

## CD8+ TILS Expression in Invasive Breast Carcinoma (No Special Type)

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### Abstract

Invasive breast carcinoma (IBC) is the most cancer in women (24%) and the cause of cancer death in women worldwide. Based on data from globocan 2018 shows the incidence of breast cancer is around 2.08 million (11.6%) which is the second rank of all cancers after lung cancer with a mortality rate of 626.6 thousand (6.6%) which is also the most common cause of death in women. The basic role of the immune system in maintaining homeostasis by immunosurveillance and initiation of the inflammatory reactions that include coordination of innate and adaptive immune cells activation against tumor cells is one of the most important in the mechanism of tumor cell elimination. Prognosis of IBC were influenced by several factors, including tumor histology grade and Tumor Infiltrating Lymphocytes (TILs). Infiltration of lymphocytes in the tumor microenvironment/TILs through CD8+ T lymphocytes is known to be an important component of adaptive immunity that eliminates tumor cells. CD8+ T cells present tumor antigens on the surface of the major histocompatibility complex class I (MHC class I). Based on its importance in clinical application and its role in biological concepts, this study was conducted to determine the expression of CD8+ in Invasive Breast Carcinoma of No Special Type (IBC-NST) grades 1,2 and 3. Analytical study with a cross-sectional design on IBC-NST samples from 2017 until 2020 at the Laboratory of Anatomic Pathology, Faculty of Medicine, Hasanuddin University Makassar from May to August 2021. Immunoexpression data were analyzed to determine its relationship with grade. Eighty samples met the inclusion and exclusion criteria, there were 17 samples (21.3%) with grade 1, 32 samples (40%) with grade 2, and 31 samples (38.8%) with grade 3. In the high CD8+ TILs group, from a total of 27 samples, 10 samples with grade 1, 6 samples with grade 2, and as many as 11 samples with grade 3. In the low CD8+ TILs group, from a total of 53 samples, there were 7 samples with grade 1, 26 samples with grade 2, and 20 samples with grade 3. Based on the Chi-square test, p value = 0.017 (p <0.05). There is a significant difference in CD8+ TILS in Invasive Breast Carcinoma of No Special Type grade 1, grade 2 and grade 3.

**Keywords:** Invasive breast carcinoma- CD8+ TILS- grade

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### Introduction

Breast carcinoma is the most frequently cancer in women (24%) and the main cause of cancer death in women worldwide. It is the second most common cancer overall in men and women (11.6%). The incidence rate has increased in the development countries in recent decades (Rakha et al., 2019). Based on data from Globocan 2018, the incidence of breast cancer is around 2.08 million (11.6%) which is the second rank of all cancers after lung cancer with a mortality rate of 626.6 thousand (6.6%) (Bray et al., 2018). Data from the Ministry of Health of the Republic of Indonesia in 2013 shows the prevalence of breast cancer is 61.682 (0.5%) (Payudara<sup>2</sup>, 2016). Data in the Anatomical Pathology Laboratory, Dr. Wahidin Sudirohusodo there were 136 cases in 2018 and 162 cases

in 2019 with breast carcinoma.

However, breast carcinoma can develop at any age, from childhood to old age (Collins, 2018). Invasive breast carcinoma (IBC) is a malignant neoplasm originating from the mammary gland epithelium, while invasive breast carcinoma of no special type (IBC-NST) is a group of IBC that cannot be classified morphologically into a specific histological type. The majority of breast carcinomas are IBC-NST, where the prognostic characteristics and management are same as with the other variant or slightly worse. The prognosis of breast carcinoma is influenced by several factors, including tumor histology grade and TILs (Rakha et al., 2019).

The basic role of the immune system is to maintain tissue homeostasis by immunosurveillance and initiation of inflammatory reactions that include coordinated activation

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of innate and adaptive immune cells (Salgado et al., 2015). Immunosurveillance is the physiological function of the immune system to recognize and destroy transformed cell before they grow into tumors, and kill the tumors that already formed (Abbas et al., 2018a). In situations where elimination is incomplete, the tumor is able to escape immune control. This process was conceptualized by the theory of cancer immunoediting, which is supported by a large amount of experimental data and clinical evidence (Salgado et al., 2015). Immunoediting is a dynamic process of immunosurveillance and tumor development, which consists of three phases: elimination, equilibration, and escape. The elimination phase is immunosurveillance, equilibrium is a latent period in which the surviving tumor will produce new variants, and escape phase is an uncontrolled tumor growth because it can eventually escape from the immune system (Dunn et al., 2004). Tumor antigens stimulate the specific adaptive immune system that can prevent or inhibit growth and spreading of tumor cells, mostly involving T cells, especially Cytotoxic T Lymphocyte (CTL) CD8+ (Abbas et al., 2018a).

CD8+ T lymphocytes are an important component of adaptive immunity that attacks tumor cells that present tumor antigens on the surface of major histocompatibility complex class I (MHC class I). CD8+ T cells produce interferon- $\gamma$  after interaction with tumor targets, then cause cell cycle inhibition, apoptosis, angiostasis and induction of tumoricidal macrophage activity (Al-Saleh et al., 2017). Based on the importance in clinical application and their role in biological concepts, it is necessary and important to determine the expression of CD8+ in IBC-NST, especially in grade 1, 2 and 3.

## Materials and Methods

In this study, we collected 80 paraffin block samples of patients with IBC-NST from the Anatomical Pathology Laboratory of Wahidin Sudirohusodo Hospital and Hasanuddin University Hospital during the period 2017 to 2020.

Unstained slides were made from paraffin blocks and CD8+ immunohistochemistry staining was performed. In

each case, slides were made from paraffin blocks then cut with a 3  $\mu$ m thick microtome. The cut in the water bath was taken using a poly-L-lysine slide, then deparaffinized. Immunohistochemical staining using CD8+ rabbit polyclonal antibody (SP16).

Assessment of CD8+ TILs expression was assessed on cell membranes using a light microscope by two pathologists based on immunohistochemical methods. The CD8+ TILs scoring method used is similar to the TILs scoring method based on the recommendations of The International TILs Working Group 2014.

The data in this study were processed using SPSS 20 for Windows software. Descriptive statistical techniques used to describe the characteristic of the basic data obtained in the form of frequency distribution. Chisquare test were used to determine CD8+ expression in low and high group based on IBC-NST grade.

## Results

Of the 80 samples, there were 17 samples (21.3%) with grade 1, 32 samples (40%) with grade 2, and 31 samples (38.8%) with grade 3, and the histopathological figures are shown in Figure 1.

Assessment of CD8+ TILs expression was performed on lymphocyte cells in the brown stained stromal compartment of the cell membrane and divided into high and low expression groups (Figure 2). The characteristics of the sample are described in Table 1.

Based on the Table 1, of the 80 samples, there were 11 samples (13,8%) in the <40 years age category and 69 samples (86,3%) in the > 40 year age category. There were 17 samples (21.3%) with grade 1, 32 samples (40%) with grade 2, and 31 samples (38.8%) with grade 3. The number of samples with high CD8+ expression were 27 samples (33,8%) and 53 samples (63,3%) of low CD8+ expression were obtained.

Assessment of CD8+ TILs expression based on histopathological grade is shown in Table 2. In the high CD8+ expression group, from a total of 27 samples, 10 samples (58,8%) were grade 1, 6 samples (18,8%) were grade 2, and 11 samples (35,5%) were grade 3. In the

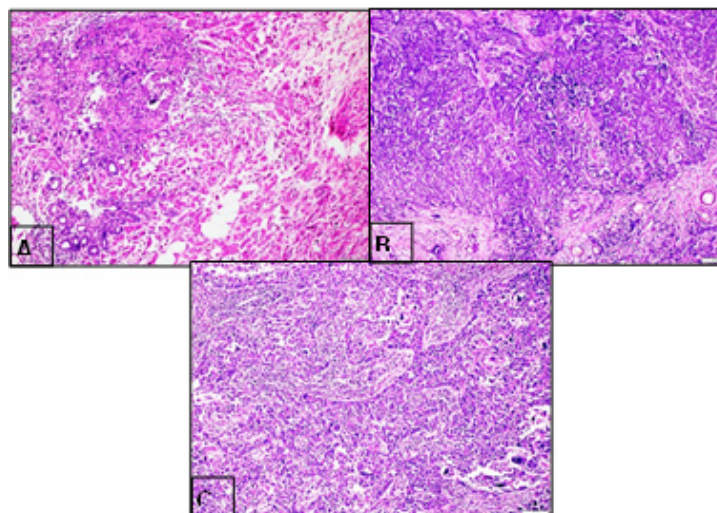


Figure 1. Histopatological Grade. A, Grade 1; B, Grade 2; C, Grade 3. (Magnification 100X)

Table 1. Sample Characteristics

Characteristics	n	(%)
Age		
< 40 years old	11	13.8
≥ 40 years old	69	86.3
Histopathological Grade		
Grade 1	17	21.3
Grade 2	32	40.0
Grade 3	31	38.8
CD8+ TILs		
High	27	33.8
Low	53	63.3

Table 2. CD8+ Expression Based on Histopathological Grade

	Histopathological Grade			Total	P
	Grade 1	Grade 2	Grade 3		
	n (%)	n (%)	n (%)	n (%)	
CD8+ TILs					
High	10 (58.8)	6 (18.8)	11 (35.5)	27 (33.8)	0.017
Low	7 (41.2)	26 (81.3)	20 (64.5)	53 (66.3)	
Total	17 (100)	32 (100)	31 (100)	80 (100)	
Chi-Square test					

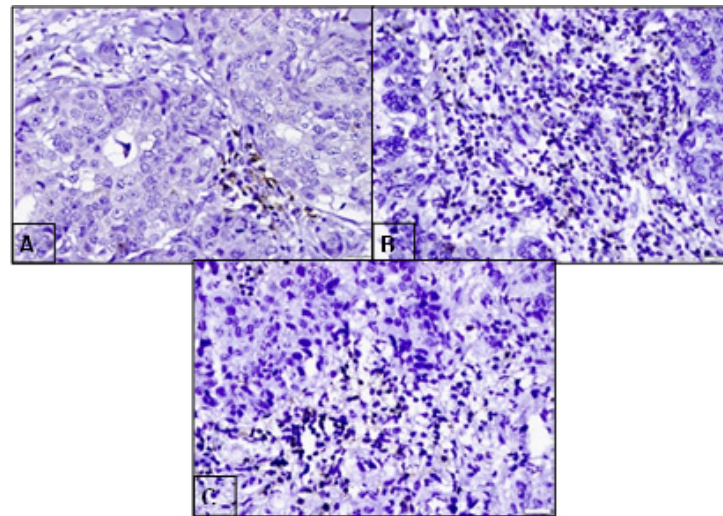


Figure 2. CD8+ Expression. A, Low CD8+ expression; B, dan; C, High CD8+ expression. (Magnification 400X)

low CD8+ expression group, from a total of 53 samples, 7 samples (41,2%) with grade 1, 26 samples (81,3%) with grade 2, and 20 samples (64,5%) with grade 3. Based on the Chi-square test, p value = 0.017 ( $p < 0.05$ ) which shows a significant difference in CD8+ TILs in the IBC-NST grade 1, grade 2 and grade 3.

## Discussion

Of the 80 IBC-NST samples, all of them were female with the age > 40 years (86.3%) more than the age <40 years group (13.8%). This is in accordance with the results of the SEER program (Surveillance, Epidemiology, and End Results) in 2009 to 2018 conducted by NCI (National Cancer Institute), the number of breast cancer patients ranged from 20 to 34 years old (1.9%), 35 to 44 years old (8.2%), 45 to 54 years old (19.2%), 55 to 64 years old (25.6%), 65 to 74 years old (26.0%), 75 to 84 years old (13.7%), and >84 years (5.4%). This is in accordance with the tendency of breast carcinoma as a malignancy of epithelial origin in general whose their incidence increases with age (Lakhani, 2012).

Tumor cells stimulate the specific adaptive immune system that can prevent or inhibit the growth and spread. Tumor cells can stimulate T cells, especially Cytotoxic T Lymphocyte (CTL) CD8+. However, tumor cells have

the ability to escape from the immune system through an immunoeediting process which consists of three phases: elimination, equilibrium, and escape. In the escape phase, the surviving tumor cells are no longer sensitive to the immune system so that tumor growth is uncontrolled. Several mechanisms by which tumor cells evade the immune response are through inhibitor molecules CTLA-4 (cytotoxic T lymphocyte-associated protein 4) or PD-1 (programmed cell death protein-1), TGF- $\beta$  cytokines, regulatory T cells, and myeloid-derived suppressor cells (MDSCs) (Abbas et al., 2018a).

CTLA-4, PD-1 and regulatory T cells inhibit tumor-specific T-cell function, TGF- $\beta$  inhibits T lymphocyte proliferation and effector function as well as macrophage activation, whereas MDSC suppresses both innate and adaptive immune responses through the secretion of immunosuppressive cytokines, such as IL-10 and TGF- $\beta$ , prostaglandins, and helps Treg differentiation. IL-10 inhibits the production of IL-12 by dendritic cells and macrophages which stimulates the secretion of IFN- $\gamma$  in innate and adaptive immunity, and inhibits the expression of MHC class II (Abbas et al., 2018a).

The histopathological grade of the tumor was assessed based on tubular formation, nuclear pleomorphism and the number of mitoses indicating differentiation and proliferation of the tumor (Rakha et al., 2019). In this

study, the high CD8+ expression group, from a total of 27 samples, 10 samples (58,8%) were grade 1, 6 samples (18,8%) were grade 2, and 11 samples (35,5%) were grade 3. In the low CD8+ expression group, from a total of 53 samples, 7 samples (41,2%) with grade 1, 26 samples (81,3%) with grade 2, and 20 samples (64,5%) with grade 3. Based on the Chi-square test, p value = 0.017 showed that there were significant differences in the expression of CD8+ TILs in each grade, where CD8+ TILs were highly expressed in low grades and low expressed in high grades. High grades show poorer differentiation and high tumor growth which can be caused by a failed immune response to inhibit the growth and development of tumor cells. Immune cells that mainly play a role in anti-tumor immunity are CD8+ CTLs. Thus, CD8+ TILs were under-expressed at higher grades indicating a failure of the immune response against tumor cells as in the results of this study. This is in line with studies conducted by Mahmoud et al (Mahmoud et al., 2011). and Peng et al (Peng et al., 2019). where the expression of CD8+ TILs was high at low grade and lymph node status was negative. In general, the prognosis of patients with breast cancer is closely related to grade, lymph node status, hormone receptor status and Ki-67 expression. Thus, high CD8+ TILs expression is associated with a better prognosis, and can be used as a potential prognostic factor in patients with breast carcinoma (Mahmoud et al., 2011; Peng et al., 2019). The expression of CD8+ TILs in IBC-NST showed a significant difference in grade, where the expression of CD8+ TILs was lower at higher grades. Immunohistochemical examination of CD8+ TILs can be used as an additional predictive and prognostic marker in IBC-NST.

## Author Contribution Statement

A, UAM, GA, SW and BJM prepared the concept, research design and data collection. A and AAZ were involved in data processing and analysis, as well as drafting articles. All authors reviewed draft articles.

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### Part of an approved

This research is part of an approved student thesis.

### Ethics Approval

This research ethics committee approved this study of the Faculty of Medicine Hasanuddin University in 2021 with approval number 128/UN4.6.5.31/PP36/2021.

### Conflict of Interest

There is no potential conflict of interest in this research.

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