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Development of a method to measure regional perfusion of the lung in anesthetized ponies using computed tomography angiography and the maximum slope model

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OBJECTIVE
To develop a method based on CT angiography and the maximum slope model (MSM) to measure regional lung perfusion in anesthetized ponies.

ANIMALS
6 ponies.

PROCEDURES
Anesthetized ponies were positioned in dorsal recumbency in the CT gantry. Contrast was injected, and the lungs were imaged while ponies were breathing spontaneously and while they were mechanically ventilated. Two observers delineated regions of interest in aerated and atelectatic lung, and perfusion in those regions was calculated with the MSM. Measurements obtained with a computerized method were compared with manual measurements, and computerized measurements were compared with previously reported measurements obtained with microspheres.

RESULTS
Perfusion measurements obtained with the MSM were similar to previously reported values obtained with the microsphere method. While ponies were spontaneously breathing, mean ± SD perfusion for aerated and atelectatic lung regions were 4.0 ± 1.9 and 5.0 ± 1.2 mL/min/g of lung tissue, respectively. During mechanical ventilation, values were 4.6 ± 1.2 and 2.7 ± 0.7 mL/min/g of lung tissue at end expiration and 4.1 ± 0.5 and 2.7 ± 0.6 mL/min/g of lung tissue at peak inspiration. Intraobserver agreement was acceptable, but interobserver agreement was lower. Computerized measurements compared well with manual measurements.

CLINICAL RELEVANCE
Findings showed that CT angiography and the MSM could be used to measure regional lung perfusion in dorsally recumbent anesthetized ponies. Measurements are repeatable, suggesting that the method could be used to determine efficacy of therapeutic interventions to improve ventilation-perfusion matching and for other studies for which measurement of regional lung perfusion is necessary.

Functional imaging of the lung, whereby the heterogeneity of perfusion in different lung regions can be measured, would be useful in studies of pulmonary physiology and could aid in the diagnosis and management of pulmonary disorders.[1] In the past, pulmonary perfusion in the equine lung has been measured with radioactive microspheres,[2–4] and ventilation-perfusion ($\dot{V}/\dot{Q}$) relationships in horses have been mapped with the multiple inert gas elimination technique.[5–8] However, both of these methods offer limited spatial resolution and are unsuitable for serial imaging, and the microsphere method necessitates euthanasia of the horse.

First-pass CT angiography (CTA) offers better spatial and temporal image resolution than other functional imaging techniques and has been used to measure regional blood flow (or perfusion) in the brain, heart, lungs, and kidneys of humans, dogs, and pigs.[9] Fundamentally, CTA is based on temporal changes in tissue attenuation following injection of a bolus of an iodine-based contrast medium.[10] The linear relationship between contrast medium concentration and tissue density changes (measured as HUs) makes mathematical modeling straightforward.[10] Multidetector CT scanners have sufficient temporal resolution to create a time-attenuation curve from a contrast bolus injected into a large vein, simplifying the analysis considerably.[11] The safety of multiple boluses of iodinated contrast medium allows serial measurements to be made following

physiological changes or therapeutic interventions. Numerous methods for analyzing CTA images, including the maximum slope model (MSM), have been described. The MSM is relatively simple, because only the maximum upslope of the time-attenuation curve is measured, and provides good repeatability.[12,13] The maximum slope of the time-attenuation curve for the first pass of a bolus of contrast medium is normalized to the arterial input function to calculate perfusion.[11,13,14]

In anesthetized horses, changes in regional perfusion within the lung and development of atelectasis lead to $\dot{V}/\dot{Q}$ mismatch and marked intrapulmonary shunting of blood in dependent lung regions.[2,15] This deoxygenated blood enters the arterial circulation, causing alterations in oxygenation and potentially hypoxemia (ie, Pa_{O_2} < 60 mm Hg).[15] We propose that in anesthetized horses, CTA could be used to study changes in lung perfusion during different modes of ventilation and to assess changes associated with interventions intended to improve $\dot{V}/\dot{Q}$ matching, potentially adding to our understanding of treatments for gas exchange derangements in anesthetized horses and other species. However, before CTA can be used for this purpose, its reliability and internal and external validity must be evaluated.

The overall purpose of the study reported here was to validate CTA with the MSM as a technique to measure pulmonary perfusion in anesthetized ponies by comparing values obtained with this method with previously reported values obtained with radioactive microspheres. More specifically, we wanted to develop a method based on CTA and the MSM to measure regional lung perfusion in anesthetized ponies, determine the intraobserver and interobserver variability of lung perfusion measurements obtained with this method, determine the variability introduced when a computer program was used to smooth the time-attenuation curves, determine whether contrast medium from repeated injections was excreted fast enough to not interfere with subsequent measurements, and identify sources of error associated with and limitations of the method. Our central hypothesis was that a method based on CTA and the MSM can be used to accurately measure regional lung perfusion in anesthetized ponies.

Materials and Methods

The study protocol was approved by the local Ethical Committee on Animal Experiments in Uppsala, Sweden (C 201/14). Six healthy ponies (5 Shetland ponies and 1 crossbred pony) with a mean weight of 190 kg (range, 150 to 241 kg) and mean age of 12 years (range, 4 to 18 years) were used in the study. The ponies comprised 1 mare, 3 geldings, and 2 stallions.

Anesthetic protocol

Ponies were premedicated with acepromazine (0.03 mg/kg, IM). A 14-gauge, 9-cm catheter was placed in the left jugular vein, and two 8.5F sheath introducers were placed in the right jugular vein following SC infiltration with lidocaine. Ponies were further premedicated with xylazine (1.1 mg/kg, IV) and butorphanol (0.025 mg/kg, IV), and general anesthesia was induced with ketamine (2.2 mg/kg, IV) and diazepam (0.05 mg/kg, IV). Once ponies were unconscious, the trachea was intubated with a 20-mm endotracheal tube, and the ponies were hoisted onto an appropriately padded CT table with their head toward the CT gantry. The endotracheal tube was connected to a large animal anesthetic circuit, and the anesthetic machine was connected to a mechanical ventilator. General anesthesia was maintained with isoflurane in oxygen, with an F_{IO_2} of approximately 0.9.

Initially, ponies were allowed to breathe spontaneously. This was then followed by a period of mechanical ventilation. The ventilation mode was continuous mandatory ventilation, with tidal volume and rate adjusted to maintain a Pa_{CO_2} between 45 and 68 mm Hg; peak inspiratory pressure was kept to < 30 cm H_2O. At the end of the experiment, ponies were hoisted into a padded stall and allowed to recover. Analgesia was provided at this time with flunixin meglumine (1.1 mg/kg, IV) and morphine (0.1 mg/kg, IM).

CT protocol

A topogram was used to identify the anatomic position of the diaphragm in relation to the field of view. A suitable slice caudal to the heart was selected in which aerated and atelectatic lung regions were well defined and sufficiently large to facilitate later analysis. Dynamic axial scans were acquired during each phase of the experiment with a third-generation, 64-slice, multidetector CT scanner (Somatom Definition AS; Siemens Medical Systems) with exposure values of 70 mAs and 120 kV, slice thickness of 15.0 mm, and rotation time of 0.33 seconds.

For injection of contrast medium, a pigtail, multihole catheter was inserted through one of the preplaced introducers, advanced into the right ventricle, and then retracted into the right atrium under pressure waveform guidance. Iodinated contrast agent was injected directly into the right atrium with an automated injector (Medrad Stellant dual-syringe CT injection system; Bayer AG) with a delay of approximately 4 seconds between the start of scanning and contrast injection to allow measurement of baseline contrast. Iohexol (Omnipaque 300 mg/mL) and iopromide (Ultravist 370 mg/mL) were used as contrast agents in pilot studies to determine whether one was superior in terms of contrast definition, and the lower-concentration contrast agent was used for the actual experiments. We also modified the method used to inject contrast medium to allow for more rapid injection and enhance CT images. This included removing the spiral extension set, using alternative tubing that was wider and shorter, using a larger volume of contrast agent, warming the contrast agent before injection to approximately 37 °C, and injecting directly into the right atrium. With these modifications, we were able to achieve an injection rate of 10 mL/s (vs 8 mL/s). The volume of contrast agent injected for each imaging sequence was 60

mL, and the catheter was immediately flushed with 30 mL of saline (0.9% NaCl) solution after injection of the contrast agent. To ensure that contrast agent did not accumulate over the course of each experiment, which involved administration of several boluses of contrast agent, line plots of baseline contrast (HU) over time were created.

CT images were acquired at end expiration while ponies were spontaneously breathing and at end expiration and peak inspiration while ponies were mechanically ventilated. Each scan sequence lasted 50 seconds. To acquire stable images without respiratory motion, it was necessary to arrest breathing either at end expiration or peak inspiration. To do this, a plumbing ball valve was positioned between the endotracheal tube and the breathing system, and the valve was used to occlude the endotracheal tube at peak inspiration or end expiration. During mechanical ventilation, the ventilator was temporarily turned off at the time the endotracheal tube was occluded.

CT analysis

After a period of training, 2 independent investigators (AA and PFL) who were blinded to the stage of the experiment, analyzed each CT scan twice, with a minimum interval between measurements of 7 days to minimize recall bias and its influence on intraobserver variability. DICOM files were exported as a series of 166 images at a rate of 3 images/s and viewed with imaging software (OsiriX 64-bit version 5.8.5; Pixmeo SARL). With standard software (ImageJ version 1.49; National Institutes of Health), lung regions were delineated by means of threshold windows (atelectatic lung, +100 to –100 HU; poorly aerated lung, –100 to –350 HU; and normally aerated and overaerated lung, –350 to –1,000 HU). A region of interest (ROI) was drawn in the pulmonary artery for evaluation of the arterial input function, and additional ROIs were drawn around atelectatic and aerated lung, as determined by the threshold windows **(Figure 1)**. Large airways and vessels were excluded from these ROIs by scrolling through the frames and observing vessel opacification. At the same time, any motion of the thoracic wall or diaphragm was noted. Time-attenuation curves were generated, and the baseline, peak, and tail were identified for each curve prior to further analysis.

Calculations and equations

From the ROI in the pulmonary artery, net attenuation used as the arterial input function was calculated by subtracting baseline HU from peak (maximum) HU. Maximum slope of the time-attenuation curves for ROIs in atelectatic and aerated lung regions were measured by use of the x and y coordinates for 2 points on the observed linear portion of the slope. The time-attenuation curves for ROIs in aerated lung regions were affected by cardiogenic pulsations (Figure 1), and in these instances, the peaks of these oscillations were used to measure maximum slope. Although breathing was stopped during image acquisition, there often was some leakage or absorption of gas from the aerated lung region, and the resulting movement of the chest wall and diaphragm was detectable. This was evident on time-attenuation curves generated from ROIs in aerated lung regions as a gradual increase in CT density over time, independent of the increase associated with contrast administration. If this occurred during the upslope of the curve, as determined by scrolling through the frames, we measured the slope of the gas loss and subtracted this from the maximum slope of the time-attenuation curve to calculate net rate of change (ie, net maximum slope). Perfusion was calculated by dividing the maximum slope of the time-attenuation curve by the maximum arterial enhancement, as indicated in the following equation[12]:

$$\text{Perfusion} = \frac{\frac{d}{dt}(c[t])_{max}}{a(t)_{max}}$$

where c = tissue concentration of the contrast agent, a = arterial concentration of the contrast agent, and t = time. Pulmonary perfusion was then normalized to tissue mass to account for spatial heterogeneity by incorporating CT attenuation values into the equation[14]:

$$\text{Normalized perfusion(mL/min/g)} = \frac{\text{Perfusion}}{\frac{HU_x - HU_{air}}{HU_{tissue} - HU_{air}}}$$

where HU_x = HU of the ROI in a non–contrast -enhanced image, HU_{air} = –1,000, and HU_{tissue} = 50.

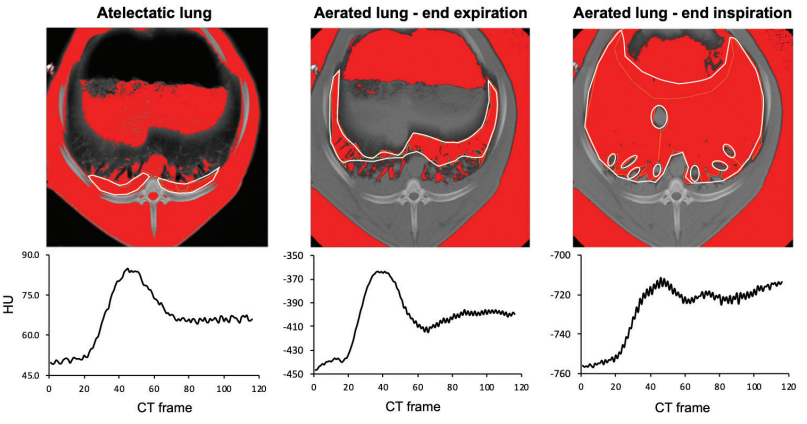

Figure 1—CT angiographic images illustrating regions of interest drawn around areas of atelectatic (+100 to –100 HU) and aerated (–350 to –1,000 HU) lung in an anesthetized pony (top row) and accompanying time-attenuation curves (bottom row) for calculation of regional lung perfusion with the maximum slope model. A white line was hand drawn around each region of interest, avoiding the diaphragm and large blood vessels. Notice that cardiogenic pulsations are quite noticeable in the time-attenuation curves for aerated lung regions and that there is a slow increase in CT attenuation over time, independent of contrast agent injection, that is represented by the second upslope. The slope of this gas loss was calculated and removed from the maximum slope calculation to obtain a measure of net maximum slope.

All calculations were performed with standard software (Excel for Mac version 16.16.27; Microsoft Corp).

Smoothed curve method

The ROI (x and y) data from 1 observer (AA) were imported in spreadsheet format into a specially coded program (Matlab; The MathWorks Inc), and this program was used to smooth time-attenuation curves from aerated regions of lung that were affected by cardiogenic pulsations. The program was able to automatically calculate maximum slope between selected points and to subtract the effect of gas loss, if present, to calculate net maximum slope. Noise removal and smoothing of the signal was performed with cubic splines[16] and a smoothing factor of 0.15. In the graphical user interface, the user marked the following epochs in the signal: before the contrast agent entered the field of view, during the upslope and downslope in the signal resulting from passage of the contrast agent, and after the contrast agent had left the field of view. Data points for the first and third epochs were fitted with a second-order polynomial (using a least-squares approach) that represented the drift in the baseline of the signal. The fitted polynomial was then removed from the signal. The point of maximum positive slope was then identified automatically and used for further calculations.

Validation of the method

As no contemporaneous measurements obtained with a gold standard method were available to validate our CT perfusion values, we compared mean perfusion values from the smoothed curve data with previously reported microsphere measurements for excised equine lungs.[2] In the microsphere study, horses were anesthetized and positioned in dorsal recumbency, and the excised lungs were divided into 4 vertical regions. We calculated mean perfusion from their data and compared our atelectatic lung perfusion with outer dorsal lung perfusion and our aerated lung perfusion with perfusion for the remaining lung fields.

Statistical analysis

Commercially available statistical software programs were used for statistical analyses (MedCalc 64-bit version 17.1; MedCalc Software; SAS version 9.4; SAS Institute Inc). Only measurements for atelectatic and aerated lung regions were compared. Correlations between the 2 observers' measurements and between measurements obtained with the smoothed curve method and those obtained by one of the observers (AA) were tested by calculating the Pearson correlation coefficient. Agreement (repeatability and reproducibility) between paired measurements was tested by means of Bland-Altman analysis. Intraobserver variability was tested by comparing paired measurements made by each observer for maximum slopes and calculated perfusion for aerated and atelectatic lung regions. Interobserver variability was tested by averaging paired measurements from each observer and comparing mean values between observers. For data processed with the smoothed curve method, agreement was assessed between mean values for original observer measurements and mean values after smoothing. Because the smoothed curve method also removed the effect of gas loss, the magnitude of gas loss removed by the smoothed curve method was compared with the gas loss calculated by the observer. Repeatability of measurements between observers and between the smoothed curve method and the original measurements of the single observer (AA) was also analyzed by calculating the intraclass correlation coefficient (ICC). Sources of variation in measurements were determined with a mixed-model ANOVA, with pony as a random effect and intervention (contrast agent injection and mode of ventilation), measurement, observer, and their interactions as fixed effects. When the effect of intervention was significant, multiple comparisons at separate time points were performed. Covariance parameter estimates from the mixed model were used to calculate the coefficient of variation between observers and between the smoothed curve method and the original measurements for the single observer to identify sources of variation between the measurements.

Results

There was little difference in tissue enhancement when using iohexol (300 mg/mL) or iopromide (370 mg/mL), and there was no evidence of allergic reactions in the ponies at any time during the study. Images suitable for analysis were acquired during the first pass of the contrast agent in all ponies during all stages of the study. Measured perfusion in poorly aerated lung regions (–100 to –350 HU) did not change between phases of the experiment and was not included in analyses of correlation and agreement. As expected, there was some interference in the time-attenuation curves from cardiac pulsations, particularly in ROIs in aerated regions of lung. Although we successfully arrested breathing with the plumbing ball valve for 50 seconds, there was some reduction in lung size over time in images acquired at peak inspiration during mechanical ventilation, either because of absorption of gas from the lung or slow leakage of gas during the time taken for image acquisition. This was evident in time-attenuation curves from ROIs in aerated lung regions as a gas loss slope (ie, a gradual increase in tissue attenuation independent of contrast over time). This occurred in approximately 55% of aerated lung image sequences acquired at peak inspiration. During spontaneous breathing, all images were acquired at end expiration, and loss of gas was not detected.

Validation of the method

Values for perfusion in atelectatic and aerated lung regions calculated with the CTA and MSM method compared well with previously reported perfusion data obtained with the microsphere method in excised equine lungs[2] **(Table 1)**.

Intra- and interobserver correlation and agreement

All paired measurements were positively correlated with high correlation coefficients **(Table 2)**.

Table 1—Mean ± SD lung perfusion (mL/min/g of lung tissue) in aerated and atelectatic regions of the lung determined by means of CT angiography (CTA) and the maximum slope model (MSM) in 6 anesthetized ponies, and comparison with previously reported values for anesthetized horses determined with a microsphere technique.[2]

	CTA and MSM method		Microsphere technique	
Phase of ventilation	**Aerated lung**	**Atelectatic lung**	**Aerated lung**	**Atelectatic lung**
Spontaneous breathing				
End expiration	4.0 ± 1.9	5.0 ± 1.2	2.3 ± 1.0	3.9 ± 0.4
Mechanical ventilation	—	—	1.8 ± 1.1	3.9 ± 0.6
Peak inspiration	4.1 ± 0.5	2.7 ± 0.6	—	—
End expiration	4.6 ± 1.2	2.7 ± 0.7	—	—

Table 2—Comparisons of measurements of the region of interest (ROI) area, maximum slope of the time-attenuation curve, and lung perfusion in 6 anesthetized ponies in which perfusion in atelectatic and aerated regions of the lung was determined by means of CTA and the MSM, and evaluation of sources of variability between measurements.

					Contribution to variability (%)		
Variable	**Comparison**	***R***	**ICC**	**CV (%)**	**Pony**	**Stage of study**	**Observer**
Atelectatic lung							
ROI area	1	0.88	0.86	8.6	85	11	4
Slope	1	0.95	0.91	9.0	70	28	2
	2	0.99	0.99	4.7	62	37	1
Perfusion	1	0.97	0.93	8.5	36	62	2
	2	0.98	0.99	5.6	32	68	0
Aerated lung							
ROI area	1	0.98	0.97	5.8	58	41	1
Slope	1	0.96	0.96	11.0	55	44	1
	2	0.93	0.94	12.3	60	40	0
Perfusion	1	0.94	0.94	12.4	31	68	1
	2	0.93	0.93	13.1	29	71	0

ICC = Intraclass correlation coefficient. R = Pearson correlation coefficient.

Comparisons were performed between (1) mean values of paired measurements performed by 2 observers (AA and PFL) at least 7 days apart (n = 54) and (2) mean values obtained by one of the observers (AA) and values obtained with a computer-based program that was used to smooth time-attenuation curves affected by cardiogenic pulsations (54). ROI areas were not compared between the human observer and the smoothed curve method because these areas were identical.

Mean difference (bias), 95% limits of agreement, and coefficients of repeatability from Bland-Altman analyses for intraobserver and interobserver comparisons and for comparisons of smoothed curve measurements with measurements from 1 observer (AA) were summarized **(Tables 3 and 4)**. For all intraobserver measurements, there was no evidence of systematic bias (ie, the 95% CI for the mean difference included 0). For atelectatic lung regions, there was some evidence of systematic bias between observers (ie, for perfusion in atelectatic lung regions, one observer tended to obtain higher values than the other). There was some variation in measurements between observers with wider 95% limits of agreement for interobserver comparisons than for intraobserver comparisons, although approximately 95% of data points were within the 95% limits of agreement.

Generally, the interobserver ICC was > 0.9 (Table 2). Most of the variation between observers was related to individual pony differences or differences in measurements at each stage of the study. Very little of the variation could be attributed to differences between observers.

Smoothed curve method correlation and agreement

All paired measurements between the smoothed curve method and the single observer (AA) were positively correlated (Table 2). The bias, 95% limits of agreement, and coefficients of repeatability were summarized (Table 4). There was some evidence of systematic bias for atelectatic lung regions (ie, the smoothed curve method tended to obtain higher value than the human observer). The 95% limits of agreement were narrower, compared with those for interobserver comparisons for atelectatic and aerated lung regions, suggesting that variability was lower. Approximately 95% of data points were within the 95% limits of agreement.

Overall, the ICCs for the smoothed curve measurements and the single observer's original measurements were > 0.9 (Table 2), and ICCs were generally higher than the interobserver ICCs. Again, most of the variation was related to individual pony differences or differences in the measurements at each stage of the study. Very little of the variation could be attributed to differences between the smoothed

Table 3—Intraobserver agreement determined by means of Bland-Altman analysis for paired measurements of perfusion in atelectatic and aerated regions of the lung determined by means of CTA and the MSM in 6 anesthetized ponies.

Variable	Observer	Bias (95% CI)	95% LOAs	COR
Atelectatic lung				
ROI area (%)	1	−0.4 (−3.0 to 2.1)	−18.8 to 17.9	848.6 mm^2
	2	−0.6 (−2.4 to 1.3)	−14.0 to 12.8	727.1 mm^2
Slope (%)	1	0.3 (−2.0 to 2.6)	−16.3 to 16.9	0.4
	2	−0.5 (−3.2 to 2.1)	−19.7 to 18.6	0.6
Perfusion (mL/min/g)	1	−0.01 (−0.1 to 0.1)	−0.6 to 0.6	0.6
	2	−0.04 (−0.1 to 0.05)	−0.7 to 0.6	0.7
Aerated lung				
ROI area (%)	1	1.0 (0.0 to 2.0)	−6.5 to 8.5	2,300.6 mm^2
	2	−0.3 (−1.9 to 1.2)	−11.5 to 10.9	3,497.7 mm^2
Slope (%)	1	1.6 (−1.5 to 4.7)	−20.6 to 23.9	0.2
	2	−2.2 (−5.7 to 1.4)	−27.6 to 23.2	0.57
Perfusion (mL/min/g)	1	−0.03 (−0.1 to 0.1)	−0.9 to 0.8	0.9
	2	−0.2 (−0.4 to 0.02)	−1.8 to 1.4	1.6

COR = Coefficient of repeatability (ie, maximum likely difference). LOAs = Limits of agreement.

Each observer (AA [1] and PFL [2]) performed measurements twice, with a minimum of 7 days between sessions (n = 108 for each observer). Bias represents the mean difference between measurements.

Table 4—Interobserver agreement determined by means of Bland-Altman analysis for paired measurements of perfusion in atelectatic and aerated regions of the lung determined by means of CTA and the MSM in 6 anesthetized ponies.

Variable	Comparison	Bias (95% CI)	95% LOAs	COR
Atelectatic lung				
ROI area (%)	1	−6.6 (−11.0 to −2.2)	−38.1 to 25.0	1,559 mm^2
Slope (%)	1	−11.1 (−14.8 to −7.5)	−37.4 to 15.2	1.01
	2	−1.6 (−3.0 to −0.1)	−16.6 to 13.4	0.4
Perfusion (mL/min/g)	1	−0.4 (−0.5 to −0.3)	−1.1 to 0.4	1.0
	2	−0.02 (−0.07 to 0.04)	−0.4 to 0.4	0.4
Aerated lung				
ROI area (%)	1	1.5 (−2.3 to 5.3)	−25.7 to 28.8	12,821 mm^2
Slope (%)	1	−0.55 (−6.0 to 4.9)	−39.6 to 38.5	0.7
	2	−1.5 (−4.5 to 1.6)	−32.8 to 29.8	0.8
Perfusion (mL/min/g)	1	−0.1 (−0.4 to 0.2)	−2.3 to 2.1	2.2
	2	−0.2 (−0.5 to 0.1)	−2.4 to 2.1	2.2
Gas loss slope (%)	2	−22.2 (−39.8 to −4.6)	−146.3 to 101.9	

Agreement was assessed between (1) mean values of paired measurements performed by 2 observers (AA and PFL) at least 7 days apart (n = 54) and (2) mean values obtained by one of the observers (AA) and values obtained with a computer-based program that was used to smooth time-attenuation curves affected by cardiogenic pulsations (n = 54). ROI areas were not compared between the human observer and the smoothed curve method because these areas were identical.

Gas loss slope (n = 52).

See Table 3 for remainder of key.

curve measurements and the single observer's original measurements.

Sources of error and limitations

Plots of baseline contrast values (HU) over time did not indicate any substantial accumulation of the contrast agent **(Figure 2)**. Agreement between slopes of gas loss calculated by the smoothed curve method and a single observer were analyzed with the Bland-Altman method (Table 4). There was systematic bias evident, in that the smoothed curve method tended to generate a gas loss slope that was greater than the slope calculated by the human observer. The 95% limits of agreement were also wide, but this reflected the

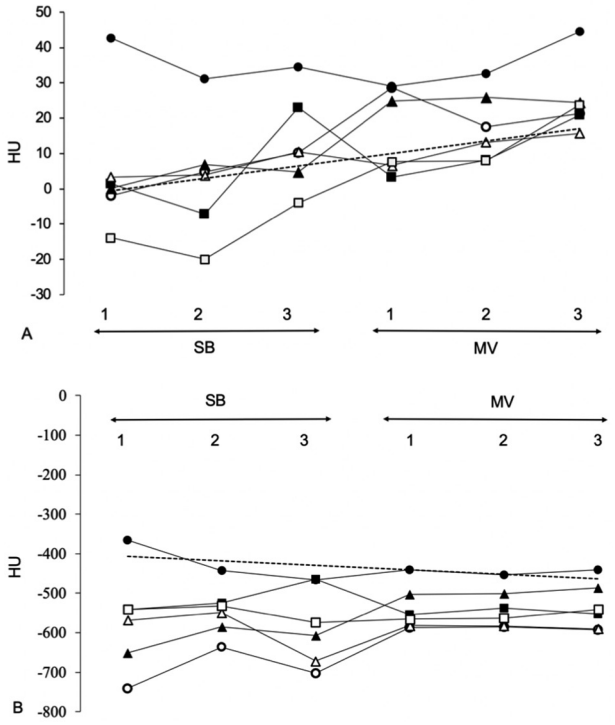

Figure 2—Change in baseline CT density (HU) over time in atelectatic (A) and aerated (B) lung regions in 6 anesthetized ponies during spontaneous breathing (SB) and mechanical ventilation (MV). Numbers (1, 2, and 3) along the x-axis represent the time course of the study, which involved injection of multiple boluses of contrast agent. Each individual pony is represented by a different symbol.

fact that the absolute value for this slope was small and agreement was expressed as a percentage difference rather than an absolute value.

Discussion

Results of the present study supported our hypothesis that CTA and the MSM could be used to accurately measure regional lung perfusion in anesthetized ponies. Our measurements of perfusion were comparable to previously reported values obtained with microspheres.[2] Additionally, values obtained by the same observer showed acceptable variability. Smoothing noisy time-attenuation curves through computerized integration simplified perfusion calculations and reduced variability, compared with manually selecting points on the time-attenuation curves. Finally, repeated injections of contrast medium did not result in sufficient accumulation to affect the results or result in toxicoses.

Injection of microspheres has been used to accurately measure the distribution of blood flow in the lungs,[17] and this technique has been used successfully in anesthetized horses.[2] However, this method necessitates euthanasia of the test subject, and because we were not euthanizing the ponies used in this study, we could not compare contemporaneous measurements of perfusion obtained with CTA and microspheres. For this reason, we used previously reported values of perfusion measured with microspheres in excised equine lungs.[2] Although perfusion values for atelectatic lung regions compared well between the 2 measurement methods, perfusion values for aerated regions showed a little more variability. This was not unexpected, because perfusion measured with the microsphere method will depend on the region of lung excised and tested, and our CTA method calculated perfusion in the entire aerated region of lung within the cross-sectional CT image. Furthermore, Dobson et al[2] used large horses (mean ± SD body weight, 508 ± 74 kg) in their study of the microsphere method, whereas we used ponies small enough to fit inside the CT gantry (range, 150 to 241 kg). Although pulmonary physiology is largely similar between horses and ponies, subtle differences may account for the small discrepancies in measurements between the 2 experimental techniques. Finally, the anesthetic maintenance agent used in our study was isoflurane, compared to halothane used in the microsphere study by Dobson et al.[2] In rats, volatile anesthetic agents affect hypoxic pulmonary vasoconstriction within the lung and therefore alter regional pulmonary perfusion, but in a similar way, at equipotent doses.[18] No similar comparative studies in horses are available, but the difference in volatile anesthetic agents may have played a role in the differences in perfusion values between the present and previous studies. Furthermore, Dobson et al[2] do not report the concentration of halothane used, and so comparisons are difficult. Overall, however, we are confident that the CTA method described in the present study provides an accurate and less invasive method of measuring regional lung perfusion in anesthetized equids.

In the present study, it was important to measure intra- and interobserver variability when calculating perfusion from manually drawn ROIs. We tried to minimize recall bias when testing intraobserver variability by ensuring there was at least 7 days between paired measurements. It is possible to obtain good intra- and interobserver reliability when analyzing CT images of the brain[19] and good intraobserver variability when analyzing CT images of lung tumors.[20] A brief training session with clear instructions, particularly when drawing ROIs, is necessary to achieve this.[19] Still, there was greater variability in our study, compared with these other studies, despite several training sessions. However, analysis of lung tissue is inherently more difficult than analysis of solid tissues such as neoplasms, owing to the nature of the image. Furthermore, automated software was used by both Sanelli et al[19] and Sauter et al,[20] making comparisons difficult.

Using 2 observers to draw ROIs and calculate perfusion introduced some variability in the present study. This was likely because ROIs are often poorly defined, thus variation in delineated ROIs is not unexpected. The variation between measurements was further investigated with a mixed-model approach and calculation of ICCs, and it was clear from these analyses that the vast majority of the variation between measurements was a result of differences be-

tween ponies or differences during the timeline of the study, both of which are expected and not related to the reliability of the measurement method. Very little of the variability could be attributed to an observer effect, but because interobserver variability was moderate, it is recommended that a single observer perform all measurements in future studies.

Measurement of the HU value in an adjacent large vessel (the pulmonary artery in our study) as the arterial input function facilitates calculation of perfusion in a ROI. Although this is a surrogate for arterial input into each voxel, it is generally well accepted as an alternative.[21] The advantage of CT over MRI in this respect is that there is a linear relationship between HU and the concentration of contrast agent, whereas with functional MRI, this linearity is not guaranteed.[11] The HU thresholds used in this study were based on work by Gattinoni et al[22] and Vieira et al.[23] Using these thresholds facilitated more repeatable and less variable ROI delineation, because it was possible to highlight atelectatic and aerated lung regions, making demarcation of ROIs much easier. Commercial CTA software is available but is expensive and designed for assessment of brain perfusion in the context of stroke. Because this software is fully automated, it is not possible to adapt it or be certain that the program is performing analyses appropriately for lung. Therefore, we did not adopt it for use in our experiments.

Because cardiac pulsations in the time-attenuation curves interfered with manual slope measurements, we used a computer program to smooth the curves and automatically calculate the maximum slope. From our results, we demonstrated that this was an acceptable method of analysis, although it introduced some variability compared with manual calculations.

In the present study, we documented a slow deflation of the lung during image acquisition at peak inspiration that was not possible to rectify. This may have been the result of slow leakage of gas from the lung via the breathing system or endotracheal tube cuff. It may also have been a consequence of ongoing absorption of gas during the 50-second image-acquisition period. This deflation was evident when scrolling through the image sequence and also on the time-attenuation curve generated from the ROI. Consequently, the density of the lung gradually increased over time, represented by an increase in HU. This was independent of the maximum upslope of the time-attenuation curve produced during injection of contrast medium. The magnitude of this gas loss slope was subtracted from the maximum slope to calculate net maximum slope. Calculation of this gas loss slope is somewhat subjective, and this introduced further variability in the final measurements and may have represented a small source of error in perfusion measurements. The variability was not great enough to adversely affect our results. However, it was evident that a single measurement technique, either manual or computer assisted, should be used in future studies.

Respiratory motion is a major problem during CTA of the lung and can lead to errors in perfusion calculations.[13] Inserting a simple plumbing ball valve almost completely eliminated this respiratory motion and was repeatable throughout the study. Respiratory hold maneuvers affect pulmonary perfusion. Perfusion measured by MRI was lower during an inspiratory hold than an expiratory hold, presumably owing to effects on flow characteristics following lung inflation.[24] Although this effect introduces a limitation into the methodology, we attempted to perform the breath hold maneuver at peak inspiration, so that the effect at each analysis time point would be similar. Images acquired at end expiration during both spontaneous breathing and mechanical ventilation may have been subject to variation due to a spontaneous inspiratory effort. The negative intrathoracic pressure generated can alter hemodynamics within the thorax. Because the maximum slope of the first pass of contrast medium occurs approximately within the first 10 seconds of imaging, spontaneous inspiratory effort is an unlikely source of error. We considered administering a peripherally acting muscle relaxant to avoid respiratory motion. However, increased lung density measured with CT is observed in humans administered succinylcholine,[25] which would have artificially altered our results. Furthermore, because repeated measurements during spontaneous breathing were made as part of another study, the use of muscle relaxants was avoided, because positive-pressure ventilation would have been necessary to support ventilation during paralysis, changing the distribution of perfusion.

It was necessary to test our images to ensure that contrast medium did not accumulate during the course of the study, because accumulation would have resulted in overestimation of perfusion.[13] By plotting baseline contrast (HU) over time for each of the 6 ponies, we were able to demonstrate that contrast medium did not accumulate significantly.

Injection characteristics of the contrast agent bolus are extremely important to consider when using the MSM.[26] During our pilot studies, tissue enhancement was barely sufficient owing to a combination of potential problems: the rate of injection, the injection site, the volume of the bolus, the concentration of iodine in the solution, and the viscosity of the contrast agent. Following our pilot experiments, the study method used a larger volume (60 mL vs 40 mL) of contrast agent and a faster rate of injection (10 mL/s vs 8 mL/s) and the contrast agent was heated to approximately 37 °C prior to injection. Extrinsic warming of iodinated contrast agents improves flow kinetics[27–29] and enabled us to achieve a faster injection rate. Furthermore, we achieved a faster injection speed by removing a spiral extension set and using alternative injection tubing that was wider and shorter.

There are specific risks associated with injection, particularly repeated injection, of iodinated contrast media. The risk of these reactions occurring is low and may be independent of dose and concentration above a certain threshold.[30,31] We did not observe any demonstrable evidence of allergic or allergic-like reactions, immediately or delayed, in the ponies used in this study. Additionally, there was no evidence of extravasation of contrast agent, and presumably this

risk was negated owing to the length and position of the administration catheter in the right atrium.

In conclusion we demonstrated that it is possible to safely and reliably measure regional lung perfusion using CTA and the MSM in anesthetized ponies. The measurements were repeatable, there was acceptable agreement between observers, and the results were comparable with results obtained with a previously described microsphere technique.[2] The method described here may be of use in physiological studies in which pulmonary perfusion must be measured and in the diagnosis and management of clinical patients with a variety of lung disorders and diseases.

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