Regional lung perfusion estimated by electrical impedance tomography in a piglet model of lung collapse

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Borges JB, Suarez-Sipmann F, Bohn SH, Tusman G, Melo A, Maripuu E, Sandström M, Park M, Costa EL, Hedenstierna G, Amato M. Regional lung perfusion estimated by electrical impedance tomography in a piglet model of lung collapse. J Appl Physiol 112: 225–236, 2012. First published September 29, 2011; doi:10.1152/japplphysiol.01090.2010.—The assessment of the regional match between alveolar ventilation and perfusion in critically ill patients requires simultaneous measurements of both parameters. Ideally, assessment of lung perfusion should be performed in real-time with an imaging technology that provides, through fast acquisition of sequential images, information about the regional dynamics or regional kinetics of an appropriate tracer. We present a novel electrical impedance tomography (EIT)-based method that quantitatively estimates regional lung perfusion based on first-pass kinetics of a bolus of hypertonic saline contrast. Pulmonary blood flow was measured in six piglets during control and unilateral or bilateral lung collapse conditions. The first-pass kinetics method showed good agreement with the estimates obtained by single-photon-emission computerized tomography (SPECT). The mean difference (SPECT minus EIT) between fractional blood flow to lung areas suffering atelectasis was −0.6%, with a SD of 2.9%. This method outperformed the estimates of lung perfusion based on impedance pulsatility. In conclusion, we describe a novel method based on EIT for estimating regional lung perfusion at the bedside. In both healthy and injured lung conditions, the distribution of pulmonary blood flow as assessed by EIT agreed well with the one obtained by SPECT. The method proposed in this study has the potential to contribute to a better understanding of the behavior of regional perfusion under different lung and therapeutic conditions.

EFFICIENT GAS EXCHANGE, the primary function of the lung, is the result of an intimate matching of regional ventilation and perfusion across alveoli. Critically ill patients under mechanical ventilation often present gross disturbances of pulmonary gas exchange. To determine the regional matching of alveolar ventilation and perfusion during pathologic conditions, a method capable of measuring ventilation and perfusion, both globally and regionally, is needed. Such measurements go beyond the structural and anatomical information provided by classical imaging technologies and require functional information like regional blood flow or the regional kinetics of a convenient tracer at the bedside. In recent publications (15, 16), the determination of regional perfusion through atelectatic lung units has been repeatedly pointed out as an important missing link in the assessment of the consequences of mechanical ventilation.

New developments in functional imaging methods such as magnetic resonance imaging (MRI; Ref. 50), X-ray computerized tomography (CT; Refs. 5, 6, 23, 32), single-photon emission computerized tomography (SPECT, Ref. 29), and positron emission tomography (PET) have provided, to various degrees, interesting insights into the heterogeneity of lung aeration and the distribution of ventilation and perfusion. From these methods, PET has established a quantitative relationship between regional ventilation and perfusion and gas exchange (52). However, the above methods lack the ability to track the frequent changes in the lung structure and function that occur in the critically ill because they do not allow for continuous monitoring at the bedside. Novel methods capable of repeated data acquisition and interpretation in real time are desirable.

Electrical impedance tomography (EIT) has emerged as a new functional imaging method potentially meeting many clinical needs (1–3, 8, 25, 51). Subjecting the chest to minute electrical currents, this radiation-free, noninvasive technique measures the electric potentials at the chest wall surface to produce two-dimensional (2-D) images that reflect the impedance distribution within the thorax. Cyclic variations in pulmonary air and blood content are the major determinants for the changes in thoracic impedance, the former usually of much larger magnitude. Because cyclic changes in local impedance mainly correspond to changes in lung aeration, recent studies (22, 27, 40, 51, 56) have shown that EIT can reliably assess imbalances in the distribution of regional ventilation in critically ill patients. Besides other features like portability and the possibility of around-the-clock monitoring, the high temporal resolution is an important aspect of bedside imaging that allows for the study not only of ventilation but also of faster physiological phenomena, such as the pulsatility of the lung during the cardiac cycle (7, 9, 17–20, 30, 37, 45, 53, 55). The measurement of lung pulsatility has been the principal method for assessing perfusion by EIT, as it correlates well with stroke volume measured by thermomobilization (53). The pulsatility...
method measures the amplitude of the cyclic perturbations in local lung impedance caused by the passage of the stroke-volume through the lung (7, 17–19, 30, 37, 45, 53, 55). Lung pulsatility, however, is also influenced to a significant degree by the distensibility of the pulmonary vessels as well as the size and patency of the pulmonary microvascular bed (42). Therefore, pulsatility-based methods might be misleading as a measure of pulmonary perfusion should collapse of small pulmonary vessels occur, as well as during any important change in parenchyma architecture.

The concept of using hypertonic solutions as an EIT contrast agent to measure perfusion was proposed a long time ago (9). More recently, lung perfusion estimated by such a method has been compared with regional static CT images (20). We herein present a modified version of the EIT-based method to estimate regional lung perfusion based on the first-pass kinetics of a bolus of hypertonic contrast. While the theoretical framework of such first-pass kinetics has been validated for CT and MRI technology (26, 28, 34, 35, 41), it requires adaptations to meet the particularities of EIT like the nature and timing of the perturbations caused by the hypertonic bolus as well as the absence of precise information about the slice thickness of the EIT image, which encompasses large portions of the lung and heart. Our hypothesis was that the novel indicator dilution method should outperform lung pulsatility as a surrogate for regional lung perfusion.

METHODS

EIT

EIT data were acquired using the enlight platform for impedance tomography, developed by the Experimental Pulmonology Laboratory and Polytechnic Institute of the University of São Paulo, in a partnership with Dixtal Biomédica (São Paulo, Brazil; Refs. 12–14). The prototype is capable of producing 50 real-time images per second. After the thoracic perimeter was measured, 32 adhesive electrodes were placed equidistantly around the circumference of the thorax just below the level of the axilla. Small electrical currents (5–12 mA; 125 kHz) were injected in a rotating sequence through pairs of electrodes, with one noninjecting electrode interposed between two injecting electrodes. During an injection pattern, the noninjecting electrodes were used to measure 29 differential voltages between interleaved electrode pairs in parallel. One complete acquisition cycle of 32 current patterns produced 928 voltage measurements comprising one “raw voltage frame” used as an input to construct a relative EIT image. These images were generated by a reconstruction algorithm for a cross section of the thorax, which is based on a sensitivity matrix derived from a three-dimensional (3-D) finite element model with ~6,000 elements, 6-cm thick, and with the approximate dimensions of a piglet’s thorax (21 × 22 cm). The relative impedance changes estimated for the midlayer of the finite element mesh (midway along the craniocaudal axis) were plotted in a matrix containing 860 valid estimated for the midlayer of the finite element mesh (midway along 6,000 elements, 6-cm thick, and with the approximate dimensions of a three-dimensional (3-D) finite element model with “raw voltage frame” used as an input to construct a relative EIT image. The average or simple sum of all pixel values within each output image was plotted against time, producing a global EIT signal. Such signal is linearly related to changes in parenchymal air content (39, 51). This concept was applied regionally within regions of interest (ROIs) producing regional EIT signals.

Concept of Estimating Regional Lung Perfusion by EIT

Due to its high conductivity, 20 % NaCl acts as an EIT contrast agent (9, 20, 49), which after injection into the right atrium, during apnea, passes through the pulmonary circulation thereby producing a dilution curve that follows typical first-pass kinetics. The resulting regional time-impedance curves are then analyzed to quantitatively assess regional perfusion. After inverting these curves (multiplying by −1), an appropriate time window lasting from the moment of bolus infusion until just before some impedance rebound was detected (usually at the middecay of the curve) was selected to avoid recirculation artifacts. This window was restricted further by using data points from the beginning of the perturbation until its peak (48), thereby making recirculation artifacts even more unlikely. The resulting curve was then fitted on a pixel-by-pixel basis by a corresponding gamma-variate function. (33, 48).

Before using such routine, however, the following additional signal processing at the pixel level had to be performed.

Subtraction of the Right Cardiac Phase

During contrast bolus injection, some EIT pixels, especially those in the right heart region, typically show a two-valley behavior in which the earlier local minimum is related to the passage of the bolus through the right heart and the later one to its passage through the lung parenchyma, with both phenomena represented within the same pixel (Fig. 1). Such behavior, known as partial volume effect, is well described for CT technology. Given the relatively large pixels and comparatively poor spatial resolution of EIT, especially along the z-axis (craniocaudal), this effect requires proper treatment. We assumed that each pixel in the image was composed of a double compartment, an earlier and faster one related to the right heart and a later and slower one to the lung. Each compartment exhibited its own first-pass kinetics and a respective gamma function. The fitting process was then performed in such a way that the sum of the two independent gamma functions had to best fit the raw curve for each pixel, allowing a certain temporal overlap between both curves. We then discarded the right heart component and continued the analysis with the net lung-perfusion signal corresponding to the expected behavior of the slow compartment and its respective gamma function (Fig. 1).

Left Cardiac Phase and Recirculation

Perfusion of the bronchial arteries and systemic recirculation cause a typical and late (8 to 12 s after bolus injection) rebound of impedance perturbation of very low magnitude. For most measurements it was easy to select a time window long enough to obtain an adequate curve fitting but short enough to avoid contamination by the above phenomena. In addition, a transient and localized impedance rebound was sometimes observed due to accumulation of contrast within the left cardiac chambers. This effect happened before recirculation and just a few frames (0.5 to 1.0 s) after the main lung-perfusion phase. Although causing impedance perturbations of much lower magnitude than the right heart, we reasoned that such phenomenon could also cause partial volume artifacts in the few pixels corresponding to the left heart region leading to an overestimation of the net lung-perfusion components. Therefore, especially because of those pixels with a very slow perfusion and those showing an early left heart perturbation, we used a method to
estimate regional blood flow that relies solely on the part of the dilution curve before its peak. For details see below, and Appendix A AND B.

Calculating Regional Flow

After extracting the net contrast curve representing lung perfusion within each pixel the following parameters were derived from the fitted gamma function: peak-value, time-to-peak, area above the curve (i.e., integral of the corresponding gamma function curve over time, also called gamma area; Ref. 48), and maximum slope within the time interval from time-to-appear to the time-to-peak.

The most widely described method for calculating regional blood flow in CT analysis, MRI, or focal angiograms is based on the central volume theorem (57), according to which the regional perfusion of a parenchymal organ is expressed by the following formula:

\[
\text{Regional Blood Flow} = \frac{\text{Area}_{\text{gamma}}}{\text{Volume}_{\text{organ}}}\]

Fig. 1. Typical behaviors of the signal in a lung and a heart/lung pixel observed after injection of a bolus of hypertonic saline. A: typical lung pixel shows 1 visible valley only, representing the passage of the contrast through the lung. Fitting of the early cardiac component was negligible and cannot be seen. Note that the nadir of the curve occurs at 3–4 s after bolus injection. B: typical pixel behavior at thoracic regions close to the heart exhibiting a 2-valley shape. Note that the first and steeper valley occurs earlier, immediately after the bolus of contrast (arrow) corresponding to the passage of the bolus through the cardiac chambers (right atrium and ventricle). A second valley can be observed later along this same pixel tracing (4–5 s after bolus injection) corresponding to the later passage of the contrast through lung regions also represented within this same pixel. Such lung regions are probably located at a different position along the craniocaudal z-axis, but at a similar location in the x-y-plane. Although fitting and integral calculations were performed after inverting the curves above, we presented the negative curves directly derived from electrical impedance tomography (EIT) images, illustrating the negative perturbation in impedance caused by the hypertonic bolus. For both pixels, the estimates for net lung perfusion (i.e., the maximum slope of the initial moment of lung perturbation) were calculated from the fitted gray curve.
Innovative Methodology

Relative blood flow $(\text{pixel}) = \frac{\text{relative blood volume (pixel)}}{\text{mean transit time (pixel)}}$

where the relative blood volume $\text{pixel}$ is directly estimated from the fitted gamma area for each pixel.

In our calculations, however, the accurate estimation for the mean transit time would require not only the removal of the signal-components related to recirculation and left cardiac phase but also the simultaneous measurement of the time-impedance curves of the feeding pulmonary artery (to be used in a deconvolution process, to correct for the fact that the bolus is not fast enough).

Due to such practical difficulties, we opted for an adaptation of an older and less frequently used method for measuring regional perfusion called the maximal slope method (28, 34). The method is based on the Fick principle of the conservation of mass to a given region of interest (28, 36). Assuming there is no venous drainage of contrast before the peak of the pulmonary artery input function, the accumulated mass of the contrast within a pixel can be calculated as the product of regional blood flow and the time integral of the pulmonary artery contrast concentration:

$$m(t)_{\text{pixel}} = \text{blood flow}_{\text{pixel}} \cdot \int_0^t \text{pulmonary artery concentration}(\tau) \cdot dt$$

(1)

Differentiating $\text{Eq. 1}$ leads to an expression of the slope or $dm(t)/dt$:

$$\frac{dm(t)}{dt} = \text{blood flow}_{\text{pixel}} \cdot \text{pulmonary artery concentration}(t)$$

(2)

Consequently, the slope will be maximal when the pulmonary artery concentration is maximal. Regional blood flow can then be calculated as:

$$\text{blood flow}_{\text{pixel}} = \frac{\left[ \frac{dm(t)}{dt} \right]_{\text{max}}}{\text{pulmonary artery concentration}(t)_{\text{max}}}$$

(3)

The maximal slope method is thus based on the concept that the speed of accumulation of contrast within a pixel, represented by the initial slope of the gamma curve, reflects perfusion as the flow of blood into that pixel or the wash-in function to that compartment. An assumption of this method is that there is no venous outflow of contrast during the period used for estimating the slope, thus requiring a high rate of contrast injection in relation to the transit times found in the lung. Another assumption is that the blood flow is constant, instead of pulsatile. For EIT, this method is especially convenient for several reasons: 1) the high temporal resolution of EIT delivers up to 50 points per second with a signal-to-noise ratio higher than in CT or MRI imaging at their common resolutions, making the curve fittings and slope determinations more reliable; 2) the bolus of hypertonic contrast is relatively small (5 ml) and can be infused in <$1 \text{ s}$; 3) we observed that pixel perturbations caused by the given amount of hypertonic contrast were well above background noise (the signal to noise ratio per pixel is higher than in CT studies of lung perfusion, for instance); 4) there was no need to measure the input function as we were not interested in absolute values of perfusion (it was enough to compare pixels within the image); and 5) we could focus on the very initial part of the gamma curve for each pixel thereby avoiding recirculation and left-heart artifacts. These artifacts can markedly affect the gamma-area calculations, but their effect on the initial slope of such gamma curve is negligible. For each pixel, we searched for the maximum slope of the gamma function from the time-to-appear to the time-to-peak of the fastest pixel (i.e., after removing the right heart perturbations, we identified the first pixel in the image to reach its peak and assumed that it represented the compartment with the fastest transit time within the lung). The relative proportion of this fastest slope, against the slopes for all other pixels, represented the relative perfusion to each lung region.

To evaluate the predicted performance of such adapted algorithm, we performed heuristic mathematical simulations (see APPENDIX A).

**EIT Perfusion Analysis Based on Local Lung Pulsatility**

At each protocol condition, an EIT perfusion analysis based on local lung pulsatility $(\Delta Z)$ (7, 17–19, 30, 37, 45, 53, 55) was performed and compared with the hypertonic saline bolus first pass kinetics method.

Briefly, this method is based on the fact that the ejection of the stroke volume from the right heart during systole causes an increase in intravascular pulmonary blood volume and pressures, which leads to the distension of the elastic pulmonary vascular bed, such bulging of blood within septa purportedly causes a decrease in local electrical impedance. It is assumed that such cyclic changes in regional impedance reflect the proportion of the stroke volume directed to such regions. Thus, by measuring the amplitude of the cyclic changes in lung impedance for each pixel, one can estimate the local distribution of right ventricular stroke volume (see supplemental video 2) or the local distribution of pulmonary blood flow. The pulsatility measurement did not require a contrast agent and was made using an ECG-gated acquisition comprising 50 consecutive cardiac cycles during a 20-s apnea. Additionally, the resulting gated signal was low-pass filtered at a frequency of 15 Hz to avoid overestimation in the $\Delta Z$ due to noise (24).

**Experimental Protocol**

The study was approved by the Animal Ethics Committee of Uppsala University (Uppsala, Sweden). Six piglets (2–3 mo old weighing 28.4 ± 2.6 kg) of mixed Hampshire, Yorkshire, and Swedish country breeds obtained from a local breeder were used and studied during various experimental conditions. All animals underwent the same routine instrumentation, intravenous anesthesia using a combination of fentanyl, ketamine and midazolam, and monitoring as previously described (46). Animals were tracheotomized and mechanically ventilated using auffed 7-mm inner diameter endotracheal tube (Mailinckrodt, Athlone, Ireland). Baseline ventilation was delivered in a volume controlled mode using a Servo-i ventilator (Maquet Critical Care, Solna, Sweden) with the following settings: tidal volume of 6 ml/kg, respiratory rate of 30 breaths/min, positive end-expiratory pressure of 10 cmH$_2$O, inspiratory-to-expiratory ratio of 1:2, and fraction of inspired oxygen of $1$. All protocol steps were performed with the animals lying in the supine position. After preparation, two major experimental conditions were studied.

1. **Unilateral lung collapse in healthy animals.** With the use of the healthy animals, the following conditions were sequentially applied: 1) bilateral lung ventilation, 2) unilateral lung ventilation due to complete contralateral lung collapse after selective intubation of the main bronchus of the ventilated lung, and 3) unilateral lung ventilation with sodium nitroprusside infusion to attenuate hypoxic pulmonary vasoconstriction.

2. **Bilateral-dependent lung collapse in injured animals.** Lung injury was induced by repeated lung lavages as previously described (31). After lung injury was established, three situations were monitored sequentially during bilateral lung ventilation: 1) ventilation using baseline settings, 2) after sodium nitroprusside infusion, and 3) after an effective lung reexpansion maintaining an “open lung condition” by a positive end-expiratory pressure of $16$ cmH$_2$O (46).

Each experimental condition lasted 45 min, and measurements were made at the end of each one.

EIT measurements of perfusion based on first-pass kinetics were performed after switching the ventilator to continuous positive airway pressure (CPAP) maintaining the same level of expiratory pressure but eliminating tidal ventilation. A 20% NaCl bolus was injected
within <1 s through a central venous line into the right atrium 5 s after starting CPAP.

**SPECT Imaging**

Pulmonary blood flow distribution was assessed by intravenous injection of $^{99m}$technetium-labeled macro-aggregated albumin ($^{99m}$Tc-MAA, Pulmocis; CISbioInternational, Gif sur Yvette, France). At the beginning of each phase, a reference SPECT was performed before each new injection of $^{99m}$Tc-MAA to subtract the remaining technetium activity from the previous scan. A CT scan (covering the same volume as the SPECT) was taken immediately after each SPECT and used for attenuation correction. Images were acquired using a SPECT/CT dual-head gamma camera (Millennium; General Electric Systems, Milwaukee, WI) with an all-purpose medium-energy collimator. SPECT acquisition was made in 60 projections (30 per head) and stored in a $128 \times 128$ matrix, resulting in a pixel size of 4.42 mm. The overall scan time for SPECT and CT was ~45 min. Data were reconstructed first on an eNTEGRA workstation and later on a Xeleris workstation (Millennium; General Electric Systems). The reconstruction was performed with an iterative model (OSEM, 4 iterations and 8 subsets) and a Hann filter (cut-off 0.85) for the postreconstruction filtering on both workstations. The 128 transverse slices, each of thickness 4.42 mm, were corrected for radiation spillover and for baseline subtraction using a HERMES workstation (Hermes Medical Solution, Stockholm, Sweden). For each reconstructed slice, the contents were analyzed by custom-made software. Before calculating activity distribution, a background subtraction of spillover and for baseline subtraction using a HERMES workstation (Hermes Medical Solution, Stockholm, Sweden). For each reconstructed slice, the contents were analyzed by custom-made software. Before calculating activity distribution, a background subtraction of spillover and for baseline subtraction using a HERMES workstation (Hermes Medical Solution, Stockholm, Sweden). For each reconstructed slice, the contents were analyzed by custom-made software. 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encompassed in ROIs; and total ($\Delta Z_{\text{pulsatile}}$) is the sum of $\Delta Z_{\text{pulsatile}}$ values of all pixels encompassed in all ROIs.

For EIT pulsatility analysis, all pixels corresponding to the heart region were previously removed. The selection of such pixels was based on a phase analysis: pixels presenting a predominant positive variation in $\Delta Z$ between two consecutive QRS complexes were considered “heart” pixels (21, 47) (Fig. 4).

RESULTS

A total of six animals are included in this analysis: three in the unilateral lung collapse protocol (complete left lung collapse in two and right lung collapse in one) and three in the bilateral dependent lung collapse protocol.

EIT-Perfusion Analysis Based On The First-Pass Kinetics

The distributions of pulmonary blood flow assessed by SPECT and EIT (first-pass kinetics) showed good agreement, as exemplified by the qualitative inspection of the images (Figs. 2 and 3) and also by the quantitative analysis using two ROIs (Fig. 5), one of them always representing the collapsed part of the lung. This was true for both healthy and injured piglets. After induction of atelectasis, both techniques consistently demonstrated an initial redistribution of blood flow to non collapsed lung areas, especially in the healthy lung preparations (see supplemental videos 1 and 3). For instance, in one of the healthy animals, the fractional pulmonary blood flow directed to the left lung decreased from ~50% down to ~20% after complete atelectasis of this lung (Figs. 2 and 5). This redirection of blood flow was attributed to hypoxic pulmonary vasoconstriction, which was later attenuated by nitroprusside infusion. We also observed an immediate increment in regional flow to dependent lung regions after recruitment of the collapsed areas in injured lungs. This increment exceeded the increment caused by nitroprusside in all animals, as demonstrated by both EIT and SPECT analysis (see Fig. 5).

Altogether, the mean difference (bias) between the estimates for the blood flow towards the atelectatic ROI in relation to the blood flow to the whole lung (henceforth called fractional blood flow), as measured by SPECT vs. EIT, was $-0.6\%$ with a SD of 2.9\% (range: $-6.7$ to $+3.8\%$).

Figure 6 shows the Bland-Altman plots of the SPECT vs. EIT measurements of fractional blood flow to four lung quadrants of the transverse images. The overall agreement was good (limits of agreement = $-10.9\%$ to $+10.5\%$) although a systematic overestimation of perfusion to the top-left quadrant was observed in EIT measurements ($P < 0.05$). We did not observe any dependence between the difference seen in SPECT and EIT-NaCl measurements and their average magnitude ($P = 0.14$).

EIT-Perfusion Analysis Based On Local Lung Pulsatility ($\Delta Z$)

Significant differences between the estimates of pulmonary blood flow distribution by SPECT and the corresponding estimates based on EIT pulsatility were observed (Figs. 5 and 6).

The discrepancy was especially magnified in the presence of whole lung atelectasis or after the infusion of nitroprusside. In all animals, SPECT analysis indicated that atelectasis caused a systematic decrease in local perfusion (mean decrease = $-23\%$ of total pulmonary blood flow; range: $-14$ to $-29\%$; $P = 0.028$, Wilcoxon rank test). The pulsatility analysis, however, showed contradictory results: atelectasis of the injured, dependent lung zones caused some decrease in $\Delta Z$ pulsatility, but atelectasis of one whole lung caused a marked increase in local $\Delta Z$ pulsatility in two animals. In Figs. 4B and 5, we show one extreme example in which EIT’s local pulsatility estimated a fractional perfusion of 74% to the atelectatic left lung, whereas the SPECT indicated just 19%.

The infusion of nitroprusside increased the blood flow through atelectatic areas in all animals. The pulsatility analysis, however, systematically suggested a decreased blood flow through atelectatic areas after nitroprusside.

Figure 6 shows the Bland-Altman plots of the SPECT vs. EIT pulsatility measurements of fractional blood flow to four lung quadrants (of axial images). There was a systematic overestimation of perfusion to the top left quadrant ($P < 0.001$) and a systematic underestimation of perfusion to the bottom right quadrant ($P < 0.01$). There was no dependence of
the difference between SPECT and EIT pulsatility in relation to their average magnitude ($P = 0.16$).

**DISCUSSION**

We herein report a novel EIT-based method for estimating regional lung perfusion at the bedside. The proposed approach is based on first-pass kinetics of an EIT-indicator dilution curve, using hypertonic saline bolus and overcoming major limitations of a pulsatility-based method. This method showed a good agreement with estimates of lung regional perfusion obtained by SPECT. Our findings also support the notion that EIT pulsatility may be a less suited method for estimating pulmonary perfusion.

A fundamental reason for the poorer performance of the EIT pulsatility, as evidenced in Fig. 4, is probably related to the fact that such method basically measures the pulsatile changes in pulmonary blood volume instead of real forward flow of blood. Changes in vascular tone and synchronous changes in air content (in opposite phase) as previously described (44, 54) might potentially affect the relationship between the cyclic pulsatile changes in regional impedance and the proportion of the stroke volume directed to those regions. Our results suggest that EIT-pulsatility data may be strongly influenced by the downstream pulmonary vascular resistance and by the distensibility of the small pulmonary vessels (19, 43). Probably, different lung conditions and airway pressures also modulated the pulsatility behavior of small pulmonary vessels. Another potential reason for the poor performance of the pulsatility method would be the inclusion of the heart region in the measurements, which we tried to avoid by identifying the heart region and excluding it from the analyses. However, the identification of pixels corresponding to the heart region might have been less than ideal in some cases, which could partially explain the overestimation of perfusion to the top left quadrant by the pulsatility method. The review of individual cases suggested that it is hard to completely exclude residual components of the left atrium from the images. The phases of the left atrium and lungs are similar enough to preclude the exclusion of the former based on phase analysis. More sophisticated methods would be required.

The biggest difference between local blood flow measured by SPECT and EIT-pulsatility images was found during unilateral collapse. Stronger pulsatile changes in impedance were found within the collapsed lung, while the amount of blood flowing to this region was significantly reduced according to Fig. 4. EIT-pulsatility (ECG gated) images of 1 animal, before removing pixels corresponding to the heart region, during 3 conditions: bilateral lung ventilation (A), unilateral healthy left lung collapse (B), and unilateral lung collapse (C) plus sodium nitroprusside infusion. A gray scale was applied, with the lighter color corresponding to higher pulsatility signal. Time-synchronized ECG plus impedance waveforms of three representative pixels are shown, with equivalent scales for both lungs (but halved for heart region). Orange pixel represents the left lung, gray represents heart, and yellow represents the right lung. After creation of atelectasis, note the global decrease in pulsatility, but especially in the right (open) lung (B), where the fractional blood flow at this moment, according to SPECT and EIT-first-pass kinetics, was maximal. Concomitantly, the left atelectatic lung gets relatively “brighter” (because of the auto-scaling of the gray images) producing the false impression of an increased perfusion. As explained in the discussion, this phenomenon may be caused by a diastolic rise of blood flow (pendelblut) from the left to the right pulmonary artery. By addition of nitroprusside infusion (C), a marked attenuation of the difference between the open and collapsed lung becomes evident, approaching the normal lung pattern with bilateral ventilation (A). Diastolic emptying of the pulmonary vessels toward the left atrium is characterized by a marked rise in $\Delta Z$ signals (amplitude = difference between maximum and minimum values of EIT signal oscillations) before the next QRS complex. Note the delay of such process within the right lung in situation (C), which is absent in A (arrows). Note also the complete opposition of phases between the heart and the lungs waveforms.
both SPECT and EIT-first-pass kinetics (Figs. 4B and 5). Our data are in agreement with the findings of Newell et al. (38), who reported a retrograde flow from atelectatic lung zones to healthy ones during the diastolic phase. Within the atelectatic lung, the elastic compartment represented by small pulmonary vessels seems to accumulate a great part of the stroke volume at the end of systole, expelling it back to the contralateral lung during diastole. The much later nadir of EIT signal oscillations in the healthy lung (suggesting a later surge in local blood volume), compared with the atelectatic one (Fig. 4C), suggested this redistribution of perfusion (“pendelblut” in analogy with the German term “pendelluft”) between both lungs. This pendelblut was not present during bilateral ventilation. We hypothesize that, in the presence of massive atelectasis, the pendelblut effect could be so marked and prolonged that the normal diastolic emptying of pulmonary vessels might be attenuated, decreasing the magnitude of ΔZ pulsatility in the healthy areas. It works as if the healthy lung was receiving an almost constant flow rather than a pulsatile, variable flow. Thus despite a higher flow rate (as evidenced by SPECT and EIT-first-pass kinetics), the healthy lung had lower pulsatility.

Interestingly, nitroprusside infusion always attenuated the relative pulsatility within the collapsed lung (Figs. 4C and 5), although systematically increasing the amount of blood shunting through these areas. This seemed to be associated with an increased pulsatility within the remaining open lung areas, rather than a true attenuation within collapsed areas.

It is important to keep in mind that, although the pulsatility method has been appealing because of its ease, it does not rely on solid mathematical foundation to really represent perfusion in a quantitative manner. In contrast, the theories behind the first-pass kinetics of a tracer and the maximum-slope approach described in this study are concepts that relate to classic physiological studies (11, 28, 34, 57) about parenchymal perfusion, having also a proven mathematical justification (see the mathematical model described in the APPENDIX A). The present data may unravel a potential limitation of the pulsatility method, especially in conditions where atelectasis may play an important role, which will have to be confirmed in further studies.

Besides the small number of animals, which prevented us from presenting definitive statistics, but rather a descriptive proof of concepts, some other limitations of this study deserve mentioning. First, due to technical reasons, SPECT and EIT image acquisitions could not be done simultaneously but sequentially. To circumvent this limitation, we always waited for

Fig. 5. Distribution of pulmonary blood flow in healthy lungs and in lungs with acute lung injury. Percentage of total pulmonary blood flow flowing through the left atelectatic lung as measured by EIT and SPECT. All animals are shown. Each panel refers to one studied animal. EIT perfusion according to the first-pass kinetics was based on the first-pass of contrast through the lung. EIT pulsatility represents the local amplitude of impedance swings during ECG-gated acquisition (see METHODS for details). Three sequential conditions in three noninjured lung preparations are shown: bilateral lung ventilation, unilateral lung collapse, and unilateral lung collapse with sodium nitroprusside infusion. Three sequential conditions in three injured lung preparations are shown: lung injury (bilateral, dependent lung atelectasis), lung injury plus sodium nitroprusside infusion, and open lung condition after lung recruitment (total reversal of atelectasis). Note the good match between the first-pass kinetics method and SPECT. Note also the opposite, artifactual changes in EIT pulsatility observed during atelectasis and after the infusion of nitroprusside.
steady-state conditions, including the effects of nitroprusside, whereby a continuous monitoring of arterial blood gases was essential. A second limitation comes from the fact that SPECT is essentially a 3-D imaging procedure, while EIT analysis is something between 2-D and 3-D (although the finite mesh is 3-D, the electrodes were placed within a single plane). Although reasonably large, the effective thickness of the EIT cross-sectional slice (~5–10 cm) varies with the size and shape of the animal (14), and one could never guarantee that most of the lung is being represented in all animals. Clinically, this limitation has to be balanced against the 20-s acquisition in EIT vs. the 45-min acquisition in SPECT. The subtraction of the cardiac component of perfusion from mixed pixels while maintaining the net lung component (Fig. 1) by using the EIT-gamma variate algorithm was an essential feature of this method. However, at the edges of the heart, some challenging overlap between the behavior of lung and heart tissue may have remained, sometimes causing uncertainties in the double-function fitting process. This phenomenon may be responsible for the lower agreement between SPECT and EIT in the top left quadrant. Although the location of the cardiac chambers in humans could make this differentiation easier in the clinical setting, more studies on this subject are needed. Fourth, the assumption of the maximal slope method that no tracer leaves the ROI before the peak artery concentration is reached could be violated in the presence of the combination of high blood volume and low blood flow. As discussed in the APPENDIX A, however, such violations in the assumption would lead to small estimation errors, provided that blood volume decreases by no more than one order of magnitude. Another potential limitation of the proposed method is the possibility of some diffusion of sodium chloride to the outside of the vessels in the lungs. In this situation, the solute that remains in the vessel will leave the lungs through the venous drainage while the diffused solute will tend to stay in the lungs, violating the conservation of mass principle (unless an extravascular compartment is accounted for). For the calculations of the maximal slope, however, the conservation of mass could still be applied correctly, since one of the assumptions is that there is no outflow of hypertonic saline before the peak of the pulmonary artery input function. In this case, what the conservation of mass implies is that all the solute that reaches the ROI (feeding vessel and extravascular compartment lumped together), irrespective of whether it remains inside the vessels or not, came through the feeding artery. Finally, one last important theoretical concern is that, due to the low spatial resolution of EIT, the maximum slope time point might be slightly displaced (in time) among subregions within the ROI, likely causing wrong estimates of spatially averaged maximum slopes. The extent of this potential limitation deserves future studies.

In conclusion, we describe a novel method based on EIT for estimating regional lung perfusion at the bedside. In both healthy and injured lung conditions, the distribution of pulmonary blood flow as assessed by EIT agreed well with the one obtained by SPECT. The method proposed in this study has the potential to contribute to a better understanding of the behavior of regional perfusion under different lung and therapeutic conditions.

APPENDIX A

Heuristic Model For Regional Blood Flow

A mathematical model representing three compartments, the right heart in series with two lung compartments in parallel (compartments A and B), was simulated using reference conditions found in normal physiology: right heart chamber volume of ~150 ml; infusion time of ~2 s; baseline blood volume for each lung compartment of ~150 ml; and total cardiac output of ~5 l/min. Each lung compartment had an inflow equal to its outflow, adjusted independently from other compartments and thus a fixed blood volume over time. This fixed blood volume could be different among compartments, depending on simulations. Equations simulating mass balance and concentration curves inside each compartment were solved numerically by assuming that...
1) the contrast was infused at the right heart chamber in a short bolus (2 s); 2) the bolus suffered immediate and ideal dispersion; and 3) blood flow was constant and nonpulsatile.

We calculated the regional percentage change in resistivity according to a modification of the formula proposed by Brown et al. (9) based on the volume fraction (V) of the indicator (the hypertonic saline) contained within the compartment at that particular moment, the conductivity of blood ($\sigma_1 = 0.62$ Sm/m), and the conductivity of the hypertonic saline ($\sigma_2 = 32$ Sm/m).

$$\frac{\rho - \rho_0}{\rho_0} = \frac{3 \cdot V \cdot (\sigma_2 - \sigma_1)}{2 \cdot \sigma_1 + \sigma_2 + 2 \cdot V \cdot (\sigma_2 - \sigma_1)}$$

This means that the instantaneous intensity of the EIT signal represented both the volume of the compartment and its final concentration of sodium.

The estimated regional lung flow was calculated as the maximal slope derived from the fitted gamma curve (34). As in our experiments, we searched for the maximal slope from the start to the time-to-peak perturbation. Percent flow directed to compartment A was calculated as the maximal slope of that compartment A divided by the sum of the slopes of compartments A and B.

We simulated changes in the perfusion to compartment A from 100% down to a minimum of 10% of the value in compartment B, which was kept constant (Fig. A1, left). The maximal slope of the perturbation found in compartment A (derived from the gamma-fitted curve) showed a strong linear correlation with the true perfusion simulated in the model ($R^2 = 1.00$). Additionally, we varied the volume of blood contained in compartment A from 100% down to 10% of the value found in compartment B, while keeping the perfusion (input flow) fixed in both compartments, to assess if the estimation of the perfusion was affected by changes in the residing blood volume (Fig. A1, right). For values of blood volume >30% of the initial value, the maximal slope measurement was not affected by the decrease in blood volume. For greater decreases in blood volume (<30% of the initial value), perfusion was underestimated, albeit to an error of <10%.

Therefore, we concluded that the maximum slope was a robust estimate of perfusion even in the presence of changes in blood volume, provided that those changes are not greater than one order of magnitude. Previous studies on blood pooling at dorsal, gravity-dependent lung regions have revealed that a difference of 10 times in blood volume can be found in normal lungs, when comparing the most extreme lung regions along the gravity axis. Under such conditions, the maximum slope approach would underestimate the perfusion of these lung regions with very low blood volume by ~10%.

**APPENDIX B**

### New Dilution Curve Fitting Method

We developed an automatic/heuristic solution that ran for every pixel in Fig. A1 in a batch process. Before the gamma fitting, we used a finite-impulse response filter in the time domain to reduce the high-frequency noise. To the filtered signal, we applied the fitting process, described in detail elsewhere (48). Of note, the fitting was noniterative and made use of only three relevant data points: the time to appear, the middle-height point, and the peak point. If the curve had two peaks, as in Fig. 1B, we localized the two peaks, even when the first one was smaller than the second one (by using the second and third derivatives), and applied this gamma-fitting to the three data points obtained till the first peak. This procedure intrinsically assumed that no contrast entered the lung before the first peak in the right heart. Then we calculated the subtraction between the raw contrast curve and the gamma-fitted curve of the right heart. We subsequently performed a second gamma fitting to this subtracted curve, obtaining the gamma-fitted curve related to the lung. This procedure was performed on a pixel-by-pixel basis. Bad fittings occurred in 5–10% of the cases and could be detected as pixels to which there was either zero or abnormally high perfusion (outliers). When this happened, we visually detected the right heart area as well as the typical-lung areas, using videos of the saline passage through the heart and lungs, and used the time to peak information of each of these two regions to establish boundaries for fitting. For instance, if the first peak of a two-peak curve occurred close to the time to peak of the typical-lung pixel, the second peak would be ignored, as opposed to considering the first and second peaks as related to the right heart and lung, respectively.

**Limitations**

In our images, due to the low spatial resolution, it was very difficult to separate the lung parenchyma from the pulmonary vessels of second or third generation. Thus our perfusion data was likely con-

Fig. A1. Model simulations for the influence of blood flow and blood volume on the estimates of lung perfusion based on the maximum-slope of contrast perturbation. Model simulations for the impedance perturbation caused by changes in instantaneous sodium concentration within 2 lung compartments (compartments A and B) after hypertonic bolus injection. Timing and amount of sodium chloride infusion (5 ml of 20% sodium chloride infused within 2 s, injected directly into a third compartment upstream, with 150 ml, simulating the right heart) was kept constant throughout the simulations. Situation of compartment B was kept constant (regional blood flow = 2.5 l/min, and regional blood volume = 150 ml), while the blood flow of compartment A changed from 2.5 down to 0.25 l/min (left). At right, we simulated a reduction in the blood volume of compartment A, from 150 ml down to 15 ml. As shown, the calculus of the maximum slope in impedance perturbations found in compartment A kept a quasilinear relationship with the true blood flow (relative perfusion) to this compartment. As also expected, pure changes in blood volume within this compartment (but with sustained blood flow) caused only a minor change (~5%) in the maximal slope. Ideally, we should see no changes related to such pure blood-volume variations.
taminated by the perfusion of the large pulmonary arteries. This is a limitation when analyzing very small regions of interest, since the inclusion or not of an artery can cause large differences in the estimates (especially if the cross section of the artery belongs to one region of interest but its feeding territory belongs to another one). However, when analyzing large regions of interest, as in our study, it is very likely that the perfusion of big arteries is mixed up with the surrounding parenchyma perfusion, and both are strongly related to the perfusion of the respective region of interest.

In theory, there might be to some extent diffusion of sodium chloride to the outside of the vessels in the lungs (characterizing the system as “open” instead of closed). In this situation, the diffused solute would tend to stay in the lungs, while the solute that remained in the vessel would leave the lungs through the venous drainage. To apply the principle of conservation of mass, we would need to consider a closed system and, therefore, we should consider the three compartments: the arterial (input), the interstitial (diffused solute), and the venous (output). Since the calculation of the maximal slope assumes that there was no outflow of hypertonic before the peak of the pulmonary artery input function, the conservation of mass would still be applied correctly if we only consider a system with two compartments. In this case, what the conservation of mass implies is that all the solute that has reached the lungs came through the feeding artery, irrespective of whether the solute has remained inside the vessels or not.

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REFERENCES


