Correlation between Reactive Stromal Expression and Histochemical Trichrome Masson Staining with Histopathological Grading in Prostate Adenocarcinoma at Adam Malik Haji Center General Hospital Medan

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ABSTRACT

Background
Adenocarcinoma of the prostate is an invasive carcinoma, composed of neoplastic prostatic epithelial cells with differentiation and secretion arranged in various histomorphological patterns.Quantitative analysis of the elements that make up the stroma in casesprostate adenocarcinomahas been linked to prognostic factors and the Gleason score, which correlates with progression and metastasis, and may contribute to new prognostic feature approaches.

Method:
Descriptive analytic study with 33 paraffin block samples diagnosed as prostate adenocarcinoma at Haji Adam Malik General Hospital Medan.Assessment is done forsee the relationship between reactive stromal expression using Trichrome Masson and histopathological grading gleason score then determine the scoring score for the stroma around the prostate gland adenocarcinoma lesion stained blue with trichrome masson, a positive score of one (+): if 0-15% stroma is stained blue, positive two (++): if >15%-<50% stroma is stained and triple positive (+++): if >50% stroma is stained blue. The histopathological grading was categorized into grade I (well differentiated: Gleason score ≤6), grade II (moderately differentiated: Gleason score 7 (3+4)), and grade III (poorly differentiated: Gleason score 7 (4+3) and 8 -10).

Results
Reactive stromal expression the most found was positive three (+++) (69.7%) and the most common histopathological grading was grade III (69.7%). There was a significant correlation between reactive stromal expression and histopathological grading in prostate adenocarcinoma (p-value=0.044), namely the worse the cell differentiation, the higher the positive score of reactive stromal expression.

Conclusion
Assessment of reactive stromal expression associated with histopathological grading can be used as an indication of prognosis in patients with prostate adenocarcinoma.

Keywords: Prostate adenocarcinoma, reactive stromal expression, gleason score, trichrome masson.

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INTRODUCTION

Prostate disorders are one of the main causes of health problems in men worldwide. This disease ranks second most frequently appearing in men after respiratory tract disorders. Currently, the dominant prostate disorders are benign prostate enlargement and prostate cancer. According to Global Cancer Statistics 2020 data, malignancy of the prostate ranks 3rd out of the 36 highest cases of malignancy in the world. Where the number of new cases is quite significant after cases of malignancy of the breast and malignancy of the lungs, with a value of 1,414,259 (7.3%) and a mortality rate of 375,304 (3.8%). In cases of specific malignancy in men, prostate malignancy is ranked the second highest after lung malignancy with a rate of 37.5/100,000.[1,2] Description of the elements that make up the stroma in the caseprostate adenocarcinomahas been linked to prognostic factors and the Gleason score, which has shown correlations in progression and metastasis, and may contribute to new prognostic feature approaches.[3]

METHOD

This research is an analytical study with a cross sectional approach. The study was conducted at Haji Adam Malik General Hospital Center Medan from November 2021 to August 2022. The population for this study consisted of all secondary data and paraffin blocks from patients who were histopathologically diagnosed as prostate adenocarcinoma who met the inclusion and exclusion criteria in Department of Anatomic Pathology, Faculty of Medicine, Universitas Sumatera Utara and Anatomic Pathology Unit of Haji Adam Malik General Hospital, Medan. The sample size in this study is the entire affordable population selected by using total sampling. The variables in this study were histopathological grading as the independent variable and reactive stromal expression as the dependent variable.

Grade prostate adenocarcinoma histopathology is assessed based on the WHO and ISUP grade grading system, which is assessed based on the Gleason architectural pattern assessment and then included in the next category, namely the grade group and histopathological grading, which are divided into 3, namely: Grade I is well differentiated: Gleason score ≤6, Grade II: Gleason score 7 (3+4). Grade III: Gleason score 7 (4+3) and Gleason score 8-10.[4]

Reactive stromal expression is the assessment of the stroma around the gland stained blue with Trichrome masson. In this study, an assessment was made of the stroma around the prostate adenocarcinoma gland lesion with a magnification of 200x, in 3 fields of view. The scoring system used to calculate the number of reactive stromal expression areas is as follows: Positive one (+): if 0-15% of the stroma around the prostate gland adenocarcinoma lesion is stained blue with trichrome masson. Positive two (++): if >15%-<50% of the stroma around the prostate adenocarcinoma lesion is stained blue with trichrome masson. Triple positive (+++): if >50% of the stroma around the gland of the prostate adenocarcinoma lesion is stained blue with trichrome masson.[5]

The collected data will be analyzed using the SPSS v 20 statistical program. Data analysis will be performed using the Somers'd Test. The p-value <0.05 was stated to be statistically significant.

RESULTS

From the 35 samples with *TUR-P* and core biopsies in this study who were diagnosed with prostate adenocarcinoma from 2021 to 2022, at Haji Adam Malik Hospital in Medan, only 33 samples had complete medical record data.

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Table 1.Characteristics of samples of patients with prostate adenocarcinoma.

Variable (n=33)	Frequency (f)	Percent (%)
Age		
≤50 years old	2	6.1
51-60 years old	9	27.3
61-70 years old	14	42.4
>70 years old	8	24.2
PSA Value Category		
Normal	7	21.2
Currently	3	9.1
High	23	69.7
Grade Group (Gleason Score)		
Grade group 1	2	6.1
Grade group 2	6	18.2
Grade group 3	2	6.1
Grade group 4	9	27.3
Grade group 5	14	42.4
Grading histopathology		
Well differentiated	2	6.1
Moderately differentiated	8	24.2
Poorly differentiated	23	69.7
Reactive stromal expression		
Positive one (+)	3	9.1
Positive two (++)	7	21.2
Positive triple (+++)	23	69.7

In this study, it was found that the sample with the youngest age of prostate adenocarcinoma was 43 years old, the oldest was 89 years old, and the mean age of the patients was 64.65 years. Most samples aged 61-70 years old, totaling 14 samples (42.4%), followed by the 2nd most aged 51–60 years old (9 people, 27.3%) and the 3rd >70 years old (8 samples, 24.2%), and ≤50 (2 samples, 6.1%). Based on the PSA value, the highest proportion of prostate adenocarcinoma samples had high PSA values, amounting to 23 samples (69.7%). Normally number 7 sample (21.2%) and PSA values being 3 in number sample (9.1%). Based on the gleason score, in this study it was found that 2 samples had grade group 1: gleason score ≤6 (6.1%), 6 samples grade group 2: gleason score 3+4=7 (18.2%), 2 samples grade group 3: gleason score 4+3=7 (6.1%), 9 samples grade group 4: glessons score 8 (4+4=8**,** 3+5=8, 5+3=8) (27.3%) and 14 samples grade group 5: gleason score 9-10 (4+5=9, 5+5=10) (42.4%). Assessment of histopathological grading by grouping into 3 groups and obtained the following results; 2 samples (6.1%) of prostate adenocarcinoma patients were determined as well differentiated. 8 samples (24.2%) were determined as moderately differentiated and 23 samples (69.7%) were determined as poorly differentiated. From the results of this study, it was found that the largest sample of 23 samples (69.7%) had a positive reactive stromal expression score of three (+++), 7 samples (21.2%) had a positive expression score of two (++) and were followed by by 3 samples (9.1%) had a positive reactive stromal expression of one (+).

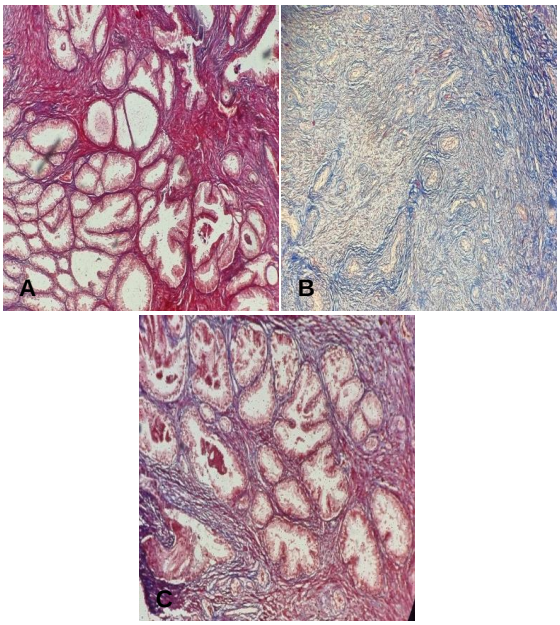

Figure 1. Prostate adenocarcinoma sample with trichrome masson stain. (A) Positive one (+) trichrome masson, 100 times magnification. (B) Positive two (++) trichrome masson, 100 times magnification. (C) Positive triple (+++) trichrome masson, 100 times magnification.

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Table 2. Relationship between reactive stromal expression and histopathological grading.

Variable	Histopathological grading category			Total	value	*p**
	Well differentiated f(%)	Moderately differentiated f(%)	Poorly differentiated f(%)	f(%)		
Reactive stromal expression						
Positive one (+)	0 (0,0)	0 (0,0)	3 (9,1)	3 (9,1)	0.351	0.044
Positive two (++)	2 (6,1)	4 (12,1)	1 (3,0)	7 (21,2)		
Positive triple (+++)	0 (0,0)	4 (12,1)	19 (57.6)	23 (69.7)		
Total	2 (6,1)	8 (24,2)	23 (69.7)	33 (100.0)		

*) Somers'd correlation test

The results of the analysis of the relationship between expressions the histopathological grade of prostatic adenocarcinoma showed that the highest one (+) positive stromal expression occurred in the Poorly differentiated category with a total of 3 samples (9.1%), the highest positive two (++) reactive stromal expression occurred in the Moderately differentiated category with a total 4 samples (12.1%). And the three highest positive reactive stromal expressions (+++) occurred in the Poorly differentiated category with a total of 19 samples (57.6%). A correlation test was performed using the Somers'd test and p-value = 0.044 (p <0.05) was obtained. With the results of this test, it was found that there was a significant correlation between reactive stromal expression and histopathological grading with a relatively weak correlation (0.21-0.40).

DISCUSSION

The number of samples of prostate adenocarcinoma that met the inclusion and exclusion criteria at the Anatomic Pathology Unit of Haji Adam Malik General Hospital Medan was 33 samples. At the same hospital, Mardiah *et al* in their research obtained prostate cancer data for 35 samples with most samples (44.1%) aged >60 years old.[6] Haji Adam Malik General Hospital Medan is a referral center for type A hospitals. The cases in this study may have occurred due to two things, namely patients with prostate cancer who have been treated at type B hospitals and patients from other regions prefer alternative treatments, due to the lack of knowledge about prostate cancer.

Prostate cancer shows a marked increase with age, that is, more than 80% of prostate cancers are diagnosed in men aged >65 years old.[7] The results of this study are in line with the theory and other studies which state that this carcinoma is very rare in men <40 years old.[1,4,6] The likelihood of developing prostate cancer increases rapidly after the age of 50 years old.[6,8-11] Prostate cancer is most often diagnosed in men aged 65-74 years old.[1,6,12] Osorio *et al*, in their study, wrote that the average age of prostate cancer patients was 64 years old.[13]

The incidence rate is nearly 60% in men over 65 years old. This happens because prostate cancer at an early stage is usually asymptomatic and often indolent.[14] Coupled with the low level of knowledge and awareness of the importance of medical examinations (screening of PSA values) for most of the Indonesian population, most of whom still have a low economy, the patient is more often detected suffering from prostate cancer after having experienced an advanced stage or elderly.

Serum PSA value before surgery is a diagnostic and prognostic parameter for prostate cancer, where the higher the PSA value, the higher the suspicion of prostate cancer.[15] PSA is produced mainly by columnar epithelial cells of the prostate gland. this PSA value can increase in BPH disease, prostate cancer, prostatitis, prostate infarction, ejaculation, after prostate massage, and after instrumentation.[16] The results of this study are similar to those of Mardiah *et al*, Putriyani *et al*, Hilbertina *et al*, and Sanni *et al*, found that the majority of prostate adenocarcinoma patients at Haji Adam Malik General Hospital Medan in the 2014–2016 period had PSA values above 4ng/ml (81.7%) and the rest ≤4ng/ml.[17]

Fang *et al*, stated that when prostate cancer develops it causes the volume of tumor tissue to increase so that the tumor tissue becomes increasingly irregular and the gleason score also increases. Irregular gland growth, gland lumen is not formed and the matrix component is reduced. PSA secretion by cancer cells will also increase as the barrier between the basal cell layer and the basement membrane is severely damaged. In addition, angiogenesis is also active at the same time, which in turn results in the leakage of PSA into

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the blood circulation and an increase in serum PSA values.[18]

Amarneel *et al,* stated that in the neoplastic process, the increase in serum PSA depends on the differentiation of tumor cells. Poorly differentiated prostate carcinoma can lead to lower PSA concentrations compared to well differentiated ones. In patients with prostate adenocarcinoma, the malignant cells produce less PSA than normal epithelial cells. Even so, due to the very large increase in the number of malignant cells in carcinoma, more PSA will be produced and the serum PSA value will increase.[19] This theory supports the results of this study that approximately 72.72% of prostate adenocarcinoma patients have high PSA values.

Gleason grading is the strongest predictor of survival after initial treatment of prostate cancer.[20] Dr. Donald Gleason created a grading system for prostate carcinoma based on different histological patterns.[21] Assessment of the grade of prostate adenocarcinoma with the gleason score system is a good predictor for determining the rate of disease progression.[22] This assessment is applied by clinicians such as the Indonesian Association of Urologists and the National Comprehensive Cancer Network.[23,24] Based on the Gleason score, in this study it was found that approx 69.7% of prostate adenocarcinoma samples were determined as poorly differentiated, approximately 24.2% moderately differentiated and 6.1% well differentiated. The results of the analysis of the relationship between expressionsreactive stromal with histopathological grading of prostate adenocarcinoma obtained p-value = 0.044 (p <0.05) which showed a significant correlation betweenreactive stromal expression with histopathological grading. The results of this study are in line with Putriyuni *et al* and Hilbertina *et al* In their research, they also found that poorly differentiated adenocarcinoma of the prostate was most commonly found, followed by moderately differentiated and well differentiated.[15] Meanwhile, based on grade group, of the 33 samples in this study, approximately 42.4% were included as grade group 5; 27.3% grade group 4; 18.2% grade group 2; 6.1% grade group 1; and 6.1% grade group 3. The results of this study are not in accordance with research. Erickson *et al*, They studied the Turku cohort 2000-2005 and found that about 36.8% of cases were assigned to grade group 1; 29.3% grade group 2; 15.3% grade group 4; 13.8% grade group 3; and only 4.8% grade group 5.[20] The difference in these results occurred due to differences in the location of the study, namely differences in countries or regions. Some of these differences have been associated with differences in socioeconomic conditions so that the environmental factors that cause adenocarcinoma of the prostate are of course also different.[14] In addition, most patients who come for treatment at Haji Adam Malik General Hospital in Medan have had prostate cancer for a long time and are in a more severe condition. so it is likely to have a higher gleason score and grade group.

Normal prostatic stroma is a smooth muscle phenotype so that the desmoplastic response of the stroma in the prostate is less obvious on histopathological preparations with hematoxylin-eosin staining. Using a trichrome masson smear can clearly distinguish between normal and reactive stroma.[25] In reactive stroma, myofibroblasts synthesize ECM components such as collagen type I, collagen type III, fibronectin isoforms, tenascin, and versican. In addition, myofibroblasts express proteases such as urokinase plasminogen activator (UPA), FAP and matrix metalloproteinases (MMPs). The production of these components results in regulation of the ECM which can stimulate the growth and migration of cancer cells. Furthermore, it is also known that myofibroblasts secrete growth factors that stimulate angiogenesis. Thus myofibroblasts are key cells involved in the formation of a reactive stromal environment that stimulates tumor growth.[26] In this study it was shown that all samples showed reactive stromal expression with different percentages of expression. From the 33 samples studied, the highest percentage of reactive stromal expression was positive three (+++) with 23 samples (69.7%), followed by positive two (++) with 7 samples (21.2%) and then positive one (+) with 3 samples (9.1%).

The limitations of this study are first, the researchers only assessed reactive stromal expression based on trichrome masson staining in cases of prostate adenocarcinoma, so they could not describe comparisons with other cases such as in cases of BPH and PIN. Second, there is limited information on clinical data in medical records, so researchers cannot

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obtain information on serum PSA values in several cases.

CONCLUSION

The most age group was aged 61-70 years old, the highest PSA value was in the high category, the most grade group was grade group 5, and the most histopathological grading was poorly differentiated. The most reactive stromal expression is the triple positive expression (+++). There is a statistically significant relationship between reactive stromal expression and gleason score histopathological grading in prostate adenocarcinoma with $p < 0.05$.

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