

Modulation of T-cell Regulators Associated with Advanced Stage of Prostate Cancer

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ABSTRACT

Background: Benign prostatic hyperplasia (BPH) and prostate cancer are the most common prostate diseases. The possible role of the immune system in the pathogenesis of BPH and prostate cancer in recent years has begun to be widely studied. Although many studies have focused on T lymphocytes on the development of BPH and prostate cancer, the role of regulatory T-cells in the pathogenesis of BPH and prostate cancer is still not well known. **Objective:** To determine the amount of regulatory T-cells in prostate cancer and BPH so that it can contribute to the concept of understanding the pathogenesis of prostate cancer and BPH. **Methods:** This study used cross-sectional design study. Total samples were 24 patients, with 13 subjects prostate cancer group, and 11 subjects BPH group. Furthermore, peripheral blood samples are taken and then the amount of regulatory T-cells is calculated. After obtaining data on the amount of CD4⁺ CD25⁺ Foxp3⁺ regulatory T-cells in the blood, data analysis was performed between groups of patients diagnosed with prostate cancer and benign prostatic hyperplasia. **Results:** The average amount of regulatory T-cells in the CRPC group was 53.44±29.43, prostate cancer group was 57.02±22.49 and the BPH group 89.71±9.31. One Way ANOVA test results showed that the average amount of regulatory T-cells between treatment groups gave a significant difference in regulatory T-cells with a p-value (0,003) <0.05. It can be concluded that there are differences in the average amount of regulatory T-cells, so we continued the testing with Tukey test. We continue to Pearson correlation study and resulted in significantly correlated with p value = 0.011 (P<0.05) and r = 0.414. **Conclusions:** It can be concluded there was significant difference between the average number of regulator T-cells in the BPH group compared with prostate cancer and CRPC patient. Further research is needed regarding the number of regulator T-cells CD4 + CD25 + FOXP3 + in prostate cancer patients (grouped according to Gleason score) and benign prostatic hyperplasia before and after therapy with bigger samples.

Keywords: Castrate Resistant Prostate Cancer, Prostate cancer, Benign Prostate Hyperplasia, T-cell regulator

1. BACKGROUND

The prostate is a male genital organ located inferiorly from the urinary bladder, in front of the rectum and enveloping the posterior urethra (1). Benign prostatic hyperplasia (BPH) is an enlarged prostate gland caused by cellular hyperplasia (2) meanwhile prostate cancer develops in the prostate gland, caused by mutations of prostate cells resulting in uncontrolled cell proliferation, as time goes by it could develop into state of androgen resistant termed castrate-resistant prostate cancer

(CRPC) (3).

Benign prostatic hyperplasia and prostate cancer are the most common prostate diseases, where BPH occurs in at least 70% of men aged 70 years (2), while prostate cancer is one of the most common malignancies that occur in men throughout the world, including Asia (4, 5). One risk factor for the pathogenesis of BPH, which also seems to play a role in the development of prostate cancer, is inflammation. Inflammation as a risk factor is evidenced by several studies where BPH progression to prostate

cancer is higher in tissues with inflammatory infiltrates than without inflammation (6).

The possible role of the immune system in the pathogenesis of BPH and prostate cancer in recent years has begun to be widely studied (7, 8). Chronic inflammation is caused by an infectious agent, causing epithelial turnover which increases the risk of malignancy by around 15%. Acute and chronic inflammation in the urogenital system shows an accumulation of immunocompetent T-cells in prostate tissue, especially T lymphocytes and macrophages. These cells secrete various cytokines, such as IL-2, IFN gamma, IL-6, IL-8, IL-15, which play a role in pathological changes and also lymphocyte activation which is characteristic of BPH and prostate cancer (7).

Although many studies have focused on T lymphocytes on the development of BPH and prostate cancer, the role of regulatory T-cells in the pathogenesis of BPH and prostate cancer is still not well known (7). Regulatory T-cells are a population of CD4⁺ T-cells with special phenotypic characteristics namely CD4⁺ CD25⁺ FOXP3⁺ which play a role in maintaining cell tolerance and maintaining tissue homeostasis (9, 10).

Cancer cells express antigens that can trigger cytotoxic T-cells, Natural Killer (NK) cells and macrophages to destroy these cells (11, 12). But if there is a failure of the immune system, it will result in tumor growth (13). Regulatory T-cells can suppress both immune cells that play a role in the humoral and cellular immune systems, by influencing the activity of surrounding immune cells (8, 14). This suppression of antitumor immunity makes these cells promoters of tumor growth (8). Obtained increased Regulatory T-cells in tumor tissue or cancer patient circulation, is evidence that these cells are involved in the pathogenesis and progression of cancer (15). Study of CD4⁺ CD25⁺ FoxP3⁺ T-cells in prostate cancer, and BPH was investigated by Mrakovic in 2014. In his research, the expression of CD4⁺ CD25⁺ FoxP3⁺ levels were higher in prostate cancer than in BPH (16). Until now the role of regulatory T-cell levels in BPH, prostate cancer, and CRPC are still inconclusive.

2. OBJECTIVE

This study aimed to find out regulatory T-cells involvement in BPH, prostate cancer, and CRPC to the concept of understanding the pathogenesis diseases.

3. MATERIAL AND METHODS

Subject

This study is a clinical observational analytic study. We include men aged to 30 to 75 years diagnosed with benign prostatic hyperplasia by PSA, ultrasound TRUS or histopathology (BPH group). Subjects were diagnosed with prostate cancer by PSA, TRUS ultrasound or histopathology, at all stages (prostate cancer group and CRPC). Subjects who have been diagnosed with prostate cancer who have been treated, but drop out within a period of 6 months, before sampling (prostate cancer group and CRPC). Understand the research objectives and research procedures, and be willing to participate in research voluntarily by signing

an informed consent agreement sheet.

We exclude subjects suffering from diseases that are likely to affect regulatory T-cell levels, such as autoimmune diseases, multiple sclerosis, type 1 diabetes, rheumatoid arthritis to minimize confounding. Subjects who have been diagnosed with prostate cancer who have received hormonal, immunosuppressive or radiation therapy for less than 6 months, before sampling. Subjects who received immunosuppressive therapy.

Measures

The research design was analytic observational with cross-sectional approach. The subjects in this study were divided into 2 groups, namely the BPH group (11 patients) and prostate cancer group (13 patients). The target population in this study were patients diagnosed with prostate cancer, benign prostatic hyperplasia and CRPC at Dr. General Hospital Saiful Anwar in the research period from October 2018 to February 2019. This research had ethical approval from Health Research Ethics Committee General Hospital Dr. Saiful Anwar Malang no. 400/180/K.3/302/2020.

Procedure methodology

All patients who had been the study sample were made complete CRPC, prostate cancer status and BPH and data collection sheets. For immunological monitoring, blood samples were taken from the patients before start of therapy. Peripheral blood was used for whole-blood analysis, or for peripheral blood mononuclear cell (PBMC) isolation by density centrifugation (Nycomed AS, Oslo, Norway). PBMC was either immediately stained and assessed by flowcytometry with lysing solution (BD Biosciences, Mountain view, CA) as described (23) in the Biomedical Laboratory of the Faculty of Medicine, Universitas Brawijaya. Cell surface antibody staining of PBMC was performed in FACS buffer for 30 min at 4°C. Intracellular CD4⁺ CD25⁺ Foxp3⁺ T-cells staining was conducted with antihuman FoxP3 staining kit (eBioscience, San Diego, CA) according to manufacturers' protocol. The following antibodies were used: fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)-, and peridinin chlorophyll protein-Cy5.5 (PerCP)-labeled antibodies directed against human CD4, CD25, and FoxP3 (all BD Bioscience). Stained cells were analyzed on a FACScalibur (BD Biosciences) using Cell Quest software.

Statistical analysis

Data were analyzed using SPSS version 17 (SPSS Inc., Chicago, IL). Characteristics of the sample are presented descriptively, using tables and narration. Analysis of data normality with the Kolmogorov-Smirnov test. If it is normal, then analyzed the data with the ANOVA Test with post hoc Tukey's test followed by Pearson Correlation test and . Value of P <0.05 was considered as the cutoff value or significance.

4. RESULTS

In this study, the total sample was 24, with 8 patients of BPH, 8 prostate cancer patients and 8 subjects of CRPC. The mean age of BPH patients was 63.55 ± 4.80, prostate cancer patients was 63.08 ± 4.92 and CRPC patients was 62.08 ± 5.12. There were no significant difference in terms

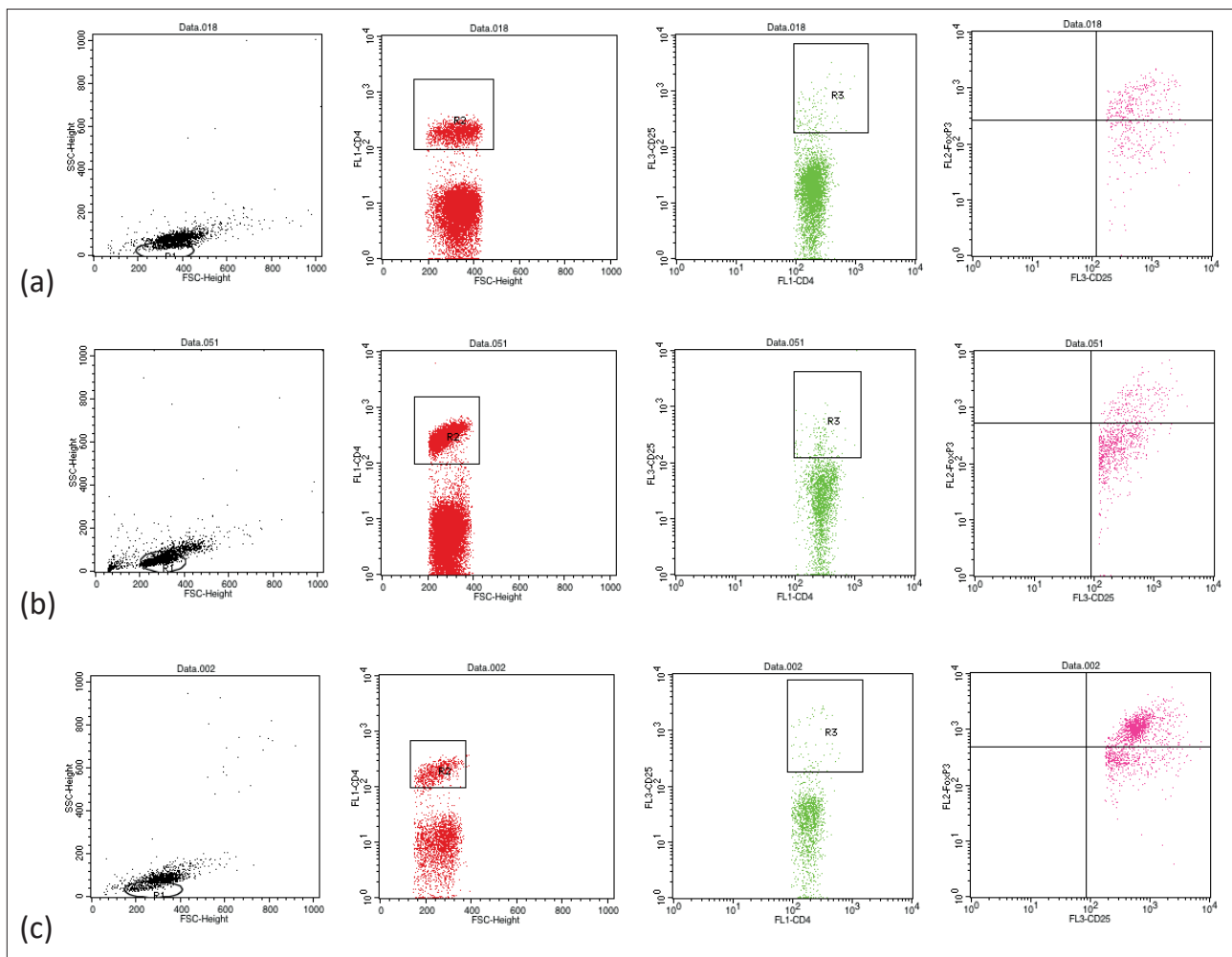


Figure 1: Flowcytometry results for BPH patient (a), prostate cancer (b), and CRPC (c)

of age. The normality test results in show the amount of Regulator T-cells fulfilled the normality assumption of 0.20 ($p > 0.05$). The homogeneity test results in also meet the assumption of homogeneity ($p > 0.05$). Therefore, regulatory T-cells was analyzed using the One Way ANOVA test.

Table 1 showed the average amount of regulatory T-cells in the BPH group was 53.44 ± 29.43 , prostate cancer group was 57.02 ± 22.49 and the CRPC group 89.71 ± 9.31 . One Way ANOVA test results showed that the average amount of regulatory T-cells between treatment groups gave a significant difference in regulatory T-cells with a p-value ($0,003$) < 0.05 . It means that there were significant differences in the average amount of regulatory T-cells. The T-test comparative study was analyzed each groups and shown in Table 2. We continue to Pearson correlation study and resulted in significantly correlated with p value = 0.011 ($P < 0.05$) and $r = 0.414$.

5. DISCUSSION

The role of the immune system in the pathogenesis of BPH and prostate cancer has recently been widely studied (7, 8). Chronic inflammation is caused by an infectious agent, causing an increase in epithelial turnover which increases the risk of malignancy. Research on acute and chronic inflammation of the urogenital system shows, the accumulation of immunocompetent T-cells in prostate

Treatment	T-reg Means	p-value
BPH	53.44 ± 29.43^a	0.003
Prostate cancer	57.02 ± 22.49^b	
CRPC	89.71 ± 9.31^c	

Table 1: The data characteristic of regulatory T-cells expression

Treatment	CRPC	Prostate Cancer	BPH
CRPC	-	0.996	0.007*
Prostate cancer	0.996	-	0.003*
BPH	0.007*	0.003*	-

Table 2: Multiple comparison of p-value of each groups using Post Hoc Test

tissue, especially T lymphocytes and macrophages. These cells secrete a variety of cytokines, which play a role in pathological changes and also lymphocyte activation which is characteristic of BPH and prostate cancer (7).

Regulatory T-cells or Regulatory Tare known to play an important role in suppressing the immune response to tumors, preventing autoimmune and balancing immune homeostasis. In cancer, regulatory T-cells density in tumors becomes a predictive factor for clinical development towards worsening, therefore it is said that Regulatory T-cells play a key role in cancer development (17). However, the role of regulatory T-cells in the pathogenesis of BPH and prostate cancer is still unknown (7).

To find out the differences in the amount of CD4⁺ CD25⁺ FOXP3⁺ T-cells in prostate cancer patients, benign prostatic hyperplasia with CRPC, we conducted a cross-sectional study, in which samples were taken from the blood vein of each subject, then the average amount of regulatory T-cells in the CRPC group was 53.44±29.43, prostate cancer group was 57.02±22.49 and the BPH group 89.71±9.31 as shown in Table 1. One Way ANOVA test results showed that the average amount of regulatory T-cells between treatment groups gave a significant difference in regulatory T-cells with a p-value (0,003) <0.05. It can be concluded that there are differences in the average amount of regulatory T-cells, continue to Pearson correlation study and resulted in significantly correlated with p value = 0.011 (P<0.05) and r = 0.414.

The increase in Regulatory T-cells obtained in the results of this study is in line with research conducted by Miller et al., in 2006 where there are a significant increase in CD4⁺ CD25⁺ T-cells in tumor tissue and peripheral blood in prostate cancer patients (18). Increased amount in regulatory T-cells in tumor tissue or cancer patient circulation, is an evidence that these cells are involved in the pathogenesis and progression of cancer (15).

An increase in Regulatory T-cells from BPH patient samples in the results of this study, following research conducted by Norstrom, where a substantial regulatory T-cell in BPH also increased. Norstrom also found signs of chronic activation supported by T-cell expression that induces activation of co-inhibitor receptors, co-stimulatory receptors, and high frequency of potential regulatory T-cells in BPH (19).

In this study, the amount of regulatory T-cells in BPH is higher than the amount of regulatory T-cells in prostate cancer with a significant difference. This can be understood, due to the prostate tissue of BPH patients with infiltration of immune cells such as T lymphocytes, NK cells, and HCV, higher than prostate cancer (7). Where it is known that the main role of regulatory T-cells is a suppression of the antitumor immune response (20).

However, this study found weaknesses, namely where the amount of samples is small so that one variable that has a distorted value will greatly affect the overall average value. In the data obtained one outrange data in the prostate cancer group, which causes the group's average value to be much lower. Therefore, further research needs to be done with larger samples.

Based on meta-analysis and recent studies have proven a strong correlation between the history of chronic prostatitis inflammation with the development of prostate cancer. The causes of prostate inflammation vary, ranging from bacteria that trigger prostatitis, sexually transmitted infections, estrogen imbalance, physical trauma, urine reflux to the prostate gland, and factors such as diet (21).

In tumors, an increase in Regulatory T-cells is consistent with its role in suppressing the antitumor immune response as evidenced in clinical and preclinical studies. This is supported by in vitro studies, where an increase in antitumor immunity after regulator T-cells has been removed (20, 22). In other studies, the elimination of CD4⁺

CD2⁺Regulatory Ts induces antitumor immunity activity in mice injected with syngeneic tumor cells (20).

The mechanism of action of regulatory T-cells is believed to mediate peripheral tolerance through suppression of self-antigen-reactive T-cells. The increase in regulatory T-cells in tumor cells aims to inhibit anti-tumor immunity, this is because most tumor antigens are self-antigens (against autoimmune is the primary function of regulatory T-cells) and Regulatory T increases to suppress inflammation. It is well known that with the ability of Regulatory T-cells to mediate suppression of reactive lymphocytes, this is thought to be a potential mechanism that explains immune failure against antitumors (20).

Finally, the results of this study showed a significant increase in Regulatory T-cells in both BPH compared to prostate cancer. This supports the idea that regulatory T-cells in tumor cells play a role in inhibiting anti-tumor immunity. The ability of these cells to mediate suppression of reactive lymphocytes forms the basis of a potential mechanism theory that explains immune failure against antitumor (20).

6. CONCLUSION

There was a statistically significant modulation of T-cells regulators in CRPC compared to the BPH and prostate cancer. Due to limited data and research available, further research is needed to support this research.

- **Patient Consent Form:** Yes the patients were informed consent and ethically approved by Health Research Ethics Committee General Hospital Dr. Saiful Anwar Malang no. 400/180/K.3/302/2020.
- **Author's Contribution:** K.P.S, B.B.P, and A.F.P gave substantial contributions to the conception or design of the work in acquisition, analysis, or interpretation of data for the work. H.K and H.S contribute in laboratory analysis and H.A.R contribute in statistical analysis. K.P.S had a part in article preparing for drafting or revising it critically for important intellectual content. K.P.S, B.B.P, and A.F.P gave final approval of the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
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