Original Article

Breast Cancer Survival Analysis Based on Immunohistochemistry Subtypes (ER/PR/HER2): a Retrospective Cohort Study

Jalal Poorolajal MD PhD[1], Nahid Nafissi MD[2], Mohammad Esmaeil Akbari MD[3], Hossein Mahjub PhD[4], Nader Esmailnasab PhD[5] Ebrahim Babaee MSc•[6]

Abstract

Background: We conducted this study to estimate the prevalence of biomarkers, including estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) among patients with breast cancer and to explore their effects on disease mortality.

Methods: We conducted this registry-based retrospective cohort study in Tehran, in 2014, using the data on 1622 patients with breast cancer, diagnosed pathologically and registered with the Comprehensive Cancer Control Center from 1998 to 2013. The outcome of interest was the survival probability of patients with breast cancer based on receptor status along with other prognostic factors such as age, histopathology, stage/grade of tumor, metastatic status, and surgical procedures using the life table, Kaplan-Meier curves, and multivariate Cox proportional hazard model. We generated different subtypes based on expression of ER, PR, and HER2, positive (+) and/or negative (–).

Results: ER+/PR+/HER2– subtype (51.5%) was the most common form of breast cancer cells. Compared to the ER+/PR+/HER– subtype, the hazard ratio (95% confidence interval) of cancer mortality was 2.14 (1.13, 4.03) for ER–/PR–/HER2– subtype, 1.92 (1.03, 3.59) for ER–/PR–/HER2+ subtype and 5.19 (1.51, 17.86) for ER–/PR+/HER2+ subtype.

Conclusion: In this study, breast cancer cases with ER–/HER2+ tumors had shorter survival than those with ER+/PR+/HER2– tumors. Triple negative tumors were the only other subtype with a statistically significant poorer prognosis. The results of this study in a middle-income country further indicate the importance of receptor status, in particular HER2 status, in the prognosis of breast cancer.

Keywords: Breast Neoplasms; Survival Analysis; Immunohistochemistry; Biological Markers; Cohort Studies; Iran

Cite this article as: Poorolajal J, Nafissi N, Akbari ME, Mahjub H, Esmailnasab N, Babaee E. Breast Cancer Survival Analysis Based on Immunohistochemistry Subtypes (ER/PR/HER2): a Retrospective Cohort Study. *Arch Iran Med*. 2016; 19(10): 680 – 686.

Introduction

Breast cancer is the most common cancer in women in both developed and developing countries.[1] Several prognostic factors for breast cancer have been well recognized.[2–5] Breast cancer is usually classified by different clinical and histological criteria for different purposes, including prognosis. The major subtypes are based on histopathological features, the grade of the tumor, the stage of the tumor, receptor status, and the gene-expression.[6] Clinicians still tend to rely on reliable and inexpensive traditional histopathological features and readily available tumor markers such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2).[7]

About 75% of all breast cancers are ER-positive and 65% are PR-positive.[8] Breast cancers containing either estrogen or progesterone receptors are called hormone receptor-positive. About two-thirds of breast cancers are hormone receptor-positive. The breast cancer cells that have neither estrogen nor progesterone receptors are called hormone receptor-negative.[9] Hormone receptor-positive cancers tend to grow more slowly and are more likely to respond to hormone therapy.

HER2 overexpression may occur in 18% to 20% of breast cancers.[9–11] HER2 overexpression is associated with worse clinical outcomes such as higher rate of recurrence and mortality in patients with breast cancer.[11,12] These breast cancers tend to be fast-growing and spread more aggressively than other breast cancers.[8,9] Thus, HER2 status should be considered in the clinical decision, along with other prognostic factors.[11] If breast cancer cells do not have ER or PR and are low in expression of HER2, they are called triple-negative. These cancers tend to occur more often in younger women and tend to grow and spread more quickly and behave more aggressively than other types of breast cancer.[13–15]

Several studies have been conducted worldwide to assess the prognostic effect of biomarkers on breast cancer. However, the prevalence and the prognostic effect of these factors may vary across countries. There is limited evidence about prognostic effects of different types of breast cancer in developing countries. This study was carried out to classify breast cancer into different subtypes based on the immunohistochemical markers ER, PR, and HER2 expression, positive (+) and/or negative (–), and to

Authors' affiliations: [1]Modeling of Noncommunicable Diseases Research Center and Department of Epidemiology, School of Public Health, Hamadan University of Medical Sciences, Hamadan, Iran. [2]Cancer Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [3]Cancer Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [4]Research Center for Health Sciences and Department of Biostatistics, School of Public Health, Hamadan University of Medical Sciences, Hamadan, Iran.[5]Kurdistan Research Center for Social Determinants of Health, School of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran. [6]Department of Epidemiology, School of Public Health, Hamadan University of Medical Sciences, Hamadan, Iran.

•**Corresponding author and reprints:** Ebrahim Babaee MSc, Department of Epidemiology, School of Public Health, Hamadan University of Medical Sciences, Hamadan 65157838695, Iran. Tel: 0098 81 38380090; Fax: 0098 81 38380509; E-mail: ebrahim_babaee@yahoo.com

Accepted for publication: 20 September 2016

explore the individual and combined effect of these markers on the disease mortality.

Materials and Methods

This registry-based retrospective cohort study was conducted in Tehran in 2014. The Research Council of Hamadan University of Medical Sciences approved the study. We used the data from patients with breast cancer (International Classification of Diseases for Oncology 3rd edition sites C50.0–C50.9)[16] diagnosed pathologically and registered with the Comprehensive Cancer Control Center (CCCC) affiliated with Shahid Beheshti University of Medical Sciences from 1998 to 2013. Cases are reported to CCCC from hospitals and any other facilities such as laboratories and clinics in Tehran. Every patient had a medical record, including medical history, demographic characteristics, clinical findings, laboratory results, surgical procedure, and dates of diagnosis, admission, and periodic visits. The patients with unknown pathology were excluded. Data were extracted from the medical records using a checklist of items according to the context of the medical records.

The outcome of interest was the survival probability of patients with breast cancer of any type from the date of diagnosis to death due to breast cancer. We contacted the patients' family to update our data. The patients who were lost to follow-up or died from causes other than breast cancer were considered as censored. The independent variables considered as predictive factors of survival probability of the patients included age, type of breast cancer (invasive ductal carcinoma (IDC), ductal carcinoma in situ (DCIS), lobular carcinoma in situ (LCIS), and invasive lobular carcinoma (ILC)), the stage of breast cancer (I, II, III, and IV), the grade of breast cancer (1, 2, and 3), the expression of hormone receptors (ER, PR, and HER2), the recurrence of tumor, metastatic status, and surgical procedure (breast-conserving surgery (BCS) and modified radical mastectomy (MRM)).

Based on the expression of ER, PR, and HER2, we generated two different classifications, the full model and the reduced model. The full model included eight possible combinations of ER, PR, and HER2, positive (+) and/or negative (–), as shown in Table 1. The reduced model included four subtypes considering either ER or PR as hormone receptor-positive as shown in Table 2. The first subtype in both models was considered as the reference group and the mortality rate of other subtypes was investigated and compared with the reference group.

The survival probability of patients with breast cancer was investigated using life table, Kaplan-Meier curves and log-rank test. The effect of prognostic factors on survival probability was explored using univariate and multivariate Cox proportional hazard model. All statistical analyses were performed at the 95% confidence level using the statistical software Stata version 11 (StataCorp, College Station, TX).

Results

Of 1741 patients with breast cancer, 119 were excluded due to unknown pathology. The analysis was based on the remaining data from 1622 patients with confirmed breast cancer. The mean (SD) age of patients at diagnosis was 48.59 (11.72) years, ranging from 17 to 98 years. The majority (34.2%) of patients were aged 40-49 years. A minority (17.0%) of the patients were cigarette smokers. IDC was the most common (89.8%) type of breast cancer. Most of the patients were diagnosed at stage II (43.5%) and III (30.8%). Most of the patients (54.3%) presented with grade 2. Almost 16.2% of the patients had distant metastasis and 14.2% had evidence of recurrence. About 70.8% of the patients were ER+, 66.6% were PR+, and 75.9% were HER2–. Almost 57.1% of the patients underwent BCS (Table 3).

The prevalence of immunohistochemical markers (ER/PR/HER2, +/–) and 1-, 5-, and 10-year survival probability of the patients based on the full and the reduce models are given in Table 4. According to the full model, ER+/PR+/HER2– and ER–/PR+/HER2+ were the most and the least common subtypes of breast cancer cells, respectively. These two subtypes had the highest and lowest 5-year survival probability among the eight

Table 1. The full model including eight possible combinations the expression of the esterogen receptor (ER), the progestron receptor (PR), and the human epidermal growth factor receptor 2 (HER2).

Esterogen	Progestron	HER2	Subtype
Positive	Positive	Positive	ER+/PR+/HER2+
Positive	Positive	Negative	ER+/PR+/HER2–
Positive	Negative	Positive	ER+/PR–/HER2+
Positive	Negative	Negative	ER+/PR–/HER2–
Negative	Positive	Positive	ER–/PR+/HER2+
Negative	Positive	Negative	ER–/PR+/HER2–
Negative	Negative	Positive	ER–/PR–/HER2+
Negative	Negative	Negative	ER–/PR–/HER2–

Table 2. The reduced model including four possible combinations based on the expression of the esterogen receptor (ER), the progestron receptor (PR), and the human epidermal growth factor receptor 2 (HER2).

Esterogen or Progestron	HER2	Subtype
Positive	Positive	ER/PR+/HER2+
Positive	Negative	ER/PR+/HER2–
Negative	Positive	ER/PR–/HER2+
Negative	Negative	ER/PR–/HER2–

Table 3. Characteristics of the patients with breast cancer (n = 1622).

Variables	Number [a]	Percentage
Age at diagnosis (yr)		
17–29	53	3.4
30–39	295	18.8
40–49	538	34.2
50–59	407	25.9
60–69	204	13.0
70–79	65	4.1
≥80	11	0.7
Cigarette smoking		
No	1013	83.0
Yes	208	17.0
Pathological type		
Ductal/lobular carcinoma in situ	90	5.5
Invasive lobular carcinoma	76	4.7
Invasive ductal carcinoma	1456	89.8
Stage		
I	300	21.2
II	617	43.5
III	436	30.8
IV	64	4.5
Grade		
1	159	12.4
2	699	54.3
3	428	33.3
Distant metastasis		
No	742	83.8
Yes	144	16.2
Recurrence		
No	775	85.8
Yes	128	14.2
Estrogen receptor		
Negative	379	29.2
Positive	921	70.8
Progesterone receptor		
Negative	432	33.4
Positive	861	66.6
Human epidermal growth factor receptor 2		
Negative	925	75.9
Positive	294	24.1
Surgical approach		
Breast-conserving surgery	825	57.1
Modified radical mastectomy	619	42.9

[a] The sum of subgroup may be less than total due to missing data.

subtypes, respectively (Figure 1). The equity of survival function was investigated using log-rank test (P = 0.0002). Based on the reduced model, ER/PR+/HER2– and ER/PR–/HER2+ were the most and the least common subtypes of breast cancer cells with the highest and the lowest 5-year survival probability among the four subtypes, respectively (Figure 2). The equity of survival function was investigated using log-rank test (P = 0.0001).

The effect of several prognostic factors on survival probability of patients with breast cancer based on the Cox proportional hazard model is given in Table 5. The proportional hazard assumption was tested using the Schoenfeld residuals test. Since the test was not significant (P = 0.305), the proportional hazards assumption was justified. According to the adjusted results, age at diagnosis (HR 1.38, 95% CI 1.12, 1.69), stage IV (HR 7.67, 2.53, 23.25), and distant metastasis (HR 11.04, 6.43, 18.96) were significantly associated with death due to cancer.

The effect of different combinations of ER, PR, and HER2 on the mortality rate of patients with breast cancer is given in Table 6. In the full model, compared to the ER+/PR+/HER– subtype as the reference group, all other subtypes had a higher risk of mortality. The HR of death due to breast cancer was 2.14 (95% CI: 1.13, 4.03) for ER–/PR–/HER2– subtype, 1.92 (95% CI: 1.03, 3.59) for ER–/PR–/HER2+ subtype and 5.19 (95% CI: 1.51, 17.86) for ER–/PR+/HER2+ subtype. In the reduced model, there was no statistically significant difference among the groups compared to ER/PR+/HER2– subtype.

Discussion

In this study, we measured the survival probability of patients with breast cancer and compared the mortality rate of the patients based on the immunohistochemical markers along with other well-known prognostic factors of breast cancer. According to our findings, the ER+/PR+/HER2– was associated with the lowest mortality rate. The risk of death was higher in the triple negative subtype (P = 0.019). The HER2+ subtype, irrespective of ER and/or PR being positive or negative, was associated with a higher risk of cancer mortality. In other words, HER2+ is an independent

Table 4. Survival probability of the patients with breast cancer by estrogen receptor (ER,) progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), positive (+) and/or negative (–) using a full model.

Time intervals (yr)	Population	Deaths	Lost	Survival	95% CI
Full model					
ER+/PR+/HER2–					
1 yr	607	7	108	0.98	0.97, 0.99
5 yr	492	25	325	0.91	0.87, 0.93
10 yr	142	15	127	0.73	0.64, 0.81
ER–/PR+/HER2–					
1 yr	24	1	5	0.95	0.71, 0.99
5 yr	18	1	11	0.87	0.57, 0.96
10 yr	6	1	5	0.62	0.13, 0.89
ER+/PR–/HER2–					
1 yr	56	0	13	1.00	1.00, 1.00
5 yr	43	5	28	0.82	0.63, 0.92
10 yr	10	3	7	0.44	0.13, 0.72
ER–/PR–/HER2–					
1 yr	206	1	20	0.99	0.96, 0.99
5 yr	185	23	104	0.82	0.74, 0.87
10 yr	58	2	56	0.76	0.65, 0.84
ER+/PR+/HER2+					
1 yr	134	0	24	1.00	1.00, 1.00
5 yr	110	13	52	0.84	0.74, 0.90
10 yr	45	10	35	0.53	0.36, 0.68
ER+/PR–/HER2+					
1 yr	30	0	4	1.00	1.00, 1.00
5 yr	26	3	16	0.83	0.56, 0.94
10 yr	7	1	6	0.62	0.18, 0.87
ER–/PR–/HER2+					
1 yr	107	0	7	1.00	1.00, 1.00
5 yr	100	21	51	0.71	0.60, 0.80
10 yr	28	7	21	0.43	0.25, 0.59
ER–/PR+/HER2+					
1 yr	10	0	1	1.00	1.00, 1.00
5 yr	9	3	6	0.50	0.11, 0.80
Reduced model					
ER/PR+/HER2–					
1 yr	687	8	126	0.98	0.97, 0.99
5 yr	553	31	364	0.90	0.87, 0.92
10 yr	158	19	139	0.71	0.62, 0.78
ER/PR–/HER2–					
1 yr	206	1	20	0.99	0.96, 0.99
5 yr	185	23	104	0.82	0.74, 0.87
10 yr	58	2	56	0.76	0.65, 0.84
ER/PR+/HER2+					
1 yr	174	0	29	1.00	1.00, 1.00
5 yr	145	19	74	0.82	0.73, 0.88
10 yr	52	11	41	0.53	0.38, 0.66
ER/PR–/HER2+					
1 yr	107	0	7	1.00	1.00, 1.00
5 yr	100	21	51	0.71	0.60, 0.80
10 yr	28	7	21	0.43	0.25, 0.59

poor prognostic factor, whether or not ER/PR is positive or negative.[17,18]

In addition, we indicated that single hormone receptor positive tumors were rare and had shorter survival than double positive or double negative cancer cells. This finding is consistent with the existing literature.[7,19,20] Maeyer *et al.* reported that only 1.5% of their primary operated cases presented the ER–/PR+ breast cancer phenotype.[19] Ng *et al.* showed that 11.6% of the breast cancer cell were a ER+/PR– and 4.6% were ER–/PR+ [20] In addition, Rakha *et al*. indicated that ER+/PR– and ER–/PR+ tumors are biologically and clinically distinct subtypes of breast cancer that are associated with more aggressive characteristics.[21]

The results of this study also demonstrated that immunohistochemical markers play an important role in the prognosis of breast cancer; however, their effect on the mortality rate of the disease diminishes when controlling for other prognostic factors, such as the pathological type of cancer, the stage of cancer, the grade of cancer, and the metastatic status.

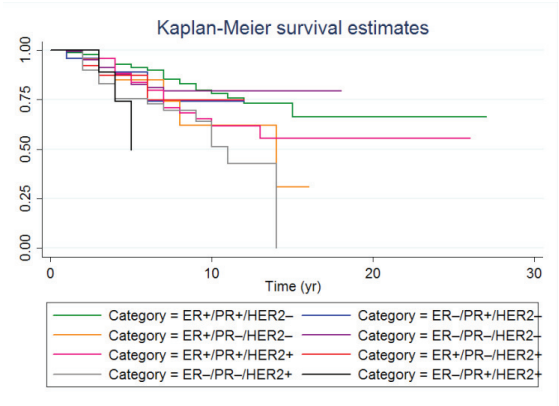

Figure 1. Survival probability of the patients with breast cancer based on immunohistochemistry markers (8 subtypes), using log-rank test (*P* = 0.0002).

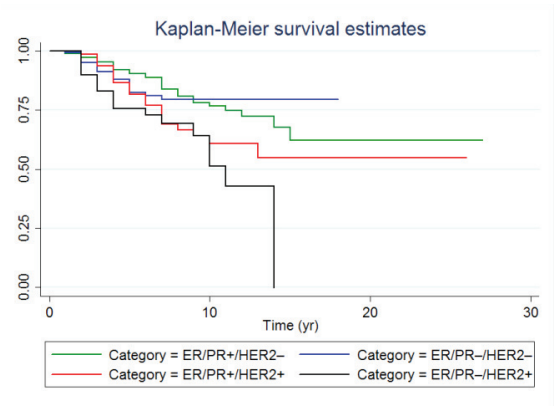

Figure 2. Survival probability of the patients with breast cancer based on immunohistochemistry markers (4 subtypes), using log-rank test (*P* = 0.0001).

Table 5. Effect of various predictive factors on survival probability of patients with breast using the univariate (unadjusted) and multivariate (adjusted) Cox regression model.

Variables	Unadjusted HR (95% CI)	*P* value	Adjusted HR (95% CI) [a]	*P* value
Age at diagnosis				
Test for trend (every 10 yrs)	1.12 (1.01, 1.25)	0.037	1.38 (1.12, 1.69)	0.002
Pathological type				
Ductal/lobular carcinoma in situ	1.00		1.00	
Invasive lobular carcinoma	6.46 (0.75, 55.38)	0.088	1.13 (0.11, 11.86)	0.917
Invasive ductal carcinoma	12.88 (1.80, 91.87)	0.011	2.06 (0.28, 15.40)	0.481
Stage				
I	1.00		1.00	
II	1.43 (0.77, 2.63)	0.248	2.60 (0.91, 7.42)	0.073
III	6.09 (3.48, 10.65)	0.001	2.66 (0.98, 7.19)	0.054
IV	24.73 (13.44, 45.51)	0.001	7.67 (2.53, 23.25)	0.001
Grade				
1	1.00		1.00	
2	2.08 (1.00, 4.32)	0.050	0.71 (0.24, 2.13)	0.545
3	5.64 (2.74, 11.61)	0.001	1.24 (0.42, 3.70)	0.696
Estrogen receptor				
Negative	1.00		1.00	
Positive	0.60 (0.44, 0.82)	0.002	0.54 (0.28, 1.05)	0.071
Progesterone receptor				
Negative	1.00		1.00	
Positive	0.61 (0.45, 0.83)	0.001	1.22 (0.63, 2.38)	0.548
Human epidermal growth factor receptor 2				
Negative	1.00		1.00	
Positive	1.94 (1.39, 2.71)	0.001	1.28 (0.81, 2.01)	0.296
Distant metastasis				
No	1.00		1.00	
Yes	11.55 (8.19, 16.30)	0.001	11.04 (6.43, 18.96)	0.001
Surgical approach				
Breast-conserving surgery	1.00		1.00	
Modified radical mastectomy	2.49 (1.82, 3.39)	0.001	1.33 (0.79, 2.24)	0.284

[a] adjusted for all variables in the table.

Table 6. Effect of various combined forms of hormone receptors, including estrogen receptor (ER) progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), on survival probability of patients with breast using the multivariate Cox regression model adjusted for age at diagnosis, stage or grade of cancer, distant metastasis, and surgical approach.

Hormone receptor	Number	Percent	Hazard ratio (95% CI)	P value
Full model (8 groups)				
ER+/PR+/HER2–	610	51.5	1.00	
ER–/PR+/HER2–	26	2.2	1.79 (0.52, 6.20)	0.359
ER+/PR–/HER2–	56	4.7	1.05 (0.40, 2.76)	0.929
ER–/PR–/HER2–	209	17.6	2.14 (1.13, 4.03)	0.019
ER+/PR+/HER2+	135	11.4	1.65 (0.83, 3.29)	0.151
ER+/PR–/HER2+	31	2.6	1.16 (0.27, 5.01)	0.844
ER–/PR–/HER2+	108	9.1	1.92 (1.03, 3.59)	0.041
ER–/PR+/HER2+	10	0.8	5.19 (1.51, 17.86)	0.009
Reduced model (4 groups)				
ER/PR+/HER2–	692	58.4	1.00	
ER/PR–/HER2–	209	17.6	1.01 (0.29, 3.49)	0.988
ER/PR+/HER2+	176	14.9	1.67 (0.95, 2.95)	0.077
ER/PR–/HER2+	108	9.1	0.90 (0.26, 3.14)	0.875

Receptor status of breast cancer cell is critical for hormone therapy. This kind of treatment lowers estrogen/progesterone levels or blocks their receptors, and is thus helpful for treatment of ER+ or PR+ cancer cells. However, treatment with hormone therapy is not helpful for hormone receptor-negative cancers. Therefore, hormone receptor-positive cancers have a better prognosis than hormone receptor-negative cancers.[9,22,23] HER2+ breast cancers tend to be aggressive and are usually associated with a worse clinical outcome and poor prognosis,[24,25] as was the case in our study. However, Herceptin in combination with chemotherapy has been shown to be associated with reduced risk of recurrence in patients with HER2+ overexpressing metastatic breast cancers and improvement of cancer-free survival.[26,27]

An important finding of the study from the viewpoint of public health is the stage of breast cancer at diagnosis. Almost 79% of the patients were diagnosed at stage II or higher and more than 35% were diagnosed at stage III or higher. This implies a long delay between the development of breast cancer and its primary diagnosis. This issue should be the focus of special attention of policymakers who plan screening and preventive programs.

We acknowledge the limitations and potential biases of this registry-based retrospective study.[28] The main limitation of this study, like any long-term cohort study, is the censoring. Although censored data are taken into account in calculation of life-table, Kaplan-Meier, and the hazard ratio, censoring may lead to overestimation or underestimation of the results. Furthermore, we excluded 119 breast cancer cases due to unknown pathology. Missing data, especially of ER, PR, and HER2, were another important problem. Incomplete or inaccurate stage/grade identification, lack of central pathology review, and lack of information about the metastatic status were other main limitations. Despite its limitation, we believe our study is of value because it was conducted in a middle-income setting where limited evidence exists and these types of investigations can highlight the prevalence and prognostic effect of immunohistochemistry markers on breast cancer.

In conclusion, we indicated that the subtype ER+/PR+/HER2– was the most common form of breast cancer. We also showed that breast cancer cases with ER–/HER2+ tumors had shorter survival than those with ER+/PR+/HER2– tumors. Triple negative tumors were the only other subtype with a statistically significant poorer prognosis. The results of this study in a middle-income country further indicate the importance of receptor status, in particular HER2 status, in the prognosis of breast cancer.

Conflict of interest statement

The authors declare that they have no conflicts of interest for this work.

Acknowledgments

This was part of the MSc thesis in Epidemiology. We would like to appreciate the Vice-chancellor of Research and Technology of the Hamadan University of Medical Sciences for financial support of this work. We also thank the staffs and managers of the Comprehensive Cancer Control Center affiliated with Shahid Beheshti University of Medical Sciences for their collaboration in this study.

References

1. World Health Organization. Breast cancer: prevention and control. 2015. Available from: URL: http://www.who.int/cancer/detection/breastcancer/en/.(Accessed 5 Feb 2015)
2. Lacey JV Jr., Kreimer AR, Buys SS, Marcus PM, Chang SC, Leitzmann MF, et al. Breast cancer epidemiology according to recognized breast cancer risk factors in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial Cohort. *BMC Cancer.* 2009; 9: 84.
3. International Agency for Research on Cancer. *World Cancer Report 2008.* Lyon: IARC; 2008.
4. Cheraghi Z, Poorolajal J, Hashem T, Esmailnasab N, Doosti Irani A. Effect of body mass index on breast cancer during premenopausal and postmenopausal periods: a meta-analysis. *Plos One.* 2012; 7(12): e51446.
5. Farhadian M, Mahjub H, Poorolajal J, Moghimbeigi A, Mansoorizadeh M. Predicting 5-year survival status of patients with breast cancer based on supervised wavelet method. *Osong Public Health Res Perspect.* 2014; 5(6): 324 – 332.
6. van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskuil DW, et al. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med.* 2002; 347(25): 1999 – 2009.
7. Parise CA, Caggiano V. Breast cancer survival defined by the ER/

PR/HER2 subtypes and a surrogate classification according to tumor grade and immunohistochemical biomarkers. *J Cancer Epidemiol.* 2014; 2014: 469251.
8. WebMD. Types of breast cancer: ER positive, HER2 positive, and triple negative. 2015. Available from: URL: http://www.webmd.com/breast-cancer/breast-cancer-types-er-positive-her2-positive. (Accessed 27 Feb 2015)
9. American Cancer Society. *Breast Cancer*. Atlanta: ACS; 2014.
10. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science.* 1987; 235(4785): 177 – 182.
11. Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Arch Pathol Lab Med.* 2007; 131(1): 18 – 43.
12. Gilcrease MZ, Woodward WA, Nicolas MM, Corley LJ, Fuller GN, Esteva FJ, et al. Even low-level HER2 expression may be associated with worse outcome in node-positive breast cancer. *Am J Surg Pathol.* 2009; 33(5): 759 – 767.
13. Foulkes WD, Smith IE, Reis-Filho JS. Triple-negative breast cancer. *N Engl J Med.* 2010; 363(20): 1938 – 1948.
14. Aghili M, Lashkari M, Farrokhpey AH, Izadi S. Triple-negative breast cancer survival in Iranian patients. *Acta Med Iran.* 2013; 51(8): 560 – 566.
15. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res.* 2007; 13(15 Pt 1): 4429 – 4434.
16. World Health Organization. *International Classification of Diseases for Oncology: ICD-O-3.* 3rd ed. Geneva: WHO; 2000.
17. Ménard S, Fortis S, Castiglioni F, Agresti R, Balsari A. HER2 as a prognostic factor in breast cancer. *Oncology.* 2001; 61(Suppl 2): 67 – 72.
18. Cheang MC, Chia SK, Voduc D, Gao D, Leung S, Snider J, et al. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. *J Natl Cancer Inst.* 2009; 101(10): 736 – 750.
19. De Maeyer L, Van Limbergen E, De Nys K, Moerman P, Pochet N, Hendrickx W, et al. Does estrogen receptor negative/progesterone receptor positive breast carcinoma exist? *J Clin Oncol.* 2008; 26(2): 335 – 336.
20. Ng CH, Pathy NB, Taib NA, Mun KS, Rhodes A, Yip CH. The estrogen receptor negative-progesterone receptor positive breast carcinoma is a biological entity and not a technical artifact. *Asian Pac J Cancer Prev.* 2012; 13(4): 1111 – 1113.
21. Rakha EA, El-Sayed ME, Green AR, Paish EC, Powe DG, Gee J, et al. Biologic and clinical characteristics of breast cancer with single hormone receptor positive phenotype. *J Clin Oncol.* 2007; 25(30): 4772 – 4778.
22. Purdie CA, Quinlan P, Jordan LB, Ashfield A, Ogston S, Dewar JA, et al. Progesterone receptor expression is an independent prognostic variable in early breast cancer: a population-based study. *Br J Cancer.* 2014; 110(3): 565 – 572.
23. Davies C, Pan H, Godwin J, Gray R, Arriagada R, Raina V, et al. Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. *Lancet.* 2013; 381(9869): 805 – 816.
24. Domingo-Domenech J, Fernandez PL, Filella X, Martinez-Fernandez A, Molina R, Fernandez E, et al. Serum HER2 extracellular domain predicts an aggressive clinical outcome and biological PSA response in hormone-independent prostate cancer patients treated with docetaxel. *Ann Oncol.* 2008; 19(2): 269 – 275.
25. Tokunaga E, Okada S, Yamashita N, Akiyoshi S, Kitao H, Morita M, et al. High incidence and frequency of LOH are associated with aggressive features of high-grade HER2 and triple-negative breast cancers. *Breast Cancer.* 2012; 19(2): 161 – 169.
26. Gijsen M, King P, Perera T, Parker PJ, Harris AL, Larijani B, et al. HER2 phosphorylation is maintained by a PKB negative feedback loop in response to anti-HER2 herceptin in breast cancer. *PLoS Biol.* 2010; 8(12): e1000563.
27. Shak S. Overview of the trastuzumab (Herceptin) anti-HER2 monoclonal antibody clinical program in HER2-overexpressing metastatic breast cancer. Herceptin Multinational Investigator Study Group. *Semin Oncol.* 1999; 26(4 Suppl 12): 71 – 77.
28. Izquierdo JN, Schoenbach VJ. The potential and limitations of data from population-based state cancer registries. *Am J Public Health.* 2000; 90(5): 695 – 698.