Whole-Body Barometric Plethysmography Characterizes Upper Airway Obstruction in 3 Brachycephalic Breeds of Dogs

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Background: A novel test using whole-body barometric plethysmography (WBBP) was developed recently to diagnose brachycephalic obstructive airway syndrome (BOAS) in unsedated French bulldogs.

Hypothesis/Objectives: The hypotheses of this study were: (1) respiratory characteristics are different between healthy non-brachycephalic dogs and brachycephalic dogs; and among pugs, French bulldogs, and bulldogs; and (2) obesity and stenotic nares are risk factors for BOAS. The main objective was to establish a diagnostic test for BOAS in these 3 breeds.

Animals: A total of 266 brachycephalic dogs (100 pugs, 100 French bulldogs, and 66 bulldogs) and 28 nonbrachycephalic dogs.

Methods: Prospective study. Exercise tolerance tests with respiratory functional grading, and WBBP were performed on all dogs. Data from WBBP were associated with functional grades to train quadratic discriminant analysis tools to assign dogs to BOAS+ and BOAS- groups. A BOAS index (0–100%) was calculated for each dog. Receiver operating characteristic (ROC) curves were used to evaluate classification ability.

Results: Minute volume was decreased significantly in asymptomatic pugs ($P = .009$), French bulldogs ($P = .026$), and bulldogs ($P < .0001$) when compared to nonbrachycephalic controls. Respiratory characteristics were different among breeds and affected dogs had a significant increase in trace variation. The BOAS index predicted BOAS status for each breed with 94–97% (95% confidence interval [CI], 88.9–100%) accuracy (area under the ROC curve). Both obesity ($P = .04$) and stenotic nares ($P = .004$) were significantly associated with BOAS.

Conclusions and Clinical Importance: The WBBP can be used as a clinical tool to diagnose BOAS noninvasively and objectively.

Key words: Brachycephalic obstructive airway syndrome; Quadratic discriminant analysis; Respiratory function test; Whole-body barometric plethysmography.

Brachycephalic obstructive airway syndrome (BOAS) is common among extremely brachycephalic breeds of dogs.[1,2] Physical examination, history, and lesion assessment under sedation or general anesthesia are used to diagnose BOAS.[3] These methods however are either subjective or invasive, which creates difficulty when evaluating disease progression and the effectiveness of treatment in a clinical setting. Hence, development of new methods for non-invasive and objective measurements of respiratory function in affected dogs is crucial.

The use of pneumotachographs along with analysis of tidal breathing flow volume loops (TBFVL), as well as the forced oscillation technique, have allowed pre-

Abbreviations:

AIC	Akaike's information criterion
BCS	body condition score
BOAS	brachycephalic obstructive airway syndrome
BW	body weight
CI	confidence interval
EMMS	electromedical measurement systems
ETT	exercise tolerance test
MV	minute volume
OR	odds ratio
PEF	peak expiratory flow rate
PENH	enhanced pause
PIF	peak inspiratory flow rate
QDA	quadratic discriminant analysis
ROC	receiver operating characteristic
RR	respiratory rate
RT	relaxation time
SD	standard deviation
TBFVL	tidal breathing flow volume loops
Te	expiratory time
Ti	inspiratory time
TV	tidal volume
WBBP	whole-body barometric plethysmography

vious measurement of respiratory function in conscious dogs and detection of airway obstructions.[4–6] However, these techniques require use of a tight-fitting facemask attached to the pneumotachograph, which is particularly difficult to apply to a brachycephalic dog's muzzle without having air leakage or causing stress in untrained dogs. Whole-body barometric plethysmography (WBBP) is a non-invasive technique of measuring respiratory function that has been validated and utilized

in unrestrained unsedated experimental mice to characterize respiratory patterns during sleep and wakefulness.[7–10] This technique has also been used in experimental and clinical studies using dogs and cats for pharmacological studies and respiratory disorders.[11–21] Our previous study on French bulldogs showed that respiratory airflow characteristics obtained from WBBP are distinguishable between BOAS-affected and clinically healthy French bulldogs.[18] Affected dogs showed high variations in respiratory parameters caused by dynamic obstructions and multiple lesion sites. Quadratic discriminant analysis (QDA) was developed as a classifier using the respiratory data obtained from WBBP and the BOAS index that was proposed can be used for diagnostic and screening purposes.

In addition to French bulldogs, pugs and bulldogs also are reported to be highly predisposed to BOAS.[1,2,22,23] Although the differences in anatomy and BOAS lesion sites among the 3 extremely brachycephalic breeds have been investigated recently,[24–27] studies on respiratory characteristics in different brachycephalic breeds are limited. A previous study investigated the differences in respiratory parameters among healthy nonbrachycephalic dogs, BOAS-affected and clinically healthy bulldogs and Boston terriers.[5] Another study compared respiratory parameters between healthy nonbrachycephalic dogs and BOAS-affected dogs (bulldogs, pugs, Bordeaux dogs, and Shar-Pei dogs) before and after upper airway corrective surgery.[11] Both studies identified significant differences in respiratory variables between healthy nonbrachycephalic dogs and BOAS-affected brachycephalic dogs, but did not show differences in most respiratory variables between clinically healthy (or BOAS-affected dogs postoperatively) and BOAS-affected brachycephalic dogs. Brachycephalic characteristics that have been associated with BOAS include stenotic nares and obesity.[1,28–30] Their effects on respiratory function, however, have not yet been assessed.

The questions asked in this study were: (1) whether respiratory characteristics are distinguishable between affected and nonaffected dogs of different breeds, and among the 3 extremely brachycephalic breeds; and (2) whether obesity and stenotic nares are risk factors for BOAS. A major purpose of this study was to develop a noninvasive diagnostic test for BOAS.

Materials and Methods

Animals

Our prospective study included pugs, French bulldogs, and bulldogs referred for upper airway consultation to the Queen's Veterinary School Hospital (QVSH), University of Cambridge (termed "clinical dogs"), as well as pet dogs of the selected 3 breeds that were volunteered by UK owners and breeders between September 2011 and June 2015 (termed "study dogs"). A detailed history of each dog was taken from owners including type, severity, frequency and circumstances of occurrence of respiratory signs. Exclusion criteria included age <1 year, previous upper airway surgery, history and clinical findings of lower airway disease, or some combination of these. Nonbrachycephalic dogs referred to the QVSH for reasons other than respiratory disease, and staff-owned

dogs were included in the study as controls. All control dogs underwent physical examination by the investigators to rule out airway disease. Dogs that were on medications that may change respiratory parameters (eg, prednisolone, other anti-inflammatory drugs) were excluded from the study. Work was performed under informed ethical consents CR62 and CR63 from the Department of Veterinary Medicine, University of Cambridge.

Methods

Respiratory Functional Grading and Risk Factor Assessments for BOAS. Each dog was graded for functional severity of BOAS using a previously established 4-point functional grading system (Table 1) based on clinical evaluation before and after a 3-minute exercise tolerance test (ETT).[18] BOAS functional *Grade 0* dogs (asymptomatic, BOAS free) and *Grade I* dogs (mild BOAS, dog shows mild respiratory noise but exercise tolerance is unaffected) were considered clinically healthy for their breed. *Grade II* dogs (moderate BOAS, dog requires medical attention such as weight control, surgical intervention or both) and *Grade III* dogs (severe BOAS, dog requires immediate surgical intervention) were considered clinically affected. These results were used in further training of the computational classifier.

In addition to body weight measurement, a standard assessment of body fat (body condition score [BCS] on a 1–9 point scale)[31] was performed on each dog. A BCS ≥ 7 was categorized as obese. Severity of nostril stenosis was examined. Open or mild stenotic nares were considered normal for the breeds, whereas moderate or severe stenosis was defined as "stenotic nares" (Fig 1).

Non-Invasive Respiratory Function Test Using Whole-Body Barometric Plethysmography. Whole-body barometric plethysmography was performed using 2 barometric chambers: ElectroMedical Measurement Systems (EMMS) model PLY370 (inner volume 175 L) for small dog breeds and model PLY360 (inner volume 280 L) for middle-sized to large breeds.[a] The chambers were equipped with 4 ports (pneumotachographs) on the upper surface. A balanced bias airflow of room air (20 L/min) was supplied (bias flow regulator BFL0404, EMMS) to maintain the O_2 concentration and prevent CO_2 accumulation. A CO_2 concentration sensor was equipped at the side of the chamber. One pole of a pressure transducer (TRD5701, EMMS) was open to the main chamber and the other pole was open to a reference chamber (chamber PLY370) or to the exterior room environment (chamber PLY 360). Transducer signals were amplified using a strain gauge amplifier and were sampled by a commercial software.[b] Calibration of the chamber pressure signal was performed dynamically before each test by injecting 50 mL of room air into the chamber and integrating under the resultant flow curve. The flow reading was tested by continuous cycles of injecting and withdrawing 10 mL, 20 mL, 30 mL of air into and from the chamber. The calibration procedure was repeated if tidal volume reading errors exceeded ±10%. The flow was measured via pseudoflow, with the animal completely unrestrained inside the chamber. The pressure transducer measured the pressure increase caused by the added temperature and humidity of the inspired air on inspiration, and the pressure decrease caused by cooling on expiration (minus any small changes caused by oxygen, CO_2, and water vapor exchange across the lung surface). This pressure difference, again measured via a reference environment, was directly proportional to flow. The pseudoflow signals were analyzed to obtain respiratory parameters including: respiratory rate (RR, breaths/minute), inspiratory time (Ti, s), expiratory time (Te, s), tidal volume (TV, mL), minute volume (MV = TV × RR, mL), peak inspiratory flow rate (PIF, mL/s), peak expiratory flow rate (PEF, mL/s), relaxation time (RT, time point when 65% of TV was expired), pause ([Te-RT]/RT), and enhanced pause (PENH = [PEF/PIF] × pause).

Table 1. Functional grading system of brachycephalic obstructive airway syndrome (BOAS) based on respiratory signs before and after an exercise tolerance test (ETT)

		Respiratory Noise[a]	Inspiratory Effort[b]	Dyspnea/Cyanosis/Syncope[c]
Grade 0	Pre-ETT	Not audible	Not present	Not present
	Post-ETT	Not audible	Not present	Not present
Grade I	Pre-ETT	Not audible or mild	Not present	Not present
	Post-ETT	Mild	Not present to mild	Not present
Grade II	Pre-ETT	Mild to moderate	Mild to moderate	Not present
	Post-ETT	Moderate to severe	Moderate to severe	Mild dyspnea; cyanosis or syncope not present
Grade III	Pre-ETT	Moderate to severe	Moderate to severe	Moderate to severe dyspnea; may or may not present cyanosis. Inability to exercise.
	Post-ETT	Severe	Severe	Severe dyspnea; may or may not present cyanosis or syncope.

The grading system was established previously.[18] The clinical grading was based on respiratory signs before (pre-ETT) and immediately after a 3-minute exercise tolerance test (post-ETT) with trotting speed of approximately 4–5 miles/hour performed by the study investigators. Presentation of at least one sign in the highest grade determines the final grading result.

[a]Respiratory noise was diagnosed by pharyngolaryngeal auscultation. Mild: only audible under auscultation; moderate: intermittent audible noise that can be heard without stethoscope; severe: constant audible noise that can be heard without stethoscope.

[b]An abnormal respiratory cycle characterized by evidence of increased effort to inhale the air with the use of diaphragm and/or accessory muscles of respiration and/or nasal flaring with an increase in breathing rate. Mild: regular breathing patterns with minimal use of diaphragm; moderate: evidence of use of diaphragm and accessary muscles of respiration; severe: marked movement of diaphragm and accessary muscles of respiration.

[c]Dogs that have had episodes of syncope and/or cyanosis as documented by owner's report are classified into Grade III without ETT. Mild dyspnea: presents sign of discomfort; moderate dyspnea: irregular breathing, signs of discomfort; severe dyspnea: irregular breathing with signs of breathing discomfort and difficulty in breathing.

Detailed protocols, data acquisition of variables, and data processing using WBBP for unrestrained, unsedated conscious dogs have been described previously.[18] Briefly, after clinical examination, each dog was placed in the chamber. Acclimatization (5–10 minutes) was followed by a recording period of 20 minutes. Dogs that were intolerant to the procedure (ie, showed signs of anxiety) after 2 attempts were excluded from the study. Each breath cycle was recognized automatically by the eDacq software.[b] All respiratory cycles that had differences >20% between inspiratory and expiratory volumes were excluded automatically. Periods of body movement, sniffing, or vocalization that caused artifacts were identified using the recorded surveillance video and eliminated manually. Twenty representative, consecutive, resting breaths during wakefulness were collected for further data analysis. An ETT for functional grading was performed after WBBP testing to avoid possible alterations of baseline respiratory function after exercise.

Classifier Design for Discrimination between Grade 0/I and Grade II/III Dogs. The classifier design was based on the model established for French bulldogs in our previous study.[18] Briefly, the 4-point functional grades of dogs were used as classes in training the QDA-based classifier.[32] The QDA generates a quadratic decision surface in the feature space to separate classes. In a probabilistic setting where 4-class QDA corresponds to minimum-error-probability classification of new samples into four multivariate Gaussian classes, this value reflects the relative prior probabilities of the 4 classes. A predictive index, the BOAS index, was generated by modeling the caudal probabilities obtained from a 4-class QDA model using 6 variables: means and standard deviations (SDs) of 3 ratios of WBBP parameters (ie, Te/Ti_m; Te/Ti_sd; PEF/PIF_m; PEF/PIF_sd; MV/BW_m and MV/BW_sd) for the 20 breaths obtained from each dog.

The parameters were used in the following Equation (1):

$$\delta_k(x) = \frac{1}{2}\log|\Sigma_k| - \frac{1}{2}(x - \mu_k)^T \Sigma_k^{-1}(x - \mu_k) + \log \pi_k \quad (1)$$

where Σ_k are the covariance matrices, μ_k are the parameter means and π_k are the prior probabilities for the different grades in the training set (data published in Data S1). The matrix x

contains the measured parameters of the actual dog so that the equation results in a matrix $\delta_k(x)$ containing logarithmic probabilities. This matrix then is corrected to contain normal probability values.

$$\delta_k(x)^{corr} = e^{\delta_k(x) - \max_k \delta_k(x)} \quad (2)$$

The final caudal probabilities are calculated using the normalized values in $\delta_k(x)^{corr}$.

$$p_k(x) = \frac{\delta_k(x)^{corr}}{\sum \delta_k(x)^{corr}} \quad (3)$$

The final caudal probability values (p_k) are calculated in percentage format for the 4 grades ($p_0 + p_I + p_{II} + p_{III} = 100\%$). The caudal probabilities are weighed to suggest relative severity of the disease. For this study, the interval of disease severity was assumed to be equal. Hence, the BOAS index (I_{BOAS}) is derived as:

$$I_{BOAS} = \frac{1}{3}p_I + \frac{2}{3}p_{II} + p_{III} \quad (4)$$

Note that p_0 is omitted from the equation because it is multiplied by 0 during the weighting.

The BOAS index ranges from 0 to 100%. The initial cut-off point of BOAS index was set at 50% to discriminate between BOAS− (ie, BOAS index <50%) and BOAS+ (ie, BOAS index ≥50%) after a binary classification. The cut-off values then were refined based on receiver operating characteristic (ROC) curves. Three breed-specific models and 1 general model were built:

- Model (PD): breed-specific model based on 100 pugs
- Model (FB): breed-specific model based on 100 French bulldogs
- Model (BD): breed-specific model based on 66 bulldogs
- Model (PFB): general model based on 266 brachycephalic dogs (100 pugs, 100 French bulldogs, and 66 bulldogs).

Statistical Analysis

Differences in subject characteristics among groups were tested using nonparametric Mann–Whitney tests. Associations of age,
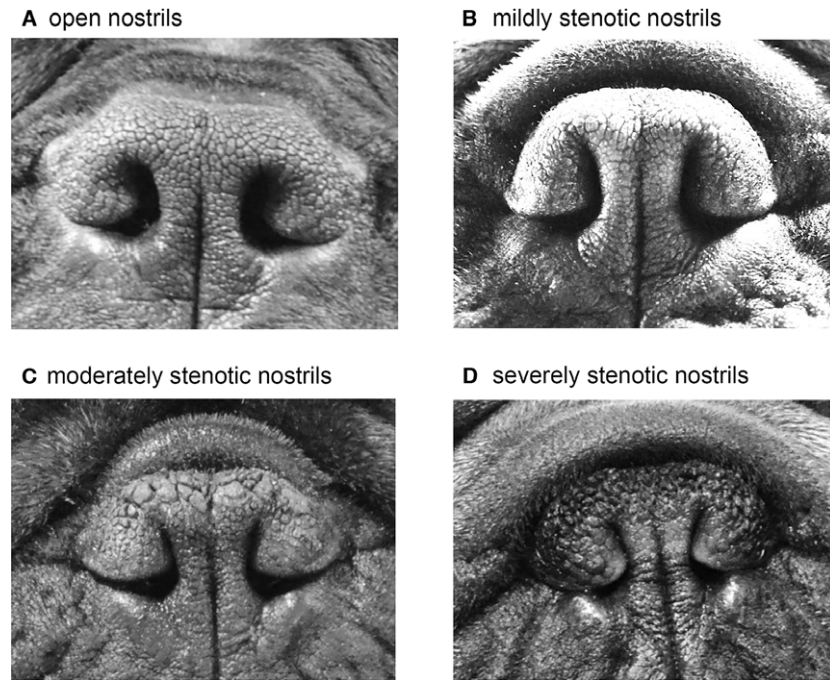
Fig 1. Definition of the degree of nostril stenosis in brachycephalic dogs. Representative nostrils of French bulldogs with different degrees of stenosis. (**A**) Open nostrils: nostrils are wide open; (**B**) Mild stenotic nostrils: slightly narrowed nostrils but the lateral nostril wall does not touch the medial nostril wall. Immediately after the exercise tolerance test (ETT), the nostril wings should move dorsolaterally to open on inspiration. (**C**) Moderately stenotic nostrils: the lateral nostril wall touches the medial nostril wall at the dorsal part of the nostrils and the nostrils are only open at the bottom. Immediately after the ETT, the nostril wings are not able to move dorsolaterally and there may be nasal flaring (ie, muscle constraction around the nose trying to enlarge the nostrils); (**D**) Severely stenotic nostrils: nostrils are almost closed. The dog may switch to oral breathing from nasal breathing with stress or very gentle exercise such as playing.

sex, obesity, and nostril stenosis with BOAS status (normal and clinically affected) were assessed using forward stepwise logistic regression. For each WBBP parameter, means and SDs of 20 representative breaths from each dog were calculated. The normality of the data within groups was assessed using descriptive statistics and the Kolmogorov–Smirnov test. Homogeneity of variance was evaluated using Levene's test. Variables of the nonbrachycephalic controls and Grade 0 brachycephalic dogs were compared using nonparametric Mann–Whitney tests. A simple 3-way QDA was used to discriminate the respiratory characteristics (Te/Ti_m, Te/Ti_sd, PEF/PIF_m, PEF/PIF_sd, MV/BW_m, and MV/BW_sd) among the 3 Grade 0 brachycephalic breeds. To compare WBBP data obtained for the 2 groups of BOAS functional Grade 0/I and Grade II/III dogs, a multi-level mixed linear regression model was used. Breed was included as a random effect encompassing both body size and morphometric differences among breeds. Model fit was assessed using Akaike's information criterion (AIC). Other predictors such as obesity (yes/no; yes = BCS 7–9; no = BCS 3–6), age (years), and sex (male/female) were tested as fixed effects or covariates. Bonferroni correction was used for adjustment for multiple comparisons except in table 3, where raw P values are indicated. The statistic analyses were performed with a commercial statistical software.[c] Significance was set at $P < .05$.

The diagnostic value of the BOAS index was assessed by calculating the area under each ROC curve. Performance metrics were computed over 2,000 bootstrap samples of the whole dataset to generate 95% CI to delineate the expected range of classifier performance. Diagnostic accuracy was estimated using sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios. The cut-off values selected from the ROC curves were those that best identified BOAS+ animals where the sensitivity and specificity were approximately equal. Computations for data pro-

cessing, feature extraction, QDA, bootstrap resampling, and ROC construction were implemented using the packages "MASS," "verification," and "caret" available in the R Project.[d]

Results

Subjects and Clinical Assessments

Characteristics of the subjects are summarized in Table 2. In total, 294 dogs were recruited including 28 nonbrachycephalic controls and 266 brachycephalic dogs (100 pugs, 100 French bulldogs, and 66 bulldogs). Of the 266 brachycephalic dogs, 44 were "clinical dogs," and the remainders were "study dogs." Body condition score (BCS) was found to be significantly higher in pugs ($P < .0001$) and bulldogs ($P = .003$) compared to nonbrachycephalic controls. This was particularly so in pugs, where 62% of dogs were diagnosed as obese (ie, BCS ≥ 7). Stenotic nares were found in >50% of pugs and French bulldogs, and approximately 40% of bulldogs. The prevalence of Grade II/III BOAS among the "study dog" population was 60% of pugs, 46% of French bulldogs, and 40% of bulldogs.

Comparison of Respiratory Parameter Measurements and Risk Factor Analysis

Respiratory parameters from nonbrachycephalic controls and Grade 0 brachycephalic dogs are presented in Table 3 and Fig 2A.

Table 2. Characteristics of the study subjects (n = 294).

	Pugs	French Bulldogs	Bulldogs	Nonbrachycephalic Controls[a]
Dog number	100	100	66	28
Clinical dogs[b]/study dogs[c]	18/82	20/80	6/60	0/28
Female %/intact %	54%/76%	62%/68%	65.2%/94%	60.71%/60.71%
Age (years)	3.13 [1–12.25]	2.5 [1–10.5]	1.83 [1–10.5]	2.75 [1–12]
Body weight (kg)	8.4 [4.6–14.4]	11.5 [8–17]	24.9 [15–32]	11.62 [6–27]
BCS (1–9), Obesity[d]%	7 [4–9][ee], 62%	5 [3–7], 13%	6 [4–8][e], 36.4%	5 [4–6], 0%
Stenotic nares %	58.2%[ee]	66.7%[ee]	40.9%[ee]	0%
Functional Grade	Grade 0: 7%	Grade 0: 10%	Grade 0: 15.2%	Grade 0: 100%
	Grade I: 26%	Grade I: 34%	Grade I: 40.9%	
	Grade II: 50%	Grade II: 41%	Grade II: 28.8%	
	Grade III: 17%	Grade III: 15%	Grade III: 15.2%	
Prevalence of BOAS in study dogs	59.8% (CI$_{95}$: 48.9–69.7%)	46.3% (CI$_{95}$: 35.8–57.1%)	40% (CI$_{95}$: 28.6–52.6%)	0%

Data are presented as median [minimum–maximum]. CI$_{95}$ = 95% confidence interval.

[a]Breeds of control dogs: Border collie (n = 1), Cairn terrier (n = 1), Cross (n = 4), Jack Russell terrier (n = 4), King Charles spaniel (n = 1); Springer spaniel (n = 2); Beagle (n = 6); West Highland white terrier (n = 1); miniature Schnauzer (n = 2); King Charles spaniel (n = 1); Labrador retriever (n = 3); American bullterrier (n = 1); Dachshund (n = 1).

[b]Clinical dogs: dogs that were referred to the Queen's Veterinary School Hospital for upper airway corrective surgery.

[c]Study dogs: dogs from owners who participated in the study voluntarily. The dogs may or may not present clinical signs of brachycephalic obstructive airway syndrome (BOAS).

[d]BCS = body condition score; obesity defined here as BCS ≥7.

[e]Significantly different from nonbrachycephalic control dogs at $P < .01$ or at [ee]$P < .001$.

In general, MV/BW_m in all the Grade 0 brachycephalic breeds was significantly decreased ($P = .009$ in pugs; $P = .026$ in French bulldogs; $P < .0001$ in bulldogs) when compared to nonbrachycephalic controls. Te/Ti_m was significantly lower in Grade 0 French bulldogs ($P < .0001$) and bulldogs ($P < .0001$), but not in pugs ($P = .284$), when compared to nonbrachycephalic controls. RR_m was significantly higher ($P = .043$) in Grade 0 French bulldogs when compared to controls.

Most of the individual respiratory variables in Grade 0 bulldogs were significantly different from those of the other 2 brachycephalic breeds, whereas respiratory characteristics in Grade 0 pugs and Grade 0 French bulldogs were more similar. Only Te/Ti_sd was significantly different ($P = .005$). Altogether, the classification results for respiratory characteristics from 3-class QDA using 6 variables (ie, Te/Ti_m, Te/Ti_sd, PEF/PIF_m, PEF/PIF_sd, MV/BW_m, and MV/BW_sd) show that the Grade 0 dogs of the 3 breeds display distinct respiratory characteristics. All dogs could be accurately classified into the correct breed (Data S2) using the QDA tool. Obesity was found in 29% and 20% of the Grade 0 pugs and bulldogs, respectively. None of the nonbrachycephalic controls and Grade 0 French bulldogs was obese. Stenotic nares only were found in 1 of the Grade 0 pugs. None of the Grade 0 French bulldogs and bulldogs had stenotic nares.

Figure 3 illustrates representative examples of baseline WBBP flow traces in different breeds with varying BOAS grades. The WBBP flow traces were clearly distinguishable between Grade 0/I and Grade II/III brachycephalic dogs. Grade 0/I dogs had smoother flow traces with relatively equal inspiratory and expiratory phases (Fig 3B). In Grade II/III dogs, the waveforms varied among breeds and also among the affected individuals within breeds (Fig 3C). Affected bulldogs commonly had dynamic obstructions with high frequency airflow fluctuations over respiratory cycles. Affected French bulldogs showed a range of different waveforms, but most of them had additional clearly identifiable waveforms where the inspiratory phase was extremely restricted with noticeable airflow fluctuations and a high peak at the early stage of expiration, followed by a gradual tapering (Fig 3C, French bulldog Type B).

Analysis of respiratory parameter measurements of Grade 0/I and Grade II/III dogs is shown in Table 4. The relationship between BOAS status and respiratory parameters varies significantly across the different breeds as shown by a decrease in AIC index when the effect of breed was added into the models. TV/BW_m, MV/BW_m, PIF/BW_m, PEF/BW_m, and PEF/PIF_m were significantly greater in Grade II/III dogs compared to the Grade 0/I dogs. SDs of all respiratory parameters were greater in Grade II/III dogs. This is reflected by the much larger scatter of breath parameters for grade III dogs, which is graphically illustrated in Fig 2B when compared to Fig 2A as well as intrabreed comparisons in Fig 2C–E. RR_m and Te/Ti_m were not significantly different between Grade 0/I and Grade II/III dogs.

Among the three brachycephalic breeds, obesity ($\beta = 0.64$, odds ratio [OR] = 1.90, 95%CI = 1.03–3.50, $P = .04$), male sex ($\beta = 0.90$, OR = 2.47, 95% CI = 1.35–4.53, $P = .004$), and stenotic nares ($\beta = 1.81$, OR = 6.09, 95%CI = 3.40–10.89, $P < .0001$) were positively associated with BOAS Grade II/III. There were

Table 3. The baseline respiratory parameters in nonbrachycephalic control dogs and Grade 0 pugs, French bulldogs, and bulldogs.

	Nonbrachycephalic controls (n = 28)	Grade 0 pugs (n = 7)	Grade 0 French bulldogs (n = 10)	Grade 0 bulldogs (n = 10)
Obesity (%)	0%	28.57%	0%	20%
Stenotic nares (%)	0%	14.29%	0%	0%
RR_m	20.81 (16.95–25.02)[‡]	22.50 (16.81–24.1)	23.16 (21.82–29.85)*	23.46 (19.81–28.13)
TV/BW_m	11.64 (9.71–12.85)[‡‡,§§§]	10.17 (8.55–13.11)[§§]	8.59 (7.83–10.32)**	6.66 (6.05–8.18)***,[††]
MV/BW_m	233.42 (224.56–254.69)[††,‡,§§§]	217.57 (190.57–219.95)**,[§§]	211.14 (190.33–235.11)*	176.97 (140.52–183.51)***,[††,‡‡]
Te/Ti_m	1.37 (1.28–1.55)[‡‡‡,§§§]	1.29 (0.83–1.66)	0.90 (0.82–1.12)***	0.95 (0.81–1.20)***
PIF/BW_m	16.40 (13.72–18.11)[‡‡‡,§§§]	13.68 (12.55–15.92)[§§]	11.90 (10.07–12.80)***	9.83 (8.10–10.56)***,[††,‡]
PEF/BW_m	13.42 (10.92–14.75)[§§§]	12.15 (10.99–14.84)[§§]	11.92 (11.19–13.46)	8.38 (7.07–9.94)***,[††,‡‡]
PEF/PIF_m	0.83 (0.75–0.89)[‡‡,§]	0.89 (0.81–1.12)	1.02 (0.96–1.10)*	0.90 (0.83–0.98)*,[‡]
RR_sd	2.14 (1.49–2.76)	2.85 (2.09–3.79)	2.37 (2.05–3.09)	2.73 (1.38–3.27)
TV/BW_sd	1.45 (1.22–1.82)[§§]	1.73 (1.17–1.85)[§]	1.35 (1.06–1.62)	0.82 (0.69–1.06)**,[†,‡]
MV/BW_sd	24.64 (15.09–31.19)[§]	23.95 (13.92–28.10)	25.23 (16.32–37.02)	14.52 (11.31–22.56)*,[‡]
Te/Ti_sd	0.24 (0.17–0.32)[‡,§]	0.32 (0.22–0.36)[‡,§]	0.16 (0.13–0.24)*,[††]	0.14 (0.11–0.22)*,[†]
PIF/BW_sd	1.89 (1.51–2.65)[‡,§§]	1.85 (1.40–2.70)[§]	1.42 (1.20–1.86)*	1.05 (0.79–1.74)**,[†]
PEF/BW_sd	1.93 (1.48–2.55)[§]	2.39 (1.90–2.72)[§]	1.92 (1.57–2.41)	1.33 (0.86–1.92)*,[†]
PEF/PIF_sd	0.11 (0.09–0.12)[†]	0.19 (0.08–0.21)*	0.13 (0.12–0.17)	0.11 (0.08–0.14)

Data are presented as median with interquartile range.

RR = respiratory rate (breath/minute); Te/Ti = expiratory time (s)/inspiratory time(s); PEF/PIF = peak expiratory flow rate (ml/s)/peak inspiratory flow rate (mL/s); MV/BW = minute volume (mL)/body weight (kg); m = mean of the parameter calculated from the 20 breaths of each dog; sd = standard deviation of the parameter calculated from the 20 breaths of each dog.

*Significantly different from the non-brachycephalic controls P(raw) < .05; **P(raw) < .01; ***P(raw) < .0001.

[†]Significantly different from the Grade 0 pugs at P(raw) < .05; [††]P(raw) < .01.

[‡]Significantly different from the Grade 0 French bulldogs at P(raw) < 0.05; [‡‡]P(raw) <0.01; [‡‡‡]P(raw) < 0.001.

[§]Significantly different from the Grade 0 bulldogs at P(raw) < 0.05; [§§]P(raw) <0.01; [§§§]P(raw) < 0.001.

no interactions between any of the factors. Obesity had a negative effect on the means of all the volume-related respiratory parameters (ie, TV/BW_m, MV/BW_m, PIF/BW_m, PEF/BW_m), but had no significant effect on the SD of any respiratory parameters. Age was not significantly associated with BOAS status. Stenotic nares were significantly associated with BOAS status in Pugs (β = 1.46, OR = 4.3, 95%CI = 1.69–10.97, P = .002), French bulldogs (β = 2.97, OR = 19.56, 95% CI = 5.48–69.80, P < .0001), and bulldogs (β = 1.15, OR = 3.147, 95%CI = 1.05–9.45, P = .011). After adjusting for the stenotic nares, male dogs were more likely to be affected if they were French Bulldogs (β = 1.82, OR = 6.17, 95%CI = 1.68–22.67, P = .006) or Bulldogs (β = 1.50, OR = 4.49, 95%CI = 1.44–14.05, P = .01), but not if they were pugs.

Quadratic Discriminant Analysis (QDA) to Classify BOAS Status

Classification results using the BOAS index for each model are presented in Table 5 and Data S3. The ROC curves derived from each BOAS index are shown in Fig 4. Each of the 3 breed-specific models, Model (PD), Model (FB) and Model (BD), had better classification results than the general model, Model (PFB). Nevertheless, positive predictive values in all models were >90%, and all final ROC curves had good or excellent discrimination: area under the curve (AUC) = 91.2% (95%CI: 87.5–94.8%) for Model (PFB), AUC = 93.9% (95%CI: 88.9–98.9%) for Model (PD), AUC = 97.2% (95%CI:

94.1–100%) for Model (FB), and AUC = 97.0% (95% CI: 91.2–100%) for Model (BD). The best cut-off points for the breed-specific models (BOAS index = 55.38% in pugs, 49.41% in French bulldogs, and 43.53% in bulldogs) were similar to the original classification setting (BOAS index = 50%), whereas the cut-off points on Model (PFB) for each breed varied (49.90% in pugs, 66.37% in French bulldogs, and 31.11% in bulldogs). The overlap of BOAS index between Grade 0/I and Grade II/III dogs in this model can be clearly seen in Data S3, but is decreased by specifying different cut-off points for each breed.

Discussion

We have developed a tool that allows quantification of BOAS severity utilizing the respiratory variables obtained from WBBP in unrestrained brachycephalic dogs. The baseline respiratory characteristics were significantly different among asymptomatic pugs, French bulldogs, bulldogs, and nonbrachycephalic breeds. In addition, the proposed BOAS index can distinguish BOAS-affected and clinically healthy brachycephalic dogs using breed-specific models. Obesity and stenotic nares in brachycephalic dogs are highly associated with BOAS.

Ours is the first study on respiratory function in BOAS-predisposed breeds that recruited a large number of both affected and clinically healthy dogs. BOAS has a high prevalence in all 3 breeds, but it often is unrecognized. In this study, approximately 40–50% of the
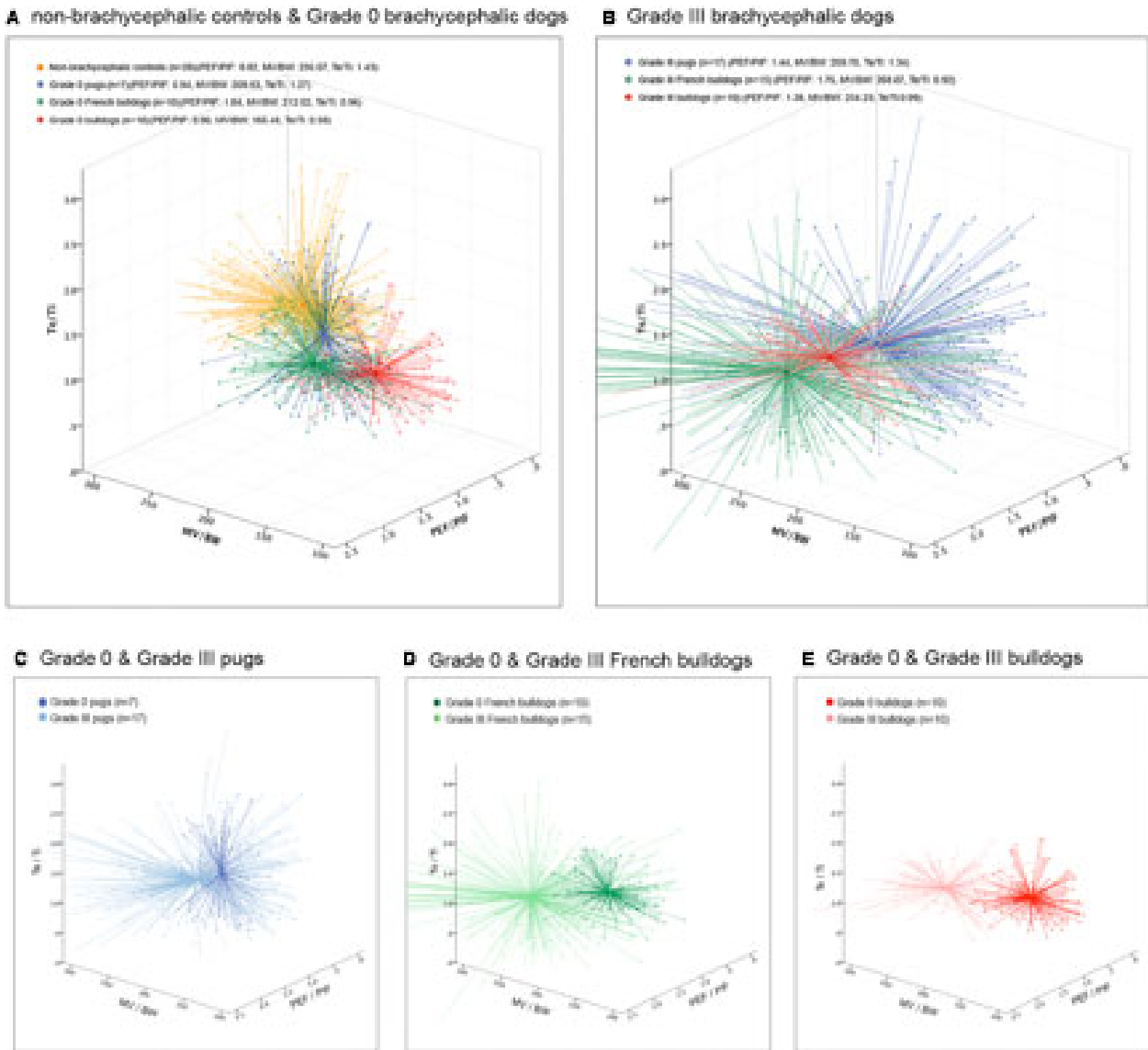
Fig 2. Breaths plotted against three selected respiratory parameters. Twenty representative breaths per dog (represented as crosses), assigned colors for each group with a marked centroid (co-ordinates of each axis). (**A**) Nonbrachycephalic control dogs vs Grade 0 brachycephalic dogs; (**B**) Grade III brachycephalic dogs to be compared with Grade 0 groups in (**A**); (**C**)–(**E**), breed-specific comparisons between Grade 0 and Grade III dogs. PEF/PIF = peak expiratory flow rate (mL/s)/peak inspiratory flow rate (mL/s); MV/BW = minute volume (mL)/bodyweight (kg); Te/Ti = expiratory time (s)/inspiratory time (s).

pet dogs in the study group that were moderately or severely affected had not been treated for the disease. French bulldogs, pugs, and bulldogs recently have become extremely popular worldwide and all were listed among the top 10 breeds registered with the United Kingdom Kennel Club in 2014. These breeds have experienced a great increase in annual registration in the last 10 years with 343% increase in pugs, 2,985% increase in French bulldogs, and 199% increase in bulldogs.[33] Respiratory function in bulldogs and French bulldogs has been characterized in a small number of dogs previously.[5,18] However, pugs have not been investigated and these 3 extremely brachycephalic breeds have not been compared.

Respiratory Characteristics Are Different among NonBrachycephalic Dogs, Pugs, French Bulldogs, and Bulldogs

In this study, Grade 0 bulldogs and French bulldogs showed a decrease in mean MV/BW and mean Te/Ti. They also had higher PEF/PIF compared to nonbrachycephalic dogs. Similar findings were obtained when breathing patterns in healthy nonbrachycephalic dogs and clinically healthy bulldogs and Boston terriers were investigated.[5] Grade 0 pugs showed relatively similar respiratory characteristics to nonbrachycephalic controls. By contrast, Grade 0 bulldogs showed changes in respiratory characteristics that indicate upper airway
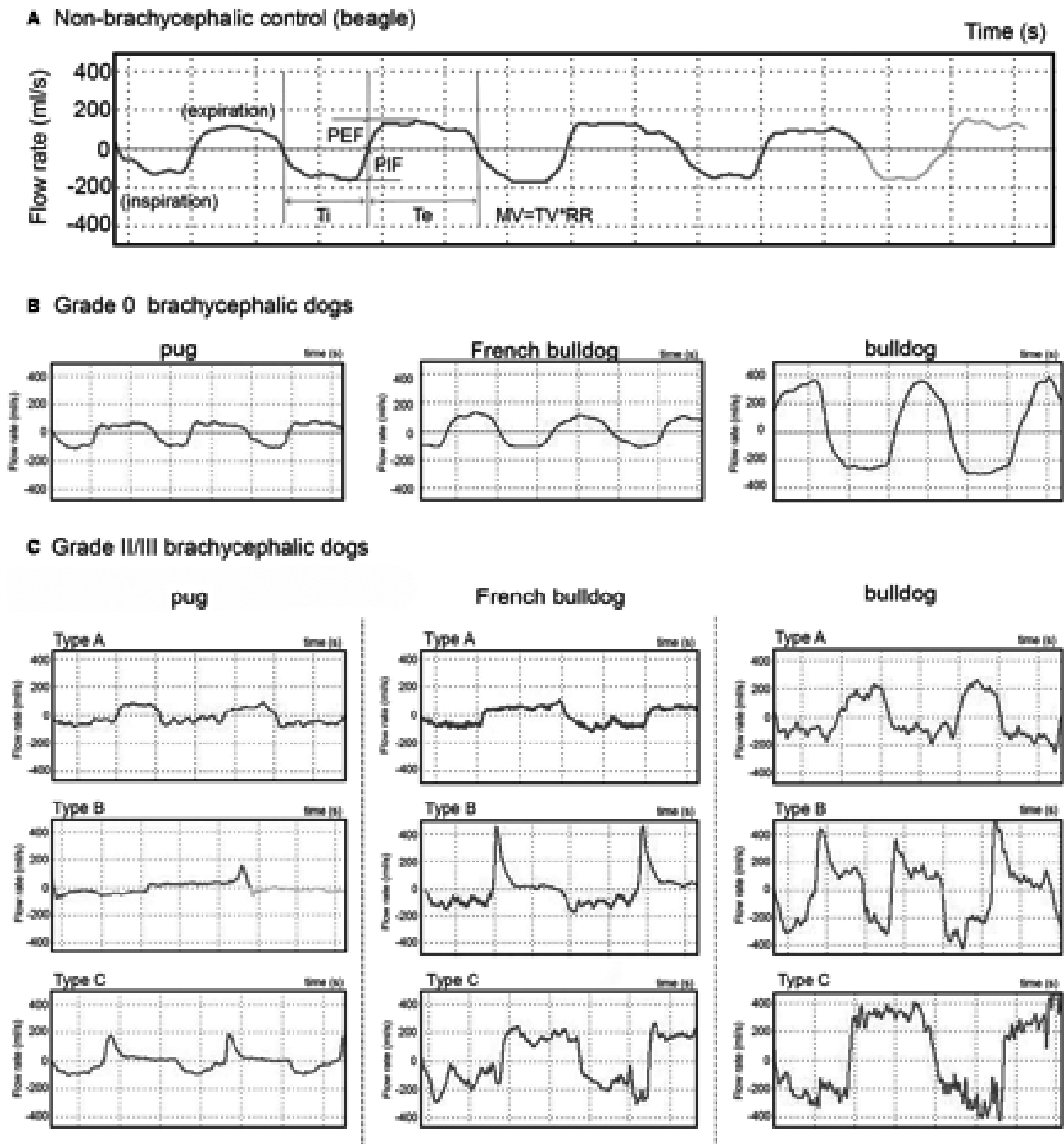
Fig 3. Respiratory flow trace samples. (**A**) Flow traces of a nonbrachycephalic control; (**B**) Flow trace samples of Grade 0 brachycephalic dogs; (**C**) Flow trace samples of Grade II/III brachycephalic dogs, showing variations in amplitude, flow pattern, and noise within the trace. Take French bulldog as an example, Type A shows extremely low amplitude when compared to Type B and C; however, the peak flow rates of inspiration and expiration are equal while they are significantly different in Type B. Noise, low amplitude high frequency fluctuations can be seen in all three types mainly during inspiration.

restrictions and dynamic obstructions. We looked further at the differences among the 3 brachycephalic breeds. A multivariable classification method using QDA showed that the respiratory characteristics are clearly distinguishable. Grade 0 bulldogs showed lower minute volume and slower flow rate during both inspiration and expiration when adjusted for weight, and relatively higher variations in most of the variables. Differences in respiratory characteristics reflect the anatomical differences among skull types and among brachycephalic breeds. Therefore, breed-specific models were developed in this study. Clinically, these Grade 0 dogs are considered "asymptomatic for BOAS." However, there is very limited evidence about whether the

Table 4. The differences in respiratory parameters between BOAS functional Grade 0/I and Grade II/III brachycephalic dogs, and the effect of obesity on respiratory parameters.

	Grade 0/I Brachycephalic Dogs (n = 114)[a]	Grade II/III Brachycephalic Dogs (n = 152)[b]
Obesity (%)	28.95%	43.42%
Stenotic nares (%)	31.37%	74.65%
RR_m	22.46 ± 5.03	22.12 ± 4.57
TV/BW_m	9.25 ± 2.49	10.28 ± 3.33**·†
MV/BW_m	195.81 ± 31.73	218.60 ± 67.18***·††
Te/Ti_m	1.08 ± 0.27	1.10 ± 0.31
PIF/BW_m	11.77 ± 2.52	13.58 ± 4.46***·††
PEF/BW_m	11.17 ± 2.66	17.58 ± 8.46**·††
PEF/PIF_m	0.96 ± 0.14	1.32 ± 0.43*
RR_sd	2.74 ± 0.94	3.12 ± 1.16**
TV/BW_sd	1.44 ± 0.58	1.95 ± 0.82***
MV/BW_sd	22.87 ± 8.49	35.15 ± 15.09***
Te/Ti_sd	0.21 ± 0.10	0.39 ± 0.15***
PIF/BW_sd	1.64 ± 0.76	2.39 ± 1.25***
PEF/BW_sd	1.79 ± 0.74	3.78 ± 2.14**
PEF/PIF_sd	0.13 ± 0.06	0.30 ± 0.16**

A linear mixed model was used with level 1 individual dog and level 2 breeds (random effect). Data are presented as mean ± standard deviation.

RR = respiratory rate (breath/minute); Te/Ti = expiratory time (s)/inspiratory time(s); PEF/PIF = peak expiratory flow rate (mL/s)/peak inspiratory flow rate (mL/s); MV/BW = minute volume (mL)/body weight (kg); m = mean of the parameter calculated from the 20 breaths of each dog; sd = standard deviation of the parameter calculated from the 20 breaths of each dog.

[a]Grade 0/I pugs = 33, Grade 0/I French bulldogs = 44, Grade 0/I bulldogs = 37.

[b]Grade II/III pugs = 67, Grade II/III French bulldogs = 56, Grade II/III bulldogs = 29.

*Significantly different from the BOAS− dogs at $P < .05$; **$P < .01$; ***$P < .001$.

†Obesity has a significant negative effect on the respiratory parameter at $P < .05$; ††$P < .01$.

respiratory changes seen in clinically healthy brachycephalic dogs will cause long-term secondary effects on health such as gastrointestinal disorders, metabolic changes, or other problems.[34,35] Large studies on gastrointestinal disorders in healthy brachycephalic dogs are needed. Hypomagnesemia and hypercoagulation were found in clinically healthy bulldogs compared to nonbrachycephalic control dogs and boxers.[36,37] Hypertension also was reported in systemically healthy pugs, Boston terrier, French bulldogs, and bulldogs.[38] These findings support the argument that the bulldog is a natural model for sleep apnea and hypopnea syndromes in humans with similar metabolic changes.[29]

Respiratory Characteristics in Grade II/III pugs, French Bulldogs, and Bulldogs

Grade II/III dogs are "awake snorers" with increased respiratory noise after exercise and labored breathing. Loss of constancy in the breathing pattern is an obvious change in respiration in BOAS (Fig 3C). In Grade II/

III dogs, the breathing appears more chaotic and requires continuous adjustment, which contrasts with the consistent airflow patterns seen in Grade 0/I dogs (Fig 3B). The TBFVL of BOAS-affected bulldogs has been recorded.[5] The study commented that the most common loop shape in brachycephalic dogs was characterized by a flattened (fixed-type obstruction) or a rounded expiratory phase (nonfixed-type) with a flattened inspiratory phase. The loop often contained bursts of high frequency flow oscillations during inspiration and occasionally during expiration. The WBBP flow traces of a bulldog were collected before and after surgery,[11] and were similar to the Type B Bulldog BOAS traces in this study. So far, interpretation of the flow waveforms in BOAS-affected dogs remains unclear. As can be seen in Fig 3C, flow waveforms of Grade II/III dogs in all 3 breeds are not uniform. Nevertheless, 1 of the advantages of using QDA is that its quadratic boundary allows inclusion of different types of traces into the models.

The BOAS Index is an Useful Tool to Discriminate Objectively between Affected and Clinically Healthy Pugs, French Bulldogs, and Bulldogs

Marked variations of respiratory characteristics in Grade II/III dogs, not only among breeds but also within breeds, were observed in this study. Such variations were taken into account in the QDA classifier, which finds a novel application in characterizing respiratory flow traces. The QDA is a classic classifier with a quadratic decision surface, generated by fitting class conditional densities to the data and using Baye's rule to perform predictions.[32] In our previous study on French bulldogs, a training group, approximately 60 dogs, was evaluated by a test dataset and found to have good sensitivity and specificity.[18] We thus used a minimal number of 60 dogs to train the QDA classifier. We further used the caudal probabilities generated from QDA to calculate a predictive BOAS index. The BOAS index proposed here is a numeric scale to quantify the relative severity of BOAS. Our previous study of 89 French bulldogs showed that once the model is trained, QDA could accurately classify new dogs.[18] After a preliminary test using a test dataset (20% of the total dog number), an internal permutation test was performed to validate the final models presented in this study. The finding that the discriminant performance is better when we use breed-specific instead of general models is consistent with the differences that exist in the respiratory pattern and anatomy of the 3 breeds. Positive predictive values are all >94% in the 3 breed-specific models. The protocol to collect WBBP data, process the data, and generate the BOAS index is straightforward and can be widely used in general practice with minimal staff training.

Obesity and Stenotic Nares are Associated with BOAS

Obese brachycephalic dogs have a higher risk of being BOAS-affected, and obesity has a significant effect

Table 5. Classification results of BOAS− and BOAS+ in pugs, French bulldogs, and bulldogs using quadratic discriminant analysis (QDA).

	Model (PD)	Model (FB)	Model (BD)	Model (PFB)
Prevalence	67%	56%	43.94%	57.14%
	(CI$_{95}$: 56.88–76.08%)	(CI$_{95}$: 45.72–65.92%)	(CI$_{95}$: 31.74–56.70%)	(CI$_{95}$: 50.96–63.17%)
Sensitivity	88.06%	94.64%	89.66%	80.92%
	(CI$_{95}$: 77.82–94.70%)	(CI$_{95}$: 85.13–98.88%)	(CI$_{95}$: 72.65–97.81%)	(CI$_{95}$: 73.76–86.83%)
Specificity	93.94%	93.18%	100%	92.98%
	(CI$_{95}$: 79.77–99.26%)	(CI$_{95}$: 81.34–98.57%)	(CI$_{95}$: 90.51–100%)	(CI$_{95}$: 86.64–96.92%)
Positive predictive value	96.72%	94.64%	100%	93.89%
	(CI$_{95}$: 88.65–99.60%)	(CI$_{95}$: 85.13–98.88%)	(CI$_{95}$: 86.77–100%)	(CI$_{95}$: 88.32–97.33%)
Negative predictive value	79.49%	93.18%	92.50%	78.52%
	(CI$_{95}$: 63.54–90.70%)	(CI$_{95}$: 81.34–98.57%)	(CI$_{95}$: 79.61–98.43%)	(CI$_{95}$: 70.63–85.12%)
Positive likelihood ratio	14.53	13.88	N/A*	11.53
	(CI$_{95}$: 3.78–55.83)	(CI$_{95}$: 4.65–41.46)		(CI$_{95}$: 5.89–22.59)
Negative likelihood ratio	0.13	0.06	0.10	0.21
	(CI$_{95}$: 0.07–0.24)	(CI$_{95}$: 0.02–0.17)	(CI$_{95}$: 0.04–0.30)	(CI$_{95}$: 0.15–0.29)

Model (PD): breed-specific model based on 100 Pugs. Model (FB): breed-specific model based on 100 French Bulldogs. Model (BD): breed-specific model based on 66 Bulldogs. Model (PFB): general model based on 266 brachycephalic dogs (100 Pugs, 100 French Bulldogs, and 66 Bulldogs).

CI$_{95}$ = 95% confidence interval.

*Not calculable as specificity = 1.

on decreasing TV/BW and MV/BW as well as PIF/BW and PEF/BW. Flow limitation during both inspiratory and expiratory phases suggests that obesity worsens respiration in brachycephalic dogs. An increase in soft tissue abutting the fixed bony structures results in a decreased airway lumen and increased stiffness of the respiratory system, which limits lung expansion.[39,40] Similarly, experimental beagles had significantly decreased TV/BW and significantly increased RR after being fed to obesity.[19] The TV/BW often is decreased in severely obese humans, and breathing follows a rapid, shallow pattern with significant decreases in PEF/BW.[39,41] We have not separated the effects of obesity and other conformational effects associated with brachycephaly on respiratory function because there were insufficient obese Grade 0 dogs in each breed. Therefore, further study into changes in respiratory function after weight loss in brachycephalic dogs is warranted. In this study, obesity was defined based on BCS. In human medicine, in addition to the use of body mass index as an indication of obesity when investigating associations between obesity and obstructive sleep apnea, measurements of waist circumference (central obesity), neck circumference, deposition of fat around specific parts of the body such as neck or the base of the tongue were reported.[42–46] Additional studies on the predictive obesity-related parameters that may increase the risk of developing BOAS are required.

Until now, it has been difficult to distinguish in BOAS the functional consequences of each individual anatomic change associated with brachycephaly. Our study shows that severe stenosis of nares in brachycephalic dogs is a very important contributor to (by restricting airflow), or consequence of BOAS (through further collapse after a period of chronic high negative pressure within the airway). For French bulldogs in particular, the risk of BOAS in dogs with stenotic nares increases about 20 times. This breed previously has been shown to be at particular risk of mucosal contact points between the plica recta and nasal septum.[28] The Starling resistor model[47] equates airway function to a hollow tube with a constriction within the nasal cavity and near the nostrils, and a caudal collapsible segment, the oropharynx. This model predicts that a nose obstruction upstream will generate a negative intraluminal pressure downstream at the oropharynx, resulting in pharyngeal collapse.[48] Even when the dog breathes orally, the majority of inspired air passes through the nose, and the expired air goes through the mouth, both during shallow thermal panting and deep panting.[49,50] Therefore, nasal obstructions not only restrict the airflow during nasal breathing at rest but also affect thermal regulation during panting in dogs.

Limitations

This study has several limitations. First, the prevalence of BOAS observed here reflects a mixed group of clinical cases and volunteer dogs and may not represent the true prevalence in the 3 breeds. However, it does not affect the aim of this study because both the numbers of affected dogs and clinically healthy dogs are sufficient for QDA. Second, we have not separated the effects of obesity and BOAS on respiratory function because there were insufficient obese Grade 0 dogs in each breed. Therefore, further study of the changes that occur in respiratory function after weight loss in brachycephalic dogs is warranted. Third, only the clinical dogs had computed tomography scans of the thorax to exclude lower airway disease. Invasive diagnostic assessments (eg, radiography, CBC, and serum biochemistry panel) on volunteer study dogs were not possible because of ethical considerations. For these dogs, lower airway disease was ruled out based on history
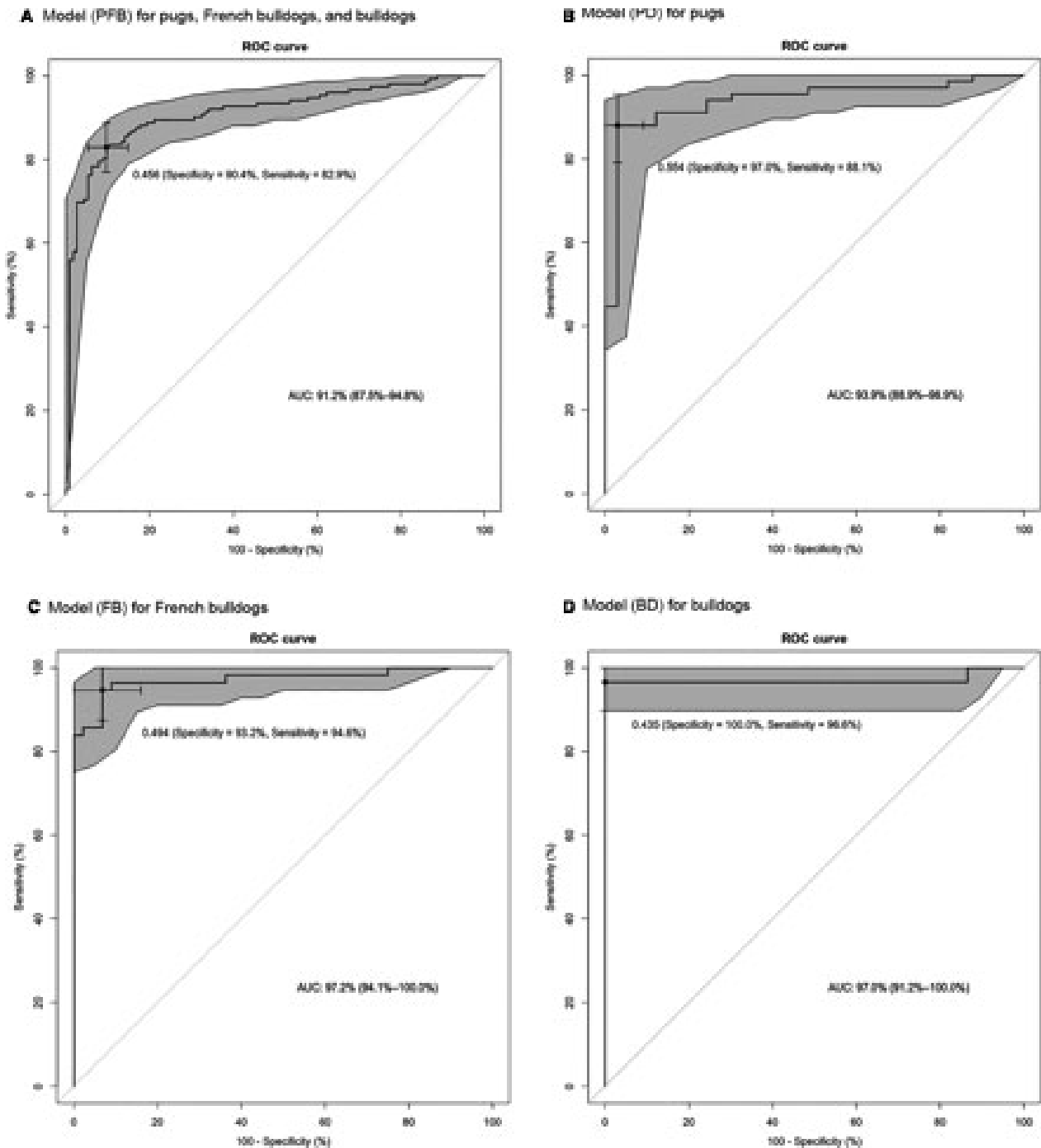
Fig 4. Receiver operating curves (ROC) assess the classification accuracy on diagnostic models for brachycephalic obstructive airway syndrome. The area between 95%CI for the curve is shaded. A cross and the associated values show the cut-off values chosen for use in the diagnostic test. AUC, area under the curve.

and chest auscultation. Mild underlying lower airway compromise may affect ventilatory variables. Nevertheless, the proposed classifier closely reflects overall functional respiration as seen in the ETT, and so is of considerable value to the owners of tested animals. Fourth, we deliberately chose dogs that were ≥1 year old after consideration of maturity in terms of body dimension and respiratory physiology. However, there

are limited studies on changes in respiratory physiology with age in dogs. Therefore, whether the model can be applied to puppies will require a cohort investigation. Last, only 3 brachycephalic breeds were included in the study. Classification models for other commonly affected breeds such as Pekingese, Japanese Chin, Boston terriers, and Shih-Tzu might require further validation.

Conclusions and Relevance of the Study

In conclusion, breed-specific WBBP-based QDA classification tools for pugs, French bulldogs, and bulldogs showed excellent discrimination accuracy between Grade 0/I and Grade II/III dogs. The use of a BOAS index represents a large advance over the subjective criteria previously used to identify BOAS. This testing has the potential to facilitate clinical practice in monitoring disease progression and evaluating surgical outcome after upper airway surgery.[51] The use of QDA may be relevant to other respiratory function studies that require characterization of respiratory flow traces.

Footnotes

Acknowledgments

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Data S1. Input data for QDA models.

Data S2. Classification results of respiratory waveforms in Grade 0 pugs, French bulldogs, and bulldogs using quadratic discriminant analysis (QDA).

Data S3. The positions of all individuals, by breed and BOAS status measured by clinically supervised exercise tolerance test, compared with their caudal probabilities on the breed specific and combined breed BOAS indices..