ARTICLE IN PRESS

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
x (x): x-x, 2026
DOI: 10.9775/kvfd.2026.38087

RESEARCH ARTICLE

CBC-Derived Composite Hematological Indices as Candidate Biomarkers for Canine Hyperadrenocorticism: A Case-Control Study

Seyedeh Zahra EBRAHIMIAN JELOUDAR [1], Mahmood AHMADI-HAMEDANI [1(*)], Hasti AZARABAD [2], Mehran BEIKHA [3]

1 Department of Clinical Sciences, Faculty of Veterinary Medicine, Semnan University, Semnan, IRAN
2 Clinical Pathology Laboratory, Fattahian Private Veterinary Hospital, Tehran, IRAN
3 Department of Clinical Sciences, Faculty of Veterinary Sciences, Islamic Azad University, Science and Research Branch, Tehran, IRAN

(*) **Corresponding author:**
Mahmood Ahmadi-Hamedani
Phone: +98-9113716709
E-mail: ahmadi.hamedani@semnan.ac.ir

How to cite this article?

Ebrahimian Jeloudar SZ, Ahmadi-Hamedani M, Azarabad H, Beikha M: CBC-Derived Composite Hematological Indices as Candidate Biomarkers for Canine Hyperadrenocorticism: A Case-Control Study. *Kafkas Univ Vet Fak Derg*, 2026 (Article in Press).
DOI: 10.9775/kvfd.2026.38087

Article ID: KVFD-2026-38087
Received: 11.09.2026
Accepted: 09.11.2026
Published Online: 11.11.2026

Abstract

Hyperadrenocorticism (HAC) in dogs involves sustained overproduction of glucocorticoids, leading to systemic inflammation and thrombo-inflammatory changes; however, the diagnostic value of composite hematological indices derived from complete blood counts (CBC) remains unexplored. This case-control study assessed the effectiveness of leukocyte-, erythrocyte-, and platelet-based composite hematological indices, along with routine biochemical parameters, for diagnosing naturally occurring HAC in dogs compared with healthy controls. We compared hematological and biochemical variables in 10 dogs with naturally occurring hyperadrenocorticism (HAC) and 10 matched healthy controls. We also computed and compared CBC-derived composite indices between the two groups. Dogs with HAC had significantly higher neutrophil counts and lower lymphocyte counts than controls (P=0.035 and P=0.018; Cohen's d=1.08 and d=-1.18, respectively). Of the composite indices, the neutrophil-to-lymphocyte ratio (NLR) had the greatest diagnostic accuracy (AUC=0.88; sensitivity 100%, specificity 70%; cut-off 3.14), then came the mean corpuscular volume-to-lymphocyte ratio (MCLR) (AUC=0.81), the mean platelet volume-to-lymphocyte ratio (MPLR) (AUC=0.83; sensitivity 100%), and the systemic immune-inflammation index (SII) (AUC=0.78). The groups did not differ significantly in the platelet-morphometric and erythrocyte-platelet indices. Serum alkaline phosphatase (ALP) demonstrated the highest overall diagnostic efficiency (AUC=0.91; sensitivity 100%, specificity 70%; cut-off 140 IU/L) but is not specific to HAC. These results suggest that NLR, MPLR, MCLR, and SII may be candidate biomarkers for canine HAC. Because of the small sample size, the findings, including the cut-off values, are preliminary and require validation in larger, independent, etiologically stratified cohorts before use as diagnostic tools.

Keywords: Biomarker, Complete blood count, Cortisol, Cushing's Syndrome, Dog, Inflammation, Neutrophil-to-lymphocyte ratio

INTRODUCTION

Hyperadrenocorticism (HAC), or Cushing's disease, is one of the most common endocrinopathies in dogs and results from chronic excessive glucocorticoid secretion [1]. Beyond its classic endocrine manifestations, HAC is increasingly recognized as a systemic disease involving chronic inflammation, endothelial dysfunction, and thromboembolic complications [2,3]. The hematologic changes typical of chronic glucocorticoid exposure are known as the "stress leukogram," which includes neutrophilia and lymphopenia due to neutrophil demargination, delayed neutrophil apoptosis, and glucocorticoid-mediated suppression of lymphocytes [4]. Cortisol is associated with changes in platelet activation and numbers, increased thrombotic risk, and altered cell-to-cell adhesion through increased thromboxane production [3]. Despite considerable progress in diagnosing and managing canine HAC, the most widely used endocrine tests, such as the ACTH stimulation test and the low-dose dexamethasone suppression test (LDDST), have practical limitations. They are expensive, require specific sampling procedures and laboratory resources, and do not always yield consistent results when monitoring treatment response [5,6]. Consequently, interest is growing in more affordable and widely available biomarkers for diagnosing and longitudinally monitoring hypercortisolemic dogs [7].

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Recently, inflammatory markers derived from routine complete blood count (CBC) have been found useful in evaluating systemic inflammation and disease severity in human and veterinary patients. Most attention has focused on the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) because they summarize the main cortisol-mediated changes (neutrophilia, lymphopenia, and thrombocytosis) in a single, simple index [4]. In patients with hypercortisolism, increased NLR and PLR values have been associated with disease presence and appear to increase with cortisol levels [8,9]. Dogs are no exception: recent findings showed that dogs with HAC had significantly higher NLR and PLR values than healthy dogs and dogs with non-adrenal disease. In addition, NLR decreased significantly after successful trilostane therapy, suggesting that CBC-derived indices may be inexpensive, readily available tools for screening and for monitoring treatment response in dogs with HAC [4].

Although that study investigated a single, well-studied population of dogs, the underlying question has broader relevance: can multi-lineage CBC-derived indices capture the circulating hematological profile associated with sustained hypercortisolism? Standard endocrine testing faces similar cost and accessibility barriers in many countries. However, the inflammatory indices currently in use are mostly ratios of white blood cells and may therefore reflect only part of the blood changes induced by chronic endocrine stress. Evidence from veterinary and human medicine suggests that combining white cell counts with red cell and platelet variables may better reflect bone marrow output and the systemic inflammatory response [10]. Composite indices that use the lymphocyte count as the denominator, such as the red cell distribution width-to-lymphocyte ratio and the mean platelet volume-to-lymphocyte ratio, have shown diagnostic and prognostic value in inflammatory conditions, possibly because they capture simultaneous changes in red cell, white cell, and platelet production [3,11]. Because canine HAC involves chronic dysregulation of both the inflammatory and hematologic systems, these multi-lineage indices may give a more comprehensive picture of the systemic changes in affected dogs. In this study, we therefore evaluated the diagnostic performance of an expanded set of leukocyte-, erythrocyte-, and platelet-derived composite hematological indices, together with routine biochemical variables, in dogs with naturally occurring HAC compared with healthy control dogs.

Material and Methods

Ethical Statement

This study was approved by the Semnan University Animal Experiments Local Ethics Committee (Approval no: IR.SU.REC.1405.42). All dog owners provided written informed consent.

Study Design and Subjects

We conducted the study from May 2023 to January 2025 at an outpatient animal clinic in Tehran, Iran, prospectively enrolling 20 privately owned pet dogs (10 with natural HAC and 10 age- and sex-matched healthy controls). Because this was an exploratory, hypothesis-generating trial and we encountered few eligible animals with naturally occurring HAC during enrollment, we did not use an a priori sample size or power calculation for any analyses; we discuss this further below. We obtained written informed consent from all dog owners.

HAC Diagnosis and Eligibility Criteria

All dogs that displayed clinical signs of HAC, such as excessive drinking and urination, excessive hunger, abdominal distention, and skin lesions, underwent workups. We administered dexamethasone intravenously at 0.01 mg/kg (Dexamethasone, Darou Pakhsh Co., Tehran, Iran). Blood samples were drawn just before injection and 4 h and 8 h after administration of dexamethasone to evaluate serum cortisol levels. The test was considered suggestive of HAC when either of the 2 results at 8 h post-dexamethasone was >1.5 µg/dL [12]. The test does not distinguish adrenal from pituitary origin, and we did not perform an ACTH stimulation test or further workup because the aim was to characterize the overall hematological profile of dogs with HAC of any origin.

Exclusion Criteria and Control Group Characteristics

We reviewed clinical histories for concurrent inflammatory, neoplastic, or infectious disorders, diabetes mellitus, or chronic renal dysfunction during the period relevant to HAC development. We used physical examination, CBC, and serum biochemistry to exclude dogs with signs of concurrent or occult disease. We did not perform urinalysis and diagnostic imaging in all dogs because these tests were omitted in some dogs evaluated for HAC; we address this in the limitations.

Hematological and Biochemical Analysis

All animals underwent cephalic venipuncture to obtain 4 mL of venous blood. Half of each sample was placed in EDTA tubes for CBC measurement; the other half in a plain tube to determine Serum constituents. We assessed CBC variables on an automated hematology analyzer (Celltac VET MEK-6550; Nihon Kohden, Tokyo, Japan) and used them to calculate derived composite indices according to the formula previously established by Kocaturk et al.[10]. These included the neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio, monocyte-to-neutrophil ratio, mean corpuscular volume-to-lymphocyte count ratio (MCLR), platelet-to-

lymphocyte ratio, and plateletcrit-to-lymphocyte count ratio (PCLR). We also calculated additional platelet-morphometric composite ratios, including the mean corpuscular volume-to-mean platelet volume ratio, hematocrit-to-plateletcrit ratio, mean platelet volume-to-platelet count ratio, mean platelet volume-to-plateletcrit ratio, mean platelet volume-to-platelet distribution width ratio, mean platelet volume-to-lymphocyte count ratio (MPLR), and platelet distribution width-to-lymphocyte count ratio. The systemic immune-inflammation index (SII, also referred to in some sources as PNLR) was calculated as platelet count × NLR. We also calculated the hemoglobin-albumin-lymphocyte-to-platelet (HALP) score.

Statistical Analysis

We performed statistical analyses using SPSS v27.0 (IBM Corp., Armonk, NY, USA). Normality was assessed with the Shapiro-Wilk test. Continuous variables were expressed as mean±pooled standard error of the mean (SEM) or as medians (IQR), depending on distribution. Differences between HAC and control dogs were assessed with an independent-samples t-test for normally distributed data or a Mann-Whitney U test otherwise. Discriminatory ability of variables that showed significant differences was examined using ROC curves; the area under the curve (AUC) was calculated and reported with 95% confidence intervals (CI), as well as the optimal cutoff point, obtained by calculating Youden's index. $P<0.05$ was considered statistically significant. No correction was made for multiple comparisons, as this was an exploratory study of a wide array of composite indices.

Therefore, interpret any reported P-values as hypothesis-generating. Effect sizes (Cohen's d) and 95% CI for comparisons in *Table 1* were derived from the provided group means and the SEM of the mean difference ($d = t\sqrt{(1/n_1+1/n_2)}$, where t = mean difference / SEM of difference). Because the cutoff values were derived and evaluated in the same small sample, without an independent validation cohort, the diagnostic performance estimates are likely to be optimistic and should be regarded as preliminary.

Results

Hematological and Biochemical Findings

Neutrophils increased significantly (10.03 vs. 6.71; P=0.035), while lymphocytes decreased significantly (1.26 vs. 2.05; P=0.018) in HAC cases relative to controls *(Table 1)*. Alkaline phosphatase in dogs with HAC was significantly higher than in controls (732.40 vs. 138.60 IU/L; P=0.003), as was albumin (P=0.043); mean total white cell count, mean platelet count, and hemoglobin level did not differ significantly. Effect sizes (Cohen's d) for most blood workups, especially lymphocyte count (d=-1.18), neutrophil count (d=1.08), alkaline phosphatase (d=1.22, approx.), and albumin (d=0.98), were larger, while effect sizes for platelets, hemoglobin, and WBC ranged between d=0.33 and 0.48; 95% confidence intervals were wide, consistent with the small sample size *(Table 1)*.

Table 1. *Mean values, standard error of the mean difference (SEM), statistical significance, and effect size (Cohen's d with 95% CI) of hematological and serum biomarkers in dogs with HAC compared to healthy dogs*

Parameter	Group	n	Mean	SEM	P	Cohen's d	95% CI (d)
PLT	HAC	10	387.10	61.81	0.340	0.45	-0.44 to 1.33
	Healthy	10	325.30				
NEU	HAC	10	10.03	1.38	0.035	1.08	0.14 to 2.01
	Healthy	10	6.71				
LYM	HAC	10	1.26	0.30	0.018	-1.18	-2.13 to -0.23
	Healthy	10	2.05				
HGB	HAC	10	15.02	1.01	0.472	0.33	-0.55 to 1.21
	Healthy	10	14.28				
ALB	HAC	10	3.23	0.15	0.043	0.98	0.06 to 1.91
	Healthy	10	2.90				
ALP[a]	HAC	10	732.40	217.75	0.003	1.22	0.26 to 2.17
	Healthy	10	138.60				
WBC	HAC	10	11.22	1.60	0.302	0.48	-0.41 to 1.37
	Healthy	10	9.51				

PLT, platelet count; NEU, neutrophil count; LYM, lymphocyte count; HGB, hemoglobin; ALB, albumin; ALP, alkaline phosphatase; WBC, white blood cell count. Cohen's d was back-calculated from the reported group means and the SEM of the mean difference ($d = t\sqrt{(1/n_1+1/n_2)}$); by convention, $|d| \approx 0.2$, 0.5, and 0.8 indicate small, medium, and large effects, respectively. [a]ALP was analyzed by the Mann-Whitney U test owing to non-normal distribution

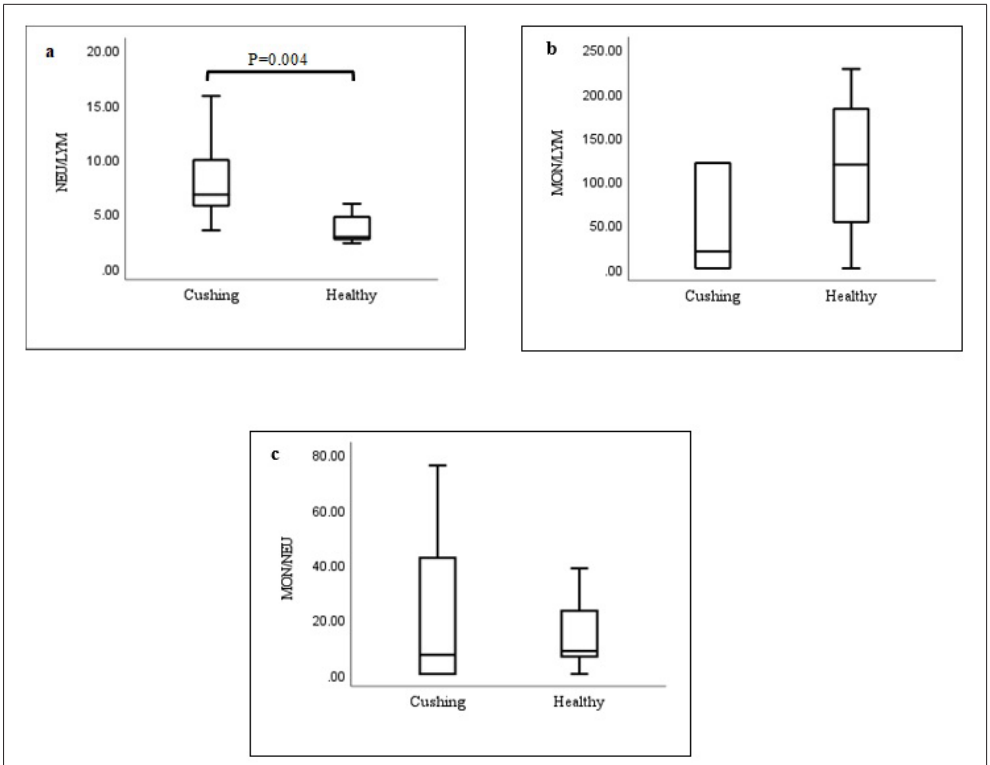


Fig 1. Comparison of white blood cell-derived indices between dogs with naturally occurring HAC and healthy controls (n=10 per group). **(A)** Neutrophil-to-lymphocyte ratio, **(B)** Monocyte-to-lymphocyte ratio, **(C)** Monocyte-to-neutrophil ratio. Boxes show the median and interquartile range, and whiskers show the range of the data. HAC, hyperadrenocorticism

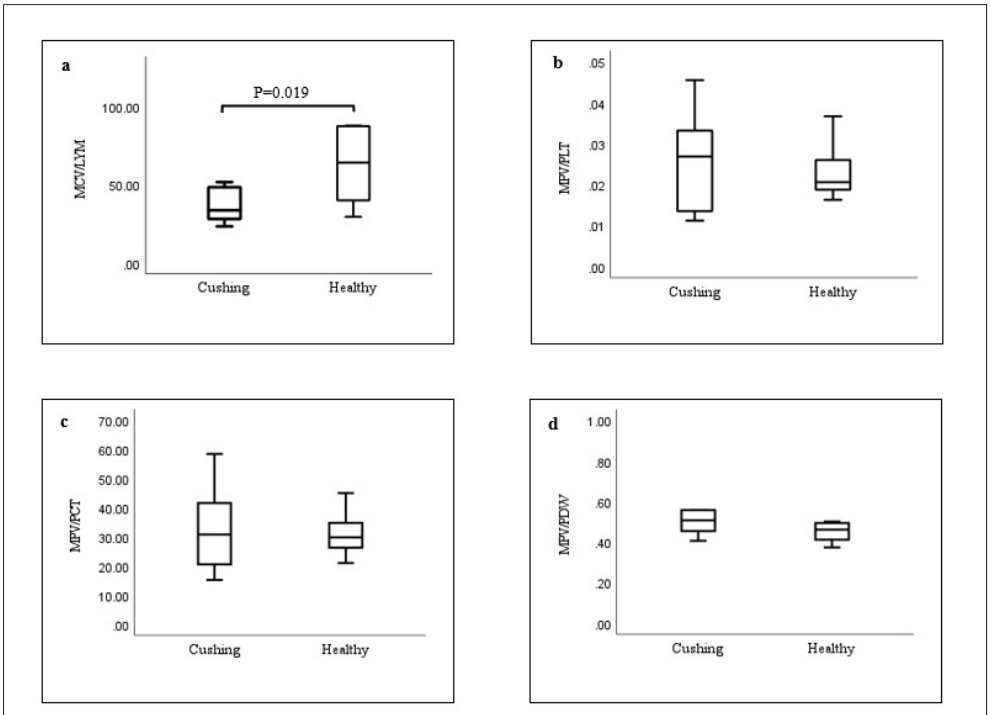


Fig 2. Comparison of erythrocyte-leukocyte and platelet-morphometric composite ratios between dogs with naturally occurring HAC and healthy controls (n=10 per group). **(A)** Mean corpuscular volume-to-lymphocyte count ratio, **(B)** Mean platelet volume-to-platelet ratio, **(C)** Mean platelet volume-to-plateletcrit ratio, **(D)** Mean platelet volume-to-platelet distribution width ratio. Boxes show the median and interquartile range, and whiskers show the range of the data. HAC, hyperadrenocorticism

White Blood Cell-Derived Indices

The NLR was significantly higher in dogs with HAC than in normal controls (P=0.004) *(Fig. 1A)*. HAC dogs showed a non-significant trend toward an increased monocyte-to-lymphocyte ratio (MLR) *(Fig. 1B)*, and there were no statistically significant differences in the monocyte-to-neutrophil ratio (MNR) between control and HAC dogs.

Erythrocyte-Leukocyte and Platelet-Morphometric Composite Ratios

MCLR was significantly higher in dogs with HAC compared with healthy controls (P=0.019) *(Fig. 2A)*. Platelet-morphometric ratios did not differ significantly between groups, although HAC dogs showed greater variability *(Fig. 2B-D)*.

Platelet-Leukocyte Composite Indices

PCLR and MPLR were significantly higher in dogs with HAC than in healthy dogs (P=0.049 and P=0.013, respectively) *(Fig. 3A and 3B)*. PLR and the platelet distribution width-to-lymphocyte count ratio did not differ significantly between groups *(Fig. 3C and 3D)*.

Erythrocyte-Platelet Composite Indices, HALP, and SII

No difference was detected between the groups in MCV/MPV (MCMPR) or Hct/Pct (HCPCR) *(Fig. 4A and 4B)*. HALP scores overlapped considerably between the groups, with a lower median in HAC dogs (approximately 0.16) than in healthy dogs (approximately 0.25) *(Fig. 4C)*. Serum albumin, a component of the HALP score, also differed significantly between groups (P=0.043; *Table 1*). SII was significantly elevated in HAC dogs (P=0.034) *(Fig. 4D)*. Box-plot analysis showed a greater range and greater individual variation for parameters such as NLR, MPLR, MCLR, SII, and PCLR in HAC dogs compared with healthy dogs *(Figs. 1A, 2A, 3A, 3B, 4D)*.

Diagnostic Performance

NLR showed the best discrimination (AUC=0.88; 95% CI 0.72-1.00; sensitivity 100%, specificity 70%; cut-off 3.14) and MCLR had good discriminative performance (AUC=0.81; 95% CI 0.62-1.00; sensitivity 80%, specificity 70%; cut-off 38.64). Among platelet-leukocyte parameters, MPLR had the highest sensitivity (100%; AUC=0.83, 95% CI 0.63-1.00; specificity 60%; cut-off 3.412), PCLR had a moderate result (AUC=0.76; 95% CI 0.55-0.97; sensitivity 70%, specificity 70%; cut-off 0.147), and SII was moderate (AUC=0.78; 95% CI 0.57-0.98; sensitivity 90%, specificity 60%; cut-off 1010.44). ALP had the highest AUC of 0.91 (95% CI 0.78-1.00), with 100% sensitivity and 70% specificity at a cut-off of 140 IU/L. In comparison, albumin showed lower discriminative capacity, with an AUC of 0.76 (95% CI 0.55-0.97) *(Table 2)*. We performed all computations using data from 10 dogs per group; the broad confidence intervals indicate considerable imprecision, and we have not validated the cut-off values in a separate sample.

Discussion

In dogs, HAC causes chronically elevated glucocorticoids and a wide range of systemic changes, including immune dysfunction, chronic low-grade inflammation, and alterations in the thrombo-inflammatory axis [13]. Although many clinicians are familiar with the stress leukogram, our findings suggest that composite CBC indices such as NLR, MCLR, MPLR, and SII may offer additional diagnostic information at low cost and from routinely available data in general clinical practice [14]. This is consistent with growing evidence from human and veterinary medicine that hypercortisolism reshapes circulating leukocyte populations, with coexisting systemic inflammation and suppressed cellular immunity [1,15].

Dogs with HAC showed a significant increase in neutrophils (P=0.035) and a decrease in lymphocytes (P=0.018). This well-known pattern results from direct glucocorticoid effects on white blood cells, largely mediated through the glucocorticoid receptor (GR). In neutrophils,

Table 2. *Optimal cut-off values of hematological indices and serum biomarkers for the diagnosis of canine HAC, including sensitivity, specificity, area under the curve (AUC), standard error (SE), and 95% CI*

Parameter	Cut-off	Sensitivity (%)	Specificity (%)	AUC	SE	95% CI
NLR	≥3.14[a]	100	70	0.88	0.08	0.72-1.00
MCLR	≥38.64[b]	80	70	0.81	0.09	0.62-1.00
PCLR	≥0.147[b]	70	70	0.76	0.11	0.55-0.97
MPLR	≥3.412[b]	100	60	0.83	0.10	0.63-1.00
SII	≥1010.44[b]	90	60	0.78	0.10	0.57-0.98
ALB	<3.15[b]	70	70	0.76	0.10	0.55-0.97
ALP	≥140[a]	100	70	0.91	0.06	0.78-1.00

NLR, neutrophil-to-lymphocyte ratio; MCLR, mean corpuscular volume-to-lymphocyte count ratio; PCLR, plateletcrit-to-lymphocyte count ratio; MPLR, mean platelet volume-to-lymphocyte count ratio; SII, systemic immune-inflammation index; ALB, albumin; ALP, alkaline phosphatase. [a]*P<0.01;* [b]*P<0.05*

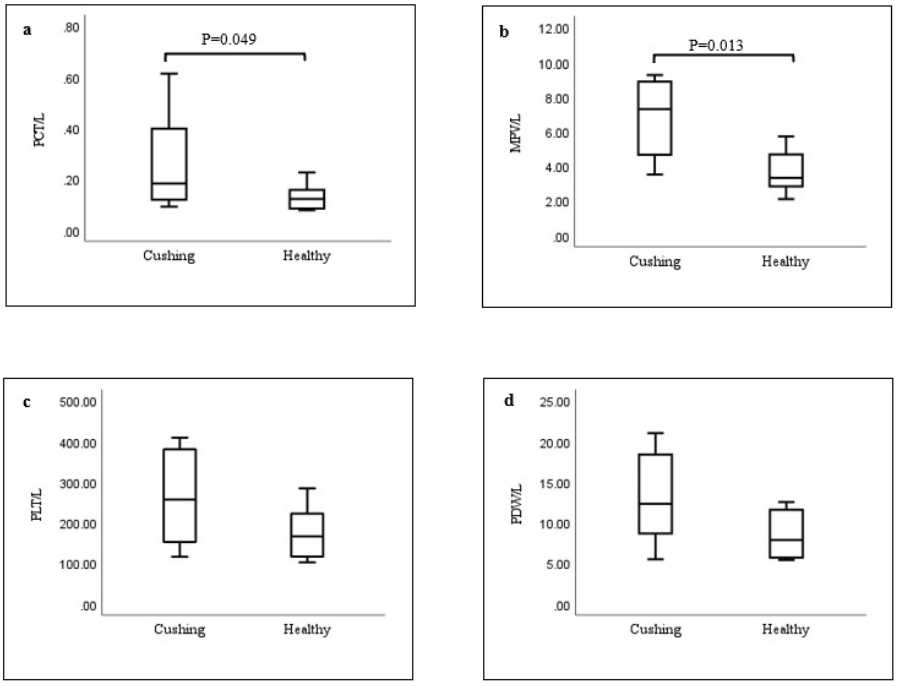


Fig 3. Comparison of platelet-leukocyte composite indices between dogs with naturally occurring HAC and healthy controls (n=10 per group). **(A)** Plateletcrit-to-lymphocyte count ratio, **(B)** Mean platelet volume-to-lymphocyte count ratio, **(C)** Platelet-to-lymphocyte ratio, **(D)** Platelet distribution width-to-lymphocyte count ratio. Boxes show the median and interquartile range, and whiskers show the range of the data. HAC, hyperadrenocorticism

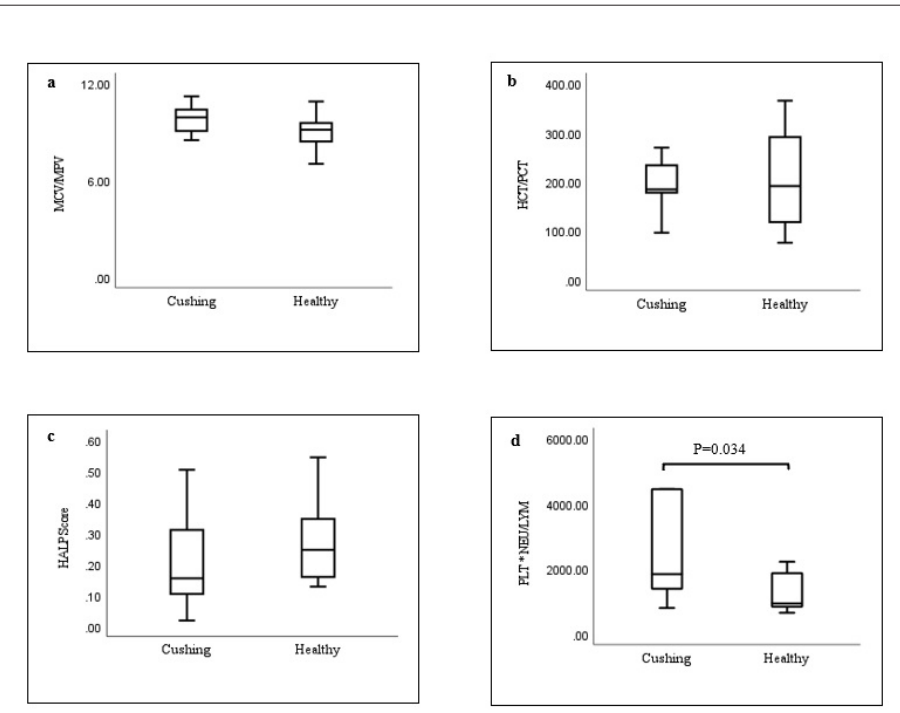


Fig 4. Comparison of erythrocyte-platelet composite indices, HALP score, and systemic immune-inflammation index between dogs with naturally occurring HAC and healthy controls (n=10 per group). **(A)** Mean corpuscular volume-to-mean platelet volume ratio, **(B)** Hematocrit-to-plateletcrit ratio, **(C)** HALP score, **(D)** Systemic immune-inflammation index. Boxes show the median and interquartile range, and whiskers show the range of the data. HAC, hyperadrenocorticism

glucocorticoids promote maturation and release from the bone marrow and induce L-selectin shedding (partly via annexin A1), which favors detachment from the endothelium (demargination) [16]. Glucocorticoids also prolong neutrophil survival by suppressing spontaneous (constitutive) apoptosis through GR-dependent up-regulation of the anti-apoptotic protein Mcl-1 (involving p38 MAPK) and IAP proteins, and down-regulation of the pro-apoptotic Fas receptor. However, this regulation depends on cellular context and microenvironment [16]. In lymphocytes, by contrast, glucocorticoids induce apoptosis. This process is initiated by, and strictly dependent on, GR binding, and glucocorticoids can engage both the intrinsic (mitochondrial, Bcl-2 family-controlled) and the extrinsic (death-receptor) apoptotic pathways; regulation of Bcl-2 family members, notably induction of pro-apoptotic BH3-only proteins such as Bim and Puma, is considered central. Depending on the availability and responsiveness of the apoptotic machinery, cell death may show either apoptotic or necrotic morphology [17]. Together with lymphocyte redistribution to peripheral lymphoid tissue, these mechanisms result in lymphopenia [18,19]. Among the leukocyte ratios, NLR had the highest discriminatory ability (AUC=0.88; sensitivity 100%, specificity 70%; cut-off 3.14). These findings agree with recent observations that NLR is markedly higher in hypercortisolic dogs than in healthy dogs and dogs with non-adrenal disease [4], suggesting that NLR may be an affordable, practical screening tool pending independent validation. A similar pattern has been reported in humans, where WBC-derived inflammatory indices, mainly NLR, have been useful for identifying patients with suspected Cushing's syndrome and assessing inflammation in Cushing's disease [18,19]. In addition to single-lineage ratios, several composite indices combining multiple cell lineages were analyzed. The erythrocyte-leukocyte index MCLR differed significantly between the groups (AUC=0.81; sensitivity 80%; specificity 70%; cut-off 38.64). Glucocorticoids modulate the proliferation and differentiation of erythroid progenitors, which could alter red cell indices during chronic glucocorticoid excess [20]. Because MCLR is the ratio of mean corpuscular volume to lymphocyte count, its higher values in HAC dogs may largely reflect the lymphopenia observed here. This study did not test whether changes in erythrocyte size contribute independently, or whether MCLR adds information beyond lymphocyte count and NLR; this remains to be determined.

Some findings may support a thrombo-inflammatory component in canine HAC, although this remains a hypothesis. Procoagulant changes and cortisol-induced disturbances in platelet function are thought to jointly increase the risk of hypercoagulability and thromboembolic disease in dogs with HAC [2,3]. This study did not assess coagulation, thromboelastography, or platelet function; thus, the analysis relies on composite indices and prior studies rather than direct findings from this investigation. In dogs with HAC, both PCLR and MPLR increased, with MPLR showing 100% sensitivity (AUC=0.83, specificity 60%, cut-off 3.412). Because both indices use lymphocyte count in the denominator, the observed increases may reflect lymphopenia rather than changes in platelet levels; platelet counts did not differ significantly between groups, mean platelet volume was not reported as a distinct measure, and platelet function assessments were not conducted. Although research in specific age groups suggests plasma cortisol is negatively associated with platelet activity [21], and chronic hypercortisolemia has also been proposed to enhance platelet activation and vascular endothelial inflammation, the relationship between cortisol and platelet activity remains unsettled and was not directly addressed in this study.

The higher SII in dogs with HAC (AUC=0.78, sensitivity 90%) may reflect the combined contribution of neutrophilia and lymphopenia, with platelet count as an additional component; any pro-thrombotic implication is speculative because platelet count did not differ between groups and platelet function was not assessed. Oxidative stress may also play a role, as it has been reported as a component of canine HAC [22], but because we did not measure it, any effect would be inferred rather than directly determined. The spread observed in the box plots (NLR, MPLR, MCLR, and SII) may reflect individual variation; however, the study provides insufficient subtype and stage information (adrenal- versus pituitary-dependent) to justify further speculation on this point.

Regarding the biochemical markers investigated, ALP showed the best combined specificity and sensitivity (AUC=0.91; sensitivity 100%, specificity 70%; cut-off 140 IU/L), which may reflect increased activity of the steroid-induced ALP isoenzyme [23]. ALP is not specific to HAC and can be elevated in various hepatobiliary, neoplastic, or drug-induced conditions; elevated ALP alone should not diagnose HAC and should be interpreted alongside clinical signs and dedicated endocrine testing. HALP combines hemoglobin, albumin, lymphocyte count, and platelet count. Its lower median in HAC dogs, despite higher albumin, may be driven mainly by lymphopenia. Because the distributions overlapped considerably, these data do not support a discriminatory value for HALP. Platelet count and hemoglobin concentration did not differ significantly between groups on their own. Composite indices including them did differ, but these differences may be driven mainly by neutrophil and lymphocyte changes; we did not test whether multi-lineage indices

add information beyond NLR or lymphocyte count, and this needs confirmation in larger samples. The thresholds reported here were estimated from only 20 dogs (using the Youden index; e.g., MPLR 3.412 and NLR 3.14) and derived and evaluated in the same sample, which tends to overestimate diagnostic performance. They are therefore preliminary and exploratory and should be interpreted with considerable caution until validated in a larger, independent cohort. For example, a sensitivity of 100% (10/10 dogs) has an exact 95% CI of approximately 69-100%, and a specificity of 70% (7/10 dogs) has an exact 95% CI of approximately 35-93%, illustrating how imprecise these estimates are.

There are some limitations to the present study that must be acknowledged. The small sample (10 dogs per group) and the absence of an a priori power calculation are the main limitations of this study. With 10 dogs per group, the study had 80% power (two-sided α=0.05) to detect only large effects (Cohen's d of approximately 1.3 or greater), and power for the smaller effects observed for platelet count, hemoglobin, and WBC (d=0.33-0.48) was below 20%. Type II errors are therefore likely, and non-significant results should not be interpreted as evidence of no difference. Effect-size estimates from small samples also tend to be inflated, and the wide 95% CIs of the AUCs (e.g., 0.72-1.00 for NLR) show that diagnostic performance was estimated imprecisely. Cut-off values, sensitivities, and specificities derived from the same small cohort are likely to be unstable and optimistic, and the findings should be regarded as preliminary and hypothesis-generating until confirmed in larger, independent, multi-center cohorts. Classification into pituitary- or adrenal-dependent types was not carried out, so generalization is difficult; dogs were paired by breed, sex, and body weight, but age differences (mean age 6-15 years) could confound the results, particularly with regard to platelet parameters. Exclusion of other diseases was based on clinical findings alone, without further testing, so subclinical illness may have been missed. No correction was made for multiple comparisons; thus, the reported P-values are exploratory. This study did not investigate platelet function, oxidative stress, or coagulation directly, and relied on the available literature to interpret these aspects. All dogs were recruited from a single clinic in Iran, so these cut-off values may not be directly applicable to other laboratory reference intervals, and the generalizability of the findings to other populations remains to be established. Further multi-center research with defined methodologies is essential to confirm these findings.

In conclusion, naturally occurring HAC in dogs showed considerable differences in specific composite hematological parameters. This research has identified NLR, MPLR, MCLR, and SII as potential additional diagnostic markers for HAC in dogs; however, these results are preliminary. Because of the limited sample size, cut-off values and estimates of diagnostic performance must be confirmed in larger, independent, etiologically diverse, multi-center studies before any clinical application.

Declarations

Availability of Data and Materials: The data supporting the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly available because they contain identifiable client-owned animal records.

Acknowledgements: The authors thank the staff of the Clinical Pathology Laboratory and the participating veterinary clinics for their assistance, and are grateful to the dog owners for their cooperation.

Funding Support: The authors received no financial support for the research, authorship, and/or publication of this article.

Competing Interests: The authors declare that they have no competing interests.

Declaration on Generative Artificial Intelligence (AI): Generative AI (Claude, Anthropic) was used solely to improve the readability and language of the manuscript. It was not used to create or modify tables or figures, or to generate or analyze data. The authors reviewed and edited all content and take full responsibility for the content of the publication.

Ethical Compliance Declaration: The authors declare that the study was conducted in accordance with all applicable animal welfare and research ethics principles, institutional and national/international regulations, and the requirements of the relevant ethics committees and funding/supporting institutions, where applicable. The authors accept full responsibility for the ethical integrity of the study.

Author Contributions: S.Z.E.J. and M.A.: conceptualization, methodology, formal analysis, writing - original draft. H.A. and M.B.: investigation, data curation. S.Z.E.J. and M.A.: writing - review and editing. All authors read and approved the final version of the manuscript.

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