RESEARCH ARTICLE

Molecular Types and Neoadjuvant Chemotherapy in Patients with Breast Cancer- While Molecular Shifting is More Common in Luminal a Tumors, The Pathologic Complete Response is Most Frequently Observed in Her-2 Like Tumors

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Abstract

Background: Pathologic complete response (pCR) is one of the most important target end-points of neoadjuvant chemotherapy (NACT) in patients with breast cancer (BC). In present study, we aimed to investigate the relationship between molecular subtypes and NACT in patients with BC. Materials and Methods: Using the Akdeniz University database, 106 patients who received NACT for operable breast cancer were retrospectively identified. Prognostic factors before and after NACT were assessed. According to the molecular subtypes, molecular shifting after NACT and tumoral and nodal response to NACT were analyzed. Results: The distribution of subtypes was: Luminal A, 28.3% (n=30); Luminal B, 31.1% (n=33); HER2-like, 24.5% (n=26); and basal like/ triple negative (BL/TN), 16.0% (n=17). According to molecular subtypes, pCR rates in both breast and axillary were 0%, 21.4%, 36.4% and 27.3% for luminal A, luminal B, HER2-like and BL/TN, respectively (p=0.018). Molecular subtype shifting was mostly seen in luminal A type (28.6%) after the NACT. The pCR rate in breast and axillary was significantly higher in patients with HER2-like type BC. Conclusions: In patients with HER-2 like type BC, NACT may be offered in early stages. Additionally, due to molecular shifting, adjuvant treatment schedule should be reviewed again, especially in the luminal A group.

Keywords: Breast cancer - molecular subtypes - neoadjuvant chemotherapy - response - molecular shifting

Asian Pac J Cancer Prev, **15 (21)**, 9379-9383

Introduction

Breast cancer is the most common cancer in women in the world. The incidence of BC is increasing in the both western and Asian world of which the incidence rates are below 40 per 100,000 (Ferlay et al., 2010; Keramatinia et al., 2014)

Locally advanced breast cancer makes up to 50% of the newly diagnosed breast cancers (Valero et al., 1996). NACT has become commonly used treatment in order to increase the chance of breast-conserving surgery in patients with large operable BC and render inoperable tumors resectable by downstaging of the tumor. Efficacy or unnecessary toxicity of chemotherapy can be evaluated by monitoring the dimensional changes of the tumor allowing the physician to continue the therapy. Also, the use of NACT may succeed in a pathological complete response, which correlates with prolonged periods of remission (Valero et al., 2002). Generally used NACT regimens are antracycline and taxane based therapies.

Receptor status was considered by reviewing each individual receptor: ER (Estrogen Receptor), PR (Progesterone Receptor), HER2 (HER2/neu Receptor); but newer approaches look at these together, along with the tumor grade, to categorize BC into several molecular subclasses (Prat and Perou, 2011) that have different prognoses (Genestie et al., 1998) and may have different responses to specific therapies. Researchers are looking for how molecular subtypes of BC may be useful in planning treatment and developing new therapies. Most studies divide breast cancer into four major molecular subtypes: luminal A (ER+ and/or PR+, HER2-,), luminal B (ER+ and/or PR+, HER2+), HER2-like (ER-, PR-, HER2+) and basal like/triple negative (ER-, PR-, HER2-). The prevalences of molecular subtypes were aproximately as follows: luminal A 40%, luminal B 20%, basal like/ triple negative 15-20% , HER2-like 10-15% (Potemski et al., 2005; Fan et al., 2006; Hu et al., 2006; Schnitt, 2010; Voduc et al., 2010; Carey, 2013). Luminal A and B groups are the most common molecular sub-groups.

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Luminal A has the best prognosis, whereas the basal-like/triple negative (BL/TN) has the worst prognosis. Although tumors with HER2-like have bad prognosis, HER2 targeted therapies have changed the outcome (Carey et al., 2006; Fan et al., 2006; Hu et al., 2006; Loi et al., 2007; Voduc et al., 2010; Carey, 2013).

In present study, we aimed to investigate the relationship between molecular subtypes and NACT in patients with BC.

Materials and Methods

Between 2002 and 2013, 106 BC patients who received NACT for operable BC were retrospectively collected from Akdeniz University database. The patients were divided into four groups; Luminal A (ER+ and/or PR+, HER2-), luminal B (ER+ and/or PR+,HER2+), HER2-like (ER-, PR-, HER2+) and BL/TN (ER-, PR-, HER2-). The age, menopausal status, performance status, histological types, tumor (T) and nodal (N) clinical stage, hormone receptor status, Ki-67 status, grade, lymphatic invasion (LI), vascular invasion (VI), perineural invasion (PNI), NACT agents, trastuzumab as part of NACT (for the patients in whom HER2 positive) and response to NACT were imported into Statistical Package for the Social Sciences version 16.0 (SPSS 16.0). Definition of pCR is the absence of invasive cancer and in-situ cancer in the breast and axillary nodes. Staging was done according to The American Joint Committee on Cancer (AJCC) Staging Manual (7th edition) (Edge et al., 2010). ER and PR status were determined by immunhistochemistry and tumors with >10% positively stained tumor cells were classified as positive for ER and PR. HER2 status was also determined by immunhistochemistry or by fluorescence in situ hybridization (FISH) analysis. HER2-positive tumors were defined as 3+ on immunhistochemistry or as

Table 1. Descriptive Statistics fort he 106 Patients According to Molecular Subtypes

		Luminal A (n:30)	Luminal B (n:33)	HER-2 Like (n:26)	BL/TN (n:17)	P value
Age(mean)		49.2±9.6	49.6±11.5	46.4±11.2	46.5±13.9	0.618
Menopausal status	Premenopausal	56.70%	60.60%	69.20%	58.80%	0.798
	Postmenopausal	43.30%	39.40%	30.80%	41.20%	
Performance Status	PS0	70%	71.90%	84.60%	82.40%	
	PS1	26.70%	28.10%	15.40%	11.80%	0.473
	PS2	3.30%	0%	0%	0	
Hystologic Type	Invazıv Ductal	76.70%	87.80%	76.90%	88.20%	
	Invazıv Lobular	3.30%	0%	0%	5.90%	
	Mıxt	6.70%	6.10%	0%	0	0.507
	Inflammatuar	3.30%	0%	7.70%	0	
	Other	10%	6.10%	15.40%	5.90%	
Clinic Stage	Stage 1	10%	0%	0%	0%	
	Stage 2	33.30%	24.20%	34.60%	11.80%	
	Stage 3	56.70%	75.80%	65.40%	88.20%	
Clinic T stage	T0	0	0	0%	11.80%	
	T1	6.70%	0	3.80%	5.90%	
	T2	46.60%	51.50%	57.70%	47.10%	0.066
	T3	26.70%	18.20%	7.70%	29.40%	
	T4	20%	30.30%	30.80%	5.90%	
Clinic N stage	N0	26.70%	15.20%	15.40%	11.80%	
	N1	36.70%	42.40%	46.20%	29.40%	0.612
	N2	30%	33.30%	30.80%	58.80%	
	N3	6.70%	9.10%	7.70%	0	
Grade	Grade 1	7.40%	3%			
	Grade 2	92.60%	36.40%	56.50%	50%	<0.001
	Grade 3	-	60.60%	43.50%	50%	
Ki67	Low	27.30%	0	0	0	
	Modarate	27.30%	0	0	0	0.100
	High	45.40%	100%	100%	100%	
Lymphatic Invasion	Yes	46.40%	33.30%	39.10%	14.30%	0.223
	No	53.60%	66.70%	60.90%	85.70%	
Vascular Invasion	Yes	25%	23.30%	26.10%	15.40%	0.897
	No	75%	76.70%	73.90%	84.60%	
Perineural İnvasion	Yes	10.70%	10%	4.30%	7.70%	0.853
	No	89.30%	90%	95.70%	92.30%	
NACT	Antracycline+Taxan	60%	72.70%	88.50%	82.40%	
	Antracycline based	30%	12.10%	7.70%	17.60%	0.047
	Taxan based	3.30%	15.20%	3.80%	0	
	Hormonal	6.70%	0	0	0	
NA trastuzumab	Yes	-	39.40%	73.10%	-	
	No	-	60.60%	26.90%	-	

positive by FISH for immunhistochemically 2+ staining. The histological grade and nuclear grade were identified according to the modified Bloom-Richardson system. LI, VI and PNI were investigated as yes/no form. To determine the features of patients with BC, frequency analysis, two independent samples t test, one way ANOVA and chi-square tests were performed and p<0.05 was considered statistically significant.

Results

The distribution of subtypes was: Luminal A: 28.30% (n=30); Luminal B: 31.13% (n=33); HER2-like: 24.52% (n=26); and BL/TN: 16.03% (n=17).The features of groups were depicted in Table 1. The mean age at diagnosis were as follows: 49.2±9.6, 49.6±11.5, 46.4±11.2 and 46.5±13.9 years for luminal A, luminal B, HER2-like and BL/TN subtypes, respectively (p=0.618). Most of the patients were premenopausal among groups. Most of the patients were performance status 0 and 1. Invasive ductal carcinoma type was the mostly seen histopathological type in all subtypes. There were not any significant differences regarding menopausal status, performance status, histopathological type, clinical stage, clinical T stage, clinical N stage, Ki67, LI, VI and PNI (p=0.798, p=0.473, p=0.507, p=0.064, p=0.066, p=0.612, p=0.100, p=0.223, p=0.897, p=0.853 respectively). There was a significant difference between the molecular subtypes for grade (p<0.001). BL/TN group mostly had N2 disease (58.8%), while other groups had generally N1 disease. The ratio of T4 stage was higher in luminal B and HER2-like tumors than luminal A and BL/TN subtypes (20%, 30.3%, 30.8%, 5.9% respectively). Concurrent or sequential Antracyclin and taxane based chemotherapy were the most used NACT regimens in all subtypes. Antracycline alone was the second most preferred regimen. 60.6% of the patients with luminal B and 26.9% of HER2-like BC had not taken trastuzumab as NACT. Molecular shifting rate was higher in luminal A type than the others after NACT; 28.6% of Luminal A, 14.3% of Luminal B, none of HER2-like, 20% of BL/TN have shifted to other subtypes (p<0.001) (Table 2). After NACT, tumoral (T) and nodal (N) downstaging ratios were shown in Figure 1 and 2.

Pathological CR in breast after NACT were as follows: 3.8% for luminal A, 35.5% for luminal B, 47.8% for HER2-like type and 61.5% for BL/TN type (p=0.001). pCR rates in axillary after NACT were as follows: 15.8% for luminal A, 38.5% for luminal B, 57.9% for HER2-like type and 693.6% for BL/TN type (p=0.022). pCR in breast and axillary was significantly higher among patients with HER2-like type than the others (p=0.018). pCR rates were shown in Table 3.

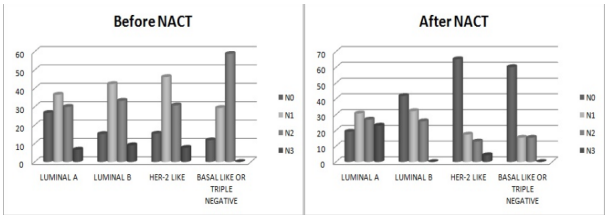

Figure 1. Tumor (T) Staging According to Luminal Types Before and After NACT (T Staging was Defined as Clinical and Pathologic Before and After NACT, Respectievely)

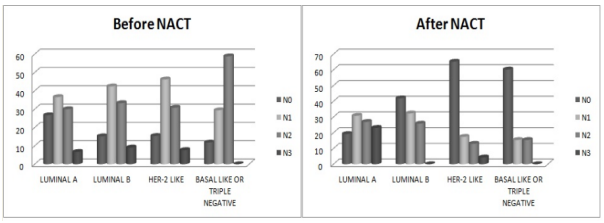

Figure 2. Node (N) Staging According to Luminal Types Before and After NACT (N Staging was Defined as Clinical and Pathologic Before and After NACT, Respectievely)

Discussion

More than 70% of patients achieve objective response (including pathological complete remission in 10-25% of cases) and many patients experience down-staging via NACT (Ferley et al., 2010).

It had been shown that luminal A group had the best disease free survival (DFS) rate while the worst DFS rate was among the HER2-like group (Najafi, 2013). It had been reported that improved survival outcomes were observed in patients with pCR compared with those with residual tumor. The association between pCR and long-term survival was weakest for luminal groups

Table 2. Luminal Types Before and After Neodjuvant Chemotherapy (Patients with pCR in Breast and Axillary or Unevaluable After NACT were Excluded) (p<0.001)

Post-NACT	Luminal A (n:14)	Luminal B (n:7)	HER-2 Like (n:5)	BL/TN (n:5)
Luminal A	71.40%	14.30%	0%	0
Luminal B	21.40%	85.70%	0%	20%
HER 2 Like	0	0	100%	0%
Triple Negative or Basal Like	7.20%	0	0%	80%

Table 3. pCR Rates in Breast, Axillary and in Both of them According to Molecular Types

	Luminal A	Luminal B	HER 2 Like	BL/TN	P value
pCR (no invasive tumor in breast)(for T>0 tumors at diagnosis)	3.80%	35.50%	47.80%	61.50%	0.001
pCR (no invasive tumor in axillary)(For N>0 tumors ar diagnosis)	15.80%	38.50%	57.90%	63.60%	0.022
pCR (no invasive tumor in breast and axillary)(For T>0 and N>0 tumors at diagnosis)	0%	21.40%	36.40%	27.30%	0.018

and low grade tumors. On the other hand it was shown that association between pCR and long-term outcome was strongest in patients with aggressive breast cancer subtypes. In some studies, it was reported that pCR was not a prognostic factor in luminal A or luminal B and HER2-positive breast cancer (Wolmark et al., 2001; von Minckwitz et al., 2011; von Minckwitz et al., 2012; Cortazar et al., 2014).

Some groups have reported that basal-like and HER2-like tumors showed the higher pathological complete response rate than luminal groups (Parker et al., 2009; Lv et al., 2011; Khokher et al., 2013). In the present study; relatively higher pCR rates were achieved locally (axillary or breast) among luminal B, HER2-like and TN/BL subtypes than that in previous studies. But there were relatively lower response rates in luminal A subtype than previous studies. There was no pCR in luminal A subtype for both breast and axillary region.

We found pCR rate was significantly (p=0.018) higher among patients with HER2-like (36.4%) and BL/TN (27.3%) than luminal tumors (0.0% and 21.4% for luminal A and B respectively) in both breast and axillary region. Similarly, it was reported that the pCR to NACT was significantly better among basal-like (27%) and HER2-like tumors versus luminal tumors (7%) (Carey et al., 2007).

In some studies with all stages, high grade was associated with non-luminal subtypes (Kadivar et al., 2012; Engstrøm et al., 2013). This observation is not consistent with present study. In this study luminal B group had the higher grade than the other subtypes. In the other studies the luminal type BC were well differentiated, low TNM profile tumors with a low Ki-67 proliferation index (Irigoyen et al., 2011; Widodo et al., 2014). On the contrary, the basal type and HER2 carcinomas presented higher TNM profile, poorly differentiated tumors with high Ki-67 proliferation indexes (Irigoyen et al., 2011; Chuthapisith et al., 2012). Similarly, in this study earlier stages (stage I-II), lower Ki-67 indexes, lesser axillary nodal involvement associated with luminal A subtype whereas non-luminal subtypes showed more aggressive tumor characteristics especially high grade, Ki-67, LVI, PNI, clinical stage (stage III) tumors.

Breast tumor response to NACT varied among the different molecular subtypes. Ruano, et al reported this response was lowest in luminal A and highest in non-luminal HER2+ group. Also HER2+ and triple-negative were were shown to be the groups with the best axillary histological response (Ruano et al., 2014). Present study was in the same line with literature as follows: primary tumor response rates were 3.8% in luminal A, 47.8% in HER2-like type and 61.5% in BL/TN type. Axillary response rates were 15.8% in luminal A, 57.9% in HER2-like, 63.6% in BL/TN type. This study revealed that breast cancer subtypes are in relation with the response to NACT (table 5). In the literature it was reported that after the NACT more than 30% reduction in primary tumor size in 74.2% of patients were observed (Egwuonwu et al., 2013). In present study the most tumoral (T) downstaging was in BL/TN subtype and nodal downstaging was higher in HER2-like and BL/TN subtpypes than luminal subtypes (Figure 1-2).

This study demonstrated that the pCR rate in breast and axillary were the significantly higher in patients with HER2-like type BC. It was known that patients with pCR had excellent prognosis. According to our study results, we speculate that NACT may be offered in early stage HER-2 like especially >2cm tumors as in Techno, NeoALLTo, NeoSphere and GeparQuinto studies (Untch et al., 2011; Baselga et al., 2012; Gianni et al., 2012; Untch et al., 2012). Molecular shifting after NACT was more frequently observed in Luminal A type tumors. Particularly in this group the treatment schedule should be rewieved again after NACT.

References

Baselga J, Bradbury I, Eidtmann H, et al (2012). NeoALTTO Study Team. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. *Lancet*, **379**, 633-40.

Carey LA, Perou CM, Livasy CA, et al (2006). Race, breast cancer subtypes, and survival in the carolina breast cancer study. *JAMA*, **295**, 2492-502.

Carey LA, Dees EC, Sawyer L, et al (2007). The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. *Clin Cancer Res*, **13**, 2329-34.

Carey LA (2013). Molecular intrinsic subtypes of breast cancer. In: UpToDate.

Chuthapisith S, Permsapaya W, Warnnissorn M, et al (2012). Breast cancer subtypes identified by the ER, PR and HER-2 status in Thai women. *Asian Pac J Cancer Prev*, **13**, 459-62.

Cortazar P, Zhang L, Untch M, et al (2014). Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. *Lancet*, **384**, 164-72.

Edge S, Byrd DR, Compton CC, et al (2010). AJCC cancer staging manual (7th ed). New York, NY: Springer.

Egwuonwu OA, Anyanwu SN, Nwofor AM (2013). Efficacy of neoadjuvant chemotherapy in down staging locally advanced pre-menopausal breast cancer in Eastern Nigeria: is four courses adequate? *J Cancer Res Ther*, **9**, 638-43.

Engstrøm MJ, Opdahl S, Hagen AI, et al Molecular subtypes, histopathological grade and survival in a historic cohort of breast cancer patients. *Breast Cancer Res Treat*, **140**, 463-73.

Fan C, Oh DS, Wessels L, et al (2006). Concordance among gene-expression-based predictors for breast cancer. *N Engl J Med*, **355**, 560-9

Ferlay J, Shin HR, Bray F etal. (2008). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*, **127**, 2893-917.

Genestie C, Zafrani B, Asselain B, et al (1998). Comparison of the prognostic value of Scarff-Bloom-Richardson and Nottingham histological grades in series of 825 cases of breast cancer:major importance of the mitotic count as a component of both grading systems. *Anticancer Res*, **18**, 571-6.

Gianni L, Pienkowski T, Im YH, et al (2012). Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. *Lancet Oncol*, **13**, 25-32.

Hu Z, Fan C, Oh DS, et al (2006). The molecular portraits of breast tumors are conserved across microarray platforms. *BMC Genomics*, **7**, 96.

Irigoyen MA, García FV, Iturriagagoitia AC, et al (2011). Molecular subtypes of breast cancer: prognostic implications and clinical and immunohistochemical characteristics. *An Sist Sanit Navar,* **34**, 219-33.

Kadivar M, Mafi N, Joulaee A, Shamshiri A, Hosseini N (2012). Breast cancer molecular subtypes and associations with clinicopathological characteristics in Iranian women, 2002-2011. *Asian Pac J Cancer Prev*, **13**, 1881-6.

Keramatinia A, Mousavi-Jarrahi SH, Hiteh M, Mosavi-Jarrahi A (2014). Trends in incidence of breast cancer among women under 40 in Asia. *Asian Pac J Cancer Prev*, **15**, 1387-90.

Khokher S, Qureshi MU, Mahmood S, Nagi AH (2013). Association of immunohistochemically defined molecular subtypes with clinical response to presurgical chemotherapy in patients with advanced breast cancer. *Asian Pac J Cancer Prev*, **14**, 3223-8.

Loi S, Haibe-Kains B, Desmedt C, et al (2007). Definition of clinically distinct molecular subtypes in estrogen receptor-positive breast carcinomas through genomic grade. *J Clin Oncol*, **25**, 1239-46.

Lv M, Li B, Li Y, et al (2011). Predictive role of molecular subtypes in response to neoadjuvant chemotherapy in breast cancer patients in Northeast China. *Asian Pac J Cancer Prev*, **12**, 2411-7.

Najafi B, Anvari S, Roshan ZA (2013). Disease free survival among molecular subtypes of early stage breast cancer between 2001 and 2010 in Iran. *Asian Pac J Cancer Prev*, **14**, 5811-6.

Parker JS, Mullins M, Cheang MC, et al (2009). Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol*, **27**, 1160-7.

Potemski P, Kusinska R, Watala C, et al (2005). Prognostic relevance of basal cytokeratin expression in operable breast cancer. *Oncology*, **69**, 478-85,

Prat A, Perou CM. (2011) Deconstructing the molecular portraits of breast cancer. *Mol Oncol*, **5**, 5-23.

Ruano R, Ramos M, García-Talavera JR, et al (2014). Sentinel node biopsy after neoadjuvant chemotherapy in breast cancer. Its relation with molecular subtypes. [Article in Spanish]. Rev Esp Med Nucl Imagen Mol. doi: 10.1016/j.remn. 2014.04.003. [Epub ahead of print]

Schnitt SJ (2010). Classification and prognosis of invasive breast cancer: from morphology to molecular taxonomy. *Mod Pathol*, **23**, 60-4.

Untch M, Fasching PA, Konecny GE, et al (2011). Pathologic complete response after neoadjuvant chemotherapy plus trastuzumab predicts favorable survival in human epidermal growth factor receptor 2-overexpressing breast cancer: results from the TECHNO trial of the AGO and GBG study groups. *J Clin Oncol*, **29**, 3351-7.

Untch M, Loibl S, Bischoff J, et al (2012). German Breast Group (GBG); Arbeitsgemeinschaft Gynäkologische Onkologie-Breast (AGO-B) Study Group. Lapatinib versus trastuzumab in combination with neoadjuvant anthracycline-taxane-based chemotherapy (GeparQuinto, GBG 44): a randomised phase 3 trial. *Lancet Oncol*, **13**, 135-44.

Valero V, Buzdar AU, Hortobagyi GN. (1996) Locally advanced breast cancer. *Oncologist*, **1**, 8-17.

Valero V, Buzdar AU, McNeese M, Singletary E, Hortobagyi GN (2003) Primary chemotherapy in the treatment of breast cancer: the University of Texas M. D. AndersonCancer Center experience. *Clin Breast Cancer*, **2**, 63-8.

Voduc KD, Cheang MC, Tyldesley Set al. (2010). Breast cancer subtypes and the risk of local and regional relapse. *J Clin Oncol*, **28**, 1684-91

von Minckwitz G, Untch M, Nuesch E, et al (2011). Impact of treatment characteristics on response of different breast cancer phenotypes: pooled analysis of the German neo-adjuvant chemotherapy trials. *Breast Cancer Res Treat*, **125**, 145-56.

von Minckwitz G, Untch M, Blohmer JU, et al (2012). Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. *J Clin Oncol*, **30**, 1796-804.

Widodo I, Dwianingsih EK, Triningsih E, Utoro T, Soeripto (2014). Clinicopathological features of Indonesian breast cancers with different molecular subtypes. *Asian Pac J Cancer Prev*, **15**, 6109-13.

Wolmark N, Wang J, Mamounas E, Bryant J, Fisher B (2001). Preoperative chemotherapy in patients with operable breast cancer: nine-year results from National Surgical Adjuvant Breast and Bowel Project B-18. *J Natl Cancer Inst Monogr*, **30**, 96-102.