Influence of lung injury on cardiac output measurement using transpulmonary ultrasound dilution: a validation study in neonatal lambs

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Editor’s key points
• Cardiac output (CO) measurement using transpulmonary ultrasound dilution (TPUD) is a promising method for cardiac output (CO) measurement in severely ill neonates. The incidence of lung injury in this population is high, which might influence CO measurement using TPUD because of altered lung perfusion. We evaluated the influence of lung injury on the accuracy and precision of CO measurement using TPUD in an animal model.

Background. Transpulmonary ultrasound dilution (TPUD) is a promising method for cardiac output (CO) measurement in severely ill neonates. The incidence of lung injury in this population is high, which might influence CO measurement using TPUD because of altered lung perfusion. We evaluated the influence of lung injury on the accuracy and precision of CO measurement using TPUD in an animal model.

Methods. In nine neonatal lambs, central venous and arterial catheters were inserted and connected to the TPUD monitor. Repeated lavages with warmed isotonic saline were performed to gradually induce lung injury. CO measurements with TPUD (COtpud) were compared with those obtained by an ultrasonic transit-time flow probe around the main pulmonary artery (COufp). An increase in oxygenation index was used as an indicator of induced lung injury during the experiment. Post-mortem lung injury was confirmed by histopathological examination.

Results. Fifty-five sessions of three paired CO measurements were analysed. The mean COufp was 1.53 litre min⁻¹ (range 0.66–2.35 litre min⁻¹), and the mean COtpud was 1.65 litre min⁻¹ (range 0.78–2.91 litre min⁻¹). The mean bias (standard deviation) between the two methods was 0.13 (0.15) litre min⁻¹ with limits of agreement of +0.29 litre min⁻¹. The overall percentage error was 19.1%. The accuracy and precision did not change significantly during progressive lung injury. Histopathological severity scores were consistent with heterogeneous lung injury. The capability to track changes in CO using TPUD was moderate to good.

Conclusions. The accuracy and precision of CO measurement using TPUD is not influenced in the presence of heterogeneous lung injury in an animal model.

Keywords: cardiac output; children; indicator dilution techniques; lung injury, monitoring; neonate

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Haemodynamic monitoring is part of standard care in critically ill children.1–3 Measurement of cardiac output (CO), haemodynamic volumes, and extravascular lung water is increasingly used to provide information about the patient’s haemodynamic state and to initiate and monitor therapy in many disease states.4 5 Pulmonary arterial thermodilution measurement is regarded as the gold standard reference method for measuring CO in the adult clinical setting, although nowadays, it is used in selected patients only.6 7 The limitations of this technique are well documented.8 9 Transpulmonary thermodilution (TPTD) has been designated as the method of preference for measuring CO in paediatric critical care.10 11 In neonatal intensive care, however, the use of CO measurement—including TPTD—is still limited due to (i) technical restraints and size limitations of the available techniques and (ii) the period of circulatory transition, when patent fetal channels might influence the accuracy of CO measurement.12 Transpulmonary ultrasound dilution (TPUD) is based on changes in ultrasound velocity in arterial blood after central venous injection of isotonic saline at body temperature.13 It is a promising technology for neonatal CO measurement, since no dedicated catheters are required. The method uses an extracorporeal arterio-venous (AV) loop with a peristaltic pump, which can be connected with any indwelling catheter, including umbilical catheters. TPUD technology has been validated in adults14–16 and children17 18 with good accuracy and precision. Neonatal and juvenile animal studies showed that TPUD is accurate, precise, and safe regarding CO measurement, also in the presence of a significant left-to-right shunt.19–21
Any indicator dilution method should fulfil the following conditions to avoid errors in CO measurement: use of an inert, soluble indicator that quickly and completely mixes after injection; constant blood flow during measurement, and preservation of indicator between the injection and detection site. With regard to potential transpulmonary loss of indicator, Moser and Kenner found in healthy, anaesthetized dogs that only 0.08% of the injected volume of isotonic saline is lost during the first pass through the pulmonary capillary bed. The incidence of respiratory failure due to respiratory distress syndrome (RDS), meconium aspiration, congenital lung diseases, and pneumonia is high in critically ill newborns. As these conditions are often associated with increased capillary permeability, lung oedema, and a ventilation/perfusion mismatch, we hypothesized that there might be an absolute or relative increase in indicator loss, potentially influencing the reliability of CO measurements. The objective of this study was to investigate whether gradually induced lung injury influenced the agreement between CO obtained by TPUD (CO_{tpud}) and an ultrasonic transit-time flow probe positioned around the main pulmonary artery (CO_{ufp}) in an experimental lamb model.

**Methods**

**Transpulmonary ultrasound dilution**

TPUD is based on the physical principle that ultrasound velocity is different in blood and isotonic saline, which is used as indicator. An extracorporeal AV loop is connected between indwelling arterial and central venous catheters. Only during measurement procedures, blood flows through this loop that is controlled by a peristaltic pump to prevent stasis of blood and to provide stable blood flow. Isotonic saline at body temperature (1.0 ml kg\(^{-1}\)) is injected into the venous limb. A venous sensor, clamped on the venous limb of the loop, calculates the exact amount and onset of injected indicator. The saline passes the venous catheter and mixes with blood while entering the superior vena cava. The circulation carries the injectate through the heart, lungs, and descending aorta where the arterial catheter is located. An arterial sensor, positioned downstream on the arterial limb of the extracorporeal loop, detects the decrease in ultrasound velocity of the blood. Specially designed software (CO-status\(^{\circledR}\), Transonic Systems Inc., Ithaca, NY, USA) is used to calculate CO and haemodynamic volumes from the obtained dilution curve. Values are displayed on the monitor. The method has previously been described extensively.

**General**

This experiment was performed in accordance with Dutch national legislation concerning guidelines for the care and use of laboratory animals, approved by the Ethical Committee on Animal Research of the Radboud University Nijmegen (RU-DEC #2010-034) and performed in nine random-bred lambs under terminal general anaesthesia. The lambs were premedicated with an i.m. injection of ketamine (10 mg kg\(^{-1}\)), atropine (0.03 mg kg\(^{-1}\)), and midazolam (0.2 mg kg\(^{-1}\)). After placing a peripheral i.v. for injection of propofol (2 mg kg\(^{-1}\)) orotracheal intubation was performed with a cuffed tracheal tube (ID 4–6 mm; Kruse, Marslev, Denmark) and the lambs were ventilated mechanically in the pressure-controlled mode using a Datex Ohmeda Excel 210 SE anaesthesia machine (GE Healthcare, Waukesha, WI, USA). Anaesthesia was maintained with inhaled isoflurane (0.5–2.0%), and i.v. sufentanil (15–25 μg kg\(^{-1}\) h\(^{-1}\)), midazolam (0.2 mg kg\(^{-1}\) h\(^{-1}\)), and pancuronium (0.02 mg kg\(^{-1}\) h\(^{-1}\) after a loading dose of 0.05 mg kg\(^{-1}\)). Ventilator settings were adjusted in order to approximate normoxaemia (SaO\(_2\) 90–95%) and normocapnia (PaCO\(_2\) 4.0–6.5 kPa). A servo-controlled heating mattress and a heating radiator were used to maintain a rectal temperature between 38 °C and 39 °C. The animals were euthanized at the end of the experiment by an overdose of pentobarbital (150 mg kg\(^{-1}\) i.v.).

**Instrumentation**

Immediately after induction of anaesthesia, intravascular catheters were surgically inserted. The tip of the arterial catheter (umbilical vessel catheter 5 Ch/35 cm/1.7 mm, Argyle\(^{TM}\), Tyco Healthcare/Kendall Ireland Limited, Tallamore, Ireland) was positioned in the abdominal aorta via the left femoral artery and connected with the arterial limb of the extracorporeal circuit for TPUD measurement and continuous arterial pressure measurement. A double-lumen central venous catheter (16 G/16 cm/1.7 cm, Arrow, Arrow International, Reading, PA, USA) was inserted via the jugular vein with the position of the tip in the superior vena cava. One of the lumina of this central venous catheter was connected to the venous limb of the AV loop for TPUD measurement. The other lumen was used for administration of fluids and medication.

A left thoracotomy was performed at the left fourth intercostal space. The pericardium was incised and the aorta located. The ascending aorta was gently separated from the pulmonary artery by a blunt surgical forceps. An adequately sized transit-time flow probe (10 or 12A, PAX series, Transonic Systems Inc.) was placed around the main pulmonary artery. We chose to place the flow probe around the main pulmonary artery and not the ascending aorta as the main pulmonary flow reflects the systemic flow even in the presence of possible extracardiac shunts. Furthermore, a flow probe around the ascending aorta does not include the coronary blood flow. Acoustic gel was added between the flow probe and the vessel to obtain a qualitative good signal. If still open, the native ductus arteriosus was ligated to prevent shunting. The pericardium and thoracic cavity were closed and the lambs were turned into a supine position. The flow probe was checked for zero value directly post-mortem.

**Induction of lung injury**

We used an RDS model, since RDS is one of the most frequently observed conditions on a neonatal intensive care
unit. Lung injury was gradually induced by repeated (broncho-alveolar) saline lavages as described by Lachmann and colleagues. Lung lavage was performed by instilling 10–30 ml kg$^{-1}$ warmed isotonic saline in 60 ml aliquots. After a 2–3 min period, the saline was drained by gravity and subsequently suctioned with a tracheal suction catheter (10 Ch/60 cm, Mully, Unomedical A/S, Denmark). Ventilator settings were adjusted during instillation in order to maintain tidal volumes of 10 ml kg$^{-1}$. Blood gas analyses were performed at baseline and after every two lavages. Lavages were repeated until a P$\text{O}_2$ < 100 torr (<13.3 kPa) was achieved at an F$\text{IO}_2$ of 1.0. The lambs remained in the supine position during the experiment to obtain a bilateral washout and to guarantee an adequate flow probe position.

**Experimental protocol**

After a stabilization period of 15 min, the study protocol was started. Sessions of CO measurement using TPUD (COtpud) were performed at baseline and after every two lavages. After every pair of lavages, a stabilization period of 15 min was taken. Every CO measurement session consisted of triplicate injections with 1.0 ml kg$^{-1}$ isotonic saline at random in the respiratory cycle.

**Determination of lung injury**

Oxygenation index (OI) was used as an indicator to assess the severity of the induced lung injury during the experiment. The OI was defined using the following formulas:

$$\text{OI} = \frac{mPAW \times FIO_2}{PaO_2}$$

(1)

where mPAW is the mean airway pressure (cm H$_2$O), FIO$_2$ the oxygen fraction (%), and PaO$_2$ the partial oxygen tension (torr) and

$$mPAW = \frac{(\text{PIP} \times \text{Tinsp}) + (\text{PEEP} \times \text{Texp})}{\text{Tinsp} + \text{Texp}}$$

(2)

where PIP is the peak inspiratory pressure (cm H$_2$O), Tinsp the inspiratory time (s), PEEP the positive end-expiratory pressure (cm H$_2$O), and Texp the expiratory time (s).

A higher OI is indicative of more severe lung injury. To confirm lung injury, post-mortem histopathological examination was performed using scoring systems described previously. The lungs were imbedded in substantial amounts of pleural fluid. Lung tissue from the right and left lower lobe was fixated in 4% buffered formalin immediately after removal. After mounting in paraffin, two slices of 4 μm thickness were stained with haematoxylin/eosin and periodic acid-Schiff (PAS), respectively. The histopathological changes in the lung tissue were scored using a semi-quantitative scoring system by a pathologist. Variables scored were alveolar and interstitial inflammation, alveolar and interstitial haemorrhage, oedema, atelectasis, and necrosis. Each variable was scored on a 0- to 4-point scale (0, no injury; 1, injury in 10% of the field; 2, injury in 20% of the field; 3, injury in 30% of the field; 4, injury in >30% of the field) (scores adapted from Hilgendorff and colleagues). The maximum possible score for each tissue sample was 28. The mean scores of the two tissue samples for every lamb were also calculated.

**Other measurements**

We used biomedical data acquisition software (Poly, Inspektor Research Systems BV, Amsterdam, The Netherlands) to store COufp with a 200 Hz sampling rate. The difference between COtpud and COufp was calculated using the mean value of three consecutive TPUD measurements and the mean value of the three corresponding flow probe measurements. Before every measurement, the adequacy of signal strength of the flow probe around the main pulmonary artery was checked.

**Statistical analysis**

A Mann–Witney test was used to demonstrate any significant difference between the OI at the beginning and the end of the experiment. The method described by Bland and Altman for repeated measurements was used to assess the agreement between COtpud and COufp. The mean bias was defined as the mean difference between COtpud and COufp. The mean bias was plotted against the mean CO of both methods [(COufp + COtpud)/2]. Precision was represented by the limits of agreement (LOA). A mixed effects linear model was used to estimate the variance component due to lamb ($\sigma^2$) and the residual variance ($\sigma^2_R$). The LOA was then calculated as: mean(bias) ± 1.96 × $\sqrt{\sigma^2 + \sigma^2_R}$. The percentage error was calculated as 100% ×$[1.96 \times \text{standard deviation (SD)}$ of the bias/mean COufp]. Measurements were also grouped into different OI categories (OI < 10, OI 10–20, OI 20–40, and OI > 40). For each OI category, the median, inter-quartile range, and 95% confidence interval (CI) of the bias were calculated and reproduced in a boxplot. To demonstrate any significant difference in the mean bias between different OI categories, a mixed effect linear model was used with the OI group as fixed effect and lamb as random effect to deal with multiple measurements per lamb. A P-value of < 0.05 was considered statistically significant. The percentage error was subsequently calculated in every OI category. Changes in CO were calculated by subtracting the consecutive CO measurements obtained after every two lavages for each method. Analysis of the agreement in CO trend monitoring between the two methods was assessed with a polar plot. Agreement between methods is shown by the deviation (or angle) from the polar axis (0) and magnitude of the change in CO by the distance from the origin (0, 0). Negative changes were converted to positive changes and central zone data (<10% changes) were excluded to facilitate the statistical analysis. The mean polar angle (angular bias), the SD of the polar angle, and the radial LOA (radial sector that contains 95% of the data points) were calculated. Good trending
ability was defined as an angular bias of \(\pm 5^\circ\) and radial LOA of \(\pm 30\%\).

SPSS 16.0.01 for Windows\textsuperscript{®} (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The polar plot was created with SigmaPlot for Windows version 7.1 (Systat Software, Inc., San Jose, CA, USA).

**Results**

Table 1 shows the characteristics of the animals studied. The mean weight was 8.9 kg (range 4.1–12.3). Post-natal age ranged from 7 to 22 days. In one animal, the native ductus arteriosus was ligated. The number of lavages per animal ranged from 6 to 14. Five lambs received inotropic drugs (dobutamine 3–6 \(\mu g\) kg\(^{-1}\) min\(^{-1}\)). Two lambs (5 and 7) died before all measurement sessions could be undertaken due to severe hypoxaemia or haemodynamic instability.

There was a significant increase in OI during the experiment \((P=0.0001)\). Figure 1 shows the histopathological findings. Figure 1a shows the histopathological scoring system for every lamb: scores for individual tissue samples varied from 4 to 15 (mean scores per lamb 6.5–12.5) compatible with heterogeneous lung injury. Figure 1b and c illustrates the histopathological findings in lambs 2 and 8, respectively.

A total of 55 measurements sessions could be analysed, as two sessions were excluded due to erroneous results. The COufp ranged from 0.66 to 2.35 litre min\(^{-1}\) (mean 1.53 litre min\(^{-1}\)), and the COtpud ranged from 0.78 to 2.91 litre min\(^{-1}\) (mean 1.65 litre min\(^{-1}\)).

The Bland–Altman plot is shown in Figure 2. The overall mean bias (SD) was 0.13 (0.15) litre min\(^{-1}\), and LOA were +0.29 litre min\(^{-1}\) with an overall percentage error of 19.1%. Figure 3 shows box and whisker plots for the median bias between COufp and COtpud with increasing OI. The accuracy did not change significantly with increasing

<table>
<thead>
<tr>
<th>Lamb</th>
<th>Weight (kg)</th>
<th>Number of lavages</th>
<th>Number of COtpud sessions</th>
<th>Range in CO: COufp (litre min(^{-1})); COtpud (litre min(^{-1}))</th>
<th>(P_{O_2}) (torr (kPa)) at end of experiment</th>
<th>OI, begin–end</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>8.1</td>
<td>10</td>
<td>7</td>
<td>1.65–2.08; 1.84–2.25</td>
<td>93 (12.3)</td>
<td>5.7–19.7</td>
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<tr>
<td>2</td>
<td>7.4</td>
<td>6</td>
<td>5</td>
<td>0.83–1.35; 1.04–1.90</td>
<td>95 (12.7)</td>
<td>6.1–12.8</td>
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<tr>
<td>3</td>
<td>4.1</td>
<td>10</td>
<td>6</td>
<td>0.66–1.21; 0.94–1.54</td>
<td>45 (6)</td>
<td>6.7–50.3</td>
</tr>
<tr>
<td>4</td>
<td>10.2</td>
<td>10</td>
<td>6</td>
<td>1.55–2.11; 1.64–2.41</td>
<td>67 (8.9)</td>
<td>3.8–45.6</td>
</tr>
<tr>
<td>5</td>
<td>9.6</td>
<td>6</td>
<td>4</td>
<td>1.08–2.11; 1.22–2.60</td>
<td>67.5 (9.0)</td>
<td>3.6–54</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>14</td>
<td>8</td>
<td>0.86–1.36; 0.78–1.43</td>
<td>96 (12.8)</td>
<td>3.5–36.1</td>
</tr>
<tr>
<td>7</td>
<td>9.9</td>
<td>6</td>
<td>4</td>
<td>1.43–2.21; 1.45–2.65</td>
<td>50 (6.7)</td>
<td>3.0–70.3</td>
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<tr>
<td>8</td>
<td>11</td>
<td>14</td>
<td>8</td>
<td>1.18–2.35; 1.35–2.67</td>
<td>41 (5.5)</td>
<td>4.9–52.2</td>
</tr>
<tr>
<td>9</td>
<td>12.3</td>
<td>6</td>
<td>9</td>
<td>1.26–1.97; 1.23–1.93</td>
<td>46.5 (6.2)</td>
<td>5.8–56.8</td>
</tr>
</tbody>
</table>

**Histopathological findings:**

(a) LLL = left lower lobe, RLL = right lower lobe, 0 - no injury, 1 - injury in 10% of the field, 2 - injury in 20% of the field, 3 - injury in 30% of the field, 4 - injury throughout the field.

(b) Lung tissue of lamb 2 (200x, PAS stain): thin proteinaceous intra-alveolar fluid (thin arrows), and hyaline membranes (broad arrows).

(c) Lung tissue of lamb 8 (200x, PAS stain): interstitial oedema (star) and intra-alveolar remnants of proteinaceous material (thin arrow) consistent with hyaline membranes. Interstitial as well as intra-alveolar inflammatory infiltrates (broad arrows).

Fig 1 Histopathological findings. Scoring system adapted from Hilgendorff and colleagues.\textsuperscript{28}
OI (P=0.95). Percentage errors for OI subgroups were 20.0% (OI<10), 15.1% (OI 10–20), 18.1% (OI 20–40), and 18.9% (OI>40). The polar plot (Fig. 4) shows moderate-to-good trending as the plot shows an angular bias of \( +8^\circ \) and radial LOA of 32%.

**Discussion**

In this study, we found that CO measurement using TPUD was reliable with acceptable accuracy and precision (percentage error <20%) in an experimental neonatal lamb model with induced, heterogeneous lung injury.

Lung injury is often associated with an increased pulmonary capillary permeability, resulting in pulmonary oedema and increased extravascular lung water. It is well known that indicator loss can occur using TPTD.\(^{33, 36}\) As the TPTD technique uses a thermal indicator (heat), the volume of the distribution is not only limited to the blood vessels, but also includes the extravascular lung water space. The TPUD technique uses a non-diffusible indicator (normothermic isotonic saline), so we assume that loss into the extravascular space is negligible. However, flow dynamics may alter in

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**Fig 2** Bland–Altman plot for repeated measurements for the assessment of agreement between TPUD CO (COtpud) and main pulmonary artery blood flow (COufp). The solid line represents the mean bias and the dotted lines represent LOA for all data (OI<10, OI 10–20, OI 20–40, and OI>40).

**Fig 3** Box and whisker plots for the median bias between CO measured by flow probe (COufp) and TPUD (COtpud) with increasing OI. The box represents the inter-quartile range of the bias: the whiskers express the 95% CI of the bias. Mixed effect linear model showed no significant difference between OI groups.

**Fig 4** Half circle polar plot representing the changes in CO measured with TPUD (COtpud) and the ultrasound flow probe around the main pulmonary artery (COufp). Central zone data (<10% changes) are excluded. The dashed line represents the mean radial bias (8°), dotted lines the 95% CI (32°). Almost all data are situated within the 30° radial limits.
the injured lung: pulmonary arterial vessels tend to constrict in response to local hypoxia, redistributing blood flow away from poorly ventilated regions and towards lung regions that are well ventilated, optimizing local ventilation/perfusion matching and minimizing intrapulmonary shunting. This could lead to differentiated transit times due to dispersion of the indicator, a change in the shape of the dilution curve, and therefore influencing CO measurements.

We did not opt for an oleic acid or a lipopolysaccharide model to induce lung injury but chose the lavage model as this model represents most of the RDS seen in neonatal patients. RDS is a heterogeneous disease with different gradations and is characterized by areas of atelectasis, inflammation, necrosis, and oedema. The degree of inflammation is not as substantial as in the other models and the permeability is less affected, although some authors found prominent oedema, indicating increased permeability. Therefore, we have to be aware that the results might not be entirely representative for other neonatal lung diseases such as meconium aspiration syndrome or neonatal pneumonia.

We used the OI to express the lung injury during the experiment. The increase in OI was statistically significant. In combination with the haemodynamic instability, severe lung injury was clinically evident. We preferred to use the gold standard (histopathology) to confirm the lung injury and used therefore a scoring system described earlier to validate the grade of RDS. Individual tissue scores (left vs right lung) varied between 4 and 15, consistent with inhomogeneous moderate lung injury (affected and non-affected areas). Although it is difficult to show a causal relationship between the clinical lung injury and the histopathology, there seems to be a trend that those lambs with the highest OI at the end (>40) also had the highest mean histopathological severity scores (>8) (cf. Fig. 1 and Table 2).

In this study, we found an overall mean bias of 0.13 litre min\(^{-1}\) with LOA of ±0.29 litre min\(^{-1}\) when comparing CO\(_{TPUD}\) and CO\(_{UFP}\). The percentage error of 19.1% is less than the clinically acceptable limit of 30% as defined by Critchley and Critchley. We should notice that we compared TPUD with the gold standard (ultrasonic transit time flow probe), which has a very high precision. Therefore, clinically acceptable limits would be around 20% rather than 30%, making this method acceptable for measuring CO in the neonatal population. This overall accuracy and precision is also comparable with results of the TPTD\(^{36-41}\) and the TPUD\(^{17,20,21,42}\) CO measurements in paediatric animal models and children without lung injury as shown in Table 2.

As the overall accuracy and precision is the result of measurements without and with different degrees of lung injury, we also calculated bias and LOA for the different OI subgroups. We found no significant change in either accuracy or precision in the subgroups with higher OI scores compared with the subgroup with OI <10 (measurements without lung injury). It can be noticed that the number of measurements in every subgroup is relatively small, and therefore, it might be possible that a statistically significant difference could
not be reached. In our opinion, this is of no clinical relevance since the percentage errors for every subgroup are below the 20% margin. Since there is no change in accuracy and precision with increasing OI (and confirmed lung injury), it can be presumed that indicator loss seems indeed to be unlikely.

When there is no obvious indicator loss, there can still be a delayed pulmonary transit time of indicator due to an altered circulation. This could result in a change in the indicator dilution curve. In non-affected areas, we speculate that the injected indicator will travel fast, passing the ultrasound sensor without delay, whereas in injured pulmonary areas, the indicator might travel more slowly, reaching the arterial sensor at a later time point. The expected dilution curve will be less high and wider compared with the initial curve. The total area under the dilution curve, however, should remain the same without hardly any change in measured CO. Dilution curves indeed changed in some of the lambs, particularly in those with the highest histopathological scores (lambs 3, 5, and 8). An example of the change in dilution curve is shown in Figure 5. This finding supports the hypothesis that the delay and differentiation in transit time does not influence CO measured by TPUD in this heterogeneous RDS model. However, there might be a condition, when the indicator release from the affected lung area is such delayed, that the indicator passes the detection sensor after the usual detection timeframe (60 s), resulting in an overestimation of CO. We did not find that in our population.

The applicability of a CO measurement technique is not only determined by the reliability of assessment of absolute CO values, but also by the capability to track changes in CO. We compared changes in CO after consecutive lung lavages between TPUD and the ultrasonic flow probe using a polar plot. In this plot, data points should lie along the dashed line of angular bias to demonstrate good trending ability as the mean CO changes, which is shown by our data, rather than being widely dispersed throughout the half circle. We found that the TPUD technique has moderate-to-good trending ability in this lung injury model as the angular bias is $\pm 8^\circ$ and radial LOA 32% (Fig. 4).

There are some limitations to this study. First, we did not rule out possible intracardiac shunts (patent foramen ovale or ventricular septum defects) which could interfere with CO measurements. On the other hand, the TPUD method is relatively good in identifying shunts, and we did not measure any shunts in the animals using TPUD. Secondly, despite extensive surfactant lavage, signs of histopathological injury were only present in maximal 30% of lung tissue sample. A possible explanation for this phenomenon is probably the relative short time between lavages and histopathological examination. With a longer interval time, microscopic changes could have been more pronounced. However, we believe that 30% histopathological injury can be considered as a sign of substantial lung injury. Other authors have also found the same degree of lung injury after extensive surfactant wash-out. Lastly, we did not measure intrapulmonary shunt flow during lung injury to confirm our hypothesis of altered lung flow. However, the changes in the dilution curves due to increased transit times suggest lung flow alterations.

We conclude that CO measurement using TPUD is reliable in the presence of moderate-to-severe RDS. Indicator loss is negligible and the delayed indicator transit time in animals with heterogeneous lung injury does not affect the reliability of CO measured by TPUD. TPUD is a promising technique to measure CO in critically ill newborns. Clinical studies in this population are warranted.

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**Declaration of interest**

None declared.
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