International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

CXCR4 Biomarker Expression in Invasive Ductal Carcinoma of the Breast and Its Relation with Axillary Lymph Node Metastasis

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Received: 10/02/2016 *Revised: 10/03/2016* *Accepted: 15/03/2016*

ABSTRACT

Introduction: CXC type 4chemokine receptor (CXCR4) has been documented as a biomarker for breast cancer since 2001. It is believed that it's over expression is related to a more aggressive phenotype of this disease. In this study, we wondered if higher rates of CXCR4 expression were associated with higher rates of axillary lymph node metastasis in patients with invasive ductal carcinoma.
Methods: We selected 68 patients with invasive ductal carcinoma (IDC) in a 3 year period and we verified if their histopathological blocks derived from their surgeries over expressed CXCR4. After cross-referencing these data with their medical records information, we checked to determine if they had positive axillary lymph node metastasis.
Results: The positive expression of CXCR4 in any level is a risk factor for axillary lymphatic metastasis in IDC (RR: 2, 50, CI: 1, 44 to 9, 00, p<0, 05). These results explain why this biomarker has been focused in many researches trying to inhibit its actions.
Conclusion: Higher expressions of CXCR4 were associated with higher rates of axillary lymph node metastasis. In the future, CXCR4 may serve as a prognostic marker and may also influence on therapy decisions.

Key-words: invasive ductal carcinoma, CXCR4, lymph nodes metastasis, breast cancer.

INTRODUCTION

Breast cancer is the second most common neoplasm in ocidental women and the first cause of cancer-related mortality among Brazilian women. [1] Because of that, its detailed study, including its biomarkers that would contribute to the disease progression, is essential. A molecular biomarker is the biomolecular characters that may be objectively measured as indicators of a disease or its biological processes. [1]

In breast cancer, there are many biomarkers already documented, such as, estrogen receptors, progesterone receptors, human epidermic growth factor and many others. [2]

CXC type 4 chemokine receptor (CXCR4) is a transmembrane proteic receptor which was identified as a tropic co-receptor between T-cell lines and human immunodeficiency virus. Its role in breast cancer metastasis was first documented in 2001 and it has been used as a biomarker for this disease since then. [1]

CXCR4 has great affinity for CXCL12 chemokine, which is made by lungs, bones and lymph nodes. The microenvironment changes that Invasive Ductal Carcinoma promotes (generally induced by hypoxia) generate an over-

regulation of CXCR4, which would culminate in its over expression. [1,3]

It was also noted that in patients that had higher levels of CXCR4 expression there are higher lymph nodes metastasis, recurrence and mortality rates for IDC. There is evidence of higher tumor growth, more advanced pTNM stages e lower survivor rate when CXCR4 is over expressed. [3] However, those findings were not verified in HER-2 positive IDC. [1] We can say that the over expression of CXCR4 in HER2 negative ICD would indicate a more aggressive phenotype in this tumor. [4,5]

These findings are so relevant that some researches already see CXCR4-CXCL12 binding inhibition as a therapeutic target in the near future. The development of AMD3100 (plerixafor-, used in multiple myeloma and other hematological neoplasms treatments), a drug that inhibits this binding and has showed a less invasive and metastatic activity in CXCR4 positive ICDS and it proves our statement. [1,6] Another drug that is being tested is POL5551 and it already showed a reduction in lymph node metastasis in CXCR4 positive ICD. [7]

In our study, we searched for evidence of CXCR4 over-expression in breast tumors beyond analysis of immunohistochemistry of patients and we searched clinical data of these patients, like age, survival time, lymph node or distant organs metastasis.

MATERIALS AND METHODS

This is a historical cohort study (observational, longitudinal and retrospective study with no control-cases) in which we selected 111 patients diagnosed with ICD that went through surgery between January 1st 2010 and April 30th 2013 in Emílio Carlos Teaching Hospital, Padre Albino Teaching Hospital and São Domingos Hospital, the three located in Catanduva, São Paulo State, Brazil. The histopathological material of each surgery was recovered from Emílio Carlos Teaching Hospital Pathology Department and Citopat Laboratory (both also located in Catanduva, Brazil) and we analyzed the glass slides correspondent of each one of the blocks. We selected only the blocks that contained ICD cells in breast and axillary lymph nodes tissue. Then, we marked the areas in which contained ICD and sent the material to AC Camargo Cancer Center's Histopathology Laboratory, in SãoPaulo, Brazil, where these localities where extracted through TMA technique (Tissue Microarray).

After obtaining TMA glass slide, we took it to University of São Paulo Medical School Immunohistochemistry Laboratory, in Ribeirão Preto, Brazil, where they applied 3'-diaminobenzadine-tetra-hydrocloride, a marker that graduates CXCR4 expression in the cancer cells. For supplementary analysis we also made immunohistochemistry for estrogen receptors (ER), progesterone receptors (PR), Her-2 and Ki-67.

The data obtained in their medical records were analyzed and added to the immunohistochemical data we collected. The records were recovered from Emílio Carlos Teaching Hospital Archives and from the Mastology Clinic Eduardo Rogério Malaquias Chagas, both in Catanduva, Brazil.

The following factors were noted in our analysis: age, tumor location, tumor size in its biggest axis, kind of surgery, number of compromised axillary lymph nodes, T stage according to pathological staging, nuclear grade, histological grade, presence of angiolymphatic invasion, presence of extensive intraductal component (EIC), expression of biomarkers (ER, PR, Ki-67, Her-2 and CXCR4), presence of therapy adjuvance (chemotherapy, radiotherapy and hormone therapy), disease-free survivor rate, global survivor rate and systemic metastasis throughout clinical follow-up.

Expression grade of CXCR4 was divided in 4 categories: negative, weak, moderate and strong. [5]

We excluded from our study the patients that had one or more of the following characteristics: insufficient

quantity of material for TMA obtaining, unreadable and/or incomplete medical records, absence of ICD diagnosis in the histopathological report, follow-up in other services and patients that gave up of the treatment.

RESULTS

From 111 patients selected, 43 had at least of the exclusion criteria and were not included in our statistics (mainly because they did their follow-up in other services, or because there was insufficient quantity of material for TMA and incomplete medical records). In total, we had 68 analyzed patients, all women.

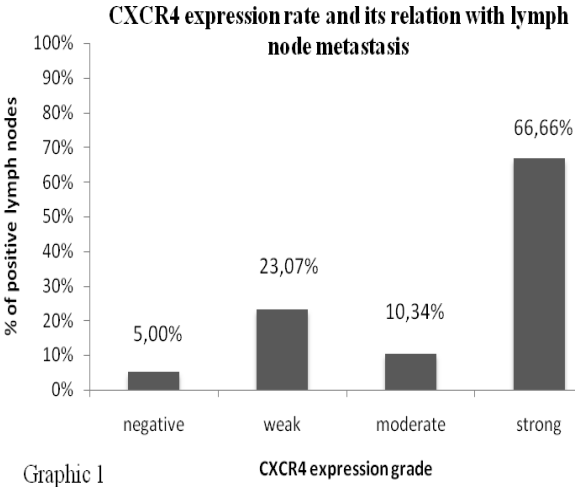
Our 68 patients were 18 SUS (Brazil's Universal and Cost-free Health System) patients (26.47%) and 50 private/health care patients (73.52%).

The mean age was 59.23±13.75 years old (youngest patient: 31. oldest patient: 83). Most cases occurred on the left breast (52.94%). The mean tumor size in its biggest axis was 2.29±1.19 cm (biggest: 8cm. smallest: 0.5cm). Quadrantectomy was performed in 50 (73.52%) of our patients. The remaining 18 (26.47%) were subjected to mastectomy. The relative risk of having a tumor with pT2 stage or higher in SUS patients compared with private/health care patients is 1.57 (CI: 1.03-117.20. $p<0.05$). There was 1 tumor with pT1a stage (1.47%). 4 with pT1b stage (5.88%). 22 with a pT1c stage (32.35%). 36 with a pT2 stage (52.94%). 2 with a pT3 stage (2.94%) and 2 with a pT4 stage (2.94%). About the nuclear grade. 12 patients had a grade 1 tumor (17.64%). 36 a grade 2 (52.94%) and 20 a grade 3 (29.41%). In the histological grade. 5 patients has a grade 1 tumor (7.35%). 41 a grade2 (60.29%) and 21 (30.88%) a grade 3.

Most women did not have angiolymphatic invasion in their histopathology study (70. 58%) and neither had EIC (74.00%). All of them were submitted to adjuvant radiation therapy. most of them were under hormone therapy (64.70%) and 47.05% was subjected or was still under chemotherapy (we did not discriminate if neoadjuvant or adjuvant).

The mean disease-free survivor rate was 44.74±22.60 months (lowest: zero. highest: 60 months). The mean global survivor rate was 45.10±11.64 months. In our sample, 5.88% of patients showed systemic metastasis during medical follow-up.

About CXCR4 expression. 20 patients expressed it negatively (29.41%). 13 expressed it weakly (19.11%). 29 expressed it moderately (42.64%) and 6 did it strongly (8.82%). It the women with negative expression. the axillary involvement was 5.00%. Between the women that expressed it weakly. the involvement rate was 23.07%. The women from the third category had a 10. 34% metastasis rate in axillary lymph nodes. And the women with strong expression had a 66. 66% rate of neoplastic involvement in axillary lymph nodes. The relative risk for axillary lymph node metastasis is 2.50 (CI=1.44 to 9.00; $p<0.05$). The graphic 1 represents the positive rates of metastatic lymph node positivity according to CXCR4 grade of expression.



Graphic 1

Meanwhile, the biomarkers had the following results: 53 (77.94%) of the patients had ER positivity. 43 (63.23%) had PR positivity. 10 (17.24%) had Her-2 positivity and 32 (47.05%) had a level of Ki-67>10%. We could not establish a statistically relevant relation that would

determine a more aggressive phenotype between tumors which were positive for both Her-2 and CXCR4.

DISCUSSION

In this study, it was possible to verify that there is a statistical relevant relation between the expression of CXCR4 in ICD and its metastasis to axillary lymph nodes. Another relation we could observe was the fact that SUS patients had a higher staging tumor rate than private/health care patients. We could also note that tumors that strongly express CXCR4 had higher presentation of axillary involvement, which matches with the findings in other studies. [8,9] Most cases of tumors with strong expression came from SUS patients. Among patients that evolve with systemic disease, we could not determine a statistical relevant relation between these condition and CXCR4 expression, although many studies could do it. [9-13]

The path through which CXCR4 contributes with ICD metastasis is being studied. There are studies that the CXCR4 expression contributes for the activation of another chemokine receptor, CXCR2, which would activate MEK-PI3K cellular-pathway that would promote lymphangiogenesis in the tumor microenvironment and would aid the cancerous cells in their implantation in the lymph nodes. Other scientists could demonstrate that CXCR4 positive cells can make the natural selection in the tumor microenvironment. This finding was made after Sun et al (Aug. 2014) injected cells that strongly express CXCR4 amongst CXCR4 negative ICD cells in vitro. Later, they verified that there has been a raise in the amount of CXCR4 positive cells and a reduction in the cells that negatively express it. [14-16]

Studies show that the prognosis of breast cancer is worsened by CXCR4 positivity. Most of these articles realized that the triple-negative tumors (the ICD with the worst prognosis) that positively express CXCR4 have an even worse outcome. [17] Two recent meta-analysis were able to demonstrate that any grade of CXCR4 expression predicts a unfavorable evolution in ICD patients. The positive expression also predicts higher mortality rates and higher staging at diagnosis. [10, 11,18]

Doubtlessly, there are consistent data that the positive expression of CXCR4 is associated to a higher rate of lymph node involvement, regardless of the histological subtype of breast cancer. [19]

Many studies with drugs that might help in the near future have been designed in response of the impacts that these findings generated. For example, Nef-M1 is a drug that antagonizes the effects of CXCR4. It stimulates the apoptosis of cells that express CXCR4 and inhibits tumor metastasis, which lowers the size of the tumor in its primary site. [20] This is not the only drug that has being tested for these porpoises. We already quoted plerixafor and POL5551; in Iran, Darakhshan et al (2014) some researchers tested an association between tamoxifen and tranilast (an anti-allergic medication that is being tested in proliferative and autoimmune disorders) and they verified that there has been a decrease of tamoxifen-resistant and CXCR4 positive cells. [21]

Other studies showed that the down regulation of CXCR4 aids in becoming the tumor silent and also reduces its metastasis, whether this is done by genic therapy, whether this is done by the use of other drugs. [9,22]

In a generic way, any interference in any molecular pathway (adhesion, proliferation, migration) that inhibits or antagonizes the expression of CXCR4 and its biochemical actions increases the survivor rate and decreases the loco-regional and systemic metastasis rates. [23,24] All these findings only reiterate the importance of studies like ours in this field.

Some scientists went beyond that discussion and showed that this subject is much more complex than just the expression of a few receptors that we search in the Immunohistochemistry of ICD on a daily basis. Recent discovers in other cancer

biomarkers showed the hypothesis that may yet exist unknown relation between these markers that contribute to the metastasis of these carcinomas [25,26] (after all, how can we explain the metastasis of cancers that are negative for all known biomarkers?). Wang et al (Mar. 2013) realized a fluctuation between the expressions of breast cancer markers throughout time, which makes us believe that not only their static levels that contribute for the cancer natural history, but maybe its temporal and biochemical evolution. [27] Andreou et al (Dec. 2012) realized that patients with higher serum levels of norepinephrine (i.e., more stressed) had lower expressions of CXCR4. This only proves that there are still a lot of unknown facts in the Immunohistochemistry of those tumors. [28]

So, the presence of expression of CXCR4 maintains several molecular pathways that assist at axillary lymph node metastasis and chemotherapy resistance during breast cancer treatment. [29,30] Another relevant association in our study is the fact that SUS patients were more likely to present at diagnosis more advanced tumors than private/health care patients. This fact may contribute for a more aggressive phenotype and with more over-expression of CXCR4. There are still many biomarkers being discovered and that may aid in a deeper analysis of ICD's natural history. We consider that studies like ours, that show the epidemiology of these biomarkers in our professional environment, will be useful in the future for target-therapy and quotidian immunohistochemistry of CXCR4 usage and these may benefit our future patients. [1,31]

CONCLUSION

It was possible to establish a relation statistically relevant between axillary lymph nodes metastasis in ICD that express CXCR4 positively. There are still many facts unknown about this subject but, doubtlessly, we hope that in the near future this biomarker contributes as a prognostic and therapeutic factor in this histologic subtype of breast cancer.

Conflicts of Interest Statement: The authors declare that they have no conflict of interest.

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How to cite this article: Pacheco de Mello MC, Chagas ERM. CXCR4 biomarker expression in invasive ductal carcinoma of the breast and its relation with axillary lymph node metastasis. Int J Health Sci Res. 2016; 6(4):65-71.

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