

Invited lecture/Scientific contribution/Original research

Brachycephalic Dogs with Brachycephalic Obstructive Airway Syndrome Have Increased Variability in Red Blood Cell Size

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Abstract:

Brachycephalic obstructive airway syndrome (BOAS) is a conformation-related respiratory disorder of dog breeds with congenitally flattened facial and skull anatomy. BOAS is characterized by chronic shortness of breath and subsequent difficulty in exercising, a tendency to overheat, increased and abnormal respiratory noise, and low oxygen levels. The aim of our retrospective study was to investigate the level of red blood cell distribution width (RDW), a biomarker of chronic hypoxemia, in groups of BOAS patients with different degrees of BOAS and a group of healthy non-brachycephalic dogs. Red blood cell distribution width provides information on the variability in the red blood cell volume. It is a simple and inexpensive variable included in the complete blood count report. Seventy-two BOAS patients and 24 non-brachycephalic dogs were included in this retrospective study. Patients with BOAS were classified into grade 1 (13 dogs), grade 2 (27 dogs), and grade 3 (32 dogs) according to the severity of the disease. In our study, a significantly ($p < 0.05$) higher RDW was found in all groups of BOAS patients compared to the non-brachycephalic dog group. However, we found no significant difference in RDW between the groups of BOAS patients. Thus, we may conclude that BOAS patients have increased variability in the size of red blood cells compared with healthy non-brachycephalic dogs. Our results warrant further studies to determine the potential utility of RDW in BOAS and to clarify the role of RDW in BOAS patients in relation to the severity of BOAS and cardiovascular risk.

Keywords: Brachycephaly; Brachycephalic obstructive airway syndrome; Dogs; Haematology; Erythrocytes; Red blood cell distribution width

1. Introduction

In recent years, brachycephalic dogs, which exhibit various characteristics of young animals have become increasingly popular internationally. Their large, round, wide-set eyes and flat, rounded faces resemble those of human infants, making these dogs a pleasure to care for. Common breeds of brachycephalic dogs include English and French bulldogs, Boston terriers and pugs; these breeds are characterised by a severe shortening and widening of the skull that results in narrowing of the airway, making them susceptible to conformational respiratory condition known as brachycephalic obstructive airway syndrome (BOAS) (Fasanella et al., 2010; Meola, 2013; O'Neill et al., 2015; Packer et al., 2015; Dupre and Heidenreich, 2016; Liu et al., 2017). Artificial selective breeding for extreme brachycephaly has resulted in bony changes and upper airway deformation, leading to increased airway resistance caused by excessive soft tissues that has not decreased proportionally with bony changes. Affected dogs present with clinical signs of respiratory dysfunction that may include inspiratory dyspnea, snoring, stertor, stridor, and panting, gastrointestinal signs such as vomiting and regurgitation, and intolerance to stress, exercise, and heat, sleep disturbances, and in severe cases, cyanosis and even syncopal episodes (Fasanella et al., 2010; Roedler et al., 2013; Packer et al., 2015; Dupre and Heidenreich, 2016; Liu et al., 2017). Brachycephalic dog breeds may have additional systemic complications (Meola, 2013). Even when these dogs are systemically healthy, they have been shown to have hypertension, and significantly higher packed cell volume and arterial pCO₂ and significantly lower arterial pO₂, compared to non-brachycephalic dog breeds (Hoareau et al., 2012). Furthermore, hypomagnesemia (Mellema and Hoareau, 2014) and hypercoagulability (Hoareau and Mellema, 2015) have also been demonstrated in clinically healthy Bulldogs. In addition, the presence of a hypercoagulable state (Crane et al., 2017), elevated levels of inflammatory markers (Rancan et al., 2013), and higher levels of cardiac troponin I have been reported in canine patients with BOAS (Planellas et al., 2012).

Brachycephalic obstructive airway syndrome shares features of obstructive sleep apnea syndrome (OSAS) (Hendricks et al., 1987; Hendricks, 1992), which is a highly prevalent breathing disorder in humans, caused by the repetitive collapse of the narrow upper airway during sleep (Eisele, 2015; Lavie, 2015). Obstructive sleep apnea syndrome is characterized by intermittent hypoxia, leading to blood hypoxaemia, hypercapnia, sleep fragmentation, augmented respiratory efforts and increased sympathetic activity. In patients with OSAS, repeated episodes of cessation of breathing, lead to hypoxia and reoxygenation, which results in increased production of reactive oxygen species/reactive nitrogen species (ROS/RNS) and thus oxidative stress. The latter adversely affect the associated cardio-/cerebro-vascular disease in OSAS patients (Eisele, 2015; Lavie, 2015).

Increased values of red blood cell distribution width (RDW) (Ozsu et al., 2012; Leon Subias et al., 2017) and an association between RDW and OSAS severity have been reported not only in adult OSAS patients with and without cardiovascular disease (Sokucu et al., 2014; Leon Subias et al., 2017), but also in children with OSAS (Morell-Garcia et al., 2020). For many years, RDW was used for the differential diagnosis of anemias. Nowadays, RDW is a parameter with numerous clinical applications (Salvagno et al., 2015). It is also considered a biomarker of chronic hypoxemia (Yčas, 2017). Red blood cell distribution width, an index of circulating red blood cell volume heterogeneity (conventionally known as anisocytosis), is included in routine complete blood count reports, making it a simple and inexpensive parameter. The RDW is a coefficient of variation of red cell volume calculated by dividing the standard deviation of red cell volumes by the mean corpuscular volume (MCV) and multiplying by 100 to express the result as a percentage. The RDW thus provides information on the variability of the volume of circulating erythrocytes. In addition, RDW is associated with several serious diseases, including most of those that cause hypoxemia. It has been reported that RDW responded to applied hypoxia (Salvagno et al., 2015; Yčas, 2017). Intermittent hypoxia, which also occurs in OSAS and BOAS patients, stimulates the synthesis and secretion of erythropoietin, which is one of the factors with great impact on the increase of RDW (Salvagno et al., 2015; Yčas, 2017; Li et al., 2018). The RDW has not been reported in BOAS patients. Therefore, our retrospective study aimed to evaluate the RDW in canine patients with different grades of BOAS admitted to the Small Animal Clinic for surgical treatment of BOAS.

2. Methods

2.1. Dogs

The present study is based on a retrospective evaluation of complete blood count reports of 72 client-owned dogs diagnosed with BOAS and 24 healthy dogs of non-brachycephalic breeds that underwent elective surgery. The 24 healthy non-brachycephalic dogs served as controls. These dogs were considered healthy based on normal history, normal clinical examination, and results of hematological and biochemical analyzes. At inclusion to the study, the history of the BOAS patients was obtained using a questionnaire about behavior, health, and lifestyle. Additionally, the owners of BOAS patients completed a preoperative owner questionnaire in order to investigate a wide range of clinical signs (respiratory and gastrointestinal signs, exercise tolerance, and sleep disorders). All dogs that showed signs of concurrent disease or had received any type of therapy or vaccination within the last month were excluded from the evaluation. The diagnosis of BOAS was based on clinical signs of upper airway obstruction and anatomical anomalies, as described elsewhere (Dupre and Heidenreich, 2016; MacPhail and Fossum, 2019). The severity of the disease was classified based on the anatomical anomalies of the airway. Patients were classified as grade 1, grade 2, and grade 3 based on the decrease in the radius of the airway at the level of the nasopharynx, oropharynx, laryngopharynx, and larynx after soft palate surgery (Erjavec et al., 2021). The patients with BOAS were scheduled for surgical treatment under general anesthesia. The overall patients' health status was assessed by history, physical examination, and the results of haematological (complete blood count with white blood cell differential count; only results on RDW are reported in this manuscript), biochemical (data not shown), and venous blood gas analyzes (data not shown).

Written informed consent was obtained from the owners. All procedures complied with the relevant Slovenian governmental regulations (Animal Protection Act, the Official Gazette of the Republic of Slovenia, 43/2007). The study was evaluated and approved by the Ethical Committee on Animal Research of the Veterinary Faculty, University of Ljubljana.

Haematological analyzes were performed within one hour after collection of blood samples using an automated laser-based haematology analyzer (ADVIA 120, Siemens, Munich, Germany) and multispecies software. Data were analyzed using commercial software (IBM SPSS 25.0, Chicago, Illinois, USA). We used the Shapiro-Wilk test to check whether the data were normally distributed. Based on the results of normality tests, the Kruskal-Wallis test followed by multiple comparisons and Mann-Whitney test (non-parametric tests) were used to compare the parameters (RDW, weight, age) between the groups of dogs. A value of $p < 0.05$ was used to determine significance.

3. Results

Baseline data for patients with BOAS and healthy non-brachycephalic dogs are shown in Table 1. No significant difference in weight was found between the control dogs and the groups of BOAS patients. However, control dogs and grade 1 BOAS patients were significantly younger than grade 3 BOAS patients. Regardless of the severity of BOAS, French bulldogs were the most common breed (37/72). Statistical analysis revealed a significantly higher RDW in all groups of BOAS patients (Table 2) compared with the group of non-brachycephalic dogs. However, there was no significant difference in the level of RDW among the three groups of BOAS patients.



Table 1: Baseline data of patients with different grades of brachycephalic obstructive airway syndrome (BOAS) and healthy non-brachycephalic dogs (Control)

	Control	Grade 1	Grade 2	Grade 3	All BOAS patients
Number	24	13	27	32	72
Sex (F/M)	11/13	7/6	16/11	21/11	44/28
Age (months)	15.0	13	31.0	35.0*	30.5
Median (IQR)	11.0 – 40.5	8.5 – 28.5	16.0 – 55.0	19.3 – 67.5	16.4 – 55.8
Weight (kg)	10.8	8.5	10.1	10.0	9.84
Median (IQR)	7.8 – 22.7	6.5 – 12.1	8.8 – 12.6	8.4 – 11.6	8.4 – 11.8
Breeds	Non-brachy	7 FB, 4 ST, 1 EB, 1 BST	13 FB, 6 BST, 6 P, 1 EB, 1 ST	17 FB, 8 P, 6 BST, 1 ST	37 FB, 14 P, 13 BST, 6 ST, 2 EB

*grade 3 patients significantly were older than grade 1 patients ($p = 0.010$) and control dogs ($p = 0.016$). BST: Boston Terrier; EB: English Bulldog; F: female; FB: French Bulldog; IQR: interquartile (25th to 75th percentile) range; M: male; P: Pug; ST: Shih Tzu

Table 2: Red blood cell distribution width (RDW) of patients with different grades of brachycephalic obstructive airway syndrome (BOAS) and healthy non-brachycephalic dogs (Control)

Group	RDW (%) Median; IQR	p values ^a
Reference range ^a	11.9 – 14.5	
Control (n = 24)	12.5; 12.1 – 13.1	
Grade 1 (n = 13)	13.0; 12.9 – 14.0	$p = 0.037$
Grade 2 (n = 27)	13.4; 13.1 – 13.9	$p < 0.001$
Grade 3 (n = 32)	13.1; 12.7 – 13.6	$p = 0.027$
All BOAS patients	13.2; 12.9 – 13.8	$p < 0.001$

*p values indicating the significant difference in comparison to control dogs

^aHematology analyzer Advia 120 (Siemens, Munich, Germany)

IQR: interquartile (25th to 75th percentile) range; n: number of dogs

4. Discussion

In this study, RDW was investigated in brachycephalic dogs with different severity grades of BOAS. We could not compare our results with those of similar studies performed in BOAS patients because to our best knowledge no papers have been published on the subject. Because of the similarity of the two syndromes (Hendricks et al., 1987; Hendricks, 1992), we discussed our results with those obtained in OSAS patients. The results of our study showed that RDW was significantly higher in all groups of BOAS patients than in healthy non-brachycephalic dogs, although the median values were within the reference range. These results suggest that BOAS patients have greater variability in the size of red blood cells compared to controls. Similar results were obtained in OSAS patients (Ozsu et al., 2012; Leon Subias et al., 2017). The higher RDW values in BOAS patients may be due to increased erythropoietin synthesis, as a consequence of the hypoxemia present in these patients (Hendricks et al., 1987; Hendricks, 1992; Hoareau et al., 2012). In addition, a high RDW values in BOAS may be due to other factors, such as inflammation (Lippi et al., 2009; Leon Subias et al., 2017). Intermittent hypoxia is one of the important factors that cause systemic inflammation (Dewan et al., 2015). In BOAS patients, plasma concentrations of pro-inflammatory and anti-inflammatory cytokines and nitric oxide were significantly higher than in control dogs and appeared to be related to disease severity (Rancan et al., 2013).

Some limitations must be considered when interpreting our results. The first and the most important limitation of our study is the lack of erythropoietin measurements, which could help us in the

conclusion of our study. The second limitation is the fact that the the control dogs and patients in BOAS grade 1 were significantly younger than the patients in BOAS grade 3. The third limitation is the gender distribution of BOAS patients. We included more females than males, 44 and 28, respectively.

5. Conclusions

Based on our results, we may conclude that BOAS patients have increased variability in red blood cell size compared with healthy, non-brachycephalic dogs. Our results warrant further studies to determine the potential utility of RDW in BOAS and to clarify the role of RDW in BOAS patients in relation to the severity of BOAS and cardiovascular risk.

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Conflicts of Interest: The authors declare no conflict of interest.

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