect a dynamism in the proliferation-reducing effect of NACT with time from termination in most breast cancers, with the potential exception of the luminal A-like tumours (unlikely to receive NACT) or the ones with good response to treatment, where the effect may be more permanent. We used different methods of assessment, including two objective ones based on sampling (IR, GRID) and a thoroughly carried out but subjective one (EB-EST), and all showed similar trends (Figure 8); we have finally selected EB-EST based on the best goodness-of-fit and its widespread use in clinical practice. The absolute values of Ki67 differed by method, and probably artificial intelligence-based quantification would have been the most robust support for substantiating our findings, but due to the current unavailability of this technology in our hands, we sought to demonstrate the similarities in the results using different methods of assessment.

There were obvious limitations during our research. In the study evaluating the change in grade and its subscores from biopsy to excision, the PST group contained a relatively low number of cases, there was no chance to analyse the data according to surrogate molecular subtypes or type of neoadjuvant chemotherapy administered. Similarly, the study examining the trend in change of Ki67 contained a suboptimal number of cases, which rendered the elimination of statistical outlier values and thus the improvement of the goodness-of-fit unfeasible.

6. CONCLUSIONS AND NEW FINDINGS

Our results reinforced the previously described concordance of grade and its subscores between CNB and corresponding EXC specimens, as well as the tendency to underestimate tubule formation (by overestimating its score), nuclear pleomorphism, mitotic rate and grade on CNBs when compared with EXC samples in discordant cases. Following primary systemic chemotherapy, concordance rates were lower and a reduction in M subscore and grade could be observed.

Our second work strengthened the prognostic value of grade in the postneoadjuvant setting, indicating that yG3 is associated with worse RFS and OS than yG1 and yG2. Therefore, a reduction in grade seen in approximately 2/3 of discordant cases, which amounted to nearly 1/4 of the patient populations treated with NACT in our first and second study may be associated with better prognosis. This result was supported by the univariable Cox regression analyses, however, as the significance is lost in multivariable analysis, the prognostication does not seem to be independent.

Our findings reinforce a drop in proliferation following NACT in most breast carcinomas evaluated, and suggest that this drop may decrease with increasing time between the last cycle of NACT and surgery. This dynamism was seen in more aggressive molecular subtypes and tumours with lesser response to treatment, and might be missing from the rare luminal A-like tumours or carcinomas with better response to treatment, though conclusions for these latter subgroups were limited by low case numbers.

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9. MAGYAR NYELVŰ ÖSSZEFOGLALÓ

Az emlőrák gyakori betegség, kezelése egyre inkább tud személyre szabott lenni. Ehhez számos paramétert kell értékelni: prognosztikai tényezőket (a kimenetelre bizonyítottan hatást gyakorló változókat) és prediktív markereket (egy adott kezelés hatékonyságát előre jelző változókat) egyaránt. A patológiai lelet tartalmazza azokat a döntő tényezőket, amelyek szerepet játszanak a legmegfelelőbb kezelési terv kiválasztásában. A leletnek számos fontos prognosztikai tényező mellett tartalmaznia kell a szövettani grade-et (G) is.

(1)

A szövettani grade a tumor differenciáltságát és biológiai viselkedését tükrözi, és emlőrák esetében a tubulusképzés (T), a magpleiomorfizmus (P) és a látótérre korrigált mitotikus ráta (M) alpontszámainak összeadásával határozzák meg; ezek mindegyike háromfokozatú skálán kerül értékelésre. Az alpontszámok összege alapján a tumor jól differenciált (G1, 3-5 pont), közepesen differenciált (G 2, 6-7 pont) vagy rosszul differenciált (G 3, 8-9 pont) lehet.

Munkánkban a Bács-Kiskun Megyei Oktatókórházban 2010 és 2022 között diagnosztizált emlőrákos eseteket vontunk be. Minden bevont esetben a szövettani differenciáció fokát (G) és annak alpontjait az eredeti leletekből nyertük ki, és az összes adatot Microsoft Excel táblázatba rögzítettük. Az eseteket az alkalmazott terápia típusa szerint csoportokba osztottuk, neoadjuváns kemoterápiában (NACT) részesülő csoportot (PST), és primer műtéttel kezelt, neoadjuváns kezelés nélküli csoportot (CHIR) különítettünk el. A szövettani grade-et a nottinghami osztályozási rendszer szerint egy emlőpatológiában jártas patológus értékelte. Minden esetben a kezelés előtti (hengerbiopsziás, CNB) és utáni (sebészi, EXC) mintából is meghatározásra került a G, valamint T, P és M értékei. A CNB és EXC minták így az adott változók első és második mérési eredményét jelentették. A CHIR és PST csoportban minden változó minden értékének eloszlását, valamint a két csoportban a konkordancia mértékét Chi-négyzet próba segítségével értékeltük mind a CNB, mind az EXC minták esetében. A változók módosulásának értékelését nemparaméteres Wilcoxon-féle előjel próbával végeztük.

Az alpontszámok és grade változását illetően CNB és EXC minták között mind a CHIR (T: 78,9%, P: 68,9%, M: 74,6%, G: 71,2%), mind a PST (T: 77%, P: 70,3%, M: 50%, G: 61,5%) csoportokban egyaránt magas egyezési arányt figyeltünk meg. Az eltérő esetekben a leggyakoribb változás egy

pontnyi különbség volt. Az egyezési arányok szignifikánsan eltértek a CHIR és a PST csoportok között a G értékében ($p = 0,024$) és az M alpontszámban ($p < 0,0001$).

A látott változások eredményeként a CHIR csoportban a grade gyakrabban emelkedett (71,2%, 95% CI: 64,7%-77,0%), míg a PST csoportban gyakrabban csökkent (63,2%, 95% CI: 50,2%-74,5%). A Wilcoxon-féle előjel próbának az eredményei statisztikailag szignifikáns változást jeleztek mindkét csoportban az összes értékelt paraméter tekintetében (CHIR-csoport: T, P, M és G, mind $p < 0,001$; PST-csoport: T, $p < 0,001$, P, M és G, $p < 0,05$).

Az eredmények értelmében a hengerbiopsziákból és a műtéti anyagokból meghatározott G általában azonos, az eltérés PST kapcsán nagyobb mértékű. Eltérés esetén a biopsziákban a G-t és a meghatározásának alapját képező tubulusképzést, magpleiomoprhismust és mitotikus aktivitást gyakrabban alulértékeljük, de NACT esetén gyakoribb, hogy a hengerbiopsziás G és az M pontszám a magasabb, azaz a kezelés, ha befolyással van a grádusra, akkor inkább csökkenti azt, éspedig a mitózisok számának csökkentése révén.

(2)

A nem áttétes emlőrák kezelésében szisztémás kemoterápia a műtét előtt és/vagy után is alkalmazható, így neoadjuváns, vagy adjuváns kemoterápiának definiálhatjuk. Az International Collaboration on Cancer Reporting (ICCR) irányelvei szerint a G-t mind a CNB, mind az EXC mintákból, továbbá NACT után is értékelni kell, bár ez utóbbi esetben prognosztikai értéke kevésbé bizonyított.

Második munkánkba azon emlőrákos betegek kerültek bevonásra, akik 1999 és 2018 között a Szegedi Tudományegyetem Szent-Györgyi Albert Klinikai Központ Onkoterápiás, illetve Sebészeti Klinikáján NACT-kezelésben, majd műtéti beavatkozásban részesültek, valamint azon betegek, akiket 2000 és 2018 között a Bács-Kiskun Megyei Oktatókórházban, Kecskeméten hasonlóan kezeltek.

A CNB alapján meghatározott G (BxG) és EXC mintából meghatározott NACT utáni yG kategóriák különböző összehasonlításaiban a teljes túlélés (OS) és a recidívamentes túlélés (RFS) közötti különbségek statisztikailag szignifikánsak voltak. Az összes eset közül 320 daganat esetében állt rendelkezésre mind a BxG-re, mind a yG-re vonatkozó adat; 221 (69%) esetben nem

változott a grade, míg 21 (7%) esetben emelkedés, 78 (24%) esetben pedig csökkenés volt megfigyelhető.

Általánosságban elmondható, hogy mind a BxG, mind az yG magasabb kategóriái kedvezőtlen prognózissal társultak az RFS és az OS becslések tekintetében. Alcsoport-elemzést is végeztünk az eltérő BxG-vel és yG-vel rendelkező esetekre, ami a magasabb yG kategória kedvezőtlen prognózisát tükrözte mind az RFS, mind az OS tekintetében. Ugyanakkor az yG prognosztikai értéke a többváltozós elemzés kapcsán nem bizonyult függetlennek.

(3)

Miután megállapítottuk, hogy a NACT hatására, ha változik a G, akkor inkább csökken, és ez leginkább a mitózisszám redukciójával függ össze, továbbá hogy változást követően az yG-nek is van prognoszikai értéke, elemezni akartuk a proliferáció csökkenésének időbeli vonatkozásait. Ehhez NACT kezelés előtti és utáni szövetminták Ki67 proliferációs markerrel való festődésének változását vizsgáltuk az utolsó szisztémás kezelés és a műtét közötti idő függvényében lineáris regresszió segítségével.

A kizárások után összesen 54 páros eset került be a tanulmányba. A helyettesítő molekuláris altípusokon alapuló alcsoport-elemzésben a HER2-pozitív ($p=0,091$, $R2=0,2589$), tripla negatív (TN; $p=0,045$, $R2=0,2563$) és luminális B-szerű ($p=0,039$, $R2=0,1961$) tumorok mind emelkedő trendvonalat mutattak, ami arra utal, hogy a NACT proliferációt gátló hatása ezekben a tumorokban az idő múlásával csökken. A luminális A-szerű daganatok mutatták a legjobb illeszkedést a lineáris regressziós modellhez ($p=0,125$, $R2=0,766$), itt csökkenő trendvonalat láttunk, ami a proliferáció gátló hatásnak a fennmaradását jelezheti, bár ez az alcsoport tartalmazta a legkevesebb esetet ($n=4$), ami alapján konklúziót levonni nem lehet.

A Residual Cancer Burden (RCB) regressziós grádus, valamint a European Working Group for Breast Screening Pathology (EWGBSP) tumour response (TR) pontszámain alapuló alcsoport-elemzés értelmében a nagyobb reziduális tumormennyiséget mutató kategóriák esetén (RCBII, RCBIII, TR2b, TR2c, TR3) a proliferáció csökkenésének időbeli csökkenésére utaló lineáris regressziós trendvonalak emelkedő tendenciáit tapasztaltuk, míg a kis reziduális volumenre utaló kategóriák esetében (RCB-I és TR2a) ez a trendvonal csökkenő volt, ami a hatás csökkenése ellen

szólt; igaz az utóbbi kategóriákban kis számú beteg volt, ami a levonható következtetéseket korlátozza.

10. APPENDIX

I. **Ádám Ferenczi**, Gábor Cserni. Changes in breast cancer grade from biopsy to excision following surgery or primary chemotherapy. Pathologica 2024; 116:22-31, doi: 10.32074/1591-951x-958

IF (2024): 2,9 (Scimagojr: Q1)

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