RESPONSE TO NEOADJUVANT THERAPY AND LONG-TERM OUTCOME IN PATIENTS WITH TRIPLE NEGATIVE BREAST CANCER

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Abstract

Background. Triple negative breast cancer (TNBC) is defined by the lack of estrogen receptors (ER), progesterone receptors (PR) and human epidermal growth factor receptor 2 (HER-2) expression. In this study we investigated response to neoadjuvant chemotherapy in TNBC patients and its impact on disease free (DFS) and overall survival (OS).

Patients and methods. We identified 134 patients with stage I-III TNBC treated at Riga East University Hospital between 2009-2012. 48 patients with TNBC received neoadjuvant chemotherapy. Correlation of clinical and pathological parameters with pathologic complete response (pCR) rate, disease-free and overall survival measurements and organ specific relapse rates were analysed.

Results. 48 patients with stage IIB-IIIC TNBC were included, 17 patients received anthracycline based, 31 patients anthracycline and taxane based neoadjuvant chemotherapy. 8 patients (16%) had pCR, 38 patients had incomplete response, 2 patients had disease progression during neoadjuvant chemotherapy. pCR correlates with primary tumor size, but not with other clinical and pathological factors. At a median follow-up of 30 months, 100% patients who reached pCR were disease-free versus 55% in those without pCR (p=0.017). Overall survival was 100% in patients who had pCR versus 50% in those without pCR (p=0.020). 2 patients are alive after disease recurrence, 20 patients died. The most common sites of disease recurrence were brain, lung and liver.

Conclusions. Patients with TNBC who have a pCR in the breast and axillary nodes have a significantly improved disease-free and overall survival rate compared with patients with residual disease after neoadjuvant chemotherapy.

Keywords: Breast cancer, triple negative, neoadjuvant chemotherapy

Introduction

Triple-negative breast cancer (TNBC) is characterized by the lack of estrogen receptor (ER), progesterone receptor (PR) expression and human epidermal growth factor receptor 2 (HER-2) expression. These cancers occur in approximately 11-20% of all patients with breast cancer (Bauer, 2007; Perou, 2000), and are associated with unfavourable prognosis (Carey, 2006; Carey, 2007). Patients with TNBC do not benefit from molecular targeted treatments like endocrine therapy or trastuzumab, because they lack specific targets for these drugs.

Neoadjuvant chemotherapy that was initially used for locally advanced breast cancer has become more common for patients with operable breast cancer. As a result more patients can undergo breast conserving surgery. In addition, neoadjuvant chemotherapy allows observing individual response to chemotherapy. It has been proved that long term outcome correlates with pathologic tumor response rates (von Minckwitz, 2012).

In this study we have analyzed 48 TNBC patients who receive neoadjuvant anthracycline or anthracycline and taxane based chemotherapy to determine the incidence of pathologic complete response (pCR), the clinical and pathologic factors associated with it, and the clinical course of patients according to the pathologic tumor response.

Patients and methods:

We identified 134 patients with stage I-III TNBC treated at Riga East University Hospital Latvian Oncology Centre (Riga, Latvia) between 2009-2012. 48 patients with TNBC who received neoadjuvant chemotherapy were included in the study. Inclusion of the patients was based on the following criteria: 1) receipt of at least 4 cycles of neoadjuvant chemotherapy; 2) availability of complete information on clinical TNM (cTNM) and pathologic stage (pTNM) in case of surgery; 3) response to treatment; and 4) no expression of ER, PR and HER-2. Patients with primary inflammatory carcinoma were not included in the analysis. Staging was performed according to American Joint Committee on Cancer guidelines (AJCC, 2010).

Pathology Assessment

Pathologic diagnosis, ER, PR and HER-2 status were determined by core biopsy prior to systemic therapy. Core biopsy samples were analyzed at the Riga East University Hospital Pathology Center. Hematoxylin and eosin stained slides were assesed for histologic type of the tumor and histologic grade. Histologic grade was assessed using the Elston-Ellis modification of the Scarff-Bloom-Richardson grading system (Elston&Ellis, 1991). ER and PR status were determined by immunohistochemistry (IHC, DAKO LSAB+ methodology, monoclonal antibodies anti-ER (clone ER-1D5) and anti-PR

(clone PR-636)), tumours with less than 1% of stained cells were considered receptor negative (Hammond, 2010). HER-2 status was assessed by IHC (HercepTest, DakoCytomation, Glostrup, Denmark) according to the manufacturer's protocol. A negative HER2 test is defined as an IHC result of 0 or 1+ (Wolff , 2007).

Pathologic response was determined by microscopic examination of the excised tumour and lymph nodes after neoadjuvant chemotherapy. Pathologic complete response (pCR) was defined as no residual invasive and in situ cancer in breast and lymph nodes (von Minckwitz, 2012).

Statistical analyses

χ^2 test was used to determine factors predictive of pCR. Parameters analyzed were age at the time of diagnosis, tumour histology, menopausal status, histologic grade, stage, clinical tumour (T) and nodal (N) score, type of neoadjuvant chemotherapy regimen, number of chemotherapy cycles, and type of surgery.

Overall survival (OS) was calculated from the date of diagnosis to the date of last follow-up or death, disease-free survival (DFS) was calculated from the date of definitive surgery to the date of last follow-up or disease relapse using the method of Kaplan and Meier. The log-rank statistic was used for univariate comparisons of survival end points.

Statistical analyses were performed using IBM SPSS Statistics for Windows 22.0.

Results:

48 patients with TNBC, who received neoadjuvant chemotherapy at our institution were included in the analysis. Patient and tumor characteristics are shown in Table 1. The mean age of patients was 49 years (range 29-67years). All tumors were ductal carcinomas.

17 patients (35%) received anthracycline based chemotherapy (FAC- doxorubicin 50mg/m², cyclophosphamide 500mg/m², fluorouracil 500mg/m² every 3 weeks), 31 patients (65%) received anthracycline and taxane based chemotherapy (AP- doxorubicin 60mg/m², paclitaxel 175mg/m² every 3 weeks). Median number of neoadjuvant chemotherapy cycles was 5 (range 4-7), patients received adjuvant chemotherapy if all planned therapy was not completed before surgery. Disease progression was observed in 2 patients; 46 patients underwent surgery, 6 of them had breast conserving surgery, 40 had mastectomy.

8 patients (17%) experienced pCR compared with 38 patients (83%) with residual disease (RD). Correlation between clinical and pathological factors and pCR is shown in Table 2. Correlation was observed between

clinical primary tumor (cT) size and pCR; there was no correlation with age, stage, clinical nodal status and grade.

Table 1. Patient Characteristics

Characteristic	No. of patients	%
Age range, years		
20-29	1	2
30-39	8	17
40-49	18	37
50-59	10	21
60-69	11	23
Mean	49.4	
Prechemotherapy T stage:		
T1	4	8
T2	20	42
T3	13	27
T4	11	23
Prechemotherapy N stage:		
N0	3	6
N1	16	33
N2	10	21
N3	19	40
Nuclear grade		
2	12	25
3	34	71
Unknown	2	4
Neoadjuvant chemotherapy regimen		
Anthracycline based	17	35
Anthracycline taxane based	31	65
Surgical therapy		
Breast-conserving therapy	6	13
Mastectomy	40	87
Adjuvant radiation		
No	13	28
Yes	33	72

Of 45 patients with node-positive disease before starting treatment, 23 (51%) were node negative at surgery. There was a trend for higher pCR rate for anthracycline and taxane based chemotherapy (19 versus 12% respectively) that did not reach statistical significance, probably because of the small sample size.

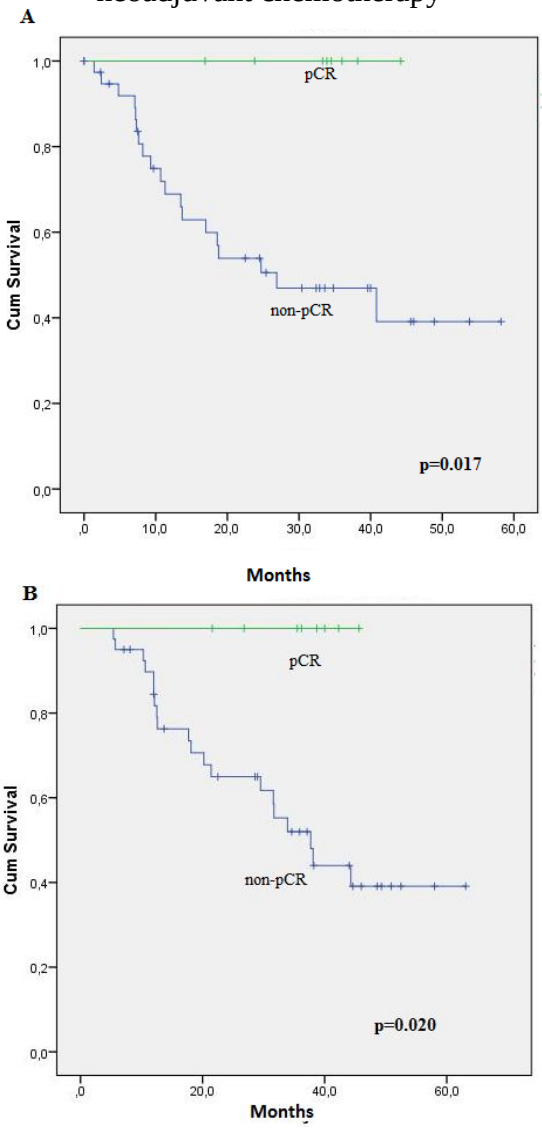
Table 2. Correlation between clinical and pathologic factors and pCR

Characteristic	pCR	Non-pCR	P value
Prechemotherapy T stage			**0.042**
T1	1	3	
T2	5	15	
T3	2	11	
T4	0	11	
Prechemotherapy N stage			0.459
N0	0	3	
N1	4	12	
N2	2	8	
N3	2	17	
Nuclear grade			0.874
2	2	10	
3	5	29	
Unknown	1	1	
Neoadjuvant chemotherapy regimen			0.584
Anthracycline based			
Anthracycline taxane based	2	15	
	6	25	
Number of neoadjuvant chemotherapy cycles			0.76
4-5	7	19	
6-7	1	19	

At a median follow-up of 30 months (range 5.4 to 60.1 months), DFS was 100% in patients who reached pCR versus 55% in those without pCR (p=0.017) (Figure 1A).

3 patients had pCR in breast, but residual disease in lymph nodes, 1of them had disease relapse. 2 patients had disease progression during neoadjuvant chemotherapy. 3 patients had local recurrence, 17 at distant sites (brain, n=6; liver, n=4; lung, n=5; bone, n=2). At a median follow-up of 30 months, 6 patients (35%) in the FAC group and 12 patients (38%) in the AP group had experienced disease relapse. Mean time to relapse was 32.1 months (95%CI 26.2 to 44.6 months) in the FAC group versus 36.4 months (95%CI 33.3 to 49.6months) in the AP group, difference is not significant (p=0.686).

Figure 1. Relationship of pathologic brimary breast tumor and axillary lymph node response to disease-free survival (A) and overall survival (B) after neoadjuvant chemotherapy



Overall survival was 100% in patients who had pCR versus 50% in those without pCR (p=0.020) (Figure 1B). 2 patients are alive after disease recurrence, 20 patients died, 7 patients (41%) in the FAC group and 13 patients (42%) in the AP group, 2 patients died without documented progression. Mean survival time was 35.4 months (95%CI 26.2 to 44.6 months) versus 41.4 months (95%CI 33.3 to 49.6 months) for FAC and AP groups respectively (p=0.551).

Discussion

TNBC is an agressive subtype of breast cancer that lacks a therapeutic target, making chemotherapy the only systemic modality used in the treatment of this disease. The use of neoadjuvant chemotherapy has increased in recent years. This strategy offers a number of advantages, one of the most important is in vivo assessment of tumor response.

Response of the tumor to neoadjuvant chemotherapy provides important prognostic information, prognosis of patients with a pCR in both the breast and axillary lymph nodes is much better than of those with residual disease (Liedtke, 2008; von Minckwitz, 2012). As shown in previous studies, patients with TNBC has higher rates of pCR following neoadjuvant chemotherapy than non-TNBC (Carey, 2007; Rastogi, 2008). Carey et al. found that TNBC and HER-2 positive breast cancers yield much higher pCR rates (27% and 36%, respectively) compared with the luminal subtypes, which had a pCR rate of 7% (p=0.01). A pCR rate of 17% was observed in our study, where majority of patients were diagnosed with locally advanced TNBC.

As pCR correlates with prognosis several strategies were evaluated to increase pCR rate. An important advance was the addition of taxanes to anthracycline-based chemotherapy. An increase in clinical responses and pCR rates has been reported in several trials with the addition of taxanes (Martin, 2011; Dieras, 2004; Bear, 2003; Vinholes, 2001). A higher pCR rate was observed with anthracycline and taxane versus anthracycline-based regimen (19% versus 12% respectively) in our study. Chemotherapy regimen without taxanes is not a current standard for neoadjuvant therapy for TNBC patients. FAC regimen was given because taxanes was not reimbursed for neoadjuvant treatment of breast cancer patients in 2009 and early 2010 in Latvia.

Interim response guided neoadjuvant strategy was tested in Gepar Trio trial (von Minckwitz, 2008). Changing neoadjuvant chemotherapy on the basis of individual patient responses to the first two cycles improved DFS and OS for patients with luminal-type, but not for triple negative or HER-2 positive breast cancer patients.

Some novel neoadjuvant treatment regimens in TNBC incorporate platinum agents, based on the hypothesis of greater susceptibility of triple negative and BRCA1/2-mutant tumors to agents that damage DNA. Adding carboplatin to a regimen of neoadjuvant chemotherapy resulted in more than 20% increase in pCR in women with TNBC (von Minckwitz, 2013). Another study confirmed that adding carboplatin or carboplatin and bevacizumab to standard neoadjuvant chemotherapy regimen significantly increased pCR rate (Sikov, 2013). In the Gepar-Quinto trial (Gerber et al., 2011), the addition of bevacizumab to standard neoadjuvant chemotherapy with a

sequential anthracycline and taxane regimen produced a significantly higher rate of pCR in the TNBC patients, no data on long-term follow-up are available yet. In a very small study involving 25 BRCA-mutation carriers 80% of whom had TNBC, 4 cycles of single agent cisplatin produced a pCR rate of 72% (Gronwald, 2009). Two additional studies evaluated the same regimen in TNBC patients and reported pCR rates of 21% (Silver, 2010) and 15% (Ryan, 2009). There is a lack of randomized trial data.

Neoadjuvant regimens without anthracyclines were evaluated in patients with TNBC. Carboplatin plus weekly paclitaxel yielded pCR rate of 67% in patient subset with TNBC (Sikov, 2009). Another study looked at a platinum-containing regimen without anthracycline or taxane in triple negative and BRCA-mutated patients (Telli, 2013). Carboplatin, gemcitabine and iniparib was given for 6 cycles, pCR rate of 36% was achieved in the overall population, but triple negative patients who also had BRCA-mutations had pCR rate of 56%.

TNBC may have higher metastatic potential, with increased probability for visceral and central nervous system metastases versus bone metastases (Liedtke, 2008; Lin, 2008). We observed similar results, of 17 patients with distant relapse, 6 had brain metastases, 5 lung, 4 liver and only 2 patients had bone metastases.

Study by Liedtke et al. showed that despite significantly higher pCR rates (22% versus 11%, p=0.034), patients with TNBC had decreased 3-year progression-free ($p<0.0001$) and 3-year overall survival rates ($p<0.0001$) compared with non-TNBC (Liedtke, 2008). If pCR was achieved, patients had similar overall survival regardless of receptor status. Our results show that TNBC patients with pCR have excellent disease free and overall survival. However in the setting of residual disease after neoadjuvant chemotherapy, the overall prognosis of the disease is poor, half of the patients had disease recurrence and died from breast cancer.

A pCR can be considered as a reliable surrogate marker for disease free and overall survival in patients with TNBC (von Minckwitz, 2011). Patients can be relieved from unfavourable prognosis in case a pCR is diagnosed, patients without pCR are at high need for new treatment options.

However it is not clear how to predict response to neoadjuvant treatment and which patients with TNBC are more likely to experience pCR.

It is proved that TNBC is not a homogeneous group. Gene expression analyses have identified molecular subtypes of TNBC (Lehmann, 2011; Rouzier, 2005). Lehmann and Bauer et al. classified TNBC into 7 subtypes by gene expression microarray. Heterogeneous response of TNBC to neoadjuvant chemotherapy suggests that different subtypes of TNBC may be associated with different pCR rates. In addition, some molecular drivers in TNBC subtypes that can be therapeutically targeted are identified. Gene

expression microarray was performed in 130 TNBC patients who received standard neoadjuvant chemotherapy (Masuda, 2013). Molecular subtypes of TNBC have different response rates to neoadjuvant chemotherapy, 28% pCR rate for all patients (range 0-52% for particular subgroups). Lehmann and Bauer's classification has a strong impact because it classifies all TNBC population into 7 homogeneous subtypes, and subtype classification had a predictive preclinical effect on the outcome of therapy that incorporate specific targeted treatments, as an androgen receptor antagonist and PI3K/mTOR inhibitor. Study by Masuda et al. showed that 7 TNBC subtypes predicted the rate of pCR to current standard chemotherapy regimens.

This study has a number of limitations. It is retrospective analysis, patient sample is small and heterogenous regarding initial tumor size, nodal status, stage and number of neoadjuvant chemotherapy cycles received. Nonetheless, it clearly showed that pCR correlates with prognosis in patients with TNBC.

Conclusion

Patients with TNBC who have a pCR in the breast and axillary nodes have a significantly improved disease-free and overall survival rate compared with patients with residual disease after neoadjuvant chemotherapy.

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