Breast cancer and neoadjuvant therapy: any predictive marker?

R. VYZULA[1], L. DUŠEK[2], J. ŽALOUDIK[1], R. DEMLOVÁ[1], D. KLIMEŠ[2], Š. SELVEKEROVÁ[1]

[1]Masaryk Memorial Cancer Institute, e-mail: vyzula@mou.cz, 656 53 Brno, Czech Republic; [2]Centre of biostatistics and analysis, Brno, Czech Republic

Received March 8, 2004

The majority of patients with breast carcinoma receive chemotherapy as a component of multimodality treatment. Over the past decade, it has become increasingly more common to deliver chemotherapy first, but this has raised new questions within all disciplines of cancer management. However, the effect of cytotoxic treatment cannot be predicted on individually specific basis, then identification of tumor characteristics associated with tumor therapeutic response and outcome is then of great clinical interest. We studied 141 patients at Masaryk Memorial Cancer Institute, who received neoadjuvant chemotherapy and/or chemotherapy + radiotherapy (CHT/CHT+RT) between 1994–2002. Tumor samples were taken prior to and after neoadjuvant therapy. We quantified the response to therapy pathologically and determined histological and molecular tumor characteristics (steroid receptors, CEA, Ca 15-3). In addition to therapeutic response as immediate outcome, event free survival (EFS) was examined as more complex primary end-point of the study. Complete remission (CR) has been achieved in 6.5%, partial remission (PR) in 49.6%, stable disease (SD) in 26.2% and progression disease (PD) in 17.7% patients. Patients were divided into two groups according to the result of neoadjuvant therapy – responders (CR+PR+SD, who successfully underwent surgery), and risk group (patients with SD or PD, who could not undergo surgery). Responders to neoadjuvant CHT/CHT+RT regimens reached statistically significant better EFS than non-responders, low tumor size (T2) and stage (II) categories were confirmed as additional predictive factors not only for EFS but for therapeutic response as well. The study primarily examined predictive power of tumor markers as CEA, Ca 15-3, and steroid receptors (ER/PR) and searched for their role in the prospective evaluation of neoadjuvant therapy. We evaluated these factors as potential predictors of EFS, independent in predictive power on therapeutic response to neoadjuvant therapy. Diagnostically valuable cut off points were proposed in ROC analysis for all these markers. Responders to the neoadjuvant therapy with Ca 15-3 <23.0 kU/l, CEA <5.0 mg/l, estrogen receptors (ER) >5.0 fmol/mg or both estrogen /progesterone receptors (ER/PR) positive had statistically significantly better EFS in comparison to patients with Ca 15-3 ≥23.0 kU/l, CEA ≥5.0 mg/l, ER ≤5.0 fmol/mg, or other cases than patients double positive in ER/PR. Marker Ca 15-3 revealed significant predictive power even within the group of non-responders, these patients with Ca 15-3 <23.0 kU/l had better EFS as compared to patients with Ca 15-3 ≥23.0 kU/l. Tumor size and low stage proved predictive value for immediate response to neoadjuvant therapy. Risk parameters for neoadjuvant therapy were T4, stage III, namely if RT was necessary. Therapeutic response to neoadjuvant therapy was independent on investigated molecular parameters, but there was strong predictive association of Ca 15-3, CEA and ER/PR receptors with event free survival development. Diagnostically valuable cut-off points were proposed and validated for sensitivity and specificity in ROC analysis.

Key words: breast cancer, neoadjuvant chemotherapy, neoadjuvant chemoradiotherapy, predictive parameters

There are several arguments for applying chemotherapy and/or chemoradiotherapy in a so-called neoadjuvant setting, prior to surgery. First, by downstaging the tumor and lymph node metastases in greater than 80% of cases, less extensive resections are needed and breast conservation becomes increasingly feasible [3, 12, 33, 48]. Second, micrometastasis that may be present are thus treated at the earliest possible moment. This could prevent changes in metastatic cells, associated with a worse prognosis: acceleration of growth upon resection of the primary tumor and development of drug-resistant subclones [22, 23, 44]. A third advantage of neoadjuvant chemotherapy is that it enables the monitoring of treatment efficacy and makes it possible to identify markers of response to chemotherapy. This as-

Table 1. Overall characteristics of sample data sets (n=141)

PATIENT/DISEASE			TUMOR		NEOADJUVANT TREATMENT	
Age (years)[1]	55 (45; 68)		Histopathology		Overall summary of applied CHT	
Age (categories)	<50 yr	30.5%	Invasive ductal	66.0%	FAC	73.0%
	50–59 yr	39.0%	Invasive lobular	18.4%	CMF	8.5%
	≥60 yr	30.5	Others	15.6%	FEC	6.4%
					AC	4.9%
Follow-up I (months)[1,2]	3.0 (1.1;4.8)		Important diagnostic examinations[1]		Others	7.2%
Follow-up II (months)[1,2]	35 (21; 74)		ER(fmol/mg)[3]	6.8 (0.0; 31.2)		
			PR (fmol/mg)[3]	5.1 (0.0; 74.3)	Applied CHT in two therapeutic regimens	
Diagnosis C50.9	60.9%		ER positivity[3]	48.9%	Reg. R1: only CHT (n=107; 75.9%)	
C50.4	17.0%		PR positivity[3]	44.7%	[FAC (72.9%); CMF (5.6%);	
C50.3	5.7%		Ca15-3 (kU/l)	19 (9; 49)	FEC (6.5%); others (14.7%)]	
C50.1	5.7%		CEA (μg/l)	2.1 (0.9; 5.0)	Reg. R2: CHT+RT (n=34; 24.1%)	
C50.7	2.9%				[FAC (73.5%); CMF (17.7%);	
C50.5	2.9%		ALB (g/l)	47.4 (43.4; 51.1)	FEC (5.9%); others (2.9%)]	
Others	4.9%		KR (μmol/l)	85 (73; 100)		
			LDH (μkat/l)	6.2 (4.9; 8.2)	Therapeut. regimens (R1/R2) and clin. stage	
Cl. stage II	12.8%		GMT (μkat/l)	0.41 (0.22; 1.06)	Clinical stage II	
IIIA	33.3%		ALT (μkat/l)	0.41 (0.22; 0.79)	R1 (only CHT): 100%	
IIIB	53.9%		AST (μkat/l)	0.47 (0.25; 0.77)	Clinical stage III	
			ALP (μkat/l)	1.32 (0.82; 1.95)	Only CHT (R1): 73.2%; CHT+RT (R2): 26.8%	
T categories T2	22.0%		Hb (g/l)	138 (121; 149)	Therapeut. regimens (R1/R2) and histology	
T3	22.7%		Ery (10^{12}/l)	4.5 (3.9; 4.9)	(only clinical stage III)	
T4	55.3%		Leu (10^9/l)	7.1 (4.7; 9.2)	Invasive ductal (n=75)	
			Lym (10^9/l)	1.9 (1.2; 2.5)	Only CHT (R1): 74.7% CHT+RT (R2): 25.3%	
N categories N0	7.1%		Neu (10^9/l)	4.5(2.3; 6.3)	Invasive lobular (n=26)	
N1	63.8%		Throm (10^9/l)	257 (166; 368)	Only CHT (R1): 65.4%; CHT+RT (R2): 34.6%	
N2	29.1%		Mo (10^9/l)	0.5 (0.3; 0.8)	Others (n=22)	
					Only CHT (R1): 77.3%; CHT+RT (R2): 22.7%	

CHT – chemotherapy, FAC (5-fluorouracil, doxorubicin, cyclophosphamide), CMF (cyclophosphamide, methotrexate, 5-fluorouracil), FEC (5-fluorouracil, epirubicin, cyclophosphamide), AC (doxorubicin, cyclophosphamide), RT – radiotherapy. [1]Quantitative parameters are summarized by estimate of median, supplied by 10% and 90% percentiles (in parentheses). [2]Follow-up I covers period from date of diagnosis to the date of final evaluation of neoadjuvant therapy (therapeutic response). Follow-up II represents overall follow-up period of the whole data set. [3]Serum level of receptors estimated either quantitatively (biochemistry, fmol/mg) or as positive findings (combined biochemical and immunohistochemical evaluation).

sessment allows the opportunity to "cross over" to a different regimen for an individual patient if there is minimal or no response to the first regimen [16, 32].

The main aim of this retrospective study was to provide further insight into breast cancer response to chemotherapy and/or chemoradiotherapy by identifying factors that can predict response of the primary tumor to neoadjuvant therapy, and further development of the disease. The effect of cytotoxic treatment cannot be predicted for individual patient and the role of potential predictors has not been sufficiently investigated in individually specific models. Therefore, the identification of tumor characteristics with predictive power is of great clinical interest [13, 16, 39, 52, 54].

Material and methods

Patients. The present study was a retrospective analysis of the Masaryk Memorial Cancer Institute, Brno, Czech Republic database. All patients who were initially treated with neoadjuvant chemotherapy and eventually radiotherapy for breast cancer were evaluated. Between 1994 and 2002, 141 patients were registered and selected according to our criteria. Histopathological diagnosis was performed in all patients on core needle biopsy specimens obtained before treatment. Parameters as age, clinical stage, TNM classification, steroid receptor status, tumor markers, CEA, Ca 15-3, applied chemotherapy, therapeutic regimens, and some biochemical values were evaluated (Tab. 1). Sample is principally stratified according to clinical stage (II–III), histology (dominated by ductal invasive tumors: 66%) and by neoadjuvant therapeutic strategy CHT/CHT+RT. Sample data were obtained by retrospective monitoring and from representative single-institution pilot study with descriptive and predictive aims. Response was assessed after neoadjuvant chemotherapy by histopathological examination. All patients received a median of four (range: 1–6) cycles of neoadjuvant chemotherapy. There were used following chemotherapy regimens: FAC (5-fluorouracil, doxorubicin, cyclophosphamide), FEC (5-fluorouracil, epirubicin, cyclophosphamide), AC (doxorubicin, cyclophosphamide), CMF (cyclophosphamide, methotrexate, 5-fluorouracil),

AT (doxorubicin, docetaxel), NVB/EPI (vinorelbine, epirubicin), EPI/T (epirubicin, docetaxel), T/NVB (paclitaxel, vinorelbine), A (doxorubicin), MITO/NVB (mitomycin, vinorelbine). Radiotherapy has been added to chemotherapy in 34 patients (24.1%).

Statistical methods.

Clinical monitoring, follow-up and censored cases. The study consisted of 141 consecutive patients with sufficient follow-up information up to the time of analyses. The sample includes both patients with sufficient therapeutic response to neoadjuvant therapy (n=116) and risk group of non-responders (cases that did not reach surgical treatment or revealed very early progression, n=25). Values of all potential predictors and basic patient's characteristics were obtained at first diagnostic visit. Follow-up data were routinely collected during clinical monitoring in agreement with standard hospital patient's protocol records. Follow-up data include date of visit, timing of complications and serious events and date of death. Values of all investigated markers (CEA, CA 15-3, ER, PR) were retrospectively validated by double controls.

The study was designed as single arm, observational and focused on the clinical praxis. That is why patients were recruited consecutively without any specifically limiting filters, in order to provide representative pattern of routine hospital admissions. Timing of hospital admissions for progression or relapse was taken as time of event and was coded as positive record for the analyses of study endpoints. Admissions for standard clinical controls were considered as time-censored points, in survival analyses censored at time of the last control visit.

Statistical analyses. All statistical tests were performed on intention-to-treat principle, no case was excluded prior to the analyses and all failure events or deaths were recorded as fully equivalent. A value $\alpha<0.05$ was taken as an universal indicative limit for statistical significance. Standard descriptive statistics were used to express differences among subgroups of cases (mean supplied with 95% confidence limits or relative frequencies). Standard univariate statistical tests were used for differences between chosen subgroups of patients: Fisher exact test in binary outcomes, ML chi-square test for ordinal categorical variables, unpaired Student's t-test for normally distributed continuous variables and Mann-Whitney test for non-normally distributed continuous variables.

The best maximum likelihood estimates of diagnostically valuable cut-off values were obtained by receiver operating characteristic curve analysis (ROC). The ROC curves were computed for each of examined predictors (CEA, CA 15-3, ER, PR) and only statistically significant cut-off values entered subsequent evaluation of relative risk. Two independent analytic strategies were applied to quantify predictive power of examined variables to predefined study endpoints: (1) time-related risk of early event that was studied by proportional Cox regression models (time-related parameter, early events were recognized as relapse or progression occurred up to 24 months of follow-up), (2) risk of insufficient therapeutic response to the neoadjuvant therapy (numerically coded as binary variable) was examined by logistic regression. Event-free survival was regarded as principal for the study, immediate therapeutic response was taken as secondary output verifying very short-term predictive power of potential predictors.

At descriptive level, stratified Kaplan-Meier product-limit method was applied to discriminate survival rates between two or more subgroups given by the cut-off values. Standard Peto-Prentice generalized log-rank test was used as comparative statistical test. Time-related probability of early event was then used to stratify patients at different level of risk. A univariate Cox proportional hazard analysis was used as final model identifying significant predictors of event-free survival. Hazard ratio was estimated within its 95% confidence limits and supported by significance level [2, 40, 58].

Results

Neoadjuvant therapeutic regimens and primary endpoints of the study. Therapeutic response to neoadjuvant therapy was basically classified into two groups: (1) responders (CR + PR + SD) with successfully applied surgery, and (2) non-responders (risk category) – i.e. people with immediate progression during neoadjuvant treatment or cases that remained in stable disease without subsequent surgery. In addition to therapeutic response, event free survival was examined as complex "early-warning" indication of further development of disease, i.e. indication of risk event (relapse, progression) up to 24 months. Period of 24 months was sufficiently covered by follow-up of individual cases and its selection as "early event risk point" is also in agreement with common clinical practice.

Characteristics of patients treated by neoadjuvant therapeutic regimens (R1/R2) are statistically summarized according to key categories and clinical stage (Tab. 2). Differences in age of treated women were statistically negligible, both quantitatively in years and in categories of age. Very significant differences were however obtained in T–N classes of primary tumor: considering stage III, regimen CHT/RT (R2) was apparently applied for significantly advanced disease, mostly in T4 category (87.8%) with positive finding in N (N1–N2: 96.9%). Lately mentioned parameter was further shown as predictive risk factor for non-responders to neoadjuvant therapy. No significant differences in histology classification occurred among therapeutic categories and – as it has already been stated – all three therapeutic categories could be compared within histological type "ductal invasive" as dominant class.

Table 2. Neoadjuvant therapeutic regimens (R1/R2) – overall characteristics

Parameters[1]	R1. Only CHT		R2. CHT/RT
	Clin. stage II	*Clin. stage III*	*Clin. stage III*
Sample size (n)	18	90	33
Age (years)[2]	53 (44; 65)	55 (44; 69)	56 (47; 68)
Age categories (%)			
<50 yr	38.9	32.2	21.2
50–59 yr	22.2	42.2	39.4
≥60 yr	38.9	25.6	39.4
T categories (%)			
T2	77.8	16.7	6.1
T3	22.2	28.9	6.1
T4	–	54.4	87.8
N categories (%)			
N0	27.8	4.4	3.1
N1	72.2	67.8	48.5
N2	–	27.8	48.4
Histopathology (%)			
Invasive ductal	100%	62.2%	57.6%
Invasive lobular	0%	18.9%	27.2%
Others	0%	18.9%	15.2%

CHT – chemotherapy, RT – radiotherapy. [1]All categorical parameters are expressed in % calculated within columns. [2]Quantitative parameters are summarized by estimate of median supplied by 10% and 90% percentiles (in parentheses).

Risk analysis relating therapeutic response to clinical characteristics. Risk analysis relating therapeutic response to clinical characteristics deals with main therapeutic results of neoadjuvant therapy, i.e. response in T and N categories of tumor and successfully applied surgery (Tab. 3). No significant difference in any of these outputs occurred comparing stage II and III as treated only by CHT, 93–94% of these tumors reached subsequent surgery and more than 50% of them revealed sufficient response in T and N categories. Significantly worse therapeutic response was reached in the case of stage III/ CHT+RT category, apparently due to more advanced status of disease.

As expected, category stage II/CHT and III/CHT revealed significantly decreased relative risk for response to neoadjuvant therapy (with non-responders rate up to 11% in both categories). The same positive development was documented for event free survival in this group. On the other hand, stage III (treated by CHT+RT) fell evidently into region of significantly increased risk both for therapeutic response (45.5% of non-responders) and event-free survival – this result was logically correlated with similar output found for high T categories of primary tumor. Histology types were of statistically negligible influence on probability of risk event, both in therapeutic response and in EFS. Regarding age categories, only women >60 yr appeared to have increased risk of early time-related event.

Overall profile of therapeutic response categories

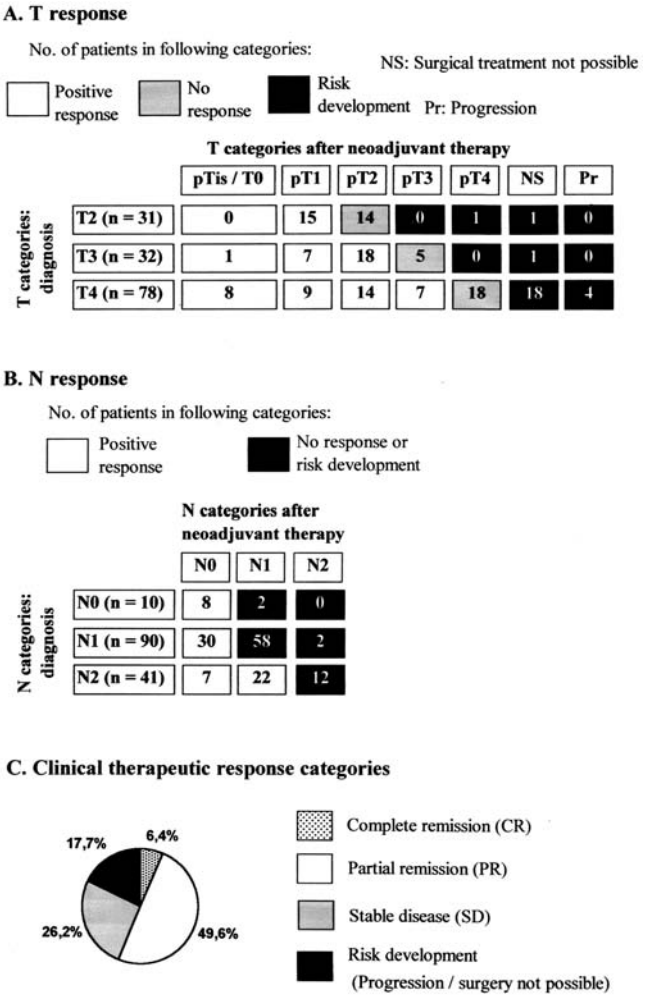

Figure 1. Overall summary of therapeutic response reached after neoadjuvant therapy.

reached after neoadjuvant treatment is displayed in the Figure 1. Complete remission was achieved in 6.5% patients, partial remission in 49.6%, stable disease in 26.2%, and progression in 17.7%.

Survival. Summarized survival analysis performed over the whole follow-up period is documented in the Figure 2, event-free survival stratified according to therapeutic response to neoadjuvant therapy is displayed in the Figure 3. Although some of the common clinical categories appeared to explain risk development in association with neoadjuvant treatment (Tab. 3), they were not able to completely describe differences occurred in subsequent survival of patients. Category Stage III/CHT+RT could be characterized by apparently worse profile of overall survival (Fig. 2). Therapeutic response reached after neoadjuvant therapy evidently separated EFS only for the already defined risk group ($p<0.05$; Fig. 3). The time-related analyses did not however bring clear separation of patients with CR/PR

Table 3. Risk analysis relating study endpoints to clinical characteristics

Examined categories	Endpoint I. Therapeutic response to neoadjuvant therapy				Endpoint II. Event free survival
	Patients with applied surgery CR+PR	SD	Risk group: no surgery in SD or progression	Odds ratio[1]	Relative risk[2]
Clinical stage/neoadjuvant therapy					
Stage II/CHT	55.6%	33.3%	11.1%	0.54(0.12;2.56)	0.39(0.12;0.86)*
Stage III/CHT	61.1%	30.0%	8.9%	0.19(0.08;0.49)**	0.84(0.47;1.48)
Stage III/CHT+RT	42.4%	12.1%	45.5%	8.21(3.15;21.18)**	1.78(1.19;3.39)**
T category of tumor					
T2	48.4%	45.2%	6.5%	0.26(0.05;0.92)*	0.92(0.44;1.90)
T3	81.3%	15.6%	3.1%	0.11(0.01;0.69)**	0.47(0.21;1.25)
T4	48.7%	23.1%	28.2%	7.85(2.20;18.42)**	1.75(1.06;3.21)*
Age categories					
<50 yr	60.5%	23.3%	16.3%	0.86(0.32;2.27)	1.03(0.55;1.92)
50–59 yr	52.7%	29.1%	18.2%	1.06(0.43;2.56)	0.71(0.39;1.34)
≥60 yr	55.8%	25.8%	18.6%	1.08(0.43;2.78)	1.76(1.22;3.12)**
Histopathology					
Invasive ductal carcinoma	52.7%	27.9%	19.4%	1.41(0.54;3.67)	1.04(0.56;1.95)
Invasive lobular carcinoma	69.2%	23.1%	7.7%	0.33(0.07;1.53)	0.84(0.38;1.89)
Others	54.6%	22.7%	22.7%	1.46(0.48;4.44)	1.13(0.50;2.52)

CHT – chemotherapy, RT – radiotherapy, CR – complete response, PR – partial response, SD – stable disease. [1]Relative risk related to the results of neoadjuvant therapy (not possible surgery, progression); estimated by univariate logistic regression and supplied by 95% confidence limits (in parentheses); [2]Relative risk of progression or relapse event; estimated by univariate Cox regression models and supplied by 95% confidence limits (in parentheses). *Values of relative risk significantly lower or higher than 1 at level $p<0.05$; **Values of relative risk significantly lower or higher than 1 at level $p<0.01$.

and cases that remained in stable disease even after neoadjuvant therapy – we can then conclude that there must be other significant factors influencing survival.

Molecular predictive parameters. Search for other predictors that are relatively independent on common clinical categories seems to be necessary to explain early progression/relapse event that occurred in all groups of patients, regardless they are stratified according to therapeutic response (Fig. 3), clinical stage or treatment strategy (Fig. 2). This study was focused on initial values of Ca 15-3, CEA, ER and PR receptors and brings analyses that proved their potentially predictive association with specified endpoints.

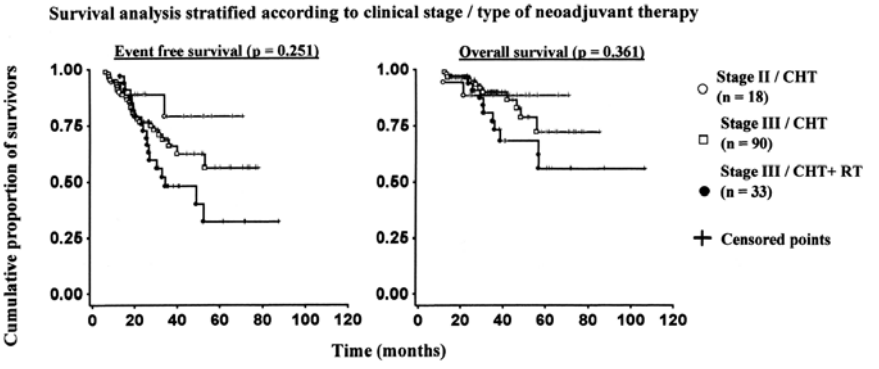

Figure 2. Survival analysis of study endpoints.

Taking early progression or relapse as principal risk event, the initial values of tumor markers and ER/PR receptor levels were subjected to the ROC analysis (Tab. 4) and diagnostically suitable cut-off values were extracted (Tab. 5). ROC analysis further validated proposed points for sensitivity and specificity working with early risk events that occurred up to 24 months as dependent variable. ROC analysis confirmed significant influence of all examined markers on early event probability and allowed to sufficiently estimate effective cut-off points for further analyses (Ca 15-3 ≥23 kU/l, CEA ≥5 mg/l; ER ≤5 fmol/mg; PR ≤5 fmol/mg). This output could be regarded as representative conclusion on significant predictive importance of the biomarkers.

We can therefore conclude, that initial values of all examined markers could be effectively related to event – free survival with relevant cut-off values (Tab. 4, 5). Subsequent Cox regression analyses further proved significant predictive power of ER/PR double positivity as factor very significantly decreasing the risk of early progression or relapse

Table 4. Initial value of tumor markers and ER/PR receptors levels in ROC analysis taking early time-related event (progression or relapse) as risk endpoint[1]

Parameters as single predictors[2]	ROC curve (Max. likelihood estimates)[3]	Critical test – result value Cut-off point	Sensitivity at cut-off	Specificity at cut-off
Ca 15-3 (kU/l)	Az: 0.84 (0.59; 0.96) a: 0.89 (0.18; 1.99) b: 0.46 (0.06; 0.86)	Ca 15.3 ≥23.0 kU/l	0.836	0.727
CEA (mg/l)	Az: 0.70 (0.49; 0.75) a: 1.25 (0.44; 1.98) b: 1.95 (0.38; 3.53)	CEA: ≥5.0 mg/l	0.702	0.651
ER (fmol/mg)	Az: 0.75 (0.52; 0.87) a: 0.82 (0.08; 1.66) b: 0.95 (0.24; 1.67)	ER: ≤5.0 fmol/mg	0.724	0.689
PR (fmol/mg)	Az: 0.75 (0.55; 0.83) a: 1.27 (0.03; 2.41) b: 2.03 (0.50; 3.55)	PR: ≤5.0 fmol/mg	0.805	0.676

ER – estrogen receptors, PR – progesterone receptors. [1]Early time-related event was exactly defined as progression or relapse that occurred up to 24 months of follow up after the end of neoadjuvant therapy. [2]Only physiological parameters providing sufficient ROC estimates of cutoff points were included in the analyses, i.e. area under the curve ≥0.65 and sensitivity or specificity of critical test at least 0.6. [3]Parameters of binormal ROC curve: a – vertical of ROC curve; b – slope of the fitted ROC curve when plotted as a straight line on normal deviate axis; both a and b were supplied with 95% confidence intervals (in parentheses). Az – Area under the ROC curve, supplied with asymmetric 95% confidence limits for binormal area estimate (in parentheses).

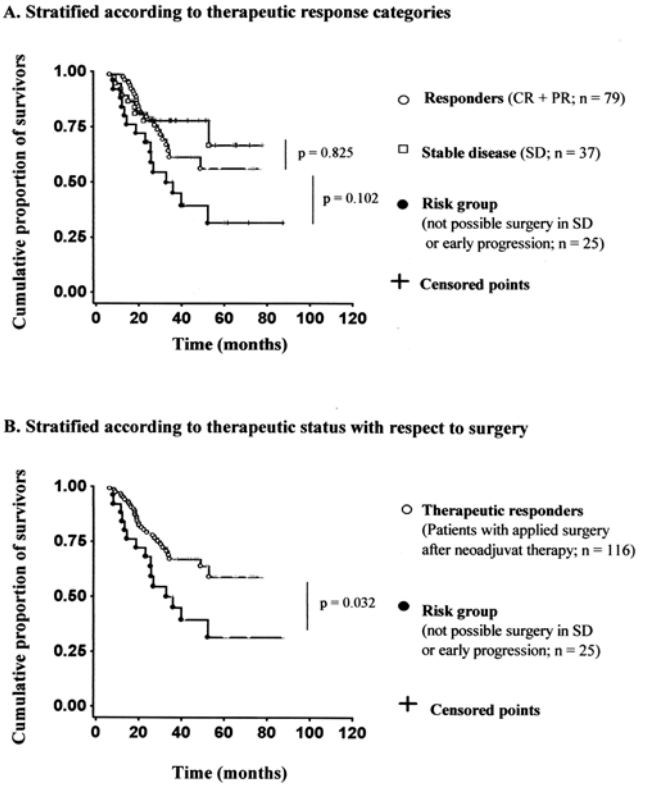

Figure 3. Event free survival in relation to therapeutic response reached after neoadjuvant therapy.

Table 5. Cut-off categories of tumor markers and ER/PR receptors as potential independent predictors in risk analysis

Examined categories	Endpoint I. Therapeutic response to neoadjuvant therapy				Endpoint II. Event free survival
	Patients with applied surgery CR+PR	SD	Risk group: no surgery in SD or progression	Odds ratio[1]	Relative risk[2]
Ca15-3 ≥23.0 kU/l (n=48)	62.5%	16.7%	20.8%	1.25(0.51;3.06)	3.36(2.32;5.21)**
CEA ≥5.0 mg/l (n=16)	50.0%	25.0%	25.0%	1.49(0.44;5.15)	2.44(1.25;4.81)*
ER ≤5.0 fmol/mg (n=72)	56.5%	27.5%	15.9%	1.27(0.53;3.06)	1.79(1.01;3.21)*
PR ≤5.0 fmol/mg (n=78)	55.2%	25.6%	19.2%	1.26(0.52;3.06)	1.93(1.07;3.49)*
ER/PR combination					
ER+/PR+ (n=55)	58.2%	27.3%	14.5%	0.69(0.27;1.74)	0.37(0.17;0.55)**

ER – estrogen receptors, PR – progesterone receptors, CR – complete response, PR – partial response, SD – stable disease. [1]Relative risk related to the results of neoadjuvant therapy (not possible surgery, progression); univariate logistic regression, supplied by 95% confidence limits (in parentheses). [2]Relative risk of progression or relapse event; estimated by univariate Cox regression models and supplied by 95% confidence limits (in parentheses). *Values of relative risk significantly lower or higher than 1 at level p<0.05. **Values of relative risk significantly lower or higher than 1 at level p<0.01.

event. Predictive potential of these parameters towards immediate therapeutic response after neoadjuvant therapy was however statistically negligible and we also found their independence on common clinical categories like stage, histology and other factors.

These findings are summarized in Table 5 by univariate logistic and Cox-regression models. Apparently, all examined biomarkers and associated cut-off points reached significant increase in relative risk only in event-free survival (confirmed by confidence limits as well). None significant risk relationship occurred in relation to therapeutic response categories (estimated as odds ratio). Very important conclusion was provided by combined modelling of risk influence of ER/PR receptors in Cox regression models. Risk potential of ER or PR receptors as single variables is significantly lower in comparison with their combination.

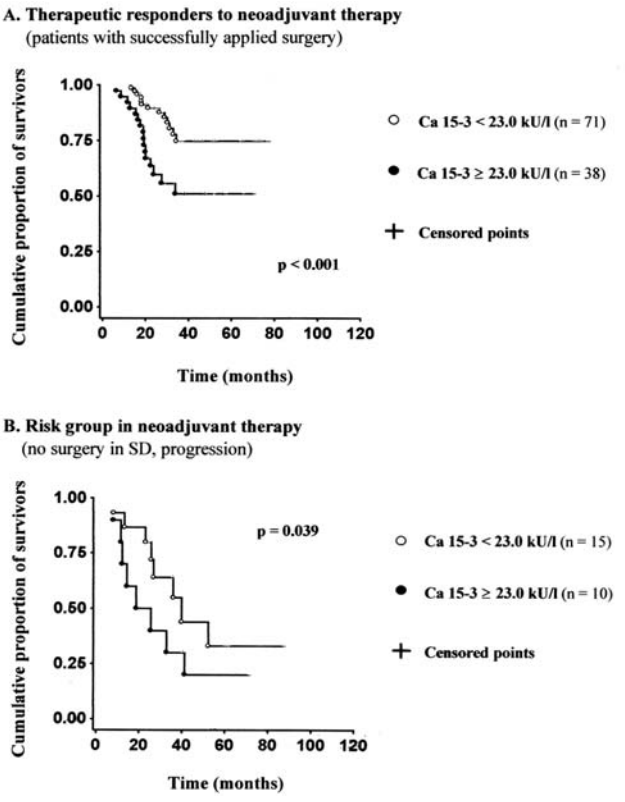

Figure 4. Ca 15-3 as factor predictively stratifying event-free survival.

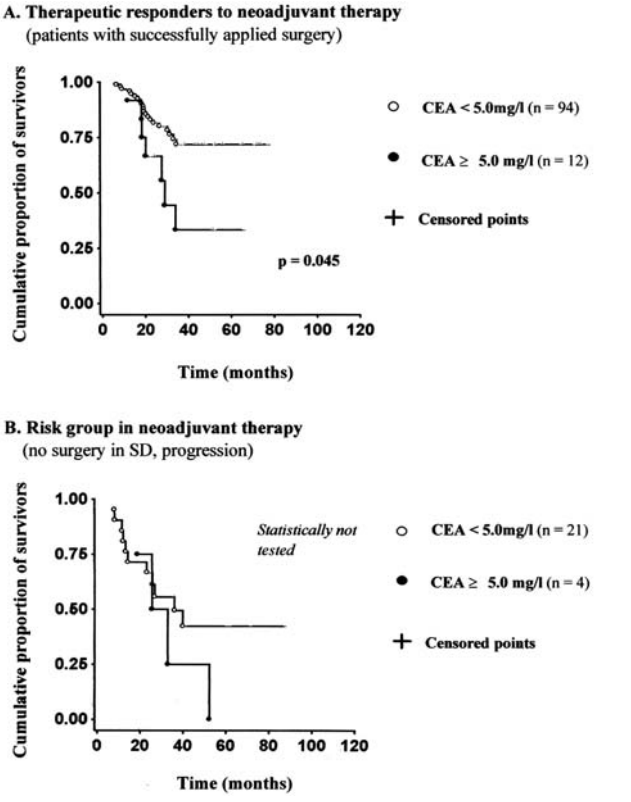

Figure 5. CEA as factor predictively stratifying event free survival

Namely combined double positivity ER+/PR+ revealed sharp decrease of relative risk in event-free survival (relative risk 0.36).

EFS analyses on Figure 4 support defined predictive potential of Ca 15-3, and verified cut-off value for both therapeutic responders and non-responders. Tumor marker Ca 15-3 <23 kU/l reached significantly better prediction for EFS. This finding strongly increases importance of Ca 15-3 as potential independent predictive parameter (based on results of Tab. 4). Initial values of CEA were significantly increasing in the following order of stage/therapy categories: II/CHT <III/CHT <III/CHT+RT. Figure 5 shows CEA as factor stratifying event free survival with better prediction for therapeutic responders with CEA <5.0 mg/l. Figure 6 shows ER as factor stratifying event free survival with risk prediction for patients with lower value than 5.0 fmol/mg in both responders and non-responders to neoadjuvant therapy. However, combination of ER/PR double positivity is shown in Figure 7 with significantly better prediction in therapeutic responders than in risk group patients.

Discussion

Neoadjuvant chemotherapy has become popular, especially for patients with advanced breast cancer [6, 8, 18, 21, 31]. The last decade has seen a surge of interest in neoadjuvant medical therapy [9, 11, 14, 15, 24, 55]. There are many of clinical studies and their summarizing with different results [17, 19, 25-31, 34, 35, 38, 42, 43, 45, 47, 50, 51, 53].Theoretically, prolongation of overall survival or relapse-free survival was expected from neoadjuvant chemotherapy, but there are still many controversies. The NSABP B-18 trial demonstrated no advantage of neoadjuvant chemotherapy in terms of mentioned parameters [23]. There is effort to find the best combination and the best cytostatics for neoadjuvant setting [32, 36, 37, 41]. The optimal schedule and number of cycles have not been determined [6, 49, 57]. NSABP B-27 has shown encouraging data in significant increases in the clinical CR rate, pCR rate, and achievement of axillary lymph node-negative status. Unfortunately the ultimate value of preoperative docetaxel will not be known until the disease-free and overall survival data are mature [5, 19]. Single agent docetaxel given on a weekly schedule appeared to be also effective, in terms of pCR [20].

The results of randomized clinical trials do not indicate a survival advantage for women treated with neoadjuvant systemic therapy but equally importantly, they do not indicate a survival disadvantage from this approach. Potential advantages include the ability to modify treatment based on the observed response of the tumor *in vivo*, the ability to select treatments according to predictive biological charac-

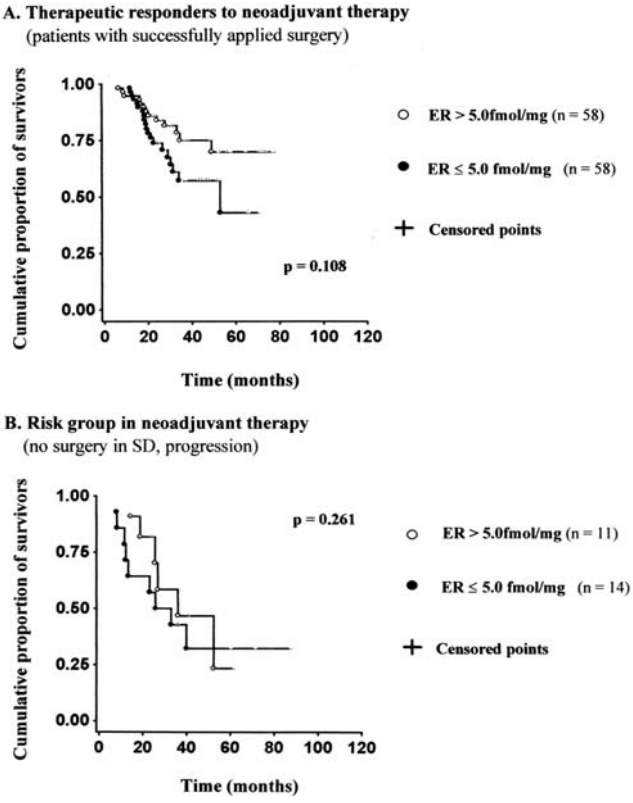

Figure 6. ER as factor stratifying predictively event free survival.

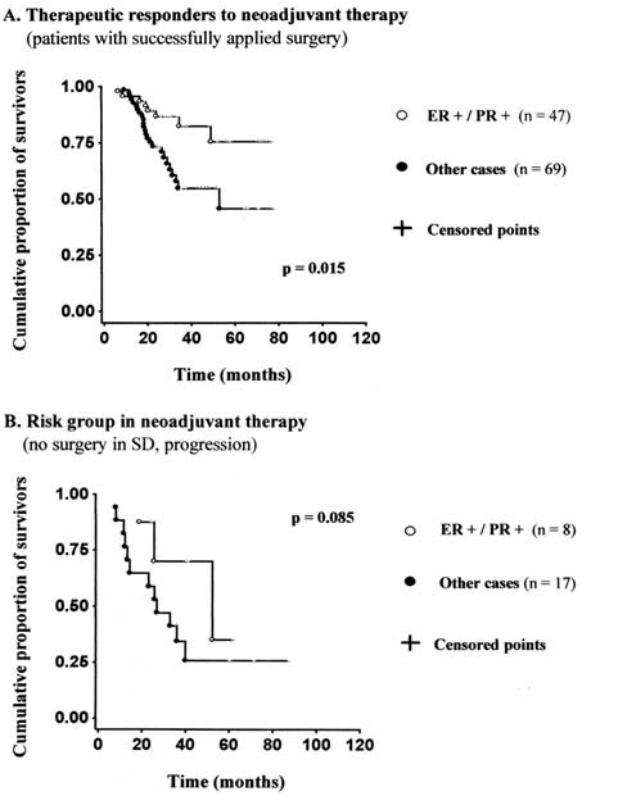

Figure 7. ER/PR double positivity as factor predictively stratifying event-free survival.

teristics of the tumor. That means to develop reliable biological and molecular markers that might predict for long-term outcome to therapy [1, 4, 7, 10, 15, 20, 22, 39, 44, 54, 56, 59]. Summarizing the current clinical trials it is evident that histological or nuclear grade has the strongest correlation with response [10, 44, 56]. Well differentiated tumors seldom, if ever, achieve a pCR, whereas nearly all of the pCR occur in patients with poorly differentiated tumors. High tumor proliferative rate assessed by mitotic index or proteins as mitosin and Ki-67 has been reported to correlate with pCR [1, 22, 49]. Some reports found that estrogen receptor negative tumors respond more often to neoadjuvant chemotherapy than ER-positive tumors [22, 44, 49]. Some other study did not find any difference in pCR for ER-positive or -negative tumors [20]. Unfavorable response has been reported with HER-2/neu overexpression, p53 mutation, and low Bax [4, 10, 22, 59]. It is clear that no good predictive factors have been determined.

Our retrospective study has confirmed some of the above mentioned results. Stage II, and most of stage III breast cancer could undergo just neoadjuvant chemotherapy to reach response, and undergo surgery. Increased risk to shorten EFS is in stage III treated by chemotherapy and radiation therapy together and tumor size T4. There is no predictive value for Ca 15-3, CEA, ER/PR status, in terms of response to neoadjuvant therapy, but there is predictive value of these parameters in terms of event free survival. We can conclude, that initial values of all these potential predictors could be effectively related to EFS development, with defined cut-off values. Very important conclusion was provided by combined modelling of risk influence of ER/PR receptors in Cox regression models. Risk potential of ER or PR receptors as single variables is significantly lower in comparison with their combination – namely combined double positivity ER+/PR+ revealed sharp decrease of risk in event-free survival.

References

[1] AAS T, GEISLER S, EIDE GE, HAUGEN DF, VARHAUG JE et al. Predictive value of tumour cell proliferation in locally advanced breast cancer treated with neoadjuvant chemotherapy. Eur J Cancer 2003; 39: 438–446.

[2] ALTMAN DG. Practical Statistics for Medical Research. London: Chapman and Hall, 619p, 1991.

[3] AMAT S, BOUGNOUX P, PENAULT-LLORCA F, FETISSOF F, CURE H et al. Neoadjuvant docetaxel for operable breast cancer induces a high pathological response and breast-conservation rate. Brit J Cancer 2003; 88: 1339–1345.

[4] ANELLI A, BRENTANI RR, GADELHA AP, DE ALBUQUERQUE

AA, SOARES F. Correlation of p53 status with outcome of neoadjuvant chemotherapy using paclitaxel and doxorubicin in stage IIIB breast cancer. Annal Oncol 2003; 14: 428–432.

[5] ANONYMOUS. Neoadjuvant docetaxel augments the efficacy of preoperative doxorubicin/cyclophosphamide in operable breast cancer: First results of NSABP B-27. Clin Breast Cancer 2002; 3: 26–28.

[6] BALTALI E, ALTUNDAG MK, ONAT DA, ABBASOGLU O, OZISIK Y et al. Neoadjuvant chemotherapy with taxotere-epirubicin-5-fluorouracil (TEF) in local-regionally advanced breast cancer: A preliminary report. Tumori 2002; 88: 474–477.

[7] BONNEFOI H, DIEBOLD-BERGER S, THERASSE P, HAMILTON A, VAN DE VIJVER M et al. Locally advanced/inflammatory breast cancers treated with intensive epirubicin-based neoadjuvant chemotherapy: Are there molecular markers in the primary tumour that predict for 5-year clinical outcome? Annal Oncol 2003; 14: 406–413.

[8] BRAUD AC, LEVY E, FEUILHADE F, OTMEZGUINE Y, CALITCHI E et al. Combination of vinorelbine, epirubicin, and cyclophosphamide as neoadjuvant chemotherapy for locally advanced breast cancer: Phase II study. Am J Clin Oncol-Cancer Clin Trials 2002; 25: 303–307.

[9] BUCHHOLZ TA, HUNT KK, WHITMAN GJ, SAHIN AA, HORTOBAGYI GN. Neoadjuvant chemotherapy for breast carcinoma – Multidisciplinary considerations of benefits and risks. Cancer 2003; 98: 1150–1160.

[10] BUCHHOLZ TA, KATZ A, STROM EA, McNEESE MD, PERKINS GH et al. Pathologic tumor size and lymph node status predict for different rates of locoregional recurrence after mastectomy for breast cancer patients treated with neoadjuvant versus adjuvant chemotherapy. Int J Radiat Oncol Biol Phys 2002; 23: 880–888.

[11] BUSTOVA I. Neoadjuvant chemotherapy with mitoxantrone in treatment of locally advanced breast cancer. Klin Onkol 2002; 15: 145–147.

[12] CANCE WG, CAREY LA, CALVO BF, SARTOR C, SAWYER L et al. Long-term outcome of neoadjuvant therapy for locally advanced breast carcinoma: Effective clinical downstaging allows breast preservation and predicts outstanding local control and survival. Ann Surg 2002; 236: 295–303.

[13] CHAKRAVARTHY A, CHARAUND P, KELLEY M, JOHNSON D, STEWART Z et al. Proteomic profiles from serial core biopsies following neoadjuvant paclitaxel/radiation for stage II/III breast cancer. Proc Am Soc Clin Oncol 2003; 22: 860, Abs 3455.

[14] CHAPUIS PO, GOFFIN J, WONG N, PERRET C, GHADIRIAN P et al. A significant response to neoadjuvant chemotherapy in BRCA1/2 related breast cancer. J Med Gen 202; 39: 608–610.

[15] CHOLLET PJM, AMAT S, PENAULT-LLOREA F, AYEVA M, MOURET-REYNIER MA et al. Neoadjuvant docetaxel: 4-year survival outcomes and prognostic factors in patients with operable breast cancer (BC). Proc Am Soc Clin Oncol 2003; 22: 48, Abs 190.

[16] CLEATOR S, PARTON M, DOWSETT M. The biology of neoadjuvant chemotherapy for breast cancer. Endocrine-Related Cancer 2002; 9: 183–195.

[17] COSKUN U, GUNEL N, ONUK E, YILMAZ E, BAYRAM O et al. Effect of different neoadjuvant chemotherapy regimens on locally advanced breast cancer. Neoplasma 2003; 50: 210–216.

[18] DE MATTEIS A, NUZZO F, D'AIUTO G, LABONIA V, LANDI G et al. Docetaxel plus epidoxorubicin as neoadjuvant treatment in patients with large operable or locally advanced carcinoma of the breast: A single-center, phase II study. Cancer 2002; 94: 895–901.

[19] D'ORAZIO AI, O'SHAUGHNESSY J, SEIDMAN AD. Neoadjuvant docetaxel augments the efficacy of preoperative docetaxel/cyclophosphamide in operable breast cancer: First results of NSABP B-27. Clin Breast Cancer 2002; 2: 266–268.

[20] ESTEVEZ LG, CUEVAS JM, ANTON A, FLORIAN J, LOPEZ-VEGA JM et al. Weekly docetaxel as neoadjuvant chemotherapy for stage II and III breast cancer: Efficacy and correlation with biological markers in a phase II, multicenter study. Clin Cancer Res 2003; 9: 686–692.

[21] EZZAT AA, RAHAL M, AJARIM D, IBRAHIM E, BAZARBASHI S et al. Dose dense neoadjuvant sequential chemotherapy in the management of locally advanced breast cancer: A phase II study. Proc Am Soc Clin Oncol 2003; 22: 51, Abs 202.

[22] FANEYTE IF, SCHRAMA JG, PETERSE JL, REMIJNSE PL, RODENHUIS S, VAN DE VIJVER MJ. Breast cancer response to neoadjuvant chemotherapy: Predictive markers and relation with outcome. Br J Cancer 2003; 88: 406–412.

[23] FISHER B, BRYANT J, WOLMARK N. Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. J Clin Oncol 1998; 16: 2672–2685.

[24] FRANCO S, SILVA O, BATEMAN D, DOLINY P, JORDA M et al. Neoadjuvant (NEO) treatment of locally advanced and inflammatory breast cancer with weekly taxotere and carboplatin in tumors that do not overexpress HER-2. Proc Am Soc Clin Oncol 2002; 21: 59b, Abs 2048.

[25] GIBSON AD, O'SHAUGHNESSY JA, BURSTEIN H, JAIN VK. Pathologic complete response with docetaxel neoadjuvant therapy for breast cancer. Clin Breast Cancer 2003; 4: 24–27.

[26] GOBLE S, BEAR HD. Emerging role of taxanes in adjuvant and neoadjuvant therapy for breast cancer: The potential and the questions. Surg Clin N Am 2003; 83: 943–971.

[27] GOGAS H, FOUNTZILAS G. The role of taxanes as a component of neoadjuvant chemotherapy for breast cancer. Annal Oncol 2003; 14: 667–674.

[28] GOGAS H, PAPADIMITRIOU C, KALOFONOS HP, BAFALOUKOS D, FOUNTZILAS G et al. Neoadjuvant chemotherapy with a combination of pegylated liposomal doxorubicin (Caelyx) and paclitaxel in locally advanced breast cancer: A phase II study by the Hellenic Cooperative Oncology Group. Annal Oncol 2002; 13: 1737–1742.

[29] HEYS SD, HUTCHEON AW, SARKAR TK, OGSTON KN, MILLER ID et al. Neoadjuvant docetaxel in breast cancer: 3-year survival results from the Aberdeen trial. Clin Breast Cancer 2002; 3 Suppl 2: S69–S74.

[30] HURLEY J, DOLINY P, SILVA O, GOMEZ-FERNANDEZ C, REIS I et al. Neoadjuvant herceptin/taxotere/cisplatin in the treatment of locally advanced and inflammatory breast cancer. Proc Am Soc Clin Oncol 2002; 21: 50a, Abs 196.

[31] HUTCHEON AW, HEYS SD, SARKAR TK. Neoadjuvant doce-

taxel in locally advanced breast cancer. Breast Cancer Res Treat 2003; 79 Suppl 1: S19–S24.

[32] IKEDA T, JINNO H, MATSU A, MASAMURA S, KITAJIMA M. The role of neoadjuvant chemotherapy for breast cancer treatment. Breast Cancer 2002; 9: 8–14.

[33] INAJI H, KOMOIKE Y, MOTOMURA K, KASUGAI T, SAWAI Y et al. Breast-conserving treatment after neoadjuvant chemotherapy in large breast cancer. Breast Cancer 2002; 9: 20–25.

[34] JACKISCH C, VON MINCKWITZ G, EIDTMANN H, DAN COSTA S, RAAB G et al. Dose-dense biweekly doxorubicin/docetaxel versus sequential neoadjuvant chemotherapy with doxorubicin/cyclophosphamide/docetaxel in operable breast cancer: Second interim analysis. Clin Breast Cancer 2002; 3: 276–280.

[35] KUMAR RLA, CLEATOR S, ASHLEY SE, POWLES TJ. 5-year follow-up of a phase 3 trial of neoadjuvant chemoendocrine therapy in operable breast cancer. Brit J Cancer 2003; 88 Suppl 1: S19, Abs 5.3.

[36] LANKESTER KJ, PHILLIPS JE, LAWTON PA. Weight gain during adjuvant and neoadjuvant chemotherapy for breast cancer: An audit of 100 women receiving FEC or CMF chemotherapy. Clin Oncol 2002; 14: 64–67.

[37] LIVINGSTON R. Current and planned trials with capecitabine in adjuvant/neoadjuvant therapy of breast cancer. Oncology (Huntington) 2002; 16: 29–32.

[38] MAMOUNAS EP. Neoadjuvant chemotherapy for operable breast cancer: Is this the future? Clin Breast Cancer 2003; 4 Suppl 1: S10–S19.

[39] McINTOSH SA, PANCHALINGAM L, PAYNE S, MILLER ID, SARKAR TK et al. Freehand core biopsy in breast cancer: An accurate predictor of tumour grade following neoadjuvant chemotherapy? Breast 2002; 11: 496–500.

[40] METZ CE. ROC methodology in radiologic imaging. Invest Radiol 1986; 21: 720–733.

[41] MORRELL L, LEE Y, REIS I, HURLEY J. MVAC as neoadjuvant therapy in locally advanced and inflammatory breast cancer: Ten year update. Proc Am Soc Clin Oncol 2003; 22: 61, Abs 244.

[42] OZMEN V, CABIOGLU N, IGCI A, DAGOGLU T, AYDINER A et al. Inflammatory breast cancer: Results of antracycline-based neoadjuvant chemotherapy. Breast J 2003; 9: 79–85.

[43] PELISSIER P, DELALOGE S, MATHIEU M-C, JEANBLANC G, PETIT T et al. Intensified anthracyclin doses do not improve clinical and pathological responses to neoadjuvant FEC for operable breast cancer (BC): results of a multicenter randomized trial. Proc Am Soc Clin Oncol 2002; 21: 64a, Abs 254.

[44] PIERGA J-Y, MOURET E, LAURENCE V, DIERAS V, SAVIGIONI A et al. Prognostic factors for survival after neoadjuvant chemotherapy in operable breast cancer: The role of clinical response. Eur J Cancer 2003; 39: 1089–1096.

[45] POWLES TJ. Neoadjuvant chemotherapy for operable breast cancer – The Green/Hortobagyi Article Reviewed. Oncology (Huntington) 2002; 16: 889–890.

[46] RAGAZ J. Neoadjuvant chemotherapy for operable breast cancer – The Green/Hortobagyi Article Reviewed. Oncology (Huntington) 2002; 16: 892–894.

[47] SANCHEZ-ROVIRA P, JAEN A, DUENAS R, PORRAS I, MARTINEZ E et al. Neoadjuvant gemcitabine therapy for breast cancer. Clin Breast Cancer 2002; 3 Suppl 1: S39–S44.

[48] SAUVEN P. The surgical management of patients following neoadjuvant chemotherapy for locally advanced breast cancer. Eur J Cancer 2002; 38: 2371–2374.

[49] SHANNON C, SMITH I. Is there still a role for neoadjuvant therapy in breast cancer? Crit Rev Oncol Hematol 2003; 45: 77–90.

[50] SMITH IC, HEYS SD, HUTCHEON AW, MILLER ID, PAYNE S et al. Neoadjuvant chemotherapy in breast cancer: Significantly enhanced response with docetaxel. J Clin Oncol 2002; 20: 1456–1466.

[51] SMITH IE, A'HERN R, COOMBES G, HICKISH T, O'BRIEN M et al. A randomized neoadjuvant chemotherapy trial of vinorelbine/epirubicin (VE) vs standard doxorubicin/cyclophosphamide (DC) in patients with 3 cm diameter operable breast cancer (TOPIC 2). Proc Am Soc Clin Oncol 2003; 22: 21, Abs 83.

[52] STEARNS V, SINGH B, TSANGARIS T, CRAWFORD JG, NOVIELLI A et al. A prospective randomized pilot study to evaluate predictors of response in serial core biopsies to single agent neoadjuvant doxorubicin or paclitaxel for patients with locally advanced breast cancer. Clin Cancer Res 2003; 9: 124–133.

[53] THERASSE P, MAURIAC L, WELNICKA-JASKIEWICZ M, BRUNING P, CUFER T et al. Final results of a randomized phase III trial comparing cyclophosphamide, epirubicin, and fluorouracil with a dose-intensified epirubicin and cyclophosphamide plus filgrastim as neoadjuvant treatment in locally advanced breast cancer: An EORTC-NCIC-SAKK multicenter study. J Clin Oncol 2003; 21: 843–850.

[54] TULBAH AM, IBRAHIM EM, EZZAT AA, AJARIM DS, RAHAL MM et al. HER-2/neu overexpression does not predict response to neoadjuvant chemotherapy or prognosticate survival in patients with locally advanced breast cancer. Med Oncol 2002; 19: 15–23.

[55] VAN PRAAGH I, CURE H, LEDUC B, CHARRIER S, LE BOUEDEC G et al. Efficacy of a primary chemotherapy regimen combining vinorelbine, epirubicin, and methotrexate (VEM) as neoadjuvant treatment in 89 patients with operable breast cancer. Oncologist 2002; 7: 418–423.

[56] WANG J, BUCHHOLZ TA, MIDDLETON LP, ALLRED DC, TUCKER SL et al. Assessment of histologic features and expression of biomarkers in predicting pathologic response to anthracycline-based neoadjuvant chemotherapy in patients with breast carcinoma. Cancer 2002; 94: 3107–3114.

[57] WENZEL C, LOCKER GJ, PLUSCHNIG U, ZIELINSKI CC, RUDAS M et al. Phase I/II trial of weekly epidoxorubicin and docetaxel (wED) in the neoadjuvant and palliative treatment of patients with breast cancer. Cancer Chemoth Pharmacol 2002; 50: 155–159.

[58] ZAR JH. Biostatistical Methods. 2nd ed. London: Prentice Hall 1984: 556p.

[59] ZHANG F, YANG Y, SMITH T, KAU S-W, McCONATHY JM et al. Correlation between HER-2 expression and response to neoadjuvant chemotherapy with 5-fluorouracil, doxorubicin, and cyclophosphamide in patients with breast carcinoma. Cancer 2003; 97: 1758–1765.