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Ultrasound Strain Measurements for Evaluating Local Pulmonary Ventilation

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Abstract

Local lung function is difficult to evaluate, because most lung function estimates are either global in nature, e.g. pulmonary function tests, or require equipment that cannot be used at a patient's bedside, such as computed tomograms. Yet, local function measurements would be highly desirable for many reasons. In a recent publication [1], we were able to track displacements of the lung surface during breathing. We have now extended these results to measuring lung strains during respiration as a means of assessing local lung ventilation. We studied two normal human volunteers and 12 mice with either normal lung function or experimentally induced pulmonary fibrosis. The difference in strains between the control, normal mice and those with pulmonary fibrosis was significant ($p < 0.02$), while the strains measured in the human volunteers closely matched linear strains predicted from the literature. Ultrasonography may be able to assess local lung ventilation.

Keywords

ultrasound strain measurement; lung ventilation; lung ventilator monitoring; pulmonary fibrosis; lung ultrasound; acute respiratory distress syndrome (ARDS)

I. Introduction

Many lung diseases are patchy or non-uniform in their distributions. Yet, these non-uniformly distributed lung diseases, such as idiopathic pulmonary fibrosis (IPF) or acute lung injury/acute respiratory distress syndrome (ARDS), are still often evaluated using methods that only provide generalized assessments of lung function [2-4]. For example, IPF evaluation involves pulmonary function studies which are global estimates of lung function that cannot assess the true distribution of the disease [2, 5-7].

Local assessments can be performed; the most common of which is the standard chest radiograph. However, because of the limitations of chest radiographs, regional evaluations of diseases with non-uniform lung involvement generally focus on computed tomography (CT) with CT generally considered the gold standard for local lung architecture [8]. Yet, CT is not perfect. CT is not a portable technique, so it cannot be used to assess lung function or mechanics in remote locations such as in intensive care units (ICU), and it is not optimal for screening/monitoring due the radiation risk. Further, CT does not provide much functional information without extensive computational efforts [9].

Magnetic resonance imaging (MRI) is another option with the potential to measure local lung ventilation/function and new developments in parallel imaging, shared echo techniques, and rotating phase encoding are making the method more viable [10]. However the technique has significant problems with signal to noise due to the low proton densities in the lungs, susceptibility artifacts, the inherent qualitative nature of the imaging itself, and the inability to do lung assessments at clinical care sites such as in ICUs [10].

The best present option for a monitoring technique for local pulmonary function/ disease is electrical impedance tomography (EIT). EIT reconstructs local estimates of pulmonary impedance, which correlate to the degree of local aeration of lung. However, EIT has several limitations: 1) it is restricted to one transverse plane through the chest, 2) it would be a difficult monitoring mode since the chest needs to be wrapped in detectors for a measurement, 3) presently the impedance estimates do not seem to correlate with CT lung density, and 4) the results are qualitative, so only relative changes can be evaluated [4].

There is now a great deal of interest in ultrasound imaging for evaluating lung disease. Many papers showing the utility of ultrasonography in diagnosing and assessing various pulmonary and intrathoracic diseases have been published [11]. Besides standard applications such as localization of pleural effusions, physicians are now using ultrasound to identify and characterize such conditions as pulmonary edema, pulmonary fibrosis, and pneumonias [12-15]. These evaluations have almost all been based on characterization of artifacts that likely occur between the pleura and lung surface. These typically manifest as linear artifacts that project from the lung surface into what would be gas-filled lung. The assessment of the underlying conditions based on the number and quality of these artifacts is qualitative or semi-quantitative at best, and none of them assess any component of lung physiology or mechanics [16].

There is now evidence that local lung strain can be estimated by ultrasound. Measuring lung strain could provide a method of monitoring local lung ventilation changes that produce

these strains. In a recent publication, we demonstrated that we could track the motion of the lung surface using ultrasound two-dimensional speckle tracking [1]. The purpose of the tracking in that study was to estimate tissue displacements for guiding radiation therapy treatments of tumors. In this publication, we use the displacement estimates on the lung surface to calculate local strains produced by the expansion and contraction of the lung during breathing in human volunteers and in a murine model of pulmonary fibrosis. As one would expect during inspiration, the strain increases and during expiration the strain decreases. This measurement could lead to an entirely new application of ultrasound to pulmonary disease.

II. Materials and Methods

A. Mouse Scans

To assess ultrasound strain measurement's ability to evaluate pulmonary function, we targeted a mouse model of pulmonary fibrosis for analysis.

Twelve C57BL/6 mice were included in the experiment. The mice were weight and age-matched (18-22 grams at 6-8 weeks of age). In six mice, a targeted Type II alveolar epithelial cell injury model was used to generate pulmonary fibrosis. Each of these mice, expressing the human diphtheria toxin (DT) receptor in an alveolar epithelial cell (AEC) restricted manner downstream of the surfactant protein C promoter (SPC-DTR+) and DTR- (wild-type) mice, was intraperitoneally injected with DT once daily for 14 days at a dose of 10.0 $\mu\text{g/kg}$ as previously described [17]. Six control mice were injected for the same duration with 100 μL of phosphate-buffered saline alone. All protocols used in this study were approved by the University of Michigan Unit for Laboratory Animal Medicine.

The mice were scanned in the prone position. The hair was removed from bilateral chest walls. The animals were either anesthetized with xylazine (5-10 mg/kg injected intraperitoneally) and ketamine (80-120 mg/kg injected intraperitoneally) or isoflurane inhalation (1% – 5% isoflurane typically titrated to about 3% with the duration of exposure modulated to effect).

All of the seven mice anesthetized with isoflurane developed collapse of the left lung. Only the right lungs were aerated presumably secondary to the effects of the anesthetics used (see below). In these cases, the single right lung measurement was considered representative of that mouse's lung dynamics.

We imaged the lungs with a commercially available ultrasound scanner (Vevo 2100, FujiFilm VisualSonics, Toronto, Canada) using 55 MHz linear array. The transducer was held in position against each mouse's chest using a restraining device provided by the manufacturer. Cine loops of respiratory motion were captured in transverse and sagittal orientation, although only the sagittal motions were evaluated in this study. Gray-scale motion in the loops was evaluated in Digital Imaging and Communications in Medicine (DICOM) format. The loops were on the order of about 1 - 3 second long and were sampled at around 300 – 400 frames per second depending on parameters such as the depth of field. The imaging loops were then transferred to a work station where commercially available

speckle tracking software (EchoInsight, Epsilon Imaging, Ann Arbor, MI) was used for analysis. The loops were evaluated interactively where two regions of interest (ROIs) were placed along the moving lung surface which was easy to identify based on its motion on the real-time loops (Fig 1a). ROI motion was tracked in 2D (laterally and axially). Tissue displacements were estimated based on the correlation peaks of the tracked speckle between frames. The lung surface strain was primarily determined by lateral motion due to acquisition geometry, which is mainly orthogonal to the standard axial strains measured in most one-dimensional applications [18].

Strain was defined as the Lagrangian strain estimated from two user defined regions of interest selected on the lung surface. $\text{Strain} = (L_f - L_i)/L_i$, where L_f is the frame to frame final distance between the centers of the regions of interest and L_i is the initial distance between the centers of the regions of interest. The continually changing strain values over time represent the breathing cycle of expansion and contraction of the underlying lung (Fig 1a). This definition is very similar to what is used for estimating deformations in cardiac echosonography [19]. The instantaneous Lagrangian strain was plotted as a function of time. (Fig. 1b).

B. Human Scans

The human scans were performed for purposes of demonstration (Fig. 2), and the imaging was performed under a University of Michigan Institutional Review Board- approved prospective study. Two normal volunteers gave written informed consent to have their lungs scanned. For the purposes of this demonstration, the human lung data was captured in rf (although this detail does not appear to be necessary.) Lung surface motion radiofrequency (rf) signals were captured at 105 frames per second using a clinical ultrasound scanner (Epsilon Imaging, Ann Arbor, MI). A 6.0 MHz linear array was used to acquire the images. The recorded lung surface motion was tracked using software specially designed for tracking heart motion in cardiac echo examinations (EchoInsight, Epsilon Imaging, Ann Arbor, MI). We tracked the rf signals using a previously described complex (real and imaginary numbers) cross-correlation algorithm [20]. The rf sampling rate was 20 MHz. The scans were performed intercostally making the image orientations dependent on the angles of the intercostal spaces relative to the cephalocaudal directions. We then rotated the probe orthogonally relative to this position so that we were scanning across the ribs. The selected ROIs for the lung surface as 2×5 mm.

Signals were temporally high-passed filtered to reduce the large static signals caused by specular reflections of the lung surface. It was observed that the specular reflections and stationary artifacts were stronger in human data compared to mouse data, perhaps due to the reduced acoustic scattering associated with the lower imaging frequency allowing for higher amplitude specular reflections. Given that the human data was rf captured, a 3-point Hanning high-pass FIR filter with coefficients (-0.25, 0.5, and -0.25) was used.

III. Statistics

We performed statistical analyses on the mouse scans. Ultimately there were 6 control mice and 6 mice with fibrosis induced by targeted type 2 alveolar epithelial death (the diphtheria

toxin model (DTR:DT)). As mentioned, we were only able to scan the right lungs in 7 of the 12 mice. We discovered that the left lungs had collapsed when the mice were anesthetized with isoflurane and oxygen. This was presumably due to the rapid diffusion of oxygen out of the anesthetized lung [21, 22]. Since it is very frequently the case that the lung analyses are made on single lungs in mouse experiments, we decided that it was appropriate to use the right lung measurements in these cases as representative of each mouse's ventilatory motion (c.f. [23-25]).

We acquired at least 3 longitudinal loops of the measured lungs during respiration, and in each longitudinal image, there could be up to 10 breaths. In parallel with the lung tracking, we also tracked the motion of the overlying soft-tissues. We used the strain of the overlying soft-tissue as a method to define when breaths were taken. We then calculated the strain for each breath by matching the soft-tissue strain for each breath with lung strain using the strain drift module in EchoInsight. We defined the strain for each breath as the difference between the maximum and minimum lung surface strain within the time segment defined by soft-tissue strain for each breath (Fig. 1b).

We then calculated the mean lung surface strain value for each cine loop; ultimately combining all of these cine loop means to calculate an overall mean lung surface strain for each mouse in each group, i.e. controls and DTR:DT. Thus each mouse provided only one mean strain estimate. After the experiment was completed, the mice were sacrificed, and lung fibrosis was assessed by quantification of hydroxyproline as previously described [26].

Each mouse was considered an independent measurement for both strain and hydroxyproline measurements. The mean values for each group of strains and hydroxyproline assay were compared using a two-tailed t-test (Microsoft Excel, Redmond, WA). $p < 0.05$ is considered significant.

IV. Results

The means and standard deviations of the strains and the means and standard deviations for the hydroxyproline assays for both groups are shown in Fig 3 and Fig 4. The differences between strains in the controls and the DTR:DT treated lungs were significant ($p < 0.02$) (Fig. 3). The difference between the hydroxyproline concentrations in the controls and DTR:DT mice was significant ($p < 0.0004$) (Fig. 4).

An example of a strain estimate from a human volunteer is shown in Fig 2. The human measurements are similar to linear strain values obtain using MRI in a previous study [27].

V. Discussion

Ultrasound scanning of the lung has been receiving increased interest recently. Besides well-known applications such as pleural effusion assessments, lung scanning has been used to assess pulmonary edema, pneumonias, and there are some ultrasound findings related to pulmonary fibrosis [12-15]. All of the criteria used in these assessments are likely multiple reflection artifacts. They can be generated between any number of different surfaces such as the lung and pleura or within fluid-filled fissures in the lungs. The appearance of the

artifacts, which have been referred to as A-lines, B-lines, and comets, [28, 29] is typically a ray of relatively high intensity line segments projecting along a beam path. Similar things are often seen elsewhere in the body projecting behind gas-filled objects [30].

Being artifacts, these objects are quite unstable, and their number and intensities can vary with the positions of the ultrasound scanhead relative to the target tissue. This variability and the fact that artifacts are the critical finding, makes quantifying these objects very difficult [16]. Therefore, scans of the lungs, whose findings are based on artifacts, are inherently qualitative. The qualitative nature of these scans still does not belie the fact that lung ultrasound scans have still found a useful niche in the imaging armamentarium.

Yet, it would be valuable to be able to make quantitative measurements of lung function using ultrasound. We have shown that quantitative measurements of lung displacements can be made by tracking the motion of the lung surface [1]. In this paper, we have preliminarily taken the next step by showing it is possible to represent local respiration using lung strain measurements in human volunteers, and we have shown that changes in lung strain can be identified in a relevant murine model of lung fibrosis.

The ability to non-invasively make such measurements in real-time would have many potential applications including the evaluation and management of patients with ARDS and lung fibrosis, or assessment of diseases in which all current applications used to measure local lung structure and function are either very difficult and hence impractical to perform, (CT and MRI), are qualitative (MRI and EIT), or are subject to substantial variation (pulmonary function studies) [9, 10, 31, 32]. Ultrasound strain estimates of local lung ventilation could overcome these difficulties and allow for the non-invasive realtime assessment of lung mechanics.

There are several limitations in this study; the largest being the small subject numbers. This was a pilot study, and numbers are relatively small. Yet the strain differences between normal and fibrotic mouse lung were large enough to be significant, and the strains correlate with the degree of lung injury based on the hydroxyproline assay. Second, we were only able to track one lung in the majority of the cases. This seems to be an unfortunate consequence of the isoflurane anesthesia that we used. However, quantitative analyses of one lung seems to be quite standard in mouse models of pulmonary pathology, so measuring strains in one lung would not be considered out of the ordinary in this context. Third, we were measuring and evaluating global changes in ventilation where the ultimate application of strain models will be applied and evaluated for local pulmonary ventilation, since methods for evaluating global lung ventilation exist and are in clinical use. Unfortunately, this is very difficult to do in mice largely because of their small size. The DTR:DT model requires intraperitoneal injections of diphtheria toxin, which produces a global effect. Thus, another non-mouse animal model of pulmonary injury would be required for assessing strain's ability to assess local ventilation. We are in the process of investigating such an animal model. However, for a preliminary study such as this, we believe that the global comparisons provided here suggest that further investigation is justified. Fourth, we only analyzed longitudinal strains in the mice. The radius of curvature of the lungs in the transverse orientation was small relative to the longitudinal direction. This led to ROIs that

extended along cords that crossed behind the lung surface. These would represent incorrect surface strain estimates. In order to overcome this, we will have to develop quantitative strain estimates that measure small increments along curved surfaces.

This is further complicated by the fact that the lung surface area is stretching and contracting, so the true strain is really an area strain. Such strains would lead to decorrelation whenever one-dimensional (1D) ultrasound transducer arrays are employed for tracking since any speckle components moving perpendicular to the scan plane cannot be tracked. This problem could be corrected with two-dimensional (2D) array transducers. However, the strain estimates in the longitudinal direction seemed to suffice for this preliminary work.

VI. Conclusion

In conclusion, we have shown that ultrasound strain imaging can potentially represent local changes in lung ventilation in real-time. There is evidence that the method can detect differences in normal and fibrotic lungs in a mouse model. We have also been able to make the measurement in human subjects. Further, study is needed to determine if ultrasound strain estimates can become a viable measure of local lung ventilation.

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Figure 1a.

Sagittal image of a control mouse showing how a lung surface strain estimate is made. The highly echogenic lung surface is visible here with two red regions of interest (ROIs) connected by a dotted red line running along the lung surface. The lung strains are measured between these two ROIs. Two connected purple ROIs are located in the superficial soft tissues to estimate that strain for reference. The yellow line at the bottom of the image represents 5 mm. Noise-filled lung is also marked.

Figure 1b.

Plot of the time-dependent strain made at the lung surface ROI in Fig 1a over a period of 0.80 sec. The red curve shows the instantaneous strain estimates of the lung surface segment shown in Fig 1a. There were three measureable breaths taken during this period. The breaths are defined by the purple curve representing the soft-tissue strain. The peak-to-peak time periods for the soft-tissue strain marked by arrows represent the boundaries of each breath. Within these boundaries, the difference between the maximum and minimum lung surface strains represents the strain for that breath. In this case, the lung surface strains range between about 17.7% and 22.8%. (There was very little strain drift in this case.)

Figure 2. Strain trace from the right lung of human volunteer. The trace encompasses one breath, and the maximum to minimum strain is at least 32.5%.

Figure 3. Mean longitudinal lung strains and standard deviations in control mice and mice with pulmonary fibrosis (DTR:DT).

Figure 4. Means and standard deviations of hydroxyproline concentrations ($\mu\text{g/ml}$) in control mice and mice with pulmonary fibrosis (DTR:DT).