Acta Clin Croat 2020; 59:439-444
doi: 10.20471/acc.2020.59.03.07

Original Scientific Paper

MAY HEMOCYTOMETER PARAMETERS BE A BIOMARKER IN DISTINGUISHING BETWEEN ADRENAL ADENOMAS AND CARCINOMAS AND IN PROGNOSIS OF ADRENOCORTICAL CARCINOMAS?

Pinar Sisman[1], Buket Bicer[2], Ozen oz Gul[3], Soner Cander[3], Canan Ersoy[3], Ozlem Saraydaroglu[4] and Erdinc Erturk[3]

[1]Medicana Hospital, Department of Endocrinology and Metabolism, Bursa, Turkey;
[2]Uludag University Medical School, Department of Internal Medicine, Bursa, Turkey;
[3]Uludag University Medical School, Department of Endocrinology and Metabolism, Bursa, Turkey;
[4]Uludag University Medical School, Department of Pathology, Bursa, Turkey

SUMMARY – Recently, studies have reported that inflammatory response and elevated platelet counts are associated with several cancers. In the present study, we aimed to evaluate hemocytometer parameters in differentiating adrenal adenoma and carcinoma, and the prognostic utility of hemocytometer parameters in adrenocortical carcinoma (ACC). We included 30 patients with nonfunctional adrenal adenoma and 13 patients with ACC having undergone surgery between 2005 and 2017 and followed up postoperatively at our centre. The neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), red blood cell distribution width (RDW), mean platelet volume (MPV) and plateletcrit (PCT) were evaluated preoperatively in all patients included in the study. There was a significant difference between the adrenal adenoma and ACC groups in terms of neutrophil and lymphocyte counts, NLR and PLR. There was no significant difference between the two groups in terms of platelet count and MPV, but PCT levels were significantly lower in ACC group. There was no statistically significant difference between recurrent and/or metastasis positive patients and negative ones according to NLR, PLR, RDW and MPV. There was a statistically significant difference in RDW levels and tumor diameter between the groups. Our study is the first to evaluate hemocytometer parameters in differentiating adrenal adenomas and carcinomas, and also in the prognosis of ACC. The present study suggested that the hemocytometer parameters may be a marker in the differential diagnosis of adrenal adenomas and carcinomas. However, our study also showed that these parameters had no prognostic value in ACC.

Key words: *Adrenal adenoma; Adrenocortical carcinoma; Hemocytometer parameters; Mean platelet volume; Neutrophil to lymphocyte ratio*

Introduction

Adrenal incidentaloma is defined as an asymptomatic adrenal mass detected on imaging not performed for suspected adrenal disease. Adrenal incidentalomas are composed of benign and malignant lesions, and benign adenomas constitute more than 90% of adrenal incidentalomas[1]. Adrenocortical carcinoma (ACC) is a rarely seen endocrine malignancy and diagnosis is generally established based on symptoms due to hypersecretion from tumor or local effects of large tumor lesions. Recently, ACC is mostly detected in the asymptomatic period owing to the increasing utilization

Correspondence to: *Pinar Sisman, MD*, Medicana Hospital, Department of Endocrinology and Metabolism, Odunluk Mah. Izmir Yolu Cad. No:41, 16110 Bursa, Turkey
E-mail: drpinarsisman@gmail.com
Received October 2, 2018, accepted February 3, 2020

of imaging methods[2]. When adrenal mass is detected on imaging, it is recommended to establish whether the adrenal mass is benign or malignant. For this purpose, performing computed tomography (CT) may be recommended. Taking biopsy material from the mass is not recommended due to the risk of implantation of tumor cells into the surrounding tissues. Until now, no biochemical parameter clearly indicating whether an adrenal mass is benign or malignant has been identified[3].

Recently, the relationship between some of the hemocytometer parameters and cancer has been investigated[4]. The neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), red blood cell distribution width (RDW) and mean platelet volume (MPV) are the most studied parameters[5]. Platelets play a pivotal role in many steps of cancer development, such as tumor growth, invasion, aberrant angiogenesis, and metastasis due to the interaction with tumor cells[6]. Platelets are rich in growth factors including platelet-derived growth factor and transforming growth factor-β. These factors are often produced by tumor cells and contribute to tumor progression[7]. Elevated platelets have been shown to be associated with poor prognosis in several cancers including gastric cancer, colorectal cancer, pancreatic cancer and gynecologic cancers. Since total platelet count depends on the balance between platelet production and consumption, platelet volume indices are more frequently used[8]. MPV, the most commonly used measure of platelet size, is an indicator of activated platelets. Altered MPV levels were found in a wide range of cancers such as gastric cancer, colon cancer, lung cancer, breast cancer and ovarian cancer[7].

The relationship between inflammation and malignancies has been known for years. Inflammation plays a role in cancer proliferation, angiogenesis and invasion. Since NLR is a biomarker of inflammation, many recent studies have shown an association between NLR and various types of cancer[4,9]. In the present study, we aimed to evaluate hemocytometer parameters in differentiating adrenal adenoma and carcinoma, and the prognostic utility of hemocytometer parameters in ACC.

Patients and Methods

The study included 13 ACC patients and 30 patients with nonfunctional adrenal adenoma having undergone surgery during the 2005-2017 period, with postoperative follow up at our centre. All study patients were evaluated for Cushing syndrome, primary hyperaldosteronism and pheochromocytoma. Cushing syndrome was diagnosed in patients with serum cortisol >1.8 μg/dL following 1 mg dexamethasone suppression test or 2 days of low-dose dexamethasone suppression test and in patients with suppressed serum adrenocorticotropic hormone (ACTH) levels (<5 pg/mL). Hyperaldosteronism was excluded if the plasma renin activity (PRA)/aldosterone ratio was <30. Similarly, pheochromocytoma was excluded in patients with normal plasma metanephrine and normetanephrine levels. Patients with Cushing syndrome, hyperaldosteronism and pheochromocytoma were excluded. The study was approved by the institutional Ethics Committee. Data on age, sex, preoperative biochemical testing and radiological imaging results of the patients included in the study were obtained from patient files. Preoperative CT was used to assess adrenal mass diameter based on the shortest and longest diameters. In addition, archival files of ACC patients were reviewed and data on tumor diameter on pathologic examination studied. Tumor diameter was evaluated based on the long and short diameters on postoperative pathologic examination. In patients with ACC, data were also collected on recurrence and metastasis development during postoperative follow up.

Patients were divided into groups with adrenal adenomas and carcinomas. The latter were further divided into two subgroups of patients with and without recurrence/metastasis. Hemoglobin, hematocrit, neutrophils, lymphocytes, platelet count, NLR, PLR, RDW, platelet distribution width (PDW), MPV and plateletcrit (PCT) performed in the last five days before operation were evaluated in all patients included in the study. The PCT = platelet count x MPV/10000 formula was used for PCT calculation[10].

Statistical analysis

Statistical evaluations were performed using IBM SPSS version 21 (IBM Acquires SPSS Inc., Somers, NY, USA). The χ^2-test was used for comparison of categorical data, whereas the Mann-Whitney U test or Student's t-test was used for comparison of numerical data, as appropriate. Spearman correlation analysis was applied for correlation analyses. Demographic characteristics and preoperative frequency data were ex-

pressed as mean and standard deviation (SD) and percent (%) where appropriate. A p value <0.05 was considered statistically significant.

Results

A total of 43 patients, 30 patients with nonfunctional adrenal adenomas and 13 patients with ACC, were included in the study. There were 27 (62.7%) female and 16 (37.3%) male patients, mean age at diagnosis 53.5±11.4 years. Demographic characteristics of the patients are shown in Table 1.

Of the 30 patients with adrenal adenoma included in the study, there were seven (23.3%) male and 23 (76.7%) female patients, mean age at diagnosis 53.0±11.2 years. The mean longest tumor diameter was 33.6±13.8 mm and 38.2±20.7 mm recorded on preoperative radiological evaluation and postoperative pathologic examination of patients, respectively. Of the 13 patients with ACC included in the study, there were nine (69.2%) male and four (30.8%) female patients, mean age at diagnosis 54.61±12.16 years. The mean longest tumor diameter was 73.4±51.1 mm on preoperative radiological evaluation and 80.3±55.8 mm on postoperative pathologic examination.

There was no significant age difference between adrenal adenoma and ACC groups. Hemoglobin and hematocrit levels were statistically lower and RDW levels higher in patients with ACC. However, there was no significant difference according to RBC level between the two groups. The mean neutrophil level was 5577±3287 K/μL in the adrenal adenoma group and 7452±2396 K/μL in the ACC group. The mean lymphocyte level was 2498±1179 K/μL and 1420±0.687 K/μL in the adrenal adenoma and ACC group, respectively. There was a significant between-group difference according to neutrophil, lymphocyte, NLR and PLR values. In our study, platelet count, MPV and PCT were also compared between the two groups. There was no significant between-group difference according to platelet count and MPV, but PCT levels were significantly lower in the ACC group.

During follow up, locoregional recurrence was observed in one patient, distant metastasis in two patients, and both locoregional recurrence and distant metastasis were observed in two patients. NLR, PLR, RDW and MPV values were 4.10±1.45, 204.77±70.76, 17.46±1.38 and 7.74±0.73 in patients with recurrent and/or metastatic disease, respectively. In patients without recurrence and/or metastasis, NLR, PLR, RDW and MPV values were 9.24±6.74, 232.62±135.97, 15.98±1.47 and 8.85±1.18, respectively. There was no statistically significant difference according to NLR, PLR, RDW and MPV between recurrent and/or metastasis positive patients and negative ones. There was a statistically significant difference in RDW levels and

Table 1. Relationship between diagnosis and hematologic parameters

	Adrenal adenoma (n=30)	Adrenocortical carcinoma (n=13)	p
Age at diagnosis (yrs)	53.0±11.2	54.6±12.1	0.62
Sex:			
Male (%)	7 (23.3)	9 (69.2)	**0.007**
Female (%)	23 (76.7)	4 (30.8)	
Neutrophil count (K/μL)	5577±3287	7452±2396	**0.03**
Lymphocyte count (K/μL)	2498±1179	1420±0.687	**0.001**
Platelet count (K/μL)	260.7±57.6	259.3±109.6	0.26
Neutrophil/ lymphocyte ratio	2.4±1.4	7.9±6.1	**0.001**
Platelet/ lymphocyte ratio	127.2±57.1	221.9±112.4	**0.006**
RDW (%)	14.8±1.7	16.5±1.5	**0.006**
MPV (fL)	8.5±1.7	8.4±1.1	0.83
PCT (%)	0.22±0.05	0.17±0.02	**0.01**
PDW (%)	17.1±1.2	17.0±1.8	0.95
Hemoglobin (g/dL)	13.3±1.4	12.1±1.7	**0.03**
Hematocrit (%)	40.0±4.1	36.9±5.3	**0.04**
Mean cell volume (MCV)	87.7±4.1	84.8±7.9	0.06
RBC (M/μU)	4.5±0.5	4.3±0.6	0.25
WBC (K/μL)	8.6±3.2	8.5±2.5	0.98

RDW = red blood cell distribution width; MPV = mean platelet volume; PCT = plateletcrit; PDW = platelet distribution width; RBC = red blood cell; WBC = white blood cell

Table 2. Comparison of hematologic parameters according to adrenocortical carcinoma groups

	Recurrence /metastasis+ (n=5)	Recurrence /metastasis- (n=8)	p
Age at diagnosis (yrs)	55.40±11.30	54,12±13.42	1.00
Gender, M/F	3/2	1/7	
NLR	4.10±1.45	9.24±6.74	0.50
PLR	204.77±70.76	232.62±135.97	0.77
RDW (%)	17.46±1.38	15.98±1.47	0.09
MPV	7.74±0.73	8.85±1.18	1.10

NLR = neutrophil/lymphocyte ratio; PLR = platelet/lymphocyte ratio; RDW = red blood cell distribution width; MPV = mean platelet volume

tumor diameter between the two groups (p=0.007) (Table 2).

Discussion

Adrenal incidentaloma is defined as a mass detected in the adrenal gland on an imaging study performed for other reasons unrelated to adrenal disease. The prevalence of adrenal incidentaloma increases with age and is seen at an equal frequency in male and female individuals. The prevalence of adrenal incidentaloma is about 4% in the middle age group and ACC accounts for approximately 8% of all adrenal incidentalomas. When adrenal incidentaloma is found, determination of excess adrenal hormone hypersecretion and assessment of the possible tumor malignancy is suggested[11].

Imaging modalities such as CT, magnetic resonance imaging (MRI) and ^{18}F-FDG-PET are mostly preferred to differentiate benign and malignant tumors of the adrenal gland[12]. The majority of adrenal adenomas contain high fat. However, malignant adrenal lesions contain almost no fat. Hounsfield unit (HU) on non-contrast CT has high sensitivity and specificity in differentiating benign and malignant lesions. The pattern obtained after contrast enhancement (called washout) is also a good way to distinguish between benign and malignant tumors[13]. Adenomas demonstrate low attenuation on unenhanced CT (≤10 HU) and absolute enhancement washout of contrast ≥60% and/or relative washout ≥40% on contrast enhanced CT. Losing of signal in opposed-phased MRI is also diagnostic of adenoma[14]. Unenhanced measurements >30 HU on CT usually indicate ACC or pheochromocytoma. Large tumor size, heterogeneous enhancement, necrosis and invasion of adjacent organs are other features suggesting malignancy[15].

Adrenocortical carcinomas are usually large (>6 cm), heterogeneous and aggressive tumors with necrosis, hemorrhage and calcifications in 30% of cases. Recurrence rate of ACC after surgery is high and prognosis is poor even in non-metastatic cases[16]. It is generally not possible to distinguish malignant tumors based only on clinical features. There is no well-known biochemical parameter predicting malignancy either. Fine needle aspiration biopsy is not useful in differentiating between adrenal adenoma and primary adrenal carcinoma[11]. Besides, in case of suspected ACC, fine needle aspiration biopsy should not be performed due to the risk of tumor spillage. It may be used to evaluate an indeterminate adrenal mass with a known primary tumor[17]. In our study, the mean longest diameter of tumors was 38.2±20.7 mm on postoperative pathologic examination in patients with adrenal adenoma. In the ACC group, pathologic examination showed a mean diameter of 80.3±55.8 mm.

Measuring the hemocytometer parameters is a relatively inexpensive and universally available method. Its implementation is easy and results are obtained quickly[7]. In recent years, it has been shown that hemostatic parameters may be beneficial both in benign-malignant differential diagnosis and in cancer prognosis[4]. In the study conducted by Cui *et al.*, reduced PDW was demonstrated to be independently associated with prognosis of non-small cell lung cancer[8]. In another study, the relationship between MPV and clinicopathological characteristics of colorectal cancer was evaluated, demonstrating that MPV was elevated in colorectal cancer[18]. Gaitanidis *et al.* also showed PLR and MPV to be independent predictors of disease progression in pancreatic neuroendocrine tumors[19]. Similar to previous literature reports, Takeuchi *et al.* demonstrated that elevated PDW/platelet count significantly reduced disease-free survival in patients with breast cancer[7]. In our study, there was no significant difference between the adrenal adenoma and ACC groups according to platelet count and MPV, but PCT levels were significantly lower in the ACC group. Our results also showed that there was no statistically significant difference according to MPV levels between recurrence and/or metastasis positive patients and negative ones.

Since inflammation plays a role in several steps of the carcinogenesis process, in recent years, NLR and PLR have been evaluated in various types of cancer[9]. Miyamoto *et al*. found preoperative NLR to be a useful predictor of both long-term and short-term outcomes in gastric cancer patients[20]. In a meta-analysis that included 8252 patients, NLR was found to be a promising prognostic biomarker for pancreatic cancer[21]. In another meta-analysis including 3028 patients, Li *et al*. have reported elevated PLR to be associated with unfavorable overall survival in pancreatic cancer[22]. RDW is also found as a strong predictor of all-cause mortality and cancer progression in prostate cancer[23].

On the other hand, Yaylacı *et al*. did not find any significant relationship between benign nodular goiter and papillary thyroid carcinoma in terms of NLR and MPV[24]. In addition to the contradictory results of previous studies, Ozyalvaclı and Yasar report on a multitude of factors affecting standardization of MPV measurement, such as blood drawing by venipuncture, type and amount of anticoagulant in the blood collection tube, and time elapsed from blood drawing to testing[25]. In our study, there were significant differences between the patients with adrenal adenomas and ACC according to neutrophil, lymphocyte, NLR and PLR. However, when NLR and PLR were compared between recurrent and/or metastasis positive patients and negative ones, no significant difference was found.

Because the majority of adrenal masses are benign adenomas, identification of novel markers for use in the preoperative period to determine whether suspected adrenal masses are benign or malignant is important. The limitations of the present study were the small number of patients and the single-centre study design. The retrospective nature of the study may also be considered as a limitation of the study. Despite these limitations, our study was the first to evaluate hemocytometer parameters in differentiating adrenal adenomas and carcinomas, as well as its potential role in the prognosis of ACC.

According to many studies in the literature, NLR, PLR, RDW and MPV seem to be associated with poor outcomes in various types of cancer. However, the studies investigating these markers are very heterogeneous and there are a number of studies advocating countervailing opinion. The present study suggested that the hemocytometer parameters may be a marker in the differential diagnosis of adrenal adenomas and carcinomas. However, our study also showed that these parameters were not useful in the prognosis of ACC. We think that more comprehensive studies in larger patient series may reveal the significance of these parameters in the diagnosis and prognosis of ACC more clearly and definitely.

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Sažetak

MOGU LI HEMOCITOMETRIJSKI PARAMETRI POSLUŽITI KAO BIOLOŠKI BILJEZI U RAZLIKOVANJU ADRENALNIH ADENOMA I KARCINOMA TE U PROGNOZI ADRENOKORTIKALNIH KARCINOMA?

P. Sisman, B. Bicer, O. oz Gul, S. Cander, C. Ersoy, O. Saraydaroglu i *E. Erturk*

Nedavna istraživanja pokazuju da su upalni odgovor i povišeni trombociti udruženi s nekim vrstama karcinoma. Cilj našega istraživanja bio je procijeniti hemocitometrijske parametre u razlikovanju adrenalnog adenoma i karcinoma, kao i prognostičku vrijednost hemocitometrijskih parametara u adrenokortikalnom karcinomu (*adrenocortical carcinoma*, ACC). U istraživanje smo uključili 30 bolesnika s nefunkcionalnim adrenalnim adenomom i 13 bolesnika s ACC operiranih između 2005. i 2017. godine i poslije operacije praćene u našoj ustanovi. Kod svih bolesnika uključenih u studiju prijeoperacijski su izmjereni sljedeći parametri: omjer neutrofila i limfocita (*neutrophil/lymphocyte ratio*, NLR), omjer trombocita i limfocita (*platelet/lymphocyte ratio*, PLR), širina distribucije eritrocita (*red blood cell distribution width*, RDW), srednji volumen trombocita (*mean platelet volume*, MPV) i pleteletkrit (PCT). Utvrđena je statistički značajna razlika između skupina bolesnika s adrenalnim adenomom i onih s ACC u broju neutrofila i limfocita, NLR i PLR. Nije bilo značajne razlike među skupinama u broju trombocita i MPV, ali su razine PCT bile značajno niže u skupini s ACC. Nije bilo statistički značajne razlike u NLR, PLR, RDW i MPV između bolesnika s opetovanim ACC i/ili metastazama i onih bez tih stanja. Statistički značajna razlika između skupina nađena je za razine RDW i promjer tumora. Naše istraživanje je prvo te vrste u kojem su se procjenjivali hemocitometrijski parametri u razlikovanju adrenalnih adenoma i karcinoma te u prognozi ACC. Rezultati studije ukazuju na to da bi se hemocitometrijski parametri mogli primijeniti kao biljezi u diferencijalnoj dijagnostici adrenalnih adenoma i karcinoma. Međutim, naše je istraživanje pokazalo kako ovi parametri nemaju prognostičku vrijednost kod ACC.

Ključne riječi: *Adrenalni adenom; Adrenokortikalni karcinom; Hemocitometrijski parametri; Srednji volumen trombocita; Omjer neutrofila i limfocita*