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

Kafkas Univ Vet Fak Derg
29 (5): 513-520, 2023
DOI: 10.9775/kvfd.2023.29599

RESEARCH ARTICLE

Evaluation of Some Systemic Inflammatory Biomarkers in Canine Malignant Mammary Tumors [1]

Zeynep Merve EKICI [1] Musa Ozgur OZYIGIT [2] Deniz NAK [1 (*)] Zehra AVCI KUPELI [2] Fikriye Ecem KURUOGLU [1] Davut KOCA [3] Talha AVCILAR [1] Ogulcan GUMUS [1] Yavuz NAK [1]

[1] *Presented in part at the International Veterinary Sciences Congress, May 12-14, 2022 in Istanbul, Türkiye*

[1] Bursa Uludag University, Veterinary Faculty, Department of Obstetrics and Gynecology, TR-16059 Gorukle, Bursa - TÜRKİYE
[2] Bursa Uludag University, Veterinary Faculty, Department of Pathology, TR-16059 Gorukle, Bursa - TÜRKİYE
[3] Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Van Yüzüncü Yıl University, Van - TÜRKİYE

(*) **Corresponding author:** Deniz NAK

Phone: +90 224 294 0823
Cellular phone: +90 532 510 0035
Fax: +90 224 294 1202

E-mail: deniznak@gmail.com

How to cite this article?

Ekici ZM, Ozyigit MO, Nak D, Avci Kupeli Z, Kuruoglu FE, Koca D, Avcilar T, Gumus O, Nak Y: Evaluation of some systemic inflammatory biomarkers in canine malignant mammary tumors. *Kafkas Univ Vet Fak Derg,* 29 (5): 513-520, 2023.
DOI: 10.9775/kvfd.2023.29599

Article ID: KVFD-2023-29599
Received: 10.04.2023
Accepted: 28.08.2023
Published Online: 05.09.2023

ABSTRACT

The aim of this study is to investigate whether neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammation index (SII), albumin-to-globulin ratio (AGR), and prognostic nutritional index (PNI) parameters could be used as biomarkers for canine malignant mammary tumors (MMTs), and the changes in these parameters according to different tumor (T), lymph node (N), and metastasis (M) stages (TNM I-II-III, TNM IV, TNM V) and the number of affected mammary glands (single, multiple). Thirty-seven with MMT and 20 healthy dogs were used in this study. Complete blood count and biochemistry analysis were performed in all dogs. Tumor material is removed by tru-cut and sent to the pathology laboratory for diagnosis. NLR, PLR, and SII values increased, and LMR and PNI values decreased in dogs with MMT. Median NLR values increased and median LMR and PNI values decreased as the TNM stage progressed. In dogs with a single MMT, median NLR, and PLR values were found to be lower than in dogs with multiple MMTs, and median LMR, SII, AGR, and PNI values were higher. The present results indicated that NLR, LMR, PLR, SII, and PNI parameters could be used as biomarkers for canine MMT. Also, NLR, LMR, PLR, SII, PNI, and AGR parameters may be valuable biomarkers that reveal the degree of systemic immune response according to different TNM stages and the number of affected mammary glands.

Keywords: Canine, Malignant mammary tumor, Inflammation, Biomarkers, Oncology

INTRODUCTION

Cancer begins when the cell becomes unable to respond to the mechanisms that control its division due to a number of structural defects in the cell [1]. Initially, cells that have undergone neoplastic transformation are detected by the host immune system and eliminated by various mechanisms. However, this effect of the immune system against tumors can be weak and insufficient, and even some components contribute to tumor development at the same time [2,3]. In fact, the tumor-associated immune response is more likely to contribute to tumor growth, progression, and immunosuppression than it is to form an effective host antitumor response [4]. Revealing the links between cancer and inflammation has implications for the prevention and treatment of cancer [3,5]. It has been stated that cancer-related inflammation is associated with changes in circulating white blood cells and some biochemical parameters both in humans and dogs [6-8]. Lymphocytes have the most important role in the immune response against cancer [9,10]. Neutrophils and monocytes also have critical roles in antitumor immunity, they exhibit their antitumor functions directly or work with lymphocytes. However, when they gain a cancer-supportive structure, they start to exhibit quite important pro-tumor functions and contribute to tumor progression, promote metastasis, and show immunosuppressive activity [11,12]. Also, neutrophils can prevent the anticancer functions of lymphocytes [13]. Platelets become active by interacting with cancer cells and show many functions that participate

in cancer progression, metastasis, and inflammation [14]. Therefore, parameters such as neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII) are calculated in many studies conducted to date. High NLR, PLR and SII values and a low LMR value are associated with advanced disease and poor prognosis. Moreover, they have been shown to be useful for the selection of the appropriate treatment method and the management of the disease in several neoplastic conditions both in humans [5,6,15-20] and dogs [21-23]. The albumin-to-globulin ratio (AGR) parameter is a useful biomarker for revealing systemic inflammation associated with malignancies and has prognostic value in various types of cancer including breast cancer in humans [24-26]. The prognostic nutritional index (PNI) is a new systemic immune-nutrition index and represents the immune and nutritional status of the host [27].

It has been demonstrated in many studies that the parameters we mentioned above can be used as biomarkers in the diagnosis, management of the disease, and prediction outcome in many cancer types, including a large population of breast cancer [15,19,24,27]. Only NLR has been reported to have a prognostic value in dogs with mammary tumors [28]. Therefore, this study aimed to investigate, firstly, whether the parameters indicating the systemic inflammatory response associated with cancer could be used as biomarkers for canine MMT, and secondly, the changes of these parameters according to different TNM stages and the number of affected mammary glands.

Material and Methods

Ethical Statement

The study was approved by the Ethics Committee on Animal Research of Bursa Uludag University (Approval No: 2020-03/03).

Animals

The first group of dogs in this study included 37 dogs with malignant mammary tumor (MMT) aged 3 to 16 years. The breeds of the dogs included Terrier (7), Golden Retriever (5), Crossbreed (5), Cocker (4), Doberman (2), Siberian Husky (2), Doberman Pinscher (1), Jack Russel Terrier (1), Dogo Argentino (1), Rottweiler (1), German Shepherd (1), Chihuahua (1), Pekingese (1), Beagle (1), Cane Corso (1), American Staffordshire Terrier (1), Kurzhaar (1) and Alabai (1). The second group of dogs in this study included 20 healthy dogs aged 2 to 10 years from different breeds (Crossbreed (11), Alabai (3), Golden Retriever (2), Labrador Retriever (1), Dogo Argentino (1), Border Collie (1) and Chow Chow (1)) as controls. None of the dogs had concurrent systemic inflammatory or immune-related diseases. Two of the dogs with MMT had cancer-related cachexia. The diet of the dogs in the study was not uniform. Characteristics of dogs with MMT and the control group are summarized in *Table 1*.

Table 1. *Characteristics of the dogs with malignant mammary tumors (MMTs) and the control group*

Characteristics	n		Percentage (%)	
	Dogs with MMTs	Control	Dogs with MMTs	Control
Age				
≤8	17/37	17/20	45.95	85
Neutering Status				
Neutered	6/37	6/20	16.22	30
Intact	30/37	14/20	81.08	70
Remnant Ovary	1/37	0	2.70	0
Pseudopregnancy History	6/37	2/20	16.22	10
Contraception History	4/37	0	10.81	0
TNM Stage				
I-II-III	14/37		37.84	
IV	15/37		40.54	
V	8/37		21.62	
Number of Tumors				
Single	10/37		27.03	
Multiple	27/37		72.97	
Histopathological Evaluation				
Scirrhous Adenocarcinoma	9/37		24.32	
Solid Adenocarcinoma	6/37		16.22	
Tubular Adenocarcinoma	5/37		13.51	
Invasive Ductal Carcinoma	3/37		8.11	
Adenocarcinoma	3/37		8.11	
Papillary Adenocarcinoma	2/37		5.41	
Malignant Mixed Tumor	2/37		5.41	
Tubulopapillary Carcinoma	2/37		5.41	
Tubular Carcinoma	2/37		5.41	
Spindle Cell Carcinoma	1/37		2.70	
Invasive Cribriform Carcinoma	1/37		2.70	
Carcinoma	1/37		2.70	

All dogs had a general examination, complete blood count, and biochemistry analysis, in addition to that thoracic radiography and intra-abdominal ultrasonography were done in dogs with mammary tumors. Ultrasound-guided biopsy samples were obtained from mammary tumors with a 14-gauge tru-cut biopsy needle. Fine-needle biopsy was performed from local lymph nodes with a 21-gauge needle. Lidocaine Hydrochloride (Jetocaine, ADEKA, Samsun, Turkey) was used at 4 mg/kg for local anesthesia before biopsies. All biopsies were sent to the pathology laboratory. 37 dogs that were found to have MMT according to the pathological examination were included in the study. All dogs with MMT were evaluated

according to the modified TNM system [TNM I-II-III ($T_{1-2-3}N_0M_0$), TNM IV ($T_{1-2-3}N_1M_0$), TNM V ($T_{1-2-3}N_{0\_1}M_1$)] as described by Goldschmidt et al.[29]. Those with tumors in one mammary gland were grouped as "single" and those with tumors in more than one mammary gland were grouped as "multiple".

Biochemical, Hematology Analyses and Biomarkers

Blood was taken from the vena cephalica antebrachii into EDTA tubes for complete blood count and into dry tubes for measurement of biochemical values. The blood samples centrifuged at 4000 rpm for 10 min and their serums were separated on the day of the examination. A complete blood count was performed with the "Hasvet VH5R, Automated Hematology Analyzer" (Urit, China) device. Total Protein (TP), Albumin (ALB), Alkaline Phosphatase (ALP), Glucose (GLU), Total Bilirubin (TBIL), Inorganic Phosphorus (IP), Total Cholesterol (TCHO), Gamma-Glutamyl Transferase (GGT), Alanine Aminotransferase (ALT), Calcium (Ca), Creatinine (CRE), Blood Urea Nitrogen (BUN), Globulin (GLOB), values were determined by "FUJI DRI-CHEM NX500V IC Chemistry Analyzer" (FUJIFILM, Japan) device.

The NLR was determined by dividing the neutrophil (10^9/L) by lymphocyte counts (10^9/L) [21]. The LMR was calculated by dividing the lymphocyte (10^9/L) by monocyte counts (10^9/L) [21]. The PLR was detected by dividing the platelet (10^9/L) by lymphocyte counts (10^9/L) [21]. The SII was calculated by multiplying the neutrophil count (10^9/L) by the platelet count (10^9/L) and dividing by the lymphocyte count (10^9/L) (N × P/L) [21]. The AGR was estimated by dividing the albumin (g/dL) by globulin (g/dL) [24]. The PNI parameter was obtained by summing 10 times serum albumin (g/dL) and 0.005 times lymphocyte count (per mm^3) (10 × ALB + 0.005 × L) [27].

Histopathological Evaluation

Biopsy samples of mammary tumors were fixed in 10% buffered formalin and embedded in paraffin. Sections of 4 µm thickness were taken from the tissues passed through alcohol and xylol and stained with hematoxylin&eosin. Tubule and mammary alveolar formation, nuclear polymorphism, pleomorphism, mitosis index, inflammatory infiltration, necrosis, adjacent tissue invasion, and lymph node metastasis were evaluated according to the World Health Organization criteria for canine mammary tumors [30].

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 28 for Windows. Shapiro-Wilk test was used to examine whether the data were normally distributed. Nonparametric tests were used for data that did not show normal distribution. The comparison of the values of the dogs with MMT and the control group was done by using the Mann-Whitney U-test, which is one of the nonparametric tests. The Kruskal-Wallis test was used for the comparison according to the TNM stage and the number of affected mammary glands. Significance values had been adjusted by the Bonferroni correction for multiple tests. Statistical significance was set at P value <0.005.

Results

The mean age of dogs with MMT and the control group was 9.59±3.02 and 6.20±2.31 years, respectively (P<0.001). 86.48% of dogs with MMT and 45% of the control group were pure-breed dogs. The most common breeds of dogs with MMT included Terriers 18.92% (7/37), Golden Retrievers 13.51% (5/37), Crossbreeds 13.51% (5/37), and Cockers 10.81% (4/37). Characteristics of dogs with MMT are summarized in *Table 1*. Hematological and biochemical data and reference intervals of dogs with MMT and control group are given in *Table 2* and *Table 3*. Radiographic images of two dogs with lung metastases and histopathological image samples of dogs with MMTs are given in *Fig. 1* and *Fig. 2*. Differences between the dogs with MMT and the control group were found statistically significant for NLR, LMR, PLR, SII and PNI parameters (P<0.05). The AGR value did not show any differences between the groups (P=0.496). The data are summarized in *Table 4*.

In the comparison made according to TNM staging, the

Table 2. *Hematological parameters of the dogs and the referance ranges*

Variable	Dogs With CMT		Control		Referance
	Median(min:max)	Mean±SEM	Median(min:max)	Mean±SEM	
NEU10^9/L	8.19 (3.47:36.1)	9.36 ± 0.90	6.05 (3.74:9.66)	6.13±0.37	2.7-9.4
MON 10^9/L	0.61 (0.12:4.02)	0.75 ± 0.11	0.32 (0.09:0.64)	0.34±0.03	0.1-1.3
LYM 10^9/L	1.55 (0.78:4.47)	1.79 ± 0.15	2.48 (1.15:4.91)	2.53±0.19	0.9-4.7
PLT 10^9/L	349 (147:693)	377.78 ± 24.51	263 (172:384)	265.95±12.35	186-545

Data expressed as median (min:max) and mean ± SEM. NEU=neutrophils, MON=monocytes, LYM=lymphocytes, PLT=platelets.

Table 3. *Biochemical parameters of the dogs and the referance ranges*

Variable	Dogs With MMTs		Control		Referance Values
	Median(min:max)	Mean±SEM	Median(min:max)	Mean±SEM	
TP g/dL	6.8 (5.6:8.2)	6.71±0.10	6.5 (5.9:7.1)	6.48±0.08	5.5-7.2
ALB g/dL	3.4 (2.3:4.5)	3.39±0.08	3.4 (3:4)	3.39±0.06	3.2-4.1
GLOB g/dL	3.1 (2.5:5.4)	3.29±0.11	3.1 (2.6:3.7)	3.09±0.06	1.9-3.7
ALP U/L	56 (14:287)	80.31±11.35	38 (19:86)	43.63±4.93	7-115
GLU mg/dL	104 (64:134)	104.2±2.38	107.5 (70:123)	101.95±3.50	68-104
TBIL mg/dL	0.3 (0.2:0.6)	0.28±0.02	0,2 (0.2:0.4)	0.24±0.02	0-0.2
IP mg/dL	3.6 (1.5:6.5)	3.61±0.17	3.5 (2.9:4.6)	3.65±0.10	2.7-5.4
TCHO mg/dL	287 (169:437)	302.18±15.49	165 (108:275)	175±12.59	136-392
GGT U/L	<10	<10±0	<10	<10±0	0-8
ALT U/L	41.5 (10:149)	45.16±3.94	36.5 (20:75)	41.25±3.25	17-95
CA mg/dL	10.6 (9.3:12.1)	10.65±0.11	11.1 (10.2:11.9)	11.15±0.11	9.4-11.1
CRE mg/dL	0.75 (0.41:1.6)	0.81±0.05	0.69 (0.43:0.97)	0.66±0.031	0.6-1.4
BUN mg/dL	14.05 (5:29)	14.62±1.09	13 (5:19.9)	12.43±1.01	9-26

Data expressed as median (min:max) and mean ± SEM. ALB=albumin, GLOB=globulin, APL=alkaline phosphatase, GLU=glucose, TBIL=total bilirubin, IP=inorganic phosphorus, TCHO=total cholesterol, GGT=gamma-glutamyl transferase, ALT=alanine aminotransferase, CA=calcium, CRE=creatinine, BUN=blood urea nitrogen

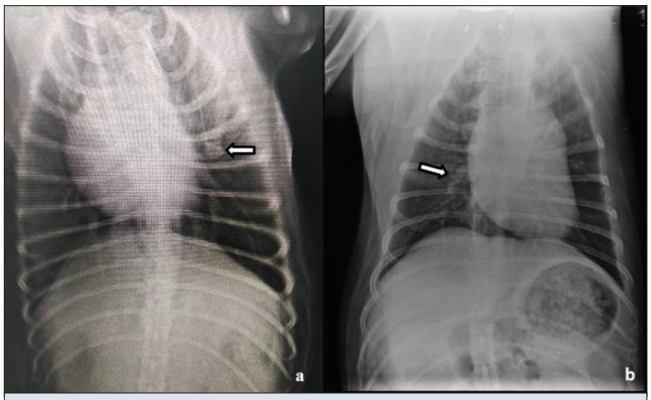

Fig 1. Ventrodorsal radiographic views of the thorax in a 5 years old Terrier (a) and in an 8 years old Cane Corso (b) with MMTs

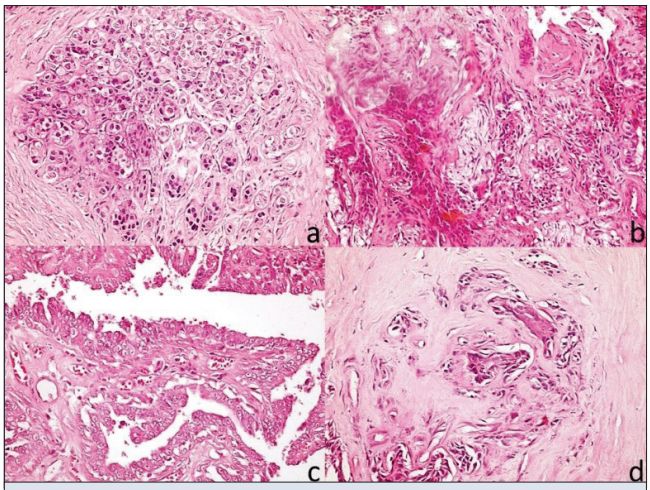

Fig 2. a- Solid Adenocarcinoma. Neoplastic cells exhibit a pronounced pleomorphism and fill the alveolar lümen, **b**- Malignant Mixed Tumor. The neoplasm contains multiple clusters of carcinoma cells as well as regions characterized by the proliferation of myoepithelial cells, **c**- Tubulopapillarycarcinoma. Neoplastic cells have a vesicular appearance and are binucleated. Tubular epithelia have formed multiple layers, **d**- Scirrhous Adenocarcinoma. Excessive increase in fibrous stroma led to deterioration of lobular structure. Alveoli and ducts are not visible

NLR value did not show statistical significance between the control group and TNM I-II-III stage (P=0.069), while the differences between each of TNM IV and TNM V and the control group were significant (P<0.001, P=0.012, respectively). The LMR values were found to be statistically significantly lower in each of the TNM I-II-III, TNM IV, and TNM V stages compared to the control group (P=0.032, P<0.001, P=0.006, respectively). The PLR values were determined significantly higher in TNM I-II-III and TNM IV stages versus the control group (P=0.03, P=0.001, respectively). However, the difference between the PLR values of the TNM V group and the control group was not significant (P=0.076). The SII values were significantly higher in TNM I-II-III, TNM IV, and TNM V stages versus the control group (P=0.018, P<0.001, P=0.038, respectively). The overall test did not show significant differences between the groups for the AGR parameter (P=0.344). The PNI values did not show statistically significant differences between TNM I-II-III and TNM V stages versus the control group (P>0.05), but the difference in the PNI value between TNM IV and control group was significant (P=0.039). There was no significant difference in the biomarkers assessed among TNM I-II-III, TNM IV, and TNM V stages *(Table 5)*.

The NLR, LMR, PLR, and SII values were found to be

Table 4. *Comparison of dogs with malignant mammary tumors (MMT) and control group*

Parameters	Dogs with MMTs		Control		P Value
	Median (min : max)	Mean ± SEM	Median (min : max)	Mean ± SEM	
NLR	5.01 (1.56:29.35)	6.34±0.80	2.77 (1.12:4.30)	2.63±0.20	<0.001
LMR	2.68 (0.31:12.83)	3.89±0.56	6.99 (3.82:27.56)	9.26±1.44	<0.001
PLR	242.11 (47.09:689.66)	262.79±25.92	107.28 (48.73:236.36)	117.80±10.66	<0.001
SII	1791.98 (383.58:12855.12)	2574.62±420.64	724.18 (199.87:1342.55)	713.05±70.23	<0.001
AGR	1.07 (0.52:1.73)	1.08±0.05	1.06 (0.92:1.54)	1.11±0.03	0.496
PNI	41.93 (31.9:56.65)	43.15±6.59	45.38 (39.75:58.55)	46.47±1.08	0.022

Data expressed as median (min:max) and mean ± SEM. NLR=neutrophil-to-lymphocyte ratio, LMR=lymphocyte-to-monocyte ratio, PLR=platelet-to-lymphocyte ratio, SII=systemic immune-inflammation index, AGR=albumin-to-globulin ratio, PNI=prognostic nutritional index.

Table 5. *Comparisons according to TNM stages of the dogs with malignant mammary tumors.*

Parameters	TNM I-II-III		TNM IV		TNM V		Control		P Value
	Median (min : max)	Mean ± SEM	Median (min : max)	Mean ± SEM	Median (min : max)	Mean ± SEM	Median (min : max)	Mean ± SEM	
NLR	4.66^{ab} (1.86:14.55)	4.84 ±3.21	5.83^{a} (3.02:29.35)	7.97±1.70	6.51^{a} (1.56:8.62)	5.9±0.92	2.77^{b} (1.12:4.30)	2.63±0.20	<0.001
LMR	3.07^{a} (0.81:12.83)	5.00±1.08	2.54^{a} (0.31:10.59)	2.99±0.60	2.27^{a} (1.07:12.25)	3.63±1.34	6.99^{b} (3.82:27.56)	9.26±1.44	<0.001
PLR	200.37^{a} (47.76:689.66)	252.21±48.37	255.45^{a} (47.09:617.50)	286.08±40.52	254.74^{ab} (55.03:431.03)	237.64±44.23	107.28^{b} (48.73:236.36)	117.80±10.66	0.001
SII	1478.84^{a} (525:8731.03)	2159.07±601.15	2165.96^{a} (538.24:12855.12)	3272.57±840.97	2082.66^{a} (383.58:3723.86)	1993.18±399.91	724.18^{b} (199.87:1342.55)	713.05±70.23	<0.001
AGR	1.19 (0.76:1.73)	1.74±0.08	0.94 (0.52:1.54)	1.02±0.10	0.94 (0.84:1.25)	1.01±0.07	1.06 (0.92:1.54)	1.11±0.03	0.344
PNI	43.88^{ac} (37:56.65)	45.56±1.61	41.25^{bc} (31.9:54.90)	41.10±1.87	40.80^{ac} (33.95:54.35)	42.10±2.54	45.38^{a} (39.75 8.55)	46.47±1.08	0.029

$^{a-c}$ *Different superscripts indicate values that within the row are significantly different. Data expressed as median (min:max) and mean ± SEM. NLR=neutrophil-to-lymphocyte ratio, LMR=lymphocyte-to-monocyte ratio, PLR=platelet-to-lymphocyte ratio, SII=systemic immune-inflammation index, AGR=albumin-to-globulin ratio, PNI=prognostic nutritional index*

Table 6. *Comparisons according to the number of affected mammary glands.*

Parameters	Single		Multiple		Control		P Value
	Median (min : max)	Mean ± SEM	Median (min : max)	Mean ± SEM	Median (min : max)	Mean ± SEM	
NLR	4.73^{a} (2.17:8.62)	4.88±0.72	5.34^{a} (1.56:29.35)	6.87±1.06	2.77^{b} (1.12:4.30)	2.63±0.20	<0.001
LMR	3.23^{a} (1.07:11.00)	4.70±1.19	2.68^{a} (0.31:12.83)	3.59±0.63	6.99^{b} (3.82:27.56)	9.26±1.44	<0.001
PLR	232.58^{a} (97.40:382.58)	240.23±32.57	242.11^{a} (47.09:689.66)	271.15±33.60	107.28^{b} (48.73:236.36)	117.80±10.66	<0.001
SII	1889.92^{a} (525.00:3723.86)	1898.28±314.00	1734.58^{a} (383.58:12855.12)	2825.12±560.49	724.18^{b} (199.87:1342.55)	713.05±70.23	<0.001
AGR	1.12 (0.52:1.62)	1.09±0.12	1.03 (0.61:1.73)	1.08±0.06	1.06 (0.92:1.54)	1.11±0.03	0.781
PNI	43.10 (33.95:56.30)	43.66±2.26	41.70 (31.90:56.65)	42.96±1.33	45.38 (39.75:58.55)	46.47±1.08	0.066

Different superscripts indicate values that within the row are significantly different. Data expressed as median (min:max) and mean ± SEM. NLR=neutrophil-to-lymphocyte ratio, LMR=lymphocyte-to-monocyte ratio, PLR=platelet-to-lymphocyte ratio, SII=systemic immune-inflammation index, AGR=albumin-to-globulin ratio, PNI=prognostic nutritional index

significantly different between dogs with a single MMT and control group (P=0.037, P=0.024, P=0.009, and P=0.002, respectively). Similarly to that, the same values of the dogs with multiple MMTs and the control group were found significantly different (P<0.001, P<0.001, P=0.001, and P<0.001, respectively). In dogs with a single MMT, the median NLR and PLR values were lower and the median LMR and SII values were higher than in dogs with multiple MMTs, but no statistical significance was found (P>0.05). The overall test did not show significant differences between the groups for the AGR and PNI parameters (P=0.781, P=0.066, respectively) *(Table 6)*.

Discussion

There are strong similarities between human breast cancer (HBC) and canine mammary tumors [31-33]. Also, neoadjuvant chemotherapy can be used in the treatment of canine mammary tumors similar to that in humans [34]. As in HBC [6,11,35], it has been noted that there are some remarkable changes in blood values in the presence of mammary tumors in dogs [7,8]. In veterinary medicine, it has been stated that NLR, LMR, PLR, and AGR, parameters may be potential biomarkers in certain malignancies of dogs [21-23,28]. But until recently, no literature data were evaluating LMR, PLR, SII and PNI parameters in dogs with MMTs. Uribe-Querol et al.[28] reported that a high NLR value (NLR<5) before treatment was associated with a lower survival rate in dogs with mammary tumors. They also stated that NLR could be used as a prognostic marker for disease severity, but AGR value did not show any predictive value on tumor malignancy. In this study, we found that in dogs with MMT, NLR, PLR, and SII values were high, and LMR and PNI values were low as in human breast cancer [15,18,27]. However, in our dogs with MMT, there was no significant difference compared to our control group in the AGR parameter [24-26]. In the study by Lallo et al.[36], it was stated that AGR values were lower in malignant MMTs. Unlike this, the AGR value did not differ in dogs with MMT when compared with healthy ones in the study by Uribe-Querol et al.[28] and in this study. We recommend evaluating the AGR parameter in larger populations.

Median NLR, PLR, LMR, and SII values show differences among TNM stages in HBC. It has been said that NLR, PLR, LMR, and SII parameters can be valuable and guide in the staging of HBC [18]. Low AGR has been found to be associated with advanced-stage of HBC and low PNI parameter has been found to be associated with advanced disease [24,27]. High NLR values have been associated with advanced or aggressive HBC [5,15,20]. It has been shown that NLR values increase as the disease progresses in TNM stages of HBC [17,18]. In our study, although not statistically significant, the median NLR values increased as TNM stages progressed (4.66, 5.83, 6.51, respectively). Low LMR is correlated with advanced disease and TNM stages in HBC [18,20]. Compatible with this, median LMR values decreased as TNM stages progressed (3.07, 2.54, 2.27, respectively) in our study, but this decrease was not statistically significant. It was shown that the PLR parameter was correlated with advanced disease and TNM stages [18,20], and another study reported that it was associated with lymph node metastasis but not with advanced T stages [16]. Unlike them, Elyasinia et al.[17] reported that there was no relationship between PLR values and different TNM stages of HBC. Similarly, PLR values did not show a significant difference among TNM stages in the dogs with MMT. The SII parameter has been found to be associated with the advanced TNM stage in HBC [18,19]. The median SII values in TNM IV and V stages were found to be higher than the median SII value in the TNM I-II-III stages in our study. Low AGR has been found to be associated with the advanced stage of HBC [24]. In our study, the highest median AGR value was observed in the TNM stage I-II-III group, in which no metastases have formed. A low PNI parameter has been found to be associated with advanced disease in HBC [27]. Although there was no statistically significant difference in our study, median PNI values decreased as TNM stages progressed (43.88, 41.25, 40.8, respectively). Similar changes were detected in NLR, LMR, PLR, and PNI parameters at TNM stages in dogs with MMT as in HBC. These biomarkers reveal the balance between the immune system and systemic inflammation. In the early stages of the disease, tumors can be detected and destroyed by the immune system. As the disease progresses, the immunogenic capacity of the tumor decreases and its inflammatory capacity increases. As detectable tumors develop, cancer cells develop different mechanisms that mimic peripheral immune tolerance to avoid tumorocidal attack [2,37]. In our study, although the changes in SII and AGR parameters according to TNM stages showed some differences from HBC, median values in TNM I-II-III stages suggested that less systemic inflammatory response occurred in the early stages of canine MMT *(Table 5)*.

Lymph node involvement seems to be the most important factor in predicting prognosis using systemic inflammatory parameters in HBC [16,19,20,25,27]. In our study, it was found that there was no significant increase in NLR values up to TNM stage IV, where the disease affected the lymph nodes for the first time. The highest NLR, SII, and median PLR, SII values, with the lowest LMR, AGR, and PNI values were obtained in TNM stage IV *(Table 5)*. The most significant P values were obtained for the NLR, LMR, PLR, and SII parameters compared to the control group and TNM stage IV. Interestingly, the PNI parameter showed a significant difference only when TNM stage IV

and the control group were compared *(Table 5)*. However, no statistical difference was found between TNM IV and V groups. According to the current literature, the rate of bilateral HBC development humans is low [38] but, multiple tumors are more common in dogs [39,40]. In dogs with a single MMT, median NLR, and PLR values were found to be lower than in dogs with multiple MMTs, and median LMR, SII, AGR, and PNI values were higher in our study *(Table 6)*. These results suggest that the systemic inflammatory response may be higher when the disease metastasizes to other mammary glands than when it is localized in a single mammary gland. More research should be done about this subject in larger populations.

Advanced clinical staging is known to be associated with hematologic parameters and provide prognostic information for canine mammary tumors [8]. The data of this study showed that some combinations (NLR, LMR, PLR, SII, and PNI) of hematological and biochemical data routinely measured for systemic inflammatory response, varied between healthy dogs and dogs with malignant mammary tumors. It is also suggested that these biomarkers could be used as biomarkers in different TNM stages of MMTs and in cases where the disease metastasizes from the mammary gland of the primary tumor to other mammary glands. However, further studies are needed to determine the value of these biomarkers in determining the prognosis of the disease, choice of treatment modality, or prediction of response to chemotherapy.

Availability of Data and Materials

Data supporting these findings are available upon request from the corresponding author (D. NAK) on reasonable request.

Acknowledgements

I would like to thank Prof. Dr. Sezgin Şentürk for his support and contributions to the study.

Funding Support

This research was not financially supported.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Statement

The study was approved by the Ethics Committee on Animal Research of Bursa Uludag University (Approval No: 2020-03/03).

Author Contributions

D.N. and Z.M.E. wrote the manuscript, conducted experiments and interpreted the results. M.O.O. and Z.A.K perfor.med the histopathological examination. F.E.K. and T.A. made a significant contribution to and supported the experiments. D.K. and O.G. participated in the experiments. Y.N. and all authors critically reviewed and revised the manuscript draft and approved the final version for publication.

References

1. Cooper GM: The cell: A molecular approach. 2nd ed., Sunderland (MA): Sinauer Associates, 2000.

2. Dunn GP, Old LJ, Schreiber RD: The immunobiology of cancer immunosurveillance and immunoediting. *Immunity,* 21 (2): 137-148, 2004. DOI: 10.1016/j.immuni.2004.07.017

3. Grivennikov SI, Greten FR, Karin M: Immunity, inflammation, and cancer. *Cell,* 140 (6): 883-899, 2010. DOI: 10.1016/j.cell.2010.01.025

4. Balkwill F, Mantovani A: Inflammation and cancer: Back to Virchow? *Lancet,* 357 (9255): 539-545, 2001. DOI: 10.1016/S0140-6736(00)04046-0

5. Guthrie GJK, Charles KA, Roxburgh CSD, Horgan PG, McMillan DC, Clarke SJ: The systemic inflammation-based neutrophil-lymphocyte ratio: Experience in patients with cancer. *Crit Rev Oncol Hematol,* 88 (1): 218-230, 2013. DOI: 10.1016/j.critrevonc.2013.03.010

6. Roxburgh CS, McMillan DC: Role of systemic inflammatory response in predicting survival in patients with primary operable cancer. *Future Oncol,* 6 (1): 149-163, 2010. DOI: 10.2217/fon.09.136

7. Duda NCB, Valle SF, Matheus JP, Angeli NC, Vieira LC, Oliveira LO, Sonne L, Gonzalez FHD: Paraneoplastic hematological, biochemical, and hemostatic abnormalities in female dogs with mammary neoplasms. *Pesq Vet Bras,* 37 (5): 479-484, 2017. DOI: 10.1590/S0100-736X2017000500009

8. Oliveira MR, Carneiro RA, Nunes FC, Teixeira SV, Vieira TC, Lavalle GE, Cassali GD: Hematological and biochemical alterations in female dogs with mammary cancer and inflammatory carcinoma. *Arq Bras Med Vet Zootec,* 74 (3): 428-436, 2022. DOI: 10.1590/1678-4162-12471

9. Martínez-Lostao L, Anel A, Pardo J: How do cytotoxic lymphocytes kill cancer cells? *Clin Cancer Res,* 21 (22): 5047-5056, 2015. DOI: 10.1158/1078-0432.CCR-15-0685

10. Seliger B: The role of the lymphocyte functional crosstalk and regulation in the context of checkpoint inhibitor treatment. *Front Immunol,* 10:2043, 2019. DOI: 10.3389/fimmu.2019.02043

11. Kiss M, Caro AA, Raes G, Laoui D: Systemic reprogramming of monocytes in cancer. *Front Oncol,* 10:1399, 2020. DOI: 10.3389/fonc.2020.01399

12. Cerezo-Wallis D, Ballesteros I: Neutrophils in cancer, a love-hate affair. *The Febs Journal,* 289 (13): 3692-3703, 2022. DOI: 10.1111/febs.16022

13. Oberg HH, Wesch D, Kalyan S, Kabelitz D: Regulatory interactions between neutrophils, tumor cells and T cells. *Front Immunol,* 10:1690, 2019. DOI: 10.3389/fimmu.2019.01690

14. Palacios-Acedo AL, Mège D, Crescence L, Dignat-George F, Dubois C, Panicot Dubois L: Platelets, thrombo-inflammation, and cancer: Collaborating with the enemy. *Front Immunol,* 10:1805, 2019. DOI: 10.3389/fimmu.2019.01805

15. Ozyalvacli G, Yesil C, Kargi E, Kizildag B, Kilitci A, Yilmaz F: Diagnostic and prognostic importance of the neutrophil lymphocyte ratio in breast cancer. *Asian Pac J Cancer Prev,* 15 (23): 10363-10366, 2014. DOI: 10.7314/APJCP.2014.15.23.10363

16. Krenn-Pilko S, Langsenlehner U, Thurner EM, Stojakovic T, Pichler M, Gerger A, Kapp KS, Langsenlehner T: The elevated preoperative platelet-to-lymphocyte ratio predicts poor prognosis in breast cancer patients. *Br J Cancer,* 110, 2524-2530, 2014. DOI: 10.1038/bjc.2014.163

17. Elyasinia F, Keramati MR, Ahmadi F, Rezaei S, Ashouri M, Parsaei R, Yaghoubi M, Elyasinia F, Aboutorabi A, Kaviani A: Neutrophil-lymphocyte ratio in different stages of breast cancer. *Acta Med Iran,* 55 (4): 228-232, 2017.

18. Sökmen FC, Karacin C: The role of peripheral blood inflammatory markers in the staging of breast cancer. *East J Med,* 26 (1): 171-174, 2021. DOI: 10.5505/ejm.2021.71542

19. Zhang Y, Sun Y, Zhang Q: Prognostic value of the systemic immune-inflammation index in patients with breast cancer: A meta-analysis. *Cancer Cell Int,* 20:224, 2020. DOI: 10.1186/s12935-020-01308-6

20. Song D, Li X, Zhang X: Expression and prognostic value of ratios of platelet lymphocyte, neutrophil lymphocyte and lymphocyte monocyte in breast cancer patients. *Am J Trans Res,* 14 (5): 3233-3239, 2022.

21. Macfarlane L, Morris J, Pratschke K, Mellor D, Scase T, Macfarlane M, Mclauchlan G: Diagnostic value of neutrophil-lymphocyte and albumin-globulin ratios in canine soft tissue sarcoma. *J Small Anim Pract,* 57 (3): 135-141. 2016, DOI: 10.1111/jsap.12435

22. Skor O, Fuchs-Baumgartinger A, Tichy A, Kleiter M, Schwendenwein I: Pretreatment leukocyte ratios and concentrations as predictors of outcome in dogs with cutaneous mast cell tumours. *Vet Comp Oncol,* 15 (4): 1333-134, 2016. DOI: 10.1111/vco.12274

23. Rejec A, Butinar J, Gawor J, Petelin M: Evaluation of complete blood count indices (NLR, PLR, MPV/PLT, and PLCRi) in healthy dogs, dogs with periodontitis, and dogs with oropharyngeal tumors as potential biomarkers of systemic inflammatory response. *J Vet Dent,* 34 (4): 231-240, 2017. DOI: 10.1177/0898756417731775

24. Azab BN, Bhatt VR, Vonfrolio S, Bachir R, Rubinshteyn V, Alkaied H, Habeshy A, Patel J, Picon AI, Bloom SW: Value of the pretreatment albumin to globulin ratio in predicting long-term mortality in breast cancer patients. *Am J Surg,* 206 (5): 764-770, 2013. DOI: 10.1016/j.amjsurg.2013.03.007

25. Chi J, Xie Q, Jia J, Liu X, Sun J, Chen J, Yi L: Prognostic value of albumin/globulin ratio in survival and lymph node metastasis in patients with cancer: A systematic review and meta-analysis. *J Cancer,* 9 (13): 2341-2348, 2018. DOI: 10.7150/jca.24889

26. Xuan Q, Yang Y, Ji H, Tang S, Zhao J, Shao J, Wang J: Combination of the preoperative albumin to globulin ratio and neutrophil to lymphocyte ratio as a novel prognostic factor in patients with triple negative breast cancer. *Cancer Manag Res,* 11, 5125-5131, 2019. DOI: 10.2147/CMAR.S195324

27. Mantzorou M, Koutelidakis A, Theocharis S, Giaginis C: Clinical value of nutritional status in cancer: What is its impact and how it affects disease progression and prognosis? *Nutr Cancer,* 69 (8): 1151-1176, 2017. DOI: 10.1080/01635581.2017.1367947

28. Uribe-Querol E, Romero-Romero L, Govezensky T, Rosales C: Neutrophil to lymphocyte ratio and principal component analysis offer prognostic advantage for dogs with mammary tumors. *Front Vet Sci,* 10, 2297-1769, 2023. DOI: 10.3389/fvets.2023.1187271

29. Goldschmidt MH, Pena L, Zappuli V: Tumors of Mammary Gland. **In,** Meuten DJ (Ed): Tumors in Domestic Animals. 5th ed., 723-725, Willey Backwell, USA, 2017.

30. Misdorp W, Else RW, Hellmen E, Lipscomb TP: World Health Organization International Histological Classification of Tumors of Domestic Animals. Washington, DC: Armed Forces Institute of Pathology, 1999.

31. Queiroga FL, Raposo T, Carvalho MI, Prada J, Pires I: Canine mammary tumours as a model to study human breast cancer: Most recent findings. *In Vivo,* 25 (3): 455-465, 2011.

32. Carvalho MI, Silva-Carvalho R, Pires I, Prada J, Bianchini R, Jensen-Jarolim E, Queiroga FL: A comparative approach of tumor-associated inflammation in mammary cancer between humans and dogs. *BioMed Res Int,* 2016:4917387, 2016. DOI: 10.1155/2016/4917387

33. Gray M, Meehan J, Martínez-Pérez C, Kay C, Turnbull AK, Morrison LR, Pang LY, Argyle D: Naturally-occurring canine mammary tumors as a translational model for human breast cancer. *Front Oncol,* 10:617, 2020. DOI: 10.3389/fonc.2020.00617

34. Kuruoglu FE, Ozyigit MO, Nak D, Avci Kupeli Z, Ekici ZM, Koca D, Avcilar T, Sahin ME, Nak Y, Shahzad AH: Efficacy and toxicity of doxorubicin and cyclophosphamide for the neoadjuvant treatment of locally advanced stage canine mammary tumors. *Kafkas Univ Vet Fak Derg,* 26 (6): 729-734, 2020. DOI: 10.9775/kvfd.2020.24112

35. Gago-Dominguez M, Matabuena M, Redondo CM, Patel SP, Carracedo A, Ponte SM, Martinez ME, Castelao JE: Neutrophil to lymphocyte ratio and breast cancer risk: Analysis by subtype and potential interactions. *Sci Rep,* 10 (1):13203, 2020. DOI: 10.1038/s41598-020-70077-z

36. Lallo MA, Ferrarias TM, Stravino A, Rodriguez JFM, Zucare RLC: Hematologic abnormalities in dogs bearing mammary tumors. *Rev Bras Sci Vet*, 23 (1-2): 3-8, 2016. DOI: 10.4322/rbcv.2016.020

37. Gonzalez H, Hagerling C, Werb Z: Roles of the immune system in cancer: from tumor initiation to metastatic progression. *Genes Dev*, 32, 1267-1284, 2018. DOI: 10.1101/gad.314617.118

38. Jobsen JJ, van der Palen J, Ong F, Riemersma S, Struikmans H: Bilateral breast cancer, synchronous and metachronous; differences and outcome. *Breast Cancer Res Treat,* 153 (2): 277-283, 2015. DOI: 10.1007/s10549-015-3538-5

39. Rodríguez J, Santana Á, Herráez P, Killick, DR, de Los Monteros AE: Epidemiology of canine mammary tumours on the Canary Archipelago in Spain. *BMC Vet Res,* 18 (1):268, 2022. DOI: 10.1186/s12917-022-03363-9

40. Zheng HH, Du CT, Yu C, Zhang YZ, Huang RL, Tang XY, Xie GH: Epidemiological investigation of canine mammary tumors in Mainland China between 2017 and 2021. *Fron Vet Sci,* 9:843390, 2022. DOI: 10.3389/fvets.2022.843390