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Assessment of Hematochemical Parameters and Canine Cognitive Dysfunction Rating Scale in Healthy Senior and Geriatric Dogs

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Abstract: Aging is a process that includes natural and time-dependent changes in the body. Dogs whose quality of life is not adversely affected are classified as healthy elderly dogs. Behavioral and cognitive status should be taken into consideration together with blood analyzes in the health evaluation of old dogs. Therefore, it was aimed to evaluate hematochemical parameters and canine cognitive dysfunction (CCD) scale in 10 healthy senior (senior group, SG) and 14 healthy geriatric (geriatric group, GG) dogs in this study. No statistical difference was determined between the groups in the CBC analysis. In the blood gases analysis, pCO_2 (P=0.006), base excess (P=0.020) and HCO_3 (P=0.041) levels were found to be lower in GG compared to the SG. In serum biochemistry analysis, BUN (P=0.016) and GGT (P=0.003) levels were found to be higher whereas LDH (P=0.008) and triglyceride (P=0.003) levels were found to be lower in GG compared to SG. The total CCD scale score was 14.50 (12-23) in the SG and 50.50 (32-68) in the GG (P<0.001). In conclusion, differences in some parameters were determined as a result of blood gases and serum biochemistry analyzes between healthy senior and geriatric dogs, and it was concluded that routine hematochemical analyzes are important also in healthy elderly dogs. In addition, it was observed that the CCD scale scores of *Terriers* were higher in this study.
Keywords: Cognitive dysfunction, dog, geriatric, hematochemical analysis, senior

Sağlıklı Senior ve Geriatrik Köpeklerde Hematokimyasal Parametrelerin ve Köpek Bilişsel Disfonksiyon Derecelendirme Ölçeğinin Değerlendirilmesi

Öz: Yaşlanma, vücutta meydana gelen doğal ve zamana bağlı değişiklikleri içeren bir süreçtir. Yaşam kalitesi olumsuz etkilenmemiş köpekler, sağlıklı yaşlı olarak sınıflandırılır. Yaşlı köpeklerin sağlık değerlendirmesinde kan analizleri ile birlikte davranışsal ve bilişsel durum da dikkate alınmalıdır. Bu nedenle bu çalışmada 10 sağlıklı yaşlı (Senior grup, SG) ve 14 sağlıklı geriatrik (Geriatrik grup, GG) köpekte hematokimyasal parametrelerin ve köpek bilişsel işlev bozukluğu (CCD) ölçeğinin değerlendirilmesi amaçlandı. Tam kan sayımı (CBC) analizinde gruplar arasında istatistiksel herhangi bir fark belirlenmedi. Kan gazı analizinde GG'de pCO_2 (P=0.006), baz açığı (P=0.020) ve HCO_3 (P=0.041) düzeyleri SG'ye göre daha düşük bulundu. Serum biyokimyası analizinde GG'de BUN (P=0.016) ve GGT (P=0.003) düzeyleri SG'ye göre daha yüksek olarak belirlenirken, LDH (P=0.008) ve trigliserit (P=0.003) düzeyleri daha düşüktü. Toplam CCD skala skoru SG'de 14.50 (12-23), GG'de 50.50 (32-68) olarak belirlendi (P=0.000). Sonuç olarak, sağlıklı yaşlı ve geriatrik köpekler arasında kan gazları ve serum biyokimya analizleri sonucunda bazı parametrelerde farklılıklar saptandı ve sağlıklı yaşlı köpeklerde de rutin hematokimyasal analizlerin önemli olduğu sonucuna varıldı. Ayrıca, bu çalışma kapsamında *Terrier* ırkı köpeklerin CCD skala skorlarının daha yüksek olduğu gözlendi.
Anahtar kelimeler: Bilişsel işlev bozukluğu, geriatrik, hematokimyasal analiz, köpek, senior

Introduction

Aging is a process that involves natural and time-dependent changes that occurs in the later stages of life in both humans and animals, rather than the pathological changes observed in the body. Owing to advanced medical care techniques, it was stated by several researchers that healthy aging is possible (Gilmer and Aldwin, 2003; Bellows et al., 2015). Healthy aging in humans has been defined as an absence of risk of disease or disease-related abnormal conditions. Healthy aging in humans includes cognitive, physical and physiological health and this scope can also be available for dogs (Rowe and Kahn, 1997; Bellows et al., 2015). Developing a valid definition for healthy aging is important for future studies in elderly dogs. In addition, it was reported that determining clinical methods that can distinguish healthy aging from disease-related conditions when evaluating old age dogs is essential (Salvin et al., 2011, Bellows et al., 2015). The definition of healthy aging in dogs can be defined as the absence of any

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clinically significant disease. As dogs age, they naturally undergo a series of changes; however, dogs whose age-related changes are minimal or whose quality of life is not adversely affected can be classified as healthy elderly dogs (Neilson et al., 2001; Bellows et al., 2015). Various factors including breed and age affect the aging process. Generally, small-medium breed dogs which are between 7-10 years old are considered senior, and dogs that are older than 11 years old are considered geriatric (Bellows et al., 2015).

Diseases of elderly dogs are often multi systemic and complex. Blood laboratory analyzes are important in the early diagnosis of the diseases in order to achieve a successful treatment and monitor the quality of life (Thrall et al., 2012; Bellows et al., 2015). For this reason, annual or 6-month blood laboratory analyzes are important not only to the early diagnosis of diseases but also to determine if there are any disease predisposition. It was reported that routine blood laboratory tests for elderly dogs should include serum biochemistry with CBC, blood gases and electrolytes analyzes (Rizzi, 2015). In the clinical examination of elderly dogs, distinguishing aging process from disease-related conditions is important (Bellows et al., 2015). Hematological and serum biochemistry findings of healthy senior and geriatric dogs that determined by the IAMS Company Pet Health and Nutrition Center® database (P&G, 2011) indicate that these findings differ compared to the general dog population (Bellows et al., 2015). For this reason, it is ideal to compare the hematological and biochemical analysis results of elderly dogs with reference ranges specifically determined for senior and geriatric dogs.

The most common disorders that can cause hematological abnormalities in elderly dogs are reported to be kidney and liver diseases, diabetes mellitus, and hyper/hypothyroidism (Stockham and Scott, 2008; Thrall et al., 2012). The ability of the kidneys to remove metabolic waste and reabsorb essential compounds decreases as dogs age, and clinical signs vary with the extent and duration of the renal function impairment. Expected clinical-pathological abnormalities in renal diseases are elevated BUN and creatinine levels along with decreased glomerular filtration rate (GFR) (Rizzi, 2015). It was reported that severe azotemia must be evaluated together with urine specific gravity (Latimer, 2011). Therefore, it was stated that performing routine laboratory analysis on common causes of hematological and/or biochemical abnormalities in elderly dogs is essential for improving the quality of life (Rizzi, 2015)

As in humans, changes associated with aging in dogs may not be considered pathological and generally do not affect negatively overall welfare and/or quality of life (Salvin et al., 2011). Nevertheless, clinical examinations are essential to eliminate other diseases. Mobility, skin and hair health, gastrointestinal, immune, hepatic, respiratory and renal assessment, as well as behavioral and cognitive status should definitely be considered in the health assessment of elderly dogs (Baldwin et al., 2010; Bellows et al., 2015). It is difficult to distinguish healthy elderly dogs from dogs with cognitive or behavioral disorders due to medical and aging findings that cause behavioral changes (Neilson et al., 2001). For this reason, it was reported that the assessment of the canine cognitive dysfunction rating scale (CCDR) along with routine hematological and biochemical analyzes is important in determining cognitive and behavioral health in healthy elderly dogs (Bellows et al., 2015). Canine cognitive dysfunction (CCD) or canine dementia is a neurobehavioral syndrome characterized by changes in learning, memory, social interaction, and sleep patterns (Landsberg et al., 2003; Salvin et al., 2011). As a result of several studies, it was demonstrated that the syndrome affects 85% of the elderly dog population and often cannot be diagnosed (Neilson et al., 2001; Osella et al., 2007; Salvin et al., 2011). Also, similarities were reported between CCD and Alzheimer's disease in terms of progressive structure, neuropathological abnormality and pharmacological response (Ruehl et al., 1995). For this reason, a CCD scale with a cut-off value of >50 (Salvin et al., 2011) was established to assess the cognitive profile of dogs. The CCD scale, which is used to evaluate the cognitive level of dogs, enables successful and timely intervention of undiagnosed or mismanaged cases (Bellows et al., 2015). Thus, with early and correct diagnosis, the general welfare and quality of life of elderly dogs can be improved. The CCD scale, which is a clinical and ethological screening and evaluation tool, is useful in distinguishing between cognitive aging and healthy aging, and the obtained results can advance the developments in the field of canine and human health (Salvin et al., 2011; Lorenzini, 2014).

Therefore, in this study, it was aimed to reveal the parameters that come forth in routine health screenings including clinical and laboratory examinations, as a result of hematochemical and CCD score evaluation in small-medium breed healthy senior and geriatric dogs.

Material and Methods

Animal material

The animal material of this study was consisted of 24 elderly dogs in total, 10 healthy senior and 14 healthy geriatric dogs from different sexes and small-medium breeds, which were brought to Selcuk University Animal Hospital of Faculty of Veterinary Medicine for a routine health check. Anamnestic data revealed that the dogs were vaccinated and treated with dewormers routinely, fed with commercial dry dog food and walked twice a day. The dogs included in the study were divided into two groups: Senior (Senior group, SG) and Geriatric (Geriatric group, GG), according to the age criteria that determined previously (Bellows et al., 2015). Dog breeds included in the study were

Terrier (n=12), Spanial Cocker (n=5) and Pointer (n=7). In the study, it was determined that the mean age of the SG was 7.10±0.73 whereas the mean age of GG was 12.14±2.76 years.
The ethical approval (2021/63) was obtained from the Selcuk University Faculty of Veterinary Medicine Ethics Committee (SÜVFEK).

Physical examinations
Physical examinations including respiratory rate, pulse, capillary refill time (CRT) and body temperature measurements, skin and hair structure inspection, evaluation of palpable lymph nodes such as mandibular, prescapular, superficial inguinal and popliteal, heart and lung auscultation, abdominal palpation, hydration status evaluation, as well as microscopic examination of stool specimens and orthopedic examinations were performed.

Inclusion and exclusion criterias
Dogs that were learned to have a history of disease or were observed to have any disease-related symptoms during physical examination were not included in the study. Also, dogs that did not meet the breed and age criteria (Bellows et al., 2015) within the scope of senior and geriatric categorization were not included in the study.

Canine cognitive dysfunction scale
The CCD scale of all dogs included in the study was obtained as a face-to-face questionnaire with the owners. In line with the answers given by the owners to the questionnaire consisting of 12 questions, the CCD scale for each dog (1=Never, 2=Once a month, 3=Once a week, 4=Once a day, 5=More than once a day) were obtained. The questionnaire and the results are presented in Table 1.

Table 1. CCD scale questionnaire results

Questions		Senior Group (SG)		Geriatric Group (GG)
1) How often does your dog speed up and slow down, circle, or walk aimlessly?	**1**	N:4 (40%)	**1**	N:1 (7.2%)
	2	N:5 (50%)	**2**	N:2 (14.3%)
	3	N:1 (10%)	**3**	N:3 (21.4%)
	4	N:0 (0%)	**4**	N:6 (42.8%)
	5	N:0 (0%)	**5**	N:2 (14.3%)
2) How often does your dog stare blankly at the wall or floor?	**1**	N:4 (40%)	**1**	N:0 (0%)
	2	N:5 (50%)	**2**	N:1 (7.2%)
	3	N:1 (10%)	**3**	N:3 (21.4%)
	4	N:0 (0%)	**4**	N:4 (28.6%)
	5	N:0 (0%)	**5**	N:6 (42.8%)
3) How often does your dog get behind objects and can't get around them?	**1**	N: 9 (90%)	**1**	N:5 (35.7%)
	2	N: 1 (10%)	**2**	N:4 (28.6%)
	3	N: 0 (0%)	**3**	N:2 (14.3%)
	4	N: 0 (0%)	**4**	N:3 (21.4%)
	5	N: 0 (0%)	**5**	N:0 (0%)
4) How often does your dog not recognize a family member or animal?	**1**	N: 10 (100%)	**1**	N:4 (28.6%)
	2	N: 0 (0%)	**2**	N:3 (21.4%)
	3	N: 0 (0%)	**3**	N:4 (28.6%)
	4	N: 0 (0%)	**4**	N:2 (14.3%)
	5	N: 0 (0%)	**5**	N:1 (7.1%)

Questions		Senior Group (SG)		Geriatric Group (GG)
5) How often does your dog walk up against walls or doors?	**1**	N:7 (70%)	**1**	N:1 (7.1%)
	2	N:3 (30%)	**2**	N:0 (0%)
	3	N:0 (0%)	**3**	N:5 (35.7%)
	4	N:0 (0%)	**4**	N:4 (28.6%)
	5	N:0 (0%)	**5**	N:4 (28.6%)
6) How often does your dog walk away when you pet?	**1**	N:8 (80%)	**1**	N:1 (7.1%)
	2	N:1 (10%)	**2**	N:0 (0%)
	3	N:1 (10%)	**3**	N:5 (35.7%)
	4	N:0 (0%)	**4**	N:4 (28.6%)
	5	N:0 (0%)	**5**	N:4 (28.6%)
7) Compared to six months ago, is your dog speeding up and slowing down, circling or walking aimlessly nowadays?	**1**	N:10 (100%)	**1**	N:3 (21.4%)
	2	N:0 (0%)	**2**	N:5 (35.8%)
	3	N:0 (0%)	**3**	N:6 (42.8%)
8) Is your dog often blankly staring at the wall or the floor nowadays compared to six months ago?	**1**	N: 5 (50%)	**1**	N:2 (14.4%)
	2	N:4 (40%)	**2**	N:6 (42.8%)
	3	N:1 (10%)	**3**	N:6 (42.8%)
9) Compared to six months ago, is your dog nowadays urinating in places where it hasn't?	**1**	N:7 (70%)	**1**	N:0 (0%)
	2	N:1 (10%)	**2**	N:4 (28.6%)
	3	N:2 (20%)	**3**	N:2 (14.2%)
	4	N:0 (0%)	**4**	N:6 (42.8%)
	5	N:0 (0%)	**5**	N:2 (14.2%)
10) Is your dog having trouble finding a dropped food nowadays compared to six months ago?	**1**	N:9 (90%)	**1**	N:4 (28.6%)
	2	N:1 (10%)	**2**	N:0 (0%)
	3	N:0 (0%)	**3**	N:2 (14.2%)
	4	N:0 (0%)	**4**	N:5 (35.8%)
	5	N:0 (0%)	**5**	N:3 (21.4%)

Questions		Senior Group (SG)		Geriatric Group (GG)
11) Is it happening nowadays that your dog is unable to recognize a family member or animal compared to six months ago?	**1**	N:7 (70%)	**1**	N:3 (21.4%)
	2	N:2 (20%)	**2**	N:8 (57.2%)
	3	N:1 (10%)	**3**	N:3 (21.4%)
12) Is your dog's active time reduced nowadays compared to six months ago?	**1**	N:7 (70%)	**1**	N:0 (0%)
	2	N:1 (10%)	**2**	N:1 (7.2%)
	3	N:2 (20%)	**3**	N:4 (28.6%)
	4	N:0 (0%)	**4**	N:6 (42.8%)
	5	N:0 (0%)	**5**	N:3 (21.4%)

1=Never, 2=Once a month, 3=Once a week, 4=Once a day, 5=More than once a day. Multiplication coefficient to be applied for questions between 7 and 12: x1 = Same, x2 = Much, x3 = Too much

Collection and measurements of blood samples

Blood samples (with anticoagulant (K_3EDTA and heparin) and without anticoagulant) from all dogs included in the study were collected by the same personnel with minimal restraint in order to not cause stress. Venous blood samples (5-10 mL) were taken from the vena cephalica venepunction. From the blood samples with K_3EDTA, CBC (WBC, Hb, PCV, RBC, thrombocyte, MCV, MCH, MCHC, Lymphocyte, Monocyte, Granulocyte, MS4 CFE 279®, Haematology Analyzer, France); from heparinized blood samples, blood gases and electrolyte analyzes (pH, pCO_2, pO_2, sodium, potassium, chlorine, lactate, base excess, and bicarbonate; ABL 90® Flex Blood Gas/ Electrolyte Analyzer, Model 5700 Radiometer, USA); from blood serum samples (5000 rpm for 5 minutes centrifugation for serum extraction), serum biochemistry analyzes (BUN, creatinine, glucose, total protein, albumin, ALT, ALP, cholesterol, triglyceride, calcium and phosphorus, Biotecnica BT 3000 Plus®, Italy) were performed. All laboratory analyzes were performed within 30 minutes after blood sampling.

Statistical analysis

Kolmogorov-Smirnov test was applied to determine whether all data were parametric or non-parametric. Parametric data were evaluated as mean±SEM with Student's t test (for the assessment for homogeneity of variance, Levene's test was used), and non-parametric data were evaluated Mann Whitney U test and as median (min-max). In addition, ROC analysis was performed to determine the cut-off values for ages and CCD scale for all groups. Statistical significance was accepted as $P<0.05$ for all data. SPSS 21.0 statistical software (SPSS for Windows®) was used for data analysis.

Results

Physical examination findings

No abnormal findings were determined in the clinical examination of the dogs included in the study. No parasite eggs were observed in the microscopic examination of the stool specimens and orthopedic examination findings were normal. The clinical examination findings and the mean ages of the groups are presented in Table 2.

Table 2. Mean ages and clinical examination findings of the dogs

Parameters	Senior Group (SG) (mean±SEM)	Geriatric Group (GG) (mean±SEM)	P value
Respiratory rate (per minute)	35.90±6.82	30.78±5.93	0.072
Heart rate (per minute)	73.80±7.64	78.92±9.47	0.157
Capillary refill time (sec)	2.30±0.48	2.64±0.49	0.106
Temperature (°C)	38.10±0.30	38.18±0.42	0.572
Age (year old)	**7.10±0.73**	**12.14±2.76**	**<0.001**

Canine cognitive dysfunction scale evaluation

The CCD scale scores which were obtained from the questionnaire were statistically different between groups (P=0.004). The total CCD scale score was 14.50 (12-23) for the SG and 50.50 (32-68) for the GG (P<0.001). The results are presented in Table 3.

Table 3. CCD Scale results

Questions	1	2	3	4	5	6	7	8	9	10	11	12	Total score
Senior Group [Median (min-max)]	2 (1-3)	1 (1-2)	1 (1-2)	1 (1-1)	1 (1-2)	1 (1-3)	2 (2-3)	1 (1-4)	1 (1-3)	1 (1-2)	1 (1-3)	1 (1-3)	14.50 (12-23)
Geriatric Group [Median (min-max)]	4 (1-5)	4 (2-5)	2 (1-4)	2.5 (1-5)	4 (1-5)	3 (1-5)	7 (2-12)	5.5 (3-15)	4 (2-8)	4 (1-5)	4 (1-9)	4 (2-12)	50.50 (32-68)
P value	<0.001	<0.001	0.004	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Hematochemical and blood gases analysis findings

The hematochemical analyzes results were evaluated considering the reference ranges determined by the IAMS Company Pet Health and Nutrition Center® (P&G, 2011). No statistical difference was determined between the groups as a result of the CBC. The detected values were within the normal reference ranges. CBC parameters are presented in Table 4 and other hematochemical parameters with statistical differences are presented in Figure 1.

Table 4. CBC analysis results

Parameters	Senior Group (SG) (mean±SEM)	Geriatric Group (GG) (mean±SEM)	P value
WBC (m/mm^3)	12.63±3.46	12.04±3.15	0.676
Lymphocyte (%)	3.24±1.27	3.83±1.89	0.374
Monocyte (%)	0.91±0.62	1.33±1.07	0.243
Granulocyte (%)	8.34±3.12	6.65±1.85	0.148
RBC (M/mm^3)	7.22±1.05	7.59±1.47	0.480
MCV (fl)	74.22±4.88	70.42±8.81	0.192
HCT (%)	54.44±9.31	52.72±9.14	0.660
MCH (pg)	27.33±17.36	20.08±2.40	0.222
MCHC (g/dL)	29.53±3.02	28.65±1.95	0.433
RDW	10.42±1.15	10.35±0.96	0.890
Hb (g/dL)	16.05±2.33	15.22±2.54	0.423

WBC: White blood cells, RBC: Red blood cells, MCV: Mean corpuscular volume, HCT: Hematocrit, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW: Reticulocytes distribution width, Hb: Hemoglobin

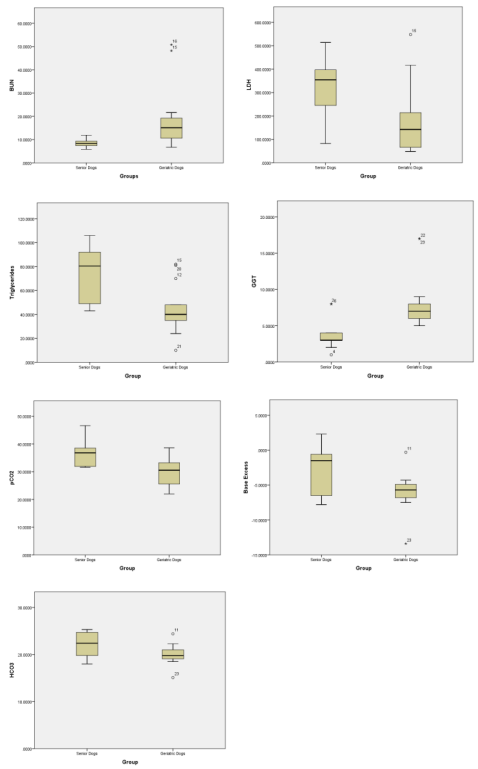

Figure 1. Boxplot graphics of BUN, LDH, Triglyceride, GGT, pCO_2, BE and HCO_3 levels.

In blood gases and electrolyte analysis, pCO_2 (P=0.006), BE (P=0.020) and HCO_3 (P=0.041) levels were found to be lower in the GG compared to SG. Other parameters were within reference ranges. The results are presented in Table 5.

Table 5. Blood gases and electrolyte analysis results

Parameters	Senior Group (SG) (mean±SEM)	Geriatric Group (GG) (mean±SEM)	P value
pH	7.38±0.04	7.38±0.04	0.809
pCO_2 (mmHg)	**37.23±5.27**	**30.45±5.26**	**0.006**
pO_2 (mmHg)	40.74±9.49	35.65±5.94	0.157
Potassium (mmol/L)	3.91±0.37	3.95±0.47	0.820
Sodium (mmol/L)	155.10±4.99	152.28±6.97	0.262
Chlorine (mmol/L)	116.10±4.40	116.78±4.29	0.708
Lactate (mmol/L)	3.10±1.50	2.75±1.50	0.580
Base excess (mmol/L)	**-2.72±3.40**	**-5.88±2.75**	**0.020**
HCO_3 (mmol/L)	**22.05±2.50**	**19.90±2.11**	**0.041**

pH: Power of hydrogen, pCO_2: Partial pressure or carbondioxide, pO_2: Partial pressure of oygen, HCO_3: Bicarbonate

In serum biochemistry analysis, BUN (P=0.016) and GGT (P=0.003) levels were found to be higher whereas LDH (P=0.008) and triglyceride (P=0.003) levels were found to be lower in the GG compared to SG. Serum biochemistry analysis results are presented in Table 6.

Also, ROC analysis of age comparison and CCD score of dogs in both groups was performed. As a result, high AUC (0.950), sensitivity (%100), specificity (%94) and cut-off value of 46.50 for CCD score, and high AUC (0.926), sensitivity (%87), specificity (%87) and cut-off value of 10.50 for CCD age were determined. ROC plots and analysis results were presented in Figure 2 and Table 7, respectively.

Table 6. Serum biochemistry analysis results

Parameters	Senior Group (SG) (mean±SEM)	Geriatric Group (GG) (mean±SEM)	P value
BUN (mg/dL)	**8.52±1.94**	**18.80±13.76**	**0.016**
Creatinine (mg/dL)	0.93±0.27	1.14±0.41	0.146
ALT (U/L)	63.10±23.20	77.42±66.23	0.465
AST (U/L)	25.70±4.83	33.85±29.70	0.330
ALP (U/L)	84.40±35.25	106.64±112.49	0.498
Amylase (U/L)	448±123.22	634.50±327.61	0.068
Glucose (mg/dL)	116.50±11.27	104.21±19.48	0.065
LDH (U/L)	**333.80±122.62**	**173.07±147.21**	**0.008**
T. bilirubin (mg/dL)	0.48±0.19	0.42±0.24	0.529
D. bilirubin (mg/dL)	0.27±0.14	0.23±0.18	0.619
Phosphorous (mg/dL)	3.74±0.81	3.72±1.35	0.967
Albumin (g/dL)	2.94±0.40	3.07±0.49	0.462
Cholesterol (mg/dL)	209±79.44	246.85±143.56	0.418
Calcium (mg/dL)	11.05±1.67	11.29±2.10	0.757
Triglyceride (mg/dL)	**75.70±22.53**	**44.28±20.73**	**0.003**
Magnesium (mg/dL)	1.95±1.05	1.51±0.51	0.248
GGT (U/L)	**3.90±2.33**	**8.24±3.89**	**0.003**
Total protein (g/dL)	6.26±0.94	7.10±1.15	0.061
CPK (U/L)	115.70±67.43	111.14±95.13	0.892

BUN: Blood urea nitrogen, ALT: Alanine aminotransferase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, LDH: Lactate dehydrogenase, GGT: Gamma-glutamyl transferase, CPK: Creatine phosphokinase

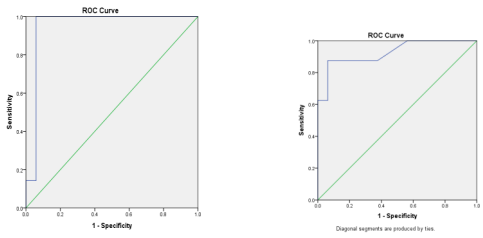

Figure 2. CCD score ROC plot (left) and CCD age ROC plot (right).

Discussion and Conclusion

Aging triggers cellular and molecular changes in mammals that increase the risk of disease development such as genomic instability and organ dysfunction. Recently, the increase in the number of elderly dogs, which constitutes 49% of almost all domestic animals worldwide, has increased the need for studies investigating the effects of biological effects of aging on the quality of life (Ülgen et al., 2015; Lee et al., 2020). Dogs are the most human-like creatures compared to other laboratory animals in terms of lifestyle, exposure to environmental pathogens, medical care, and physical activity (Lorenzini, 2014). The

Table 7. CCD Score ROC analysis results

Parameters	AUC	Std. Error	P value	Asymp. %95 CI		Cut-off	Sensitivity (%)	Specifity (%)
				Lower Bound	Upper Bound			
CCD Score	0.950	0.050	0.001	0.851	1.000	46.50	100	94
CCD Age	0.926	0.061	0.001	0.805	1.000	10.50	87	87

AUC: Area under curve, Std error: standart error CI: confidence interval

need to improve the welfare of domestic dogs is also beneficial for the development of geriatric research in humans (Lee et al., 2020). Aging is an ontogenetic process that begins with birth and ends with death (Maggio et al., 2006). Recent studies have identified cellular and molecular parameters associated with aging, such as altered intercellular communication, genomic instability, mitochondrial dysfunction, and epigenetic changes in mammals (Lopez-Otin et al., 2013; Lee et al., 2020).

Aging in animals as in humans occurs by development of predisposition to diseases as a result of a breakdown of the relationship between body systems due to acquired diseases and environmental conditions (Ülgen et al., 2015). In previous studies, it has been reported that CBC and biochemical profile analyzes are objective and more specific compared to routine physical examination in dogs, and are important in the follow-up and analysis of changing physiological characteristics (Maggio et al., 2006). In addition, it was reported in hematological phenotype studies in dogs of various breeds that dogs are the ideal model for hematological characterization of mammals (Lawrance et al., 2013). Therefore, hematological and biochemical profile analyzes are essential for the diagnosis and screening of diseases in veterinary medicine (Lee et al., 2020; Miglio et al., 2020). Low MCV and MPV levels were reported in CBC findings of senior and geriatric dogs (Lee et al., 2020). However, in some previous studies, no significant age-related hematological differences were reported (Strasser et al., 1993). In this study, no statistical differences were observed neither in the physical examination nor in the CBC findings between senior and geriatric dogs. These findings might be related to the hydration status (Lowseth et al., 1990) of the dogs of the present study. Also, low number of healthy elderly dogs in the present study should be kept in mind. Many diseases cause abnormal acid-base balance through changes in ventilation or metabolic disorders (Bellows et al., 2015; Lee et al., 2020). Both conditions stimulate compensation mechanisms that provide acid-base balance. Base excess is controversial as it is affected by respiratory changes in vivo. This situation is due to free ion exchange between blood and interstitial fluid. Therefore, as pCO_2 changes in vivo, HCO_3 also changes along with base excess and other ions provide ion balance between the blood and interstitial space (Schlichtig et al., 1998). Elevations in BUN are an infrequent observation in the geriatric dog despite the morphological and functional changes that occur in the aging kidney (Lowseth et al., 1990). The elevated BUN level in GG of the current study (P=0.016) was consistent with the previously reported findings due to decreased GFR as a result of up to 75% loss of functional renal mass in geriatric dogs (Rizzi, 2015). Thus, the numerically higher BUN levels of geriatric dogs compared to senior dogs of the present study may indicate greater renal mass loss (Lowseth et al., 1990; Strasser et al., 1993). Also, when compared to SG, low pCO_2 (P=0.006), base excess (P=0.020) and HCO_3 (P=0.041) levels of GG in blood gases were interpreted depending on the renal compensation mechanism. These findings were consistent with previous reports (Lowseth et al., 1990; Strasser et al., 1993; Willems et al., 2017). However, assessment of renal clearance is required to detect abnormal renal function changes (Strasser et al., 1993).

In previous reports, due to the high reserve capacity of the liver AST, ALT and GLDH showed no age-related changes, whereas GGT increased (Strasser et al., 1993). Gamma glutamyl transferase (GGT) is an enzyme found in blood serum and on the surface of different organ cells such as liver, pancreas, intestine, lung and kidney (Bulusu and Sharma, 2016). Except that serum GGT is a traditional biomarker of hepatobiliary diseases, its high level is associated with cardiovascular diseases, diabetes mellitus, hypertension and metabolic syndrome (Targher, 2010; Caravaca-Fontan et al., 2017). It has also been reported that high GGT levels are associated with endothelial dysfunction (Yılmaz et al., 2013). The high GGT level (P=0.003) determined in GG of the study may be associated with subclinical organ dysfunction including intestines, kidney and liver (Stockham and Scott, 2008; Thrall et al., 2012), which may cause hematological abnormalities in geriatric dogs. Also, it was reported that the membrane located GGT increases with age, possibly indicating changes in the parenchyma of the ageing liver (Strasser et al., 1993). Although there was no statistical difference, numerical values of some parameters (BUN: 18.80±13.76, ALT: 77.42±66.23 AST: 33.85±29.70,

ALP: 106.64±112.49) of GG compared to SG may support the fact that the liver parenchyma has had undergone further changes in geriatric dogs.

Triglycerides are synthesized in intestinal and liver cells and used as an energy source (Hegele 2009). Similarly, the blood concentration of lactate dehydrogenase (LDH), a glycolytic enzyme that used as an energy source, decreases in cases where cellular energy metabolism is disrupted (Marconato et al., 2009). Low LDH (P=0.008) and triglyceride (P=0.003) levels of GG compared to SG, were interpreted as a result of mitochondrial dysfunction (Catic, 2018), which plays an important role in the aging process. In addition, the low triglyceride level may be associated with long life span (Liu et al., 2017). Also, in a previous study, it was reported that triglyceride levels were lower in 14-year-old dogs compared with the 12-year-old dogs (Lowseth et al., 1990). Furthermore, significantly higher triglyceride levels without any consequences for the blood vessels, the heart and the circulation in rats were reported. The findings of the present study were consistent with the previous reports and the fact that animals' life expectancy is short in terms of development of signs of arteriosclerosis in senior dogs compared to geriatric ones (Strasser et al., 1993; Willems et al., 2017).

Canine cognitive dysfunction is a common problem in geriatric dogs (Neilson et al., 2001, Osella et al., 2007). It has been reported that routine hematochemical analyzes and CCD evaluation are important in determining cognitive and behavioral health in healthy elderly dogs (Bellows et al., 2015), with a cut-off value of >50 (Salvin et al., 2011). The highest CCD scores in GG of the study were 59, 68, 57, 58 and 64 in the 5^{th}, 7^{th}, 10^{th}, 13^{th}, and 14^{th} dogs, respectively. The breed of all dogs with the highest CCD scores was *Terrier* and the average age was determined to be 14.4 years. Although the CCD score of Terrier dogs (n=7 in the GG, 12 in total) was found to be higher than other breeds in this study, in order to make inferences about predisposition of cognitive disorders studies with a larger number of animals are required, considering the number of animals included in the present study. In addition, it was determined that the cut-off value for the CCD score was 46.50, and the age cut-off value for cognitive disorders was 10.5 in the ROC analyses of the current study. These findings were similar to previously reported threshold values and methods of evaluation of cognitive decline (Salvin et al., 2011; Ülgen et al., 2015).

The limitations of this study are the low number of animal material, undetermined glomerular filtration rate, and the lack of evaluation of specific biomarkers (such as cardiac troponins, coagulation parameters and symmetric dimethylarginine) for the diagnosis of undetermined comorbid diseases.

In this study, significant differences were determined in BUN, LDH, GGT and triglyceride levels between healthy senior and geriatric dogs. As a result of hematochemical analyzes, it was observed that differences in blood gases and electrolyte analysis such as lower BE and HCO_3 levels between the senior and geriatric dogs might be due to altered renal compensation mechanisms and serum biochemistry differences such as elevated BUN, decreased LDH and GGT levels might be resulting from decreased glomerular filtration rate and disruption of cellular energy metabolism or mitochondrial dysfunction. Beside hematochemical differences between the groups, *Terriers* had higher CCD scores. It was concluded that as many organ functions decline with age, regular health screening by blood tests and canine cognitive dysfunction rating scale may raise owner awareness for potentially important clinical signs in aging dogs.

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