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Title: Predictive Significance of Preoperative Neutrophil to Lymphocyte Ratio versus Platelet to Lymphocyte Ratio for Gleason score in Prostate Cancer Patients

Running Head: NLR and PLR in Prostate Cancer Prognosis

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ABSTRACT

Objective: Inflammation has critical role in development and progression of cancer. Neutrophil to lymphocyte ratio (NLR) and Platelet to lymphocyte ratio (PLR) are easily accessible basic inflammatory parameters. Here, we aimed to analyze the association between NLR, PLR and Gleason score of prostate cancer that is main parameter for prognosis of prostate cancer

Materials and Methods: A total 173 prostate cancer patients (mean age 63±6.2 years) who were performed radical prostatectomy included in to this retrospective study. The NLR and PLR were derived from the complete blood cell count results at preoperative period. Patients were divided in to two groups as low-grade prostate cancer [Gleason score ≤7 (3-4)] and high-grade [Gleason score≥ 7(4+3)]. Logistic regression analysis was performed to determine the association.

Results: Univariate logistic regression analysis showed that Ln PSA [1.83, 95% CI (1.01, 3.3) p=0.04], Lnlymphocyte [0.38, 95% CI (0.15, 0.94) p=0.03] and LnNLR [1.9, 95%CI 1.9 (1.13, 3.38) p=0.01] levels were significantly associated with high-grade Gleason score. However, lnPLR levels revealed association with marginal statistically significance [2.06, 95 % CI (0.95, 4.4) p=0.06]. In multiple analyses, after adjusting the analysis for age, Ln NLR [1.96, 95% CI (1.12, 3.42) p=0.01 and Ln lymphocyte levels [0.38, 95% CI (0.15, 0.97) p= 0.04] were still statistically significantly associated with high-grade prostate cancer

Conclusion: Higher NLR levels were significantly associated with high-grade prostate cancer. However, PLR levels were not significant predictor for higher Gleason scores.

Keywords: Neutrophil to lymphocyte ratio, platelet to lymphocyte ratio, prostate carcinoma, Gleason score

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INTRODUCTION

Prostate cancer exists as the second most common cancer and sixth leading cause of cancer death among men worldwide. By aging and increasing use of prostate specific antigen (PSA) as screening marker substantial increase of prostate cancer diagnosis has been documented in many countries (1). In Turkey, several reports have been documented the prevalence of prostate cancer is almost two to three times higher than Asian population and similar rates with Europe (2). Also, first multicenter, population based report from Turkey, documented that prostate cancer incidence rate is 35 cases per 100.000, highlighting the importance of its burden for both economic and health related quality of life of the patients (3).

Chronic inflammation has imperative role in the development of cancer (4). The traditional risk factors for prostate cancer are age, genetic and western lifestyle (4). Besides genetic, environmental factors that lead to the chronic inflammation in prostate such as infection, diet or other exposures are important in prostate cancer etiopathogenesis (4). Moreover, chronic inflammation has considerable effect on progression and metastasis through angiogenesis and epithelial mesenchymal transition (EMT), impacting the dynamics of the tumor microenvironment in prostate cancer (5).

Neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) are inflammatory parameters are easily reachable from routine complete blood count and have been reported as prognostic value in solid organ cancers (6, 7-10). They also have been suggested as an emerging marker of systemic inflammation, tumor hypoxia and necrosis. Several reports have documented to use these basic clinic parameters to differentiate malign form benign in prostate lesions (11, 12). However, evidence about the prognostic values of NLR and PLR in prostate cancer is scarce. Hence, in this retrospective study we aimed

to analyze to prediction effect of preoperative NLR and PLR levels for prostate carcinoma histological grade using Gleason scoring system by radical prostatectomy specimens.

MATERIALS and METHODS

Study Population

A total of 173 men whom diagnosed biopsy proven prostate carcinoma, and underwent robot assisted radical prostatectomy between October 2012 and January 2018 at Yuksek Ihtisas University Faculty of Medicine Koru Ankara Hospital Department of Urology were included in to these retrospective analyses. We have excluded the patients with infectious or inflammatory disease (i.e. active connective tissue disorder, HIV, any other proven infections) or having no sufficient medical record. Also, none of the patients received anticancer therapy before operation. The Institutional Review Board of Yuksek Ihtisas University Faculty of Medicine Koru Ankara hospital approved the study protocol.

Clinical and Laboratory Analyses

The NLR was calculated using the neutrophil and lymphocyte counts by complete blood count (CBC) obtained before surgery and PLR was calculated platelet divided by lymphocyte as in same CBC results. All surgeries performed by the same surgery team. Tumor grade of the radical prostatectomy specimens was determined according to the ISUP consensus on Gleason grading (13).

Statistical Analysis

Continuous variables were presented as mean ± SD or median (IQR) according to the distributions and categorical variables as frequencies and percentage. The comparison of variables between two groups were performed according to the normality, either t-test or Mann-Whitney U test for continuous variables, chi-square test for categorical variables. Logistic regression analyses were used to evaluate the

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possible association between NLR, PLR and Gleason score. All leukocyte, neutrophil, lymphocyte, platelet, PSA, NLR and PLR values were log-transformed to reach the normal distribution and transformed values were used in the regression analysis. Since BMI values were unable to obtain for all patients multiple analyses were adjusted by age. Independent variables haven't put together in the model for possible interaction. Gleason score results were grouped as low grade and high grade for the analyses. A p value under 0.05 was considered as statistically significant. Statistical analysis was carried out using SPSS, version 25.0.

RESULTS

Our study group comprised of 173 men with biopsy proven prostate carcinoma. The demographics of study population are depicted in **Table 1**. The mean age was 63±6.2 years, with median preoperative PSA of 8.4 (5.1, 16.4) ng/mL. All patients underwent robotic assisted radical prostatectomy and surgery specimens' pathology report showed that 106 patients had low-grade tumor [GLEASON under 7(3+4)] and 67 patients had high-grade tumor [GLEASON higher than 7(4+3)]. Only 9 patients had lymph node metastasis. Among 173 patients 41 (24%) of those had positive resection margin.

Median leukocyte level was 7420 (6320, 8840) / µL, median neutrophil level was 4380 (3700, 5890) / µL, median lymphocyte level was 2110 (1760, 2560) / µL, median platelet level was 222000 (181.000, 262000) / µL and mean Hb level was 14.5± 1.2 g/dL. The median NLR was 2.1 (1.6, 2.9) and PLR was 103.4 (84.7, 135.4) (**Table 1**).

We have also compared all characteristics among study subgroups assigned as low grade and high grade according to the Gleason Scoring system. The comparison analysis depicted in **Table 2**. The comparison regarding serum PSA levels showed that high-grade group has statistically significantly higher values than

low grade (p=0.04) (Table 2). Although both NLR and PLR values were higher in high-grade group, the difference in NLR showed marginal statistical significance (p=0.06), however PLR was not significantly different (p=0.14) (**Figure 1**).

Association Between NLR, PLR and GLEASON Score

Univariate logistic regression analysis showed that Ln PSA [1.83, 95% CI (1.01, 3.3) p=0.04], Lnlymphocyte [0.38, 95% CI (0.15, 0.94) p=0.03] and LnNLR [1.9, 95%CI 1.9 (1.13, 3.38) p=0.01] levels were statistically significantly associated with high-grade Gleason score. However, lnPLR levels revealed association with marginal statistically significance [2.06, 95 % CI (0.95, 4.4) p=0.06]. In multiple analyses, after adjusting the analysis for age, Ln NLR [1.96, 95% CI (1.12, 3.42) p=0.01 and Ln lymphocyte levels [0.38, 95% CI(0.15, 0.97)p= 0.04] were still statistically significantly associated with high-grade prostat cancer. Also the slight association for LnPSA [1.79, 95% CI (1.79, 0.99, 3.26) p=0.05] and LnPLR [2.1, 95 % CI (0.96, 4.6) p= 0.06] were still observed in multiple analyses. All results were summarized at **Table 3**.

DISCUSSION

Recently, there are several studies that aimed to find parameters, which are inexpensive, easily available and practical in clinical use for diagnosis, follow-up and prediction of prognosis on solid organ cancers. Measuring serum PSA levels, rectal digital examination and prostate biopsy are standard techniques for the diagnosis among the men who are suspected for prostate cancer. The possibility of finding prostate cancer is ranging between 20-67 % by trans-rectal prostate biopsy (14). However, false negative results have been reported as high as 23% in first prostate biopsy. It is known that repeated biopsies are needed to detect cancer especially for the patients have previous pathology reports as atypical small acinar proliferation (ASAP) or high-grade prostate intraepithelial neoplasia (HGPIN) (15, 16). Thus, several tests

have been developed to clarify diagnosis prior to biopsy such as magnetic resonance imaging. However, those tests are not easy to perform and expensive and also increase the expenditure. Accordingly, all research area has been focused on to develop markers that are cheaper, easy to perform in clinical practice.

Inflammation is crucial in prostatic carcinogenesis and tumor progression by immune cell infiltration in prostate tissue and fibroblast activation along with such several different mechanisms (4, 17). Neutrophil to lymphocyte ratio and PLR are inexpensive and practical parameters that can be performed by one complete blood count in routine clinic visits. Neutrophil to lymphocyte ratio has been studied in several different solid organ tumor areas[18] and found predictive for both development and prognosis of cancer. In our study, we have demonstrated that NLR levels were independent predictor for high-grade prostate carcinoma in both univariate and multiple analyses with almost same prediction level with serum PSA levels. Similar with our findings, previous studies have also found NLR as an independent prognostic marker in prostate carcinoma (10, 19). Gleason scoring system correlates closely clinical feature of the prostate carcinoma (13). Higher scores indicate worse outcome of the cancer (20, 21). Hence, finding of the close association between high NLR levels and high Gleason score is suggestive for the usefulness of the NLR regarding the prediction of high-grade histology respecting poor tumor prognosis. Our results are similar with the previous report by Lu et al, demonstrating the higher the levels of NLR, the higher the degree of Gleason score and malignancy of prostate cancer (22). Langsenlehner et al. (23) have also reported similar findings by demonstrating high NLR is associated with prostate carcinogenesis and concluded that increased neutrophil associated inflammation and decreased lymphocyte associated tumor response might be a part of carcinogenesis. Jang et al. (24) demonstrated the higher NLR levels obtained before radical prostatectomy is associated with higher biochemical relapse along with poor survival among

2067 prostate cancer patients. Taken all these together, large, multicenter validation studies, which analyze the certain cut-off level of NLR in prediction of grading prostate carcinoma, would serve NLR as an inexpensive, accessible and promising marker for estimating the cancer clinical behavior in these patients. The other simple inflammation based parameter that we studied here is PLR, which is also obtainable from complete blood count. Many studies disclosed the higher pretreatment levels of PLR is associated with poor prognosis in several type of solid cancers (25-27). In our study, although the patients with high-grade prostate carcinoma tend to have much higher levels of PLR and in regression analysis showed positive relation with Gleason score, we were unable to find statistically significant association. The reports in the literature regarding the prediction value of PLR for diagnosis and prognosis of prostate carcinoma are conflicting. Yuksel et al. (12) suggested PLR is an additional predictor marker for distinguishing prostate lesions benign from malign nature. Wang et al. (10) also suggested to use PLR as an additional marker for prediction of prognosis in prostate cancer patients. Also in another report, which PLR and NLR levels studied together in urological cancers, both inflammatory parameters were found in association with poor prognosis in prostate carcinoma (28). The evidences recommends the higher PLR level reflects the elevated platelet dependent tumor growth (pro-tumor reaction) and decreased lymphocyte mediated anti-tumor immune response and both attributed to progression and poor outcome in tumors (17, 23). On the contrary, Zanaty et al. (29) have studied both the predictive effect of preoperative NLR and PLR levels among organ confined prostate cancer patients and were unable to find any significant association for both markers concluding the localized tumors might not trigger the systemic inflammatory response. Likewise, we were unable to find strong association between PLR and Gleason degree in our study. The inflammatory pathways in tissue level and its reflection in clinical laboratory results might not be always correlated. The lack of power of the relation between PLR and histological status in our study might be a

signal for neutrophils has more imperative role in development and progression in malignancy rather than platelets. However, this issue should be further clarified with the studies performing simultaneous blood and specimen evaluation.

We believed that we have performed our analysis among substantial sample size. However, the design of our study is retrospective, which might limit our results causality interpretation, although the data were obtained in prospective manner. Here, our goal was to determine the relation with histological grading of prostate cancer, but longer follow-up to see the link with long-term clinical prognosis would provide more accurate and clinically applicable results.

CONCLUSION

Overall, our study confirms that higher NLR levels are indicator of high-grade prostate carcinoma suggesting significant clinical significance for these patients. We were unable to reach statistical significance regarding the association with PLR levels. Further large scale, follow-up studies are needed to validate these results with certain cut-off levels of these inflammatory parameters.

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Conflict Of Interest: The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Table 1. Characteristics of the study patients

	n=173
Age (years, mean±SD)	63±6.2
PSA (ng/mL, median IQR)	8.4 (5.1, 16.4)
GLEASON SCORE (N, %)	
Low Grade [6(3+3), 7(3+4)]	106 (61%)
High Grade [7(4+4) and higher]	67 (39 %)
Lymph node metastasis (n, %)	9 (5.1%)
Positive resection margin (n, %)	41 (24%)
Hemoglobin (g/dL, mean±SD)	14.5±1.2
Leukocyte (/ µL, median, IQR)	7420 (6320, 8840)
Neutrophil (/ µL, median, IQR)	4380 (3700, 5890)
Lymphocyte (/ µL, median, IQR)	2110 (1760, 2560)
Platelet (/ µL, median, IQR)	222000 (181000, 262000)
NLR (median, IQR)	2.1(1.6, 2.9)
PLR (median , IQR)	103.4 (84.7, 135.4)

PSA; prostate specific antigen, SD; standard deviation, IQR; interquartile range, NLR; neutrophil to lymphocyte ratio, PLR; platelet to lymphocyte ratio

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Table 2. The comparison of Parameters between study groups

	Low Grade [GLEASON ≤ 7(3+4)] n=106	High Grade [GLEASON ≥7(4+3)] n=67	P
Age (years, mean±SD)	62±6	63±6	0.17
PSA (ng/mL, median IQR)	6.6 (4.8, 11.9)	11.1(6.3, 32.2)	**0.04**
Lymph node metastasis (N, %)	0	9	<0.001
Positive resection margin (N, %)	18	23	0.01
Hemoglobin (g/dL, mean±SD)	14.6±1.2	14.2±1.3	0.05
Leukocyte (/ µL, median, IQR)	7470(6352, 8767)	4680(6160, 8850)	0.81
Neutrophil (/ µL, median, IQR)	4375(3800, 5800)	4680(3560, 6020)	0.80
Lymphocyte (/ µL, median, IQR)	2160(1785, 2632)	2100(1670, 2450)	0.11
Platelet (/ µL, median, IQR)	227000(192000,269000)	217000(188000,257000)	0.46
NLR (median, IQR)	2.03(1.6, 2.6)	2.3(1.6, 3.3)	**0.06**
PLR (median , IQR)	102(83, 129)	109(89, 149)	0.14

PSA; prostate specific antigen, SD; standard deviation, IQR; interquartile range, NLR; neutrophil to lymphocyte ratio, PLR; platelet to lymphocyte ratio

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Table 3. Associations between gleason score group for prostate carcinoma and inflammatory parameters (univariate and multiple logistic regression analyses)

	Univariate		Multiple*	
	B (95% CI)	**p**	**B (95% CI)**	**p**
Age (years, mean±SD)	1.03 (0.98, 1.08)	0.17	-	
Ln-PSA (ng/mL, median IQR)	1.83 (1.01, 3.3)	**0.04**	1.79 (0.99, 3.26)	**0.05**
Ln-Leukocyte (/ µL, median, IQR)	0.90 (0.42, 1.96)	0.80	0.91 (0.42, 1.98)	0.81
Ln-Neutrophil (/ µL, median, IQR)	1.41(0.69, 2.87)	0.33	1.40 (0.68, 2.87)	0.35
Ln-Lymphocyte (/ µL, median, IQR)	0.38 (0.15, 0.94)	**0.03**	0.38 (0.15, 0.97)	**0.04**
Ln-Platelet (/ µL, median, IQR)	1.09 (0.38, 3.08)	0.86	1.15 (0.39, 3.36)	0.79
Ln-NLR (median, IQR)	1.9 (1.13, 3.38)	**0.01**	1.96 (1.12, 3.42)	**0.01**
Ln-PLR (median , IQR)	2.06 (0.95, 4.4)	**0.06**	2.1 (0.96, 4.6)	**0.06**

- *age adjusted multiple analyses.
- PSA; prostate specific antigen, NLR; neutrophil to lymphocyte ratio, PLr; platelet to lymphocyte ratio, LN; log transformed

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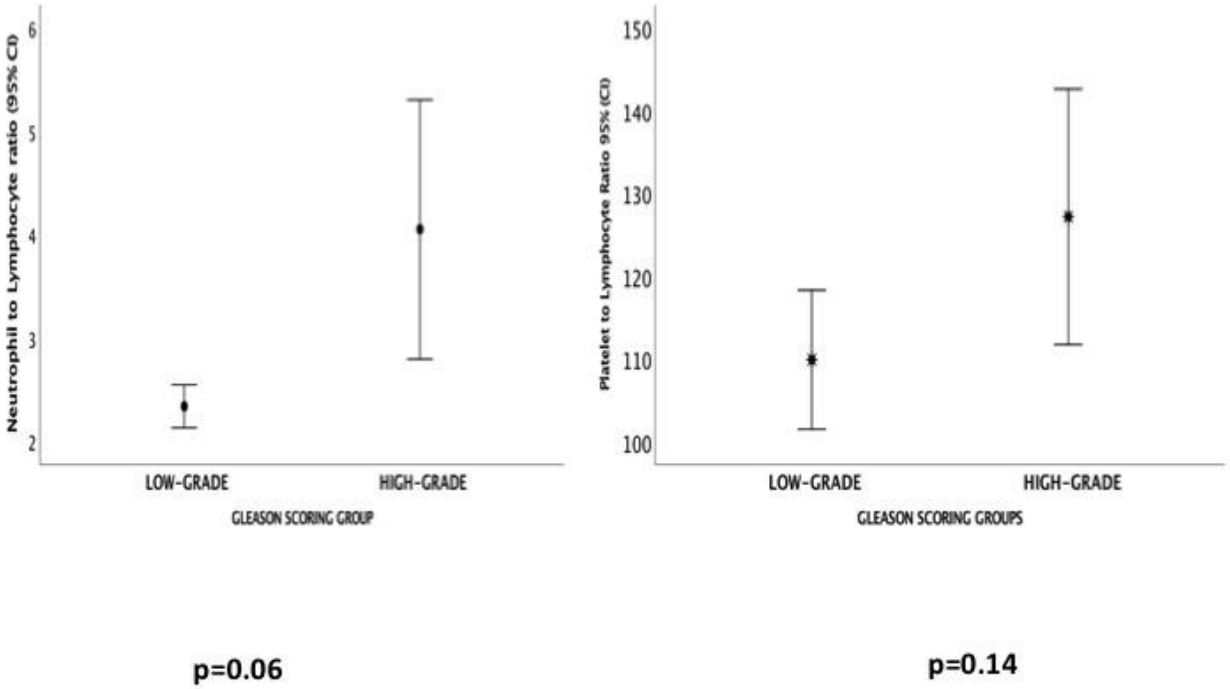

Figure 1. NLR and LR values among study subgroups. Both NLR and PLR values were higher in high-grade group, the difference in NLR showed near statistical significance (p=0.06), however PLR was not significantly different (p=0.14)

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