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Mechanisms associated with progression from lobar inflammation to bilateral lung injury: an experimental study

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Abstract

Background: Localized lung insult, such as pneumonia, can increase respiratory drive and effort. The interplay between the initial injury, the inflammatory reaction and increased drive and effort could damage other areas and progress to bilateral lung injury.

We aimed to describe the pathophysiological mechanisms causing injury in previously healthy regions of contralateral lung, when respiratory drive and effort were increased by lobar inflammation.

Methods: Localized pulmonary inflammation was induced through instillation of 0.5 mg/kg lipopolysaccharide (LPS) in the left lower lobe of 18 pigs, while 5 animals served as sham controls. After 24 hours of spontaneous breathing, the severity of right lung injury was assessed by the validated histological score and correlated with potential physiological and biological determinants.

Results: Animals challenged with lobar inflammation developed bilateral lung injury, associated with increased respiratory drive and effort.

Histological score of the right lung was characterized by wide inter-individual variability (median 11 [8-14], range 3-25). Right lung injury score was correlated with respiratory drive and effort; with respiratory rate and minute ventilation, but not with tidal volume; with peak inspiratory and driving transpulmonary pressures, and with EIT-derived lung strain; with lower sub-atmospheric alveolar pressure and with more negative end-expiratory transpulmonary pressure.

Right lung injury score was also correlated with inflammatory plasma cytokines: higher SDF-1α and lower IL-1Ra, IL-5 and GM-CSF.

Conclusions: An experimental model of localized lung inflammation allowed us to investigate the role of specific pathophysiological mechanisms for the development of injury in previously healthy lung regions.

Keywords: respiratory effort; self-inflicted lung injury; inflammation; spontaneous breathing

Introduction

Progression from local insult (e.g., pneumonia or contusion) to bilateral lung injury could result from multiple pathophysiological mechanisms, triggered by elevated inspiratory drive and effort (1). Increased spontaneous respiratory activity has been associated with worsening bilateral lung injury, (2–5) while studies on its role in the progression from local to bilateral injury lack. In patients with the acute respiratory distress syndrome (ARDS), the association between increased respiratory effort, worsening injury and poor outcomes is supported by recent pilot studies (3, 6–12). In milder bilateral lung injury, instead, spontaneous breathing could protect the lung, likely because of lower inspiratory effort and less susceptibility (3, 13). Studies focused on the development of injury in previously healthy lung regions, with a longer observation period and comprehensive assessment of its pathophysiological mechanisms are scant, if any. An early pivotal experimental study showed that hyperventilation could induce injury in healthy lungs (14). However, respiratory drive was increased by acidification of the cerebrospinal fluid, which may not translate to patients with pneumonia, in whom localized pulmonary injury, inflammation and respiratory effort likely act synergistically in the progression to diffuse injury.

Both self-inflicted lung injury (SILI) and ventilation-induced lung injury (VILI) are caused by excessive mechanical stress and strain, but with some differences. Large drops in pleural pressure generated by strong inspiratory efforts are uneven, with risk of regional injury due to high local transpulmonary pressures and focal alveolar overdistension exacerbated by gas shift within the lungs (pendelluft). They also facilitate pulmonary edema formation through increased pulmonary capillary hydrostatic pressure and transvascular pressure gradient.

In the present study, we reasoned that understanding mechanisms involved in the progression from localized inflammation to bilateral lung injury (i.e., as in clinical evolution from pneumonia to ARDS) could have both pathophysiological and clinical significance, given the

association between worsening lung injury, the risk for intubation and mortality (9, 15). We developed an experimental model of localized pulmonary inflammation induced through the instillation of lipopolysaccharide (LPS) in the left lower lobe of healthy swine. The main endpoint was the description of mechanisms leading to the development of injury at 24 hours in the initially healthy right lung. Briefly, we described the development of right lung injury, but with large variability in terms of severity. Then, we investigated the correlation between the severity of right lung injury and candidate pathophysiological mechanisms, including increased respiratory drive and effort, lung stress and strain, alveolar and transpulmonary pressure and pendelluft measured by advanced respiratory monitoring, such as esophageal pressure (Pes) and electrical impedance tomography (EIT). Third, we explored the correlation of plasma proteomics profile with the severity of right lung injury and with effort.

Methods

This experimental study in a porcine model was approved by the Italian Ministry of Health, Rome, Italy (Ref. n° 855/2024-PR prot. 568EB.49) and conducted according to the European Directive 2010/63/EU on the protection of animals used for scientific studies and the Italian decree 26/2014.

Animal preparation. Healthy female pigs were anesthetized, tracheostomized and instrumented with pulmonary artery and carotid artery catheters to monitor hemodynamics and collect blood samples. An EIT monitoring belt (PulmoVista 500; Drager, Lubeck, Germany) was placed around the thorax and Pes catheter was inserted and calibrated. See the Online Supplement for further details.

Study protocol. Eighteen deeply sedated pigs lying on the left side and on controlled ventilation (LPS group) underwent bronchoscopic instillation of 0.5 mg/kg of E. coli LPS

(LPS 055:B5; Product no. L2880; Sigma Aldrich, Saint Louis, MO) into the inferior lobe of the left lung. Bronchoscopy was performed by experienced thoracic surgeon. Five pigs underwent the same protocol (preparation, anesthesia, instrumentation and management) but did not receive LPS (Sham group).

Then, animals were positioned prone, and sedation was decreased to the minimal propofol dose useful to maintain autonomous respiratory drive, without excessive movements for safety reasons. The experiment started upon start of spontaneous breathing (pressure support ventilation with PEEP 5 cmH_2O, support 5 cmH_2O, FiO_2 0.5) and lasted for 24 hours.

At the end of the experiment, blood samples were collected and processed for plasma proteomics (see below). Three lung tissue samples from each lung were taken for histological analysis and immunohistochemistry. Severity of lung injury was then quantified by validated lung histological score (16) performed by a blinded pathologist.

Measurements. Measurements were performed after 6 and 12 hours after start of the experiment, as well as the end. A 5-minute continuous recording of airway, esophageal and transpulmonary pressure waveforms including short end-inspiratory and end expiratory occlusions was collected, synchronized with EIT ventilation data. Data were analyzed offline to obtain average values of: respiratory rate (RR), tidal volume (Vt) and minute ventilation (MV); airway occlusion pressure (P0.1), esophageal pressure swing (ΔPes), airway pressure deflection (ΔPocc); transpulmonary driving pressure (ΔP_L = [Pplat – $Pes_{end\text{-}insp}$] – [PEEP– $Pes_{end\text{-}exp}$]); dynamic end-inspiratory transpulmonary pressure (P_{Linsp}); Pressure-Time Product (PTP, calculated as the time integral of the difference between Pes and the static recoil pressure of the chest wall during inspiration, given that Pes reflects the difference between muscular pressure, Pmus, and elastic recoil pressure of the chest wall (17)), both per minute (PTPmin) and per breath (PTPbreath).

From offline analysis of EIT data we obtained: global and regional ventilation (18), tidal deformation of the lung (a surrogate for lung strain) (19), and occult pendelluft (20), as previously described. Alveolar pressures were estimated by recursive least square fitting of transpulmonary pressure and EIT data (21).

Systemic and pulmonary hemodynamics, arterial and mixed venous blood gas analyses, diuresis, fluid balance and dose of sedative drugs were also collected.

At the end of the experiment, plasmatic levels of 105 inflammatory mediators were evaluated with the Human XL Cytokine Array Kit (R&D system, Minneapolis, MN USA).

See the online supplement for details on physiologic and EIT measurements, histology and proteomic analyses.

Statistical analysis. Variables are presented as mean and SD or median and interquartile range [IQR], according to their distribution, as assessed using the Shapiro-Wilk test. Comparisons of continuous data between groups were done using one-way ANOVA, if performed only at one time point, or mixed ANOVA for repeated measures to assess the effect of time. Univariate linear mixed-effect model was performed for each candidate physiological and biological variable with the right lung injury histological score as the dependent variable. Linearity, homoscedasticity and normality of residuals were tested for all models. Correlation coefficients (r) and associated p-values were calculated. Statistical analysis was conducted with JMP® Pro software, Version 16 (SAS Institute Inc., Cary, NC) and results were considered statistically significant at $p < 0.05$.

Results

Development of bilateral lung injury. Instillation of LPS in the left lower lobe, followed by 24 hours of spontaneous breathing, resulted in the development of bilateral lung injury in the

LPS group compared to Sham. Table 1 shows the main pathophysiological differences between study groups: in the LPS group, histological lung injury was bilateral, with mild deterioration in gas exchange and more relevant impairment of respiratory mechanics.
In comparison to Sham, respiratory drive and effort, lung stress and minute ventilation were increased in the LPS group, with equal dose of sedative drugs. Lower $PaCO_2$ indicated more activated respiratory drive, too. Hemodynamics were stable and comparable in both groups (apart from higher heart rate due to inflammation and higher work of breathing), as well as fluid balance (Table 1).

Histopathological characteristics of right lung injury. Figure 1 shows the variability in the severity of right lung injury: the histological score assessed by blinded pathologist and extensively validated showed a normal distribution (Shapiro-Wilk $p = 0.27$) across a wide range of values (3 to 25). Of note, within the relatively short observation period (24 hours), 50% of the animals already developed moderate-severe right lung injury based on previous publications (i.e., histological injury score of the right lung >10).
Figure 2 shows representative histological images of right lung SILI, with two degrees of severity (right lung injury score ≤ 10 for milder vs. > 10 for more severe), compared to Sham. Mild alveolar epithelial injury with granulocyte infiltration characterized less severe right lung injury (Figure 2b), while alveolar disruption and massive exudate altered the lung structure in more severe right injury (Figure 2a). Immunohistochemistry assay showed progressive abundancy of macrophages, with aggregation in clusters in more severe right lung injury (Figure 2d and 2e).

Pathophysiological mechanisms of progression to right lung injury. In terms of ventilatory pattern, we observed correlations between right histological score, respiratory rate ($r = 0.47$, $p = 0.048$) and minute ventilation ($r = 0.56$, $p = 0.016$), but not with inspired tidal volume ($r = 0.16$ $p = 0.54$) (Figure 3a-c). When we analyzed inspiratory drive and effort, right lung injury

score was correlated with P0.1 (r = 0.54 p = 0.021), ΔPocc (r = 0.63 p = 0.005) and ΔPes (r = 0.74 p < 0.001) (Figure 4a-c). The metabolic work of breathing per minute (PTPmin) was correlated to higher histological score (r = 0.72 p = 0.007), too, likely indicating a potential additional role for respiratory rate (Figure 3d). The right lung injury score was also correlated with lower value of alveolar pressure (Palv) measured during inspiration (r = -0.48 p = 0.042), which increases stretch of the alveolar wall cells and the risk for fluids extravasation (Supplemental Figure 1a). Indeed, Palv ranged from -3 to + 4 cmH_2O, reaching sub-atmospheric values in animals with more severe right lung involvement.

Higher right lung injury score was correlated with inspiratory mechanical lung stress and strain (Figure 5a-b). Dynamic inspiratory transpulmonary pressure (P_{Linsp}) and driving transpulmonary pressure (ΔP_L) (i.e., the main measures of inspiratory stress) were correlated with its severity (r = 0.54 p = 0.019 and r = 0.74 p < 0.001, respectively). In addition, tidal deformation of the right lung (i.e., the regional lung strain measured by EIT, see Figure 2 g-i) was strongly correlated with the right injury score (r = 0.88 p < 0.001), too (Figure 5c). Animals ventilated with PEEP values lower than Pes end-exp (i.e. negative values of $P_L$end-exp, reflecting higher risk of alveolar collapse) developed more severe right lung injury (r = -0.59 p = 0.009) (Supplemental Figure 1b).

At variance from previous works (4), we did not observe an association between occult pendelluft during inspiration and right lung injury score (r = 0.36 p = 0.138) (Fig 5d), possibly indicating a difference in the injurious mechanisms involved in progression from lobar insult to bilateral injury as compared to those involved in worsening of severe ARDS. Hemodynamics, both central and systemic, were not correlated with the right lung injury score (Online Supplement Table E1). Finally, in contrast with recent data (17), fluid balance was not correlated with the progression of injury (r = -0.09 p = 0.729).

***Time-course of pathophysiological mechanisms of progression to bilateral injury*.** We compared the relevant variables in animals developing milder vs. more severe right lung injury (median value of right lung SILI score ≤10 vs. >10) at T6 and T12. The respiratory drive and effort parameters, P0.1, ΔPes and driving transpulmonary pressure started to separate at 12 hours (Online Supplemental Table E2 and Figure 2). These data could support clinical observations that worsening respiratory failure in spontaneously breathing patients is best predicted at 12 hours (18). Indeed, the correlation between ΔPes and right lung injury was significant already at 12 hours ($r = 0.85$ $p < 0.001$).

Plasma proteomics and development of right lung injury. The correlation between the circulating levels of inflammatory mediators and the right lung injury score was explored by proteomic approach (Supplemental figure 3). Four out of 105 tested proteins showed a significant correlation (figure 6a-d). Higher levels of the chemokine stromal cell derived factor-1 alfa (SDF-1a) correlated with higher histological score ($r = 0.58$ $p = 0.038$). The three proteins with a negative regression coefficient with right injury score are involved in the regulation of the inflammatory response: interleukin-1 receptor antagonist (IL-1Ra: $r = -0.65$ $p = 0.023$), interleukin-5 (IL-5: $r = -0.61$ $p = 0.027$) and granulocyte-macrophages colony-stimulating factor (GM-CSF: $r = -0.64$ $p = 0.018$).

Interestingly, right lung injury score was not correlated with circulating levels of LPS ($r = 0.12$ $p = 0.659$) (Supplemental Figure 4).

Systemic inflammation, drive and effort. Our experimental study showed that respiratory effort and drive are directly correlated with systemic inflammation. Both ΔPes and ΔP_L were correlated with IGFBP-3 ($r = 0.66$ $p = 0.019$ and $r = 0.69$ $p = 0.009$), TNF-alfa ($r = 0.68$ $p = 0.011$ and $r = 0.6$ $p = 0.015$), IL-13 ($r = 0.59$ $p = 0.033$ and $r = 0.62$ $p = 0.023$) and IL-1beta ($r = 0.68$ $p = 0.011$ and $r = 0.64$ $p = 0.019$) (Figure 7a-d), while P0.1 was correlated with IL-31 ($r = 0.71$ $p = 0.007$) (figure 7e).

Discussion

The main findings of this experimental study on an animal model of bilateral lung injury initiated by a pro-inflammatory insult to a single lobe are: a) despite standardized methods to induce the primary left lobar insult, severity of right lung injury varies widely among animals; b) development of injury in previously healthy right lung results from specific pathophysiological mechanisms related to increased respiratory drive and effort; c) explorative proteomic analysis suggests specific biomarkers with injurious and/or protective effects for right lung injury, and highlights the effects of systemic inflammation on increased respiratory drive and effort.

Pneumonia is the leading cause of ARDS in clinical studies (22). Our experimental model replicated the pathophysiological combination of localized pulmonary inflammation and increased drive and effort which likely characterizes the progression from pneumonia to ARDS. On the other hand, it presumably represents just a simplified explorative approach, as other important factors like different etiologic agents, primary lobe involved, insult intensity, patient characteristics, etc. still need to be properly addressed.

Inter-individual variability of right lung injury score occurring in this standardized animal model could suggest a role for genetic factors which modulate the response to the initial pro-inflammatory stimulus in many ways (23). Activation of lung inflammation, due for example to cytokines genes variations, could affect the progression to bilateral injury, both directly and through increased respiratory drive and effort (24)(25). Genetic variations could have also led to more intense respiratory effort for the same inflammatory activation. Indeed, genetic polymorphisms linked to inflammatory pathways may alter both the susceptibility to ARDS and severity (26).

We observed correlations between right lung injury and increased drive and effort, as assessed by P0.1, ΔPes and ΔPocc, and with the downstream increase in lung stress. This has important consequences on the monitoring of spontaneously breathing patients with pneumonia at risk of progression to ARDS.

Surprisingly, not all the consequences of increased drive and effort resulted relevant for right lung injury. As far as ventilatory pattern is concerned, we found that larger Vt did not correlate with the right histological score. Tidal volume is considered a surrogate target for lung protection in the clinical management of spontaneously breathing ARDS patients undergoing non-invasive ventilation (11). However, Vt does not constantly and predictably reflect respiratory effort because of the interaction with lung compliance and heterogeneous distribution of ventilation. At variance, tidal strain, measuring the increase of lung volume normalized by starting inflation, correlated well with right injury score in our model of initially localized pulmonary insult. Monitoring of more specific variables such as driving pressure and strain could increase our ability to protect the lungs.

The correlation between respiratory rate and severity of right lung injury suggests that activation of respiratory drive resulted in tachypnea in this animal model; more importantly, it supported the role of respiratory rate (and thus of mechanical power) in the progression to bilateral lung injury (27). Pressure-time product, indeed, is a metabolic measurement of respiratory work which incorporates per breath effort and respiratory rate (28) and was correlated with right lung injury score, potentially suggesting its role as monitor of lung protection.

Our data confirm the hypothesis that negative (sub-atmospheric) values of Palv during inspiration contribute to progression to bilateral injury (29). This, and the protective role of positive transpulmonary pressure at end-expiration, also confirm that use of higher PEEP

could be protective, by attenuating capillary leak, preventing cyclic alveolar collapse and/or limiting diaphragm efficiency (5).

The explorative proteomic analysis identified biomarkers with physiologically sound correlations with right lung injury severity. For example, the positive correlation between SDF-1 and histological score is supported by the role of this chemokine in the sustained recruitment of neutrophils involved in the progression of lung injury in murine models (30). Among the three "protective" biomarkers negatively correlated with right lung injury score, IL-1Ra and IL-5 protect against the adverse effects of excessive inflammatory response. Indeed, an experimental study showed that IL-1Ra modulates the susceptibility to LPS-induced lung injury in mice (31). As for GM-CSF, this mediator could limit early epithelial cell injury and maintain alveolar macrophage function in the lung (32, 33). Blockade of injurious biomarkers, like SDF-1, or administration of protective factors, like GM-CSF, represent fascinating pharmacological perspectives to modulate progression to bilateral injury, or even to prevent it. Early use might be key to their efficacy, as some of these approaches failed to improve clinical outcome in patients with established ARDS (34).

Regulation of inflammatory response might also allow effective control of respiratory drive and effort. Stimulation of brain respiratory centers could possibly occur in two ways: systemic release of inflammatory mediators from the insulted lobe and/or neural signaling from the inflamed lung via the vagus nerve (35). Plasmatic levels of inflammatory cytokines were correlated with ΔPes and ΔPL in our model; while, at variance from observations in patients, we could not find a correlation between most inflammatory cytokines and P0.1, except IL-31. Overall, these data suggest that lung inflammation is associated with increased respiratory drive and effort.

Our study has many limitations. First, generalizability of this model of localized lung injury could be questioned since lobar inflammation by LPS could differ from bacterial, viral or

aspiration pneumonia, in terms of intensity, evolution, systemic reaction. Second, right lung injury was quantified by histological score at the end, while in-vivo assessment along the experiment might improve our understanding on how and when lobar inflammation starts affecting surrounding lung regions and improve human translation. Third, proteomic analysis was performed with commercially available human kits: while the consistent signal suggest that results are reliable, specific reporting standards are lacking. Finally, correlation does not imply causation: conclusive evidence demonstrating whether increased drive and effort are determinants of injury, consequences of injury, or both, is still lacking.

In conclusion, we developed a novel experimental model of bilateral lung injury from localized lobar inflammation induced by LPS: this resulted in increased respiratory drive and effort and in the development of contralateral lung damage within 24 hours, albeit with significant inter-animal variability. We described specific pathophysiological mechanisms associated with progression to right lung injury, deriving from increased respiratory effort, which stands out as principal responsible for the injury and as target parameter for early monitoring. The explorative proteomic analysis suggests candidate pathways of inflammatory response, whose modulation could potentially prevent progression to bilateral injury.

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Figure legends

Figure 1. Distribution of the severity of right lung injury. The figure shows the distribution of right lung injury score in both LPS and Sham animals. The score was in the lower ranges in Sham animals, while it was normally distributed across a wide interval in LPS group, indicating inter-individual variability. * $p < 0.05$ SHAM vs. LPS

Figure 2. Representative images for histology, immunohistochemistry and strain by EIT in animals with different right lung injury severity, compared to Sham. Animals with a right lung histological score > 10 (A) showed significant thickening of the alveolar walls and alveolar lining cell injury, culminating in complete pulmonary hepatization, with fluids exudate containing immune cells, red blood cells and fibrin. Animals with a right histological score ≤ 10 (b) revealed some loss of alveolar integrity, haemorrhage and granulocyte infiltration. The alveolar wall with a single layer of alveolar epithelial cells was preserved in sham animals (c).

Immunohistochemistry for ionized calcium-binding adapter molecule-1 (IBA-1) showed clusters of macrophages (stained in brown), diffuse within the lung parenchyma of LPS animals with more severe right lung injury (d), while macrophages are distributed in the alveolar wall, but at a lower density in milder cases (e). Few resident macrophages are present in sham animals (f).

EIT-derived strain, measuring tidal deformation indicized by the starting lung unit volume, showed progressively higher intensity in sham, mild and more severe right lung injury (g-i).

Figure 3. Correlations between right lung injury score and respiratory pattern. Higher histological score is correlated with higher respiratory rate, minute ventilation and pressure-time product, but not with tidal volume.

Figure 4. Correlations between right lung histological score and respiratory drive and effort. The score is correlated with P0.1, ΔPes and ΔPocc

Figure 5. Correlations between right lung histological score and stress, strain and pendelluft. Maximal stress (dynamic inspiratory transpulmonary pressure), tidal stress (transpulmonary driving pressure) and tidal strain (EIT-derived), but not occult pendelluft, are correlated with the histological right lung score.

Figure 6. Correlations between systemic inflammation and injury. Significant correlation with higher histological scores was disclosed for stromal cell derived factor-1 alfa (SDF-1a), thus indicating a possible role in the worsening of acute lung damage. Conversely, interleukin-1 receptor antagonist (IL-1Ra), interleukin-5 (IL-5), granulocyte-macrophages colony-stimulating factor (GM-CSF) showed a negative correlation, suggesting a possible protective effect.

Figure 7. Correlations between systemic inflammation and respiratory effort and drive

A positive correlation was found between ΔPes and four inflammatory proteins: Insulin-like Growth Factor Binding Protein 3 (IGFBP-3), IL-1beta, IL-13 and TNF-alfa (Figure 7 a-d), while P0.1 was correlated with IL-31 (7 e).

Table 1. Physiological differences between LPS and Sham groups

	Sham (n=5)	LPS (n=18)	P-value
Histological score			
Global	11 [6-16]	37 [31-44]	0.003
Left lung	6 [3-9]	27 [22-31]	0.001
Right lung (SILI score)	6 [3-9]	10.5 [8-14]	0.045
Gas exchanges			
PaO_2/FiO_2 (mmHg)	454 [336—488]	361 [326- 398]	0.061
Ventilatory ratio	1.6 [1.2-1.9]	2.2 [1.8-2.7]	0.013
$PaCO_2$ (mmHg)	49 [46-54]	39 [36-44]	0.007
pH	7.43 [7.42-7.44]	7.48 [7.45-7.50]	0.004
Ventilation, drive and effort			
MV (L/min)	3.7 [2.6-5.3]	7.6 [6-9.6]	0.032
Vt (ml/kg)	5.4 [4.5-6.4]	5.9 [4.7-8]	0.480
ΔP_L (cmH_2O)	7.2 [6.0-8.3]	12.4 [9.4-15.7]	0.004
ΔPes (cmH_2O)	2.96 [1.54-3.64]	8.1 [4.9-10.8]	0.004
RR (bpm)	21 [13-28]	31 [27-47]	0.025
P0.1 (cmH_2O)	0.95 [1.39-1.49]	3.5 [1.99-5.32]	0.004
Sedation			
Propofol (mg/kg/h)	128 [86-131]	104 [88-122]	0.434
Hemodynamics			
SAP (mmHg)	97 [95-102]	104 [96-121]	0.260
MAP (mmHg)	77 [74-85]	79 [73-94]	0.650
DAP (mmHg)	56 [53-66]	62 [51-77]	0.460
Heart rate (bpm)	76 [76-92]	107 [98-116]	0.003
PAPs (mmHg)	23 [23-25]	23 [19-24]	0.240
PAPm (mmHg)	18 [16-19]	18 [14-20]	0.620
PAPd (mmHg)	12 [10-14]	10 [7-15]	0.450
PCWP (mmHg)	6 [5-7]	8 [7-12]	0.031
Cardiac Output (L/min)	3.4 [3.4-4.1]	4.2 [3.9-4.5]	0.060
Fluid Balance at T 24 (ml)	315 [259-446]	303 [200-620]	0.910
Arterial Lactates (mmol/l)	0.7 [0.7-1.1]	0.7 [0.7-1.1]	0.940

Abbreviations: PaO_2: arterial oxygen partial pressure; $PaCO_2$: arterial carbon dioxide partial pressure; MV: minute ventilation; RR: respiratory rate, Vt: tidal volume; ΔP_L: transpulmonary driving pressure; ΔPes: esophageal pressure swing; P0.1: airway occlusion pressure; SAP: systolic arterial pressure; MAP: mean arterial pressure; DAP: diastolic arterial pressure; PAPs/m/d: pulmonary arterial pressure systolic/mean/diastolic; PCWP: pulmonary capillary wedge pressure

Figure 1.

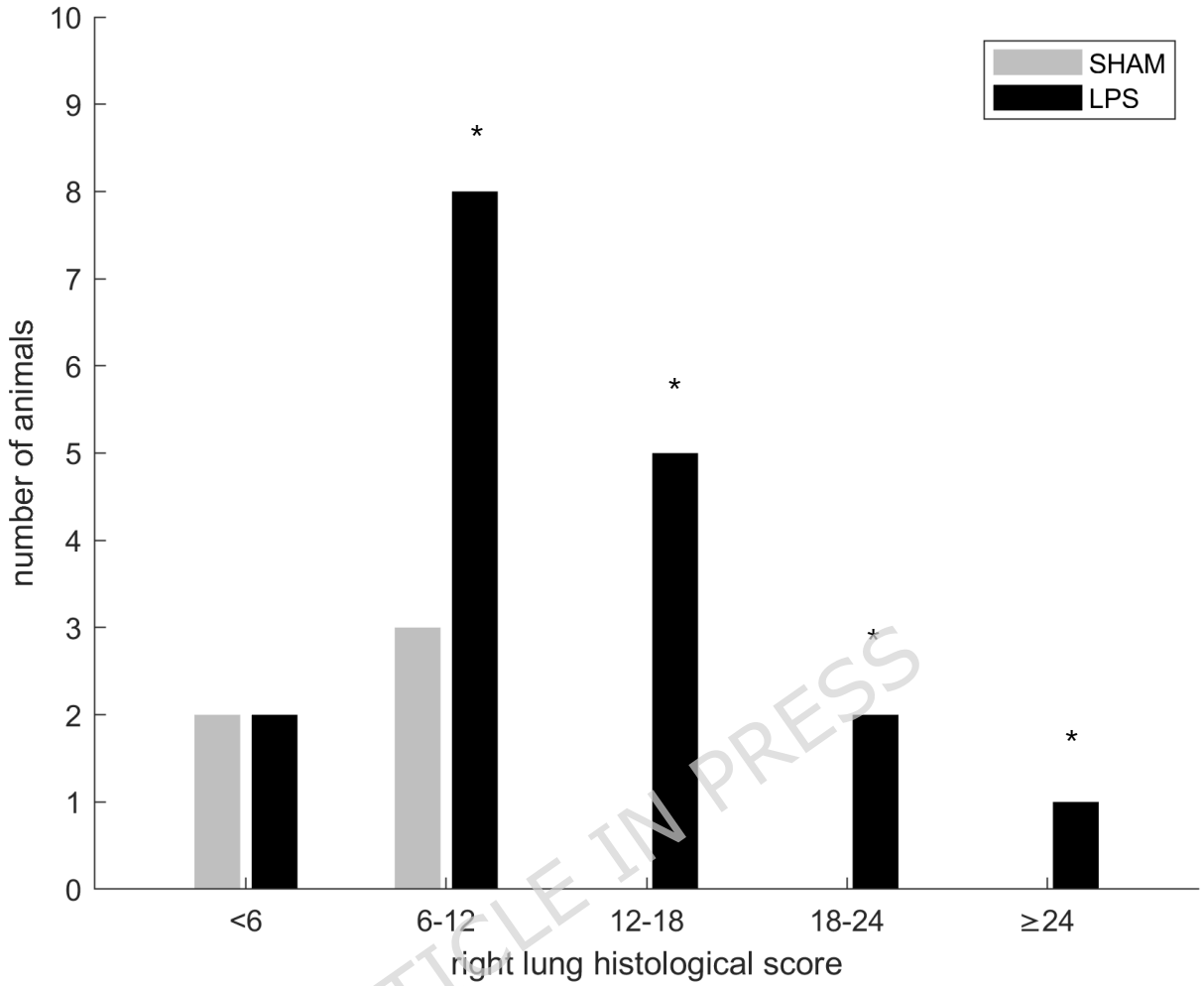


Figure 2.

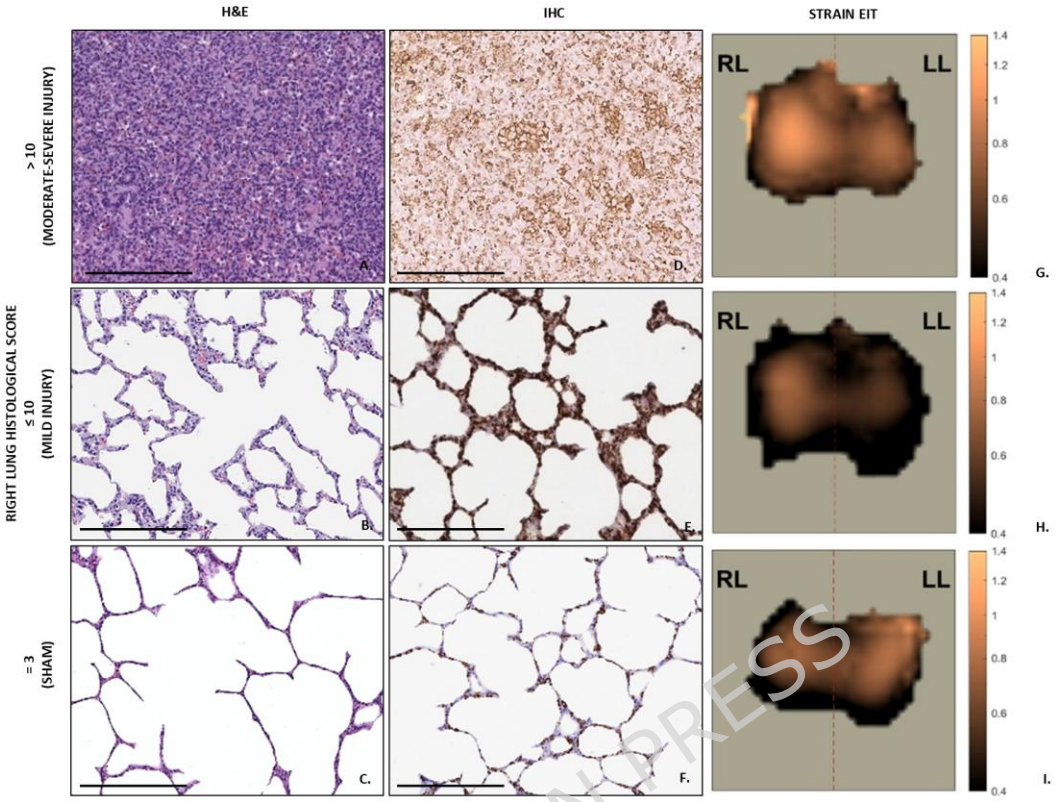


Figure 3.

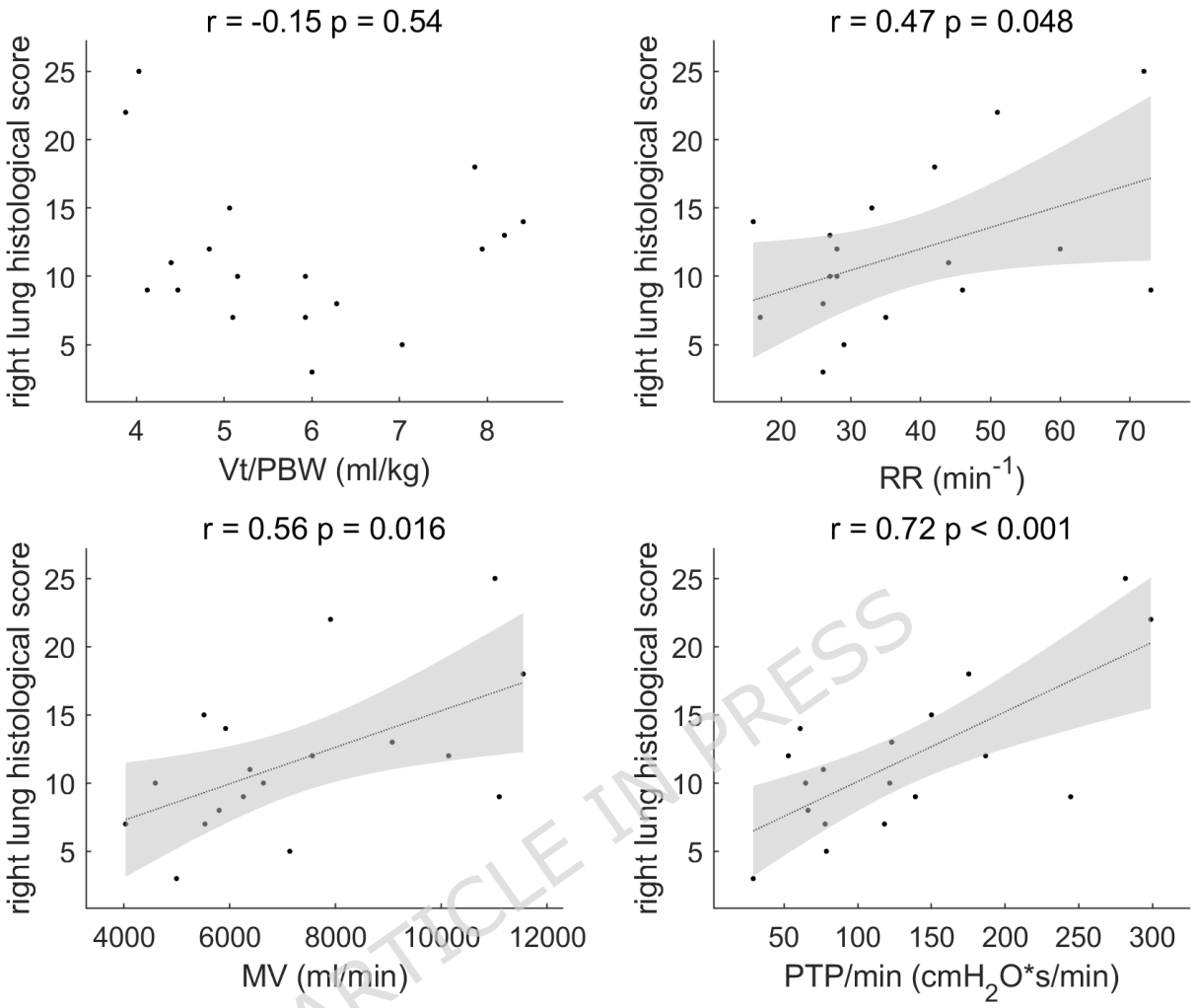
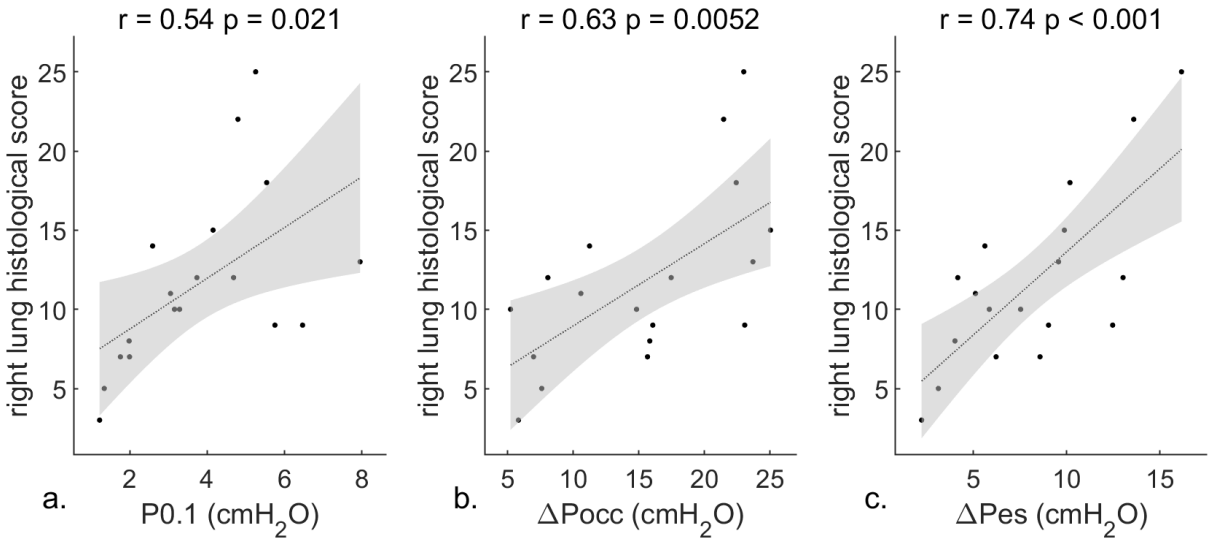


Figure 4.



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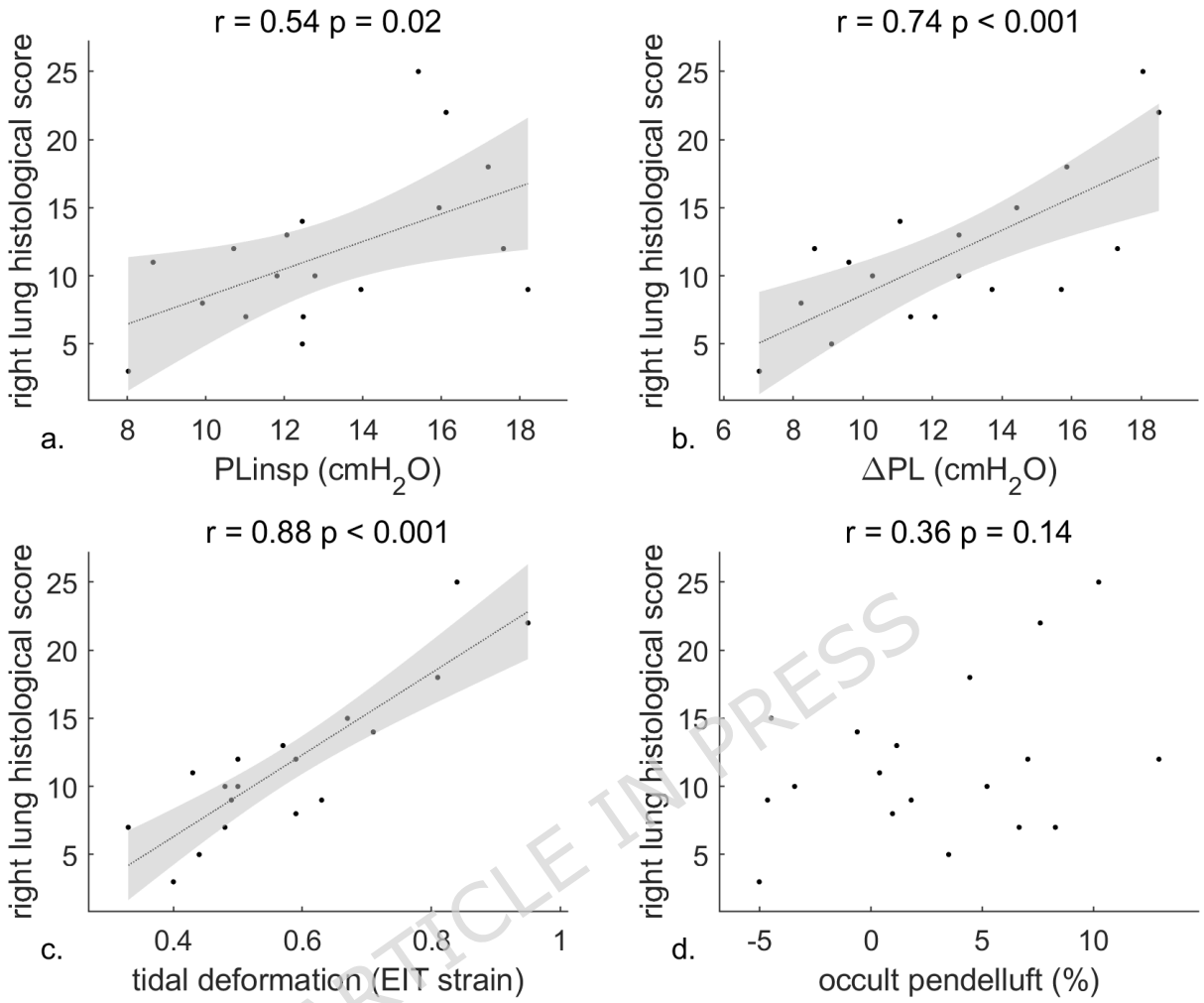


Figure 6.

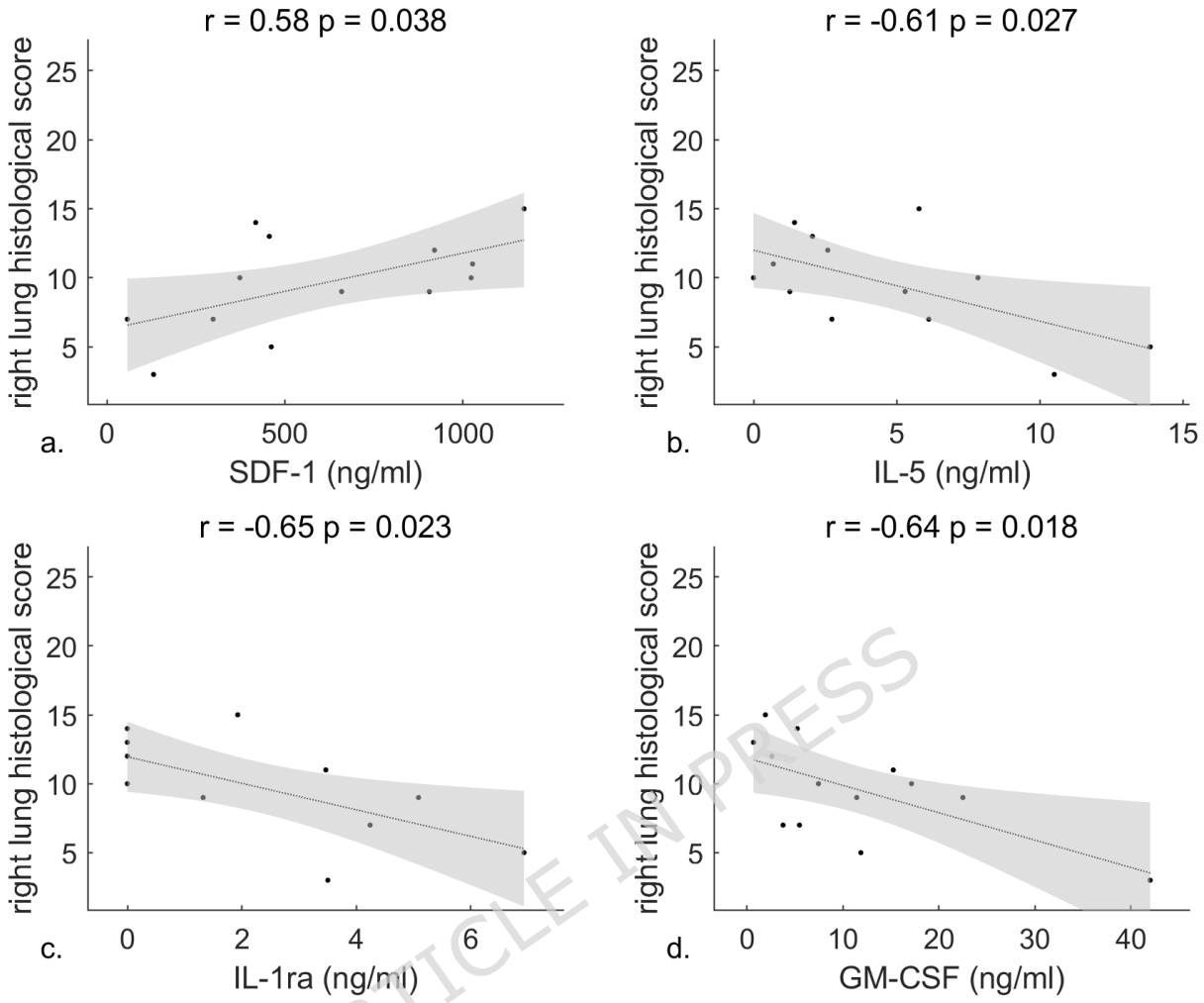
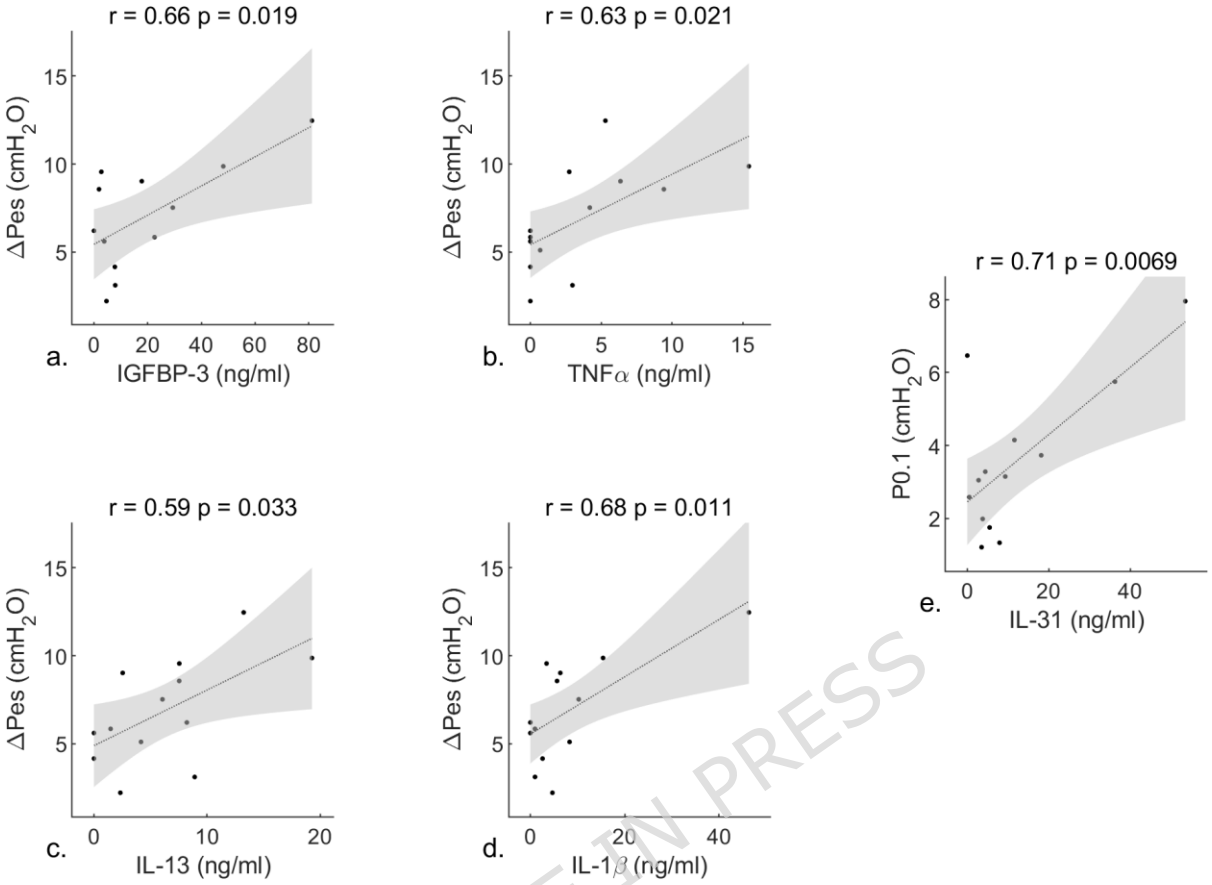


Figure 7.



Declarations:

Ethics approval: the study protocol was approved by the Italian Ministry of Health, Rome, Italy (Ref. n° 855/2024-PR prot. 568EB.49)

Consent for publication: not applicable

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Additional material: Additional study details can be found in the online Supplement