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Relationship between hematological and biochemical tests and senility in dogs

Relação entre exames hematológicos, bioquímicos e a senilidade em cães

Relación entre exámenes hematológicos, bioquímicos y senilidad en perros

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Abstract
Morphological and functional changes during senility give rise to changes to organs and tissues. Even in healthy dogs, laboratory parameters may differ, thus making preventive treatment difficult. The aim of this study was to evaluate hematological and biochemical parameters in elderly and geriatric dogs and to compare them for follow-up and possible worsening of prognosis. Thirty-four apparently healthy dogs living in the city of Ribeirão Pires, SP, were included in this study, divided into two groups: elderly (8-11,9 years) and geriatric (12 years or more). Two blood samples were taken with an interval of 20 days between them, to determine blood counts and urea, creatinine, alkaline phosphatase, alanine aminotransferase and blood glucose levels. The statistical analysis showed that this was a homogeneous group, since the only difference found between the elderly and geriatric dogs was in urea values (elderly > geriatric, $p = 0.010$), while in the hematological tests there were no differences between the groups or collection times. Even so, changes in test results occurred: 53% in hematological tests and 74% in biochemical tests, respectively. This highlights the importance of laboratory evaluations for prognoses and, consequently, improves the quality of life and wellbeing of dogs.
Keywords: Biochemistry; Dogs; Elderly; Geriatric; Hematology.

Resumo
Mudanças morfológicas e funcionais durante a senilidade incorrem em modificações de órgãos e tecidos. Mesmo em cães saudáveis, os parâmetros laboratoriais podem diferir, dificultando o tratamento preventivo. O objetivo deste estudo foi avaliar parâmetros hematológicos e bioquímicos em cães idosos e geriátricos e compará-los para acompanhamento e possível agravamento em prognóstico. Trinta e quatro cães aparentemente saudáveis, residentes na cidade de Ribeirão Pires –SP, foram incluídos no estudo, sendo divididos em 2 grupos: o grupo idoso (8-11,9 anos) e geriátrico (12 anos ou mais). Foram realizadas 2 coletas sanguíneas com intervalo de 20 dias entre elas, para exames de hemograma e

bioquímicos, ureia, creatinina, fosfatase alcalina, alanina aminotransferase e dosagem de glicemia. Com a análise estatística mostrou-se que se tratava de um grupo homogêneo, já que a única diferença encontrada entre cães idosos e geriátricos foi em valor de ureia (idosos > geriátricos, p = 0,010), enquanto nos exames hematológicos não houve diferenças entre grupos ou coletas. Mesmo assim, as alterações em exames apresentaram ocorrências, correspondendo a 53% em exames hematológicos e 74% em exames bioquímicos, respectivamente. O que ressalta a importância da avaliação laboratorial como prognóstico e, consequentemente melhora na qualidade de vida e bem-estar dos cães.
Palavras-chave: Bioquímica; Cães; Idosos; Geriátricos; Hematologia.

Resumen

Los cambios morfológicos y funcionales durante la senilidad dan lugar a cambios en órganos y tejidos. Incluso en perros sanos, los parámetros de laboratorio pueden diferir, lo que dificulta el tratamiento preventivo. Objetivo: El objetivo de este estudio fue evaluar parámetros hematológicos y bioquímicos en perros ancianos y geriátricos y compararlos para el seguimiento y posible empeoramiento del pronóstico. Treinta y cuatro perros aparentemente sanos residentes en la ciudad de Ribeirão Pires, SP, fueron incluidos en este estudio, divididos en dos grupos: ancianos (8-11,9 años) y geriátricos (12 años o más). Se tomaron dos muestras de sangre con un intervalo de 20 días entre ellas, para determinar hemogramas y niveles de urea, creatinina, fosfatasa alcalina, alanina aminotransferasa y glucosa en sangre. El análisis estadístico mostró que se trataba de un grupo homogéneo, ya que la única diferencia encontrada entre los perros ancianos y geriátricos fue en los valores de urea (ancianos > geriátricos, p = 0,010), mientras que en las pruebas hematológicas no hubo diferencias entre los grupos. o Horarios de recogida. Aun así, se produjeron cambios en los resultados de las pruebas: 53% en pruebas hematológicas y 74% en pruebas bioquímicas, respectivamente. Esto resalta la importancia de las evaluaciones de laboratorio para el pronóstico y, en consecuencia, mejora la calidad de vida y el bienestar de los perros.
Palabras clave: Bioquímica; Perros; Ancianos; Geriátrico; Hematología.

1. Introduction

Aging is a natural, non-pathological process, composed of several factors that may be endogenous or exogenous and involve morphological and functional changes to all organs, thus differentiating the current body condition from its condition at previous times of life (Bellows, et al., 2015). Elderly dogs often present a variety of abnormalities in multiple systems that impair their quality of life and, when sick, they may depend on continuous-use medications for chronic diseases (Metzger & Rebar, 2012).

In humans the age phases are well established, but in dogs there is differentiation according to the size and life expectancy of the animal. It has been estimated that senility accounts for 25% of the estimated lifespan of dogs up to the end of life (Creevy, et al., 2019). A study has shown that laboratory abnormalities are common in apparently healthy elderly dogs (Willems, et al., 2016).

Older dogs present reduced phagocytosis by peripheral blood neutrophils and decreased neutrophil response, compared with puppies. This change in innate immunity may contribute to increased morbidity and mortality (Hall, et al., 2010).

Moreover, one of the most frequent findings in elderly dogs is anemia, which may be due to iron deficiency, secondary to chronic kidney disease or even idiopathic disease (Radakovich, et al., 2017).

The vulnerability of the health of elderly dogs emphasizes the importance of performing laboratory tests as part of the routine geriatric evaluation of elderly dogs, with the aim of ensuring their wellbeing (Bellows, et al., 2015). The cause-effect relationship between advanced age among dogs and laboratory alterations is still a matter of debate, although it is a topic of great relevance for clinical veterinary medicine.

The objectives of this study were: 1) to evaluate and compare hematological and biochemical parameters in apparently healthy elderly and geriatric dogs; 2) to conduct follow-up in order to track the evolution of these dogs' health as they aged.

2. Methodology

Subject

We included in the study 34 apparently healthy dogs between 8 and 15 years of age, of both sexes and without defined breeds, that were living in the city of Ribeirão Pires, SP, Brazil. The exclusion criteria were situations of sickness or treatment with medications.

This study was approved by the Animal Use Research Committee of Santo Amaro University, under number 25/2019. A free and informed consent statement was signed and handed over by the keeper of each dog.

Experimental design

The study consisted of observational and quantitative in a non-probabilistic sample for convenience (Marotti, et al. 2008; Zangirolami-Raimundo, et al., 2018). The dogs were divided into two groups according to their age group: group 1 consisted of 18 dogs aged 8 and 11.9 years, named the elderly group; and group 2 consisted of 16 dogs aged 12 years and over, named the geriatric group (Fernandes, et al., 2013).

A blood sample of 5 ml was collected from each dog by means of cephalic vein puncture, after an eight-hour fasting period. Each sample was divided into two parts, placed in a sterile tube with anticoagulant (EDTA K3) and in a sterile tube with coagulation activator (dry tube). Blood glucose was measured through the use of the Freestyle Neo Optium® (Abbott, Ireland).

All the samples were collected between 8 and 11 a.m. and then sent to the Clinical Laboratory of the Veterinary Hospital of Santo Amaro University in isothermal boxes.

In hematological tests, the hemoglobin concentration, globular volume, hematometry, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and range of variation of erythrocytes (red cell distribution width, RDW) were evaluated. Platelet measurements and mean platelet volume (MPV) were determined in an automated cell counter (model BC-2800 Vet®, Mindray, China) (Martins, et al., 2013).

A differential leukocyte count was performed by means of optical microscopy, on blood smears stained using the rapid panoptic method, in accordance with the hematological staining principles established by Romanowsky, acting for 15 seconds.

The biochemical tests performed were urea, creatinine, alkaline phosphatase and alanine aminotransferase, using the Bioplus BIO-200® device (Bioplus, Brazil).

Twenty days later, new blood sampling was performed in order to ascertain whether any differences in test values had appeared (Galvão & Carvalho, 2015).

Statistical analysis

The Levene test was performed to evaluate homoscedasticity, skewness test to analyze symmetry, kurtosis test to assess curve design and Shapiro-Wilk test to check on normality of distribution. These tests confirmed that parametric statistical analysis could be used.

Thus, Student's t test for independent samples was used to compare differences between groups (intergroup analysis) and Student's t test for paired samples was used to compare times (intragroup analysis).

All the statistical tests used were two-tailed and the significance level was established at 5% ($p < 0.05$). The statistical analyses were performed using the SPSS 25.0 software.

3. Results

The mean age of the elderly group was 9.16 ± 1.10 years, minimum = 8.00, maximum = 11.00; while in the geriatric group it was 13.19 ± 1.42, minimum = 12.00, maximum = 15.00.

Analysis between the groups was performed at two times: the first sample collection time and 20 days later. Laboratory analyses from the two samplings were performed immediately after each collection time.

The dogs presented high frequencies of alterations in the tests: 53% in hematological tests and 74% in biochemical tests, in relation to the reference values used.

The hematological values were similar in the two groups (elderly and geriatric) (Table 1).

Table 1. Statistical analysis (Student's t test) on hematological tests among elderly and geriatric dogs, presented as means and standard deviations ($p < 0.05$).

Variables	References	Groups	Mean ± standard deviation	P-value
Hematocrit_1	37 - 55%	Elderly	44.1111 ± 9.08709	0.613
		Geriatric	45.5333 ± 6.35685	
Hematocrit_2	37 - 55%	Elderly	45.2667 ± 8.68057	0.671
		Geriatric	44.0667 ± 6.44168	
Hemoglobin_1	12 - 18 (g/dl)	Elderly	16.4444 ± 3.60232	0.742
		Geriatric	16.8113 ± 2.52407	
Hemoglobin_2	12 - 18 (g/dl)	Elderly	17.0667 ± 3.68465	0.734
		Geriatric	16.6733 ± 2.47544	
Erythrocytes_1	5.5 – 8.5 (x10^6/µl)	Elderly	6.6967 ± 1.36495	0.682
		Geriatric	6.8667 ± 0.89378	
Erythrocytes_2	5,5 - 8,5 (x10^6/µl)	Elderly	6.8467 ± 1.23092	0.653
		Geriatric	6.6627 ± 0.96897	
Mean corpuscular volume_1	60 – 72 (fl)	Elderly	0.65973637 ± 0.043236122	0.794
		Geriatric	0.66377633 ± 0.044616348	
Mean corpuscular volume_2	60 – 72 (fl)	Elderly	0.65957728 ± 0.038584907	0.869
		Geriatric	0.66164233 ± 0.028454447	
Mean corpuscular hemoglobin concentration_1	31 – 34 (g/dl)	Elderly	0.00371844 ± 0.000140243	0.561
		Geriatric	0.00368904 ± 0.000146215	
Mean corpuscular hemoglobin concentration_2	31 – 34 (g/dl)	Elderly	0.00374757 ± 0.000139881	0.444
		Geriatric	0.00378478 ± 0.000122221	
Platelets_1	200 -500 (x10^3/µl)	Elderly	243166.67 ± 88260.477	0.139
		Geriatric	295666.67 ± 110333.693	
Platelets_2	200 -500 (x10^3/µl)	Elderly	274800 ± 88178.553	0.230
		Geriatric	313933.33 ± 86654.047	
Leukocytes_1	6000 – 17000 µl	Elderly	12733.33 ± 4709.69	0.774
		Geriatric	12326.67 ± 2973.806	
Leukocytes_2	6000 – 17000 µl	Elderly	13220 ± 4862.275	0.542
		Geriatric	12320 ± 2869. 594	
Neutrophils_1	55 - 80%	Elderly	72.7778 ± 8.39857	0.626
		Geriatric	71.4667 ± 6.56687	
Neutrophils_2	55 - 80%	Elderly	72.8667 ± 10.75617	0.920
		Geriatric	72.5333 ± 6.9577	
Lymphocytes_1	12 - 30%	Elderly	19.4444 ± 8.88636	0.913
		Geriatric	19.1333 ± 6.9577	

Lymphocytes_2	12 - 30%	Elderly	19 ± 10.94793	0.842
		Geriatric	18.3333 ± 6.74713	
Monocytes_1	3 - 10%	Elderly	6.6111 ± 1.81947	0.060
		Geriatric	8.0667 ± 2.46306	
Monocytes_2	3 - 10%	Elderly	7.1333 ± 2.06559	0.243
		Geriatric	7.9333 ± 1.57963	
Eosinophils_1	2 - 10%	Elderly	1.1667 ± 0.70711	0.510
		Geriatric	1.3333 ± 0.72375	
Eosinophils_2	2 - 10%	Elderly	1.0 ± 1.06904	0.562
		Geriatric	1.2 ± 0.7746	

_1: first evaluation; 2: second evaluation. Source: Jain, (1993); Cowell, (2004); Thrall, (2015); Tesser, et al, (2016).

In the biochemical analysis, it could be seen that this was a homogeneous group of dogs, since the only difference between the elderly and geriatric dogs was found in the variable of urea (elderly > geriatric, p = 0.010) (Table 2).

Table 2. Statistical analysis (Student's t test) on biochemical tests among elderly and geriatric dogs, presented as means and standard deviations ($p < 0.05$).

Variables	References	Groups	Mean ± standard deviation	P-value
Urea_1	7.0 - 28.0 (mg/dl)	Elderly	36.4833 ± 11.50965	0.220
		Geriatric	31.6125 ± 11.12582	
Urea_2	7.0 - 28.0 (mg/dl)	Elderly	42.8167 ± 13.81662	0.010*
		Geriatric	30.0687 ± 13.37429	
Creatinine_1	0.9 - 1.7 (mg/dl)	Elderly	1.0611 ± 0.26153	0.989
		Geriatric	1.0625 ± 0.31172	
Creatinine_2	0.9 - 1.7 (mg/dl)	Elderly	0.9111 ± 0.20548	0.458
		Geriatric	0.8625 ± 0.16683	
Alkaline phosphatase_1	16 - 140 (IU/l)	Elderly	71.75 ± 32.79053	0.310
		Geriatric	83.4938 ± 33.48607	
Alkaline phosphatase_2	16 - 140 (IU/l)	Elderly	126.8944 ± 225.59949	0.460
		Geriatric	84.175 ± 35.15272	
Alanine aminotransferase_1	10 - 120 (IU/l)	Elderly	47.5556 ± 11.09967	0.870
		Geriatric	48.4375 ± 18.55431	
Alanine aminotransferase_2	10 - 120 (IU/l)	Elderly	57.0556 ± 15.18696	0.061
		Geriatric	47.25 ± 14.0594	
Capillary hematocrit_1	37 - 55%	Elderly	47.4444 ± 5.8834	0.424
		Geriatric	45.6667 ± 6.7153	
Capillary hematocrit_2	37 - 55%	Elderly	45.5625 ± 7.76289	0.868
		Geriatric	46 ± 6.66548	
Plasma protein_1	6.0 -8.0 g/dl	Elderly	7.3222 ± 0.58265	0.956
		Geriatric	7.3067 ± 1.01662	
Plasma protein_2	6.0 -8.0 g/dl	Elderly	7.5625 ± 0.82371	0.184
		Geriatric	7.1 ± 1.05898	
Glycemia_1	75 - 130 (mg/dl)	Elderly	64.06 ± 6.655	0.082
		Geriatric	68.88 ± 8.951	
Glycemia_2	75 - 130 (mg/dl)	Elderly	65.28 ± 11.811	0.626
		Geriatric	67.31 ± 12.289	
Weight_1		Elderly	21.8889 ± 5.38439	0.481
		Geriatric	20.175 ± 8.46715	

Weight_2	Elderly	22.2222 ± 6.00094	0.380
	Geriatric	19.9938 ± 8.51215	

_1: first evaluation; 2: second evaluation. Source: Jain, (1993); Cowell, (2004); Thrall, (2015); Tesser, et al, (2016).

4. Discussion

In dogs, senility varies according to the breed and size of the animal. Large and giant dogs are considered elderly between 6 and 8 years and geriatric from 9 years of age onwards, while medium and small dogs are considered elderly between 7 and 10 years and geriatric from 11 years of age onwards (Bellows, et al., 2015). In this study, because the dogs were without defined breed, it was proposed to divide them into two groups. Thus, they were considered elderly if they were between 7 and 11.9 years of age and geriatric if they were 12 years of age and over.

In a study conducted among healthy middle-aged dogs, it was demonstrated that approximately 6% had alterations in hematological and biochemical tests (Dell'Osa & Jaensch, 2016). However, in the present study approximately 53% of the dogs presented alterations in hematological tests and 76% in biochemical tests, although all the dogs were apparently healthy. Nonetheless, even with a high percentage of alterations, to values slightly outside of the references (both higher and lower values), the means for the parameters ended up being borderline, and the values for these parameters of the dogs remained within the reference means for the species.

This divergence in reference values was also seen in a study on laboratory tests, in which on average 27% of the dogs presented changes in hematological values and 30.7% in leukocytes (Tesser, et al., 2016).

Senility gives rise to progressive changes to tissues and cells, which leads to susceptibility to increased occurrence of some diseases, such as heart diseases, renal failure and cognitive dysfunction (Nabi, et al., 2010). Oxidative stress is one of the factors that has been studied in elderly dogs, and this has been correlated with the neurodegenerative process and with neuromuscular decline, which compromise the quality of life of geriatric animas (Rofina, et al., 2006) and may increase liver enzyme levels. However, the animals in this study did not show any difference between the groups.

The most common clinical manifestations among sick geriatric dogs are anorexia, vomiting, diarrhea, weight loss, obesity, vision loss, tooth loss, lethargy, incoordination, coughing, dyspnea, polyuria, urinary incontinence and neoplasia (Srikala, et al., 2019). However, although the animals in the present study were at advanced ages, they did not have any of these symptoms

Geriatric groups of dogs have been seen to have a tendency towards reduced hemoglobin and erythrocyte profiles (Pati, et al., 2015). This was corroborated in another study in which reductions in hematocrit, MCV and lymphocytes were observed with aging, while platelets increased with advancing age (Radakovich, et al., 2017). Moreover, lymphocyte values were found to be lower, while monocytes and platelets were higher in elderly dogs (over 10 years), compared with adults between 2 and 6 years of age; and these changes were correlated with physiological, immunological and biological changes to the body due to aging (Sanjeeta, et al., 2013). This may be due to decreased bone marrow production, splenomegaly and decreased erythrocyte production (Radakovich, et al., 2017). In the present study, this alteration was not observed in relation to elderly and geriatric animals, although all the dogs studied were older than 8 years of age.

The mean urea value was found in another study to be higher in geriatric dogs than in elderly dogs (Radakovich, et al., 2017). That finding differed from what was seen in the present study, in which the urea level was lower in geriatric dogs, but both values were within the reference standards for adult dogs.

In another previous study, serum glucose, GH and IGF-1 presented higher levels in young dogs than in elderly dogs (Lee, et al. 2020). In the present study, there was no difference in blood glucose levels between the groups of dogs. The mean blood glucose level was slightly lower than the reference that was used. This had already been seen in other studies, in which it

was suggested that the reduction was specifically age-related or was due to age-related reduction of hepatic glycogen stores, such that glucose levels became lower in older individuals (Lowseth, et al., 1990; Lee, et al., 2020; Radakovich, et al., 2017). A difference in mean blood glucose levels between healthy young dogs and adults who received the same diet in pre-anesthesia fasting has also been found ($p < 0.05$) (Lecheta, et al., 2019).

In addition to changes in blood tests, a study among 28 healthy dogs showed that older dogs had higher numbers of nuclei per cell in liver cytological analyses ($p < 0.05$), compared with middle-aged and young dogs, which could contribute to biochemical changes (Stockhaus, et al., 2002).

The results from hematological tests were similar during 12 months of follow-up with monthly collections for blood donor dogs (Santos, et al., 2013). In the present study, the samples from the first and second collections, with an interval of 20 days between them, also showed similar values. The only exception was the urea value in the second sample, which was higher in the elderly dogs than in the geriatric dogs, although the values were still within the reference parameters for adult dogs.

In another study, the average survival of dogs at 12 years was estimated. Death was correlated with the presence of clinical signs or reduction in quality of life (Gates, et al., 2017). However, during the present study, none of the dogs showed worsening of their clinical condition or died.

5. Conclusion

It was possible to observe that geriatric dogs showed a reduction in urea levels in relation to elderly dogs. No changes in the mean values of the other parameters, whether hematological or biochemical, were observed between the groups.

Even in apparently healthy dogs, a high frequency of changes in examinations was found. This emphasizes the importance of laboratory evaluations for making a prognosis and early diagnosis, and consequently for improving the quality of life and wellbeing of dogs.

New research is needed in search of physiological improvement in this phase of life of animals, this group intends to advance in this line.

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