RESEARCH ARTICLE

Vascular Invasion as an Independent Prognostic Factor in Lymph Node Negative Invasive Breast Cancer

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Abstract

Introduction: Identification of simple and measurable prognostic factors is an important issue in treatment evaluation of breast cancer. The present study was conducted to evaluate the prognostic role of vascular invasion in lymph node negative breast cancer patients. Methods: in a retrospective design, we analyzed the recorded profiles of the 1,640 patients treated in the breast cancer department of Motahari clinic affiliated to Shiraz University of Medical Sciences, Shiraz, Iran, from January 1999 to December 2012. Overall and adjusted survivals were evaluated by the Cox proportional hazard model. All the hypotheses were considered two-sided and a p-value of 0.05 or less was considered as statistically significant. Results: Mean age in lymph node negative and positive patients was 50.0 and 49.8 respectively. In lymph node negative patients, the number of nodes, tumor size, lymphatic invasion, vascular invasion, progesterone receptor, and nuclear grade were significant predictors. In lymph node and lymphatic negative patients, vascular invasion also played a significant prognostic role in the survival which was not evident in lymph node negative patients with lymphatic invasion. Discussion: The results of our large cohort study, with long term follow up and using multivariate Cox proportional model and comparative design showed a significant prognostic role of vascular invasion in early breast cancer patients. Vascular invasion as an independent prognostic factor in lymph node negative invasive breast cancer

Keywords: Node negative - breast cancer - vascular invasion - prognostic factor - survival

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Introduction

Breast cancer is the most common cancer affecting the women. The major cause of short survival in breast cancer is dispersion of malignant cells from the primary location leading to formation of metastases. Metastasis of a malignant tumor occurs by the tumoral cells passing from sequential stages known as the metastatic cascade. One of the important steps in this process is the invasion of tumoral cells in the lymphovascular component around the cancer cellular matrix. Although no single special prognostic factor has been reported for breast cancer, many useful clinicopathological indicators have been identified (Bekir, 2003; Goldhrisch et al., 2005).

Identification of clinically predictive and prognostic factors is considered as an important issue in treatment evaluation of breast cancer. Moreover, an ideal prognostic factor would be capable of predicting the development of metastasis and overall survival in all patients and can be measurable in the primary tumor following the initial treatment (Bekir, 2003). A number of host and disease related factors, such as age at diagnosis time, menstrual and menopausal age, tumor size, nuclear grade, surgical margin status, estrogen and progesterone receptors, lymphovascular invasion around tumoral matrix, and axillary lymph node status, are well-established prognostic factors in breast cancer survival (Goldhirsch et al., 2003; 2005; 2007).

In lymph node positive patients, involvement of axillary lymph node is a signal of local invasion of the tumor and may be accompanied by metastasis. It has also been considered as an independent predictor in a large number of studies (Carol et al., 2002; Kim et al., 2011; Song et al., 2011). In addition, involvement of axillary lymph node is an important determinant in patient staging in TNM system. On the other hand, logically speaking, a correlation is expected to exist between lymphatic component invasions and axillary lymph node involvement. It has also been shown that lymphovascular space invasion is positively correlated with regional lymph node metastasis and a greater recurrence rate in many cancers.

In Lymph node negative patients, the scenario is much more different. In these patients, finding relevant and more accurate prognostic factors indicating the progress of the disease in the primary stages can play a major role in increasing the survival rate and designing the best treatment protocol. One of the most suitable factors notably evaluated by the researchers is lymphovascular invasion. Up to now, a great number of studies have been conducted in order to find the prognostic role of lymphovascular invasion in breast cancer, especially in

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node negative patients (Sebastian et al., 2004; Pauline et al., 2005; Gurleyik et al., 2011). It seems that this factor may be an adverse prognostic factor for survival in patients with breast cancer, especially those with the node negative disease. Although many reports have suggested that lymphovascular, vessels involvement may have a more important role in the initial dissemination of breast cancer, whether vascular or lymph vessels invasion or both have equal roles in prognosis and prediction, especially in lymph node negative breast cancer, is still a remarkable question.

The present study aims to evaluate the prognostic role of vascular invasion in invasive breast cancer, especially in lymph node negative patients. The researchers attempt to consider and control the effect of confounder and intermediated prognostic risk factors, which had been traditionally, entered into multivariate survival analysis models for assessing the unbiased effect of vascular invasion on the overall survival rate.

Materials and Methods

The present study was in fact a retrospective analysis of the recorded profiles of the patients treated in breast cancer department of Motahari clinic affiliated to Shiraz University of Medical Sciences, Shiraz, Iran. The study data were extracted from a prospective database of 2,863 patients with stage I, II, or III invasive breast cancer. We retrospectively reviewed all the profiles of the patients who had undergone surgical treatment at the department from January 1999 to December 2012 and 1,640 records which were more eligible were selected for the final analysis. In this study, the overall survival rate refers to the 12-year survival rate.

All the patients were visited in the hospital at least every 6 months for 5 years and then at least once a year. The follow-up after diagnosis was done according to the protocols established at the clinic, including hospital charts, physician records, and approved cancer registries. Afterwards, the data were entered into the SPSS statistical software (v. 15) using the double entry approach and the entered data were randomly rechecked by an independent observer. In order to increase the accuracy of the data records and survival status of the patients, 620 patients' records were randomly selected and followed up through phone procedure by the expert clinic personnel. Finally, the selected variables were extracted from the patients' records and divided into two categories of host- and disease- related variables.

The only host-related variables entered into the models were age at primary operation (<45 or ≥45 year) and the number of months the patients lived after the operation. On the other hand, the disease-related variables included the tumor size (cm), lymph node status (either involved or free), number of removed lymph node (<22, ≥22), nuclear grade (well, moderate, poor), and presence or absence of lymphatic, vascular, or lymphovascular invasion. Pathological lymphovascular invasion was defined as the presence of tumor emboli within peritumoral endothelial-lined spaces and could distinguish between lymphatic and blood vessels components. Practically, lymphovascular invasion was considered positive if vascular or lymphatic invasion was present. According to the protocol established in the cancer registry center, tumor size was defined and measured as the largest diameter of the invasive component. Moreover, hormonal receptor status was determined through radioimmunoassay or immunohistochemistry methods. Lymph nodes were stained with hematoxylin and eosin and examined for tumor cell metastasis. In addition, in case 3 or more axillary lymph nodes were positive, the patients were considered as lymph node positive, while those with less than 3 positive axillary lymph nodes were considered as lymph node negative patients.

Hormone receptors (estrogen and progesterone receptors) status was determined by immunohistochemical analysis using a tissue microarray. Hormone receptors were considered positive if the expression was ≥10%. It should be noted that estrogen and progesterone receptor results were entered into the models as binary variables (positive, negative). In this study, the herceptin peptide expression results were obtained through immunohistochemical analysis, scored as 0, 1+, 2+, or 3+, and entered into the models with positive or negative codes. The results were considered positive if the patients had obtained a score of 2+ or 3+. Finally, the survival time was defined as the period between the operation and death and the patients who were alive at the end time of the study were considered as the censored patients. All the patients were managed and operated by one expert surgeon.

Statistical methods

Categorical variables were presented as frequency (percent), continuous normally distributed variables as mean±SD, and those not normally distributed as the median (±inter-quartile range). Before entering all the variables into the multivariate analysis, inter-correlation between important prognostic factors, such as lymphatic, vascular, lymphovascular invasion, and the patients' clinicopathological data, was analyzed using appropriated correlation coefficient and chi-square test. Besides, overall survival (OS), adjusted overall survival rate for lymph node status, and tumor size were evaluated through the Kaplan–Meier method. After selecting the variables in univariate analysis, overall survival and adjusted survival rates were evaluated by Cox proportional hazard model. All proportional hazard assumptions were verified using the Grambsch-Therneau test. The log-rank test was also used in order to compare the curves of two or more groups. All the hypotheses were considered two-sided and a p-value of 0.05 or less was considered as statistically significant.

Results

The mean age of the patients was 49.85 (49.33-50.42) years and their median age was 49 (34-64) years. Mean and standard error of age in lymph node negative and positive patients was 49.96-0.384 and 49.78-0.4, respectively.

The mean and 95% confidence interval follow up period was 60.93 (59.35-62.51) months and the median

Table 1. The Results of Univariate Survival Analysis by the Kaplan-meier Model in the Two Groups

Predictor	Level	Lymph node negative			Lymph node positive		
		12 year survive	Log rank	P value Chi square	12 year survive	Log rank Chi square	P value
Number of node	> 22	82.3	0.72	0.39	79.6	5.89	0.015*
	≤22	80.7			66.5		
Age group(year)	<45	76.4	1.28	0.26	72.6	1.82	0.177
	≥45	79.2			68		
Tumor size(cm)	<2	79	7.61	0.022***	77	11.39	0.003*
	2-5	76.5			63.9		
	>5	71.6			73.4		
Lymphatic invasion	Free	82.9	19.72	0.000***	80.9	4.27	0.038*
	Involved	69.9			66		
Vascular invasion	Free	82.6	16.39	0.000***	73	9.2	0.002*
	Involved	73.4			62.3		
Estrogen receptor	Positive	84.1	2.04	0.36	74.6	8.3	0.016*
	Negative	75.3			63		
Progesterone receptor	Positive	79	3.59	0.166	74	12	0.002*
	Negative	73.8			63		
Herceptin protein	Negative	75.4	0.57	0.45	75.1	9.67	0.002*
	Positive	79.3			59.7		
Nuclear grad	One	78.1	6.9	0.032***	75.7	6.8	0.033*
	Two	76.4			67		
	Three	74.8			65.3		
Lymphovascular invasion	Free	81.7	24.2	0.000***	78.2	15.84	0.000*
	Involved	73.6			71.1		

and inter-quartile follow up period was 52.77 (5.7-99.9) months. In addition, the mean and 95% confidence interval of follow up time in lymph node negative and positive patients was 63.15 (60.9-65.39) and 58.67 (56.45-60.89) months, respectively. Besides, the minimum and maximum periods of the patients' presence in the study were 1.97 and 154.6 month, respectively. Contingency correlation coefficient between axillary lymph node and lymphovascular invasion was 0.63 and 0.8, respectively and was statistically significant (p value≤0.05). The results of univariate survival analysis are shown in Table 1.

In univariate survival analysis through the Kaplan-meier method, the overall survival rate was estimated as 75% with 95% confidence interval (71.2-78.9). Moreover, the mean survival time was estimated as 132.18 and the confidence interval was 129.33-135.04. The study results revealed a significant difference between the overall survival rates of different levels of tumor size, nuclear grade, lymphatic, vascular, lymphovascular invasion, and axillary lymph node status; however, no significant difference was observed between the age groups regarding the overall survival rate (Table 1).

In lymph node negative and positive patients, survival rates were significantly different between the subgroups of vascular, lymphatic and lymphovascular invasion. In lymph node and lymphatic negative patients, vascular invasion played a significant prognostic role in the survival rate (Mantel-Cox=8.21 p-value=0.004), while it did not have any significant prognostic roles in the survival rate of lymph node negative patients with lymphatic invasion (mantel- Cox=2.88 p-value=0.09).

After univariate analysis, variables, such as tumor size, estrogen receptor, nuclear grade, and lymphatic and vascular invasion in lymph negative patients and all the predictor except for age group in lymph node positive ones were eligible for being entered into the multivariate Cox model.

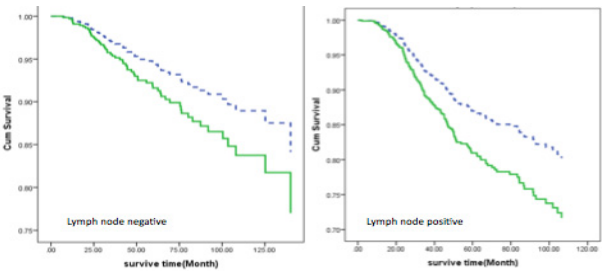

Figure 1. Adjusted Cox Proportional Survival Functions in Lymph Node Negative and Positive Patients Treated Separately

Finally, in lymph node negative patients (Table 2), the number of nodes, tumor size, lymphatic invasion, vascular invasion, progesterone receptor, and nuclear grade were significant predictors in multivariate Cox proportional hazard model. In lymph node positive group, on the other hand, the number of nodes, tumor size, progesterone receptor, and herceptin peptide were the significant predictors which remained in the model.

Adjusted Cox proportional survival functions in lymph node negative and positive patients are separately shown in Figure 1.

Table 2. Overall Survival Rate Comparison in Sub Groups of Lymph Node Negative

Lymphatic status	Vascular invasion	12- year survival	Mantel- Cox	df	p-value
Free	Free	84.0	8.21	1	0.004*
	Invasion	69.9			
Invasion	Free	69.7	2.88	1	0.090**
	Invasion	67.0			

*Statistically significant. **Statistically not significant but clinically notable

Table 3. Results of Cox Regression Survival Analysis

Variable	Lymph node negative					Lymph node positive				
	HR	Se	CI		P value	HR	Se	CI		P value
Age group	1.38	0.406	0.775	2.46	0.273	1.14	0.193	0.818	1.591	0.438
Estrogen receptor	0.778	0.245	0.419	1.444	0.427	1.203	0.211	0.852	1.697	0.294
Nuclear grade	1.269	0.272	0.833	1.933	0.266	1.254	0.153	0.987	1.593	0.064
Number of node	0.757	0.202	0.449	1.28	0.3	1.589	0.309	1.085	2.328	0.017*
Tumor size	1.449	0.323	0.936	2.244	0.096**	1.439	0.195	1.103	1.878	0.007*
Herceptin protein	1.409	0.396	0.812	2.446	0.222	1.678	0.275	1.216	2.316	0.002*
Lymphatic involvement	2.765	0.819	1.548	4.941	0.001*	1.443	0.305	0.953	2.184	0.083**
Vascular involvement	1.968	0.569	1.116	3.472	0.019*	1.356	0.229	0.973	1.889	0.072**
Progesterone receptor	1.891	0.565	1.053	3.396	0.033*	1.353	0.275	1.21	2.316	0.069**

*Statistically significant. **Statistically not significant but clinically notable

Discussion

Since many years ago, five traditional prognostic factors have been used for decision therapy in breast cancer: age at disease diagnosis, lymph node status, tumor size, estrogen receptor, and nuclear grade. Of course, performing scientific research works still continues in order to identify more prognostic factors in node negative breast cancer. The St. Gallen consensus meeting suggested differentiating between the high and low risk groups in node negative patients who may benefit from adjuvant therapy (Goldhrisch et al., 2005). Our study demonstrated that in lymph node negative patients, both lymphovascular invasion and vascular as well as lymphatic invasion, as separate risk factors, have independent prognostic roles in 12-year survival.

The results of both univariate and multivariate analyses in the present study showed that the total removed lymph node only played a significant role in overall survival rate in lymph node positive patient, which is consistent with the results of the studies such as (Bekir et al., 2003; Polednak 2003; Yu et al., 2008; Port et al., 2010; Young, 2011), while in contrast with those of the study by Kim et al. (2011).

In line with the results of the present study, there is a global consensus about the prognostic role of Tumor size in survival of node negative patients (Carter et al., 1989; Collett et al., 1994; Fisher et al., 1997; Goldhirsch et al., 2001; Carol et al., 2002; Bekir et al., 2003; Polednak 2003; Sebastian et al., 2004; Gurleyik et al., 2007; Park et al., 2008; Lee et al., 2009; Song et al.,, 2011; Clayton et al., 2012). In this study, the 12-year survival rate was equal to 87 % in less or equal to 20 mm tumor size in comparison to 71.6% in greater than 50 mm tumor size (Table 1), which is in contrast with the results of the study conducted by Sebastian et al. (2004).

Consistent with the results of a large number of studies, our study findings showed that nuclear grade played a prognostic role in node negative patients (Yuan et al., 1992; Gasparini et al., 1994; Carlmagno et al., 1996; Andrulis et al., 1998; Carol et al., 2002; Bekir et al., 2003; Polednak, 2003; Sebastian et al., 2004a; 2004b; Gurleyik et al., 2007; Lee et al., 2009; Nimeus-Malmstrom et al., 2010; Song et al., 2011; Clayton et al., 2012). In the current study, the survival rate was obtained as 74.8% in grade three compared to 87.1% in grade one (Table 1), which is in contrast with the study by Ashwini et al. (2008).

On the contrary to the findings of many studies, no significant role was found for age in the 12-year survival rate in negative or positive lymph node patients (Yuan et al., 1992; Bekir, 2003; Polednak, 2003; Ashwini et al., 2008; Florence et al., 2010). This finding is consistent with the results of a few studies conducted on the issue (Gasparini et al., 1994; Carol et al., 2002; Polednak, 2003; Sebastian et al., 2004a; 2004b; Nimeus-Malmstrom et al., 2010; Song et al., 2011).

Our study results showed a significant role for estrogen receptor only in node positive patients; nevertheless, this role did not remain significant in multivariate Cox analysis (Table 3). It seems that estrogen receptor status has a predictive value in combination with adjuvant therapy; however, the effect of estrogen receptor status alone on the survival rate is questionable (Sebastian et al., 2004a; 2004b). This finding is in contrast with those of a great number of studies (Yuan et al., 1992; Carol et al., 2002; Gurleyik et al., 2007; Florence et al., 2010).

In our study, progesterone receptor did not show any significant roles in prognosis and survival rate in lymph node negative patients, while it revealed a significant role in 12-year survival in lymph node positive patients. These findings are in line with the results of a large number of studies (Yuan et al., 1992; Gasparini et al., 1994; Carlmagno et al., 1996; Carol et al., 2002; Gurleyik et al., 2007; Florence et al., 2010), while in contrast with those of Androlis' study (Andrulis et al., 1998). Moreover, Herceptin protein had only a significant effect on survival in lymph node positive patients, which is in agreement with the results of the study by Flourence et al. (2010).

Although few studies have reported controversial roles for lymphovascular invasion as a prognostic factor (3, 14, 21), a great number of studies have confirmed the important role lymphovascular invasion plays in survival prognosis of breast cancer patients (Neville et al., 1992; Lauria et al., 1995; Camp et al., 2000; Voogd, 2001; Woo et al., 2002; Kuru et al., 2003; Sebastian et al., 2004; Pauline et al., 2005; Ashwini et al., 2008). The results of the present study are in line with those of the most recent studies and show the important role lymphovascular invasion plays as an independent prognostic risk factor in both lymph node negative and positive patients.

On the other hand, although lymph node negative breast cancer patients have favorable prognosis in short and long time, determining highly specific prognostic factors which could distinguish between low- and high-risk groups is important in clinical decision-making

(Bekir, 2003; Goldhrisch et al., 2005).

Although the prognostic role of lymphovascular invasion has been known since many years ago, many researchers have tried to distinguish between vascular and lymphatic components, especially as independent factors, in lymph node negative patients (Teel, 1964; Fujimori et al., 1968; Pinder et al., 1994; Mohammed et al., 2009). In spite of these studies, vascular invasion was not entered into the risk categories for adjuvant therapy in the final report of the 8th St. Gallen international meeting in 2003. In 9th St. Gallen meeting in 2005 (Goldhrisch et al., 2005), in spite of the agreement of the majority of the panelists regarding the vascular invasion's being a risk factor, it was added to the risk categories as the controversial risk factor. Therefore, it is necessary to conduct more accurate cohort studies using multivariate analysis in order to assess the prognostic role of vascular invasion in breast cancer. The results of our large cohort study (1,640 patient) with long term follow up and using multivariate Cox proportional model and comparative design showed the significant prognostic role of vascular invasion in early breast cancer patients.

In this study, we could not find any studies focusing on vascular invasion as a prognostic factor especially on lymph node negative patients. Our study results suggested lymphovascular invasion as an independent prognostic factor in breast cancer and vascular component as a separate factor which plays a prognostic role in the survival rate of the patients, particularly lymph node negative patients. These results are more strongly supported by this finding that the prognostic role of vascular invasion had remained significant in lymph node and lymphatic negative patients, while vascular invasion played no significant roles in the survival rate of lymph node negative, lymphatic positive patients (Table 2 and 3).

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