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# Tidal breathing flow volume loop analysis of 21 healthy, unsedated, young adult male Beagle dogs

KK Adamama-Moraitou,[a] D Pardali,[a]* G Menexes,[b] LV Athanasiou,[c] G Kazakos[d] and TS Rallis[a]

**Background** Reference values for dogs regarding tidal breathing flow volume loop (TBFVL) parameters are scarce in the veterinary literature, so each new study requires a healthy reference population to be studied simultaneously with the diseased one.

**Objectives** To establish reference values for TBFVL parameters in healthy dogs, to detect any potential variability in loop shape and in various TBFVL parameters and to propose clinically useful parameters for TBFVL analysis.

**Design** Cross-sectional, prospective study.

**Methods** Twenty-one healthy, unsedated, untrained, young adult, male Beagle dogs, with minimum variability in body weight and somatometry were used. Their good health status was confirmed by physical examination, complete blood count, serum biochemistry, urinalysis, serology and parasitology for *Dirofilaria immitis*, faecal examination, arterial blood gas analysis, electrocardiography, and radiographic and endoscopic examinations of the respiratory tract. The shape of the TBFVLs was assessed initially. Volume, time and flow parameters, as well as their ratios, were calculated; in total 44 parameters were evaluated. Statistical indices, including Cronbach's $\alpha$, discrimination index, coefficient of variation (CV) and 95% confidence intervals were estimated for each parameter.

**Results** One consistently reproducible type of TBFVL shape was identified that had a similar appearance to the letter D. Statistical analysis showed that only two parameters were found to have Cronbach's $\alpha$ lower than 0.80. The CV for the TBFVL parameters ranged from 1.5% to 49%, but the vast majority had values lower than 20%. Eight parameters had very low CV, indicating increased homogeneity.

**Conclusions** A large number of clinically applicable TBFVL parameters were identified. Parameters related to flow and time were considered to correlate more objectively to the functional capacity of the respiratory system of healthy, unsedated dogs.

**Keywords** dogs; pulmonary function tests; reference values

**Abbreviations** AUC, area under the curve; CV, coefficient of variation; DI, discrimination index; EF, expiratory flow rate; FEV, forced expiratory volume; IF, inspiratory flow rate; MEFV, maximum expiratory flow-volume; MIFV, maximum inspiratory flow-volume; PEF, peak expiratory flow rate; PIF, peak inspiratory flow rate; RR, respiratory rate; TBEV, tidal breathing expiratory volume; TBFVL, tidal breathing flow-volume loop; TE, expiratory time; TI, inspiratory time; TTOT, total respiratory time; VPEF, volume until peak expiratory flow; $V_T$, tidal volume

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Pulmonary function tests are used routinely in human medicine to evaluate the type and severity of respiratory disease, to decide on clinical management strategies and assess the response to treatment (conservative or surgical) over time.[1] In general, adult patients are willing and able to cooperate and complete the forced/maximum expiratory and/or inspiratory manoeuvres that are necessary to accomplish the maximum expiratory and inspiratory flow-volume (MEFV, MIFV) curves. However, human infants, as well as severely decompensated adult patients, may be uncooperative, unable to coordinate or feel discomfort because of the procedure or their disease, which prevents them from accomplishing a maximum-effort manoeuvre.[2] The use of tidal flow patterns has been proposed for the diagnosis of airway narrowing and laryngotracheal disease.[2,3]

In veterinary medicine, the evaluation of the tidal breathing flow volume loop (TBFVL) was initially proposed for the clinical assessment of airway obstruction in conscious dogs.[4] Since then, various studies have been conducted and reported in the veterinary literature on the use of TBFVL for the diagnosis and/or management of canine upper or lower respiratory diseases.[5–10]

As with any other diagnostic tests, the findings of TBFVL analysis should be compared with established normal findings,[11] which are created by the application of a given diagnostic test in a healthy representative population.[12] In the veterinary literature, reference values for TBFVL parameters in canine patients are scarce,[1] so in all veterinary studies the authors have used a healthy population to create their own database of reference values. However, this is not always adequate, because healthy populations are sometimes difficult to find and in most of the studies the epidemiological data (age, weight, sex, breed, environmental and living conditions) of the healthy population differed significantly from that of the animals suffering from the disease studied.[11]

The aims of the present cross-sectional, prospective study were to establish reference values for TBFVL parameters for healthy, unsedated and untrained Beagle dogs of the same age, sex and living conditions, with a very low variability in body weight and somatometric measurements, to detect any potential variability in loop shape

*Corresponding author.
[a]Companion Animal Clinic (Medicine), Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, 11Stavrou Voutira str., 54627, Thessaloniki, Greece; didipardali@yahoo.gr
[b]Laboratory of Agronomy, School of Agriculture, Aristotle University of Thessaloniki, University Campus, Thessaloniki, Greece
[c]Clinic of Medicine, School of Veterinary Medicine, University of Thessaly, Karditsa, Greece
[d]Companion Animal Clinic (Anaesthiology and Intensive Care), Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

and various TBFVL parameters, and to propose clinically useful parameters for TBFVL analysis.

## Materials and methods

The study population consisted of 21 intact male, purebred Beagle dogs, 2 years of age, weighing between 12 and 20.3 kg (mean ± SD 15.59 ± 2.34 kg) and living in the same environmental conditions. All the study dogs belonged to the research colony of the School of Veterinary Medicine, University of Thessaly. Use of these animals was in compliance with the European Communities Council Directive 86/609/EEC and Greek laws. The experimentation on these animals was approved by State authorities.

The health status of all the study dogs was assessed by physical examination followed by complete blood count, serum biochemistry, urinalysis, serology (Snap Canine Heartworm PF, IDEXX, Westbrook, ME, USA) and parasitology (modified Knott's test) for *Dirofilaria immitis* infection, faecal examination with Baermann and flotation and sedimentation methods for lung worms, electrocardiography (Simpliscriptor EK 100, Hellige, Freiburg, Germany), radiographic examination (Polydoros 80, Siemens, Munich, Germany) of the thorax and endoscopic examination (Karl Storz 60003VB, Karl Storz GmbH Co., Tuttlingen, Germany) of the respiratory tract. Arterial blood gas analysis was performed in all dogs with a bed-side analyser (i-STAT$_{TM,}$ SDI, Abbott Laboratories, Northbrook, IL, USA); samples were obtained under non-stressful conditions from the femoral artery and were handled in accordance with the manufacturer's instructions.

According to their medical records, none of the dogs had ever been injured, had surgery performed on the respiratory system or been affected by any cardiac or respiratory disease. In addition, none of them had received any medication that could influence respiratory function for at least 15 days before the evaluation or had suffered from any painful condition up to the time of the study.

In order for radiography and endoscopy to be performed, dogs were premedicated with acetylpromazine (0.05 mg/kg IM; Acepromazine, Agroseed Candilidis, Athens, Greece) in combination with butorphanol (0.2 mg/kg IM; Butomidor, Richter-Pharma, Wels, Austria) and anaesthesia was induced and maintained by IV administration of propofol (Propofol Abbott 1%; Abbott Laboratories, Karachi, Pakistan). Radiographic examination of the respiratory tract consisted of plain lateral, ventrodorsal and dorsoventral radiographs of the neck and thorax, as well as a lateral radiograph of the head, intraoral dorsoventral radiograph of the nasal cavities and rostrodorsal–caudodorsal oblique radiograph of the frontal sinuses.

Endoscopic evaluation of the respiratory tract was performed in all dogs immediately after radiographic examination. The aim of the procedure was to further assess the structural integrity of both nasal cavities and the nasopharyngeal area, as well as the functional and structural integrity of the larynx, trachea and bronchi, using a flexible fibre optic endoscope. The dogs were not intubated. Bronchoalveolar lavage was also performed using warm sterile isotonic saline under aseptic conditions. The retrieved fluid was processed immediately after sampling for nucleated cell count, cytological differentiation and culture for bacteria and fungi.

Somatometric measurements of the thorax and the jaw were made for each study dog, comprising measurement of the circumferential diameter of the thorax directly behind the forelimbs at the widest point, the length of the sternum and the length of the mandible from the symphysis menti to its angle. Body condition score was estimated at the same time.

TBFVL measurement was performed in all 21 dogs while they were alert, calm, unsedated, in a standing position, breathing room air, with a normal body temperature and in the same quiet, non-stressful environment. It was always conducted 1 day prior to the radiographic and endoscopic evaluation in order to avoid the influence of general anaesthesia and irritation of the respiratory tract by the endoscope. Care was taken not to compress the neck, thorax or abdomen of the dog. In addition, the head was kept in a normal position with the mandibles at an angle of 45–90° to the neck. No attempts were made to train any of the study dogs to accustom them to the procedure. A solid, plastic, tight-fitting face mask with its own latex seal (Eickemeyer, Tuttlingen, Germany) of appropriate size was placed over the mouth, including the lip commissures. Because no rebreathing was detected, the dead space of the mask was considered unremarkable. The dogs were allowed to adapt for 2–3 min and then the facemask was attached to a pressure sensor (D-lite$^{TM}$, Datex Ohmeda, Louisville, KY, USA) connected to a high-sensitivity transducer (Spirotransducer, Infoproject, Thessaloniki, Greece) that transforms pressure signals into electrical signals (0–5 V). The analogue signals were digitised with a data acquisition card (PCL-711B, PC-Multilab Card, sample rate 100/s; Advantech Europe, Eindhoven, The Netherlands) and analysed with specially designed software (Spirometer, Infoproject). During the procedure, the software displayed flow–volume loops and flow versus time curves. The system was calibrated before each measurement with a standard volume syringe (model 1000 CalSTAT, Volume Calibration Standard, Electronics Inc., Med Science, St Louis, MO, USA) by the injection of a known volume of air through the system and the integration of the flow with that volume.[9,10] For each dog, TBFVL measurements were recorded for approximately 10 min and data from 10 representative breaths were obtained. The criteria for the selection of loops were the same as those used in previous studies and included a lack of artefacts (e.g. movement, vocalisation) and a respiratory rate (RR) ≤60 breaths/min; integrator drift associated with high amplifier sensitivity was solved by selecting loops with a difference ≤5% between inspiratory and expiratory volumes.[4,9,13]

Loops were initially assessed with respect to their shape. Subsequently, tidal volume ($V_T$), tidal expiratory volume at 0.1 and 0.5 s after the beginning of expiration (TBEV 0.1, TBEV 0.5), RR, expiratory and inspiratory peak flows (PEF, PIF), mid-tidal expiratory and inspiratory flows ($EF_{50}$, $IF_{50}$), expiratory and inspiratory flow rates at end-tidal volume plus 12.5%, 25%, and 75% end-tidal volume ($EF_{12.5}$, $IF_{12.5}$, $EF_{25}$, $IF_{25}$, $EF_{75}$, $IF_{75}$), as well as their ratios, were calculated. Expiratory time (TE), inspiratory time (TI) and total respiratory time (TTOT) and their ratios were determined. Finally, the area under the curve (AUC) at peak, 50% and 25% of expiratory and inspiratory flows (AUC PEF, AUC PIF, AUC 50% EF, AUC 50% IF, AUC 25% EF, AUC 25% IF), as well as the total area under the expiratory and inspiratory curve and selected ratios of these parameters, were calculated, as proposed for dogs, cats and humans for the diagnosis of various diseases related to the respiratory system such as chronic bronchitis, laryngeal paralysis and tracheal collapse.

Data were summarised by calculating statistical indices of central tendency, variance and reliability. More specifically, statistical indices (min, max, mean, median, standard error of mean, standard deviation, coefficient of variation (CV), Cronbach's α, discrimination index) for each parameter were calculated and 95% confidence intervals for the mean values were also determined. Cronbach's α was used to determine the reliability of the 10 repeatable TBFVL measurements for each dog.[14,15] Values ≥0.80 were considered satisfactory. The DI represents the consistency of the 10 repeatable TBFVL measurements for each dog and more specifically the correlation of each measurement with the mean value of the 10 measurements per dog.[15] Median DI values ≥0.20 were considered satisfactory. All statistical analyses were performed using SPSS v15.0 (Chicago, IL, USA) statistical software.

## Results

Physical examination of the study dogs did not reveal any abnormalities. The complete blood count, serum biochemistry, urinalysis and arterial blood gas analysis were within reference values. Serology and parasitology for *D. immitis*, as well as faecal examination for parasites, were negative. Electrocardiographic measurements were within the reference ranges for adult Beagle dogs. Radiographic and endoscopic examinations did not reveal any abnormalities. Nucleated cell count and cytological differentiation of the lavage fluid were within normal ranges and cultures for bacteria and fungi were negative.

Somatometric measurements were estimated in centimetres. Circumferential diameter of the thorax directly behind the forelimbs at its widest was 59.09 ± 2.36 (mean ± SD), the length of the sternum was 24.08 ± 2.36 (mean ± SD) and the length of the mandible bone from symphysis menti to its angle was 15.71 ± 1.03 (mean ± SD). The body condition score was found to be 3/5 for all the study dogs by the same clinician.

Only one type of TBFVL shape was identified in all the study dogs and it was consistently reproducible in all of them, as well as in all respiratory cycles that were recorded for each dog. The loop shape had a similar appearance to the letter D. Inspiratory gas flow rates increased gradually, reaching a plateau at approximately 40% inspiratory $V_T$. Peak inspiratory flow occurred late in inspiration, usually at 85% inspiratory $V_T$, after which it decreased rapidly and returned to zero. Expiratory flow rates increased rapidly at the beginning of expiration and approximately at end-expiratory volume plus 75% of $V_T$ and then decreased gradually to zero without showing any plateau (Figure 1). Absolute values for all 44 TBFVL parameters were calculated and are presented in Tables 1–4; 24 of them were calculated directly from the TBFVL, whereas the remainder (20 parameters) were derived ratios.

Mean and standard deviation, as well as 95% confidence intervals for the mean values of all parameters, are presented in Tables 1–4. Only the TI and TI/TTOL parameters were found to have Cronbach's α <0.80 (data not shown). DI was >0.52 for time variables, and >0.85 for all other measurements (data not shown). The CV for the TBFVL parameters ranged from 1.5% for TI/TTOL to 49% for AUC 25% PEF. The vast majority of the parameters had CV <20% (data not shown). The minimum, maximum and mean values of parameters with very low CVs (<0.10: PIF/IF50, IF50/IF25, IF25/IF12.5, EF50/EF25, TI, TE, TE/TI, and TI/TTOL), which demonstrated increased homogeneity, are presented separately in Figure 2.

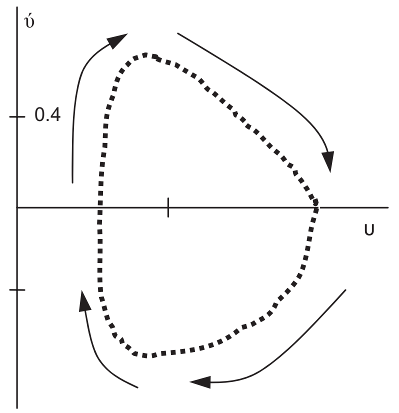


**Figure 1.** Tidal breathing flow volume loop from an alert, healthy, 2-year-old, intact male unsedated Beagle dog. The loop shape has a similar appearance to the letter D. Inspiratory gas flow rates increases gradually, reaching a plateau at 40% inspiratory $V_T$. Peak inspiratory flow occurs late in inspiration and then ceases rapidly and returns to zero. Expiratory flow rate increases rapidly at the beginning of expiration and then ceases gradually to zero without showing any plateau. U, volume; ύ, flow; $V_T$, tidal volume.

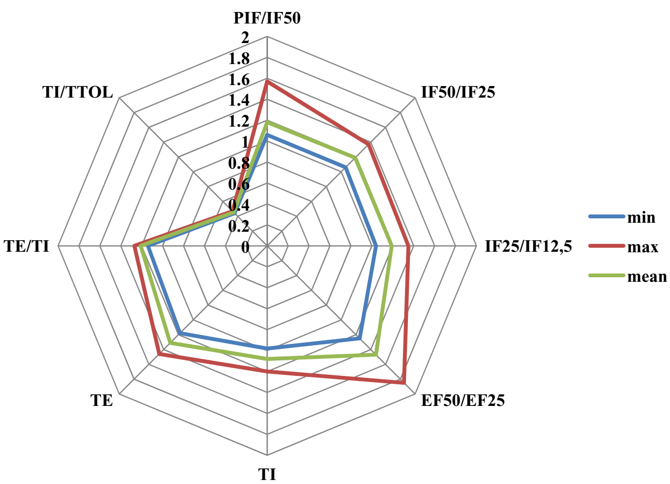


**Figure 2.** Radar plot of minimum, maximum and mean values of the eight tidal breathing flow volume loop parameters (PIF/IF50, IF50/IF25, IF25/IF12.5, EF50/EF25, TI, TE, TE/TI, and TI/TTOL) from 21 alert, healthy, 2-year-old, intact male, unsedated Beagle dogs that showed an increased homogeneity (coefficient of variance <0.10). PIF, peak inspiratory flow rate; IF, inspiratory flow rate; EF, expiratory flow rate; TI, inspiratory time; TE, expiratory time; TTOT, total respiratory time.

## Discussion

Despite the fact that TBFVL analysis has been used in veterinary medicine since 1986, there are no studies that have aimed specifically

**Table 1.** Inspiratory variables derived from tidal breathing flow volume loop from 21 healthy, young adult, intact male, unsedated Beagle dogs

| | Mean | SD | 95% CI | |
|---|---|---|---|---|
| | | | Low | Upper |
| Peak inspiratory flow (PIF, mL/s) | 221.87 | 30.68 | 207.81 | 235.93 |
| Inspiratory flow at end-tidal volume plus 75% end-tidal volume ($IF_{75}$, mL/s) | 209.95 | 29.42 | 196.47 | 223.43 |
| Inspiratory flow at mid-tidal volume ($IF_{50}$, mL/s) | 187.84 | 26.02 | 175.92 | 199.77 |
| Inspiratory flow at end-tidal volume plus 25% end-tidal volume ($IF_{25}$, mL/s) | 158.11 | 23.89 | 147.17 | 169.06 |
| Inspiratory flow at end-tidal volume plus 12.5% end-tidal volume ($IF_{12.5}$, mL/s) | 133.07 | 25.11 | 121.56 | 144.57 |
| Peak inspiratory flow divided by inspiratory flow at mid-tidal volume ($PIF/IF_{50}$) | 1.18 | 0.11 | 1.13 | 1.23 |
| Peak inspiratory flow divided by inspiratory flow at end-tidal volume plus 25% end-tidal volume ($PIF/IF_{25}$) | 1.42 | 0.17 | 1.35 | 1.50 |
| Peak inspiratory flow divided by inspiratory flow at end-tidal volume plus 12.5% end-tidal volume ($PIF/IF_{12.5}$) | 1.71 | 0.30 | 1.57 | 1.84 |
| Inspiratory flow at midtidal volume divided by inspiratory flow at end-tidal volume plus 25% end-tidal volume ($IF_{50}/IF_{25}$) | 1.19 | 0.08 | 1.16 | 1.23 |
| Inspiratory flow at end-tidal volume plus 25% end-tidal volume divided by inspiratory flow at end-tidal volume plus 12.5% end-tidal volume ($IF_{25}/IF_{12.5}$) | 1.19 | 0.09 | 1.15 | 1.24 |

SD, standard deviation; 95% CI, 95% confidence interval.

**Table 2.** Expiratory variables derived from tidal breathing flow volume loop from 21 healthy, young adult, intact male, unsedated Beagle dogs

| | Mean | SD | 95% CI | |
|---|---|---|---|---|
| | | | Low | Upper |
| Peak expiratory flow (PEF, mL/s) | 241.63 | 44.40 | 221.28 | 261.98 |
| Expiratory flow at end-tidal volume plus 75% end-tidal volume ($EF_{75}$, mL/s) | 226.42 | 41.87 | 207.23 | 245.61 |
| Expiratory flow at mid-tidal volume ($EF_{50}$, mL/s) | 179.79 | 46.36 | 158.54 | 201.03 |
| Expiratory flow at end-tidal volume plus 25% end-tidal volume ($EF_{25}$, mL/s) | 123.97 | 34.47 | 108.18 | 139.77 |
| Expiratory flow at end-tidal volume plus 12.5% end-tidal volume ($EF_{12.5}$, mL/s) | 82.98 | 34.52 | 67.17 | 98.80 |
| Peak expiratory flow divided by expiratory flow at mid-tidal volume ($PEF/EF_{50}$) | 1.39 | 0.23 | 1.29 | 1.50 |
| Peak expiratory flow divided by expiratory flow at end-tidal volume plus 25% end-tidal volume ($PEF/EF_{25}$) | 2.01 | 0.39 | 1.83 | 2.19 |
| Peak expiratory flow divided by expiratory flow at end-tidal volume plus 12.5% end-tidal volume ($PEF/EF_{12.5}$) | 3.15 | 0.86 | 2.76 | 3.55 |
| Expiratory flow at midtidal volume divided by expiratory flow at end-tidal volume plus 25% end-tidal volume ($EF_{50}/EF_{25}$) | 1.47 | 0.14 | 1.41 | 1.53 |
| Expiratory flow at end-tidal volume plus 25% end-tidal volume divided by expiratory flow at end-tidal volume plus 12.5% end-tidal volume ($EF_{25}/EF_{12.5}$) | 1.56 | 0.25 | 1.45 | 1.67 |

SD, standard deviation; 95% CI, 95% confidence interval.

to develop a database of reference values for healthy dogs.[4] In an attempt to bypass this problem, all existing reports use their own normal reference population.[5–7,9,10] The present study aimed to take a fundamental step in the development of a database. Beagle dogs were selected as the most appropriate experimental breed because of their size, passive nature and also their routine use in experimental veterinary studies.

It is well known from human medicine that reference values for pulmonary function tests are influenced by the so-called major reference variables, which are age, sex, stature and ethnic group.[12] In veterinary medicine, these variables are well-correlated with age, sex, somatometric measurements and breed. In our study, all the dogs were of the same age, sex and breed, and the somatometric measurements of the body parts related to the respiratory system did not show any major differences (data not shown). There are also a variety of other minor variables, usually defined by environmental factors (e.g. pollution, occupation, tobacco smoking).[12] Given that all the study dogs grew up and lived under the same environmental conditions and were healthy individuals, it is unlikely that environmental factors had a

**Table 3.** Ratios of expiratory to inspiratory variables derived from tidal breathing flow volume loop from 21 healthy, young adult, intact male, unsedated Beagle dogs

| | Mean | SD | 95% CI | |
|---|---|---|---|---|
| | | | Low | Upper |
| Peak expiratory flow divided by peak inspiratory flow (PEF/PIF) | 1.08 | 0.16 | 1.01 | 1.15 |
| Expiratory flow at end-tidal volume plus 75% end-tidal volume divided by inspiratory flow at end-tidal volume plus 75% end-tidal volume ($EF_{75}/IF_{75}$) | 1.09 | 0.17 | 1.01 | 1.16 |
| Expiratory flow at midtidal volume divided by inspiratory flow at mid-tidal volume ($EF_{50}/IF_{50}$) | 0.94 | 0.18 | 0.85 | 1.02 |
| Expiratory flow at end-tidal volume plus 25% end-tidal volume divided by inspiratory flow at end-tidal volume plus 25% end-tidal volume ($EF_{25}/IF_{25}$) | 0.78 | 0.19 | 0.69 | 0.87 |
| Expiratory flow at end-tidal volume plus 12.5% end-tidal volume divided by inspiratory flow at end-tidal volume plus 12.5% end-tidal volume ($EF_{12.5}/IF_{12.5}$) | 0.64 | 0.26 | 0.52 | 0.76 |
| Time variables | | | | |
| Inspiratory time in seconds (TI) | 1.08 | 0.05 | 1.06 | 1.10 |
| Expiratory time in seconds (TE) | 1.31 | 0.06 | 1.29 | 1.33 |
| Expiratory time divided by inspiratory time (TE/TI) | 1.21 | 0.03 | 1.19 | 1.23 |
| Inspiratory time divided by total respiratory time (TI/TTOL) | 0.453 | 0.007 | 0.45 | 0.46 |

SD, standard deviation; 95% CI, 95% confidence interval.

**Table 4.** Global variables derived from tidal breathing flow volume loop from 21 healthy, young adult, intact male, unsedated Beagle dogs

| Parameters of TBFVL | Mean | SD | 95% CI | |
|---|---|---|---|---|
| | | | Low | Upper |
| Tidal volume (VT, mL) | 327.09 | 81.71 | 289.64 | 364.53 |
| Tidal volume divided by inspiratory time (VT/TI) | 304.05 | 83.32 | 266.13 | 341.97 |
| Respiratory rate (RR, breaths/min) | 32.12 | 5.43 | 29.63 | 34.61 |
| Tidal expiratory volume at 0.1 s after the beginning of the expiratory phase ($TBEV_{0.1}$, mL) | 23.58 | 4.21 | 21.65 | 25.51 |
| Tidal expiratory volume at 0.5 s after the beginning of expiration ($TBEV_{0.5}$, mL) | 151.29 | 47.01 | 129.75 | 172.83 |
| Area under the expiratory curve from peak expiratory flow to end-tidal volume divided by area under the inspiratory curve from the peak inspiratory flow to the beginning of the breath (AUC PEF/AUC PIF) | 0.87 | 0.16 | 0.80 | 0.94 |
| Area under total expiratory curve divided by area under total inspiratory curve | 0.92 | 0.15 | 0.85 | 0.99 |
| Area under the inspiratory curve from the peak inspiratory flow to the beginning of the breath (AUC PIF) | 47,943.39 | 16,878.42 | 40,208.72 | 55,678.06 |
| Area under the inspiratory curve from 50% of peak inspiratory flow to the beginning of the breath (AUC 50% PIF) | 24,180.66 | 9,002.55 | 20,055.17 | 28,306.14 |
| Area under the inspiratory curve from 25% of peak inspiratory flow to the beginning of the breath (AUC 25% PIF) | 9,887.53 | 3,980.96 | 8,063.22 | 11,711.83 |
| Area under total inspiratory curve | 56,982.37 | 19,694.32 | 47,957.29 | 66,007.44 |
| Area under the expiratory curve from peak expiratory flow to end-tidal volume (AUC PEF) | 41,565.14 | 16,417.13 | 34,041.87 | 49,088.42 |
| Area under the expiratory curve from 50% of peak expiratory flow to end-tidal volume (AUC 50% PEF) | 18,866.76 | 7,872.89 | 15,258.94 | 22,474.57 |
| Area under the expiratory curve from 25% of peak expiratory flow to end-tidal volume (AUC 25% PEF) | 6,360.36 | 3,113.45 | 4,933.60 | 7,787.12 |
| Area under total expiratory curve | 52,178.07 | 19,855.3 | 43,079.23 | 61,276.91 |

SD, standard deviation; 95% CI, 95% confidence interval.

major influence on our results, and thus none of these factors was taken into account during statistical analysis.

The loop shape was comparable to that already reported previously by others as the normal loop shape of dolichocephalic and normocephalic breeds of dogs during tidal breathing.[4] The estimation of the area under the flow-volume curve was initially used for the evaluation of bronchodilators in humans with chronic bronchitic.[16] In veterinary medicine, it was first applied in cats for the diagnosis of feline asthma[13] and in dogs for the diagnosis and staging of tracheal collapse.[9] In this study, we decided to include AUC measurements because they can be used as predictors of obstructive airway diseases.[13,16] Similar to a previous study, the CVs for all AUC parameters were quite high, ranging from 34.6% for AUC PIF to 41.7% for AUC and 50% for PEF, perhaps reflecting their low reproducibility.[16] It is noteworthy that the CVs of the AUC ratios were dramatically lower from those of the absolute values and we recommend their use instead of the latter. More studies regarding obstructive airway diseases are needed in order to decide whether AUC-related parameters are worth calculating.

The tidal volume showed a relatively wide range of values and the CV was found to be 25%. This finding is compatible with that reported originally for the variation of $V_T$ according to differences in body mass in mammals.[17] Dogs with greater body weight and somatometric measurements also had increased $V_T$ (data not shown). Despite the fact that RR is also well-correlated with body mass,[4] this was not confirmed from the results of the present study. Respiratory rate did not show fluctuations in either the absolute or CV values, which is compatible with previous reports in cats and probably reflects the fact that RR is not a very sensitive index under conditions of narrow body weight variation.[13]

According to the findings of our study, TE was higher than TI and TE/TI was calculated to be 1.21 $\pm$ 0.03. Amis and Kurpershoek also reported that TE was greater than TI when calculated for healthy normocephalic dogs, and thus the TE/TI was approximately 1.26.[4] Nevertheless, in healthy normocephalic cats, the average TE/TI ratio is 1.0 ($\pm$ 0.15).[13] When TE/TI increases, the most probable cause is expiratory airflow limitation, whereas when TE/TI decreases, inspiratory airflow limitation is suspected.[1] When a disease process affects both the inspiratory and expiratory phases of respiration, the ratio may be found within the reference values, because variations in the absolute values of TE and TI could be masked when expressed as a ratio; therefore, absolute values should always be calculated.

The ratio of TI/TTOL was also estimated. This ratio is used mainly for the evaluation of the capability of lung tissue to stretch.[13] Upon inspiration, the respiratory muscles stretch the elastic and collagen tissues of the lung, the energy produced is stored in these structures and used during expiration for the restoration of the lung to its initial volume.[12] When lung volume is increased by a deep breath, the end of expiration and eventually the onset of the next inspiration, is delayed and thus TE and TTOL are increased, but the absolute values of TI may remain stable.[13]

In healthy animals, the $V_T$ values are always in proportion with RR, TI and TE, and consequently minute ventilation is stable and permits the constant partial arterial blood pressures of $O_2$ and $CO_2$ to be achieved.[1,10] Changes in $V_T$ values should be accompanied by proportional changes in RR, TE and TI values because otherwise occult hypoxemia with or without hypercapnia may be apparent.[1]

Apart from the estimation of absolute values of flow, volume, time and AUC parameters, their ratios, in various combinations, were also calculated. This was deemed necessary because the breathing effort of each dog can vary even in tidal breathing[1] and thus flow, volume, time and AUC-related parameters of two dogs of the same breed, age, size and sex may vary. The use of ratios instead of absolute values may normalise this fluctuation.[4] In addition, ratios have been proven to be more sensitive indicators of a mild deterioration of the functional capacity of the respiratory system, especially if a given disease has affected only expiration or inspiration.[12]

The tidal breathing expiratory volumes at 0.1 and 0.5 s after the beginning of the expiration are two parameters that have been used in the diagnosis of feline asthma.[13] They are similar to the forced expiratory volume (FEV) in maximum expiratory curves and the volume until peak flow (VPEF) in tidal expiratory curves used for human adult and infant subjects, respectively.[18,19] The two parameters have been found to correlate well with bronchial and bronchiolar airflow obstruction in both humans and cats[13,19,20] and in the future they may be shown to be useful for studies evaluating the clinical efficacy of bronchodilator therapy in dogs.

Both inspiratory and expiratory flow-related parameters are well correlated with TBFVL shape.[6] In particular, mid-tidal flow parameters have been proposed as the most sensitive indicator of lower airway obstructive disease in cats[13] and dogs[4] and for laryngotracheal disease in neonates.[3] This is attributed to the fact that mid-tidal flows are effort-dependent at all lung volumes. In addition, according to the results of the present study, the expiratory and inspiratory flow parameters at 12.5% of tidal volume, as well their ratios, showed high CVs and probably are not as reliable as other parameters. When the resistance in an airway increases above the normal value, respiratory force decreases and thus the flow decreases as well. This can be observed in both phases of respiration.[3] However, it has been reported that such changes are not always detected in dogs with airway obstruction, probably because the flow depends on multiple factors such as $V_T$, time, pressure, effort and resistance.[10] Additionally, diseases that can influence intrathoracic pressure during both respiratory phases may cause the flow-related inspiratory and expiratory parameters to have values within the reference ranges.[6,21,22] The clinician should bypass this phenomenon of 'false-negative diagnosis' by taking into account many TBFVL parameters and not just the most commonly known flow-related parameters when writing the final report for a case.

## Conclusion

TBFVL analysis is a quick, simple, non-invasive diagnostic method, with minimum risk for the patient. The clinician is able to gather valuable and objective information concerning the functional capacity of the respiratory system just by applying a face mask to the animal for a few minutes. The lack of reference values, however, has made this simple procedure inapplicable for everyday use. Despite the fact that this study did not cover all possible reference populations, it has made the first steps in this direction by using a homogeneous population. In addition, it involved the measurement of a large number of clinically applicable TBFVL parameters in the dog. In the future, further studies in other breeds of dog should be performed in an attempt to complete the global database of TBFVL reference values.

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