Background: Prostate cancer is one of the most common malignancies in men worldwide, and acinar adenocarcinoma is the most prevalent one. Gleason score and grade group is recently used to predict the therapy and prognosis based on Gleason pattern. Her-2 and Ki-67 are markers that are widely studied in breast cancer, but their role in prostate cancer is still unclear. Her-2 is a transmembrane protein involved in oncogenesis. We hope by using the basic determinant Gleason pattern to analyze the expression of these two markers, it will contribute to understand its carcinogenesis and prognosis. The aim of the study is to determine the expression of Her-2 and Ki-67 in Gleason grade of prostate acinar adenocarcinoma.

Methods: An analytic observational study with cross-sectional design was done using 31 paraffin-embedded tissues of prostate acinar adenocarcinoma from the Anatomic Pathology Laboratory of Dr. Kariadi General Hospital, focusing on 48 areas classified into pattern 3, 4, and 5. Immunohistochemical staining was performed using Her-2 and Ki-67 antibodies to all tissues and the data were analyzed using the Kruskal Wallis test and the Spearman test.

Results: There are significant differences and correlations between the expression of Ki-67 and Gleason grade, but there are neither significant differences nor correlation between Her-2 expression and Gleason grade.

Conclusion: Her-2 expression is almost the same among Gleason grade as basic determinant of prostate acinar adenocarcinoma.

Keywords: acinar adenocarcinoma, prostate, Her-2, Ki-67, gleason grade.


INTRODUCTION

Prostate cancer was the most common malignancy (non-skin) in men worldwide according to the International Agency for Research, GLOBOCAN Database 2018, accounted around 2% of all cancer-related deaths in the Asia-Pacific region in 2008.¹ The prevalence of prostate cancer in Indonesia in 2013 was 0.2 % or estimated as many as 25,012 patients based on data and information center of the Indonesian Ministry of Health in 2015.² Age, genetics, smoking, and family history can be a causative risk factors for prostate cancer. Conventional adenocarcinoma accounts for more than 90% of all prostate epithelial malignancies.³ Our data in Dr. Kariadi General Hospital shows acinar adenocarcinoma as the most common histological feature.

Gleason score generally applied to the histological feature of adenocarcinoma and determining the management therapy and prognostic factor of acinar adenocarcinoma, is based on Gleason grade/pattern.⁴ Due to the prognostic marker above has limited value to each individual, it is necessary to develop some other molecular prognostic markers to complete determination of prostate cancer management therapy. Her-2 protein oncogene and a proliferative marker Ki-67 are rarely used and remain unclear in prostate adenocarcinoma.

As a member of the class I tyrosine kinase receptor, Her-2 over-expression or amplification can occur in various human tumor epithelium, especially in breast cancer which is already established as a management therapy. Conversely, the involvement of Her-2 overexpression in prostate cancer is controversial. Several studies show a relationship between over-expression Her-2 with advanced stages and higher Gleason grades, but some are the opposite. Her-2 expression has also been associated to advanced disease, metastasis, short survival, poor response to chemotherapy, and even failure of endocrine therapy.⁶

Human Ki-67 widely used as marker of cell proliferation. The Ki-67 index is higher in prostate adenocarcinoma than benign hyperplasia and also in metastases than in non-metastases cases. Therefore, increased Ki-67 index may indicate a poor disease prognosis.⁷ However, its role as an independent prognostic marker among patients with prostate acinar adenocarcinoma is still controversial.
The expression of these two markers on prostate acinar adenocarcinoma and their correlation with Gleason grade, was conducted in this study. Gleason grade as the original data of microscopic feature to determine Gleason score and grade group score is expected to increase the sensitivity and specificity of their involvement in this prostate cancer, Her-2 as a oncoprotein and Ki-67 as regulator protein.

**METHOD**

Using observational analytic study with cross-sectional design, 31 sample formalin-fixed paraffin blocks of prostate acinar adenocarcinoma specimen were collected from the Anatomical Pathology Laboratory of Dr. Kariadi General Hospital, Semarang, Indonesia from January 1, 2015 to January 1, 2018. The tissue samples obtained during transurethral resection prostate (TURP) and radical prostatectomy were considered. Representative hematoxylin and eosin (H&E) stained sections of each paraffin block were examined microscopically to confirm and evaluate the Gleason grade on 48 areas classified according to Gleason grade into pattern 3, pattern 4, and pattern 5. Brief clinical data were noted from medical records, including age at diagnosis, serum PSA levels, and metastasis.

Immunohistochemical (IHC) profile was assessed by subjecting one section each from a representative block to

**Figure 1.** Gleason histologic grade of prostate acinar adenocarcinoma (HE, 100X): A. Pattern 3. Tubular neoplastic gland accompanied with lumen, varying in size, discrete. B. Pattern 4. Fusiform glands, irregular in shape; C. Pattern 5. Neoplastic cells with no glandular features, solid, cord-like growth pattern.

**Figure 2.** Her-2 expressions (400X): A. Score 0 is negative. There is no staining seen. B. Score 1: Faint staining in more than 10% of tumor cells. C. Score 2: weak to moderate staining, more than 10% of tumor cells. D. Score 3: Strong positive of more than 30% of tumor cells.

Her-2 (Polyclonal Rabbit Anti-Human c-erb-B-2 Oncoprotein. Code A0485. Daco Denmark) and Ki-67 (Monoclonal Mouse Anti-Human Ki-67. REF PA0118. LOT 64313. Leica. United Kingdom) immunostain. IHC was performed on 3-5 µm thick section from 10% formalin-fixed paraffin-embedded specimens,
according to the streptavidin-biotin immunoperoxidase technique (Biocare). Positive control was applied to both antibodies. Positive control for Her-2 was from Her-2 positive (+3) in breast carcinoma and reactive lymphoid for Ki-67.

The immunoquantification was performed using percentage of tumor cells that react with the antibody. Each slide was evaluated at 5 fields of 400x magnification randomly, and analyzed by two pathologists. The parameter evaluation of Her-2 expression in this study based on membrane staining classified into 4 groups: 0 (negative) No staining is observed or membrane staining is observed in less than 10% of the tumor cells, 1+ (negative): A faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane, 2+ (weak positive): A weak to moderate complete membrane staining is observed in more than 10% of the tumor cells, 3+ (strong positive): A strong complete membrane staining is observed in more than 30% (formerly 10%) of the tumor cells.

The parameters for evaluating the expression of Ki-67 in this study are based on the percentage of Ki-67 positive in the nucleus (painted brown or black, diffus / granular) is divided into 4 groups: 0 (negative) = 0%, + 1 (positive 1) = 1–2.5%, +2 (positive 2) = 2.6–4%, +3 (positive 3) ≥ 5%.

Data analysis was using Windows SPSS version 17. Categorical scale data are described in numbers and percentages. Kruskal Wallis non-parametric statistical test analysis was performed to determine the differences between the 2 variables and the Spearman's correlation test to determine the relationship between the 2 variables, with the significance value $p < 0.05$.

## RESULTS

This study involved 31 patients are mostly found in the age range of 61-70 years, as many as 16 sample (51.61%), whereas in the age range 41-50 years, only 1 person (3.23%) was found at the age of 50 years. Three patients were found in aged over 80 years.

Prostate acinar adenocarcinoma patients were categorized according to Gleason pattern/grade, consisting of 48 Gleason pattern. Each sample was taken 2 dominant areas, consisting of 15 areas (31.25%) of pattern 3, 19 areas (39.58%) of pattern 4, and 14 areas (29.17%) of pattern 5 (Figure 1).

All of 15 areas of pattern 3 have Her-2 positive in 11 areas (73.33%), consist of Her-2 score 1 on 2 areas (13.33%), score 2 on 7 areas (46.67%) and score 3 on 2 areas (13.33%). Two of 19 areas (10.53%) of pattern 4 showed negative while the rest are score 1 to 3. Score 3 is on 6 of 19 areas (31.58%) of pattern 4 and 4 out of 14 areas (28.57%) of pattern 5 showed negative, while 10 areas of pattern 5 (71.43%) revealed from score 1 to 3, including 3 areas (21.43%) show score 3 (Figure 2).

All of 48 areas were expressed nuclear positive for Ki-67. Ten of 15 areas (66.67%) of grade 3 show score 1 (1–2.5%), meanwhile 6 of 19 areas of pattern 4, and 6 of 14 areas of pattern 5, showed score 3 for Ki-67 expression (Figure 3).

The Kruskal Wallis test for Her-2 showed a difference in expression at each pattern, but it was not significant. In Ki-67, there was a significant difference

### Table 1. Expression of Her-2 and Ki-67 based on grade.

<table>
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<th>4</th>
<th>5</th>
<th>$p^*$</th>
<th>$p^{**}$</th>
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* Kruskal wallis test; ** Spearman test

![Figure 3](image1.png)  
**Figure 3.** Ki-67 expression in 400x magnification: A. Score 1: 1–2.5%, B. Score 2: 2.6–4%, C. Score 3: ≥5%.
between the expression of Ki-67 and grade. Spearman's Correlation Test showed no correlation between increasing grade and increased Her-2 expression. On the other hand, increasing grade is followed by increasing Ki-67 expression with significant correlation (Table 1).

DISCUSSION

Dealing with age as one of the main risk factors of prostate cancer, 75% of prostate cancer occur on over 65 years old as well as data from WHO. The range age of 61-70 years in this study is the most dominant number of 16 samples (51.61%) with an average age of 66.56 years, as well as the studies of Mardiana et al. and Verma et al. There is one youngest age patient 50 years old in this study with history of bone metastasis. Some studies suggest that young age is associated with aggressive disease behavior and higher mortality rate.

In this study, we use Gleason pattern on prostate acinar adenocarcinoma to analyze the expression of Her-2 and Ki-67. Grade/pattern analysis is expected to increase the level of sensitivity and specificity of immunohistochemical examination. Pattern ≤ 2 were excluded, because we did not find the pattern in all sample, and according to Chen et al. pattern 1 and 2 are very rare and even if there are exist, the pattern is no longer assigned on biopsy as well as similar to and considered as atypical adenomatous hyperplasia (AAH) rather than showing a feature of prostate adenocarcinoma.

Her-2 expressions with scores 2 and 3 showed mostly on pattern 4, whereas its expressions on patterns 3 and 5 are almost the same areas number. Gleason pattern 4 was fusiform glands, irregular in gland shape and cribriform gland which looks like pattern 3 with unusual atypical glands. Regarding to the gland formation and each epithelial cell border still exists, Her-2 staining to each epithelial cell membrane of the gland, can be attached to the membrane of the cells and easily detected. There are 15 out of 48 (31.25%) areas with grade 3 appearance, consist of 9 out of 15 areas (60%) express Her-2 score 2 and 3. Meanwhile, in grade 4, show 19 out of 48 areas (39.58%) with Her-2 expression score 2 and 3 dominate as 15 out of 19 areas (78.95%). Her-2 expression on grade 5 established 14 areas out of 48 areas (29.16%).

The expression differences of Her-2 on various patterns using the Kruskal Wallis test, shows insignificant values, and data analysis with the Spearman's correlation test, showed no relationship between increasing Her-2 expression and pattern. This is consistent with research that has been done by Nishio et al. that there was no significant difference in PSA levels, advanced-stage disease, Gleason score, T-stage, or N-stage between Her-2 positive and Her-2 negative. However, this study found that the 5-year specific survival rate was significantly lower in Her2 positive than Her2 negative, the 3-year non-recurrence rate in Her2 positive was also lower than Her2 negative. In line with Mardiana et al. prove that there is no significant relationship among Her-2, Gleason score, and the incidence of bone metastases.

Several studies show different results, related to the relationship between Her-2 expression and Gleason grading system. Signoretti et al. found higher Her-2 expression in patients with total androgen ablation therapy, compared with patients with surgical therapy alone, as well as the expression obtained in failure androgen ablation therapy patients failing are higher than in patients treated by surgery alone. In addition, this study states a positive relationship between Her-2 expression and higher Gleason pattern. The same thing was found in the study of Ross et al. revealed an association between Her-2 overexpression and tumor grade. The two studies above using immunohistochemistry to see Her-2 expression but also used FISH (Fluorescent In Situ Hybridization), to confirm gene amplification. The results obtained from this study, gene amplification was found in 41% of patients, and has a relationship with high-grade tumors and in these patients, 2.3 times higher for tumor recurrence.

Prostate adenocarcinoma patients with Her-2 overexpression can be related to androgen-independent status. In the absence of androgens, overexpression of Her-2 activates PSA transcription. Her-2 mediated PSA activation, which is required AR that is not inhibited by anti-androgen drugs. Thus, overexpression of Her-2 in prostate cancer cells activates the AR pathway in the absence of ligands, stimulating androgen independence from prostate cancer cells. Her-2 initially causes survival to tumor cells by activating the AR pathway in an androgen-independent way. However, these same cells may still need androgens for proliferation. This hypothesis is supported by the observation that, although most secretory cells undergo apoptosis after androgen withdrawal, the remaining secretory cells retained in the prostate exhibit high levels of Her-2 protein. Increased expression of Her-2, is thought to be prostate-specific rather than tumor-specific mechanism for cell survival in androgen-deficient environments.

Regarding the relationship of metastasis with the Her-2 expression, positive values were found in all metastatic samples such as +1 of 1 sample, +2 of 5 samples, +3 of 3 samples. According to Murray et al., at least 85% of men with advanced disease will have bone metastasis, with an increase in the number of metastatic-free patients at the time of initial treatment having hidden micrometastasis. Furthermore, 30% to 50% of men with localized prostate cancer will develop biochemical failure with an increased PSA at 10 years. This occurs because conventional methods do not detect the spread of cancer cells at the onset of the disease. Cancer cells spread first through neural invasion, then to the blood and move to other tissues by passing capillary endothelium, invading, and forming micrometastasis. Tumor invasion is considered an unregulated physiological activity, with similarities between the molecular events of tumor invasion and normal processes such as angiogenesis and wound healing. Morote et al. who used 70 adenocarcinoma patients with metastases, reported overexpression of 64% in prostate adenocarcinoma patients with metastases. However there were no significant differences, related to Gleason score or Her-2 expression, either in bone metastases or outside the bone. Nishio et al. argue that Her-2 overexpression in prostate cancer patients with bone metastases can be a marker of poor prognosis for predicting recurrence and outcome intervals after endocrine therapy.
The difference between our study and other studies, can be caused by several factors. Our study used minimal number of samples, 3-year-length of study, manual Her-2 staining, TURP samples which have limited field to observe although TURP is most samples in our hospital that sent by the urologist due to minimal invasive management, and some limited clinical information such as PSA level and androgen status.

Analysis of Ki-67 expression data to grade with the Kruskal Wallis difference test showed a significant difference and the Spearman’s correlation test, showing a relationship between increased Ki-67 expression and Gleason pattern. This is consistent with studies that have been conducted, such as Richardsen et al. study, proving that there is a relationship between increased Ki-67 expression and Gleason grade, tumor size, and pT stage. Studies conducted by Verma et al. revealed that correlation between Ki-67 and Gleason grade, with p-value 0.002, it is said that every 1% increase in Ki-67 levels associated with Gleason Score is associated with a 12% increase in mortality related to prognosis. In the Fisher et al. cohort study, which aims to look at the value of the prognosis of Ki-67 against deaths from prostate cancer, in the univariate analysis obtained Gleason score, PSA, widespread disease, age at diagnosis, and Ki-67 score, are significant predictors for deaths from prostate cancer.

This is consistent with the theory that the marker of Ki-67 proliferation shows tumor cell proliferation, which is associated with progression, metastasis, and prognosis in several malignancies. Ki-67 is a regulatory protein related to cell cycles in the nucleus, and its expression can be detected during interphase in the nucleus in tumor epithelial cells (G1, S, G2, and mitosis), and is not found in resting cells (G0), thereby making Ki-67 excellent markers for assessing tumor growth fraction.

In this study, the expression Ki-67 with score 1, 2, 3 were found in all metastatic samples. The study by Diaconescu et al. proved that the expression of Ki-67 as a proliferation marker has a predictive value in patients with prostate cancer with lymph node metastasis is evidenced by the very significant difference in Ki-67 expression between primary tumors without metastasis with metastasis lymph nodes, although there are also those who get the opposite results such as Vis et al. studies, which have a low suitability and sensitivity for the expression of Ki-67 as a prognosis for disease aggressiveness.

The difference result above can be caused by several factors, studies that provide significant results, using samples from radical prostatectomy preparations, so that the area examined to see a tumor cell proliferation index is greater. In studies that yielded the opposite results, using needle biopsy, and only 1-2 cores were examined so that it did not represent the primary tumor, besides the heterogeneous pattern of prostate adenocarcinoma, making it difficult to analyze, because it mixed with benign gland. Besides, there is no standard cut-off point for assessing Ki-67 expression, making it difficult to set standards for Ki-67 levels in prostate cancer. Fisher et al. revealed that a 10% cut-off point was more significant compared to 5%, in terms of prognosis of death, and biochemical recurrence.

CONCLUSION

The expression of Her-2 on Gleason pattern prostate acinar adenocarcinoma is not significantly different and has no correlation. This study revealed significant difference of expression and correlation between Ki-67 positivity and increased Gleason pattern. Therefore we propose that these markers can be applied along with other prostate cancer progressive factors as on breast cancer and lymphoma usually done.

Reporting percentage of Gleason pattern by analyzing histopathological pattern is very important, especially for therapy management and predicting further prognosis for acinar adenocarcinoma patients. Her-2 expression by immunohistochemical is greatly influenced by several factor, such as serum PSA levels, androgen status, and complete clinical data is very needed to support more satisfying results.

ETHICAL APPROVAL

The study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Diponegoro, with number 554/EC/FK-RSDK/VIII/2018.

CONFLICTS OF INTEREST

The authors affirm no conflict of interest in this study.

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AUTHOR CONTRIBUTIONS

Conceptualization, Ika Pawitra Miranti and Putri Ajeng Ayu Larasati; methodology, Vega Karlowee and Indra Wijaya; software, Hermawan Istiadi; validation, Ika Pawitra Miranti and Putri Ajeng Ayu Larasati; formal analysis, Ika Pawitra Miranti; investigation, Ika Pawitra Miranti and Putri Ajeng Ayu Larasati; resources, Dik Puspasari; data curation, Hermawan Istiadi and Putri Ajeng Ayu Larasati; writing—original draft preparation, Ika Pawitra Miranti and Putri Ajeng Ayu Larasati; writing—review and editing, Ika Pawitra Miranti and Hermawan Istiadi; visualization, Dik Puspasari; supervision, Indra Wijaya; project administration, Vega Karlowee; funding acquisition, Ika Pawitra Miranti.

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