RESEARCH ARTICLE

Association of Immunohistochemically Defined Molecular Subtypes with Clinical Response to Presurgical Chemotherapy in Patients with Advanced Breast Cancer

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Abstract

Gene expression profiling (GEP) has identified several molecular subtypes of breast cancer, with different clinico-pathologic features and exhibiting different responses to chemotherapy. However, GEP is expensive and not available in the developing countries where the majority of patients present at advanced stage. The St Gallen Consensus in 2011 proposed use of a simplified, four immunohistochemical (IHC) biomarker panel (ER, PR, HER2, Ki67/Tumor Grade) for molecular classification. The present study was conducted in 75 newly diagnosed patients of breast cancer with large (>5cm) tumors to evaluate the association of IHC surrogate molecular subtype with the clinical response to presurgical chemotherapy, evaluated by the WHO criteria, 3 weeks after the third cycle of 5 flourouracil, adriamycin, cyclophosphamide (FAC regimen). The subtypes of luminal, basal-like and HER2 enriched were found to account for 36.0 % (27/75), 34.7 % (26/75) and 29.3% (22/75) of patients respectively. Ten were luminal A and 14 luminal B (8 HER2 negative and 6HER2 positive). The triple negative breast cancer (TNBC) was most sensitive to chemotherapy with 19% achieving clinical-complete-response (cCR) followed by HER2 enriched (2/22 (9%) cCR), luminal B (1/6 (7%) cCR) and luminal A (0/10 (0%) cCR). Heterogeneity was observed within each subgroup, being most marked in the TNBC although the most responding tumors, 8% developing clinical-progressive-disease. The study supports association of molecular subtypes with response to chemotherapy in patients with advanced breast cancer and the existence of further heterogeneity within subtypes.

Keywords: Breast cancer - molecular subtypes - chemotherapy response - heterogeneity - IHC surrogate

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Introduction

Breast cancer is the most frequently diagnosed cancer in the women worldwide. Enormous molecular and biological heterogeneity with impact on clinical course and response to chemotherapy has been reported. Gene Expression Profiling (GEP) has demonstrated that breast cancer consists of at least five distinct molecular subtypes (Perou et al., 2000; Sorlie et al., 2001). These subtypes have different risk factors (Phipps et al., 2011) and clinico-pathologic features (Chang et al., 2008; Cheang et al., 2008; Liedtke et al., 2008; Smid et al., 2008; Onitilio et al., 2009; Chuthapisith et al 2012). GEP is expensive and not widely available even in the developed world. The facility of GEP is nearly non-existent in the developing countries. It has not yet replaced the immunohistochemical (IHC) analysis of the classical morphological tumor biomarkers estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) in the clinical practice. A strong correlation has been demonstrated between the conventional histopathological features reported on the formalin fixed paraffin-embedded (FFPE) tumor tissue and the molecular subclass identified by GEP on fresh tumor tissue (Cheang et al., 2008).

St Gallen Consensus has proposed a simplified, four IHC based biomarker panel (ER, PR, HER2, Ki67) for the molecular classification, which can be used as a shorthand and convenient approximation of intrinsic molecular subtypes of breast cancer. It has been proposed that if reliable Ki67 labeling index is not available, some alternative measure of proliferation like histological grade (G) may be used in making distinction between Luminal A and Luminal B subtypes (Goldhirsch et al., 2011). The surrogate IHC markers for molecular breast cancer subtypes have therefore emerged as a practical clinical tool for an approximate molecular classification of breast cancer patients in the developing countries.

Advanced breast cancer (ABC) is a significant public health problem in Pakistan (Bhurgri et al., 2006; Khokher et al., 2012) and other developing countries (Moore et al., 2009). A vast majority of these patients present with advanced bulky disease with visually obvious and

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ulcerated masses in the breast. Presurgical chemotherapy, also known as Neoadjuvant or Primary chemotherapy is the current standard of care in these patients. It has been implicated that molecular characteristics of the cancer affect sensitivity to chemotherapy and clinically and pathologically similar tumors may exhibit different response to chemotherapy (Rouzier et al., 2005; Andre and Pusztai 2006; Carey et al., 2007; Colleoni et al., 2009; Coates et al., 2012). The molecular classification of breast cancer therefore provides a new framework for the study of responsiveness to chemotherapy in patients with ABC. A subgroup of patients in a previously published study (Khokher et al., 2011) having clinically evaluable tumor (Tumor size >5 cm) and tumor response evaluation to standard chemotherapy according to WHO criteria, was analyzed in the present study to evaluate the association of IHC surrogate molecular subclass of breast cancer with the clinical response to chemotherapy in patients with advanced breast cancer. We here report the association of IHC defined molecular subclass with the clinical response to chemotherapy in these patients.

Materials and Methods

The prospectively maintained data of a previously published study (Khokher et al., 2011) was reviewed and the data of a subset of patients fulfilling the inclusion and exclusion criteria of the present study were analyzed. These newly diagnosed patients of breast cancer had clinically evaluable breast tumors (Tumor size >5cm) and were treated with FAC (5 Flourouracil, Adriamycin, Cyclophosphamide) in the presurgical setting. The patients with known histopathology, IHC analysis of ER, PR, HER2 and clinical response data were sorted and selected. Attempts were made to retrieve FFPE blocks of more patients with known response to presurgical chemotherapy and unknown ER, PR and HER2 status. The retrieved blocks were submitted to the pathologist for IHC analysis of these receptors. ER and PR were considered positive when >1% cells stained positive. Hercep Score 3+ was taken as Positve, 2+ as equivocal, and 0 or 1+ as Negative for HER2. The patients with equivocal HER2 status on IHC were excluded. The patients were divided into the molecular subclasses according to the St Gallen consensus (Goldhirsch et al., 2011). Luminal A (ER/PgR+, HER2 Negative and G 1or 2), Luminal B HER2 Negative (ER/PgR+, HER2 Negative and G3), Luminal B HER2 Positive (ER/PgR+, HER2 positive and any Tumor Grade), HER2 enriched or Non luminal HER2 positive (ER/PgR Negative, HER2 positive and any Tumor Grade) and Triple Negative or Basal-like (ER/PgR Negative, HER2 Negative and any Tumor Grade) types were identified. ER/PgR+, HER2 Negative and unknown Grade were labeled as Luminal nonspecific.

Tumor measurements were made in centimeters using calipers and a tape measure (Kuerer et al., 2000) and were recorded prior to the first cycle and 3 weeks after the third cycle of FAC chemotherapy. In case of multiple or bilateral lesions measurements of the largest lesion alone were recorded. Based upon the percentage change in the product of two tumor dimensions, 3 weeks after the third course of chemotherapy, patients were divided into four response groups according to the WHO criteria (Miller et al., 1981). Clinical Complete Response (cCR) was defined as no residual clinically detectable tumor, Clinical Partial Response (cPR) as a reduction of 50% or more in the product of the maximum perpendicular diameters of the tumor, Clinical Stable Disease (cSD) as less than 50% decrease or less than 25% increase in the product of the tumor dimensions and Clinical progressive Disease (cPD) as 25% or more increase in the product of tumor dimensions. Patients exhibiting cCR or cPR were grouped as "Responders" and those exhibiting cSD or cPD were grouped as "Non responders".

Descriptive statistics were used for finding the frequency distribution of various molecular subtypes of breast cancer and Chi square test for the correlation studies between the molecular subtype and clinical response to NACT. Study was approved by the institutional review and ethical committee of University of Health Sciences and INMOL hospital, Lahore.

Table 1. Baseline Patient and Tumor Characteristics

Parameter	Group	Frequency
Age	20-29	4
	30-39	18
	40-49	22
	50-59	21
	≥60	10
Menopausal status	Pre	42
	Post	33
Histopathology	IDC	66
	ILC	8
	Mixed	1
Grade	I	1
	II	23
	III	29
	unknown	22
Stage	II B	2
	III A	11
	III B	26
	III C	6
	IV	30
ER	Positive	25
	Negative	50
PR	Positive	24
	Negative	51
HER 2	3 positive	30
	Negative & 1 Positive	45
Base Line Tumor size (cm)	5-10	21
	10.1-15	33
	15.1-20	15
	>20	6
IHC Surrogate of Molecular Subtype		
	Luminal A	10
	Luminal B Her 2 Neg	6
	Luminal B Her 2 Pos	8
	Luminal Non Specific	3
	Her2 enriched	22
	Basal-like	26

Results

Sixty five patients of the previously published study

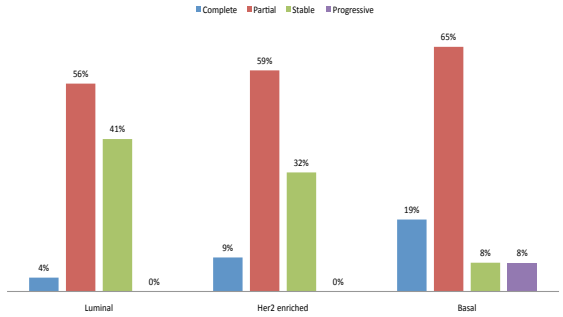

Figure 1. Response of Molecular Subtypes to Chemotherapy

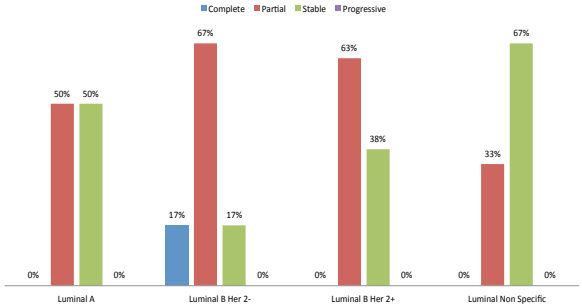

Figure 2. Response of Luminal Subtypes to Chemotherapy

(Khokher et al., 2011) met the inclusion criteria of the present study. FFPE blocks of 22 more patients of this study were retrieved and submitted to the pathologist for IHC analysis of ER, PR and HER2. Ten patients in the former group and two in the latter, having equivocal (2 positive) status of HER2 receptors were excluded. The remaining 75 patients were categorized as IHC surrogate molecular subclasses according to the St Gallen criteria already described. The baseline patient and tumor characteristics of these patients are shown in Table 1. Distribution of these patients in the three basic IHC surrogate subtypes of Luminal, Basal-like and HER2 enriched subtypes was 36% (27/75), 34.7% (26/75) and 29.3% (22/75) respectively. Among the Luminal group, 10 were Luminal A and 14 were Luminal B (8 were HER2 Negative and 6 were HER2 Positive). Three tumors were Luminal nonspecific. Figure 1 shows the response of the three basic IHC surrogate groups to presurgical chemotherapy and Figure 2 shows the response of the four subtypes of Luminal group to the presurgical chemotherapy according to the WHO criteria. Among the Luminal group only one patient (4%) had cCR and it was Luminal B HER2 negative. As a group 19% (5/26), 9% (2/22), 7% (1/6) and 0% (0/10) tumors of Basal-like, HER2 enriched, Luminal B and Luminal A respectively, achieved cCR to the standard presurgical chemotherapy.

Discussion

We have classified the advanced breast cancer patients into the four molecular classes according to the St Gallen criteria (Goldhirsch et al., 2011) and have added the term of Luminal nonspecific for the ER, PR positive HER2 negative Luminal type with unknown Grade. Luminal A, Luminal B, HER2 enriched and Basal-like subtypes thereby defined, showed distinctive sensitivity patterns for the response to chemotherapy. The basal-like triple negative disease was the most sensitive to chemotherapy as 19% patients achieved cCR compared to 9% of HER 2 enriched and 7% of Luminal B group. Among the Luminal tumors, 4% had clinical complete response and they were of the Luminal B subtype. The basal-like group showed the maximum degree of heterogeneity in the response to chemotherapy as although 19% patients achieved cCR, 8% patients developed cPD. The present study has the limitation of small number of patients and therefore minor differences in the responses could have been undermined.

Advanced Breast Cancer (ABC) is a clinical entity and includes a wide range of presentations. It may be a biologically aggressive tumor with rapid growth leading to advanced disease or a biologically indolent, slow growing tumor presenting at advanced stage, years after its first appearance because of delayed report to diagnostic facility and/or the lack of adequate treatment. Primary or presurgical chemotherapy is the current standard of care for the treatment of these patients. Primary chemotherapy was first introduced in 1970s for the down staging of inoperable disease and for the rapidly progressing inflammatory breast cancer (De Lena et al., 1981). Later its use was extended to include the operable disease to increase the rate of breast conservation surgery (BCS). The presurgical chemotherapy allows the in-vivo assessment of tumor response and therefore it is the model being used for efficacy trials of the newly developed drugs in the developed countries. However the primary objective for the use of presurgical chemotherapy in the developing countries is still down staging of the disease to achieve operability rather than increasing the rate of BCS. This is because of the limited treatment options and the late stage presentation of breast cancer in these countries. The clinical complete response to presurgical chemotherapy is a surrogate of pCR. Most of the patients with cCR are found to have pCR on histopathology of the operated specimen. Furthermore pCR is an established surrogate of DFS and OS (El-Tahir et al., 1998; Keurer et al., 1999; Pierga et al., 2003). In the context of adjuvant chemotherapy, response to chemotherapy means increased DFS and OS, while in the context of presurgical chemotherapy response means a significant reduction (50% or more) in the size of the tumor mass.

Current research and GEP demonstrates that breast cancer is a heterogeneous disease and consists of at least five distinct molecular subtypes. These subtypes are defined by genetic array testing (Perou et al., 2000; Sorlie et al., 2001) and are distinctive in risk factors (Phipps et al., 2011), natural histories, clinicopathological features (Chang et al., 2008; Cheang et al., 2008; Liedtke et al., 2008; Smid et al., 2008; Onitilio et al., 2009), survival patterns (Nguyen et al., 2008; Huber et al., 2009) and response to chemotherapy (Rouzier et al., 2005; Andre and Pusztai 2006; Carey et al., 2007; Colleoni et al., 2009; Lv et al., 2011; Coates et al., 2012). Clinical studies of response to chemotherapy in patients with locally advanced breast cancer have shown enormous heterogeneity ranging from 7 to 65% cCR and 4 to 29% pCR (Mauri et al., 2005; El-Saghir et al., 2008). This is a reflection of the heterogeneity within the advanced cases

of breast cancer, studied in these trials. The response to presurgical chemotherapy is unpredictable in an individual patient and lower rates have been reported in patients of the developing countries (Khokher et al., 2010). Resource constraints as well as the advanced nature of the disease seen in these countries imply the use of expensive drugs in the potential responders only. Molecular predictors have been utilized for the cost effective administration of targeted therapies to vulnerable population with cost savings of up to $920,000 in a cohort of patients with early breast cancer (Liang et al., 2007). These molecular predictors have not been tested for the prediction of response in patients with advanced breast cancer and are not available to these patients in the developing countries. So far no single or combinatorial marker has been found to be sufficiently predictive of the response to chemotherapy in the clinical setting. Many patients in the developing countries have locally advanced visually obvious, bulky, ulcerating, inoperable or borderline operable breast tumors. When they are treated with primary chemotherapy even a small increase in size may convert a borderline operable to inoperable tumor and add to the misery of an already inoperable disease. Prediction of response to chemotherapy with some single or combinatorial clinical parameter is therefore of extreme significance in these patients. Response to the first course of chemotherapy has been shown to have high predictive value (Khokher et al., 2011) but is not available at base line, before the exposure to chemotherapy. The histopathology and IHC analysis of the three receptors (ER, PR, HER2) are the essential components of diagnostic workup of all patients with invasive breast cancer (Wolff et al., 2007; NCCN, 2013). Four IHC based surrogate groups of molecular subclasses have been described using four clinically available parameters (ER, PR, HER2, Tumor Grade or Ki67) in the St Gallen Consensus conference (Goldhirsch et al., 2011; Fumagalli et al., 2012). These subclasses therefore act as a useful clinical tool with some degree of predictive value for the efficacy of chemotherapy in these patients. There is a consensus between the clinicians and the pathologists that GEP or IHC analysis of other markers are neither required nor recommended in the routine clinical setting (Lester et al., 2009).

The Triple Negative Breast Cancers (TNBC) is found in about 25% of all breast cancers and it has the worst overall and disease free survival. They pursue an aggressive course and are more frequent in the premenopausal and African American women (Stead et al., 2009). They are described as Triple Negative because of the lack of ER, PR and HER2 expression. It is a focus of recent investigations because owing to lack of any targeted therapy for them, they are primarily treated with chemotherapy. The triple negative receptor profile on IHC is a surrogate or close approximation of the Basal type of breast cancer on GEP. They are characterized by the expression of proliferation and basal cluster of genes. They lack ER, PR, HER2 expression and express CK5/6 and/or EGFR (Rakha et al., 2007). Tests for Cytokeratin 5/6 or EGFR/HER1 by IHC have been described in addition to ER, PR and HER2, for the determination of basal type of breast cancer (Nielson et al., 2004; Carey et al., 2006). The St Gallen panel however did not recommend their incorporation to define the basal-like tumors for the clinical decision making and guide to the therapeutic choices (Goldhirsch et al., 2011). Basal-like breast carcinomas account for 8% to 20% of all breast cancers. The two terms are not synonymous and there is some degree of overlap between basal-like and TNBC types of breast cancer. Approximately 25% of TNBC are not basal-like and about the same percentage of basal like are not TNBC. Recently claudin-low type of breast cancers has also been described in the spectrum of TNBC. They represent 5% of all breast cancers and are characterized by the expression of stem cell features (Carey, 2011). Our study of advanced breast cancer patients shows varying degrees of response within this subgroup of patients. This group showed the largest proportion (19%) of tumors achieving complete disappearance of the clinical disease as well as overall response to chemotherapy (84%) which is in consistence with the other studies (Rouzier et al., 2005; Carey et al., 2007; Liedtke et al., 2009; Lv et al., 2011). The TNBC was however the only subgroup of IHC surrogate of molecular types in our study, which showed development of disease progression with chemotherapy. This implies the existence of many further subgroups in the TNBC group of patients with advanced breast cancer and supports the need to search for new biomarkers to enable further sub classification according to the sensitivity to chemotherapy. This group is already known as a heterogeneous group in biology, response to chemotherapy and survival patterns. TNBC has further been classified into 8 subtypes (Nguyen et al., 2008) and 16 subtypes (Onitilio et al., 2009) in some studies. The heterogeneity of drug sensitivity and response outcome in the TNBC necessitates the search of a clinically useful predictor for the most cost effective use of chemotherapy.

Heterogeneity of response to presurgical chemotherapy was observed within the Luminal group of IHC surrogates of molecular subtypes in the present study. Majority of patients with Luminal subclass showed some degree of response to chemotherapy but no patient had clinical progressive disease. This is in consistence with the previous reports (Sorlie et al., 2001; 2003; Sotiriou et al., 2003). The Luminal B HER2 negative subtype was found to be the most sensitive to chemotherapy (84% responded) followed by the Luminal B HER2 positive (63% responded) tumors. Luminal A were the least responding tumors as none of the patients had cCR and only 50% patients achieved partial response in this subtype. The only patient among the Luminal group exhibiting cCR had the Luminal B HER2 negative subtype. This heterogeneity of response to chemotherapy is in consistence with the genomic heterogeneity reported for the Luminal A and Luminal B types of breast cancers (Yanagawa et al., 2012). Lack of efficacy of neoadjuvant chemotherapy in patients with ER positive tumors and Luminal A tumors is well documented (Carey et al., 2007; Colleoni et al., 2009; Lv et al., 2011) and the use of adjuvant chemotherapy has been debated in the Luminal A subclass of patients with early breast cancer (Coates et al., 2012). No case of pathologic complete response was observed among the patients with high ER expression at the European Institute of Oncology. Similarly the international breast cancer study group VIII

and IX trials found no benefit of adding chemotherapy in patients with ER positive disease (Aebi et al., 2011; Karlsson et al., 2011).

The HER2 enriched subtype in the present study showed intermediate sensitivity to chemotherapy. This subtype has two known markers of sensitivity to chemotherapy in breast cancer; HER2 over expression and ER negativity. HER2 over expression has been associated with better response to adriamycin based chemotherapy (Colleoni et al., 2008; Pritchard et al., 2008). Whether this sensitivity is linked to the coexpression of Topo2 alpha is however debated (Leo and Isola, 2003). The ER negativity has independently been associated with higher pCR (Guarneri et al., 2006; VonMinckwitz et al., 2009; 2011). The incorporation of these two response predictors in the HER2 enriched subtype has been shown to be translated into higher rates of cCR and pCR by various studies (Carey et al., 2007).

Presurgical chemotherapy decisions need to be individualized in patients with ABC. The "one size fits all" approach for treatment of these patients needs to be changed with the same force and effect as that for the patients with early breast cancer. Clinical trials like MINDACT, TAILORx and RxPONDER, are being conducted in the developed countries to identify a group of patients who are optimally treated without chemotherapy (Cianfrocca and Gradishar, 2009; Ramsey et al., 2013). Similar trials need to be designed and conducted in the developing countries for patients with advanced breast cancer. We need to identify the patients among them, who are not likely to respond, as adding treatment toxicity of ineffective treatment is actually adding "fuel to fire" with adverse effects on quality of life. This is waste of resources in general and negation of or delaying of alternative treatment strategy for an individual patient in particular. It is of crucial importance in these patients because surgical upstaging in these patients with bulky disease will either render the disease inoperable or require more extensive surgery for local control. We also need to identify the potential responders for the judicial use of already limited resources. These patients in countries with limited resources also deserve to benefit from the increasing knowledge of biologic heterogeneity of breast cancer. The treatment needs to be tailored according to the molecular subtype rather than the bulk of the disease.

In conclusion, advanced breast cancer is a heterogeneous entity exhibiting varying responses to the presurgical chemotherapy. The varied response to chemotherapy in our patients with bulky breast tumors supports the fact that it is the tumor biology and not the tumor bulk which determines the response to chemotherapy. Classification of breast cancer into the various IHC based molecular subtypes is useful to predict the response to chemotherapy to some extent and there is heterogeneity within each subgroup. Luminal A is the least responding group while TNBC is the most responding. TNBC features extreme degrees of response, exhibiting highest rates of cCR and cPD. There is a need to explore predictive biomarkers with ability to identify potential responders Vs non responders of chemotherapy, so that the best strategy for treatment with minimum cost and toxicity can be planned in an individual patient with advanced breast cancer. Further study of various subgroups of these patients in a larger population may lead to the discovery of cost effective clinically useful parameter or tool predictive of response to chemotherapy specific to these patients.

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