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Federal Department of Home Affairs FDHA
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Section **Fields (of activity)**

Do blood tests mirror recurrent dyspnea of brachycephalic dogs? Verification if blood tests are suitable as screening parameters based on clinical findings and of data gathered by whole-body plethysmography.

Aline Steiner[1], Anna Bogdanova[2], Regina Hoffmann-Lehmann[3], Sonja Hartnack[4], Iris Margaret Reichler[1]
[1]Clinic of Reproductive Medicine, [2]Red Blood Cell Research Group, Institute of Veterinary Physiology, [3] Department of Clinical Diagnostics and Services, [4]Section of Epidemiology, Vetsuisse Faculty, University Zurich

Key words
Brachycephaly, respiratory function grading, whole body plethysmography, brachycephalic obstructive airway syndrome severity, red blood cells, blood markers, stress, hypoxia

Aim of the study
i) To evaluate hypoxia and stress markers in the blood of brachycephalic dogs in relation to their severity of Brachycephalic Obstructive Airway Syndrome (BOAS) as assessed by respiratory function grading before and after exercise tests as well as by whole-body plethysmography. ii) To identify blood markers, which are alone or in combination suitable to discriminate BOAS severity grades by analysis of variance, principal component analysis, varrank algorithm and the Caret in R. iii) To propose the best marker or a combination of markers as possible predictors of BOAS severity, offering thereby an alternative to current subjective or invasive screen-ing methods. Screening methods that are easy to carry out but at the same time reliable are necessary to implement the existing legal requirements in breeding and thus assure animal welfare.

Material and methods
Privately owned French bulldogs, at least 1 year of age and without circulatory, orthopedic, and respiratory dsease other than BOAS were recruited. On presentation, after a detailed history and a general clinical examination, a blood sample was taken and transferred into coded blood tubes. The code enabled later blind analysis of the data. Hematology and biochemistry profiles were performed, additionally, reticulocyte counts and maturation status as well as redox state markers, deformability, RBC hydration and membrane osmotic stability were analyzed. Following blood sampling, respiratory function was assessed by i) whole body plethysmography (WBBP) with the dog sitting in a transparent Perspex chamber$_1$ and ii) a standardized grading of respiratory signs before and after exercise tests, i.e., a 5-minute walking test and a 3-minute trotting test.[2] The anatomical proportions of the head and body as well as the opening width of the nostrils were assessed using standardized soft-tape measurements and the standardized grading of Liu et al.[3] Four degrees of BOAS signs (absent (0), mild (1), moderate (2), severe (3)) were distinguished. Absent and mild BOAS signs were grouped as clinically unaffected (BOASΘ), moderate and severe signs as clinically affected (BOAS⊕).[4]

Results and significance
Of the 102 dogs that were recruited, 84 completed the study. Slightly more than half of the dogs were grouped BOASΘ according to their respiratory function analysis before and after exercise test: 13 dogs showed no respiratory noise (BOAS 0) and 31 dogs only mild, moderate intermittent stertor or nasal whistle (BOAS 1). The remaining 40 dogs were grouped to BOAS⊕, however, only a minority of them (n=5) were severely affected (BOAS 3). This might have affected the validity of the statistical analyses. Most of the dogs (n=35) showed moderate respiratory signs before and/or moderate to severe respiratory signs after the trotting test (BOAS 2). Among the anatomical features studied, an ordered logistic regression identified more stenotic nostrils, a larger distance between the eyes, a broader skull, and a shorter neck as risk factors for BOAS.
Unfortunately, the initial analysis of the WBBP data with the Cambridge web application resulted in implausible results (LIU, personal communication) and therefore could not be used for BOAS grading as intended. Instead,

a quadratic discriminant analysis of the WBBP data using the BOAS status, i.e., BOAS⊕ versus BOASΘ, resulting from the trotting test was performed and resulted in a model with 79.1% accuracy of classification. Thus, it appears that cut-off values need to be specific to the settings and populations in which they are used. For WBBP to be used as an independent measure of BOAS status, calibration in a larger data set would be required. Consequently, in contrast to our study design, BOAS grading based only on ET. A clear association between blood parameters and BOAS-grade was only shown for carboxyhemoglobin (CO-HB): Progressing severity of BOAS was associated with an increase in CO-HB (P=0.034), while at the same time, hemoglobin oxygenation only tends to drop. Hemoglobin binds CO much stronger than oxygen, thereby blood oxygen-carrying capacity and delivery to the tissues might be reduced. Increased CO-HB concentrations have been reported in humans with respiratory impairment [5] and higher levels of CO-HB were found in non-survivors than in survivors of SARS-COV-2 infection.[6] These higher CO-HB levels could be explained by reduced CO elimination but also by increased CO production. The impaired breathing of the patients reduces CO-elimination, while the oxidative stress-mediated inflammatory state of the patients favors release of heme oxygenase and thereby increased endogenous CO production. Heme oxygenase activity is also triggered by increased or early lysis of red blood cells; thus, CO-HB is used as a marker for hemolysis. The potential of CO-HB as marker in inflammatory pulmonary disease and also as a prognostic tool in humans was investigated. [7] However, in both, humans with COVID and dogs with BOAS, further research is needed to establish CO-HB as a predictive marker. Multiple parameters in our large data set showed only a trend of association with the BOAS severity grade. To reduce the number of variables, while preserving as much information as possible, we performed Principal component analysis (PCA). The initial dataset included animal characteristics including BOAS grade and the most promising blood parameters. Reticulocyte count was the most prominent contributor to the variance and was removed from further analysis to allow differentiating the contributions of other parameters. The contribution of various parameters into components 1 and 2 was more balanced. Three clusters of parameters with more prominent contributions into component 1 could be identified. One, including O2-Hb and CO-Hb was associated with O2 carrying capacity of blood, the other with redox state of red cells (GSH and Mbbr fluorescent intensity) and the third one, associated with RBC hydration state (Omin and Ohyper). The contribution of mean platelet volume and monocyte count into the first component was smaller than that of the other parameters. We performed additionally varrank analysis to select the most representative variable and to rank the variables of our dataset with respect to BOAS grading: The redox parameters (GSH and fluorescence intensity of Mbbr) were listed on the top, followed by CO-HB and the reticulocytes' count and properties (maturation state and hemoglobin). Furthermore, the caret model, which was applied to build predictive relationships between blood parameters and BOAS-severity scoring, revealed as most important O2-HB, followed by mean platelet volume and CO-HB. Three different statistical approaches thus identified overlapping sets of parameters as most relevant.

Concluding our results: Unfortunately, none of the investigated blood parameters were alone or in combination suitable to discriminate BOAS severity grades in one single breed. The diversity of affected breeds might even increase the high variances found in the analyzed blood parameters in the French bulldog. Much more data are needed to determine whether blood values are ultimately suitable as predictors of BOAS-severity, which is a prerequisite to the targeted selective breeding for healthier phenotype in brachycephalic breeds. According to our study results in French bulldogs, promising candidates are CO-hemoglobin, reticulocyte counts, and their maturation as well as RBC redox markers. To avoid the animal welfare-related consequences of extreme brachycephalic breeding, we recommend for the time being, that at least the French bulldog, the pug and the English bulldog undergo ET by trained veterinarians analogous to the Respiratory Function Grading Scheme of the Kennel Club and University of Cambridge's, prior to both dog shows and breeding. As soon as reference values are presented for other brachycephalic breeds, screenings should also be implemented for those. Veterinarians should take a stand against the torture breeding problem and follow the Guidelines for reproductive medicine services and surgery in dogs and cats [8] to which the veterinarians working in reproductive medicine for dogs and cats at the veterinary faculties in Germany, Austria and Switzerland have committed themselves.

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Project 2.21.02

Project duration *1.2.2021-31.10.2023*