

## Nuclear size and pleiomorphism of breast cancer cells at high and low Ki-67 proliferation index

Alfia Indah Dewi Alfina <sup>1</sup>, Willy Sandhika <sup>2,\*</sup> and Desak Gede Agung Suprabawati <sup>3</sup>

<sup>1</sup> Faculty of Medicine Universitas Airlangga Surabaya, Indonesia.

<sup>2</sup> Department of Anatomic Pathology, Faculty of Medicine Universitas Airlangga / Dr. Soetomo General Academic Hospital Surabaya, Indonesia.

<sup>3</sup> Department of Surgery, Faculty of Medicine Universitas Airlangga / Dr. Soetomo General Academic Hospital Surabaya, Indonesia.

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### Abstract

**Background:** Breast cancer cells are characterized by enlargement of nuclei and variation of nuclear size. The more anaplastic of cell nuclei reflect more aggressive and malignant cells that affect increase proliferation rate. The aim of this study is to reveal whether there was any difference of anaplastic characteristic of breast cancer cells in high and low degree of proliferation rate.

**Material and Methods:** Twenty-three cases of breast cancer were collected from archive of Pathology Department composed of high and low Ki-67 proliferations index. Anaplastic characteristics of cancer cells were obtained by measuring the area and pleomorphism of breast cancer cells in cytologic specimen from aspiration biopsy material.

**Result and Discussion:** There were no differences between breast cancer cells' size between high and low Ki-67 proliferation index while there was a difference pleomorphism between high and low Ki67.

**Conclusion:** The degree of anaplasia in breast cancer cells has a relationship with cancer cells proliferation. Further research should be made to reveal the mechanism of this phenomenon.

**Keywords:** Breast Cancer; Ki-67 Proliferation Index; Nuclear Cells Pleomorphism; Anaplastic Cells

### 1. Introduction

Breast cancer cells are characterized by enlargement of nuclei and variation of nuclear size. Breast cancer cells exhibit a spectrum of nuclear grades, which can be quantitatively assessed and are correlated with the malignancy level of the cancer. A larger cell nucleus, accompanied by variability in cell shape and size, may indicate that the cell is undergoing malignant proliferation [1]. Breast cancer cells exhibit a spectrum of nuclear grades, which can be quantitatively assessed and are correlated with the malignancy level of the cancer. A larger cell nucleus, accompanied by variability in cell shape and size, may indicate that the cell is undergoing malignant proliferation [2]. The more anaplastic of cell nuclei reflect more aggressive and malignant cells that affect increase proliferation rate. This paper aims to investigate the interrelation between the degree of malignancy as gauged by the nuclear grade and the proliferation index Ki-67, employing quantitative assessments of the nuclear measurements (Areas and Pleomorphism) of breast cancer cell nuclei and the enumeration of the proliferation index Ki-67, conducted through comprehensive manual analysis.

\* Corresponding author: Willy Sandhika

## 2. Material and Methods

### 2.1. Research Types and Design

This study employs a descriptive analytic approach with a cross-sectional design to investigate breast cancer cell malignancy, specifically examining the correlation between nuclear grade and the Ki-67 proliferation index. Descriptive analytic research provides a detailed numerical portrayal of the subject based on collected data or samples. This facilitates a thorough understanding of the research variables. The cross-sectional design of the research enables the analysis of collected data across a defined time span within the sample population, supporting the development of hypotheses pertaining to causal relationships for subsequent analytic examination.

### 2.2. Data and Sampling

The sample in this study is a subset of the breast cancer microscopic slide population from Dr. Soetomo Regional General Hospital (RSUD) Surabaya patients, namely those who underwent Fine Needle Aspiration Biopsy (FNAB) and Immunohistochemistry at Dr. Soetomo RSUD.

The number of patients with breast cancer at Dr. Soetomo RSUD in the year 2022 was 1297 patients. After reviewing, the patients who met the inclusion criteria numbered 119. With limited time, we took a sample of 23 patients with breast cancer with appropriate microscopic slide pictures and met the research sample inclusion criteria within the time frame of the year 2022.

### 2.3. Data Collection and Sampling

The sampling technique used for this research is Purposive Sampling. This technique is used to take samples from people who meet the required inclusion criteria as data from this research. In this study, data from twelve patients with a high Ki-67 proliferation index and twelve patients with a low Ki-67 proliferation index were used, taken from the patient's medical record history. This sampling was reviewed from the medical record criteria, FNAB slides, and patient Ki-67 IRS.

Initially, patient records from the hospital database were reviewed to gather data on individuals diagnosed with breast cancer, with a specific focus on extracting information pertaining to their pre-chemotherapy and post-chemotherapy status. The Anatomical Pathology Laboratory was tasked with providing the essential laboratory numbers that would uniquely identify each patient's records and facilitate subsequent analyses. Following data acquisition, the corresponding microscopic slides for the identified patients were obtained from the Anatomical Pathology Laboratory. The Fine Needle Aspiration Biopsy was stained with Hematoxylin-Eosin. These slides were then photographed using a high-resolution microscope camera, ensuring that each image captured would be suitable for detailed examination. Quantitative assessment began with the measurement of the nuclear area of the cellular structures. This involved printing the captured images onto millimeter block paper to accurately delineate and calculate the area of interest within 20 cells per patient. A standardized calibration protocol was applied to ensure the consistency and precision of measurements.

The study examined the Ki-67 proliferation index, a crucial biomarker for determining cell proliferation rates. This Ki-67 was taken and staining with immunohistochemistry method. High-quality microscopic images were printed onto standard A4 paper, and a quantitative assessment was performed by counting at least 100 cells expressing Ki-67. This count was compared to the baseline of normal cells to establish the Ki-67 index for each patient. These two principal metrics—the nuclear area and the Ki-67 index—were integrated to create a comprehensive dataset for analysis. Utilizing SPSS 26, we subjected the data to statistical tests, including the Shapiro-Wilk test to verify data normality and independent sample test to compare the data group characteristics between high and low value of Ki-67 groups.

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## 3. Results

### 3.1. Nuclear Size

Nuclear size is measured by the area and diameter. The measurement of nuclear size is conducting manually by printed-photo slide of Fine Needle Aspiration Biopsy on a millimeters block paper. The area and diameter are counted by the amount of the cubicles that hatching the nucleus on the paper. The measurement for each patient is at least twenty nucleus. Process calculating the nuclear size by using the formula of area and diameter  $x$  and  $y$ . All the collected data is subjected to a series of statistical tests to extract information and information that exist in them. To determine which

method to be used, normality of the data first needs to be determined. Hence normality test using SPSS26 is used. Since the data is below  $n = 50$ , Shapiro-Wilk is used as the statistical method to verify. The group statistics both of Low and High Ki-67 (Low defined as having Ki-67 percentage of 10-30% and High being 70-90%). The result of the Shapiro-Wilk normality test is the significance of both nuclear area, coefficient variance and nuclear diameter is above  $p$ -value 0.05, hence the data can be treated as normally distributed datapoints. The result of the measurement of nuclear size declare equal variances is not significant with result of 0.153 ( $p$ -value  $< 0.05$ ). The area mean value of the Low category of Ki-67 is approximately 129,469 and the area mean value of High category of Ki-67 is approximately 145,900. It can be concluded that in nuclear size has no significant difference between category High and Low Ki-67, it leads to there is no relationship between the nuclear size and anaplasia degree of breast cancer.

### 3.2. Nuclear Pleomorphism

Nuclear pleomorphism is counted by the coefficient variance and variance. The calculation of nuclear pleomorphism is conducting manually by printed-photo slide of Ki-67 IRS slides on papers. We do count the comparison between the expressed nucleus and the normal nucleus to finding the amount of Ki-67 proliferation index. For each patient at least has one-hundred cells counted in this field. The coefficient variance and variance are counted by the formula on SPSS ver. 26. Process calculating the nuclear size by using the formula of area and diameter  $x$  and  $y$ . The result of pleomorphism is significant where it can be inferred that the difference between high and low group of ki-67 is statistically significant with result of 0.006 ( $p$ -value  $< 0.05$ ). It can be concluded that there is a significant difference pleomorphism between category High and Low of proliferation index.

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## 4. Discussion

Studies show that the nuclear and cytoplasmic staining properties in retinoblastoma tumor cells are associated with the level of anaplasia. It was observed that the intensity of hematoxylin-eosin staining, which highlights cell nuclei, was notably increased in cells with severe anaplasia compared to those with less anaplasia. Conversely, the intensity of Eosin staining, targeting the cytoplasm, was significantly diminished in cells exhibiting severe anaplastic features as opposed to cells with lesser degrees of anaplasia [3]. Anaplastic cells are characterized by several distinctive features, such as hyperchromatic nuclei, pronounced nucleoli, and a nucleus-to-cytoplasm ratio approximating 1:1. These cells are marked by an elevated rate of mitotic division. Within a eukaryotic cell, the nucleus houses chromosomes, which are condensed forms of chromatin composed of DNA and proteins. In the context of anaplastic cells, hyperchromatism indicates an overabundance of chromatin, manifesting as intensified nuclear staining. Anaplastic cells and tissues, therefore, exhibit a visually distinct appearance from non-anaplastic counterparts, primarily due to their undifferentiated nature and potential for varying cell sizes, including the presence of giant cells. Furthermore, the nuclei in anaplastic cells tend to be darker and larger than those in non-anaplastic cells, a result of hyperchromatism. Additionally, anaplastic tissues may show altered cellular orientation and organization, with increased evidence of mitotic activity compared to non-anaplastic cells [4]. Histopathological tumor grading entails the evaluation of anaplasia in cellular specimens acquired through biopsy or surgical resection. This grading process hinges on categorizing the degree of resemblance between cancerous cells and their healthy counterparts in both morphology and functionality within the same tissue type. Specifically, a well-differentiated squamous cell carcinoma (SCC), or grade 1, exhibits only slight basal or parabasal cellular atypicality. In contrast, poorly differentiated or grade 3 SCCs are characterized by minimal to absent structural and cellular congruence with normal tissue. Grade 2 SCCs are those that do not conform to the specified characteristics of either grade 1 or grade 3. Notably, squamous cell carcinomas may display heterogeneity within a single neoplasm, presenting regions with varying levels of differentiation [5]. In breast cancer research, most studies demonstrate a statistically significant correlation with clinical outcomes, evident in both univariate and multivariate analyses. A strong relationship has been noted between the percentage of Ki-67-positive cells and factors such as nuclear grading, patient age, and mitotic rate. Additionally, several studies have indicated that Ki-67 expression is found to be higher on the young age [6]. This finding also related to other studies show that Ki-67 proliferation index has a strong relation with poorly differentiated breast cancer and the tumor grading [7].

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## 5. Conclusion

There were no differences between breast cancer cells' size between high and low Ki-67 proliferation index while there was a difference pleomorphism between high and low Ki67. The degree of anaplasia in breast cancer cells has a relationship with cancer cells proliferation.

## Compliance with ethical standards

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### *Disclosure of Conflict of interest*

The authors declare there is no conflict of interest in this study.

### *Statement of Ethical approval*

This study used data from Dr. Soetomo General Academic Hospital Surabaya. This study has received permission and approval from the Health Research Ethics Committee of Dr. Soetomo General Academic Hospital with number 1155/LOE/301.4.2/XII/2022

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## References

- [1] Meyer JS, Alvarez C, Milikowski C, Olson N, Russo I, Russo J, Glass A, Zehnbauer BA, Lister K, Parwaresch R. Breast carcinoma malignancy grading by Bloom–Richardson system vs proliferation index: reproducibility of grade and advantages of proliferation index. *Modern pathology*. 2005 Aug 1;18(8):1067-78.
- [2] Mendoza PR, Specht CS, Hubbard GB, Wells JR, Lynn MJ, Zhang Q, Kong J, Grossniklaus HE. Histopathologic grading of anaplasia in retinoblastoma. *American journal of ophthalmology*. 2015 Apr 1;159(4):764-76.
- [3] Yeung AM, Grossniklaus H, Mendoza P, D'Lorm R, Stålhammar G. Morphometric Analysis of Anaplasia in Retinoblastoma. *Investigative Ophthalmology & Visual Science*. 2020 Jun 10;61(7):2804-.
- [4] Yokoyama M. Features of anaplastic cells [Internet]. *News Medical Life Sciences*; 2018 [cited 2024 Jan 5]. Available from: <https://www.news-medical.net/life-sciences/Features-of-Anaplastic-Cells.aspx>
- [5] Gerald VDT. Squamous Cell Carcinoma of The Penis and Prepuce. In: Kim A. Sprayberry, N. Edward Robinson, Robinson's Current Therapy in Equine Medicine 7<sup>th</sup> ed. W.B Saunders; 2015. p. 418-423.
- [6] Leksono Rb. Hubungan Ekspresi Ki-67 Dengan Grading Histopatologi, Ukuran Tumor, Disease Free Survival Dan Overall Survival Pada Wanita Penderita Kanker Payudara = Correlation Between Expression of KI67 with Histopathological Grading, Tumor Size, Disease Free Survival and Overall Survival in Breast Cancer Patient (Doctoral dissertation, Universitas Hasanuddin).
- [7] Devi DGAP, Purwanto H, Sandhika W. Changes in Ki67 expression and clinical response to neoadjuvant chemotherapy in locally advanced breast cancer (LABC). *Bali Medical Journal*. 2021 Nov 12;10(3):925-31.