RESEARCH ARTICLE

Disease Free Survival among Molecular Subtypes of Early Stage Breast Cancer between 2001 and 2010 in Iran

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Abstract

Background: Breast cancer is the most common cancer among women. Molecular subtypes are important in determining prognosis. This study evaluated five-year disease-free survival among four molecular subtypes in patients with early stages of breast cancer. Materials and Methods: In this retrospective descriptive-analytical study, information on patients with breast cancer between 2001-2010 was evaluated. Five hundred ninety two patients in the early stages of breast cancer (stages 1 and 2) were selected to undergo anthracycline-based chemotherapy. Relapse, death or absence (censor) were considered as the end of the study. Patients based on ER, PR and HER-2 expression were divided into four subtypes (luminal A, luminal B, HER-2 enriched and triple negative). Information based upon questionnaire was analysed. To show the patients survival rate, life table and Kaplan-Meyer methods were used, and for comparing mean survival among different groups, the Log-Rank test was utilized. Results: Mean age at diagnosis was 47.9±9.6. Out of the 592 patients, 586 were female (99%) and 6 were male (1%). Considering breast cancer molecular subtypes, 361 patients were in the luminal A group (61%), 49 patients in the luminal B group (8.3%), 48 patients in the HER-2 enriched group (8.1%) and 134 in the triple negative group (22.6%). Mean disease-free survival was 53.7 months overall, 55.4 months for the luminal A group, 48.3 months for the luminal B group, 43 months for the HER-2enriched group and 54.6 months for the triple negatives. Disease free survival differed significantly among the molecular subtypes (p value=0.0001). Conclusions: The best disease-free survival rate was among the luminal A subgroup and the worst disease-free survival rate was among the HER-2 enriched subgroup. Disease free survival rate in the HER-2 positive groups (luminal B and HER-2 enriched) was worse than the HER-2 negative groups (luminal A and triple negative).

Keywords: Breast cancer - molecular subtypes - disease free survival rate - early stages - anthracycline chemotherapy

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Introduction

Breast cancer is the most prevalent cancer among women and the second leading cause of cancer death in women and the major cause of death among women between 40-59 years old (Siegel et al., 2011). Breast cancer is a polygenic disease with a variable phenotype.The breast cancer is made of several biologic subtypes with different behaviors and responses to treatment (Bair and Tibshirani, 2004). The main breast cancer subtypes are due to different genetic expression patterns.In the molecular classification as well as the conventional use of nuclear grading, pathology, immunohistochemical analysis of the hormone receptor and overexpression of epidermal growth factor receptore (HER-2), differentiation in gene expression are also used for determining breast cancer, which the results would constitute the major subtypes specification. Gene expression studies have defined several subtypes of breast cancer. These include three subtypes of tumors with ER negative consisting of basal like [ER negative, PR negative, HER-2 negative and positive for CK5/6 and/or epidermal growth factor receptor (EGFR)], HER-2 enriched (ER negative, PR negative and HER-2 positive) and normal like (characteristics similar to the normal breast tissue) and two subtypes of tumors with ER positive, luminal A (ER positive or PR positive and HER-2 negative) and Luminal B (ER positive or PR positive and HER-2 negative). Also the sixth subtype of breast cancer is defined as low claudin which is determined and characterized by low or no expression of cell-cell epithelial adhesion genes (claudin 3, 4, 7 and cadherin E), differentiated luminal cell surface markers (MUC-1, EpCAm) and large amount of mesenchimal to epithelial differentiation markers,cancer immune receptor genes and stem cell like factor (ALDH1A1), CD24, CD44. These subtypes are significantly different in prognosis and response to treatment targets (Prat et al., 2010). Intrinsic subtypes are devided into two major groups related to hormone receptor dependent gene expression. Luminal A and luminal B cancers are named so because they are characterized by expression of the same genes as being expressed by the common breast epithelial

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cells overlapping with ER positive breast cancers. There are also several subgroups,which are characterized by low expression of hormone receptor related genes(ER negative), one is named HER-2 enriched (previously nominated ER-/HER-2+) and the other is named triple negative.Triple negative subtype are named so due to expressing several determining genes of normal breast epithelial cells (Sørlie et al., 2001). Intrinsic subtypes are developed just for determining biologicaly related and not for the prognosis. Although in several independent data bases,These subtypes are related to the prognosis. Generally, the best prognosis was observed in luminal A group. The patients with positive hormone receptors, luminal B, has an obviously worse prognosis. The triple negative groups and HER-2 enriched have the worst prognosis. Eventually, HER-2 targeted therapy has changed the outcomes of the cancers, HER-2 enrichrd and luminal/HER-2+. Immunohistochemistry can be performed on thin blocks fixed with formalin blocks which is covered with paraffin or frozen sections. The amount of existing estrogen or progestron receptor protein is estimated according to nuclei color which might exist in malignant epithelium. A similar process is utilized for diagnosing of HER-2 protein molecules on the cell membrane (King and Greene, 1984; Layfield et al., 1998).

Materials and Methods

In this retrospective discriptive epidemiologic study,the data concerning patients with early stages of breast cancer (stage 1 and S2) between 2001 and 2010 was collected by refering to computerized information registration system.On the base of questionnaire forms, the following information was collected: age, sex, stage, size, lymph node, chemotherapy regimen and molecular receptors status. Files containing incompelete detailed information were excluded. Regarding the goal of study, the patients with stage 1, 2 according to TNM system (based on AJCC, seventh edition) who underwent anthracycline-base chemotherapy were enrolled and others were excluded. Time of relapse was considered as an end point for the study and disease-free survival was determined as the pathologic based diagnosis interval until the time of disease relapse. Withdrawal from treatment and missing relapse information would be considered as censor and the last visit would be calculated as the end point of the study. Information was analysed according to questionnaire sheets andSPSS software was used.(SPSS18 for Windows, IBM corp. Armonk, New York, United states). Analysis of survival was done with Kaplan-Meyer method and then comparison among different molecular subgroups (luminalA, luminalB, HER-2 enriched and triple negative) was done by using Log-Rank test. Clinicopathologic characteristics in each subtype were assessed with Chi-squere test. Predictive value equal or less than 5% was considered statistically significant.Luminal A was defined as (ER+ or PR+ and HER-2-), Luminal B was defined as (ER+ or PR+ and HER-2+), HER-2 was defined as (ER- and PR- and HER-2+), triple negative was defined as (ER- and PR- and HER-2-), Table 1. Determination of nuclear differentiation grade was done based on Nottingham scoring. HER-2 positive was considered as a 3+ test. Out of 1175 patients suffering breast cancer were visited between 2001-2010, 1048 patients had the sufficient data,which after excluding patients without the necessary conditions, 592 were entered in the our study.

Table 1. Immunohistochemical Characterization of Molecular Subtypes of Breast Cancer

Immunohitochemical characterization	Molecular subtype
ER+and orPR+and HER-2 -	Luminal A
ER+and orPR+and HER-2 +	Luminal B
ER-and PR-and HER-2 +	HER-2 enriched
ER-and PR-and HER-2 -	Basal

Results

Out of 1048 patients of whom the data was registered

Table 2. Description of Characteristics of the Study Population

Characteristics		No. of cases	(%)
Mean age±SD(years)		47.9±9.6	
Age (years)	<35	53	9
	35-50	323	54.5
	>50	216	36.5
Sex	Female	586	99
	Male	6	1
Histological grade	I	29	4.9
	II	297	50.2
	III	155	26.1
	Undetermined	111	18.8
Average tumor size±SD (cm)		3.1±1.2	
Tumor size (cm)	<2	144	24.3
	2-5	420	70.9
	>5	28	4.8
AJCC stage	I	59	10
	IIa	251	42.5
	IIb	282	47.6
Histologic type	Invasive ductal carcinoma	489	82.6
	Invasive lobular carcinoma	30	5.1
	Invasive ductal&lobular carcinoma	13	2.2
	Other types	14	2.4
	Undetermined	46	7.7
Site of invlvement	Right	198	33.5
	Left	256	43.5
	Bilateral	1	6
	Undetermined	132	22.3
Lymph node status	N0	245	42.3
	N+	334	57.7
	Total	579	100
Mean lymph node invovlment±SD (lymph node)			
	Vascular involvment	310	52.4
	Lymphatic involvment	312	52.7
	Perineural involvment	194	32.8
	Margine involvment	7	1.2
	Skin involvment	12	2
Metastasis status	M0	537	90.7
	M1	55	9.3
Site of metastasis	Bone	38	6.4
	Lung	21	3.5
	Liver	15	2.5
	Lymph node	3	0.5
	Brain	7	1.2
	Local reccerence	8	1.4
	HR positive	410	69.2
	ER positive	394	66.6
	PR positive	359	60.6
	Her2 positive	97	16.4

Table 3. Prevalence of Intrinsic Subtypes and Clinico-pathological Characteristics

Characteristics		LuminalA		LuminalB		HER-2+		Triple -		p value		Total
		No	(%)	No	(%)	No	(%)	No	(%)	No	(%)	
Patient		361	61	49	8.3	48	8.1	134	23	592	100	
Mean age±SD (years)		48.9±9.4		47.1±9.2		46.4±8.8		46.2±10.3			-	0.023
Age (years)	<35	25	6.9	3	6.1	2	4.2	23	17.2	9	53	0.05
	35-50	193	53.5	32	65.3	31	64.6	67	50	323	54.6	
	>50	143	39.6	14	28.6	15	31.3	44	32.8	216	36.4	
Histological grade	I	26	9	2	4.8	1	2.8	0	0	29	6	0.0001
	II	201	69.3	27	64.3	16	44.4	53	46.9	297	61.7	
	III	63	21.7	13	31	19	52.8	60	53.1	155	32.2	
Average tumor size±SD (cm)		2.9±1.1		3.3±1.2		3.2±1.2		3.2±1.2			-	-
Tumor size (cm)	<2	97	26.9	9	18.4	10	20.8	28	20.9	144	24.3	0.333
	2-5	252	69.8	37	75.5	35	72.9	96	71.6	420	70.9	
	>5	12	3.3	3	6.1	3	6.3	10	7.5	28	4.7	
AJCC stage	I	37	10.2	3	6.1	5	10.4	14	10.4	59	10	0.129
	IIa	151	41.8	16	32.7	16	33.3	68	50.7	251	42.4	
	IIb	173	47.9	30	61.2	27	56.3	52	38.8	282	47.6	
Histologic type												
Invasive ductal carcinoma		296	88.1	43	95.6	39	92.9	111	90.2	489	89.6	0.0001
Invasive lobular carcinoma		27	8	0	0	1	2.4	2	1.6	30	5.5	
Invasive ductal and lobular carcinoma		10	3	2	4.4	1	2.4	0	0	13	2.4	
Other types		3	0.9	0	0	1	2.4	10	8.1	14	2.6	
Site of invlvement	Right	123	43.6	17	43.6	9	24.3	49	48	198	43	0.185
	Left	154	54.6	22	56.4	27	73	53	52	256	55.7	
	Bilateral	5	1.8	0	0	1	2.7	0	0	6	1.3	
Lymph node status	N0	133	37.8	16	32.7	20	41.7	76	58	334	57.7	0.03
	N+	218	62.2	33	67.3	28	58.3	55	42	245	42.3	
	Vascular involvment	194	53.7	22	44.9	23	47.9	71	53	310	52.4	0.621
Lymphatic involvment		196	54.3	22	44.9	23	47.9	71	53	312	52.7	0.57
Perineural involvment		131	36.3	16	32.7	9	18.8	38	28.4	194	32.8	0.058
Margine involvment		5	1.4	0	0	0	0	2	1.5	7	1.2	0.706
Skin involvment		8	2.2	1	2	1	2.1	2	1.5	12	2	0.968
M+		28	7.8	12	6	25		9	6.7	55	9.3	0.001
Site of metastasis	Bone	20	5.5	12.5	6	12.2	6	6	4.5	38	6.4	0.071
	Lung	11	3	8.3	1	2	4	5	3.7	21	3.5	0.282
	Liver	10	2.8	2.1	3	6.1	1	1	0.7	15	2.5	0.22
	Lymph node	1	0.3	2.1	0	0	1	1	0.7	3	0.5	0.37
	Brain	2	0.6	6.3	0	0	3	2	1.5	7	1.2	0.006
Local reccerence		3	0.8	4.2	0	0	2	3	2.2	8	1.4	0.168

according the questionnaire, 796 patients underwent chemotherapy with anthracycline base of which 592 were in the early stages of breast cancer. The patients' mean age was 47.9±9.6 at the time of diagnosis. Fifty three patients were under 35 years old (9%), 323 patients between 35-50 years old (54.6%), and 216 patients were over 50 years old (36.5%). The age range was between 24 and 72 years olds. 59 patients were in stage 1 (4.9%), 251 patients were in stage 2a (42.4%) and 282 patients were in stage 2b (47.6%). The most common pathologic diagnoses were ductal (82.6%), lobular (5.1%), ductal and lobular (2.2%) and others (2.4%) respectively. The smallest tumor size was 0.5 cm and the biggest one was 9.5 cm with the mean 3.1±1.2.334 patients had lymph node involvement (57.7%). Twenty nine patients had grade 1,297 patients had grade 2 and 155 patients had grade 3 and there was no data in 111 cases. Fifty five patients finally had relapsed due to distant metastasis. Eight patients had local reccurence and finally 63 patients had relapse during the follow up. Other clinicopathologic characteristics are shown in Table 2.

Three hundred sixty one patients were in luminal A subtype (61%),49 patients were in luminal B subtype (8.3%), 48 patients were in HER-2 enriched subtype (8.1%), 134 patients were in Triple negative subtype (22.61%). Mean age of patients at the time of diagnosis in luminal A was 48.9±9.4, in luminal B was 47.1±9.2, in HER-2 enriched was 46.4±8.8, and in triple negative was 46.2±10.3 which was statistically significant (p=0.023). In patients under 35 years old triple negative group had the most quantity (17.2%) and the HER-2 enriched group had the least quantity (4.2%). The difference among the groups was significant (p=0.05). Luminal B tumors had the biggest mean size 3.3±1.2 cm and luminal A tumors had the smallest mean size 2.9±1.1 which this difference was not significant (p=0.061). Most tumors were between 2-5 cm (70.9%). Most tumors under 2 cm were in the luminal A group and most tumors over 5 cm were in the triple negative group which this difference was not significant among the groups (p=0.333). Concerning grading a significant difference was among the groups (p=0.0001).In grade 1 luminal A was the highest (9%) and triple negative was the least (zero). In grade 2 luminal A was the most (69.3%) and HER-2 enriched was the least (44.4%). In grade 3 triple negative was the most (53.1%) and luminal A was the least (21.7%). Regarding to the stage there was no significant difference among the groups (p=0.129). Most patients were diagnosed at stage 2b (p=47.6%). About lymph node there was a significant difference among the groups (p=0.030).The lowest involvement rate was in triple negative (42%) and the highest involvement rate was in luminal B group (67.3%). In relapsed group

with distant metastasis there was a significant difference among the groups (p=0.001). The lowest rate was in triple negative group (6.7%) and the highest rate was in HER-2 enriched group (25%). Other characteristics are shown in Table 3.

Mean disease-free survival of the four molecular subtypes is 53.7 months, During a 60 months of study. survival in luminal A was 55.4 months,in luminal B it was 48.3 months,in HER-2 enriched it was 43 months and in triple negative it was 54.6 months. Disease-free survival diagram in patients with early stages of breast cancer is shown in Figure 1. The overall comparison of disease-free survival rate based on Log-Rank test is significant (p=0.0001). Disease-free survival rate based on log rank test among the four molecular subtypes is shown in Table 4 and the survival is shown in Table 5. Disease free survival diagram among molecular subtypes (Kaplan-Meyer) is shown in Figure 2.

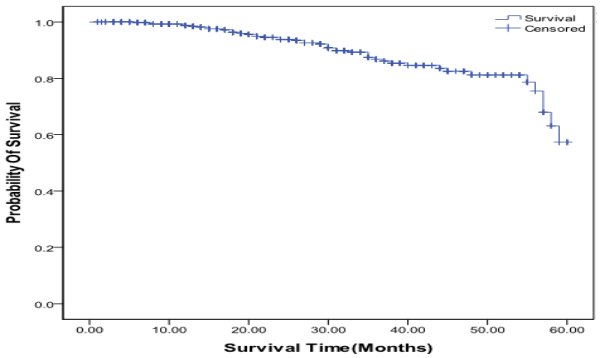

Figure 1. Disease Free Survival Diagram in Patients with Early Stages of Breast Cancer

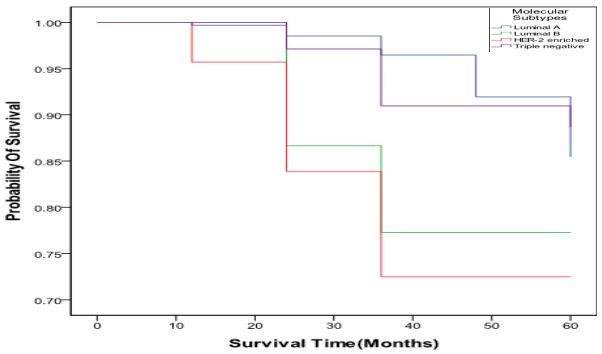

Figure 2. Disease Free Survival Diagram among Molecular Subtypes (Kaplan-Meyer)

Table 4. Disease-free Survival Rate Based on Log Rank Test Among the four Molecular Subtypes

Luminal A	Luminal B	0.001
	HER-2 enriched	0.0001
	Triple negative	0.114
Luminal B	HER-2 enriched	0.244
	Triple negative	0.077
HER-2 enriched	Triple negative	0.001

Table 5. Mean of Disease free Survival Rate among Molecular Subtypes

Molecular subtype	Mean	Standard error	Confidence interval
Luminal A	55.4	0.9	53.6-57.2
Luminal B	48.3	3.7	41.0-55.7
HER-2 enriched	43	3.7	35.5-50.4
Triple negtive	54.6	1.6	51.4-57.8
Total	53.7	0.8	52.1-55.3

Discussion

As a result of using screening mammography and efficient adjuvant therapy, the mortality rate has been declined and most women with breast cancer would be survived longer. The knowledge about periods of risks and common site of disease relapse could be used as a guide for focusing on clinical history and physical exams during the follow up visits and screening tests which provide the most information. Mean age of diagnosis in our study was 47.9±9.6 which was lower comparing to study of Su et al. (2010) and Kadivar et al. (2012) with a mean age of 53±10.4 years old at the time of diagnosis but Bennis et al. (2012) similar to Tunisian study in which the mean age of diagnosis was reported 46.8±12 and Haghighat et al. (2012) was higher comparing to Iranian study with a mean age of 45.9±10.5. In our patients 9% were under 35 years old and 63.5% were over 50 years old,similar to other Iranian study, patients under 35 years old were 12% and patients over 50 years old were 42% (Kadivar et al., 2012), with regard to study of Webster et al. (2008) patients under 35 years old were 6.2% and patients over 50 years old were 37.8% (Webster et al., 2008), our patients had breast cancer at a younger age,possibily due to ethnic and genetic differences. In our study luminal A group was seen at an older age and triple negative group was seen at a younger age. Lymph node involvement was 57.7%, distant metastasis was 15.5% which the results were similar to study of Bennis et al. (2012) 53% and 17.5% respectively, but a higher lymph node involvement comparing to other studies 41% and 39% respectively (Millar et al., 2009; Voduc et al., 2009), could be due to a delay of diagnosis comparing to western studies because of a lack of knowledge in our patients or a defect of screening or lack of appropriate medical services.The most common pathologic types in our study were invasive ductal carcinoma similar to other studies in the world (Chen et al., 2012; Kadivar et al., 2012). Estrogen hormonal receptor existed in 66.6% of patients similar to different papers (Allred et al., 1998; Kadivar et al., 2012).HER-2 overexpression incidence rate in our study was 16.4%, minimally lower than the reported rate in other studies (Ross et al., 2004), subsequently the difference may be due to a difference in laboratory methodes.In our study prevalence of luminal A was 61%, triple negative 22.6%, luminal B 8.3%, and HER-2 enriched was 8.1% similar to Iranian and Chinese study (Chen et al., 2010; Kadivar et al., 2012). As the same as other studies most of tumors under 2 cm were in luminal A group (Voduc et al., 2009; Kadivar et al., 2012). In our study most of tumors over 5 cm were in triple negative group while in the mentioned study, the most common cases were in HER-2 enriched (7%);(16.3) and then triple negative (6%);(10.7) groups respectively (Voduc et al., 2009; Kadivar et al., 2012), that the difference is little. The most tumors with grade 3 and the least tumors with grade 1 were triple negative,similar to Korean study (Noh et al., 2011) and this could be due

to the nature of these tumors.The triple negative group had fewer lymph node positive cases comparing to other groups (Voduc et al., 2009), and this could be due to more blood spreading in these tumors.The luminal A group had a lowest metastasis rate (7.8%) and the HER-2 enriched group had the highest metastasis rate (25%) while in study of Bennis et al. (2012) the highest distant metastasis rate was in group luminal B (24%) and the lowest was in group, HER-2 enriched (13%) (Bennis et al., 2012), which according to the survival in the studied groups,the results have been reasonable and logical. In a period of 60 months in Kaplan-meyer curve,survival rate respectively from highest to lowest was luminal A, triple negative, luminal B and HER-2 enriched,which these findings were similar to other studies with a better disease free survival rate in luminal A,luminal B,triple negative and HER-2 enriched respectively (Voduc et al., 2009; Chen et al., 2010; Noh et al., 2011; Caudle et al., 2012) and the difference in our study was due to a better prognosis in triple negative comparing to luminal B, and this finding indicates the importance of HER-2 overexpression in predicting disease relapse.The overall comparison of disease-free survival rate among molecular subtypes was significant based on Log-Rank method, similar difference of disease-free survival rate and mortality rate among molecular subtypes exists (Millar et al., 2009). Disease-free survival rate between the two HER-2 negative subgroups (luminal A, triple negative) was not significant and between the two HER-2 positive subgroups (luminal B, HER-2 enriched) was not significant but among the subtypes of the two mentioned groups was significant and this difference emphasizes the importance of HER-2 determination marker in patient`s prognosis. Better survival was observed for the patients with HER-2 negative tumors compared to HER-2 positive tumors (Jana et al., 2012). Relapse in subgroup luminal A was 8.6%, luminal B 12.2%, HER-2 enriched 29.1% and triple negative 8.9% of patients which was similar to study of Noh et al. (2011) the rate of relapse for HER-2 was the highest (Noh et al., 2011). In study of Voduc et al. (2009) relapse rate increased in luminal B and triple negative and HER-2 enriched (Voduc et al., 2009) and in study of Chen et al. (2012) relapse rate increased in triple negative and HER-2 enriched groups (Chen et al., 2012) and this could be due to a better response to the treatment including hormonal therapy in luminal A group.Regarding to study of Blows et al. (2010) in the first 5 year follow up,non luminal tumor had a poorer prognosis and gradually increased during the follow up period,prognosis in luminal groups,specially in luminal with HER-2 positive,was worsened (Blows et al., 2010) which matched with this study. HER-2 enriched group consists half of HER-2 positive breast cancers, clinicaly, and the other half of them includes both HER-2 and luminal gene branch overexpression and belongs to luminal B group. Before HER-2 targeted therapy, this group had a poor prognosis and now this natural history has reversed and has significantly been influenced by HER-2 efficient advanced treatments.In this study for patients ,direct HER-2 therapy was not performed which had a ngative effect on the survival of the patients with HER-2 positive (luminal B and HER-2 enriched). As mentioned studies,triple negative breast cancers were sensitive to modern chemotherapy (Rouzier et al., 2005; Carey et al., 2007; Khokher et al., 2013).

In conclusion, we have defined the main characteristics and prognosis in breast cancer in major subgroups by measuring ER and PR and HER-2 receptors. Finally we can conclude that disease free survival rate is the highest in luminal A and the lowest in HER-2 enriched subgroups. Disease free survival rate in HER-2 positive groups (luminal B and HER-2enriched) was lower than the HER-2 negative groups (luminal A and triple negative) and this study shows the prevalence of breast cancer at a younger age in our reigon which this study explains the importance of informing and education for women and considering proper screening facilities in these groups.

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References

Allred DC, Harvey JM, Berardo M, Clark GM (1998). Prognostic and predictive factors in breast cancer by immunohistochemical analysis. *Mod Pathol*, **2**, 155-68.

Bair E, Tibshirani R (2004). Semi-supervised methods to predict patient survival from gene expression data. *PLoS Biol*, **4**, 108.

Bennis S, Abbass F, Akasbi Y, et al (2012). Prevalence of molecular subtypes and prognosis of invasive breast cancer in north-east of Morocco: retrospective study. *BMC Res Notes*, **5**, 436.

Blows FM, Driver KE, Schmidt MK, et al (2010). Subtyping of breast cancer by immunohistochemistry to investigate a relationship between subtype and short and long term survival: a collaborative analysis of data for 10,159 cases from 12 studies. *PLoS Med*, **5**, 1000279.

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.

Caudle AS, Yu TK, Tucker SL, et al (2012). Local-regional control according to surrogate markers of breast cancer subtypes and response to neoadjuvant chemotherapy in breast cancer patients undergoing breast conserving therapy. *Breast Cancer Res*, **14**, 83.

Chen XS, Ma CD, Wu JY, et al (2010). Molecular subtype approximated by quantitative estrogen receptor, progesterone receptor and Her2 can predict the prognosis of breast cancer. *Tumori*, **96**, 103-10.

Haghighat S, ME Akbari ME, Ghaffari S, Yavari P (2012). Standardized breast cancer mortality rate compared to the general female population of Iran. *Asian Pac Cancer Prev*, **13**, 5525-8.

Jana D, Mandal S, Mukhopadhyay M, et al (2012). Prognostic Significance of HER-2/neu and survival of breast cancer patients attending a specialized breast clinic in Kolkata, Eastern India. *Asian Pac J Cancer Prev*, **13**, 3851-5.

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

Khokher S, Qureshi MU, Mahmood S, Nagi AH (2013). Association of immunoistochemically defined molecular subtypes with clinical response to presurgical chemotherapy

in patients with advanced breast cancer. *Asian Pac J Cancer Prev*, **14**, 3223-8.

King WJ, Greene GL (1984). Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells. *Nature*, **307**, 745-7.

Layfield LJ, Saria E, Mooney EE, Liu K, Dodge RR (1998). Tissue heterogeneity of immunohistochemically detected estrogen receptor. Implications for image analysis quantification. *Am J Clin Pathol*, **110**, 758-64.

Millar EK, Graham PH, O'Toole SA, et al (2009). Prediction of local recurrence, distant metastases, and death after breast-conserving therapy in early-stage invasive breast cancer using a five-biomarker panel. *J Clin Oncol*, **27**, 4701-8.

Noh JM, Choi DH, Huh SJ, et al (2011). Patterns of recurrence after breast-conserving treatment for early stage breast cancer by molecular subtype. *J Breast Cancer*, **14**, 46-51.

Prat A, Parker JS, Karginova O, et al (2010). Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. *Breast Cancer Res*, **12**, 68.

Ross JS, Fletcher JA, Bloom KJ, et al (2004). Targeted therapy in breast cancer: the HER-2/neu gene and protein. *Mol Cell Proteomics*, **3**, 379-98.

Rouzier R, Perou CM, Symmans WF, et al (2005). Breast cancer molecular subtypes respond differently to preoperative chemotherapy. *Clin Cancer Res*, **11**, 5678-85.

Sørlie T, Perou CM, Tibshirani R, et al (2001). Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA*, **98**, 10869-74.

Su Y, Zheng Y, Zheng W, et al (2010). Distinct distribution and prognostic significance of molecular subtypes of breast cancer in Chinese women: a population-based cohort study. *BMC Cancer*, **11**, 292.

Voduc KD, Cheang MCU, Tyldesley S, et al (2010). Breast cancer subtypes and the risk of local and regional relapse. *JCO*, **28**,1684-91.

Webster LR, Lee SF, Ringland C, et al (2008). Poor-prognosis estrogen receptor–positive breast cancer identified by histopathologic subclassification. *Clin Cancer Res*, **14**, 6625-33.