Original Article

J Breast Health 2017; 13: 5-9
DOI: 10.5152/tjbh.2016.3142

Association of ICAM-1, VCAM-1, CYCLIN D1 and Cathepsin D with Clinicopathological Parameters in Breast Carcinoma; an Immunohistochemical Study

Özgür Külahcı[1], H. Hasan Esen[2], Elife Asut[1], Salim Güngör[2]
[1]Clinic of Pathology, Adana Numune Training and Research Hospital, Adana, Turkey
[2]Department of Pathology, Necmettin Erbakan University Meram School of Medicine, Konya, Turkey

ABSTRACT

Objective: Breast carcinoma is the most common malignant tumor detected in women. The hypothesis that increased levels of adhesion molecules and Cathepsin D affect cancerous cells moving away the primary tumor and contributes to migration of the cancerous cell and may cause remote organ metastases is defended. The aim of the present study was to search the association of intracellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), Cyclin D1, cathepsin D immunohistochemically with clinicopathological parameters in the patients diagnosed with invasive ductal breast carcinoma.

Materials and Methods: The pathological slides of 153 patients diagnosed with invasive ductal carcinoma were evaluated retrospectively. Three groups were created. Group 1 consisted of patients with positive lymph node metastasis and extranodal tumor invasion; Group 2 consisted of patients with positive axillary lymph node metastasis and negative extranodal tumor invasion and Group 3 consisted of the patients with negative axillary lymph node metastasis. In all groups, 20 paraffin blocks belonging to the primary tumor in the breast were stained by ICAM-1, VCAM-1, Cyclin D1 and Cathepsin D. Findings were examined by comparing with clinicopathological parameters.

Results: The highest number of metastatic axillary lymph nodes and the highest rate of cathepsin D staining were statistically found in the cases with positive axillary lymph node metastasis and extranodal tumor invasion. CerbB2 was negative in the cases with negative ICAM-1 whereas estrogen receptor and progesterone receptor were positive in the cases with positive VCAM-1.

Conclusion: The present study reveals significant results for the patients diagnosed with invasive ductal carcinoma through breast biopsy especially before mastectomy in terms of increased number of metastatic axillary lymph nodes and extranodal tumor invasion by immunohistochemical Cathepsin D stain without any additional invasive intervention. Results of the present study may contribute to monitoring and treatment of the patients in the future.

Keywords: Breast carcinoma, Cathepsin D, immunohistochemistry, metastasis

Introduction

Breast cancer is the most common malignant tumor observed in women and 70 to 80% of the cases with breast cancer have invasive ductal carcinoma (1). Among the prognostic factors for survey, the most important accepted prognostic parameter is metastasis of the axillary lymph node. Genetic and molecular studies enabling early diagnosis and treatment of the breast cancer accelerated because of high mortality rate and incidence of breast cancer (2). It is reported that adhesion molecules of endothelial cell have a very important role in tumor formation and defense mechanism of the host. Major adhesion molecules are intracellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM) and E-Selectin. The hypothesis that increased levels of adhesion molecules affect cancerous cells moving away the primary tumor and contributes to migration of the cancerous cell and may cause remote organ metastases is defended. This appears as an important factor to determine survey of the patients (3). Cyclin D1 has a critical role in progression of the cell cycle of the protein. Disruption in expression regulation of this protein is held responsible from development of many malignant tumors including breast cancer. Since cyclin D1 is a major oncogene for human and appears as a pathogenic headstone for many breast cancer types, benefit of anti- cyclin D1 therapy is predicted on selected eligible tumors (4, 5). Cathepsin D is a lysosomal aspartyl protease which acts as a mature active enzyme in the lysosomes in an acidic pH and synthesized by stromal reactive cells and macrophages. There are studies suggesting that this protein may have a prognostic value to determine the survey in the patients with breast cancer by facilitating tumor progression by invasion followed by metastasis through breaking down the proteoglycan substances on the stromal matrix and basal membrane (6, 7). The aim of the present study is to search the

Address for Correspondence :
Özgür Külahcı, e-mail: ozgurkulahci@hotmail.com

Received: 23.05.2016
Accepted: 03.10.2016

association of ICAM-1, VCAM-1, Cyclin D1 and Cathepsin D immunohistochemically with clinicopathological parameters in the patients diagnosed with invasive ductal breast carcinoma.

Material and Methods

This study was approved by Selçuk University Scientific Research Project Coordinatorship of the ethics committee. The pathological slides of 153 patients diagnosed with invasive ductal carcinoma were evaluated retrospectively. All of the cases were women diagnosed with invasive ductal carcinoma. All of the patients had modified radical mastectomy and axillary lymph node dissection.

Microscopic Examination

Slides stained with Hematoxylen & Eosin (H&E) were examined under a microscope. Clinicopathological parameters were age, location on the right or left breast, tumor diameter, Paget's disease, axillary lymph node metastasis, number of metastatic axillary lymph nodes, extranodal tumor invasion, histological grade (Nottingham Histological Score), multifocality, DCIS, quadrant location, imunohistochemically stained estrogen receptor (ER) and progesterone receptor (PR), human epidermal growth factor receptor (HER-2/cerbB2) slides. Three individual groups were created. Group 1 consisted of patients with positive lymph node metastasis and extranodal tumor invasion; Group 2 consisted of patients with positive axillary lymph node metastasis and negative extranodal tumor invasion and Group 3 consisted of the patients with negative axillary lymph node metastasis. In all groups, 20 paraffin blocks belonging to the primary tumor in the breast were stained by ICAM-1, VCAM-1, cyclin D1 and cathepsin D. Slides were examined by comparing the aforesaid clinicopathological parameters.

The Immunohistochemical Examination

Twenty cases were selected randomly from three groups 60 slides were stained immunohistochemically with Thermo Fisher Scientific-Lab Vision CD54/ICAM-1 Ab-4 (Clone 23G12), CD106/VCAM-1 Ab-3 (Clone 1.4C3), Cyclin D1/Bcl-1 (SP4), Cathepsin D Ab-1 antibodies. Cytoplasmic staining for ICAM-1 and VCAM-1 was accepted positive and the following classification was done for staining; grade:0 for <10% of the cells stained, 1+ for 11% to 20% of the cells stained, 2+ for 21%to 75% of the cells stained, 3+ for >75% of the cells stained (8). For cyclin D1, nuclei of the cells were classified as 0 for no staining, 1+ for up to 50% of the cells stained, 2+ for >50% of the cells stained according to nuclear staining grade (9). Cytoplasmic staining for cathepsin D was accepted positive and the following classification was done for staining grade:0 for no staining, 1+ for <10% cells stained, 2+ for 10-50% of the cells stained, 3+for >50% of the cells stained (9). Nuclear staining for ER/PR was accepted positive and the following classification was done for staining grade; scoring system 0 negative for receptor, 1+ borderline, 2+ to 3+ positive for receptor; criteria 0: 0% nuclear staining, 1+ <10% nuclear staining, 2+ 10% to 75% nuclear staining, 3+ >75% nuclear staining. Cytoplasmic membrane staining for cerbB2 was accepted positive and the following classification was done for staining grade: Scoring system 0 Negative, 1+ Negative, 2+ Weak positive, 3+ Positive;

Criteria:

0 Negative. No staining is observed, or membrane staining is <10% of the tumor cells.

1+ Negative. A faint /barely perceptible membrane staining is detected in >10% of the tumor cells. The cells are only stained in part of the membrane.

2+ Weak positive. A weak to moderate complete membrane staining is observed in >10% of the tumor cells.

3+ Positive. A strong complete membrane staining is observed in >10% of the tumor cells (10).

For positive controls, tonsil tissue for ICAM-1, placenta for VCAM-1, an intestinal tissue diagnosed with mantle zone lymphoma for cyclin D1, a hepatic tissue for cathepsin D, breast tissue for ER/PR and positive stained breast carcinoma for cerbB2 were used. For such four antibodies, 1000 cells were counted under 40x magnification conditions on most densely stained areas under a light microscope and values were expressed in percentages.

Statistical analysis

The Statistical Package for the Social Sciences version 10.0 (SPSS Inc.; Chicago, IL, USA) was used to analyze the statistical data. Along with descriptive statistical methods (Mean, Standard deviation), Kruskal-Wallis test was used to compare the quantitative data for evaluation of the study data. Ki-square test was used for comparison of the qualitative data. Results were evaluated within 95% confidence interval with a statistical significance value of $p<0.05$.

Results

The number and percentage of patients in group1, group 2 and group 3 were 24% (n=37), 46% (n=70) and 30% (n=46), respectively (Table 1). A statistically significant difference was found between the groups for number of metastatic axillary lymph nodes (Table 2). It was noted that the metastatic axillary lymph node count was more in group 1 with four or more lymph nodes. A statistically significant difference was found between the groups for cathepsin D (Table 3). Cathepsin D positivity was noted in group 1 (Figure 1). There was not any statistical association between the groups and ICAM-1, VCAM-1 and cyclin D1 ($p>0.05$). A significant difference was found between the groups with negative and positive cathepsin D staining in terms of extranodal tumor invasion in metastatic axillary lymph nodes (Table 4). Cathepsin D was found positive in the cases with positive extranodal tumor invasion. There was not any statistical association in the metastatic axillary lymph nodes by ICAM-1 VCAM-1, cyclin D1 in terms of extranodal tumor invasion ($p>0.05$). A statistically significant difference was found between negative and positive groups stained by VCAM-1 and ER staining ($p=0.019$). It was noted that VCAM-1 positive cases were also positive with ER (Figure 2). There was not any statistical association between the groups ICAM-1, cyclin D1, cathepsin D and ER ($p>0.05$). A statistically significant difference was found between negative and positive groups stained by VCAM-1 and PR staining ($p=0.006$). It was noted that VCAM-1 positive cases were also positive with PR. There was not any statistical association between the groups ICAM-1, cyclin D1 and cathepsin D and PR ($p>0.05$). A statistically significant difference was found between positively and negatively stained by ICAM-1 and cerbB2 ($p=0.03$). Negative cerbB2 values were noted in ICAM-1 negative cases. There was not any statistical association between VCAM-1, cyclin D1, cathepsin D and cerbB2 ($p>0.05$).

Discussion and Conclusion

Genetic molecular factors on cell cycle, proliferation and apoptosis are predominantly considered by many researchers. Increased levels of adhesion molecules cause cancerous cells to move away from the primary tumor and contributes to migration of the cancerous cell and may cause remote organ metastases. This appears as an important factor to determine survey of the patients (3). Metastasis of a tumor is a complex process including several stages. Invasion to the extracellular

Table 1. The association between the groups and clinicopathological parameters

	Group I: (n=37)	Group II: (n=70)	Group III: (n=46)
Age	51.85±12.47 (min: 32, max: 79)	60.55±14.17 (min: 31, max: 78)	50.40±8.16 (min: 33, max: 63)
Right breast	49% (n=18)	51% (n=36)	52% (n=24)
Left breast	51% (n=19)	49% (n=34)	48% (n=22)
Tumor diameter			
≤2cm	19% (n=7)	29% (n=20)	48% (n=22)
2.1-4.9 cm	43% (n=16)	50% (n=35)	39% (n=18)
≥5 cm	38% (n=14)	21% (n=15)	13% (n=6)
Paget's disease			
None	89% (n=33)	97% (n=68)	93% (n=43)
Present	11% (n=4)	3% (n=2)	7% (n=3)
Lymph node metastasis			
None	0% (n=0)	0% (n=0)	100% (n=46)
1-3	16% (n=6)	70% (n=49)	
≥4	84% (n=31)	30% (n=21)	
Histological grade			
I	11% (n=4)	30% (n=21)	41% (n=19)
II	78% (n=29)	60% (n=42)	46% (n=21)
III	11% (n=4)	10% (n=7)	13% (n=6)
Number of foci			
1	92% (n=34)	94% (n=66)	100% (n=46)
2	5% (n=2)	6% (n=4)	
3	3% (n=1)		
DCIS			
None	54% (n=20)	80% (n=56)	61% (n=28)
Present	46% (n=17)	20% (n=14)	39% (n=18)

Table 2. The association between the groups and number of metastatic axillary lymph nodes

	Group 1		Group 2		Group 3	
Axillary lymph node metastasis	**n**	**%**	**n**	**%**	**n**	**%**
None	0		0		46	100
1-3	6	16	49	70	0	
>4	31	84	21	30	0	
p=0.001						

matrix is required for participation of a tumor cell into the circulation. Function of E-cadherin disappeared in all epithelial cancers by mutation-induced inactivation of E-cadherin genes or activation of β-cathenin genes. Modifications appeared in VCAM-1 and ICAM-1 which are adhesion molecules in the immunoglobulin family contribute to the invasion (11).

Table 3. The association of CD staining between the groups

	Group 1		Group 2		Group 3	
CD staining	**n**	**%**	**n**	**%**	**n**	**%**
Negative	1	5	9	45	4	20
1 (+)	3	15	5	25	3	15
2 (+)	4	20	5	25	2	10
3 (+)	12	60	1	5	11	55
p=0.001						

Table 4. The association between CD staining and extranodal tumor invasion in the metastatic axillary lymph nodes

	CD negative		CD positive	
Capsule invasion	**n**	**%**	**n**	**%**
None	13	32	27	68
Present	1	5	19	95
p=0.040				

A statistically significant association was found between ICAM-1 and cerbB2. Negative cerbB2 values were noted in ICAM-1 negative cases. There are different outcomes addressing the association between ICAM-1 and prognostic factors of the breast cancer in the literature. On the other hand, more studies indicating the association between ICAM-1 and cerbB2 are required. A statistically significant association was found between VCAM-1 and ER and PR staining in the present study. Positive ER and PR results were noted in VCAM-1 positive cases. Similarly, there are different outcomes addressing the association between VCAM-1 and prognostic factors of the breast cancer in the literature.

Cyclin D1 staining rate was found 75%, 45% and 65% in group1, group2 and group 3, respectively and such results were consistent with the studies in the literature.

Cathepsin D is a proteolytic enzyme which was first identified in 1980 and released by cancerous and stromal cells. There are studies suggesting that this protein may have a prognostic value to determine the survey in the patients with breast cancer by facilitating tumor progression by invasion followed by metastasis through breaking down the proteoglycan substances on the stromal matrix and basal membrane (6). Cathepsin D is an immunohistochemical marker which may be used to determine the survey rate for the patients with breast cancer; clinical studies showed that increased cathepsin D level in the breast cancer cells is an independent determinant for early recurrence and death rates (12). There are studies indicating that high cathepsin D levels show poor prognosis. Some studies showed an increase in cathepsin D expression rate in the stroma cells by increase of tumor grade in invasive ductal carcinoma (13). Furthermore, a study detected a positive correlation between cathepsin D expression of tumor cells immunohistochemically and survey of the patients with breast cancer (14); however, some studies showed negative correlation (12). A statistically significant association was found between cathepsin D and extranodal

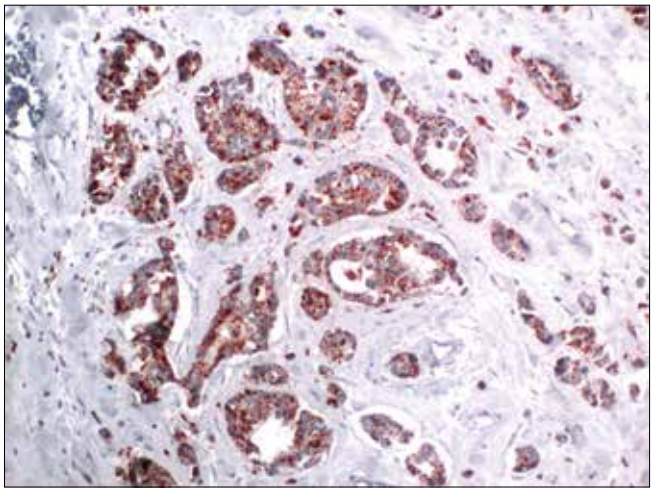

Figure 1. Positive cytoplasmic staining with Cathepsin D in the tumor cells (x100)

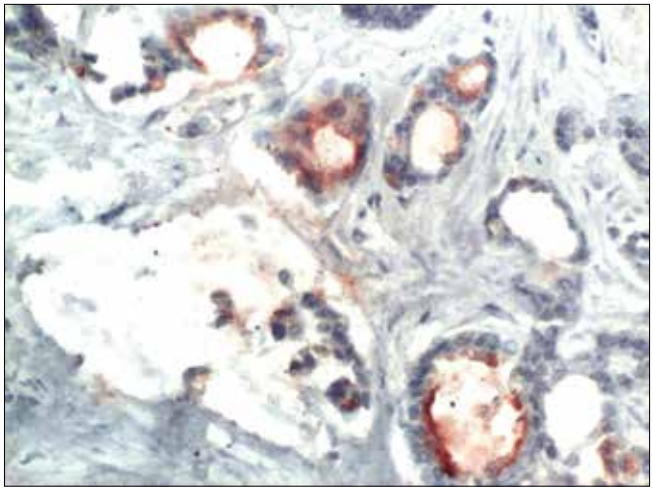

Figure 2. Positive cytoplasmic staining with VCAM-1 in the tumor cells (x200)

tumor invasion in the metastatic axillary lymph nodes in the present study. Cathepsin D was detected positive in the cases with positive extranodal tumor invasion. Fisher et al. (15) reported the extracapsullary axillary lymph node invasion as an important prognostic factor for survey and disease-independent survey and suggested radiotherapy on to the breast/chest wall and supraclavicular region for the patients with extranodal invasion findings who had positive level 1 and 2 axillary lymph node dissection regardless of the lymph node count. Yılmaz et al. (9) compared expression rates of cathepsin D, thymidine kinase 1 and cyclin D1 antibodies in benign and malignant cells in 2008; and showed increased expression in the malignant cells; such finding was considered that such three antibodies may help to detect malignant characteristics of the lesion. They reported that cathepsin D expression plays an important role for determination of biological behavior of the tumor to show possible effect on invasion of the tumor cells (7). A recent study conducted by Jacobson-Raber et al. (16) showed that a prognostic model depending on staining density scores of cathepsin D and E-cadherin immunohistochemically in breast tumors may be useful to decide on the treatment of the patients with early breast carcinoma. Dian et al. (17) considered that increased cathepsin D expression in primary breast carcinomas may be associated with recurrence and metastasis. It was reported that positive cathepsin D results may help to determine the patients with risk and to select more or less aggressive adjuvant therapy for the patients with early stage breast cancer during first 3-year follow-up (18). Huang et al. (19) showed triple-negative breast carcinoma were frequently associated with overexpression of cathepsin-D, and with aggressive disease course through lymph node invasion and high cancer cell proliferation/Ki-67 index. Guerra et al. (20) reported high level of p53 and cathepsin D, together with down-regulation of Bcl-2 is correlated with aggressiveness in breast carcinoma. A statistically significant difference was not found between the groups stained negatively or positively by cathepsin D in terms of location on the right or left breast, tumor diameter, Paget's disease, histological grade, multifocality, DCIS, quadrant location, ER, PR, cerbB2 staining in the present study. On the other hand, a statistically significant difference was detected for number of metastatic axillary lymph nodes in the groups of our study. The metastatic axillary lymph node count with 4 or more lymph nodes was detected higher in group 1 when compared with other groups.

A statistically significant difference was detected for cathepsin D staining between the groups in the present study. Cathepsin D positivity was noted in Group 1. As a result, number of metastatic axillary lymph nodes and extranodal tumor invasion in the lymph nodes were found higher in the group with positive cathepsin D staining. Cathepsin D can be used instead of sentinel lymph node biopsy or as an auxiliary method for sentinel lymph node biopsy in the management of breast cancer patients. There are limited number of studies about the association between number of metastatic axillary lymph nodes and extranodal tumor invasion. Extranodal tumor invasion is an increased risk factor for axillary recurrence. The present study reveals significant results for the patients diagnosed with invasive ductal carcinoma through breast biopsy especially before mastectomy in terms of increased number of metastatic axillary lymph nodes and extranodal tumor invasion by immunohistochemical cathepsin D stain without any additional invasive intervention. Results of the present study may contribute to monitoring and treatment of the patients in the future. More and new studies on this topic are required.

Ethics Committee Approval: Ethics committee approval was received for this study from Selçuk University Scientific Research Project Coordinatorship.

Informed Consent: Written informed consent was not received due to the retrospective nature of this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - Ö.K., H.H.E.; Design - Ö.K., H.H.E.; Supervision - Ö.K., S.G.; Funding - Ö.K., S.G.; Materials - Ö.K., H.H.E., S.G.; Data Collection and/or Processing - Ö.K., H.H.E., E.A.; Analysis and/or Interpretation - Ö.K., H.H.E., E.A.; Literature Review - Ö.K., H.H.E., E.A.; Writing - Ö.K., H.H.E.; Critical Review - Ö.K., E.A.

Acknowledgement: The authors thanks to Prof. Oktay Irkorucu for his valuable scientific contribution to over study.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial support: The present study was supported by Selçuk Scientific Research Project Coordinatorship fund with the project code of 09202070.

References

1. Greenlee RT, Murray T, Bolden S, Wingo PA. Cancer Statistics, 2000. CA Cancer J Clin 2000; 50:7-33. (PMID: 10735013) [CrossRef]

2. Gillett C, Fantl V, Smith R, Fisher C, Bartek J, Dickson C, Barnes D, Peters G. Amplification and overexpression of cyclin D1 in breast cancer detected by immunohistochemical staining. Cancer Res 1994; 54:1812-1817. (PMID: 8137296)
3. Velikova G, Banks RE, Gearing A, Hemingway I, Forbes MA, Preston SR, Jones M, Wyatt J, Miller K, Ward U, Al-Maskatti J, Singh SM, Ambrose NS, Primrose JN, Selby PJ. Circulating soluble adhesion molecules E-cadherin, E-selectin, intracellular adhesion molecule-1 (ICAM-1) and vasculer cell adhesion molecule-1 (VCAM-1) in patiens vith gastric cancer. Br J Cancer 1997; 76:1398-1404. (PMID: 9400933) [CrossRef]
4. Fantl V, Richards MA, Smith R, Lammie GA, Johnstone G, Allen D, Gregory W, Peters G, Dickson C, Barnes DM. Gene amplification on chromosome band 11q13 and estrogen receptor status in breast cancer. Eur J Cancer 1990; 26:423-429. (PMID: 2141507) [CrossRef]
5. Arnold A, Papanikolaou A. Cyclin D1 in breast cancer pathogenesis. J Clin Oncol 2005; 23:4215-4224. (PMID: 15961768) [CrossRef]
6. Ferrandina G, Scambia G, Bardelli F, Benedetti Panici P, Mancuso S, Messori A.Relationship between cathepsin D content and disease-free survival in node-negative breast cancer patients: a meta-analysis. Br J Cancer 1997; 76:661-666. (PMID: 9303368) [CrossRef]
7. Rochefort H. Biological and clinical significance of cathepsin D in breast cancer. Acta Oncol. 1992; 31:125-130. (PMID: 1622626) [CrossRef]
8. Yusufu M, Changwen W, Munire M, Jie T, Bangxing H, Jing Z, Tao H. Overexpression of VCAM-1 is correlated with poor survival of patients with breast cancer. Int J Clin Exp Pathol 2016; 9:7451-7457.
9. Yılmaz G, Geyik P, Uluoğlu Ö, Prognostic significance of immunohistochemical expression of cathepsin D, cyclin D1 and thymidine kinase 1 in invasive ductal breast carcinomas with a predominant intraductal component. Gazi Medical Journal 2008; 19:112-120.
10. Onitilo AA, Engel JM, Greenlee RT, Mukesh BN. Breast cancer subtypes based on ER/PR and Her2 expression: comparison of clinicopathologic features and survival. Clin Med Res 2009; 7:4-13. (PMID: 19574486) [CrossRef]
11. Charpin C, Garcia S, Andrac L, Horschowski N, Choux R, Lavaut MN. VCAM(IGSF) Adhesion Molecule Expression in Breast Carcinomas Detected by Automated and Quantitative Immunocytochemical Assays. Hum Pathol 1998; 29:896-903. (PMID: 9744304) [CrossRef]
12. Henry JA, McCarthy AL, Angus B, Westley BR, May FE, Nicholson S, Cairns J, Harris AL Horne CH. Prognostic significance of the estrogen regulated protein, cathepsin D, in breast cancer. An immunohistochemical study. Cancer 1990; 65:265-271. (PMID: 2295049) [CrossRef]
13. Duffy MJ, Brouillet JP, Reilly D, McDermott E, O'Higgins N, Fennelly JJ, Maudelonde T, Rochefort H. Cathepsin D concentration in breast cancer cytosols: correlation with biochemical, histological and clinical findings. Clin Chem 1991; 37:101-104. (PMID: 1899058)
14. Têtu B, Brisson J, Wang CS, Lapointe H, Beaudry G, Blanchette C Expression of cathepsin D, stromelysin-3, and urokinase by reactive stromal cells on breast carcinoma prognosis. Cancer 2001; 92:2957-2964. (PMID: 11753972) [CrossRef]
15. Fisher BJ, Perera FE, Cooke AL, Opeitum A, Dar AR, Venkatesan VM, Stitt L, Radwan JS. Extracapsular axillary node extension in patients receiving adjuvant systemic therapy: an indication for radiotherapy? Int J Radiat Oncol Biol Phys 1997; 38:551-559. (PMID: 9231679) [CrossRef]
16. Jacobson-Raber G, Lazarev I, Novack V, Mermershtein W, Baumfeld Y, Geffen DB, Sion-Vardy N, Ariad S. The prognostic importance of cathepsin D and E-cadherin in early breast cancer: A single-institution experience, Oncol Lett 2011; 2:1183-1190. (PMID: 22848286)
17. Dian D, Vrekoussis T, Shabani N, Mylonas I, Kuhn C, Schindlbeck C, Navrozoglou I, Friese K, Makrigiannakis A, Jeschke U. Expression of cathepsin-D in primary breast cancer and corresponding local recurrence or metastasis: an immunohistochemical study. Anticancer Research 2012; 32:901-906. (PMID: 22399610)
18. Markićević M, Kanjer K, Mandušić V, Buta M, Nešković-Konstantinović Z, Nikolić-Vukosavljević D.Cathepsin D as an indicator of clinical outcome in early breast carcinoma during the first 3 years of follow-up. Biomark Med 2013; 7:747-758. (PMID: 24044567) [CrossRef]
19. Huang L, Liu Z, Chen S, Liu Y, Shao Z. A prognostic model for triple-negative breast cancer patients based on node status, cathepsin-D and Ki-67 index. PLoS One 2013; 8:12. (PMID: 24340082) [CrossRef]
20. Guerra E, Cimadamore A, Simeone P, Vacca G, Lattanzio R, Botti G, Gatta V, D'Aurora M, Simionati B, Piantelli M, Alberti S. p53, cathepsin D, Bcl-2 are joint prognostic indicators of breast cancer metastatic spreading. BMC Cancer 2016; 16:649. (PMID: 27538498)[CrossRef]