The Correlation Of Immunochemistry Expression Anaplastic Lymphoma Kinase (ALK) With Level Of Stromal Tumor Infiltrating Lymphocytes (STILS) In Prostate Adenocarcinoma

M Taufik Siregar*, M.Nadjib Dahlan Lubis, Delyuzar, Joko S.Lukito, Soekimin

Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia. *Corresponding Author

DOI: 10.29322/IJSRP.10.09.2020.p10584
http://dx.doi.org/10.29322/IJSRP.10.09.2020.p10584

Abstract- Prostate cancer is currently the fifth leading cause of death in men. Now immunotherapy is used as a new paradigm of prostate treatment, where the therapeutic response is aimed at the role of Tumor Infiltrating Lymphocytes (TILs). Anaplastic Lymphoma Kinase (ALK) gene fusion has found in several types of malignancies has been clinically proven to demonstrate an effective therapeutic target response for patients with positive ALK. Currently, combination therapy continues to be developed, especially in cases of recurrence and metastasis, so it is necessary to research on prostate adenocarcinoma. The paraffin block of 32 prostate adenocarcinoma tissue was used to assess the level of stromal TILs and ALK immunohistochemistry. Results were analyzed using SPSS 22 version. ALK expressed in 87,5% of the prostate adenocarcinoma. There is no significant correlation between of ALK immunohistochemistry expression with the level of sTILs. but there is a tendency that grade group V shows a broad percentage of expression and strong intensity. Positive ALK expression in adenocarcinoma with high grade can be used as a prognosis and a optional treatment in prostate adenocarcinoma. The evaluation of the subset of TILs can be investigated further to assess its relationship to ALK expression.
Index Terms- prostate adenocarcinoma, stromal TILs, anaplastic lymphoma kinase.

I. Introduction

Prostate cancer is one of the leading causes of impaired quality of men life in worldwide.[1] Now it is currently the second and fifth leading cause of cancer death in men. Based on GLOBOCAN 2018, an estimated 1.3 million new cases of prostate cancer, of which there are 359.000 deaths in worldwide. Cancer in men is most often diagnosed in 105 of 185 countries in the world, especially America, Northern and Western Europe, Australia / New Zealand, and most of Sub-Saharan Africa.[2] Indonesian Society of Urologic Oncology (ISUO) 2011 reported 971 prostate cancer patients in Indonesia from 2006 - 2010, where as many as 50.5% of the cases were prostate adenocarcinoma stage 4.[3,4] Adam Malik Hospital, Medan in 2015-2016 reported 39 cases were diagnosed as prostate adenocarcinoma.[5] Prostate adenocarcinoma is an invasive carcinoma consisting of neoplasms of prostate epithelial cells with differentiation of secretory cells. The histomorphological form consists of glands, in the form of sheets of cells or single cells that experience neoplasia.[6] Most prostate cancer occurs in the peripheral area, often without symptoms and usually new symptoms appear after reaching an advanced stage or metastasis.[7]

Tumor infiltrating lymphocytes (TILs) are the migration of lymphocytes into tumor or peritumoral cells.[8,9] Many studies have reported the benefits of stromal TILs on tumor cells, where effective in inhibiting tumor progression, although the mechanism is still opposing. Histopathological measurement of stromal TILs is carried out to see the immune reaction against tumors, where can also affect the stage and grading of various types of cancer.[10]

ALK is an enzyme with tyrosine kinase activation, has pleiotrophin (PTN) and midkine (MDK) receptors, which secrete growth factors, known to bind and activate ALK downstream signaling.[10-13] Several studies have shown that Nucleophosmin (NPM1-ALK) and Echinoderm Microtubule Associated Protein-like 4 (EML4-ALK) are found in Non-Small-Cell Lung Cancer (NSCLC), where treatment has shown a good response for patients with positive ALK.[13-17]

Recent preclinical data have also revealed that immune checkpoint proteins can induce ALK-positive NSCLC tumors, whereas currently combination checkpoint therapy (PD-1 / PD-L1, CTLA-4) and ALK inhibitors are being studied clinically in NSCLC patients with ALK positive.[18] Based on the description above, the researcher is interested in assessing how "The Correlation Anaplastic Lymphoma Kinase (ALK) Immunohistochemical Expression and the degree of stromal Tumor Infiltrating Lymphocytes (sTILs) in Prostate Adenocarcinoma.

II. Material and Methods

Sample selection

This study was selected cross-sectionally, consist of 32 cases of prostate adenocarcinoma. The samples were obtained through TUR-P and core biopsy. Inclusion criteria are age from medical record, slides and paraffin were diagnosed prostate adenocarcinoma by histopathology.

Histopathological grading is a scale for determining the prognosis of a prostate adenocarcinoma based on histopathological assessment and the Gleason scale (WHO, 2016) which is categorized as:

1. Grade group 1, Gleason score ≤ 6 : consists of a combination of glands with well differentiation.
2. Grade group 2, Gleason score 3+4=7: More well differentiation glands, followed by fusion and cribriform of glands.
3. Grade group 3, Gleason score 4+3=7: More fusion/cribriform glands, followed well-differentiated glands.
4. Grade group 4, Gleason score 4+4=8, 3+5=8, 5+3=8: Consists of both poorly differentiation and fusion gland patterns, well differentiation followed by poorly differentiation gland, or more poorly differentiation glands followed by well differentiation.
5. Grade group 5, Gleason score 9-10: poorly differentiation gland forms with or without necrosis or both poorly / solid differentiation.[4]

Stromal Tumor-Infiltrating Lymphocytes (sTILs) are defined as mononuclear inflammatory cells that are in the stromal tissue between the nests of cancer cells, and are not directly related to cancer cells. TILs are assess the percentage of stromal area alone without including calculations tumor cells at 400 magnification. The assessment is based on the focus of the most dense lymphocyte infiltration, divided by TILs working group 2013:

1. Mild : 0-10 % stromal TILs (HPFs 200-400x)
2. Moderate : 11-49 % stromal TILs (HPFs 200-400x)
3. Severe : ≥ 50 % stromal TILs (HPFs 200-400x).[19]

ALK expression was determined by assessing the clinical score determined based on the percentage of cells expressed on immunohistochemistry (scale 0 to 3) and staining intensity (scale 0 to 3), where the intensity of staining is given a value of 0 if not stained, 1 if stained weakly, 2 if moderate, 3 if strong. The percentage of cells expressed as 0 if <10%, 1 if 11–40%, 2 if 41–70%, and 3 if ≥71%. The two scores are then added together with the result:

1 = Negative if the total score is 0-3.
2 = Positive if the total score is ≥4.[20]

Statistical analysis was performed using SPSS software version 22.0 (SPSS Inc., Chicago). To analyze data on Correlation of Anaplastic Lymphoma Kinase (ALK) Immunohistochemical Expression and the level of stromal Tumor Infiltrating Lymphocytes (sTILs) in Prostate Adenocarcinoma researchers used Mann-whitney U Test, p-value < 0.05 was considered significant.

III. RESULT

The youngest age distribution was 50 years old and the oldest was 82 years old with a mean age of 68.2. The largest age group was ≥ 71 years as many as 18 cases (56.3%). The most stromal level distribution of TILs was mild (<10%) as many as 22 cases (68.7%). The distribution of the grade group in prostate adenocarcinoma, the highest was grade V as many as 14 cases (43.8%) and the smallest was grade 1 in 1 case (3.1%). The distribution of ALK immunohistochemical expression was the most positive expression in 28 cases (87.5%). Data from the statistical test of this study showed p = 0.050, where the p value> 0.05 or there was no significant difference in the grade group.

Tabel 1. Correlation of ALK Expression and Grading Histopathology of Prostate Adenocarcinoma.

No	Grade group	ALK Expression				P-value*
		Negative		Positif		
		n	%	n	%	
1.	I	1	25,0	0	0	0,050
2.	II	1	25,0	3	10,7	
3.	III	0	0	5	17,9	
4.	IV	2	50,0	6	21,4	
5.	V	0	0	14	50,0	

* Mann-Whitney U test

The statistical test of Kruskal-Wallis was carried out to assess the correlation of level sTILs and the histopathological grade of prostate adenocarcinoma, it was obtained p-value = 0.718 (p> 0.05) which indicated no significant correlations.

Table 2. Correlation level of stromal TILS with Histopathological Grading of Prostate Adenocarcinoma.

No.	Grade group	Level of sTILs						P- value*
		<10%		10 – 49%		≥ 50%		
		n	%	n	%	n	%	
1.	I	1	4,5	0	0	0	0	0,718
2.	II	2	9,1	2	25,0	0	0	
3.	III	4	18,2	1	12,5	0	0	
4.	IV	7	31,8	0	0	1	50,0	
5.	V	8	36,4	5	62,5	1	50,0	

* Kruskal-Wallis Test

The Mann-Whitney U statistical test to assess the correlation between the level of sTILs and the ALK expression obtained p-value = 0.758 (p> 0.05), which indicates no significant correlation.

Table 3. Correlation between Stromal TILs and ALK Expression

No	Level of sTILs	ALK expressions				P-value*
		Negative		Positif		
		n	%	n	%	
1.	<10%	3	13,6	19	86,4	0,758
2.	10 – 49%	1	12,5	7	87,5	
3.	≥ 50%	0	0	2	100	

* Mann-Whitney U Test

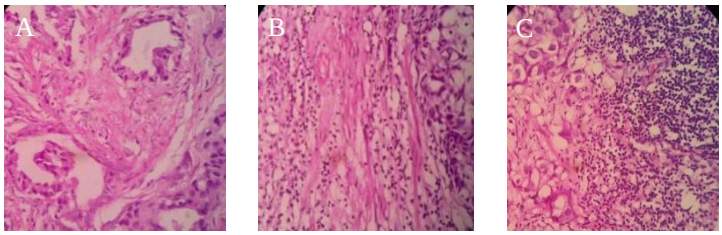

Figure 1. Infiltration of level stromal TILs of BPH. A. Mild 0-10%, B. Moderate 11-49%, C. Severe > 50%.

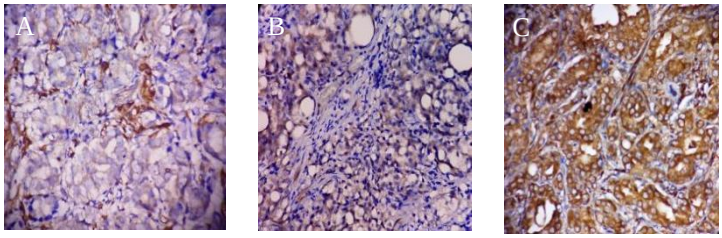

Figure 2. ALK Expressions, A. Weak, B. Medium, C. Strong

IV. DISCUSSION

Prostate Adenocarcinoma is the most common invasive carcinoma of the prostate epithelial cells. Clinical observations have proven that several factors, including androgens, heredity, environment, and somatic mutations have a role in the pathogenesis of prostate cancer.[6] Most prostate cancers arise in the peripheral area, often without symptoms and usually new symptoms appear after reaching an advanced stage or metastasis.[7] In this study the mean age for patients with prostate adenocarcinoma was 68.2 years. The data in this study are consistent with previous studies by Andreas MI et al., which found that nearly 90% of sufferers were over 60 years of age, of which two thirds of the deaths were over 75 years of age. The risk of prostate adenocarcinoma is closely related to age, where in men aged 70-79 years have an almost 7 times higher risk of developing prostate adenocarcinoma compared to men aged 50-59 years and have a 21 times higher risk of death.[21] WHO also states that it is most detected at age> 60 years, of which only 1% detected on clinical examination were <50 years old. This is closely related to lifestyle and diet which are known to cause prostate cancer at a younger age. More and more evidence shows that glandular epithelial cell injury by carcinogens, estrogen or oxidants as a trigger for chronic inflammation is a step towards cancer cell development.[22]

Gleason score is a strong predictor for determining the progression and prognosis of a prostate adenocarcinoma. However, recent studies are currently focused on the identification and validation of molecular markers that are very promising for prognosis in prostate cancer. Special attention to the gleason score and analysis of the development of cancer biology is very important in prostate cancer therapy. Furthermore, comparative oncogenomics with a valid function of a biological process have resulted in significant differences in prostate cancer patients in determining prognosis at first diagnosis or after undergoing surgery. This biological marker is still being developed with various trials to be more reliable in prostate cancer.[23] This study found 28 samples with positive ALK immunohistochemical expression and 4 samples with negative expression. Although there was no significant difference, from 28 samples found 14 samples (50%) with grade V group, where all samples expressed positive. This suggests a trend that ALK expression can be associated with grading of prostate adenocarcinoma

ALK is an enzyme that activates tyrosine kinase, by catalyzing the gamma phosphate group (derived from adenosine triphospate) in to tyrosine residues on protein substrates.[24] ALK gene deviations in cancer are generally due to chromosomal rearrangements that produce gene fusion, which has the potential to rapidly increase oncogenes. This situation has been proven by the presence of NPM1-ALK fusion and EML4-ALK fusion in Non-Small-Cell Lung Cancer (NSCLC). Increasing the number of point mutations that activate protein kinases will also activate oncogens in ALK.[14-16] Several tumor types are known to express the full length ALK Receptor Tyrosine Kinase (RTK). The protein receptors of the full length ALK RTK have been found on various tumor cells and tissues including neuroblastoma, neuroectodermal tumor, glioblastoma, and melanoma. Furthermore, full-length ALK cDNA derived from cloned RH30 rhabdomyosarcoma cDNA, which expresses full-length protein has also been reported to occur in rhabdomyosarcoma tumors. Initially ALK inhibitor immunoreactivity has been observed in several malignant tissue stains, such as breast carcinoma, malignant peripheral nerve sheath tumor, and lipogenic tumors, which exhibit more ALK fusion than full length receptors. It has been suggested that autocrine and / or paracrine growth figures involving PTN and MK can direct tumor growth expressing the normal full-length ALK receptor, and this mechanism has been investigated in glioblastoma, but has not been fully confirmed for other tumor types. Next Generation Sequencing (NGS) and ctDNA from a 39 year old small cell carcinoma prostate primary tumor have identified the ALK F1174C mutation. This analysis indicated that ALK amplification was associated with poor outcome.[25]

Previously, there have been many studies showing that the tyrosine kinase receptor is a strong biomarker and a good therapeutic target for a large number of malignancies.[26,27] The presence of ALK fusion and the ALK component tyrosine kinase activity has been used as a targeted therapy for several malignancies.[28] Initially ALK inhibitor used in NSCLC is crizotinib, whose potential small molecule has shown an overall response rate (ORR) of 65% compared with 20% docetaxel in patients who failed platinum-based therapy. Furthermore, crizotinib is associated with disease control in NSCLC patients with positive ALK fusion brain metastases.[24,29,30] Various problems in prostate cancer suffers are the increased recurrence rate and the occurrence of metastases which results in an increasing rate of prostate cancer mortality. The results of this study indicate that ALK expression is very strong in grade V group or high grade so that it can be an alternative choice in the treatment of prostate adenocarcinoma.

Tumor infiltrating lymphocytes (TILs) is the migration of lymphocytes into tumor or peritumoral cells.[31,32] Now, many types of prostate cancer have not known cause, but some literature reveals that genetic and environmental factors are considered to play a role. Some researchers suggest that inflammation can play a role in the formation of prostate cancer. This has been reported in the development of other cancers such as gastric, colon and liver.[33,34] Tumor growth from the interaction between the tumor cell complex and microenvironment, including immunity cell infiltration, fibroblasts, endothelium, blood vessels, and it produces include cytokines, chemokines and their metabolites.[35,36] Lymphocytes are known to be key mediators in adaptive immunity. In principle, lymphocytes are divided into T lymphocytes (cell mediate immunity) and B lymphocytes (humoral immunity). T lymphocytes secrete growth factor B lymphocytes, which stimulate differentiation and proliferation of B lymphocytes. B lymphocyte activation

produces plasma cells, secretes antibodies, lymphotoxins, and combines with NK cells to destroy malignant cells.[31,36] T cells can be in the stromal and intraepithelial. The T cells in the stromal area CD4+ helper / inducer cells, while the intraepithelial cells are CD8+ cytotoxic / suppressor cells. CD4+ T cells are classified according to the cytokine profile into T helper (Th) -1 and T helper (Th) -2. Th1 expresses T-beta and interferon (IFN)-γ. Th2 expresses Gata-3 and IL-4. Other T cells selectively produce IL-17 and the transcription factor RORγt (Th17), and finally T cells are identified based on the production of Th9 and Th22 cell cytokines. Cytotoxic T cells are the lumen's first line of defense against foreign agents. Changes in the phenotype of helper T cells are important in determining which T cells develop in inflammation. The fact is that the immune response stimulates prostate cancer, as shown also from histological data which reveals the presence of CD4 + T cells, CD8 + T cells, Natural Killer (NK) cells, dendritic cells, and macrophages in tumors.[37-40] There was no significant relationship between the level of sTILs and ALK expression and the association of sTILs with the grade of the prostate adenocarcinoma group, therefore further research is needed to assess the TILs subsets for prognosis and immunotherapy treatment in prostate adenocarcinoma.

V. CONCLUSION

There is a no correlation between the immunohistochemistry of ALK with level of stromal TILs in prostate adenocarcinoma

COMPETING INTERESTS

The author has no financial interests relevant to the product or company described in this article.

ACKNOWLEDGMENT

We would like to thank all staff of the Anatomical Pathology Department University of North Sumatra, Medan, Indonesia for all their assistance and cooperation.

ETHICAL APPROVAL

Health Research Ethical Committee, Universitas Sumatera Utara, Medan, Indonesia approved this study.

REFERENCES

[1] Hoffman M. Prostate Conditions, Anatomy picture of the prostate, 2014 WebMD, LLC.

[2] American Cancer Society. Global Cancer Facts & Figures 4th Edition. Atlanta: American Cancer Society; 2018.p 28-31.

[3] Umbas R, Hardjowinoto S, Mochtar CA, Safriadi F, Soesanto WD, Soedarso M A, Danarto, et al., Pedoman Nasional Pelayanan Kanker Prostat. Kementerian Kesehatan Republik Indonesia. Ikatan Ahli Urologi Indonesia. 2015

[4] Merani S, Payne J, Padwal R, Hudson D, Widder S, Khadaroo R. Predictors of in-hospital mortality and complications in very elderly patients undergoing emergency surgery. World J Emerg Surg. 2014;9(1):43.

[5] Siregar T, Delyuzar, Laksmi L I. Correlation of Stromal Tumor-Infiltrating Lymphocytes (TILs) Degrees with Total Prostate Specific Antigen (PSA) in Benign Prostate Hyperplasia and Prostate Adenocarcinoma. International Journal of Scientific and Research Publications.2019. p 490-493.

[6] Epstein JI. Chapter 21 The Lower Urinary Tract and Male Genital System. In: Kumar V, Abbas AK, Fausto N, Aster JC. Robbin and Cotran Pathologic Basis of Disease.China : Saunders Elsevier; 2010. P993-1001

[7] Caliskan S. Koca O, Akyuz M, Ozturk M, Karaman M. Clinical Significance of Single Microscopic Focus of Adenocarcinoma at Prostate Biopsy. Prostate Int; 2015, 3: 132-4

[8] Karja V, Altomaa S, Lipponen P, Isotalo T, Talja M And Risto Mokka. Tumour-infiltrating Lymphocytes: A Prognostic Factor of PSA-free Survival in Patients with Local Prostate Carcinoma Treated by Radical Prostatectomy. Anticancer Research *25*: 4435-4438 (2005)

[9] Weiss SA, Han SW, Lui K, Tchack J, Shapiro R, Berman R et al. Immunologic heterogeneity of tumor infiltrating lymphocyte composition in primary melanoma. Human Pathology. 2016; 57: 116-125.

[10] Gooden MJM, De Bock GH, Leffers N, Daemen T, Nijman HW. The prognostic influence of tumour-infiltrating lymphocytes in cancer: a systematic review with meta-analysis British Journal of Cancer (2011) 105, 93 – 103 Huang H. Review Anaplastic Lymphoma Kinase (ALK) Receptor Tyrosine Kinase: A Catalytic Receptor with Many Faces. International Journal of Molecular Sciences. 2018. P 1-20

[11] Cao Z, Gao Q, Fu M, Ni N, Pei Y, Bin-ou W. Anaplastic lymphoma kinase fusions: Roles in cancer and therapeutic perspectives (Review). Oncology Letters. 2019.17: p. 2020-2030.

[12] Holla V R, Elamin Y, Bailey A, Jhonson A, Litzenburger B, Khotskaya Y,et al. ALK: a tyrosine kinase target for cancer therapy. Cold Spring Harbor Molecular Case Study. 2017.

[13] Trigg R, Turner S. Review ALK in Neuroblastoma: Biological and Therapeutic Implications. Cancers 2018.

[14] Yang J, Dong L, Du H, Li X, Liang Y, Liu G. ALK-TPM3 rearrangement in adult renal cell carcinoma: a case report and literature review. Diagnostic Pathology. 2019. 14:11

[15] Santarpia M, Daffina M, D'aveni A, Marabello G, Liguori A, Giovanetti E. Spotlight on ceritinib in the treatment of ALK+ NSCLC: design, development and place in therapy. Drug Design, Development and Therapy. 2017.

[16] Hanna M, Najfeld V, Irie H, Tripodi J, Nayak A. Analysis of ALK gene in 133 patients with breast cancer revealed polysomy of chromosome 2 and no ALK amplification. SpringerPlus (2015) 4:43

[17] Doloksaribu R F, Delyuzar, Alferally T I. Hubungan Ekspresi *ALK* dengan *grading* histopatologi dan subtipe molekuler pada penderita karsinoma payudara invasif. 2019.

[18] Cao Z, Gao Q, Fu M, Ni N, Pei Y, Bin-ou W. Anaplastic lymphoma kinase fusions: Roles in cancer and therapeutic perspectives (Review). Oncology Letters. 2019.17: p. 2020-2030.

[19] Salgado R, Denkert C, Demaria S, Sirtane N, Klauschen F. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol. 2015; 26(2) : 259-271.

[20] To K, Tong JHM, Yeung KSF, Lung RWM, Law PPY, Chau SL. Detection of ALK Rearrangement by Immunohistochemistry in Lung Adenocarcinoma and the Identification of a Novel EML4-ALK Variant. J Thorac Oncol. 2013;8: 883-891.

[21] Andreas MI, Alvarino, Hilbertina N Gambaran Karsinoma Prostat di RSUP Dr. M. Djamil Padang tahun 2010-2013. Jurnal Kesehatan Andalas. 2017; 6(2)

[22] Humprey PA, Amin MB, Berney DM, Billis A, Cao D, Cheng L, Delahunt B, et al., Acinar Adenocarcinoma. In: Moch H, Humphrey PA, Ulbright TM, Reuter VE (eds). WHO Classification of Tumours of the Urinary System and Male Genital Organs 4th Edition. Lyon: IARC; 2016. p 138-180.

[23] Martin E, Mucci L A, Loda M, Depinho R. Prognostic Determinants in Prostate Cancer. Cancer J. 2011 November ; 17(6): 429–437

[24] Huang H. Review Anaplastic Lymphoma Kinase (ALK) Receptor Tyrosine Kinase: A Catalytic Receptor with Many Faces. International Journal of Molecular Sciences. 2018. P 1-20

[25] Carneiro B, Pamarthy S, Shah A, Sagar V, Unno K, Han H, et al. Anaplastic lymphoma kinase mutation (*ALK* F1174C) in small cell carcinoma of the prostate and molecular response to alectinib. Clin Cancer Res. 2018. 15; 24(12): 2732–2739

[26] Huo L, Jennifer LH, Mien-Chie H. Receptor tyrosine kinases in the nucleus: Nuclear functions and therapeutic implications in cancers. In: Kumar R, editor. Nuclear Signaling Pathways and Targeting Transcription in Cancer.1st ed. New York: Humana Press; 2014. p. 189-229.

[27] Maruyama IN. Mechanisms of activation of receptor tyrosine kinases: Monomers or dimers. Cells 2014;3:304-30.

[28] Della Corte, Viscardi G, Di liello R, Fasano M, Martinelli E. Role and targeting of anaplastic lymphoma kinase in cancer. Molecular Cancer (2018) 17:3

[29] Rothenstein J M, Chooback N. ALK inhibitors, resistance development, clinical trials. Curr Oncol. 2018 Jun;25(S1): S59-S67.

[30] Carperter EL, Haglund EA, Mace EM, Deng D, Martinez D, Wood AC, et al. Antibody targeting of anaplastic lymphoma kinase induces cytotoxicity of human neuroblastoma. Oncogene. 2012 November 15; 31(46): 4859–4867.

[31] Woo JR, Liss MA, Muldong MT, Palazzi K, Strasners A. et al.: Tumor infiltrating B-cells are increased in prostate cancer tissue. Journal of Translational Medicine. 2014 12:30

[32] Nelson W G, Carter H B, DeWeese T L, Emmanuel S. Antonarakis, and Mario A. Eisenberger. Prostate Cancer; Specific Malignancies part III, Elsevier 2018.

[33] Drake CG. Prostate cancer as a model for tumour immunotherapy. Nature Reviews Immunology 2010. P 580-593

[34] Kumar S, Singh R, Malik S, Manne U, Mishra M. Prostate cancer health disparities: An immuno-biological perspective. Cancer Letters 414 (2018) 153e165

[35] Shen H, Sun T, Hoang HH, Burchfield JS, Hamilton F et al., Enhancing cancer immunotherapy through nanotechnology-mediated tumor infiltration and activation of immune cells. Seminars in Immunology 34 (2017) 114–122.

[36] Abbas AK, Lichtman AH, Pillai S. Basic Immunology. Functions And Disorders Of The Immune System. Fifth Edition. 2016

[37] Silvestri I, Cattarino S, Aglianò AM, Collalti G, Sciarra A. Beyond the Immune Suppression: The Immunotherapy in Prostate Cancer. Hindawi Publishing Corporation BioMed Research International. 2015

[38] Bruno TC. Pardoll D, Siliciano R, Cox A, Vignali D. Immune Checkpoints In Tumor Infiltrating Lymphocytes. 2010.

[39] Male D, Brostoff J, Roth DB, Roitt I. Immunology, seventh edition. Mosby Elsevier. 2016.

[40] Maiaa MC, Hansenb AR, A comprehensive review of immunotherapies in prostate cancer. Critical Reviews in Oncology/Hematology 113 (2017) 292–303

AUTHORS

First Author – dr. M.Taufik Siregar, Resident of Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia, **email ID**: tdrsiregar@gmail.com

Second Author – Prof. dr. M.Nadjib Dahlan Lubis, Sp.PA(K), Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.

Third Author – DR. dr. Delyuzar, M.Ked(PA), Sp.PA(K), Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.

Fourth Author – dr. Joko S. Lukito Sp.PA(K), Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.

Fifth Author – dr. Soekimin Sp.PA(K), Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.

Correspondence Author – dr. M.Taufik Siregar, Resident of Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia, **email ID**: tdrsiregar@gmail.com