



Pre-operative Plateletcrit vs. Neutrophil to Lymphocyte Ratio in Predicting Biological Tumor Behavior in Patients with T1-4, N0, M0 Renal Cell Carcinoma: A Retrospective Analysis

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Abstract

Background: Renal cell carcinoma (RCC) is an aggressive cancer prevalent worldwide.

Objectives: We investigated whether preoperative serum levels of plateletcrit (PCT) can predict tumor stages and pathological grades in patients, who were operated on for T1-4, N0, and M0 RCC. Additionally, we compared it with neutrophil to lymphocyte ratio (NLR).

Methods: We conducted a retrospective evaluation of 196 patients, who had undergone nephrectomy (radical or partial) for T1-4, N0, and M0 RCC at our hospital from January 2016 to December 2022. Based on their histopathology results, tumor stages (T) and WHO-ISUP grades (G) were identified. The patients were separated into two categories depending on their T-stage (T1-T2 and T3-T4) and pathological grade (G1-2 and G3-4). The study compared the NLR, PCT, and their combined values to determine their role in predicting aggressiveness based on pathological stage and grade of tumors.

Results: The mean cut-off values for NLR and PCT were found to be 2.108 and 0.273, for the high tumor stage and 2.237 and 0.252 for high-grade tumors, respectively. The statistical analysis showed that NLR ($P = 0.031$) and PCT ($P = 0.006$) were significant predictors of high tumor stage, while only PCT ($P = 0.022$) was a significant predictor of high WHO-ISUP grade. The combination of both NLR and PCT helps improve the sensitivity for detecting high-grade tumors.

Conclusions: NLR and PCT can be predictive markers of the tumor stage. However, only PCT can predict the tumor grade in patients with RCC. In addition, combining the PCT and NLR scores improved the predictive ability of each parameter, especially for identifying high-grade tumors.

Keywords: Platelets, Tumor Stage, Tumour Grade, NLR, Renal Cell Carcinoma, Mean Platelet Value

1. Background

Renal cell carcinoma (RCC) is an aggressive cancer prevalent worldwide, constituting 2% to 3% of all cancers (1, 2). It predominantly affects men in the range of 60 and 70 years of age (1). Typically, surgery in the form of partial or radical nephrectomy is the preferred treatment for localized RCC. After surgical treatment, disease recurrence is observed in 10% to 50% of patients with a poor prognosis (3). It is crucial to identify novel preoperative markers that can help predict tumor behavior, thereby playing an important role in the prognostication of renal tumors.

Also, it is crucial to consider the prognostic factors for RCC, such as age, performance status, tumor stage, histological subtypes, tumor necrosis, and WHO-ISUP grade to make informed treatment decisions (4).

It is known that the development and progression of cancer are often influenced by systemic inflammation, which is also a predictor of oncological prognosis. Certain inflammatory markers obtained from blood samples, including neutrophil to lymphocyte ratio (NLR), PLR, and LMR have been identified as prognostic markers for RCC (5, 6).

Platelets are essential in hemostasis and maintaining blood vessel integrity. The MPV, or mean platelet volume, is commonly used as a measure of platelet activation and is frequently associated with inflammatory disorders and advanced cancer stages. This value is a routine component of a complete blood count (CBC) and is readily available for analysis (7). Prokopowicz et al. concluded that out of the several parameters analyzed as potential prognostic factors, only MPV and platelet count (PC) could predict the prognosis in non-metastatic RCC (8). Plateletcrit (PCT) represents the volume occupied by platelets in the blood and is derived using the formula $\text{plateletcrit (PCT)} = \text{platelet count (PC)} \times \text{mean platelet volume (MPV)} / 10\,000$. The typical range for PCT is between 0.22% and 0.24% (9). PCT is closely linked to platelet count and has significant clinical implications. Previous studies have examined its role in Crohn's disease, and acute cholecystitis, as well as its relationship with varicocele (10-12). It has already been used as a biomarker for predicting survival in patients with non-small cell lung cancer and papillary thyroid carcinoma (13, 14). However, there is limited research on the PCT's relationship with RCC.

2. Objectives

Our study aimed at determining the predictive abilities of PCT and NLR in patients with RCC, who underwent partial or radical nephrectomy, specifically in predicting tumor stage and pathological grade.

3. Methods

A retrospective data analysis of T1-4, N0, and M0 RCC who underwent partial or radical nephrectomy between January 2016 and December 2022 was conducted. Individuals with severe inflammatory conditions, hematologic disease, and distant metastasis were excluded (Figure 1). CBC analysis reports of the study group and tumor pathological details were obtained from the medical records department. Then, the NLR and PCT values were calculated, using the formula: $\text{Absolute neutrophil count} / \text{absolute lymphocyte count}$ and $\text{platelet count} \times \text{MPV} / 10\,000$, respectively. The patients were separated into two categories depending on their T-stage (T1 - T2 and T3 - T4) and pathological grade (G1 - 2 and G3 - 4). The NLR and PCT were compared between the groups. The study compared the NLR, PCT, and their combined values to determine their role in predicting aggressiveness based on pathological stage and grade of tumors.

3.1. Statistical Analysis

In this study, quantitative variables were summarized, using the median (range), while categorical variables were described in terms of frequency and percentages. The diagnostic efficacy of NLR and PCT for advanced stage and high grade was assessed by analyzing the receiver operating characteristic (ROC) curves. Also, binary logistic regression was used to combine inflammatory markers and calculate the AUC, sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV). Youden index was utilized to calculate the cut-off values to categorize the parameters. Additionally, the relationship between NLR and PCT with other clinical variables such as age, sex, stage, grade, size, tumor necrosis, type of nephrectomy (radical or partial), and histological subtype were analyzed. Normal distribution variables were compared by independent t test and percentage variables and the chi-square test. A P-value of < 0.05 was considered statistically significant. The study utilized jamovi 2.3.28 software for conducting statistical analyses.

4. Results

A total of 196 cases of RCC, confirmed through a histopathological analysis meeting the inclusion criteria, were studied. Table 1 displays the demographic details of the patients and their histopathology results. Out of the total patients, 128 (65.30%) were in the G1 - 2 group, while 68 (34.69%) were in the G3 - 4 group. Furthermore, 108 (55.10%) patients had a T1 - T2 tumor, while 88 (44.89%) patients had a T3 - T4 tumor.

The mean NLR and PCT cut-off values for the T stage were 2.108 and 0.273, respectively, while for the G group, they were 2.237 and 0.252. Tables 2 and 3 provide the tumor stages and pathological grades sensitivity, specificity, PPV, and NPV values associated with NLR and PCT values and their combined values. The study found that NLR values greater than 2.108 and PCT ratios greater than 0.273 were significant predictors of a high pathological T stage. Additionally, a PCT ratio greater than 0.252 was a statistically significant predictor of high grade. However, an NLR value greater than 2.237 was not found to be a significant predictor of high grade. Table 4 shows the relationship between NLR and PCT between clinicopathological characteristics of patients.

5. Discussion

The purpose of this research was to investigate the relationship between PCT and the clinicopathological features in patients with non-metastatic RCC, who

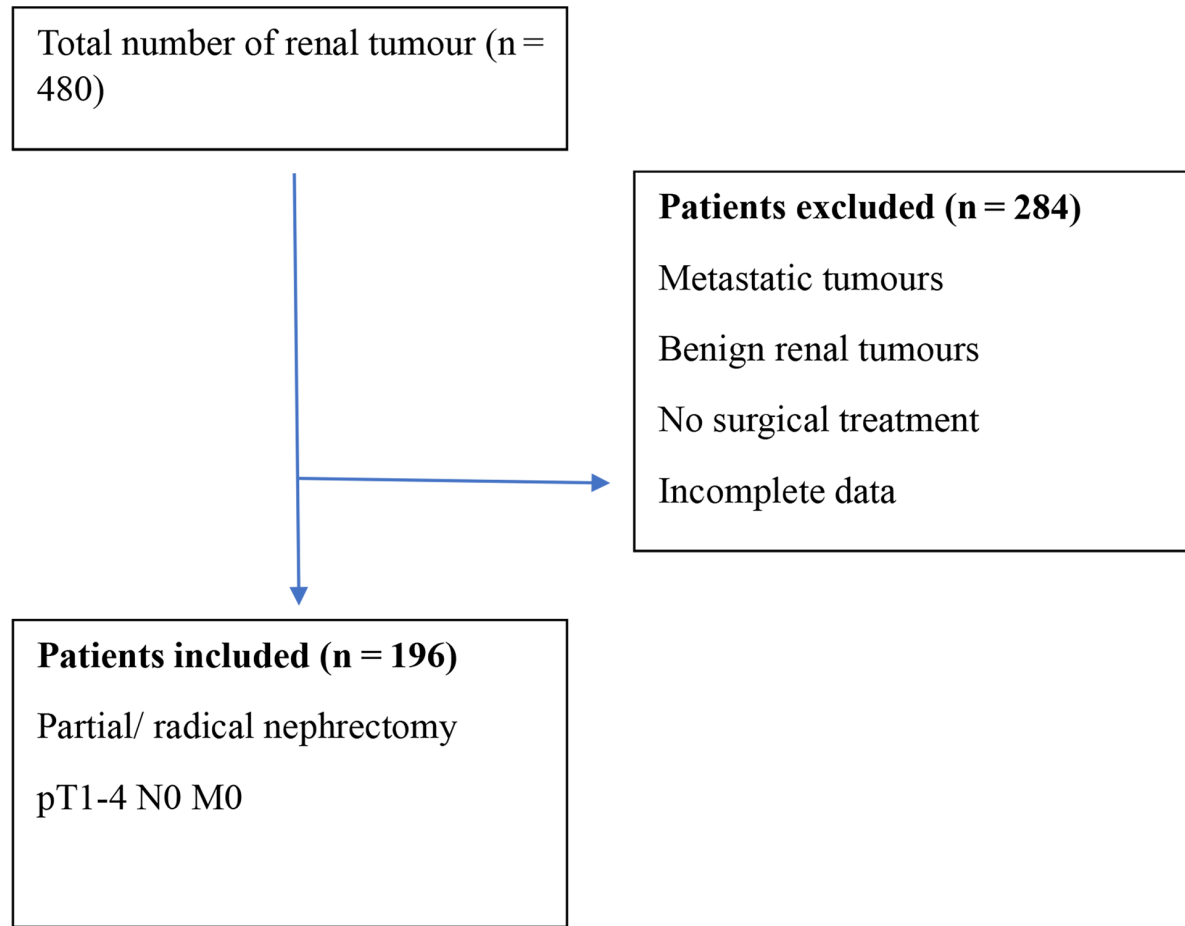


Figure 1. Flowchart of patients who met the inclusion criteria.

underwent surgery. Furthermore, this study aimed at comparing these findings with those of the commonly employed inflammatory marker NLR.

The literature has shown a correlation between the pathological grade and tumor stages of patients diagnosed with RCC after radical or partial nephrectomy and their NLR and PLR values (15, 16). The results of our study indicate that preoperative PCT may be used as a biomarker to predict higher stages and grades of RCC in patients, who undergo curative resections. There was no correlation between PCT and NLR, indicating them to be independent inflammatory response markers. We found that combining the scores of PCT and NLR has enhanced the prognostic ability of each parameter, especially in identifying high grades. As far as we know, this study is one of the earliest to examine the link between PCT and clinicopathological features in individuals with RCC.

It is recognized that platelets have a crucial role in

the progression and dissemination of malignancies (17). Platelet indices are cost-effective, reproducible, and easily accessible in clinical settings. There is increasing evidence that platelet indices could be used as markers to diagnose and predict the behavior of various types of cancer (18).

Platelets in the bloodstream can clump together and adhere to the walls of blood vessels and tumor cells. This facilitates the evasion of tumors from the immune system of the body. Upon activation, platelets release microparticles that contain growth factors that can promote the growth of tumors (19). Furthermore, the endothelial lining of tumor blood vessels can promote platelet adherence, and when stimulated, these cells can accelerate neovascularization. Cytokines directly impact the production and size of platelets, as well as the maturation of bone marrow cells, megakaryopoiesis, and thrombopoiesis. However, platelets are consumed at a higher rate in cases of inflammation or cancer, leading to

Table 1. Demographic Details of the Patients and Their Histopathology Results ^a

Parameters	Values
Number of patients	196
Mean age (y)	57.6 ± 11.4
Median tumor size (cm)	6.50 ± 2.80
Sex	
Male	130 (66.32)
Female	66 (33.67)
Laterality	
Right	106 (54.08)
Left	90 (45.91)
Histology	
Clear cell RCCs	174 (88.78)
Nonclear cell RCCs (papillary, medullary, rhabdoid, and sarcomatoid)	22 (11.22)
Operation type	
Radical	172 (87.75)
Partial	24 (12.24)
Median NLR	3.405
Median PCT	0.217
Pathological tumour stage	
T1 - T2	108 (55.10)
T3 - T4	88 (44.89)
WHO-ISUP grade	
G1 - G2	128 (65.30)
G3 - G4	68 (34.69)

^a Values are expressed as mean ± SD or No. (%).

fluctuations in platelet count. Therefore, platelet count alone may not always be a reliable indicator of disease progression (20).

Larger platelets can be easily stimulated and depleted in the tumor microenvironment, leading to a change in the MPV of circulating platelets. MPV levels act as an early indicator of platelet activation, and reductions in MPV have been observed in a few cases of neoplasm or tissue proliferation (21). While higher MPV levels are typically associated with infectious diseases, diabetes, or obesity, it is important to note that MPV changes can also indicate serious health conditions (22). Studies have shown that analyzing each parameter separately is not as effective as using it together, which takes into account both the quantity and morphology of platelets (22).

In ROC curve analysis, a larger AUC indicates a better diagnostic efficiency. Kisa E et al. found that PCT had a significant association with a high stage of RCC, whereas

it was not associated with a high grade of tumor (23). Concordantly, when we used ROC curves to analyze the performance of PCT and NLR for distinguishing higher stage and grade of tumor, we found that high PCT was significantly associated with high stage and grade. For the stage group, even though the AUC of NLR was higher, along with better sensitivity and specificity, the comparison between NLR and PCT was statistically insignificant. On combining NLR and PCT values, AUC was higher but with poor sensitivity compared to NLR alone. For the grade group, the AUC of PCT was higher and was statistically significant, unlike that of NLR. Also, we found out that on combining NLR with PCT, AUC increased with better sensitivity. There was no significant difference in the predictive ability between NLR and PCT in predicting grade group, though. Therefore, we believe that PCT may be a useful marker for the prognostication of RCC. Patients with high grade and stage of cancer showed significantly higher PCT values, which is evident by higher PCT values linked to radical nephrectomy rather than partial nephrectomy and were also associated with tumor necrosis, indicating a more aggressive tumor biology. It is currently unclear why elevated levels of PCT are associated with aggressive tumor biology. One possibility is that this association could be due to PCT's relationship with platelet activation (24). These results suggest that assessing clinicopathological characteristics of RCC with platelet index has potential prognostic importance. Accordingly, we believe that utilizing PCT as a biomarker for RCC could be a promising approach. It is a simple and cost-effective parameter that has the potential to be used in a prognostic system for RCC.

5.1. Limitations

This study is limited by its retrospective design, which may not fully address certain confounding factors and could lead to a degree of deviation. Moreover, pathology results were evaluated by multiple uropathologists; so, conclusions must be validated by large-scale multicenter clinical studies. Finally, we were unable to conduct follow-ups with patients, who had RCC and assess their disease recurrence or post-surgery status.

5.2. Conclusions

This research establishes the pivotal role of inflammation in the development of RCC. Combining the PCT and NLR scores has enhanced each parameter's prognostic ability, specifically for identifying high grades. It is recommended that PCT values be included in prognostic models for future studies.

Table 2. Best Cut-Off Values, In Which PCT and NLR Can Predict Tumor Stage, Sensitivity, Specificity, PPV, and NPV

Stage Group	AUC	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Youden's Index	Cut-Point	P-Value
PCT	0.577	29.55	88.89	68.42	60.76	0.184	0.273	0.031
NLR	0.603	90.91	37.04	54.05	83.33	0.279	2.108	0.006
Combined	0.636	88.9	29.5	60.8	68.4	-	0.5	0.015
Stage Group	AUC Difference	CI (Lower)		CI (Upper)		P-Value		
PCT vs NLR	0.026	-0.088		0.139		0.657		

Abbreviations: AUC, area under the curve; PPV, positive predictive values; NPV, negative predictive values; PCT, plateletcrit; NLR, neutrophil to lymphocyte ratio.

Table 3. Best Cut-Off Values, in Which PCT and NLR Can Predict WHO-ISUP Grade, Sensitivity, Specificity, PPV, and NPV

Grade Group	AUC	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Youden's Index	Cut-Point	P-Value
PCT	0.592	44.12	81.25	55.56	73.24	0.254	0.252	0.022
NLR	0.555	82.35	39.06	41.79	80.65	0.214	2.237	0.098
Combined	0.597	93.8	5.8	65.2	33.3	-	0.5	< 0.001
Grade Group	AUC Difference	CI (Lower)		CI (Upper)		P-Value		
PCT vs NLR	-0.037	-0.152		0.077		0.524		

Abbreviations: AUC, area under the curve; PPV, positive predictive values; NPV, negative predictive values; PCT, plateletcrit; NLR, neutrophil to lymphocyte ratio.

Table 4. Relationship Between NLR and PCT Between Clinicopathological Characteristics of Patients ^a

Variables	N	NLR	P-Value	PCT	P-Value
Age (y)			0.031		0.503
< 60	112	4.714 ± 0.413		0.227 ± 0.005	
> 60	84	6.486 ± 0.765		0.234 ± 0.008	
Gender			0.053		0.105
Female	66	4.365 ± 0.422		0.241 ± 0.007	
Male	130	6.036 ± 0.571		0.224 ± 0.006	
Type of nephrectomy			0.297		0.024
Partial	24	6.616 ± 1.058		0.200 ± 0.009	
Radical	172	5.314 ± 0.44		0.234 ± 0.005	
Size (cm)			0.357		0.843
< 7	122	5.767 ± 0.553		0.231 ± 0.007	
> 7	74	4.990 ± 0.578		0.229 ± 0.006	
Stage			0.063		0.058
T1 - T2	108	4.788 ± 0.495		0.221 ± 0.006	
T3 - T4	88	6.315 ± 0.667		0.240 ± 0.007	
Grade			0.454		0.023
G1 - G2	128	5.250 ± 0.475		0.222 ± 0.005	
G3 - G4	68	5.894 ± 0.765		0.245 ± 0.010	
Histopathology subtype			0.052		0.290
Non-clear cell	22	7.705 ± 1.836		0.215 ± 0.006	
Clear cell	174	5.191 ± 0.394		0.232 ± 0.005	
Tumor necrosis			0.029		0.044
No	62	4.170 ± 0.418		0.215 ± 0.007	
Yes	134	6.077 ± 0.558		0.237 ± 0.006	

^a Values are expressed as mean ± SD.

Footnotes

Authors' Contribution: Study concept and design: SPM, and SKR; analysis and interpretation of data: SKR and KV; drafting of the manuscript: SPM, critical revision of the manuscript for important intellectual content: SKR, AC, and AS; statistical analysis: MR.

Conflict of Interests: The authors declare no conflict of interest.

Data Availability: The data presented in this study are uploaded during submission as a supplementary file and are openly available for readers upon request.

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References

- Chow WH, Dong LM, Devesa SS. Epidemiology and risk factors for kidney cancer. *Nat Rev Urol*. 2010;7(5):245–57. [PubMed ID: 20448658]. [PubMed Central ID: PMC3012455]. <https://doi.org/10.1038/nrurol.2010.46>.
- Ljungberg B, Bensalah K, Canfield S, Dabestani S, Hofmann F, Hora M, et al. EAU guidelines on renal cell carcinoma: 2014 update. *Eur Urol*. 2015;67(5):913–24. [PubMed ID: 25616710]. <https://doi.org/10.1016/j.eururo.2015.01.005>.
- Egger SE, Yossepowitch O, Pettus JA, Snyder ME, Motzer RJ, Russo P. Renal cell carcinoma recurrence after nephrectomy for localized disease: predicting survival from time of recurrence. *J Clin Oncol*. 2006;24(19):3101–6. [PubMed ID: 16809736]. <https://doi.org/10.1200/JCO.2005.04.8280>.
- Mattila KE, Vainio P, Jaakkola PM. Prognostic Factors for Localized Clear Cell Renal Cell Carcinoma and Their Application in Adjuvant Therapy. *Cancers (Basel)*. 2022;14(1). [PubMed ID: 35008402]. [PubMed Central ID: PMC8750145]. <https://doi.org/10.3390/cancers14010239>.
- Huszno J, Kolosza Z, Mrochem-Kwarcia J, Rutkowski T, Skladowski K. The Role of Neutrophil-Lymphocyte Ratio, Platelet-Lymphocyte Ratio, and Platelets in the Prognosis of Metastatic Renal Cell Carcinoma. *Oncology*. 2019;97(1):7–17. [PubMed ID: 31048577]. <https://doi.org/10.1159/000498943>.
- Hutterer GC, Stoeckigt C, Stojakovic T, Jesche J, Eberhard K, Pummer K, et al. Low preoperative lymphocyte-monocyte ratio (LMR) represents a potentially poor prognostic factor in nonmetastatic clear cell renal cell carcinoma. *Urol Oncol*. 2014;32(7):1041–8. [PubMed ID: 25027686]. <https://doi.org/10.1016/j.urolonc.2014.04.001>.
- Bancroft AJ, Abel EW, McLaren M, Belch JJ. Mean platelet volume is a useful parameter: a reproducible routine method using a modified Coulter thrombocytometer. *Platelets*. 2000;11(7):379–87. [PubMed ID: 11132104]. <https://doi.org/10.1080/09537100020008311>.
- Prokopowicz G, Zyczkowski M, Nowakowski K, Bogacki R, Bryniarski P, Paradysz A. Basic Parameters of Blood Count as Prognostic Factors for Renal Cell Carcinoma. *Biomed Res Int*. 2016;2016:8687575. [PubMed ID: 28105437]. [PubMed Central ID: PMC5220455]. <https://doi.org/10.1155/2016/8687575>.
- Wiwanitkit V. Plateletcrit, mean platelet volume, platelet distribution width: its expected values and correlation with parallel red blood cell parameters. *Clin Appl Thromb Hemost*. 2004;10(2):175–8. [PubMed ID: 15094938]. <https://doi.org/10.1177/107602960401000208>.
- Tang J, Gao X, Zhi M, Zhou HM, Zhang M, Chen HW, et al. Plateletcrit: a sensitive biomarker for evaluating disease activity in Crohn's disease with low hs-CRP. *J Dig Dis*. 2015;16(3):118–24. [PubMed ID: 25565427]. <https://doi.org/10.1111/1751-2980.12225>.
- Zhang QF, Liang JH, He TH, Huang ZX, Liu QL, Zhang X, et al. Relationship between varicocele and platelet indices: changes of mean platelet volume before and after operation. *Andrology*. 2019;7(6):846–51. [PubMed ID: 30969016]. <https://doi.org/10.1111/andr.12605>.
- Budak YU, Polat M, Huysal K. The use of platelet indices, plateletcrit, mean platelet volume and platelet distribution width in emergency non-traumatic abdominal surgery: a systematic review. *Biochem Med (Zagreb)*. 2016;26(2):178–93. [PubMed ID: 27346963]. [PubMed Central ID: PMC4910273]. <https://doi.org/10.11613/BM.2016.020>.
- Dincel O, Bayraktar C. Evaluation of platelet indices as a useful marker in papillary thyroid carcinoma. *Bratisl Lek Listy*. 2017;118(3):153–5. [PubMed ID: 28319410]. <https://doi.org/10.4149/BLL.2017.030>.
- Hur JY, Lee HY, Chang HJ, Choi CW, Kim DH, Eo WK. Preoperative plateletcrit is a Prognostic Biomarker for Survival in Patients with Non-Small Cell Lung Cancer. *J Cancer*. 2020;11(10):2800–7. [PubMed ID: 32226498]. [PubMed Central ID: PMC7086273]. <https://doi.org/10.7150/jca.41122>.
- Shao Y, Wu B, Jia W, Zhang Z, Chen Q, Wang D. Prognostic value of pretreatment neutrophil-to-lymphocyte ratio in renal cell carcinoma: a systematic review and meta-analysis. *BMC Urol*. 2020;20(1):90. [PubMed ID: 32631294]. [PubMed Central ID: PMC7339475]. <https://doi.org/10.1186/s12894-020-00665-8>.
- Zhou X, Luo G. A meta-analysis of the platelet-lymphocyte ratio: A notable prognostic factor in renal cell carcinoma. *Int J Biol Markers*. 2022;37(2):123–33. [PubMed ID: 35238678]. <https://doi.org/10.1177/03936155221081536>.
- Buergy D, Wenz F, Groden C, Brockmann MA. Tumor-platelet interaction in solid tumors. *Int J Cancer*. 2012;130(12):2747–60. [PubMed ID: 22261860]. <https://doi.org/10.1002/ijc.27441>.
- Zhu X, Cao Y, Lu P, Kang Y, Lin Z, Hao T, et al. Evaluation of platelet indices as diagnostic biomarkers for colorectal cancer. *Sci Rep*. 2018;8(1):11814. [PubMed ID: 30087357]. [PubMed Central ID: PMC6081379]. <https://doi.org/10.1038/s41598-018-29293-x>.
- Weyrich AS, Schwartz H, Kraiss LW, Zimmerman GA. Protein synthesis by platelets: historical and new perspectives. *J Thromb Haemost*. 2009;7(2):241–6. [PubMed ID: 18983498]. [PubMed Central ID: PMC3027201]. <https://doi.org/10.1111/j.1538-7836.2008.03211.x>.
- Giannakeas V, Kotsopoulos J, Brooks JD, Cheung MC, Rosella L, Lipscombe L, et al. Platelet Count and Survival after Cancer. *Cancers (Basel)*. 2022;14(3). [PubMed ID: 35158817]. [PubMed Central ID: PMC8833779]. <https://doi.org/10.3390/cancers14030549>.
- Korniluk A, Koper-Lenkiewicz OM, Kaminska J, Kemona H, Dymicka-Piekarska V. Mean Platelet Volume (MPV): New Perspectives for an Old Marker in the Course and Prognosis of Inflammatory Conditions. *Mediators Inflamm*. 2019;2019:9213074. [PubMed ID: 31148950]. [PubMed Central ID: PMC6501263]. <https://doi.org/10.1155/2019/9213074>.
- Ding Q, Wang F, Guo X, Liang M. The relationship between mean platelet volume and metabolic syndrome in patients with type 2 diabetes mellitus: A retrospective study. *Medicine (Baltimore)*. 2021;100(13). e25303. [PubMed ID: 33787620]. [PubMed Central ID: PMC8021380]. <https://doi.org/10.1097/MD.00000000000025303>.
- Kisa E, Yucel C, Keskin MZ, Karabicak M, Yalcin MY, Cakmak O, et al. The Role of Hematological Parameters in Predicting Fuhrman Grade and Tumor Stage in Renal Cell Carcinoma Patients Undergoing Nephrectomy. *Medicina (Kaunas)*. 2019;55(6). [PubMed ID: 31216752]. [PubMed Central ID: PMC6630220]. <https://doi.org/10.3390/medicina55060287>.
- Wang L, Sheng L, Liu P. The independent association of platelet parameters with overall survival in pancreatic adenocarcinoma receiving intensity-modulated radiation therapy. *Int J Clin Exp Med*. 2015;8(11):21215–21. [PubMed ID: 26885057]. [PubMed Central ID: PMC4723902].