Deterioration of Regional Lung Strain and Inflammation during Early Lung Injury

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Abstract

Rationale: The contribution of aeration heterogeneity to lung injury during early mechanical ventilation of uninjured lungs is unknown.

Objectives: To test the hypotheses that a strategy consistent with clinical practice does not protect from worsening in lung strains during the first 24 hours of ventilation of initially normal lungs exposed to mild systemic endotoxemia in supine versus prone position, and that local neutrophilic inflammation is associated with local strain and blood volume at global strains below a proposed injurious threshold.

Methods: Voxel-level aeration and tidal strain were assessed by computed tomography in sheep ventilated with low V_T and positive end-expiratory pressure while receiving intravenous endotoxin. Regional inflammation and blood volume were estimated from 2-deoxy-2-[(18)F] fluoro-D-glucose (¹⁸F-FDG) positron emission tomography.

Measurements and Main Results: Spatial heterogeneity of aeration and strain increased only in supine lungs ($P < 0.001$), with higher strains and atelectasis than prone at 24 hours. Absolute

strains were lower than those considered globally injurious. Strains redistributed to higher aeration areas as lung injury progressed in supine lungs. At 24 hours, tissue-normalized ¹⁸F-FDG uptake increased more in atelectatic and moderately high-aeration regions ($>70\%$) than in normally aerated regions ($P < 0.01$), with differential mechanistically relevant regional gene expression. ¹⁸F-FDG phosphorylation rate was associated with strain and blood volume. Imaging findings were confirmed in ventilated patients with sepsis.

Conclusions: Mechanical ventilation consistent with clinical practice did not generate excessive regional strain in heterogeneously aerated supine lungs. However, it allowed worsening of spatial strain distribution in these lungs, associated with increased inflammation. Our results support the implementation of early aeration homogenization in normal lungs.

Keywords: ventilator-induced lung injury; mechanical ventilation; positron emission tomography computed tomography; endotoxemia; acute respiratory distress syndrome

Recent clinical trials and large registry studies have shown benefits of protective ventilatory strategies in patients without lung injury at the onset of mechanical

ventilation in intensive care units (1–5) and operating rooms (6–10). However, the mechanisms causing lung injury remain unclear, and there is no known optimal

strategy to prevent injury in these patients. Limiting V_T has been proposed as the main intervention (11, 12). In contrast, data are conflicting for strategies aiming at

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At a Glance Commentary

Scientific Knowledge on the

Subject: Protective ventilatory strategies improve outcomes in mechanically ventilated patients without lung injury. Although limiting V_T is considered an important intervention, data are conflicting on the role of homogenization of lung expansion.

What This Study Adds to the

Field: Use of current protective principles does not prevent worsening of lung strains and their distribution in the first 24 hours of mechanical ventilation of heterogeneously expanded (supine) lungs. Nonaerated regions are more inflamed than normally aerated areas within the first 24 hours. As lung injury progresses, tidal strains are increasingly distributed to regions of relatively high aeration. These regions also present higher inflammation *in vivo*, and expression of stretch and chemotaxis-related genes. Human imaging supports the presence of these inflamed relatively high strain-aeration regions in mechanically ventilated patients with sepsis. Early homogenization of lung aeration during mechanical ventilation of initially uninjured lungs may protect from the development of significant biomechanical deterioration and inflammation.

homogenizing lung aeration, such as prone position and higher positive end-expiratory pressure (PEEP). In critically ill patients, these interventions are predominantly directed to severe cases of acute respiratory distress syndrome (ARDS) despite calls for early use (13, 14) and adverse results of aggressive PEEP strategies (15). In surgical patients, although intraoperative low PEEP is still prevalent worldwide (16), intermediate levels have been associated with better pulmonary outcomes (17).

At least three factors could contribute to the injurious effect of heterogeneous lung aeration in initially uninjured lungs. First, regional parenchymal stretch could exceed the global strain (change in total lung volume/resting volume) during tidal breathing because of atelectasis, airway closure, or heterogeneity in regional ventilation. Data are

scant in this field, and it has been argued that experimental global strain thresholds for lung injury ($1.5\text{--}2.0$ applying $V_T \geq 20$ ml/kg for 28 ± 17 h) (18) would not occur in human healthy lungs even with moderate V_T (19). Second, sepsis, surgical inflammatory response, and other injurious insults could increase the susceptibility of lung parenchyma to mechanical stretch (two-hit mechanism). Third, a mechanism linked to regional blood volume or flow could affect susceptibility to injury or its progression (20). Knowledge on such factors is essential to guide early management and prevent ARDS in those patients. Although animal experiments provided information on the first two factors (21, 22), a combination of exaggerated V_T , short experimental times, and use of small animals limit their extrapolation to conditions comparable with those present in humans.

Computed tomography (CT) allows for high-resolution assessment of regional lung deformation (23–26). Neutrophilic inflammation is a major process in early lung injury quantifiable with positron emission tomography (PET) (27–31). Using hybrid PET/CT methods, we tested the hypotheses that mechanical ventilation strategies consistent with clinical practice do not protect from worsening in regional lung strains during early lung injury, and local neutrophilic inflammation is associated with local strain and blood volume at global strains below an injurious threshold (18). We investigated these hypotheses in a large animal model comparable with human lung inflation pursuing the following goals: to quantify the spatial distribution of aeration and tidal strain during the first 24 hours of mild endotoxemia and mechanical ventilation. To study the effect of aeration heterogeneity on strain distribution, we compared the usual heterogeneous lung aeration (supine) with a standard homogeneous condition (prone); and to establish the topographical relationship between regional neutrophilic inflammation and gene expression markers of lung inflammation and injury with strain and blood volume. Our results, reinforced by data in critically ill patients, support the concept of applying lung homogenization strategies before the onset of ARDS. Some results have been reported as abstracts (32–34).

Methods

The Subcommittee on Research Animal Care at the Massachusetts General Hospital approved the experimental protocol. Ten female sheep (15.7 ± 2.6 kg) were anesthetized, paralyzed, intubated, and mechanically ventilated for 24 hours using the ARDSNet low-PEEP table (35) and low V_T adjusted to reach an alveolar plateau in the volumetric capnogram. After prone lung recruitment, animals were divided into supine ($n = 5$) or prone ($n = 5$) positions. Intravenous endotoxin was started after baseline data collection (2.5 ng/kg/min; *Escherichia coli* O55: B5, List Biologic Laboratories Inc.). Methods are detailed in the online supplement.

CT Imaging

At baseline, 6 hours, and 24 hours, CT images were acquired during end-inspiratory and end-expiratory breath holds for aeration and strain analysis; and at mean lung volume (tidal breathing, 2 min) for PET attenuation correction and delineation of regions-of-interest. Voxel gas fraction was quantified considering air Hounsfield units of -1000 and tissue Hounsfield units of 0 as $F_{\text{gas}} = \text{voxel Hounsfield units} / -1000$.

We used image registration of the end-inspiratory to the end-expiratory CT images to calculate voxel-level tidal strain (voxel volume change/end-expiratory volume including lung tissue + air) (23–26). Registration accuracy was validated using landmarks (see Figure E2 in the online supplement). To compare animals and time points, strains were normalized by CT-measured global inspired air volume. Heterogeneity of aeration and tidal strain were assessed by the variance normalized by the squared mean.

PET Imaging

At baseline and 24 hours, we acquired dynamic PET images of 2-deoxy-2-[(18)F] fluoro-D-glucose (^{18}F -FDG) to measure tissue glucose metabolism, a biomarker of early ARDS (30). Kinetics parameters reflective of net uptake, phosphorylation rate, volume of distribution, and blood volume (36) were calculated for the whole lung and multiple small cylindrical regions-of-interest (~ 1.4 ml).

Tissue Samples

At 24 hours, lung tissue was sampled from ventral, mid, and dorsal regions.

From the left lung we assessed histologic lung injury score (37) and from the right lung, wet-to-dry ratios and gene expression.

Gene expression analysis. Using real-time reverse transcription quantitative PCR, we measured tissue expression of genes related to inflammatory cytokines (IL-1 β , IL-6, and CXCL-8), neutrophilic inflammation (ICAM-1 and CD11B), and epithelial (RAGE) and endothelial (amphiregulin and PAI-1) cell injury.

Human Data

PET/CT images were acquired for two patients with sepsis after informed consent. Both were mechanically ventilated (<96 h) in supine position with low V_T (5.0 and 7.0 ml/kg) and moderate PEEP (8 and 13 cm H_2O).

Statistical Analysis

Data are mean $\pm$ SD or median (25th–75th percentile) as appropriate. Two-way repeated-measures ANOVA was used for comparisons within and

between groups, applying aligned rank transformation if needed. Multiple comparisons P values were calculated from a multivariate t -distribution or adjusted by Benjamini-Hochberg. Determinants of change in ^{18}F -FDG phosphorylation rate from baseline to 24 hours were assessed through mixed-effects linear regression. Tests were two tailed and performed in R 3.3.1 (R Foundation for Statistical Computing). Significance was set at P less than 0.05.

Figure 1. (A and B) Ratios of PaO_2/FiO_2 (A) and peripheral blood neutrophil counts (B) at baseline and after 6 and 24 hours of low V_T mechanical ventilation and mild endotoxemia. (C) Driving pressure ($E_{RS} \cdot V_T$, solid line) and delta transpulmonary pressure ($E_L \cdot V_T$, dashed line). The equal driving pressure with different transpulmonary pressure in supine and prone positions was explained by the difference in E_L (D) and E_{CW} (E). ^prone and vsupine versus baseline. #prone and *supine versus 6 hours. One symbol indicates $P < 0.05$; two symbols indicate $P < 0.01$; three symbols indicate $P < 0.001$. Open gray square = prone; solid black circle = supine. Histologic findings showed regional differences in lung injury. (F) Box plot of lung injury score evaluated in three regions from ventral to dorsal in supine (black) and prone (gray) animals. # $P < 0.05$ versus ventral region. (G) Examples of hematoxylin and eosin staining in high-power fields for each one of the regions in one supine and one prone animal. Scale bars, 10 μm . E_{CW} = chest wall elastance; E_L = lung elastance; E_{RS} = respiratory system elastance.

Results

Mechanical Ventilation and Mild Endotoxemia Produced Mild ARDS in 24 Hours

$\text{PaO}_2/\text{FiO}_2$ decreased in supine animals with gas exchange criteria for mild ARDS reached at 6 hours and worsening despite increased FiO_2 (Figure 1A; *see* Tables E3 and E4). Supine animals presented lower systemic blood pressure than prone despite higher cardiac output (*see* Table E5).

Histology showed mild–moderate injury scores in both groups, with more marked ventral–dorsal score gradients in supine animals (Figure 1F). The main factor for those scores was neutrophilic infiltration (interstitium > air spaces) (Figure 1G; *see* Table E6). Hyaline membranes, although infrequent, were more prevalent in dorsal areas of supine sheep. Wet-to-dry ratios showed no group effect (supine, 5.9 [5.7–6.1]; prone, 5.6 [5.4–5.8]).

Transpulmonary Pressures Were Higher in Supine Lungs in the First 24 Hours of Lung Injury

Transpulmonary and driving pressures increased continuously during the 24 hours. Transpulmonary pressures were higher in supine than prone sheep (Figure 1C). In contrast, no difference was observed for driving pressures (Figure 1C). This was explained by the higher lung elastance in supine, whereas prone sheep with supported abdomen had higher chest wall elastance (Figures 1D and 1E). Only after 6 hours lung elastance in prone (48.7 ± 24.8 cm $\text{H}_2\text{O}/\text{L}$) deteriorated to values comparable with those present at baseline in supine animals (44.2 ± 7.9 cm $\text{H}_2\text{O}/\text{L}$). Resistances were comparable between groups (*see* Table E7).

Deterioration in the Spatial Distribution of Aeration Was Substantial in Supine but Not in Prone Animals

Nonaerated tissue fraction in supine animals increased slightly in the first 6 hours and evidently at 24 hours, particularly in dependent regions (Figures 2A and 2B). In contrast, nonaerated regions were absent in prone animals (Figure 2C). Hyperaeration was minimal in both groups even at end-inspiration (*see* Table E8).

The effect of position was even more marked on the progression of spatial heterogeneity of aeration. This was not only substantially higher in supine than prone

Figure 2. (A) Lung aeration decreased faster in supine than prone conditions when a mechanical ventilation strategy compatible with clinical practice using low positive end-expiratory pressure and V_T was applied for 24 hours to mild endotoxemic animals. (B and C) In the supine lung, on average 48% of regions are either nonaerated or poorly aerated at the end of 24 hours of mechanical ventilation and endotoxemia (B), whereas in prone animals 27% of the lung is poorly aerated and none nonaerated (C). Nonaerated, fraction of gas (F_{gas}) < 0.1; poorly aerated, $0.1 \leq \text{F}_{\text{gas}} < 0.5$; normally aerated, $0.5 \leq \text{F}_{\text{gas}} < 0.9$; and hyperaerated, $0.9 \leq \text{F}_{\text{gas}}$. (D) Aeration heterogeneity, measured as the variance normalized by the squared mean along time. There is marked contrast between the progressions of aeration heterogeneity in supine versus prone animals, with increase significantly only in supine. Values were computed at end-expiration. Group effect is indicated in the figure. $^{**}P < 0.01$ and $^{***}P < 0.001$, comparison among time points. Prone animals had no difference between time points. Open gray squares = prone; solid black circles = supine. All data refer to the whole lung.

animals, but worsened over 24 hours more in supine (Figures 2D). Importantly, the difference in aeration heterogeneity between groups was only partially explained by larger gravitational effects in

supine animals, with $32 \pm 9\%$ represented by nongravitational aeration heterogeneity (*see* Figure E3), suggesting effects of gravity-dependent heterogeneity extended to the isogravitational level.

Spatial Heterogeneity of Lung Strain Increased Predominantly in Supine Lungs with Maximal Strain Still below Measures of Excessive Lung Strain at 24 Hours

Spatial heterogeneity of tidal strain computed at the voxel level had a significantly different time course in supine versus prone sheep. Strain heterogeneity was similar for groups at baseline (Figure 3B), despite their marked differences in aeration distribution (Figure 2D). Yet, at 24 hours

strain heterogeneity increased substantially in supine but not in prone animals (Figure 3B). This resulted in larger regional tidal strains in supine than prone sheep at 24 hours, with the 95th percentile of the strain distribution increasing from 2.0 ± 0.1 to 2.4 ± 0.1 times the mean strain in supine animals (Figure 3C). Instead, prone sheep had no change (Figure 3C). Absolute strains (i.e., regional volume change/regional volume) for that 95th percentile were 0.25 ± 0.03 , substantially

lower than currently proposed global injurious values. Spatial distribution of tidal strain differed between groups, with variability in isogravitational regions (Figure 4; see Figure E4). Regions of relative high and low strain were large and concentrated in supine, and small and homogeneously distributed in prone (Figures 3A and 4).

To study the relationship between regional strain and aeration, we examined density plots of those variables computed at

Figure 3. Normalized strain computed from measurements at the voxel level in supine and prone animals at baseline and after 6 and 24 hours of low-V_T mechanical ventilation and mild endotoxemia. (A) Transverse slice at approximately two-thirds of the cephalocaudal axis is presented along time showing voxel-level strain in a cold-to-hot color scale (dark blue = compression/no strain; red = higher strain value within the image) superimposed on the computed tomography scan. Note the heterogeneous spatial distribution of strains in supine animals, in contrast to the more homogeneous distribution in prone sheep, also shown in the normalized strain distribution presented for each animal (different colors). (B and C) Heterogeneity (variance normalized by squared mean strain) increased in the supine position (B), leading to an increase in the ratio of maximum (95th percentile) to mean strain in this group (C). ###*P* < 0.001.

Figure 4. Normalized strain in one supine (top) and one prone (bottom) animal at baseline and after 24 hours of low- V_T mechanical ventilation and mild endotoxemia. Strains are color coded from low and slight compression ($-1/L$; blue) to expansion ($+3/L$; yellow). Values at the ends of the scale were highlighted making the center ($1/L$) transparent with a gradual increase in opacity for both sides, as shown in the color bars associated with the strain histograms on the right. In both groups, there was heterogeneity in isogravitational levels. Note the larger heterogeneity in the spatial distribution of strains in the supine position, with more extreme values seen in subdiaphragmatic (dark blue) and nondependent (yellow) regions, whereas a more homogeneous pattern is observed in prone conditions.

the voxel level (Figure 5). Supine animals presented a reproducible inverted U-shaped curve (Figure 5 for end-inspiratory aeration; see Figure E5 for end-expiratory): strains increased with aeration from low-aerated toward normally aerated regions. As regional aeration exceeded approximately 0.6, strains decreased with aeration (Figure 5). Compared with baseline (dashed line, Figure 5), at 24 hours median strains decreased in poorly aerated areas and increased in areas of normal-high aeration. In contrast, prone animals displayed a markedly homogeneous spatial distribution of strain versus aeration, slightly spread at 24 hours (Figure 5). Parametric response maps showed irreversibly low gas content regions present only in supine animals (see Figure E6).

Increased Lung Tissue ^{18}F -FDG Uptake at 24 Hours of Lung Injury Was Associated with Regional High Aeration, Blood Volume, and Tidal Strain

Whole-lung metabolic activity estimated from ^{18}F -FDG uptake increased

significantly at 24 hours in both groups (0.23 ± 0.04 to $0.34 \pm 0.09 \cdot 10^{-2}/\text{min}$) (Figure 6A). This increase was mostly caused by an increased phosphorylation rate (1.9 ± 0.2 to $2.6 \pm 0.5 \cdot 10^{-2}/\text{min}$) (Figure 6B), indicating more cellular activation than increased ^{18}F -FDG volume of distribution (Figure 6C). The circulating neutrophil counts increased in both groups (Figure 1B).

Spatial distribution of ^{18}F -FDG uptake rate was homogeneous for both groups at baseline. At 24 hours, it remained homogenous in prone, but showed a vertical gradient for supine sheep (Figure 6D; see Figure E7). This gradient was not only caused by higher density in dependent atelectasis, because tissue-normalized uptake increased more in atelectatic than in continuously normally aerated regions ($F_{\text{gas}} = 0.5\text{--}0.7$) (Figure 6E). Interestingly, the highly aerated regions ($F_{\text{gas}} > 0.7$) also had higher normalized uptake increase (Figure 6E). In both aerated regions, normalized uptake increased equally for the high and low tertile of tidal strains (average of baseline and 24 h) (Figure 6F).

To study the determinants of regional inflammation in aerated areas ($F_{\text{gas}} > 0.1$) we tested the effect of regional aeration, strain (mean and SD, $\text{SD}_{\text{strain}}$) and blood volume on the change in phosphorylation rate from baseline to 24 hours. The interaction between blood volume and strain (blood volume $\times$ strain; $P = 0.010$), strain ($P < 0.001$), and $\text{SD}_{\text{strain}}$ ($P < 0.001$) were related to phosphorylation rate change. Such finding suggests that higher regional blood volume increased tissue metabolic response to strain (see DETERMINANTS OF INFLAMMATION in online supplement).

Regional Gene Expression at 24 Hours Was Heterogeneously Distributed in the Lungs and Consistent with Activation of Locally Distinct Inflammatory Pathways

Regional lung tissue gene expression of a subset of markers relevant to lung injury was heterogeneous and consistent with heterogeneous local mechanical forces and blood volume. ICAM-1 (adhesion molecule) gene expression was larger in dorsal than ventral regions in supine animals and not in prone (region-group interaction; $P = 0.028$) (Figure 7). This pattern was also present in the expression of the leukocyte adhesion mediator CD11b ($P = 0.274$), which was upregulated in all animals (Figure 7). CXCL8 (encodes neutrophil chemokine IL-8) expression was consistently lower in prone than supine animals, and largest in ventral and dorsal regions. PAI-1 (secreted by the endothelium and thought to promote inflammation-mediated tissue thrombosis) expression was highest in ventral regions (high aeration, moderate strain, low blood volume) (see Figure E8). The RAGE (believed partake in barrier disruption in lung injury) gene, although in general downregulated, was more expressed also in ventral and dorsal regions, with a trend to higher expression in ventral than dorsal regions of supine sheep (four of five animals; $P = 0.073$). IL-6 was upregulated in ventral and dorsal supine regions. IL-1 β (proinflammatory cytokine) was upregulated nearly twofold, although it seemed to have lower expression in prone animals. In contrast, amphiregulin (modulator that might dampen the inflammatory response) expression was downregulated.

Figure 5. Voxel-level normalized strain versus end-inspiratory aeration (fraction of gas [F_{gas}]) at baseline and after 6 and 24 hours of mild endotoxemia and low- V_T mechanical ventilation. Data refer to all animals in supine (top) and prone (bottom). The boxes represent median and interquartile range of strains for voxels in the aeration intervals: <0.1 , $0.1\text{--}0.3$, $0.3\text{--}0.5$, $0.5\text{--}0.7$, $0.7\text{--}0.9$, and >0.9 , centered in the mean aeration within each aeration interval. Voxels between the 5th and 95th strain percentiles are depicted in a two-dimensional histogram, with the gray scale indicating the fraction of total lung volume represented by a pair of strain and aeration (black is highest). Gray scale is the same within groups. In the supine animals (top), strain-aeration relationships showed an inverted U-shaped pattern. Strain increased with aeration up to an F_{gas} approximately 0.6 followed by a decrease with aeration for F_{gas} above that value. This pattern was consistent across time points, with progressive decrease in median strain at low aerations and increase at high aerations when compared with median strain at baseline (dashed line). The decrease in gray scale for prone (bottom) at 24 hours indicates a slight spread of the distribution.

Patients with Sepsis Mechanically Ventilated for Less Than 96 Hours Showed Elevated Strain with High Normalized ^{18}F -FDG Uptake in the Highest-Aeration Regions

A later stage of injury was studied in two patients with sepsis. Both had a bimodal aeration distribution with predominance of high-normal aeration and no aeration (see Table E9 and Figure E9). Consistent with the animal experiments, the median of the strain-aeration distribution increased from low toward normal aeration (Figure 8A). Of note, median strain continued to increase with aeration at highly aerated regions including hyperaerated ($F_{gas} > 0.9$) areas (Figure 8A). Such trend matches the increased median strains at higher aeration observed at 24 hours in supine animals (Figure 5) indicating a progressive distribution of tidal strains to regions of larger static strains (aeration). Both patients had elevated whole lung ^{18}F -FDG uptake

(0.37 and $0.40 \times 10^{-2}/\text{min}$). Regions of higher normal aeration ($F_{gas} > 0.7$) had larger tissue-normalized blood volume (Figure 8C) and uptake than those in the lower half of normal aeration ($F_{gas} = 0.5\text{--}0.7$) (Figure 8B).

Discussion

In the first 24 hours of lung injury produced by mild systemic endotoxemia and mechanical ventilation using typical clinical PEEP and V_T in animals with lung size comparable with that of humans the following was found: 1) spatial heterogeneity of tidal strain worsened in supine but not in prone lungs, with increased regions of relative high strain-aeration and of derecruitment; 2) these regions presented biologic signs of injury with increased *in vivo* metabolic activity, representative of inflammation.

Derecruited regions showed increased gene expression of adhesion molecules and inflammatory cytokines. In relative high strain-aeration regions, gene expression of endothelial and epithelial stretch markers, and neutrophil chemokine were increased; 3) remarkably, these regions were more frequent in two patients with sepsis in the first 96 hours of mechanical ventilation and associated with increased regional inflammation; and 4) regional strain-blood volume interaction and small length-scale strain heterogeneity were associated with neutrophilic inflammation. These results indicate that current mechanical ventilation methods do not prevent lung biomechanical deterioration and support the relevance of interventions preceding ARDS aiming at homogenizing lung aeration to minimize lung injury.

Optimizing mechanical ventilation in patients with normal lungs is greatly important because approximately 20% of ventilated patients in intensive care have no primary lung disease (38) and approximately 230 million patients undergo major surgeries worldwide (39). Although there is agreement on the benefit of limiting V_T and driving pressures, less is known on interventions aimed at homogenizing lung aeration. In critical care, prone position and evidence for high PEEP benefit are restricted to severe ARDS (40, 41). Low stretch strategies with low PEEP have actually been defended (15, 42). In contrast, early prone position has been proposed for comatose (14) and recently for all ARDS patients (13). In surgical patients, a recent metaanalysis concluded that recruitment maneuvers and PEEP should accompany low V_T (43). Yet, permissive atelectasis with low PEEP has been defended for open abdominal surgery (12). Also, despite recommendations in favor of postoperative lung expansion (44), its implementation is poor with important negative consequences (45).

Using CT measurements, we found a markedly different spatial distribution of strains in supine versus prone lungs. Importantly, our assessment of strain was based on voxel-level measurements of distances between lung structures in contrast to previous quantifications based on gas volumes and fractions in larger regions of interest (46). In supine, the inverted U-shaped pattern of tidal strain-aeration was consistent with local sigmoid-shaped pressure-volume curves having maximal slope (compliance) and

Figure 6. (A) After 24 hours of low- V_T mechanical ventilation and mild endotoxemia, 2-deoxy-2-[(18)F]fluoro-D-glucose uptake rate, a marker of inflammation, was increased relative to baseline both in animals in supine and prone positions. (B and C) This increase was caused more by an increase in the phosphorylation rate (B) than by volume of distribution (C), indicating predominance of cellular metabolic activation. At baseline, 2-deoxy-2-[(18)F]fluoro-D-glucose uptake rate spatial distribution was mostly homogeneous in both groups. (D) After 24 hours, it remained homogeneous in prone but showed a vertical gradient in supine. (E) Not only did the tissue density increase, but also regions that became atelectatic (<0.1) had a higher increase in tissue-normalized uptake when compared with regions of constant normal (0.5–0.7) aeration. (F) Within aerated regions, first (red) and third (blue) tertile of strain had no difference in normalized uptake increase. ** $P < 0.01$ and *** $P < 0.001$ versus normally aerated region. Fgas = fraction of gas.

strain at normal aeration approximately 0.6, and lower compliance and strain at low (derecruitment) and high (hyperinflation) aeration.

Notably, worsening of strain heterogeneity along 24 hours was pronounced only in supine lungs. Increased

strain heterogeneity was paralleled by increased no aeration and poor aeration, indicating redistribution of V_T from regions of low to higher aeration. This reveals the inability of PEEP levels prescribed in the utilized protocol, usual in clinical care (15, 47), to protect the lungs against progressive

biomechanical deterioration. As a result, at 24 hours lungs showed larger atelectatic regions and increased median tidal strains in regions of higher aeration.

Atelectatic regions at 24 hours had higher increase in normalized ^{18}F -FDG uptake and expression of ICAM-1 gene,

Figure 7. Gene expression in three regions with different aeration, blood volume, and strain conditions after 24 hours of low- V_T mechanical ventilation and mild endotoxemia. In prone animals, sampled regions were selected to match the three regions along the gravitational axis sampled in the supine animals. Note that mid regions have more samples than dorsal and ventral because tissue was sampled at mid and caudal zones, which showed no difference in a paired Wilcoxon test and were treated as one region. Points are a dot plot representation of fold change relative to β -actin and a control noninjured animal measured with RT-qPCR, and gray lines indicate median (horizontal) and first and third quartiles. Comparison between regions in the same group: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

suggestive of neutrophil infiltration and inflammatory activity, than normally aerated regions. Such finding implies that a strategy exclusively based on limitation of global cyclic stretch (V_T) does not eliminate lung injury risk, in contrast

to a recent metaanalysis (12). Given that derecruitment and aeration heterogeneity were associated with cellular activation and worsening of strain heterogeneity, early interventions aiming at homogenizing lung aeration are likely important to mitigate

injury progress (48, 49). Importantly, ^{18}F -FDG measurements with PET provide a more comprehensive assessment of inflammation than local measurements from tissue samples or bronchoalveolar lavage.

The two patients had inflamed lungs (50) and large fractions of relative high strain-aeration regions. This was consistent with the progression of those regions along the 24 hours in supine sheep. Of note, such regions had larger normalized ^{18}F -FDG uptake than normally aerated regions in both patients. This suggests local superposition of relative high tidal and static strains as a factor for lung injury, potentially enhanced by the increased blood volume. Indeed, at 24 hours sheep had ventral regions with increased gene expression of markers previously associated with injurious mechanical ventilation: PAI-1, RAGE, and IL-8 (22, 29, 51–53) implying a biologic response to mechanical forces and onset of inflammation with neutrophil recruitment. These findings align with the 20-hour inflammatory differential gene expression response of ventral regions in a moderate endotoxemia model (30).

Previous experiments in initially normal lungs pointed to a protective role of prone versus supine (54–56). Yet, the applied exaggerated V_T (≥ 15 ml/kg) and short duration limited their clinical translation. An underlying assumption for those V_T is that heterogeneous aeration results in regional strains conducive to mechanical injury despite low global strain. Surprisingly, although 5% of our supine lungs were submitted to tidal strains above approximately 2.4 times mean values, the absolute magnitudes were below global strains reported as necessary to produce injury exclusively by stretch (18). Consequently, at the resolution of our CT images (2.5 mm), exaggerated strains do not occur when healthy or mild-endotoxemic lungs are ventilated with typical clinical settings.

Regional metabolic activity was associated with the interaction of strain and blood volume. Blood volume and flow (^{13}N -saline PET [57]) were correlated in seven studied sheep ($r = 0.77$; $P < 0.001$). Accordingly, both could have contributed to that interaction. High regional blood volume could indicate larger endothelial surface exposed to circulating inflammatory cells, mediators, and endotoxin, and high blood flow could enhance their local

Figure 8. Two patients with sepsis at the first 96 hours of mechanical ventilation had relationships between voxel-level normalized strain and end-inspiratory aeration (fraction of gas) consistent with the relationships observed in the animal experiments. (A) Strain increased from lowest aeration toward normal aeration but did not decrease in more aerated regions. The boxes represent median and interquartile range of strains for voxels in the aeration intervals: <0.1, 0.1–0.3, 0.3–0.5, 0.5–0.7, 0.7–0.9, and >0.9, centered in mean aeration within ranges. Voxels between the 5th and 95th percentiles of strain are depicted in a two-dimensional histogram, with the gray scale representing the fraction of total lung volume in each bin (black is highest). End-inspiratory aeration was chosen to emphasize hyperaerated regions. Inspiratory images were transformed to the expiratory computed tomography scan references using the same transformation estimated by elastic image registration to calculate the voxel-level strain. (B and C) Normalized 2-deoxy-2-[(18)F] fluoro-D-glucose uptake (B) and blood volume per gram of tissue (C) in atelectatic (<0.1), normal (0.5–0.7), and high-aeration (>0.7) regions. In both patients, regions of high aeration and potential hyperinflation have higher normalized uptake and blood volume than normally aerated regions. Fgas = fraction of gas.

delivery. These interaction results suggest that during early injury with limited V_T and endotoxemia, regional differences in the vascular component magnify the effect of strain on inflammation (“two-hit injury”). Such mechanisms apparently differ from those present during high- V_T injury (58). Therefore, interventions beyond mechanical ventilation could prevent the development of injury. The additional contribution of strain heterogeneity to regional inflammation indicates the potential effect of concentration of mechanical forces and possibly even larger microscopic strain heterogeneity. Finally, because transpulmonary pressures were higher in supine than prone sheep,

higher energy delivered to lung tissue could have additionally contributed to injury (59).

Study Limitations

The imaged lung may have changed from expiration to inspiration. We compensated for this by visually matching the first and last CT slices. Tidal strain computed with breath holds could misrepresent fast dynamic phenomena. Also, because of a short inspiratory pause, plateau pressures and therefore inspiratory CT lung volume could have been overestimated. Hence, we compared strains per unit of inspired volume and used measured V_T to calculate absolute strains. Our V_T range was above

6 ml/kg. It was within clinical practice (47), and necessary because of high anatomic dead space in our sheep. Importantly, lower V_T would further decrease regional tidal strains, reinforcing our finding of strains below the proposed injurious limit. Image registration is an ill-posed problem, the required regularization could underestimate heterogeneity. Additionally, the translation from voxel to cell deformation is unknown. ^{18}F -FDG is a glucose analogue and consequently not strictly specific to neutrophilic activation.

In conclusion, during mild-endotoxemic lung injury, a mechanical ventilation strategy that minimizes tidal strain without homogenizing aeration

distribution from the beginning of ventilation, before established ARDS, resulted in worsening of levels and spatial distribution of strain and aeration. Atelectatic and relatively high-aeration regions showed increased neutrophilic inflammation. This was dependent on the blood volume-strain interaction and strain heterogeneity. Redistribution of V_T increased strains in those relatively high-aeration regions, which presented gene expression consistent with a

stretch response and distinct from atelectatic areas. Those regions were also observed in mechanically ventilated patients with sepsis and associated with increased metabolic activation, indicative of inflammatory activity. Accordingly, provided safe interactions with other organs, strategies that homogenize lung aeration are likely beneficial at the earlier stages of mechanical ventilation in the presence of systemic inflammation. ■

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