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ORIGINAL ARTICLE

High Ki67 as negative predictor for response to concurrent radiotherapy plus Capecitabine in chemo-resistant advanced breast cancer

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Summary

Purpose: *The purpose of this study was to evaluate Ki67 as a biomarker for response to concurrent chemo-radiotherapy in previously treated patients with standard chemotherapy protocols in the neoadjuvant setting (NACT).*

Methods: *Evaluated were 33 patients treated concurrently with radiotherapy and capecitabine. All patients had residual disease after anthracycline-docetaxel based NACT, verified with imaging techniques and clinical exams. Response rate (RR) was evaluated 3 months after completion of the concurrent treatment, and was correlated to tumor immune-histochemical characteristics. Binary logical regression was used for model testing and correlation of Ki67 and RR. An Omnibus test showed the model to be statistically significant and that a set of depending variables can be used as predictors for treatment response with p=0.021. Model -2 log likelihood with Nagelkerke R Square were used to define significance of other tumor characteristics besides Ki67.*

Results: *Only Ki67 showed statistically significant correlation with RR, as high Ki67 predicts that there will be no response to concurrent capecitabine - radiotherapy treatment in chemo-resistant advanced breast cancer. Other characteristics such as histological grade, estrogen or progesterone receptors, HER2 overexpression or lymphovascular or perineural invasion showed no significance.*

Conclusion: *High value of Ki67 is a negative predictor for response in concurrent capecitabine-radiotherapy treatment in chemo-resistant advanced breast cancer.*

Key words: *breast cancer, capecitabine, concurrent, Ki67, predictor, radiotherapy*

Introduction

Breast cancer diagnosis represents a whole spectrum of malignant diseases with variable biological behavior in terms of how rapid the growth of the primary tumor is, regional lymph node involvement and metastatic potential. Classification based on histology (ductal, lobular, medullary, papillary, micropapillary, etc.), histological tumor grade (G1-G3) and presence or absence of a lymphovascular and/or neural invasion provides basic information about tumor aggressiveness.

Clinical experience shows that different patients in the same stage of breast cancer will have a different course of disease and different response to treatment. The initial study of Perou et al ex-

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plained breast cancer profiling based on gene expression arrays and defined several basically different breast subtypes [1]. Since genetic arrays are not widely available, Cheang et al proposed a simplified, clinically useful breast cancer subtype distinction based on immunochemistry assays [2]. Immunohistochemical definition of an estrogen receptor (ER) and progesterone receptor (PR) expression, overexpression or amplified human epidermal growth factor receptor 2 (HER2) oncogene and Ki67 labeling index are used as approximation to intrinsic subtypes. Determination of ER, PR and HER2 are defined by guidelines, however cutoff value of Ki67 labeling index is still under discussion [3,4]. The consensus is that Ki67 value is considered as low, and is considered as high [5,6]. Based on this, in 2011 and 2013 St. Gallen Consensus Conference of early breast cancer treatment defined four breast cancer subtypes and a treatment related to a specific subtype [7,8]. Notably Ki67, as a nuclear marker of tumor cell proliferation, is the main parameter to distinguish Luminal A from Luminal B (HER2 negative) carcinomas. Although the panel of experts in St. Gallen in 2011 agreed that the Ki67 cutoff value will be 14% for low or for high, in 2013 the cutoff of 20 - 25% was considered more appropriate [7,8]. Ki67 has been recognized as prognostic and predictive factor for breast cancer treated with chemotherapy as well as with antihormonal therapy [9-14].

Regardless of type and subtype, standard of care for locally advanced breast cancer (LABC) is anthracycline-taxane (A/T) based NACT [8–18]. Achieving a complete pathological response (ypCR) either in primary tumor or in regional lymph nodes, and especially in both sites, is predictive of better treatment outcome for disease free, event free and overall survival [19-22]. Patients who achieve partial response (PR) can differ in treatment outcome, depending on tumor subtype, but residual disease is associated with increased recurrence and poor prognosis [21]. Patients with operable or inoperable LABC resistant to NACT, achieving stable disease (SD) or progressive disease (PD) have very poor prognosis and very limited treatment options.

Since capecitabine, prodrug of 5-fluorouracil (5-FU), is recognized as well tolerated radiosensitizer and is widely used in gastrointestinal tumors, several studies analyzed concurrent use of capecitabine and radiotherapy (Cap-RT) preoperatively for LABC or recurrent disease and concurrent Cap -RT in adjuvant setting [23-34].

The aim of this study was to evaluate the treatment response to concurrent capecitabine in addition to radiotherapy in LABC or metastatic disease resistant to A/T NACT and correlate it with Ki67.

Methods

This was a single-institution retrospective study. It included all patients treated with concurrent chemoradiotherapy (cC-RT) from 2016-2019. Before any specific oncological treatment, all patients were discussed in the multidisciplinary tumor board for a final treatment decision. Patients either had an operable or an inoperable LABC or metastatic disease (M1) and were treated with concurrent chemoradiotherapy (cC-RT). Patients eligible for cC-RT were those who had:

1. Histopathological confirmation of invasive primary breast cancer confirmed on core needle biopsy specimen.
2. Immunohistochemical ± in situ hybridization determination of ER, PR, HER2 and Ki 67.
3. Ultrasound and/or mammography verification of breast tumor and additional metastatic diagnostic procedures.
4. Operable or inoperable locoregionally advanced breast cancer (LABC) or metastatic disease (M1).
5. Poor response to previous neoadjuvant or first-line antracycline/taxane based chemotherapy with trastuzumab when indicated.
6. ECOG performance status 0 - 1.

Poor response to chemotherapy was defined as:

1. Progressive disease (PD).
2. Stable disease (SD) in initially inoperable stage.
3. Residual disease after surgery: R1 resection or nodal disease after complete surgical resection.

Methodology

In all patients included in the study, the proliferation index Ki67 was determined routinely on pretreatment core needle biopsies. All biopsy specimens were obtained with 14G needles, and the average number of samples was 3-5. Biopsy specimens were fixed in formalin and molded in paraffin. The duration of fixation ranged from 12 to 24h. DAKO Monoclonal Mouse Anti-Human Ki67 Antigen Clone MIB-1 Ready-to-Use was used for immunohistochemical analysis, and the analysis was performed on DAKO Autostainer Link 4800 device. The interpretation of the obtained immunohistochemical analyses were determined only in the component of invasive malignant tumor, and the total number of tumor cells on which the proliferation index was determined depended on the amount of tumor tissue present in the biopsy. The minimum number of cells from which the results were interpreted was 500, and for most biopsy samples the evaluation was performed on 1000 tumor cells by examining whole biopsy samples at low microscopic magnification and selecting the counting field. The counting was done at the highest microscopic magnification, and all positive nuclei were counted which implied any intensity of the nuclear reaction relative to the negative nuclei. The results were interpreted as the percentage of positive tumor cell nuclei in all selected counting fields in relation to the total number of all invasive tumor cell nuclei from which the analysis was performed.

Initially, all patients had a three-dimensional computed tomography (3D-CT) simulation on a 16 slice - CT machine (GE Light speed RT) and Orfit immobilization equipment, supine position with an inclination of 15 degrees, hands above the head.

Volume delineation for radiotherapy was performed according to ESTRO-ACROP consensus guideline for elective breast irradiation [35]. Treatment planning used Eclipse V13.6 software. Map Check was used for plan verification.

All LABC patients received capecitabine 825mg/m^2, b.i.d., 5 days a week during the working week concomitant with radiotherapy. Capecitabine was taken orally, approximately 1 to 2 h before radiotherapy.

Radiotherapy was delivered to a chest wall or breast planning treatment volume with or without regional lymph nodes depending on whether they were involved or not. The radiotherapy technique was 3D conformal radiotherapy. For breast irradiation tangential coplanar fields were used with or without third anterior field for dose homogenization and to encompass the whole breast. Axilla and supraclavicular region were irradiated in two anterior and one posterior field. Total dose (TD) to both volumes was 45 Gy in 25 fractions, daily dose of 1.8

Table 1. Demographic and baseline disease characteristics

Characteristics	*LABC*	*M1*
Median age, years (range)	67 (84 - 44)	58 (35 – 72)
No. of patients (%)	20 (64)	13 (36)
Ki67 mean (±SD)	50 (±18)	60 (±16)
Ki67 range	15 – 90	30 – 90
Menopausal status		
Pre-n (%)	2 (10)	2 (15)
Post-n (%)	18 (90)	11 (85)
T stage (clinical), n (%)		
T1	0	0
T2	3 (15)	2 (15)
T3	0	1 (8)
T4	17 (85)	10 (77)
N stage (clinical), n (%)		
Positive	21 (100)	11 (85)
Negative	0	2 (15)
Histologic grade, n (%)		
G1	0	0
G2	12 (60)	8 (62)
G3	8 (40)	5 (38)
Lymphovascular invasion, n (%)		
Present	8 (40)	8 (62)
Absent	12 (60)	5 (38)
Perineural invasion, n (%)		
Present	6 (30)	8 (62)
Absent	14 (70)	5 (38)
Receptor status, n (%)		
ER+/PR+/HER2 -	6 (30)	8 (62)
ER+/PR-/HER2 -	2 (10)	1 (8)
ER-/PR+/HER2-	2 (10)	
Triple negative	7 (35)	
ER+/PR+/HER2 +	2 (10)	1 (8)
ER+/PR-/HER2 +	-	
ER-/PR+/HER2+	-	1 (8)
ER-/PR-/HER2 +	1 (5)	2 (15)
Surgery post NACT*, n (%)	10 (50)	5 (38)

LABC: locally advanced breast cancer; M1: metastatic disease; ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2; Ki67: proliferating marker; NACT: neoadjuvant chemotherapy; *some patients with LABC were converted to operable and some patients had surgery after NACT but was diagnosed as metastatic M1 disease after surgery, and before radiotherapy.

Gy. All plans were peer-reviewed and discussed during radiation oncologists and medical-physicists daily meetings. All patients were followed up in a weekly regimen and toxicity was recorded. During weekends no chemo and no radiotherapy were administered.

After finishing the Cap-RT treatment patients were followed up. Response and local control were evaluated by clinical examination, ultrasound, and distant dissemination was evaluated with CT of the brain, neck, thorax and abdomen. According to response, patients were divided into 2 groups:

1. Response (partial/PR, or complete response/CR).
2. No-response (SD or PD).

Treatment outcome was defined as response rate (RR) and encoded as "0" if there was no response to treatment (stable disease or progressive disease by RECIST criteria V1.1) and "1" if there was a response to treatment (partial or complete response by RECIST criteria V1.1) [36].

Ethical statement

This study has been performed in accordance with the ethical standards of the Declaration of Helsinki. Ethical Committee of Center of Radiotherapy and Ethical Committee of University Clinical Center of Republic of Srpska gave their approval on conducting this research.

Statistics

Standard descriptive statistics were used to define demographic and baseline disease characteristics. Omnibus test was used to show if the model was statistically significant and if the set of depending variables could be used as predictor for treatment response. Model -2 log likelihood with Nagelkerke R Square was used to define significance of all variables represented set of tumor characteristics as follows: Ki67, histologic type, histologic grade, lympho-vascular invasion, perineural invasion, estrogen receptor status, progesterone receptor status,

Table 2. Omnibus tests of coefficients model calculation

		Chi-square	*Df*	*p*
Step 1	Step	5.362	1	0.021
	Block	5.362	1	0.021
	Model	5.362	1	0.021

Table 3. Model -2 log likelihood with Nagelkerke R Square calculation for dependent characteristics

Step	-2 Log likelihood	*Cox & Snell R Square*	*Nagelkerke R Square*
1	39.25	0.150	0.202

Table 4. Patients classified in two groups by response to treatment. Value 0 means that there is no response. Value 1 means that there was a response to treatment

Classification table[a]					
		Observed	*Predicted*		*Percentage correct*
			RR		
			0	*1*	
Step 1	RR	0	17	2	89.5
		1	8	6	42.9
	Overall Percentage			69.7	

[a]The cut value is .500

Table 5. Correlation between Ki67 and response rate

Variables for Equation		B	*S.E.*	*Wald*	*Df*	*p*	*Exp(B)*	*95% C.I.for EXP(B)*	
								Lower	*Upper*
Step 1[a]	Ki67	-.051	0.025	4.235	1	0.040	0.950	0.905	0.998
	Constant	2.441	1.371	3.168	1	0.075	11.481		

[a]Variable(s) entered on step 1: Ki67

HER2 expression status, metastatic disease at the time of concurrent Cap-RT measurable residual disease at the time of concurrent Cap-RT. Binary logical regression was used for model testing and correlation between Ki67 and RR. RR is considered as an independent variable. A 5% level of statistical significance was used for variables ($p<0.05$). Data was analyzed using SPSS 23 statistical package.

Results

Out of the 33 included patients, 21 were in LABC and 12 were in M1 stage of disease. Of 21 LABC, 7 were operable and surgery was performed. Five patients in M1 group had surgery after planned NACT, but was diagnosed as metastatic disease after surgery, and before radiotherapy. Patients characteristics are shown in Table 1.

All 33 patients met the criteria, and none were excluded from the study. Omnibus test showed that the model is statistically significant and that the set of dependent variables can be used as predictive for treatment response with p=0.021 (Table 2).

Dependent characteristics: histologic type, histologic grade, lympho-vascular invasion, perineural invasion, estrogen receptor status, progesterone receptor status, HER2 expression status, metastatic disease at the time of concurrent Cap-RT measurable residual disease at the time of concurrent Cap-RT had no impact on treatment outcome, Nagelkerke R Square 0.202 (Table 3).

Whole model has 69.7% possibility for prediction if some parameters are influencing treatment outcome and 89.5% possibility to predict if there will be no response (Table 4).

Final analysis with logistic regression models evaluated tumor characteristics and their correlation to observed event, that is response rate (Table 5). Only Ki67 with Wald's statistics 4.235 and p=0.040 showed statistically significant impact on treatment outcome. This model shows that value Exp (B) is 0.95 which means that there is 95% chance that patients with high Ki67 will have no response to treatment.

Discussion

This is the first study that showed the proliferating factor Ki67 is negative predictor for response to concurrent chemo-radiotherapy, which means that there is a negative correlation between Ki67 and treatment response. Although the Omnibus test showed that the model in this study was statistically significant, the power of this study and conclusion strength are limited by its small sample size. Combined treatment that was the subject of this study is not a standard of care, even though capecitabine and radiotherapy are both standard treatments in locally advanced and metastatic setting. Inclusion criteria were exceptionally strict and a few patients were eligible, and that had an impact on the total number of patients. This problem could be solved in prospective multicentric trial. Like the Ohno et al study showed, there were some implications that suggested the patients we observed, who had high Ki67, ranging from 30-60%, were more likely to respond to treatment, and those with very high Ki67, ranging 60-90%, were more likely not to respond, further stratification of total number of patients was not recommended due to sample size [12]. Further investigation should be toward multicentric prospective assessment of the role and importance Ki67, possible stratification of Ki67 in "high" and "very high", determination of Ki67 before and after treatment and also investigation of other tumor features such as tumor grade or hormonal status.

Evaluating Ki67 as a predictive factor came from results of multiple studies which confirmed Ki67 as a prognostic and predictive breast cancer factor [9-14]. Yerushalmi et al showed positive correlation between high Ki67 and ypCR rate after NACT [9]. But Caudle et al evaluated progression during NACT and concluded that there was no linear dependency between Ki67 and ypCR since patients who progressed during NACT had higher Ki67 than those who responded with ypCR [10]. Ohno et al conducted a multicenter randomized open study assessing Ki67 as a predictive biomarker for a response in the neoadjuvant setting adding capecitabine to standard anthracycline-docetaxel based chemotherapy in early stage breast cancer. There were no significant differences in pCR, OS and DFS between docetaxel+capecitabine and docetaxel alone (p=0.748). However, they noticed that patients with mid-range Ki67 10-20% showed a trend towards pCR in docetaxel+capecitabine arm. Furthermore, multivariate logistic regression analysis showed that pretreatment Ki67 is a significant predictor of pCR in docetaxel+capecitabine neoadjuvant setting and concluded that pretreatment Ki67 value can identify patients likely to respond to this treatment. Their study was the first multicentric randomized study showing that pretreatment and posttreatment value of Ki67 can predict pCR and DFS in patients with early breast cancer treated with NACT docetaxel with or without capecitabine [12].

Several studies revealed that residual disease is a poor prognostic factor [37-42]. In triple negative breast cancer, residual disease after neoadjuvant treatment will result in 50% risk of recurrence, regardless to adjuvant treatment [34,37].

Adding capecitabine to radiotherapy in this study was based on the results of other clinical trials revealing that capecitabine can be safely applied with RT and that capecitabine improves treatment outcome in breast cancer. Masuda et al in the CREATE-X, phase III clinical trial, randomized patients with chemo-resistant disease to adjuvant capecitabine plus standard of care versus standard of care alone. Sequencing of capecitabine in CREATE-X was not uniform: some patients received capecitabine before and some after radiotherapy. But overall, CREATE-X showed that adding capecitabine in the adjuvant treatment significantly improved DFS [hazard ratio (HR) 0.58; 95% confidence interval (CI) 0.39-0.87] and overall survival (HR 0.52; 95% CI 0.30-0.90) for patients who had TNBC. Adjuvant capecitabine becomes standard of treatment for triple negative breast cancer (TNBC) [42].

Natori et al evaluated the treatment outcome of adding capecitabine to standard chemotherapy in neoadjuvant setting in a meta-analysis of controlled randomized trials and concluded that adding capecitabine improved OS and DFS in TNBC, but they also noticed that patients had increased toxicity [37]. Adding capecitabine to NACT was also investigated by O'Shaugnessy et al in a phase III study to determine whether patients with early breast cancer will benefit from adding capecitabine to standard AC protocol followed by docetaxel. The two arms consisted of AC followed by T vs. AC followed by TX, for a total 8 cycles. This randomized study did not demonstrate an improvement in DFS with AC-XT vs. AC-T after a median follow up of 5 and 7 years, but there was a better OS in the AC-XT arm. They concluded that patients with ER positive and low Ki67 cancers have very low event rate regardless of nodal status. They also noticed that Ki67 is significant and independent marker for early recurrence in TNBC. Higher Ki67 implied worse DFS in ER positive/HER2 negative cancers across treatment arms and better DFS in TNBC with higher Ki67. Eventually they raised a question if invasive lobular and mixed ductal/lobular breast cancers are as sensitive as ductal cancers are to NACT. Exploratory analysis suggested that patients with lobular/mixed breast cancer in this study benefit from adjuvant capecitabine [38].

A study of Sherry et al investigated the feasibility, safety and toxicity of combining adjuvant radiotherapy with capecitabine in chemotherapy-resistant breast cancer. They conducted a single-institutional retrospective matched cohort study from 2012-2019 and enrolled 64 patients, including 16 patients who received adjuvant Cap-RT matched 1:3 with 48 patients who received radiotherapy only. The results showed that capecitabine-based chemo-radiotherapy is safe, with toxicity similar to radiotherapy alone [34].

Woodward et al from M.D. Anderson Cancer Center conducted a single institution prospective phase II study of preoperative concomitant capecitabine-radiotherapy in advanced breast cancer to examine the response rate of gross chemo-refractory breast cancer. Patients selection was similar to our study and included inoperable disease after chemotherapy, residual nodal disease after definitive surgical resection, unresectable chest wall or nodal recurrence after prior mastectomy or oligometastatic disease. Similarly to this study, RR was evaluated using RECIST criteria, after 45Gy and capecitabine was administered in a dose of 825mg/m^2 b.i.d. From 2009-2012, 32 patients were accrued in total. Median follow-up was 12.9 months, 19 patients (73%) had partial or complete response, 14 patients (53.9%) experienced non-dermatitis toxicity related to capecitabine dose. Three out of 4 inoperable patients became operable. The study was stopped early after interim analysis suggested futility independent of response. That means that RR was significant, but treatment had no impact in OS or DFS since 9 out of 10 patients with TNBC were operable, but M1 immediately after the surgery versus 6 out of 16 patients with non-TNBC, p=0.014. Median OS and 1-year local recurrence-free survival among non-TNBC vs. TNBC was 22.8 vs. 5.1 and 63% vs. 20% (p=0.007). They concluded that capecitabine could be safely administered on radiation days and was associated with encouraging response in chemo-resistant breast cancer. However, TNBC patients had poor outcome even when response was achieved and they've suggested further investigation in non-TNBC [29].

Effectiveness of concurrent chemo-radiotherapy following NACT in LABC investigated in the study of Alvarado-Miranda et al included 112 patients treated with FAC protocol vs. AC protocol followed by concurrent chemo-radiotherapy up to total dose 60 Gy with mitomycin and 5-FU weekly vs. cisplatin and gemcitabine and surgery 6-8 weeks afterwards. Patients with ER positive status received 4 adjuvant cycles of FAC or AC. Breast tumor pCR was achieved in 42% (95% CI 33.2-50.5%), and breast plus nodal pCR was achieved in 29.5% (95% CI 21.4-37.5%). Multivariate analysis showed that ER negative status was predictive for pCR (HR= 3.8; 95% CI 1.5-9; p=0.016). No relationship between pCR and DFS was found. And only one patient had local recurrence. Until that study, loco-regional relapse was found in 30-40% of LABC despite multimodal treatment [43].

A negative study for concurrent capecitabine-radiotherapy was conducted by Liu et al from Me-

morial Sloan Kettering Cancer Center. The authors examined concurrent use of capecitabine as a radiosensitizer and its association with event-free survival (EFS) and overall survival (OS) in women with residual disease after NACT. This retrospective study included patients with breast cancer who received A/T based NACT from 2004-2016, and only 21 received concurrent Cap-RT. To assess OS they selected a clinical control cohort of 57 patients based on criteria used for Cap-RT treatment and 2:1 matched cohort matching tumor subtype, pathological stage and age. The majority of patients received Cap-RT were 50 years old and in disease stage III, hormone receptor positive, HER2 negative breast cancer and more residual disease burden after NACT. In Cap-RT group there were 9 events vs. 14 events in clinical and 10 in matched controls. Cap-RT showed a trend towards worse EFS than in clinical (HR 2.41; 95% CI 0.86-6.74, p=0.09) and matched controls (HR 2.68; 95% CI 0.91-7.90, p=0.07). Compared to clinical controls Cap-RT patients were more likely to have LVI (75 vs. 46%, p=0.03) and had larger tumors (43 vs. 23% tumor size 5 cm, p=0.08). Compared to matched controls Cap-RT also were more likely to have LVI (75 vs. 46%, p=0.04) and larger tumors (43 vs. 19% tumor size 5 cm, p= 0.05). In this study concurrent Cap-RT after NACT was associated with worse survival. It also suggested that supraclavicular and internal mammary lymph node involvement may have been more critical than the number of positive lymph nodes [32].

Brackstone et al evaluated 32 patients in a prospective phase II trial of concurrent NACT and radiotherapy in LABC from 2009-2011. Patients received NACT, 3 cycles of FEC protocol followed by docetaxel weekly for 9 weeks and had concurrent radiotherapy with docetaxel in total dose 45 Gy/25 fractions plus boost 5.4 Gy/3 fractions and surgery afterwards. Posthoc patients were matched to concurrent cohort treated with NACT, surgery and adjuvant radiotherapy. The authors concluded that neoadjuvant radiosensitizing chemotherapy with concurrent radiation in LABC significantly improved pCR (22.6 vs. 14.9%, p=0.019) but did not have significant difference in OS (HR 0.46 in favor of concurrent chemo-radiotherapy cohort (95% CI 0.16-1.36, p=0.16) at 3 years follow up. None of the cC-RT cohort with pCR had a recurrence, while 36% of patients who did not achieve pCR recurred and died of their disease within 36 months. The study was designed to accrue 52 patients, but was closed prematurely due to treatment-related deaths and high rates of radiation pneumonitis, with 32 patients accrued [30].

In conclusion, only Ki67 showed statistically significant correlation with RR, as high Ki67 predicts that there will be no response to concurrent capecitabine-radiotherapy. Other characteristics such as histological grade, estrogen or progesterone receptors, HER2 overexpression or lymphovascular or perineural invasion showed no significance.

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Conflict of interests

The authors declare no conflict of interests.

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