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# The Relationship of CXC Chemokine Receptor 4 (CXCR4) Expression with Histopathological Grading of Invasive Breast Cancer

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**Abstract**

**Introduction:** The histological grade of the tumor influences the prognosis of breast cancer. In metastatic breast cancer, the stromal cells produce a chemokine (C-X-C motif chemokine 12/CXCL12) or the so-called stromal-derived factor-1 (SDF-1) as a chemoattractant, which binds to the chemokine receptor (C-X-C chemokine receptor type 4/CXCR4) which is expressed by breast cancer cells. This study aimed to determine the expression of CXCR4 in invasive breast cancer associated with histopathological grading of invasive breast cancer.

**Methods:** This study is observational with a retrospective approach using a paraffin block archive sample diagnosed with Invasive Breast Cancer. Immunohistochemical staining using CXCR4 antibody and expression analysis was performed by light microscopy. The data were statistically analyzed by the Chi-Square test and presented in the table. The statistical test results were significant if the p-value was <0.05.

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**Results:** Showed no significant relationship between CXCR4 expression and histopathological grading in invasive breast cancer with a p-value = 0.467.

**Conclusion:** The data of this study showed that the expression of CXCR4 in Invasive Breast Cancer varied and did not support its role in determining histopathological grading.

***Keywords:*** CXCR4; Invasive Breast Cancer; histopathological grading.

## 1. Introduction

In Indonesia, breast cancer is in the first place, with an incidence of 68.8 per 100,000 population with an average death rate of 22.4 per 100,000 population [1,2]. Invasive Breast Cancer (IBC) refers to a large and heterogeneous group of malignant neoplasms originating from the mammary gland epithelium [3]. The prognosis of breast cancer is influenced by several factors, including the histological grade of the tumor, vascular invasion, and the status of lymph node metastases. If tumor cell metastases are found in the lymph nodes, the patient's life expectancy will decrease from 90% to 20% [4–7].On the migration and spread of breast cancer, stromal cells produce a chemokine (C-X-C motif chemokine 12/CXCL12) or what is called stromal-derived factor-1 (SDF-1) as a chemoattractant, which binds to a chemokine receptor (C-X-C chemokine receptor type 4/ CXCR4) which is expressed by breast cancer cells [8]. An experimental study found that CXCR4 mediates cancer cell migration, invasion, and adhesion. CXCR4 also plays a role in the metastasis of cancer cells to other organs expressing SDF-1/CXCL12 [6,9].PCR and immunohistochemical examination can do CXCR4 expression analysis. CXCR4 expression can be used to determine prognostic and therapeutic targets for cancer patients [9]. This study aimed to determine the relationship between CXCR4 expression and histopathological grade by comparing the CXCR4 expression scores in grades 1, 2, and 3 invasive breast cancers.

## 2. Methods

This study was cross-sectional to assess the expression of CXCR4 in invasive breast cancer. This research was conducted at the Anatomical Pathology Laboratory, Hasanuddin University Hospital, Makassar, Indonesia, from June to November 2021. The population of this study was paraffin block from mastectomy resection tissue sent to Hasanuddin University hospital, Dr Wahidin Sudirohusodo hospital, and Sentra Diagnostik Patologia Makassar Laboratory. The protocol of the study was approved by the Institutional Review Board at our institution (no. 0619/UN4.6.4.5.31/PP36/2021).The sample is the entire affordable population that meets the inclusion criteria. The sampling technique used was the consecutive sampling technique, and with the Lemeshow formula, the minimum sample size was 58 samples. Inclusion criteria (a) a mastectomy breast tumor tissue diagnosed by the pathologist as grade 1, 2, and 3 invasive breast cancers, (b) Paraffin block of grade 1, 2, and 3 invasive breast cancer tissue processed according to standards for examination immunohistochemistry of CXCR4 and (c) Examination and the researcher and two anatomical pathologists carried out interpretation of the results of immunohistochemistry of CXCR4. Exclusion criteria (a) Paraffin blocks the preparation of invasive breast cancer resection tissue damaged during reprocessing for CXCR4 immunohistochemical examination.

### *2.1. Hematoxylin Eosin stain*

It was stained with Hematoxylin-Eosin solution from MERCK (Darmstadt, Germany) with catalog no. 115938 and 115935, respectively, made according to a standard protocol.

### *2.2. Immunohistochemical Staining Procedure*

Immunohistochemical staining of the sample was using CXCR4 polyclonal antibody with 1:50 dilution with BIOENZY (Rabbit Anti-CXCR4 Polyclonal antibody catalog no. BZ-0813320F-AP) according to the standard protocol.

### *2.3. Interpretation of Immunohistochemical results*

Expressions were calculated using a scoring system based on the intensity of the color and the proportion of stained cells. Visually, we observed the expression locations in tumor cells' membrane, cytoplasm, and nucleus (Figure 1).

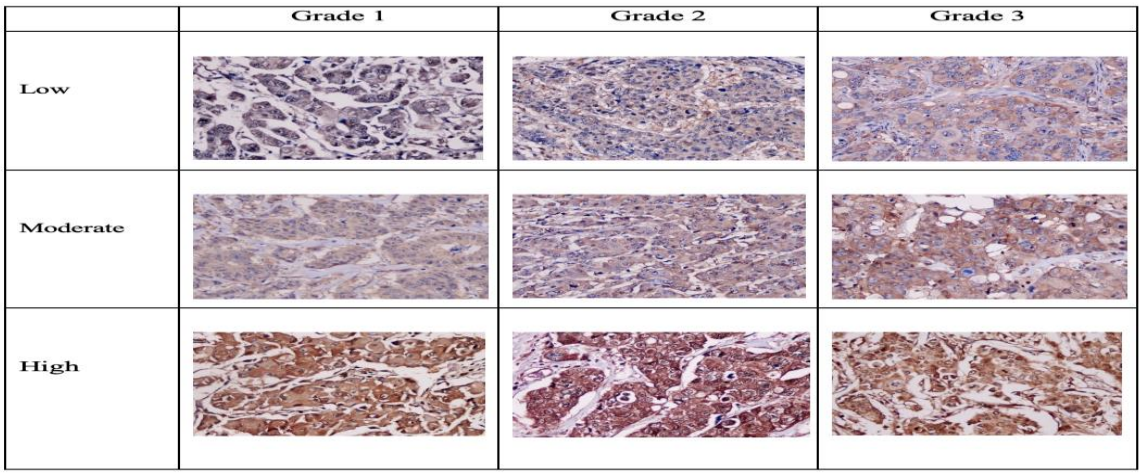


**Figure 1:** Expression of CXCR4 in Invasive Breast Cancer. Expression of CXCR4 on the membrane, cytoplasm, and nucleus of tumor cells (magnification x40)

### *2.4. Invasive breast cancer*

Invasive breast cancer with morphology or immunohistochemistry indicating mammary epithelial origin. Diagnosis of exclusion, lacking the histologic characteristics to categorize morphologically as a specific subtype of breast cancer [10]. The diagnosis was assessed by histopathological examination using HE staining and viewed with an Olympus CX-43 light microscope (Tokyo, Japan).

### *2.5. Grading*

Cancer's grade is determined by how the cells appear under a microscope based on tubular shape, nuclear pleomorphism, and several mitoses using a semi-quantitative method, obtained through HE staining [11].

### *2.6. CXCR4 expression score*

CXCR4 expression score is the total immunostaining score (0-12) obtained by multiplying the score of the proportion of tumor area stained positively (0-4) with the intensity score of CXCR4 staining (0-3) [5].

### *2.7. Determination of the degree of histopathology*

It was determined by semi-quantitative assessment using the "Elston and Ellis modification of the Scarff-Bloom-Richardson" method to assess the histological grade of breast tumors (table 1) [4,12,13].

**Table 1:** Histological criteria

| | Score | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Tubule formation | >75% | 10-75% | <10% |
| Nuclear pleomorphism | Small regular nuclei; similar to normal ductal nuclei | Intermediate size; 1.5-2 times the size of normal ductal nuclei | High-grade nuclei; more than twice the size of normal ductal nuclei |
| Mitotic count | ≤12 | 13-24 | ≥25 |
| Total score | 3-5 | Grade 1 | |
| | 6-7 | Grade 2 | |
| | 8-9 | Grade 3 | |

### *2.8. Immunoexpression of CXCR4*

Expressed in a semi-quantitative estimate with a scoring system assessing the intensity of the stained area and the proportion of the stained area [14,15].

### *2.9. Immunoreactive Score*

It is a minimum value of 0 and a maximum value of 12. It has been obtained by multiplying the intensity by the proportion of the colored area. The CXCR4 expression was declared strong if the score was five and weak if the score was ≤ 4 [14,16].

### *2.10. Statistical analysis*

The data obtained from the study results were recorded, then analyzed, and presented in tabular form. The Chi-Square test using SPSS 18 software (Armonk, NY: IBM Corp.) was used to compare the CXCR4 expression score with histopathological grading of invasive breast cancer. The statistical test results were significant if the p-value was <0.05.

## 3. Results

In our study, the mean age of invasive breast cancer patients was 51 years with a standard deviation of 10.98 years, of which 55 (55.0%) were dominated by patients aged over 50 years. The distribution of samples based

on histopathological diagnosis showed that there were 40 cases of invasive breast cancer grade 2 (40.0%), followed by grade 3 invasive breast cancer with 35 subjects (35.0%) and grade 1 in 25 subjects (25.0%) (Table 2). The results of CXCR4 expression were obtained, with the majority high expressed in 77 subjects (77.0%).

**Table 2:** Characteristics of subjects

| Variable | n | % |
|---|---|---|
| Age (years) | | |
| Mean + SD | 51+10.98 | |
| <50 | 45 | 45.0 |
| >50 | 55 | 55.0 |
| Histopathological Grading | | |
| Grade 1 | 25 | 25.0 |
| Grade 2 | 40 | 40.0 |
| Grade 3 | 35 | 35.0 |
| CXCR4 expression | | |
| High | 77 | 77.0 |
| Low | 23 | 23.0 |

### *3.1. Analysis of Differences in CXCR4 Expression in Invasive Breast Cancer Based on Histopathological Grading*

Table 3 shows that of the 77 cases of invasive breast cancer with high CXCR4 expression, it consisted of 17 subjects (19.3%) of grade 1 invasive breast cancer, 32 subjects (30.8%) of grade 2 invasive breast cancer, and 28 cases (27.0%) invasive breast cancer grade 3. Meanwhile, the total number of cases of invasive breast cancer with low CXCR4 expression was 23 cases, consisting of 8 subjects (5.8%) of grade 1 invasive breast cancer, eight subjects (9, 2%) of grade 2 invasive breast cancer, and seven subjects (8.1%) grade 3 invasive breast cancer. The Chi-Square test was obtained, with the p-value = 0.467, which means there is no significant difference in CXCR4 expression in invasive breast cancer based on histopathological grading.

**Table 3:** Differences in CXCR4 expression based on histopathological grading

| CXCR4 expression | Histopathological grading | | | p-value* |
|---|---|---|---|---|
| | Grade 1 n (%) | Grade 2 n (%) | Grade 3 n (%) | |
| High | 17 (19.3) | 32 (30.8) | 28 (27.0) | 0.467 |
| Low | 8 (5.8) | 8 (9.2) | 7 (8.1) | |

## 4. Discussion

To determine the prognostic value of breast cancer, we need a new marker that has a strong predictive value and is independent. The chemokine receptor CXCR4 is a transmembrane protein belonging to the paired G protein receptors group, and CXCR4 will bind to a specific ligand, namely CXCL12. The binding between CXCR4 and CXCL12 will activate various intracellular signal transductions that can affect the invasiveness and metastasis of tumor cells [17–19].In this study, the number of patients with invasive breast cancer was dominated by the age group above 50. These data tend to suffer from invasive breast cancer more in the older age group. The results of studies such as those conducted by Andre and his colleagues [20] showed that the age group above 50 was more dominant in suffering from invasive breast cancer. The incidence of breast cancer is higher in women

with more prolonged estrogen exposure due to early menarche, late menopause, nulliparity, and the late age of first pregnancy (after the age of 30 years) [4]. Several studies researched the location of CXCR4 expression in tumor cells and its association with clinicopathological parameters and cure rates in various cancers. Cabioglu and his colleagues [21] and Yasuoka and his colleagues [22] showed that the expression of CXCR4 in the cytoplasm of tumor cells had a significant relationship with the incidence of axillary lymph node metastases in breast cancer. In contrast, the expression of CXCR4 in the nucleus of tumor cells did not have a significant relationship with the incidence of lymph node metastases. Further studies conducted by Salvucci and his colleagues [23] on breast cancer tissue showed that tumor cells with CXCR4 in the nucleus were associated with higher grade, advanced stage, and lower survival. The mechanism of CXCR4 expression in the nucleus of tumor cells is still unclear, and further research needs to be done. Several researchers stated that the binding of CXCR4 with CXCL12 could induce the translocation of CXCR4 into the cytoplasm and nucleus of cells. Translocation of CXCR4 into the cell nucleus can activate transcription factors in the nucleus [24]. Another study suggested that endosomes facilitate CXCR4 translocation through clathrin-coated pits and require the Hsc73 molecule [25].In our study, statistical analysis using the Chi-Square test compared the expression of CXCR4 in invasive breast cancer based on histopathological grading. From this analysis, the value of $p = 0.467$ ($p>0.05$) means no significant difference in the expression of CXCR4 in breast cancer based on also found no correlation between CXCR4 expression in invasive breast cancer based on histopathological tumor grading, histologic type, and TNM staging. Our study is in line with another study that did not find a significant relationship between CXCR4 expression and histopathological grading and type [26]. Binding between CXCR4 and CXCL12 leads to the activation of various intracellular signaling transduction pathways and downstream effectors that mediate cell survival, proliferation, chemotaxis, migration, and adhesion [27].The limitation of this study was the use of a single marker cancer stem cell (CSC) with one immunohistochemical modality. It causes the expression analysis to be less accurate and unable to analyze the complexity and relationship between the CSC marker CXCR4 and other molecules involved in the invasion and metastasis of invasive breast cancer.

## 5. Conclusion

There was no significant difference in the expression of CXCR4 in grade 1, grade 2, and grade 3 invasive breast cancers. Further research is needed to correlate the CXCR4 expression in invasive breast cancer with the hormonal status of ER, PR, and Her2.

## 6. Consent

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The patients have given their written informed consent on admission to use their prospective database and files for research work.

## 7. Conflicts of interest

The authors declare that they have no conflict of interest.

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