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Lung recruitment assessed by respiratory mechanics and by CT scan: What is the relationship?

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At a Glance Commentary

Scientific Knowledge on the Subject

Recruitability is highly variable in the ARDS lung. Its assessment may be a rational strategy for setting PEEP. The CT scan can indicate how much lung tissue is recruited at two pressure levels. CT scan-based methods, however, are demanding and not practical. Bedside respiratory mechanics methods assume that changes in respiratory system compliance are due to recruitment and measure it accordingly.

What This Study Adds to the Field

Respiratory mechanics-based methods measure not only the gas entering previously empty pulmonary units, but also the gas entering already open units. Consequently they depict an overall improvement of inflation but do not measure, as the CT scan (threshold -100) the collapsed/recruitable lung tissue.

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ABSTRACT

Rationale. The assessment of lung recruitability in ARDS patients may be important for planning recruitment maneuvers and setting positive end-expiratory pressure (PEEP). **Objectives.** To determine whether lung recruitment measured by respiratory mechanics is comparable with lung recruitment measured by CT scan.

Methods. In 22 ARDS patients lung recruitment was assessed at 5 and 15 cmH_2O PEEP by respiratory mechanics-based methods: A) increase in gas volume between two pressure-volume curves (**P-Vrs curve**); B) increase in gas volume measured and predicted from respiratory system compliance (**EELV-Cst,rs**), and by CT scan; C) decrease in non-inflated lung tissue **(CT (not inflated))**, and D) decrease in non-inflated and poorly-inflated tissue (**CT (not+poorly inflated)**).

Measurements and Main Results. The P-Vrs curve recruitment was significantly higher than EELV-Cst,rs recruitment (423±223 vs. 315±201 mL, $P<0.001$) but significantly related each other ($R^2=0.93$, $P<0.001$). CT (not inflated) recruitment was 77±86 g and CT (not+poorly inflated) 80±67 g, P=0.856 and were also significantly related each other ($R^2=0.20$, P = 0.04). Recruitment measured by respiratory mechanics was 54±28% (A-**P-Vrs curve**) and 39±25% (B-**EELV-Cst,rs**) of the gas volume at 5 cmH_2O PEEP. Recruitment measured by CT scan was 5±5% (C-**CT (not inflated)**) and 6±6% (D-**CT (not+poorly inflated)**) of lung tissue.

Conclusions. Respiratory mechanics and CT measure, under the same word "recruitment", two different entities. The respiratory mechanics-based methods include gas entering in already open pulmonary units which improve their mechanical properties at higher PEEP. Consequently they assess the overall improvement of inflation. The CT scan measures the amount collapsed tissue which regains inflation.

Abstract word count 250

Key words (4-6) ARDS, lung recruitment, pressure-volume curve, end-expiratory lung

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INTRODUCTION

Recruitment can be defined as the enrollment of pulmonary units in a new status of inflation. Using computed tomography (CT), recruitment at two pressure levels has been defined either as the amount of not-inflated tissue at a given pressure that re-inflates at higher pressure (1) or as the difference in not-inflated plus poorly-inflated tissue at the two pressures (2). The recruitment measured by a CT scan usually refers to "tissue recruitment" and is expressed as grams of tissue re-inflating, or as a fraction of the total lung weight. Its routine use in clinical practice, however, is problematic, on account of the x-ray exposure, the risks of transferring patients to the CT scan facilities, and the time-consuming work to complete the necessary computations (3).

Other methods, therefore, have been suggested(4, 5). The most popular are based on changes of respiratory mechanics. As an example, to estimate recruitment between 5 and 15 cmH_2O PEEP, it has been suggested that the expected end-expiratory lung volumes (EELV) at 15 cmH_2O airway pressure, computed from respiratory system compliance, should be compared with the measured EELV, and the difference is the recruitment. (5, 6) Another approach is to trace two pressure-volume (PV) curves, starting at different EELV and pressures (5 and 15 cmH_2O) and comparing the gas differences between the two curves at 20 cmH_2O (4, 7). If there is more gas at this pressure in the PV curve starting at higher volume, this is considered recruitment. The recruitment measured by respiratory mechanics is "gas recruitment" and is expressed as the absolute amount of gas or as a fraction of the total gas content.

In this study, we compared CT scan-based and respiratory mechanics-based methods to assess to what degree they are interchangeable. Recruitability may be important in clinical practice for assessing the severity of ARDS, planning recruitment maneuvers and setting adequate PEEP levels during mechanical ventilation (8, 9).

MATERIALS and METHODS

The study protocol is summarized in the Online Data Supplement.

Study population

This study was approved by the institutional review board of our hospital, and informed consent was obtained according to Italian regulations. We enrolled 22 ARDS patients, classified according to tertiles of PaO_2/FiO_2 (at 5 cmH_2O PEEP) to obtain balanced groups. We used the tertiles because classification according to the Berlin definition at standard PEEP (10) would have produced unbalanced groups (5 mild, 15 moderate and 2 severe ARDS).

Lung recruitment assessment

CTscan

The voxels in the whole lungs were grouped in 11 CT compartments at 100 HU steps, from >0 (totally not inflated) to -1000 (only gas). According to the CT distribution of normal lung we defined four lung inflation statuses: not inflated (HU >-100), poorly-inflated (-100 >-500 HU), well inflated (-500 >-900 HU) and over-inflated (HU < -900). (11)

Lungrecruitmentmeasuredbycomputedtomography

Lung recruitment was computed as the amount of lung tissue (not-inflated or not-inflated + poorly-inflated) in which the inflation changed on raising PEEP from 5 to 15 cmH_2O. Recruitment was expressed as grams of tissue or as a percentage of total lung tissue weight:

Method A: recruitment (g) = $NI_5 – NI_{15}$

Method B: recruitment (g) = $(NI_5 + PI_5) – (NI_{15} + PI_5)$

where NI_5 and NI_{15} are grams of not-inflated tissue at 5 and 15 cmH_2O PEEP, PI_5 and PI_{15} are grams of poorly-inflated tissue at 5 and 15 cmH_2O PEEP.

AJRCCM Articles in Press. Published on 23-December-2015 as 10.1164/rccm.201507-1413OC

Gasvolumeenteringnewly-recruitedtissue

To estimate how much gas is “recruited” per gram of tissue recruited on raising PEEP from 5 to 15 cmH_2O we assumed that recruited units at PEEP 15 cmH_2O had the same gas:tissue ratio as the units already open at the same pressure. Therefore we estimated the CT-scan recruited gas as:

Method A gas $rec_{5\text{-}15} = (NI_5 - NI_{15})*g/t_{15}$

Method B gas $rec_{5\text{-}15} = [(PI_5 + NI_5) - (PI_{15} + NI_{15})]*g/t_{15}$

where gas $rec_{5\text{-}15}$ is the gas in the tissue recruited from 5 to 15 cmH_2O PEEP and g/t_{15} is the total gas/(over + well + poorly) inflated tissue CT (g) at 15 cmH_2O PEEP.

The gas in recruited tissue was expressed in absolute terms (mL) and as percentages of the total gas at PEEP 5 cmH_2O.

Estimation of lung recruitment with a pressure-volume curve (P-Vrs curve method)

The PV curves of the respiratory system were traced starting from 5 and 15 cmH_2O PEEP and from the corresponding EELVs measured by the helium dilution technique. (12, 13) The PV curves were fitted to a sigmoid model as proposed by Venegas et al. (14) and the lung recruitment was computed as the gas difference measured on the two PV curves at 15 cmH_2O. (4, 7) Lung recruitment was expressed in absolute terms (mL) and as a percentage of EELV at PEEP 5 cmH_2O.

CalculationoflungrecruitmentbyEELVandstaticcomplianceoftherespiratorysystem (EELV-Cst,rsmethod)

Lung recruitment was calculated as the difference between the EELV at 15 cmH_2O and the volume expected after raising the pressure from 5 to 15 cmH_2O PEEP (5, 6, 12), according to this equation:

$$\text{Gas recruited} = EELV_{PEEP\ 15\ cmH2O} - (EELV_{PEEP\ 5\ cmH2O} + (Cst,rs_{\ PEEP\ 5\ cmH2O} * 10\ cmH_2O))$$

where the static respiratory system compliance was measured as:

$Cst,rs_{\ PEEP\ 5\ cmH2O}$ = tidal volume (mL)/(plateau pressure$_{\text{starting from PEEP 5 cmH2O}}$–end-expiratory pressure at PEEP 5 cmH_2O)

Lung recruitment was expressed in absolute terms (mL) and as a percentage of EELV at PEEP 5 cmH_2O.

Statistical analysis

Data are expressed as mean ± SD or median [IQ range]. For comparisons we used one-way ANOVA or one-way ANOVA on ranks when variables did not appear normally distributed. Two-way repeated measures ANOVA (one factor repetition) or two-way repeated measures ANOVA (one factor repetition) on ranks (when variables did not appear normally distributed) were used to compare tertiles of PaO_2/FiO_2 and the PEEP levels. The chi-square test was used for comparing categorical variables. Agreement between the different methods for measuring recruitment was checked by linear regression and Bland-Altman analysis. (15) Two-way repeated measures ANOVA was used to compare the 11 compartments of tissue and gas distribution. Correlations between recruitment at 5 and 15 cmH_2O PEEP and the baseline physiological and CT scan variables were established with linear regression. $P <0.05$ was considered significant. All statistical tests were done with SAS(R) 9.2, (SAS Institute Inc, Cary, North Carolina, USA) or SigmaPlot 11.0.

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RESULTS

Patients

We studied 22 patients 4.2±3.2 days after the onset of ARDS. Table 1 shows their baseline characteristics according to tertiles of PaO_2/FiO_2 at 5 cmH_2O PEEP. The first tertile (median [IQ range] PaO_2/FiO_2 216 [193-273]) comprised five mild and two moderate patients, as per the Berlin definition, the second (PaO_2/FiO_2 161 [152-169]) eight moderate ARDS patients, the third (PaO_2/FiO_2 126 [86-140]) five moderate and two severe cases. Their main physiological and CT variables at 5 and 15 cmH_2O PEEP are reported in Table 2. Most of the variables improved with the higher PEEP. The results according to the ARDS Berlin classification (unbalanced groups) are reported in the Online Data Supplement.

Recruitment

CT gas and tissue distribution at 5 and 15 cmH_2O

Figure 1 depicts the tissue (left panel) and gas distribution (right panel) in the different CT compartments. On raising the PEEP from 5 to 15 cmH_2O, there was significantly less tissue in the completely degassed (> 0 HU) and almost completely degassed CT compartments (0 to -100 HU), i.e. not inflated lung compartment. The amounts of tissue in the compartments from -100 up to -600 HU did not change with PEEP 5 or 15 cmH_2O PEEP while the tissue between -700 and -900 significantly increased.

The gas distribution (right panel) was similar in CT compartments between 0 and -700 at 5 and 15 cmH_2O PEEP. In contrast, the gas in the CT compartments already inflated between CT -700 and -900 at 5 cmH_2O PEEP significantly increased at 15 cmH_2O PEEP. A median of 72% [52-104%] of the total gas due to the higher PEEP entered these two CT compartments, with the remainder distributed in the others (see Online Data Supplement for details).

Recruitment thresholds

Recruitment at the HU thresholds of 0, -100, -200 and -300 was respectively 49±83 g, 77±87 g, 86±85 g and 87±78 g. As shown, the recruiment at the commonily used threshold of -100 HU (Method A) was similar to the one computed at -200 HU or -300 HU (see Online Data Supplement). The recruitment computed at the threshold -500 HU (Method B) averaged 80±67 grams and was weakly correlated with the recruitment measured at the traditional threshold of -100 HU (Table 3 and Figure 2).

Respiratory mechanics-based gas lung recruitment

The gas recruitment measured by the P-Vrs curve was significantly higher than with the EELV-Cst_{rs} method (Table 3). Although the two methods provided significantly different amounts of recruitment, they were closely correlated (R^2=0.93, p<0.0001), as shown in Figure 3, which reports the relation between the two methods (panel A) and the Bland-Altman analysis (panel B).

Comparison of the methods

Table 3 summarizes the recruitment with the four methods. Measured with respiratory mechanics-based methods recruitment averaged 423±223 mL (54±28%) with multiple PV curves and 315±201 mL (39±25%) with the EELV-Cst,rs method. Previously degassed lung tissue (threshold -100 HU) re-inflated with PEEP was 77±86 g (5±5%) with 129±148 mL of gas (16±20%). Applying a threshold of -500 the tissue recruited was 80±67 (6±6%) g with 163±165 (16±13%) mL of gas. Recruitments measured by CT scan expressed either as grams of tissue or milliliters of gas entering that tissue were unrelated to the recruitment measured by the respiratory mechanics methods (Figure 4).

Recruitment and baseline CT scan variables

Recruitment computed by the respiratory mechanics methods was significantly related to the amount of well-inflated tissue at 5 cmH_2O PEEP (r^2=0.25, p=0.02), see Figure 5 Panel

AJRCCM Articles in Press. Published on 23-December-2015 as 10.1164/rccm.201507-1413OC

B and C. In contrast, recruitment computed from the CT scan (threshold -100 HU) was significantly related to the amount of not-inflated tissue at 5 cmH_2O PEEP (r^2=0.44, p<0.001), see Figure 5 Panel A.

Recruitment and gas exchange

At constant FiO_2, the PaO_2 improved and the venous admixture decreased significantly when PEEP was raised from 5 to 15 cmH_2O (Table 2). CO_2 clearance slightly deteriorated with significant increases in $PaCO_2$ and dead space. The improvement in gas exchange was unrelated to recruitment, however measured. The sole exception was the weak but significant relationship between the recruitment measured with the CT scan (threshold -100 HU) and the PaO_2 improvement (r^2=0.26, p=0.01) (see Online Data Supplement for all the regressions).

DISCUSSION

The word "recruitment" over the years has come to signify different concepts, each primarily relying on one of the simultaneous phenomena occurring when pressures are applied to an ARDS lung, such as the opening of new pulmonary units (16) or better inflation of already open units (2). Consequently different methods to measure "recruitment" have led to different results just because the word, as interpreted by different authors, does refer to different entities (2, 17). Basically, the CT scan method measures the recruitment of lung tissue to a new inflation status (16) whose extent depends on the threshold used, while the respiratory mechanics method measures both the gas entering the newly recruited lung units and that entering already open units whose mechanical properties are improved at higher PEEP.

The CT scan methods are based on voxel-by-voxel analysis. Each voxel of the dimension we used (0.002625 mL – 2.625 mm^3) may include up to 10-15 completely collapsed pulmonary acini or 1/30th of a single acinus at total lung capacity. We grouped (see Figure 1) all the voxels in the whole lung contour in 11 compartments of decreasing density (100-HU steps) from >0 HU (i.e. fully degassed) to -1000 HU (only air). In the Method A we applied a threshold of -100 HU. We introduced this threshold decades ago (11) and it has been widely adopted up to now (see Table E2 in the Online Data Supplement). However, in the non-inflated tissue it includes pulmonary units with inflation up to 10%(11). We arbitrarily set this limit to account for the pulmonary units collapsing because of distal airway compression, in which some gas is left behind the occlusion, requiring lower opening pressures and probably undergoing intra-tidal collapse and de-collapse. In our series (see Figure 1) the total recruitment measured at -100 HU, 77±86 g, included 49±83 g of completely degassed tissue (threhsold > 0 HU) and 28±41 g of tissue nearly degassed [0 to -100 HU].

Reske et al.(18), and Mush et al.(19) using positron emission tomography, recently proposed thresholds of respectively -200 or -300 HU to define recruitment. Applying these thresholds to our patient population, the recruitment did not change significantly (see Figure 1 and Online

AJRCCM Articles in Press. Published on 23-December-2015 as 10.1164/rccm.201507-1413OC

Data Supplement). These findings first of all suggest: (1) that a threshold up to -200 or -300 only marginally changes the recruitment calculation; and (2) that within this threshold range the recruitment of completely degassed or nearly degassed units is quantitatively small, averaging only 10% of the lung tissue. This fraction, however, probably undergoes intra-tidal collapse and de-collapse if adequate PEEP is not provided. (20, 21)

Extending the CT threshold to -500 HU, adding the poorly-inflated tissue to the recruitment, introduced a confounding factor. In fact, this Method B does not distinguish the tissue that is presumably opening and closing as inflation up to 50% includes pulmonary units which are always open. The Method B, however, is different from the method proposed by Rouby(2), althought they use the same HU threshold (-500 HU). The Method B we applied in this study used a voxel-by-voxel analysis while the Rouby method measures how much gas enters in given anatomical lung region where the contiguous voxels have an HU < -500 at ZEEP(2). In theory, if in a given anatomical region applying PEEP increases the inflation from 10% to 49.9% the Rouby method would measure a considerable recruitment while the voxel-by-voxel analysis with -500 threshold would find recruitment equal to zero, as all the inflation changes occur within the poorly-aerated compartment. Therefore Rouby's method measures both the opening of pulmonary units and better inflation of already open units.

The first method based on respiratory mechanics we applied in this study requires measurement of the end-expiratory lung volume at 5 and 15 cmH_2O PEEP (helium dilution), and of cord compliance at 5 cmH_2O PEEP. If the compliance does not change at 5 and 15 cmH_2O PEEP the expected end-expiratory lung volume at 15 cmH_2O, i.e. EELV 5 cmH_2O + compliance x (15 – 5 cmH_2O) should be equal to the measured one. If the measured volume is higher than expected it implies that compliance from 5 to 15 improved, and this has been primarily attributed to recruitment. The dual PV curve method, proposed by several authors(4, 7), measures as recruitment the gas difference at the same pressure between two PV curves starting from different PEEP levels. A positive difference indicates that compliance is increased and the increase has been attributed to the recruitment of new pulmonary units.

Being based on the same principle, i.e. the improvement in compliance, the two methods provide similar recruitment figures and are extremely well related. The key issue, however, is that the respiratory system compliance, when the lung volume increases, may improve for reasons other than enrollment of new units.

That open pulmonary units starting from different volumes inflate differently is implicit in the sigmoid PV curve of the normal lungs. At the beginning of the inflation, it takes more pressure to reach a given volume starting from low volume than from a higher one. This is due, independently of recruitment, to differences in surface forces and lung tissue resistances at different volumes. (22) Therefore, the gas increase for a given pressure increase reflects not only the possible recruitment but also the greater natural inflation of the units starting at higher volume. Actually, in the present study we found that recruitment measured by the PV curve was proportional to the amount of well-inflated lung, as is also suggested by gas distribution at higher PEEP in already well-aerated compartments (Figure 1). Therefore our data suggest that the CT scan method at threshold -100 HU (and possibly -200 and -300) measures as recruitment the amount of tissue completely degassed or nearly degassed which re-inflates with higher PEEP. The respiratory mechanics method, instead, measures as recruitment the amount of gas entering newly opened units and the amount which inflates better, according to the improvement of mechanical properties of some pulmonary units at higher volume Not surprisingly, these “recruitments” are quantitatively different and unrelated. The original method proposed by Rouby(2), which measures all gas entering the previously poorly/not-inflated lung regions, would measure a recruitment similar to that given by the PV curve, i.e. newly opened units and better mechanical properties of already open lung units going from ZEEP to 15 cmH_2O PEEP,as shown in the comparative study by Lu et al. However, in that study, where the PV curve and Rouby’s method were very well related, the changes in not-inflated tissue (threshold -100) were unrelated to the PV curve recruitment as we found in our study.

Many studies found a positive relationship between recruitment and oxygenation

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improvement (4, 23, 24). In our population, although the oxygenation increased with PEEP, its changes were weakly related only to the recruitment measured with CT scan (threshold -100 HU). This is not surprising as the PEEP may affect oxygenation with mechanisms different from recruitment as Va/Q changes, PvO_2 levels(25), total cardiac output(26), its distribution(27), true shunt changes, etc(28). These data suggest caution in equating any improvement in oxygenation to recruitment while setting PEEP.

In conclusion, we found that the different methods used to measure recruitment actually measure different phenomena related to the pressure increase. CT scan methods, at a -100 HU threshold, measure tissue that is potentially opening and closing. At a lower threshold, -500 HU, applying voxel-by-voxel analysis introduces a confounding factor with no apparent advantage. The respiratory mechanics methods clarify to what degree raising PEEP may improve overall inflation by increasing lung compliance through enrollment of new units and possible mechanical improvement of the already open ones.

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FIGURE LEGENDS

Figure 1.

Panel A: tissue distribution (grams) in 11 CT compartments from >0 to -1000 (-100 HU steps). Black bars represent PEEP 5 and gray bars PEEP 15 cmH_2O. * $p < 0.05$ ** $p < 0.01$ comparing CT scans performed at PEEP 5 and 15 cmH_2O in the same HU range.

Panel B: gas distribution (gas) in 11 CT compartments from >0 to -1000 (-100 HU steps). Black bars represent PEEP 5 and gray bars PEEP 15 cmH_2O. * $p < 0.05$ ** $p < 0.01$ comparing CT scans performed at PEEP 5 and 15 cmH_2O in the same HU range.

Figure 2.

Panel A: relationship between lung recruitment estimated by Computed Tomography (tissue methods). The grey solid line represents linear regression:

CT (not+poorly inflated tissue) recruitment = 53 + 0.35 × CT (not inflated tissue) recruitment, $P = 0.037$, $R^2 = 0.20$.

Panel B: Bland – Altman analysis of lung recruitment computed by Computed Tomography (tissue methods). X-axis represents the mean of the two measurements, while Y-axis represents the difference between the recruitment assessed by CT methods. Horizontal grey lines are the mean difference (solid), and at the limits of agreement (mean difference plus and minus 1.96 times the standard deviation of the differences, medium dashed lines).

Figure 3.

Panel A: relationship between lung recruitment estimated by Pressure-Volume curve (P-Vrs curve recruitment) and by EELV and static compliance of respiratory system (EELV-Cst,rs). The grey solid line represents linear regression:

EELV-Cst,rs recruitment = -52 + 0.87 × P-Vrs curve recruitment, $P<0.0001$, $R^2=0.93$

Panel B: Bland – Altman analysis of lung recruitment computed with the P-Vrs curve and with EELV-Cst,rs methods. X-axis represents the mean of the two measurements, while Y-axis represents the difference between the P-Vrs curve and CT. Horizontal grey lines are the mean difference (solid), and at the limits of agreement (mean difference plus and minus 1.96 times the standard deviation of the differences, medium dashed lines).

Figure 4.

Comparison of Respiratory mechanics-based methds and CT scan-based methods expressed as milliliters of gas. Solid grey lines represent linear regressions.

Panel A: gas associated to recruited tissue (not-inflated) *versus* P-Vrs curve recruitment.

$Y = 31 + 0.23 \times X$, $R^2 = 0.12$, $P = 0.11$

Panel B: gas associated to recruited tissue (not-inflated) *versus* EELV-Cst,rs recruitment.

$Y = 35 + 0.30 \times X$, $R^2 = 0.17$, $P = 0.06$

Panel C: gas associated to recruited tissue (not- + poorly-inflated) *versus* P-Vrs curve recruitment.

$Y = 78 + 0.20 \times X$, $R^2 = 0.07$, $P = 0.22$

Panel D: gas associated to recruited tissue (not- + poorly-inflated) *versus* EELV-Cst,rs recruitment.

$Y = 88 + 0.24 \times X$, $R^2 = 0.19$, $P = 0.08$

Individual patients are identified by different symbols.

Figure 5.

Panel A: recruited tissue (not-inflated) *versus* not inflated tissue at PEEP 5 cmH_2O

$Y = -4.47 + 0.12$ x X, $R^2 = 0.44$, $P < 0.001$

Panel B: P-Vrs curve recruitment v*ersus* well inflated tissue at PEEP 5 cmH_2O

$Y = 157 + 0.85$ x X, $R^2 = 0.25$, $P = 0.02$

Panel C: recruited tissue (not- + poorly-inflated) v*ersus* not inflated tissue at PEEP 5 cmH_2O

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$R^2 = 0$, $P = 0.84$

Panel D: EELV-Cst,rs recruitment v*ersus* well inflated tissue at PEEP 5 cmH_2O

$Y = 86 + 0.71 \times X$, $R^2 = 0.24$, $P = 0.02$

TABLES

Table 1. *Baseline Characteristics of the Study Population - patients classified according to Berlin definition of ARDS at 5 cmH_2O*

Characteristics	Overall population (N=22)	1st Tertile	2nd Tertile	3rd Tertile	P value
PaO_2/FiO_2 at 5 cmH_2O PEEP	161 [140-193]	216 [193-273]	161 [152-169]	126 [86-140]	
Age (years)	67.5 ± 11.7	65.6 ± 16.4	71.8 ± 10.9	64.4 ± 6.0	0.445
Male sex, *n* (%)	15 (68%)	4 (57%)	4 (50%)	7 (100%)	0.087
Body Mass Index (kg/m^2)	27.4 ± 7.1	24.8 ± 5.6	29.4 ± 9.3	27.6 ± 5.6	0.485
Tidal volume/ Actual body weight (mL/kg)	8.1 [7.0-9.8]	8.3 [7.5-9.8]	8.9 [8.0-9.8]	7.0 [6.3-7.9]	0.153
Respiratory rate (bpm)	12.5 [12.0-15.0]	12.0 [10.0-17.0]	13.5 [12.0-16.0]	13.0 [12.0-15.0]	0.708
Minute ventilation (L/min)	7.1 ± 1.4	7.3 ± 2.0	7.1 ± 1.3	6.9 ± 1.1	0.881
PEEP (cmH_2O)	10.0 [10.0-12.0]	10.0 [5.0-10.0]	10.0 [10.0-10.5]	12.0 [10.0-15.0]	0.058
Static compliance of respiratory system (mL/cmH_2O)	43.8 ± 17.9	53.1 ± 26.0	36.1 ± 10.3	43.3 ± 11.6	0.185
Intensive care mortality, *n* (%)	13 (59%)	4 (57%)	6 (75%)	3 (43%)	0.447
Causes of ARDS:					
Pneumonia	13	3	5	5	0.210
• Sepsis	4	2	0	2	
• Aspiration	3	2	1	0	
• Other	2	0	2	0	
PaO_2/FiO_2 ratio	195 ± 37	222 ± 12	198 ± 38	165 ± 34*	0.010
$PaCO_2$ (mmHg)	40.2 [36.1-44.5]	35.7 [32.3-41.0]	42.7 [38.5-48.4]	42.4 [39.0-44.5]	0.043

Data are expressed as mean ± SD or median [IQ range] as appropriate. ARDS acute respiratory distress syndrome, PEEP positive end-expiratory pressure, $PaCO_2$ partial pressure of carbon dioxide oxygen, PaO_2 partial pressure of oxygen, FiO_2 inspired oxygen fraction. P values: One Way Analysis of Variance or Kruskal-Wallis One Way Analysis of Variance on Ranks.

* P<0.05 vs 1st Tertile

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Table 2. *Gas exchange, partitioned respiratory mechanics and CT scan variables*

Characteristics	PEEP	Overall population (N=22)	1st Tertile (N=7)	2nd Tertile (N=8)	3rd Tertile (N=7)	P value Group	P value PEEP
PaO_2/FiO_2 at 5 cmH_2O PEEP		161 [140-193]	216 [193-273]	161 [152-169]	126 [86-140]		
PaO_2(mmHg):							
	5 cmH_2O	81±17	97±16	80±11	66±7	0.094	<0.001
	15 cmH_2O	112±28	120±24	109±32	107±28		
SvO_2 (%):							
	5 cmH_2O	77.6 [69.4-79.4]	78.6 [63.3-79.6]	77.6 [73.7-81.7]	74.6 [69.4-79.1]	0.685	0.046
	15 cmH_2O	79.5 [72.9-83.6]	76.1 [69.8-80.2]	80.8 [72.6-83.5]	80.0 [72.9-86.5]		
Venous admixture (%) (p interaction = 0.005):							
	5 cmH_2O	37.3±14.7	24.7±6.3	37.0±15.5‡§	50.3±7.0*†§	<0.001	<0.001
	15 cmH_2O	27.2±7.8	20.1±3.7	28.3±8.9	33.1±2.7*		
$D_{AV}O_2$ (mL/100 cc):							
	5 cmH_2O	2.7 [2.1-3.3]	2.9 [2.6-4.1]	2.4 [2.1-3.4]	2.3 [1.7-2.9]	0.080	0.050
	15 cmH_2O	2.4 [2.0-3.1]	3.1 [2.2-4.2]	2.4 [2.0-3.0]	2.2 [1.6-2.6]		
$PaCO_2$ (mmHg):							
	5 cmH_2O	46±10	40±6	46±9	51±12	0.196	0.002
	15 cmH_2O	48±11	43±9	49±11	52±12		
Dead Space (Vd/Vt) (%) (p interaction = 0.014)							
	5 cmH_2O	60±11	55±10§	62±9§	63±14	0.553	<0.001
	15 cmH_2O	63±11	60±11	65±10	63±14		
Static Compliance of Respiratory System (Cst,rs) (mL/cmH_2O):							
	5 cmH_2O	43.7±13.7	50.5±16.1	39.8±9.9	41.2±14.1	0.446	0.035
	15 cmH_2O	38.9±15	42.3±16.7	34.8±11.9	40.3±17.4		
Static Compliance of the Lung (Cst,L) (mL/cmH_2O):							

	5 cmH2O	57.8±20.6	65.5±24.3	52.3±15.2	56.2±22.5	0.397	0.065
	15 cmH2O	50.0±19.9	56.9±24.0	44.0±16.5	50.1±19.5		
Static Compliance of the Chest Wall (Cst,cw) (mL/cmH2O):							
	5 cmH2O	201 [123-251]	196 [172-251]	196 [120-241]	206 [101-280]	0.997	0.239
	15 cmH2O	186 [123-242]	175 [111-227]	185 [129-298]	197 [107-273]		
End Expiratory Lung Volume (EELV)(mL):							
	5 cmH2O	811±269	892±234	839±354	697±170	0.363	<0.001
	15 cmH2O	1563±493	1792±521	1484±549	1423±370		
Lung weight (g)							
	5 cmH2O	1378±432	1177±168	1164±231	1825±472	<0.001*‡	0.011
	15 cmH2O	1426±4551	1205±168	1195±203	1912±483		

Data are expressed as means±SD or median [IQ range]. ARDS acute respiratory distress syndrome, PEEP positive end-expiratory pressure,$PaCO_2$ partial pressure of carbon dioxide oxygen, PaO_2 partial pressure of oxygen, FiO_2 inspired oxygen fraction, SvO_2 venous oxygen saturation, $D_{AV}O_2$ arteriovenous oxygen difference . Two Way Repeated Measures ANOVA (one factor repetition) or Two Way Repeated Measures ANOVA (one factor repetition) on ranks was used to compare the physiological values obtained among the groups and within each PEEP applied. Interaction was reported only when significant.

*P<0.05 1st vs 3rd tertile; †P<0.05 2nd vs 1st tertile; ‡P<0.05 3rd vs 2nd tertile; §P<0.05 PEEP 5 cmH_2O vs. PEEP 15 cmH_2O

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Table 3. *Comparison between different methods in assessing lung recruitment*

	Methods	Overall population (N=22)	1st Tertile (N=7)	2nd Tertile (N=8)	3rd Tertile N=7)	P value Tertile	P value Interaction
PaO_2/FiO_2 at 5 cmH_2O PEEP		161 [140-193]	216 [193-273]	161 [152-169]	126 [86-140]		
Respiratory Mechanics (gas)	P-Vrs curve, mL (%)	423±223 (54±28%)	499±247 (57±26%)	333±233 (43±31%)	450±178 (65±25%)	0.364 (0.356)	0.296 (0.223)
	EELV-Cst,rs, mL (%)	315±201 (39±25%)	395±230 (45±24%)	247±200 (29±26%)	323±167 (45±25%)		
p value Methods		<0.001 (<0.001)					
CT scan (tissue)	CT (not inflated), g (%)	77±86 (5±5%)	49±77* (4±7%)*	69±61 (6±5%)	114±115* (5±5%)	0.983 (0.549)	0.012 (0.019)
	CT (not+poorly inflated), g (%)	80±67 (6±6%)	108±95 (9±9%)	82±51 (7±4%)	50±43 (3±2%)		
P value Methods		0.856 (0.298)					
CT scan (gas)	CT (not inflated gas), mL (%)	129±148 (16±20%)	134±205 (10±15%*)	138±128 (12±10%)	114±123 (25±29%*)	0.496 (0.834)	0.070 (0.014)
	CT (not + poorly inflated), mL (%)	163±165 (16±13%)	236±230 (22±19%)	179±133 (14±9%)	73±74 (10±9%)		
P value Methods		0.160 (0.990)					

Data as absolute values, as percentage of total lung volume (EELV for Respiratory Mechanics derived variables and Total Gas from CT scan for Gas recruited by CT scan) and lung weight (for tissue) (%) are express as mean ± SD. PaO_2/FiO_2 at 5 cmH_2O PEEP is expressed as median [IQ range] ARDS, acute respiratory distress syndrome; P-Vrs curve, pressure-volume curve of the respiratory system; EELV, end-expiratory lung volume; Cst,rs, static compliance of the respiratory system; CT, computed tomography. Two Way Repeated Measures ANOVA (One Factor Repetition) was performed to obtained p values and All Pairwise Multiple Comparison Procedures (Bonferroni t-test).*P <0.05 first vs second method.

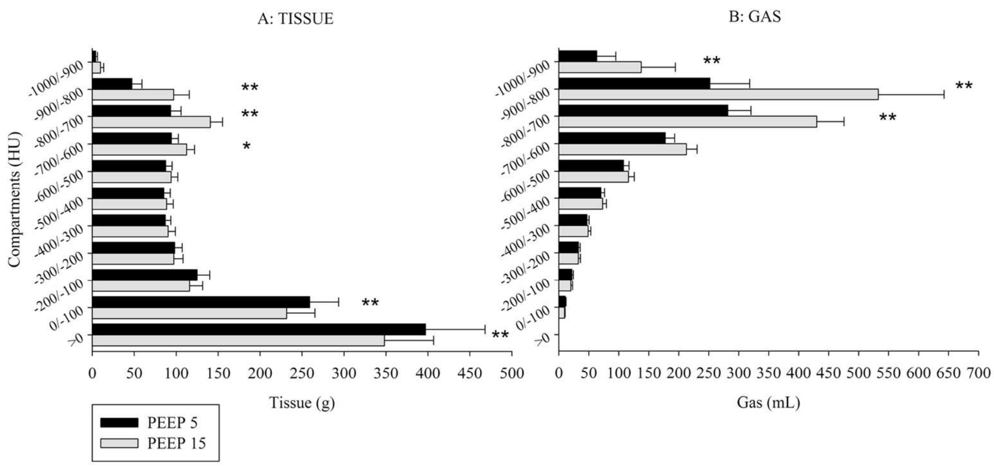

Figure 1.
Panel A: tissue distribution (grams) in 11 CT compartments from >0 to -1000 (-100 HU steps). Black bars represent PEEP 5 and gray bars PEEP 15 cmH2O. * p < 0.05 ** p < 0.01 comparing CT scans performed at PEEP 5 and 15 cmH2O in the same HU range.

Panel B: gas distribution (gas) in 11 CT compartments from >0 to -1000 (-100 HU steps). Black bars represent PEEP 5 and gray bars PEEP 15 cmH2O. * p < 0.05 ** p < 0.01 comparing CT scans performed at PEEP 5 and 15 cmH2O in the same HU range.
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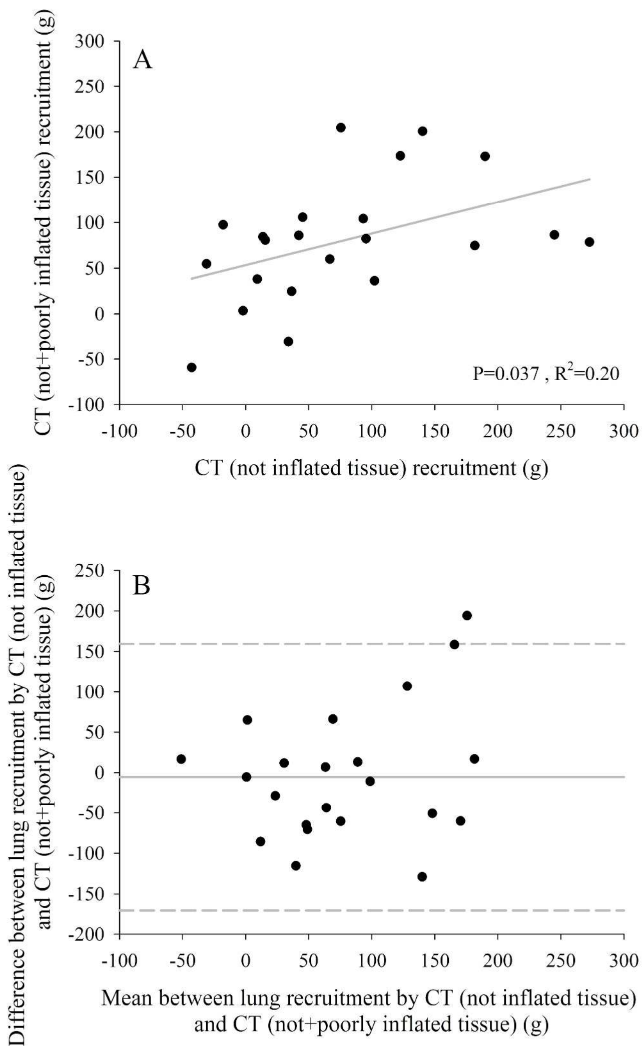
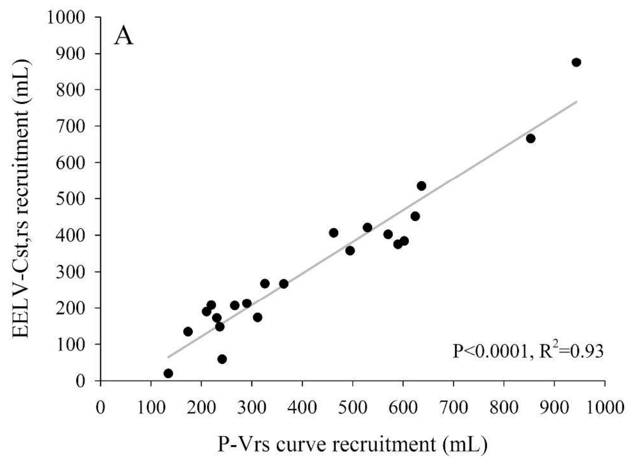


Figure 2.
Panel A: relationship between lung recruitment estimated by Computed Tomography (tissue methods). The grey solid line represents linear regression:
CT (not+poorly inflated tissue) recruitment = 53 + 0.35 × CT (not inflated tissue) recruitment, P = 0.037 , R2 = 0.20.
Panel B: Bland – Altman analysis of lung recruitment computed by Computed Tomography (tissue methods). X-axis represents the mean of the two measurements, while Y-axis represents the difference between the recruitment assessed by CT methods. Horizontal grey lines are the mean difference (solid), and at the limits of agreement (mean difference plus and minus 1.96 times the standard deviation of the differences, medium dashed lines).
297x421mm (300 x 300 DPI)



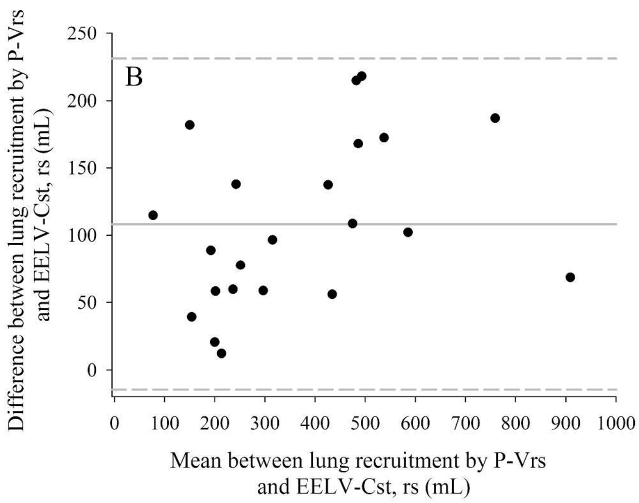


Figure 3.
Panel A: relationship between lung recruitment estimated by Pressure-Volume curve (P-Vrs curve recruitment) and by EELV and static compliance of respiratory system (EELV-Cst,rs). The grey solid line represents linear regression:
EELV-Cst,rs recruitment = -52 + 0.87 × P-Vrs curve recruitment, $P<0.0001$, R2=0.93
Panel B: Bland – Altman analysis of lung recruitment computed with the P-Vrs curve and with EELV-Cst,rs methods. X-axis represents the mean of the two measurements, while Y-axis represents the difference between the P-Vrs curve and CT. Horizontal grey lines are the mean difference (solid), and at the limits of agreement (mean difference plus and minus 1.96 times the standard deviation of the differences, medium dashed lines).
297x421mm (300 x 300 DPI)

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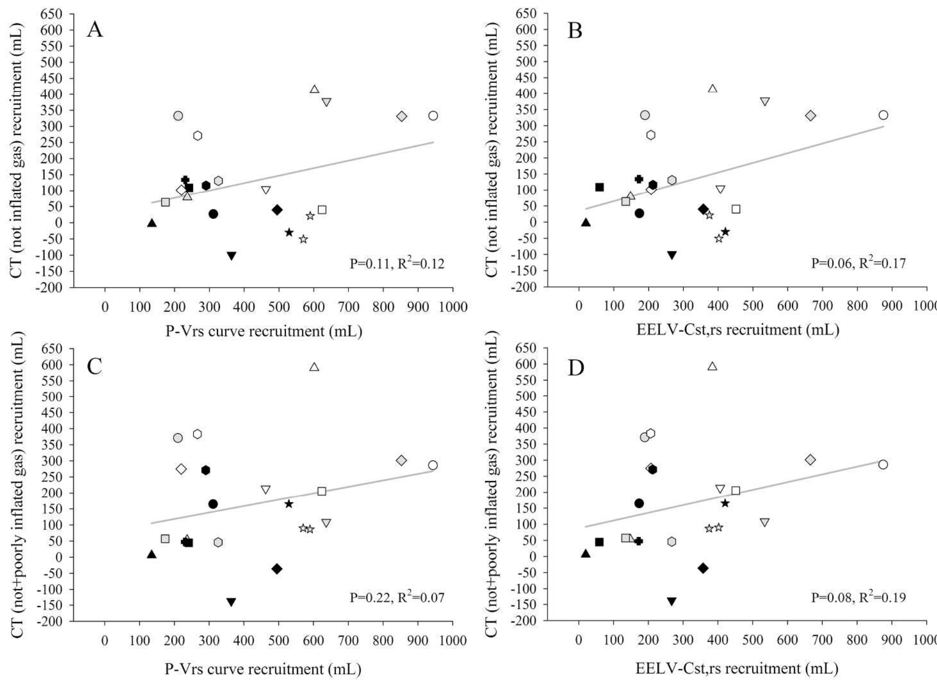

Figure 4.
Comparison of Respiratory mechanics-based methds and CT scan-based methods expressed as milliliters of gas. Solid grey lines represent linear regressions.
Panel A: gas associated to recruited tissue (not-inflated) versus P-Vrs curve recruitment.
Y = 31 + 0.23 × X, R2 = 0.12, P = 0.11
Panel B: gas associated to recruited tissue (not-inflated) versus EELV-Cst,rs recruitment.
Y = 35 + 0.30 × X, R2 = 0.17, P = 0.06
Panel C: gas associated to recruited tissue (not- + poorly-inflated) versus P-Vrs curve recruitment.
Y = 78 + 0.20 × X, R2 = 0.07, P = 0.22
Panel D: gas associated to recruited tissue (not- + poorly-inflated) versus EELV-Cst,rs recruitment.
Y = 88 + 0.24 × X, R2 = 0.19, P = 0.08
Individual patients are identified by different symbols.
210x148mm (300 x 300 DPI)

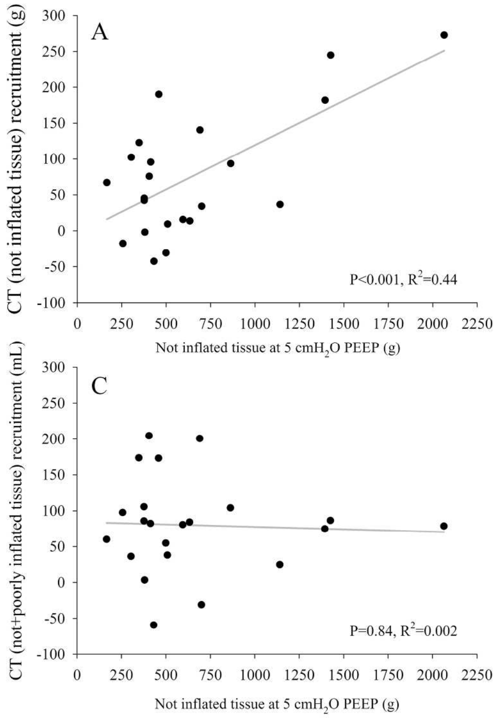

Figure 5.
Panel A: recruited tissue (not-inflated) versus not inflated tissue at PEEP 5 cmH2O
Y = -4.47 + 0.12 x X, R2 = 0.44, P < 0.001
Panel B: P-Vrs curve recruitment versus well inflated tissue at PEEP 5 cmH2O
Y = 157 + 0.85 x X, R2 = 0.25, P = 0.02
Panel C: recruited tissue (not- + poorly-inflated) versus not inflated tissue at PEEP 5 cmH2O
R2 = 0, P = 0.84
Panel D: EELV-Cst,rs recruitment versus well inflated tissue at PEEP 5 cmH2O
Y = 86 + 0.71 x X, R2 = 0.24, P = 0.02
212x159mm (300 x 300 DPI)

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Lung recruitment assessed by respiratory mechanics and by CT scan: What is the relationship?

Online Data Supplement

D. Chiumello, A. Marino, M. Brioni, I. Cigada, F. Menga, A. Colombo, F. Crimella, I. Algieri, M. Cressoni, E. Carlesso and L. Gattinoni

ONLINE MATERIALS and METHODS

Study protocol

All enrolled patients in supine position were deeply sedated and paralyzed. The anesthesia was maintained with infusion with Midazolam (0.05-0.1 mg/Kg) and Fentanyl (2-3 µg/Kg) and paralysis with Vecuronium (0.05-0.1 mg/kg). The clinical characteristics of the patients, respiratory variables, and ventilator settings were recorded before the study. The sequence of the protocol is summarized in the Figure here below). Immediately before each step of the PEEP trial and CT session, to standardize the lung volume history, a recruitment maneuver was performed for two minutes (1). The recruitment maneuver was performed in pressure control mode with PEEP 5 cmH_2O, pressure above PEEP 40 cmH_2O, respiratory rate 10 breaths/min, I/E ratio 1:1 (2). After the recruitment maneuver, 5 and 15 cmH_2O of PEEP were randomly applied. All patients were ventilated in volume controlled mode with a tidal volume of 8-10 mL per kilogram of ideal body weight throughout the study protocol. The oxygen fraction and respiratory rate were maintained unchanged for the entire study. At each PEEP level, after 30 minutes, respiratory mechanics, blood gas analyses, end expiratory lug volume (EELV) and dead space were measured. Subsequently a P-V curve was obtained. The P-V curves were obtained at each PEEP level without disconnecting the ventilator, with a low flow insufflation (6-8 L/min) (3) using a Servo I mechanical ventilator in volume controlled mode to reach an inspiratory airway pressure between 30-35 cmH_2O.

At the end the patient was transported to radiological department for lung CT scan at 5 ad 15 cmH_2O of PEEP. The whole study protocol lasted approximately 3 hours. A summary of the study is reported in Figure E1.

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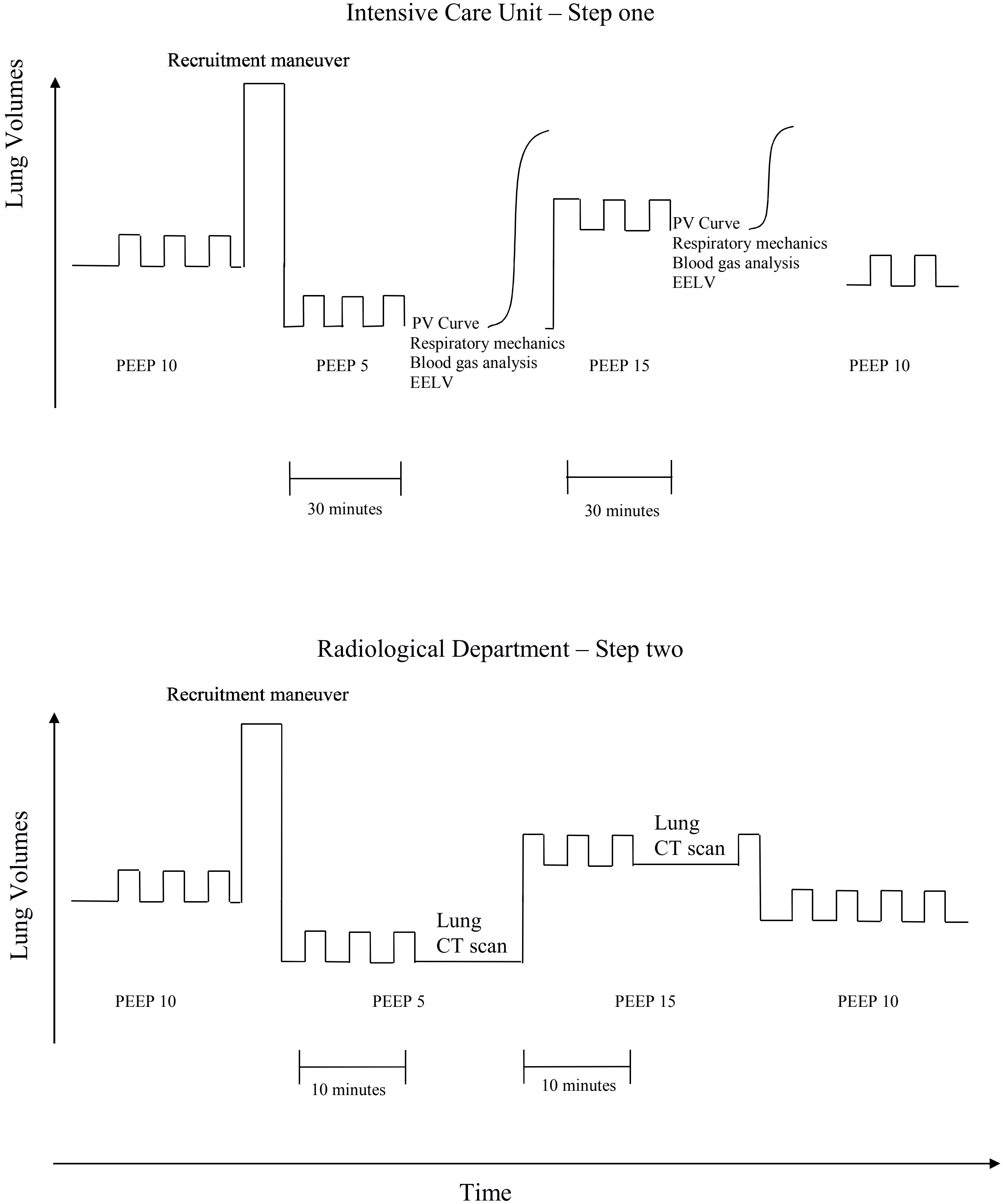
Figure E1: *Study Protocol*



This study was approved by the institutional review board of our hospital, and informed consent was obtained according to the Italian national regulations. Mechanically ventilated patients with ARDS (i.e., PaO_2/FiO_2 <300) admitted to the intensive care unit (ICU) that were scheduled for clinical reason to lung CT scan, were enrolled. Patients younger than 18 years, with hemodynamic instability and documented barotrauma were excluded. The study was initially conceived to classify patients according to Berlin definition of ARDS at the clinical PEEP, however we found that Berlin classification better reflects severity if assessed at standard PEEP of 5 cmH_2O (4, 5). Unfortunately this would have produced unbalanced groups (5 Mild, 15 Moderate and 2 Severe ARDS patients). We finally decided to classified the twenty-two ARDS patients enrolled according to tertiles of PaO_2/FiO_2 (at 5 cmH_2O PEEP) to obtain balanced groups of patients of increasing severity.

Table E1. *PaO_2/FiO_2 at standard PEEP of 5 cmH_2O distributions in the tertiles*

PaO_2/FiO_2 at 5 cmH_2O PEEP	**N**	**Median**	**Minimum**	**25° Percentile**	**75° Percentile**	**Maximum**
1st Tertile	7 (5 Mild/2 Moderate)	216.0	186.5	193.3	272.5	300.0
2nd Tertile	8 (8 Moderate)	160.7	146.4	152.1	169.0	177.3
3rd Tertile	7 (5 Moderate/2 Severe)	126.4	76.0	86.5	139.8	141.3

Study protocol and Measurements

Respiratory mechanics, EELV and dead space

The respiratory flow rate was measured with a heated pneumotachograph (Fleisch n°2, Fleisch). Volume was measured as integration of the flow. Airway pressure was measured proximally to the endotracheal tube with a dedicated pressure transducer (MPX 2010 DP. Motorola). Esophageal pressure was measured with a radio-opaque balloon (SmartCath Bicore) inflated with 1.0-1.5 mL of air connected to a pressure transducer and processed on a dedicated data acquisition system (Colligo

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and Computo, www.elekton.it). The esophageal balloon was positioned in the lower third of the esophagus. During an airway occlusion the concordant changes of airway and esophageal pressure were verified to check the correct position of the balloon (6). The static airway plateau pressure and esophageal pressure were measured at end inspiration/end expiration during a rapid airway occlusion (6). The static compliance of respiratory system, lung and chest wall were computed as previously reported (6).

The EELVs were measured at two levels of PEEP during an end expiratory pause with a simplified closed circuit helium dilution method. After ten manual breaths with an anesthesia bag, filled with 1.5 liters of 13% helium in oxygen, connected to the airway opening, the helium concentration was measured with a helium analyser (Pk Morgan Ltd, Chatam, UK). The EELV was computed using a standard formula (6).

The end tidal partial pressure of carbon dioxide and exhaled CO_2 in one minute were measured by means of continuous expiratory air sampling (CO_2SMO PLUS 8100; Novametrix Medical System). The physiologic dead space fraction (V_D/V_T) was computed according to the following formula: $V_D/V_T = (PaCO_2 - PeCO_2)/PaCO_2$, where $PeCO_2$ is the mixed expired carbon dioxide partial pressure.

Quantitative analysis of computed tomography

The CT scan measures the reduction of the radiation intensity upon passage through matter, which is called "linear attenuation coefficient" (μ) which, in turn, depends on the electron density of the tissue (7). It follows that it is possible to measure exactly the weight of a physical body with CT scan, as shown by Mull et al. in 1984 (8). For biological tissues there is a linear relationship between physical density, linear attenuation coefficient and CT numbers. To compute CT number (Hounsefield – HU units) relative linear attenuation coefficient (μ) is normalized for the reference material (water). This result is multiplyed by a magnifying constant (K) to get the CT number. Dense (compact) bone is assigned a CT number +K and air –K (8).

(1) $\text{CT number} = K([\mu - \mu_{\omega}]/\mu_{\omega})$

K is set equal to 1000

μ = attenuation number

μ_{ω} = water attenuation number

Although a number of CT number determination artifacts have been described the design features incorporated into modern CT scanners minimize some types of artifacts, and some can be partially corrected by the scanner software. It is reasonable to assume that, if CT scan images are properly acquired CT numbers correspond to tissue physical densities. Lung gas and tissue volumes were accurately estimated in foam lung models (9), ex-vivo lungs (10), frozen lungs (11) and surgically excised lobes (12). Protti et al. found a bias between methods of -9 g (-4%) and limits of agreement of −36 (-11%) and 17 g (3%) between the weight of excised lungs measured with a scale and with CT scan (13).

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We assumed (14) that lung parenchyma is composed by two compartments with very different density: air with a CT number of -1000 HU and "tissue" with a CT number of 0 HU. The tissue compartment includes lung tissue, blood and edema in ARDS patients. In each voxel gas fraction can be computed as:

(2) gas fraction = CT number/(-1000)

(3) tissue fraction = 1 – gas fraction

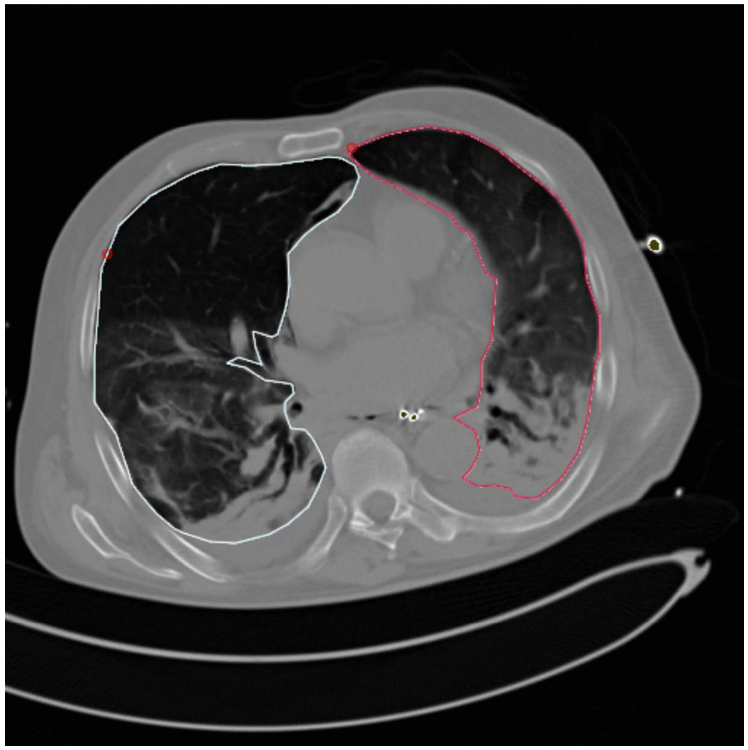
Multiplying gas fraction and tissue fraction for the voxel volume it is possible to compute gas volume (ml) and tissue volume (ml or grams as water density is equal to 1). Lung tissue volume is the sum of the tissue volumes of all voxels and gas volume is the sum of the gas volumes of all voxels.

Relationship between voxel size and size of anatomical lung structures

In the present manuscript voxels were reconstructed with a dimension of 0.73x0.73x5 mm with a voxel volume of 0.002625 mm^3. The discriminat power of the CT with this voxel per voxel analysis is remarkable. One voxel, in fact, may include from about 1/30 of a normal acinus inflated at total lung capacity to 4 to 5 whole acini completely collapsed (the dimensions of a normal acinus at FRC are between 16 to 22 mm^3 (15). As on acinus contains approximately 2000 alveoli at total lung inflation one voxel would include ~70 alveoli while in a completely degassed status up to 10000 alveoli may be included in a single voxel.

Lung profiles segmentation

Lung profiles were manually delineated following the internal profile of ribs excluding pleural effusion and main vessels/bronchi. Chiumello et al. tested the accuracy of manual segmentation of CT scan (16) in 12 ALI/ARDS patients finding that it was highly reproduciple (bias 2%).



Thresholds for tissue analysis

Voxels included in the lung profile are individually analyzed and classified according to their CT number (and gas /tissue ratio) as (14, 17, 18):

- CT > -100: not inflated tissue
- -100 > CT > -500: poorly inflated tissue
- -500 > CT > -900: well inflated tissue
- CT < -900: over inflated tissue

Table E2 presents the CT threshold used in ARDS literature.

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Table E2 - *CT threshold used in ARDS literature*

First Author	Year	Reference	Hounsfield Units Threshold				Rationale or Citations
			Not Aerated	Poorly Aerated	Normally Aerated	Hyperinflated	
Gattinoni L	1986	*Intensive Care Med* 12:137-142	+100 / -400	-	-400 / -1000	-	
Gattinoni L	1987	*Am Rev Respir Dis* 136:730-736	+100 / -100	-100 / -500	-500 / -900	-	
Gattinoni L	1988	*Anesthesiology* 69:824-832	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	
Lindberg P	1992	*Acta Anaest Scand* 36:546-553	+100 / -100	-	-	-	Study on postoperative atelectasis
Hachenberg T	1993	*Acta Anaest Scand* 37:549-555	-	-	-700 / -900	-	Study on postoperative atelectasis. Normal CT number range in the lung fields
Lundwigs U	1994	*Chest* 106:925-931	+100 / -100	-100 / -200	-	-	
Lundquist H	1995	*Acta Radiol* 36:626-632	+100 / -100	-	-	-	Suggested threshold for atelectasis
Lundwigs U	1995	*Chest* 108:804-809	+100 / -100	-100 / -200	-	-	
Rothen HU	1995	*The Lancet* 345:1387-1391	+100 / -100	-	-	-	Study on postoperative atelectasis
Rothen HU	1995	*Acta Anaest Scand* 39:118-125	+100 / -100	-	-	-	Study on postoperative atelectasis
Rothen HU	1996	*Acta Anaest Scand* 40:524-529	+100 / -100	-	-	-	Study on atelectasis during anesthesia
Reber A	1996	*Br J Anaesth* 76:760-766	+100 / -100	Poorly: -100 / -200 Reduced: -200 / -500	-500 / -900	-900 / -1000	Gattinoni L 1988: Ludquist H 1995
Umamaheswara Rao GS	1997	*Anesthesiology* 87:823-824	+100 / -100	-100 / -500	-500 / -1000	-	Gattinoni L 1986-1987-1988
Dambrosio M	1997	*Anesthesiology* 87:495-503	+100 / -150	-150 / -800		-800 / -1000	Arbitrary thresholds. Densities around zero are absent in normal lungs and indicates atelectasis or lung condensation. Densities -800 or lower correlate with hyperinflation or emphysema
Vieira SRR	1998	*Am J Respir Crit Care Med* 158:1571-1577	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 1987
Puybasset L	1998	*Am J Respir Crit Care Med* 158:1644-1655	+100 / -100	-100 / -500	-500 / -1000	-	Gattinoni L 1987
Rothen A	1998	*BJA* 81:681-686	+100 / -100	-	-	-	Study on atelectasis during anesthesia
Ludquist H	1998	*Acta Anaest Scand* 43:295-301	+100 / -100	-	-	-900 / -1000	Ludquist H 1995;
Reber A	1998	*Anaesthesia* 53:1054-1061	+100 / -100	-	-	-	Study on atelectasis during anesthesia
Neumann P	1998	*Am J Respir Crit Care Med* 158:1636-1643	+100 / -100	-	-	-	Gattinoni L 1988; Lundquist H 1995
Neumann P	1998	*J Appl Physiol* 85:1533-1543	+100 / -100	+100 / -500	-	-	Gattinoni L 1988; Lundquist H 1995 Poorly aerated tissue = lung parenchyma with aeration ≤ 50%
Tenling A	1998	*Anesthesiology* 89:371-378	+100 / -100	-	-	-	Ludquist H 1995 Study on atelectasis after cardiac surgery;
Vieira SRR	1999	*Am J Respir Crit Care Med* 159:1612-1623	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 1987; Vieira SRR 1998
Rothen HU	1999	*Br J Anaesth* 82:551-556	+100 / -100	-	-	-	Lundquist 1995 Study on atelectasis during anesthesia.
Puybasset L	2000	*Intensive Care Med* 26:857-869	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 1987; Vieira SRR 1998-1999
Puybasset L	2000	*Intensive Care Med* 26:1215-1227	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 1987; Vieira SRR 1998-1999
Neumann P	2000	*Am J Respir Crit Care Med* 161:1537-1545	+100 / -100	-100 / -500	-	-	Gattinoni L 1988; Lunquist H 1995
Malbuisson LM	2001	*Am J Respir Crit Care Med* 163:1444-1450	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Vieira SRR 1998
Gattinoni L	2001	*Am J Respir Crit Care Med* 164:1701-1711	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 1986-1987-1988; Umamaheswara Rao GS 1997; Dambrosio M 1997; Vieira SRR 1998; Puybasset L 1998
Lu Q	2001	*Intensive Care Med* 27:1504-1510	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Vieira SSR 1998
Bugedo G	2003	*Intensive Care Med* 29:218-225	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 1988; Vieira SRR 1998; Puybasset L 1998

Luecke T	2003	*Intensive Care Med* 29:2026-2033	+300 / -100	-100 / -500	-500 / -900	-900 / -1000	Vieira SRR 1998
Rouby JJ	2003	*Crit Care Med* 31 (4S): S285-S295	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Puybasset 2000
Rouby JJ	2003	*Eur Respir J Suppl* 43:26s-37s	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Puybasset 2000
Rusca M	2003	*Anesth Analg* 97:1835-1839	+100 / -100	-	-	-	Study on atelectasis during anesthesia
Wrigge H	2003	*Anesthesiology* 99:376-384	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 1988; Lundquist H 1995; Vieira SRR 1998
Luecke T	2003	*Anesthesiology* 99:1313-1322	+300 / -100	-100 / -500	-500 / -900	-900 / -1000	Vieira SRR 1999
Rylander C	2004	*Acta Anaest Scand* 48:1123-1129	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 1988; Vieira SRR 1998
Quintel M	2004	*Am J Respir Crit Care Med* 169:534-541	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 1987
Rylander C	2004	*Anesth Analg* 98:782-789	+100 / -100	Aerated: -100 / -1000			Gattinoni L 1988. This study considers only the difference between collapsed and aerated lung tissue.
Roth H	2004	*Acta Anaest Scand* 48:851-861	+300 / -100	-100 / -500	-500 / -900	-900 / -1000	Vieira SRR 1998
Albaiceta GM	2004	*Am J Respir Crit Care Med* 170:1066-1072	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	
Schreiter D	2004	*Crit Care Med* 32:968-975	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 2001
Nietzowska A	2004	*Crit Care Med* 32:1496-1593	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	
Luecke T	2004	*Acta Anaesthesiol Scand* 48:82-92	+300 / -100	-100 / -500	-500 / -900	-900 / -1000	
Grasso S	2004	*Crit Care Med* 32:1018-1027	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Neumann P 1998
Vieira SSR	2005	*Crit Care Med* 33:741-749	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Vieira SRR 1998-1999
Wrigge H	2005	*Crit Care* 9:R780-R789	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 1988; Lundquist H 1995; Vieira SRR 1998
Westerdahl E	2005	*Chest* 128:3482-2488	+100 / -100	Aerated: -100 / -1000			Gattinoni L 1988; Lunquist H 1995 Study on atelectasis after cardiac surgery
Zinserling J	2005	*Chest* 128:2963-2970	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Lundquist H 1995; Vieira SRR 1998
Gattinoni L	2006	*N Eng J Med* 354:1775-1786	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	
Karmrodt J	2006	*Br J Anaesth* 97:883-895	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 1988; Vieira SRR 1998
Borges JB	2006	*Am J Respir Crit Care Med* 174:268-278	+100 / -100	-100 / -500	-500 / -850	-850 / -1000	Gattinoni L 1987-1988; Dambrosio L 1997; A higher-than-usual threshold between normally aerated and hyperinflated compartments was intentionally chosen to increase sensitivity for detection of hyperinflated areas
Lu Q	2006	*Crit Care* 10:R95	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Malbuisson LM 2001
Henzler D	2006	*Eur Radiol* 16:1351-1359	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Puybasset L 2000
Galiatsu E	2006	*Am J Respir Crit Care Med* 174:187-197	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 1988
Carvalho AR	2006	*Crit Care* 10:R122	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Vieria SRR 1998; Gattinoni L 2001
Tusman G	2006	*Intensive Care Med* 32:1863-1871	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 2001
Luecke T	2006	*Chest* 130:392-401	+300 / -100	-100 / -500	-500 / -900	-900 / -1000	Luecke T 2003
Kyeongman J	2007	*J Korean Med Sci* 22:476-483	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Puybasset L 2000
Terragni PP	2007	*Am J Respir Crit Care Med* 175:160-166	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 2001; Rouby JJ 2003
Henzler D	2007	*Anaest Analg* 105:1072-1078	+100 / -100	-100 / -900		-900 / -1000	Henzler D 2006; in this study they don't differentiate between normal and poorly aerated lung to reduce statistical procedures
Wrigge H	2008	*Crit Care Med* 36:903-909	+100 / -100	-	-	-	Gattinoni L 1988; Lundquist H 1995; Vieira SRR 1998
Reske AW	2008	*Intensive Care Med* 34:2044-2053	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Vieira SRR 1998; Malbuisson L 2001; Gattinoni L 2001-2006
Dellacà RL	2009	*Intensive Care Med* 35:2164-2172	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Vieira SRR 1998
Grasso S	2009	*Am J Respir Crit Care Med* 180:415-423	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Puybasset L 2000

AJRCCM Articles in Press. Published on 23-December-2015 as 10.1164/rccm.201507-1413OC

Reinius H	2009	*Anesthesiology* 111:979-987	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	
Fernandez-Bustamante A	2009	*Crit Care Med* 37:2402-2411	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 1987; Vieira SRR 1998
Kozian A	2009	*Br J Anaesth* 102:551-560	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	
Stang CM	2009	*Br J Anaesth* 103:298-303	+100 / -100	-100 / -500	-500 / -850	-850 / -1000	
Reinius H	2009	*Anesthesiology* 111:979-987	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Study on atelecatasis during anesthesia
Bellani G	2009	*Crit Care Med* 37:2216-2222	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	
Constantin JM	2010	*Crit Care Med* 38:1108-1117	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Mlabuisson LM 2001
Lu Q	2010	*Crit Care* 14:R135	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Malbuisson LM 2001
Reske AW	2010	*Intensive Care Med* 36:1836-1844	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Puybasset L 2000; Gattinoni L 2001
Bruhn A	2011	*Minerva Anestesiol* 77:418-426	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L NEJM 2006
Graham MR	2011	*Can J Anaesth* 58:740-750	> -100	-100 / -500	-500 / -900	-900 / -1000	Vieira SRR 1998
Ruth Graham M	2011	*Crit Care Med* 39:1721-1730	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 2001
Kozian A	2011	*Anesthesiology* 114:1025-1035	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	
Hanson A	2011	*Pediatr Crit care Med* 12:e362-e368	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Vieira SRR 1998
Rodrigues RR	2011	*Braz J Med Biol Res* 44:598-605	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Vieira SRR 1998; Puybasset L 1998
Vena A	2011	*Intensive Care Med* 37:1378-1383	+100 / -100	-100 / -500	-	-	Gattinoni L 1988
Edmark L	2011	*Acta Anaesthesiol Scand* 55:75-81	+100 / -100	-	-	-	Lundquist H 1995; Study on atelectasis during anesthesia
Hanson A	2011	*Pediatr Crit Care Med* 12:e362-e368	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Vieira SRR 1998
Dellacà RL	2011	*Intensive Care Med* 37:1021-1030	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Vieira SRR 1998; Gattinoni L 2001
Reske AW	2011	*Crit Care* 15:R71	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 2001-2006
Reske AW	2011	*Crit Care* 15:R279	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 2006; Borges JB 2006
Mentzelopoulos SD	2011	*Intensive care Med* 37:990-999	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 2006
Bellani G	2011	*Am J Respir Crit Care Med* 183:1193-1199	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 2001
Muders T	2012	*Crit Care Med* 40:903-911	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 2001
Hanson A	2012	*Paediatr Anaesth* 2:172-179	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Vieira SRR 1998
Zannin E	2012	*Crit Care* 16:R127	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Vieira SRR 1998; Gattinoni L 2001
de Matos GF	2012	*Crit Care* 16:R4	+100 / -100	-	-	-	Borges JB 2006; Study on lung recruitability
Cereda M	2013	*Crit Care Med* 41:527-535	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 1987; Vieira SRR 1998
Bayat S	2013	*Anesthesiology* 119:89-100	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Vieira SRR 1998
Neves FH	2013	*PloS One* 8:e78643	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 1987; Vieira SRR 1998; Puybasset L 1998
Derosa S	2013	*J Appl Physiol* 115:1464-1473	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Borges JB 2006
Edmark L	2014	*Acta Anaesthesiol Scand* 58:681-688	+100 / -100	-	-	-	Study on postoperative atelectasis
Edmark L	2014	*Ups J Med Sci* 119:242-250	+100 / -100	-	-	-	Study on postoperative atelectasis
Zhang F	2014	*BioMed Eng Online* 13:30	> -100	-100 / -500	-500 / -900	< -900	Puybasset L 2000; Malbuisson LM 2001
Yang Y	2014	*Mol Biol Rep* 41:1325-1333	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 2001
Perchiazzi G	2014	*Respir Physiol Neurobiol* 201:60-70	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 1988; Vieira SRR 1998
Borges JB	2014	*Crit Care Med* 4s:e279-e287	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Borges JB 2006
Borges JB	2015	*Crit Care Med* 43:e123-e132	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 2001
Borges JB	2015	*Crit Care Med* 43:e404-e411	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Gattinoni L 2001
Wolf SJ	2015	*PloS One* 10:e0135272	+100 / -100	-100 / -500	-500 / -900	-900 / -1000	Lundquist H 1995; Reske AW 2011. "The use of the extended HU-window of -200 to +100 HU for definition of atelectasis in CT did not consistently improve the strength of correlation with PaO2 or lnPaO2"

Assessment of lung recruitment

Lung recruitment had been defined as the lung parenchyma, which is degassed at lower PEEP and regains inflation at higher PEEP. To define not inflated tissue a threshold of -100 HU is usually selected (19) including also voxels with a small gas fraction (less than 10%) to take in account gas trapped in the lung parenchyma after the closure of small airways. Lung recruitment can be expressed as absolute value (g) and as percentage of total lung tissue weight PEEP 5 cmH2O:

(4) lung recruitment (grams) = not inflated tissue (g) $_{\text{lower pressure}}$ - not inflated tissue (g) $_{\text{higher pressure}}$

(5) lung recruitment (fraction of lung parenchyma) = (not inflated tissue (g) $_{\text{lower pressure}}$ - not inflated tissue (g) $_{\text{higher pressure}}$)/total lung tissue $_{\text{lower pressure}}$(g)

Rouby method (20)

Rouby et al defined recruitment as the gas entering in the not inflated (CT up to -100) and poorly inflated (-100 > CT > -500) lung tissue. The lung parenchyma is manually partitioned in 2 compartments: a well inflated and a not inflated/poorly inflated. Rouby et al. manually delineated, on the CT scan performed at ZEEP, the anatomical lung region corresponding to the not inflated and poorly inflated lung tissue. The lung region was identified on the CT scan performed at higher PEEP using anatomical landmarks like pulmonary vessels or segmental bronchi and its content computed. Recruitment was defined as the gas volume entering in that anatomically determined lung region.

This analysis cannot be performed separately on poorly and non aerated lung regions because anatomical landmarks are not visible in the absence of pulmonary aeration. Poorly and non inflated lung regions can not be separated.

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Estimation of lung recruitment by Pressure-Volume curve (P-Vrs curve method)

The PV curves were obtained at each PEEP level without disconnecting the ventilator, with a low flow insufflation (6-8 L/min) (21) using a Servo I mechanical ventilator in volume controlled mode to reach an inspiratory airway pressure between 30-35 cmH_2O.

The PV curves were traced starting from the two PEEP levels and from the corresponding EELV (8). The PV curve of respiratory system were obtained from the plot of volume against airway (9, 10). Data pairs of airway pressure and volume of the PV curve at 5 and 15 cmH_2O of PEEP were fitted to a sigmoid model as proposed by Venegas et al. (11). Lung recruitment is computed as the vertical difference between the two curves at 15 cmH_2O (12–14). Lung recruitment was expressed as absolute value (mL) and as percentage of EELV at PEEP 5 cmH_2O.

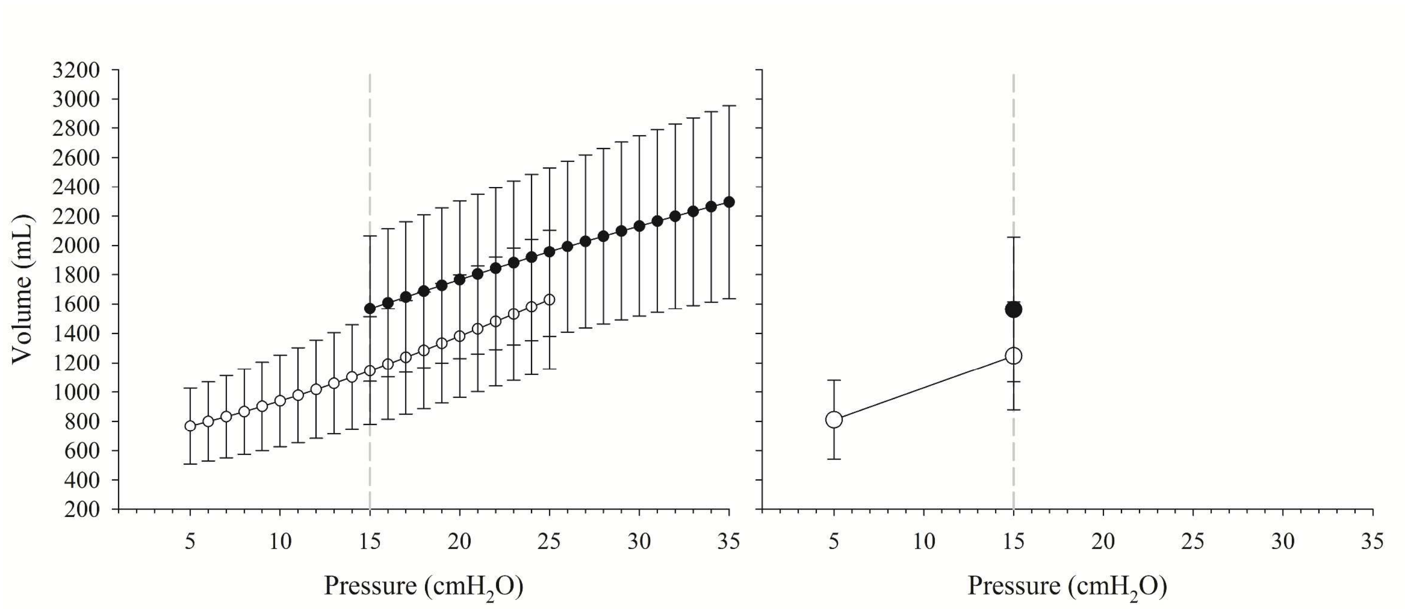
Estimation of lung recruitment by EELV and static compliance of respiratory system

The lung recruitment was estimated as the difference between the EELV measured at 15 cmH_2O and the predicted volume due to the 10 cmH_2O pressure increase from 5 cmH_2O PEEP (8, 15, 16), according to this equation:

$Gas = EELV_{PEEP\ 15\ cmH2O} – (EELV_{PEEP\ 5\ cmH2O} + (Cst,rs_{\ PEEP\ 5\ cmH2O} * 10\ cmH_2O))$

Lung recruitment was expressed as absolute value (mL) and as percentage of EELV at PEEP 5 cmH_2O.

Figure E2:



LEFT PANEL: the PV curve of each patient was fit according to Venegas (21) and volumes were computed from equations at 1 cmH_2O intervals. Figure show the average PV curves at 5 cmH_2O (open circles) and 15 cmH_2O (filled circles). Bars represent standard deviations. The vertical dashed line indicates 15 cmH_2O pressure where the recruitment was computed.

RIGHT PANEL: Figure shows, as mean and standard deviation, the EELV measured at 5 cmH_2O (open circle on the left), the expected volume at 15 cmH_2O computed as

$$Gas = EELV_{PEEP\ 15\ cmH2O} - (EELV_{PEEP\ 5\ cmH2O} + (Cst,rs_{\ PEEP\ 5\ cmH2O} * 10\ cmH_2O))$$

(open circle on the right) and the measured EELV at PEEP 15 cmH_2O (filled circle). The vertical dashed line indicates 15 cmH_2O pressure where the recruitment was computed.

Note that, for clarity, the scales of the two panels are different.

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Table E3. *Baseline characteristics of the Study Population - patients classified according to Berlin definition of ARDS at 5 cmH_2O*

Characteristics	Overall population (N=22)	Mild ARDS (N=5)	Moderate ARDS (N=15)	Severe ARDS (N=2)	P value
Age (years)	67.5 ± 11.7	60.2 ± 15.6	70.0 ± 10.4	69.0 ± 7.1	0.303
Male sex, *n* (%)	15 (68%)	2 (40%)	11 (73%)	2 (100%)	0.229
Body Mass Index (kg/m^2)	27.4 ± 7.1	23.0 ± 3.9	29.2 ± 7.7	24.5 ± 2.3	0.212
Tidal volume/ Actual body weight (mL/kg)	8.1 [7.0-9.8]	7.7 [6.8-9.1]	8.3 [7.7-9.8]	6.0 [5.6-6.3]	0.049
Respiratory rate (bpm)	12.5 [12.0-15.0]	12.0 [11.0-20.5]	12.0 [12.0-15.0]	17.0 [14.0-20.0]	0.352
Minute ventilation (L/min)	7.1 ± 1.4	7.5 ± 2.3	7.0 ± 1.2	7.2 ± 1.2	0.852
PEEP (cmH_2O)	10.0 [10.0-12.0]	7 [5.0-10.0] 3*	10.0 [10.0-12.0]	13.5 [12.0-15.0]	0.006
Static compliance of respiratory system (mL/cmH_2O)	43.8 ± 17.9	48.5 ± 17.3	43.1 ± 19.3	37.2 ± 10.2	0.568
Intensive care mortality, *n* (%)	13 (59%)	4 (80%)	7 (47%)	2 (100%)	0.197
Causes of ARDS:					
• Pneumonia	13	2	9	2	0.663
• Sepsis	4	2	2	0	
• Aspiration	3	1	2	0	
• Other	2	0	2	0	
PaO_2/FiO_2 ratio	195 ± 37	223 ± 14*	194 ± 35*	130.9 ± 6	0.007
$PaCO_2$ (mmHg)	40.2 [36.1-44.5]	34.5 [31.6-39.2]*	41.0 [38.8-46.0]	56.6 [44.3-68.9]	0.024

Data are expressed as mean ± SD or median [IQ range] as appropriate. ARDS acute respiratory distress syndrome, PEEP positive end-expiratory pressure, $PaCO_2$ partial pressure of carbon dioxide oxygen, PaO_2 partial pressure of oxygen, FiO_2 inspired oxygen fraction. P values: One Way Analysis of Variance or Kruskal-Wallis One Way Analysis of Variance on Ranks.

* P<0.05 vs severe

Table E4. *Gas exchange, partitioned respiratory mechanics and CT scan variables*

Characteristics	PEEP	Overall population (N=22)	Mild ARDS (N=5)	Moderate ARDS (N=15)	Severe ARDS (N=2)	P value Group	P value PEEP
PaO_2(mmHg):							
	5 cmH_2O	81 ± 17	106 ± 10	75 ± 10	63±14	0.020	<0.001
	15 cmH_2O	112 ± 28	128 ± 24	107 ± 29	111±22		
PaO_2/FiO_2:							
	5 cmH_2O	170 ± 54	248 ± 38	155 ± 21	81±7	<0.001*†‡	<0.001
	15 cmH_2O	231 ± 67	300 ± 59	220 ± 37	142±9		
SvO_2 (%):							
	5 cmH_2O	77.6 [69.4-79.4]	79.2 [78.6-79.6]	77.2 [69.4-79.4]	74.3 [69.4-79.1]	0.796	0.012
	15 cmH_2O	79.5 [72.9-83.6]	78.7 [76.1-80.2]	79.0 [69.8-83.6]	83.3 [80.0-86.5]		
Venous admixture (%):							
	5 cmH_2O	37.3±14.7	25.0±7.6	39.6±14.8	50.9±4.7	0.054	<0.001
	15 cmH_2O	27.2±7.8	21.1±4.0	28.3±8.1	34.2±0.2		
$D_{AV}O_2$ (mL/100 cc):							
	5 cmH_2O	2.7 [2.1-3.3]	2.8 [2.6-2.9]	2.8 [2.1-4.0]	2.0 [1.7-2.3]	0.342	0.306
	15 cmH_2O	2.4 [2.0-3.1]	2.5 [2.2-3.1]	2.4 [1.9-3.2]	2.0 [1.8-2.2]		
$PaCO_2$ (mmHg):							
	5 cmH_2O	46 ± 10	42 ± 7	45 ± 8	63±15	0.029*†	0.011
	15 cmH_2O	48 ± 11	45 ± 9	46 ± 10	65±15		
Dead Space (Vd/Vt) (%):							
	5 cmH_2O	60 ± 11	58 ± 10	58 ± 10	77±8	0.095	0.004
	15 cmH_2O	63 ± 11	63 ± 11	60 ± 11	78±6		

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Static Compliance of Respiratory System (Cst,rs) (mL/cmH_2O):							
	5 cmH_2O	43.7±13.7	47.4 ± 16.9	44.6 ± 11.7	27.5±16.4	0.307	0.098
	15 cmH_2O	38.9 ± 15.0	36.9 ± 16.4	41.3 ± 14.9	26.7±11.1		
Static Compliance of the Lung (Cst,L) (mL/cmH_2O):							
	5 cmH_2O	57.8 ±20.6	60.0 ± 22.0	60.5 ± 18.9	32.0±19.4	0.212	0.267
	15 cmH_2O	50.0 ± 19.9	49.0 ± 22.6	52.7 ± 19.5	32.5±13.3		
Static Compliance of the Chest Wall (Cst,cw) (mL/cmH_2O):							
	5 cmH_2O	201 [123-251]	189 [172-196]§	215 [118-251]	195 [121-269]§	0.678	0.025
	15 cmH_2O	186 [123-242]	129 [111-175]	203 [135-273]	149 [102-197]		
End Expiratory Lung Volume (EELV)(mL):							
	5 cmH_2O	811 ± 269	887 ± 218	811 ± 297	625±35	0.510	<0.001
	15 cmH_2O	1563 ± 493	1756 ± 577	1533 ± 488	1301±317		
Lung weight (g)							
	5 cmH_2O	1378±432	1107±114	1348±344	2283±450	<0.001*†	0.026
	15 cmH_2O	1426±451	1146±165	1393±331	2378±645		
Not inflated tissue (g, (%)) (p interaction = 0.002)							
	5 cmH_2O	656±463 (44±16%)	371±71 (34±7%)	606±324 (43±14%)§	1745±451 (76±5%)§	<0.001*†	<0.001
	15 cmH_2O	579±411 (37±15%)	324±93 (28±8%)	543±310 (37±15%)	1486±432 (62±1%)		
Poorly inflated tissue (g, (%)) (p interaction <0.001)							
	5 cmH_2O	396±145 (30±11%)	342±113 (31±10%)	410±195 (31±11%)	426±108 (20±9%)§	0.311	0.040
	15 cmH_2O	392±182 (28±10%)	289±55 (25±4%)	399±195 (29±12%)	603±83 (27±11%)		
Well inflated tissue (g, (%))							
	5 cmH_2O	322±139 (26±12%)	390±128 (35±9%)	328±124 (26±10%)	112±107 (5±4%)	0.098	<0.001
	15 cmH_2O	444±156 (34±15%)	525±98 (46±4%)	438±147 (33±12%)	289±296 (11±10%)		

Over inflated tissue (g, (%))							
	5 cmH_2O	4±9 (0.3±0.7%)	4.68± 6.67 (0.4±0.6%)	4.73±10.38 (0.3±0.8%)	0.02±0.03 (0.0±0.0)	0.719	0.288
	15 cmH_2O	10±17 (0.7±1.3%)	8.91±17.95 (0.7±1.4%)	11.57±18.66 (0.8±1.4%)	0.12±0.16 (0.0±0.0)		

PEEP positive end-expiratory pressure,$PaCO_2$ partial pressure of carbon dioxide oxygen, PaO_2 partial pressure of oxygen, FiO_2 inspired oxygen fraction, SvO_2 venous oxygen saturation, $D_{AV}O_2$ arteriovenous oxygen difference. Two Way Repeated Measures ANOVA (one factor repetition) or Two Way Repeated Measures ANOVA (one factor repetition) on ranks was used to compare the physiological values obtained among the groups and within each PEEP applied. Interaction was reported only when significant.

*P<0.05 mild vs severe; †P<0.05 moderate vs severe; ‡P<0.05 mild vs moderate; §P<0.05 PEEP 5 cmH_2O vs. PEEP 15 cmH_2O

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Table E5. *Comparison between different methods in assessing lung recruitment*

	Methods	Overall population (N=22)	Mild ARDS (N=5)	Moderate ARDS (N=15)	Severe ARDS (N=2)	P value Severity	P value Interaction
Respiratory Mechanics (gas)	**P-Vrs curve, mL (%)**	423±223 (54±28%)	464±293 (51±27%)	402±212 (52±28%)	481±220 (78±40%)	0.642 (0.373)	0.173 (0.100)
	EELV-Cst,rs, mL (%)	315±201 (39±25%)	395±282 (44±27%)	277±174 (34±23%)	401±189 (65±34%)		
P value Methods		<0.001 (<0.001)					
Tissue recruited (CT scan)	**CT (not inflated), g (%)**	77±86 (5±5%)	47±73* (4±7%)†	63±66* (5±5%)	259±20† (11±1%)†	0.107 (0.671)	<0.001 (0.013)
	CT (not+poorly inflated), g (%)	80±67 (6±6%)	100±103 (9±10%)	73±60 (6±4%)	83±6 (4±1%)		
P value Methods		0.040 (0.651)					
Gas associated to recruited tissue (CT scan)	**CT (not inflated gas), mL (%)**	129±148 (16±20%)	115±186 (10±15%)*†	117±135 (11±11%)*	254±175† (65±14%)†	0.966 (0.007)	0.018 (<0.001)
	CT (not + poorly inflated), g (%)	163±165 (16±13%)	194±201 (22±22%)	165±166 (13±10%)	78±44 (21±1%)		
P value Methods		0.578 (0.004)					

Data as absolute values, as percentage of total lung volume (EELV for Respiratory Mechanics derived variables and Total Gas from CT scan for Gas recruited by CT scan) and lung weight (for tissue) (%) are express as mean ± SD. ARDS, acute respiratory distress syndrome; P-Vrs curve, pressure-volume curve of the respiratory system; EELV, end-expiratory lung volume; Cst,rs, static compliance of the respiratory system; CT, computed tomography. Two Way Repeated Measures

ANOVA was used to compare the physiological values obtained with different methods and among the classes of the Berlin definition. Bonferroni's t-test was used as post-hoc analysis.

* $P<0.05$ vs severe; † $P<0.05$ vs second method

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Table E6: *Applying the threshold up to -300 did not change the overall results*

	Methods	Overall population (N=22)	1st Tertile (N=7)	2nd Tertile (N=8)	3rd Tertile (N=7)
PaO_2/FiO_2 at 5 cmH_2O PEEP		161 [140-193]	216 [193-273]	161 [152-169]	126 [86-140]
CT scan (tissue)	**CT (not inflated -100), g (%)**	77±86 (5±5%)	49±77 (4±7%)	69±61 (6±5%)	114±115 (5±5%)
	CT (not inflated -200), g (%)	86±85 (6±6%)	64±85 (5±7%)	83±81 (7±7%)	109±96 (5±4%)
	CT (not inflated -300), g (%)	87±78 (6±6%)	78±89 (7±8%)	88±82 (7±7%)	93±72 (5±3%)
	CT (not inflated -400), g (%)	83±68 (6±6%)	92±91 (8±8%)	85±67 (7±6%)	71±51 (4±2%)
	CT (not+poorly inflated), g (%)	80±67 (6±6%)	108±95 (9±9%)	82±51 (7±4%)	50±43 (3±2%)

Figure E3: *relationships between recruitment measured at -100, -200 and -300 HU thresholds*

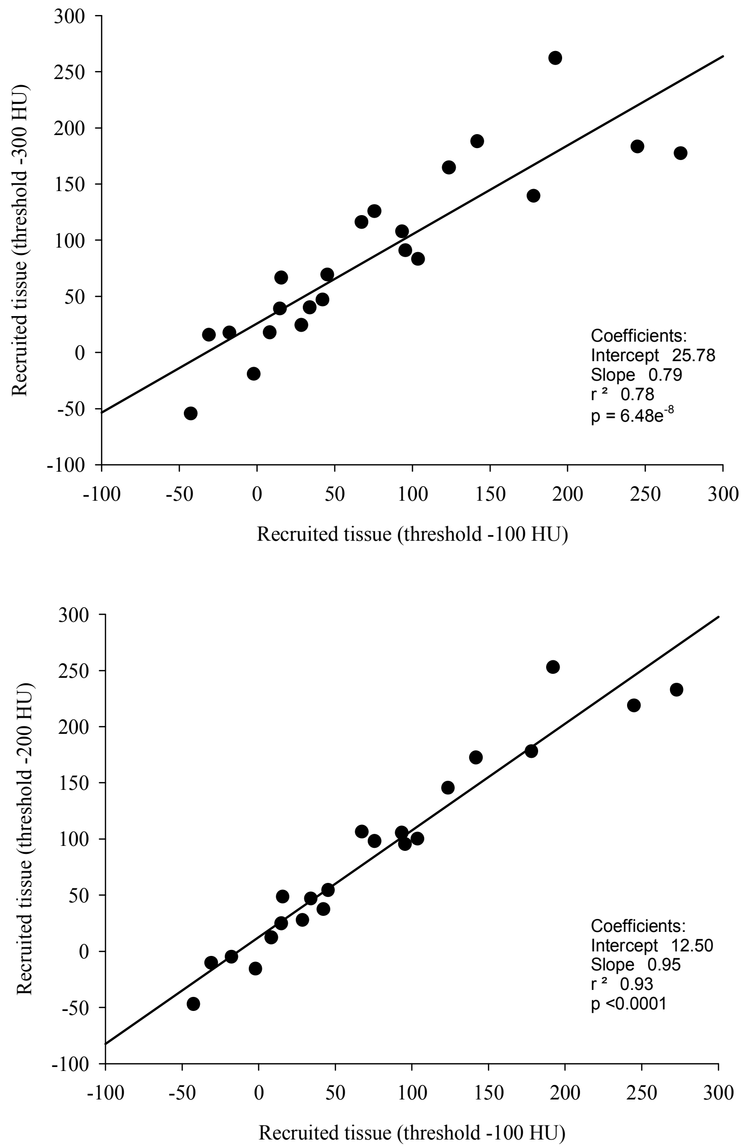

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Figure E4: *Recruitment computed with CT threshold of -200 (grams) and recruitment computed from multiple PV curves (ml)*

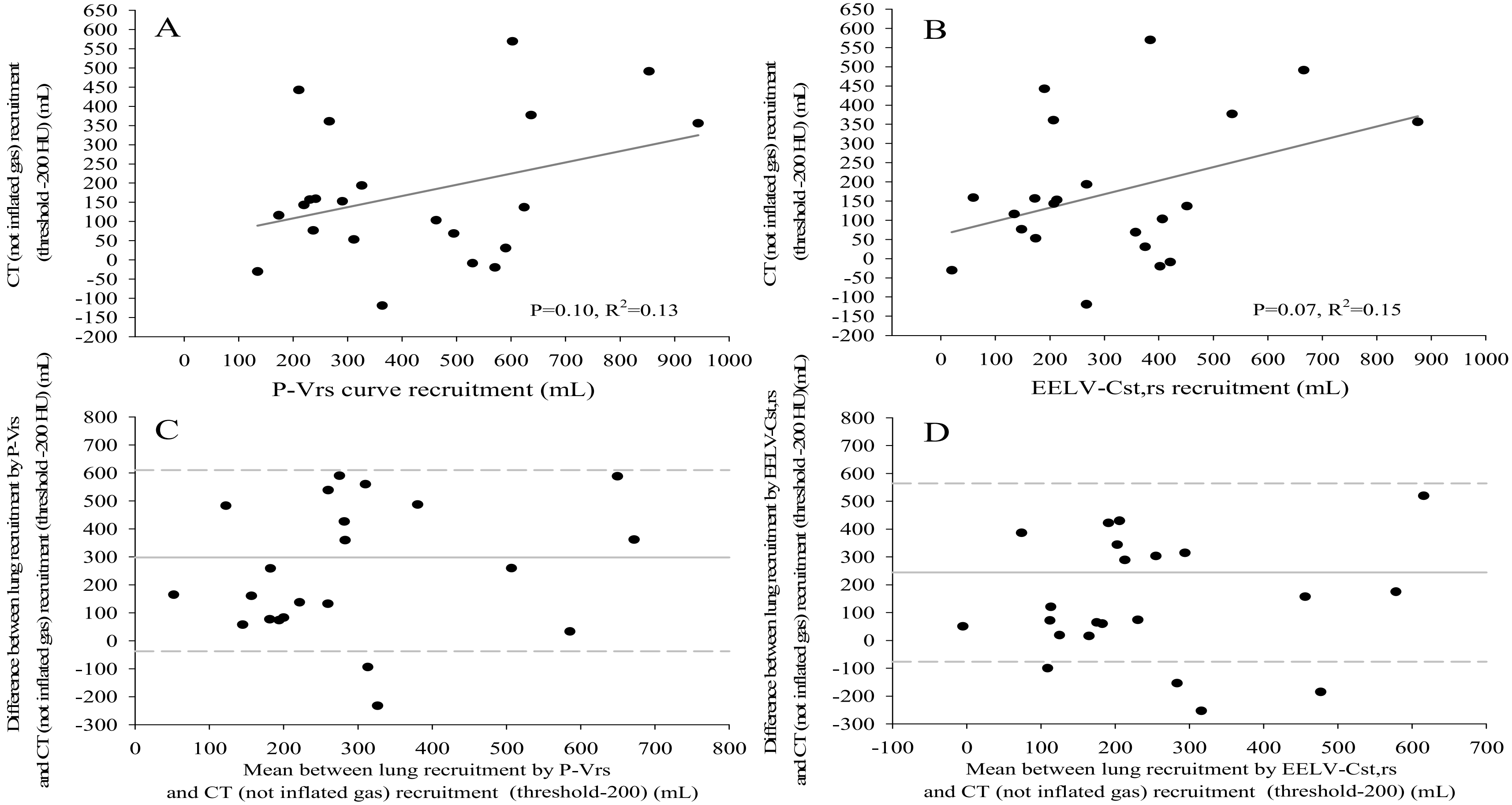

Figure E5: *Recruitment computed with CT threshold of -300 (grams) and recruitment computed from multiple PV curves (ml)*

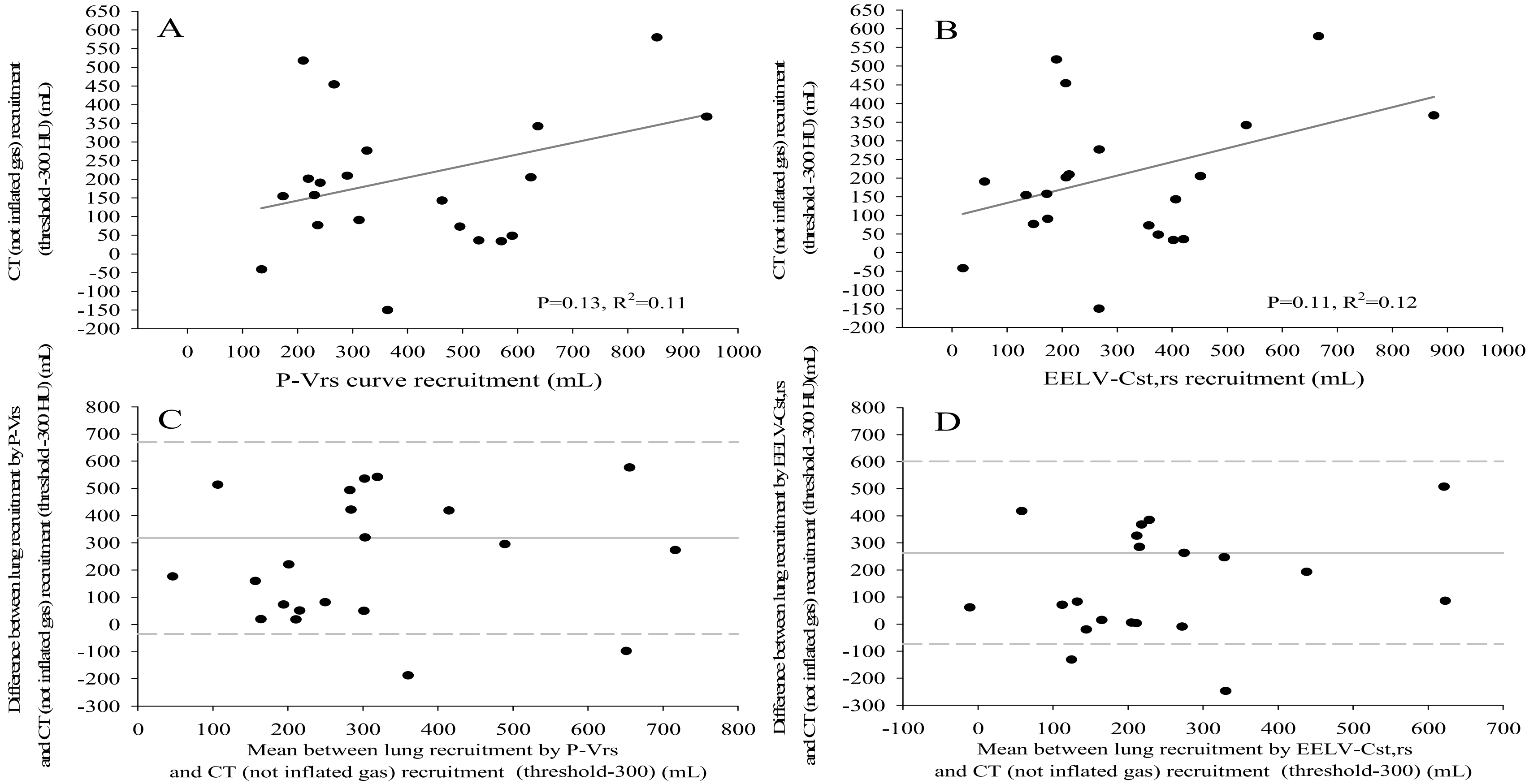

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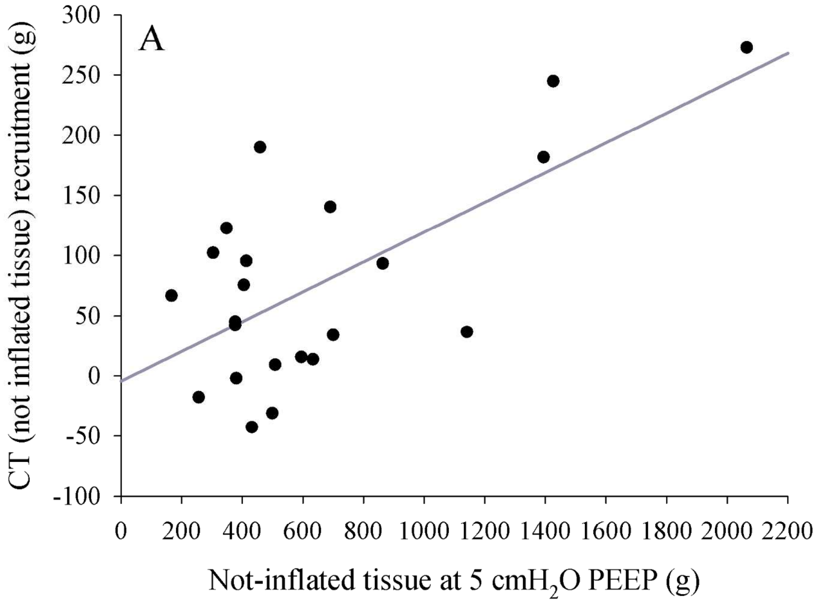
Figure E6. *CT scan-based recruitment (not-inflated tissue) and severity*



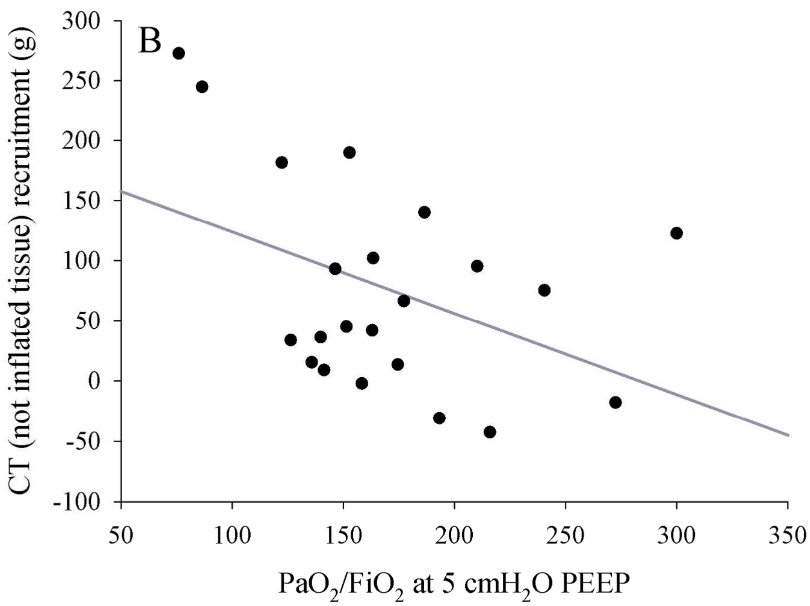
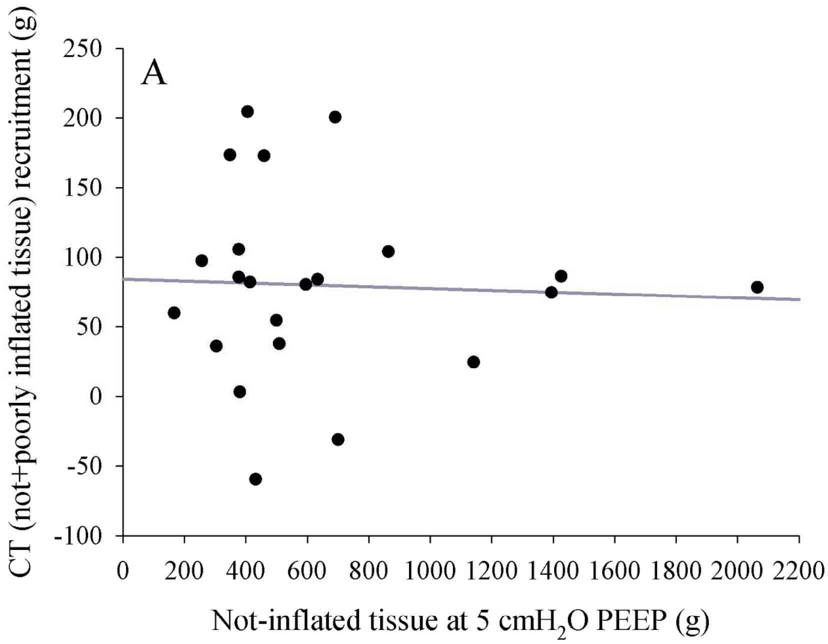


Figure shows the relationships between CT scan-based recruitment, computed by not-inflated tissue only, as a function of severity as expressed by not-inflated tissue at 5 cmH_2O (panel A) and PaO_2/FiO_2 at 5 cmH_2O (panel B).

Recruitment = -4.47 + 0.12 × Not-inflated tissue, P=0.05, R^2=0.18

Recruitment = 191.6 - 0.68 × PaO_2/FiO_2, P<0.001, R^2=0.44

Figure E7. *CT scan-based recruitment (not+poorly-inflated tissue) and severity*



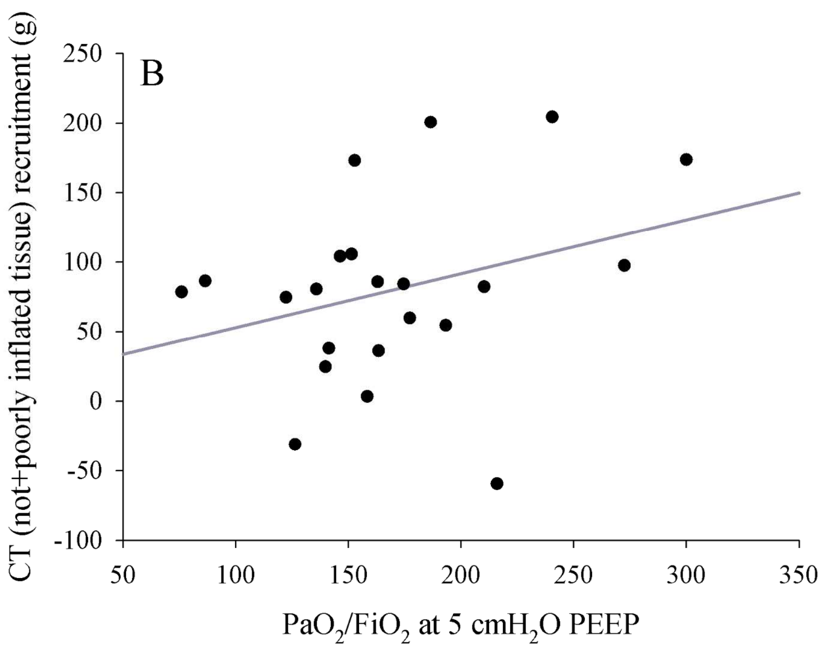


Figure shows the relationships between CT scan-based recruitment, computed by not + poorly-inflated tissue, as a function of severity as expressed by not-inflated tissue at 5 cmH_2O (panel A) and PaO_2/FiO_2 at 5 cmH_2O (panel B).

Recruitment = 84.4 - 0.007 × Not-inflated tissue, P=0.84, R^2=0.00

Recruitment = 14.2 + 0.39 × PaO_2/FiO_2, P=0.16, R^2=0.09

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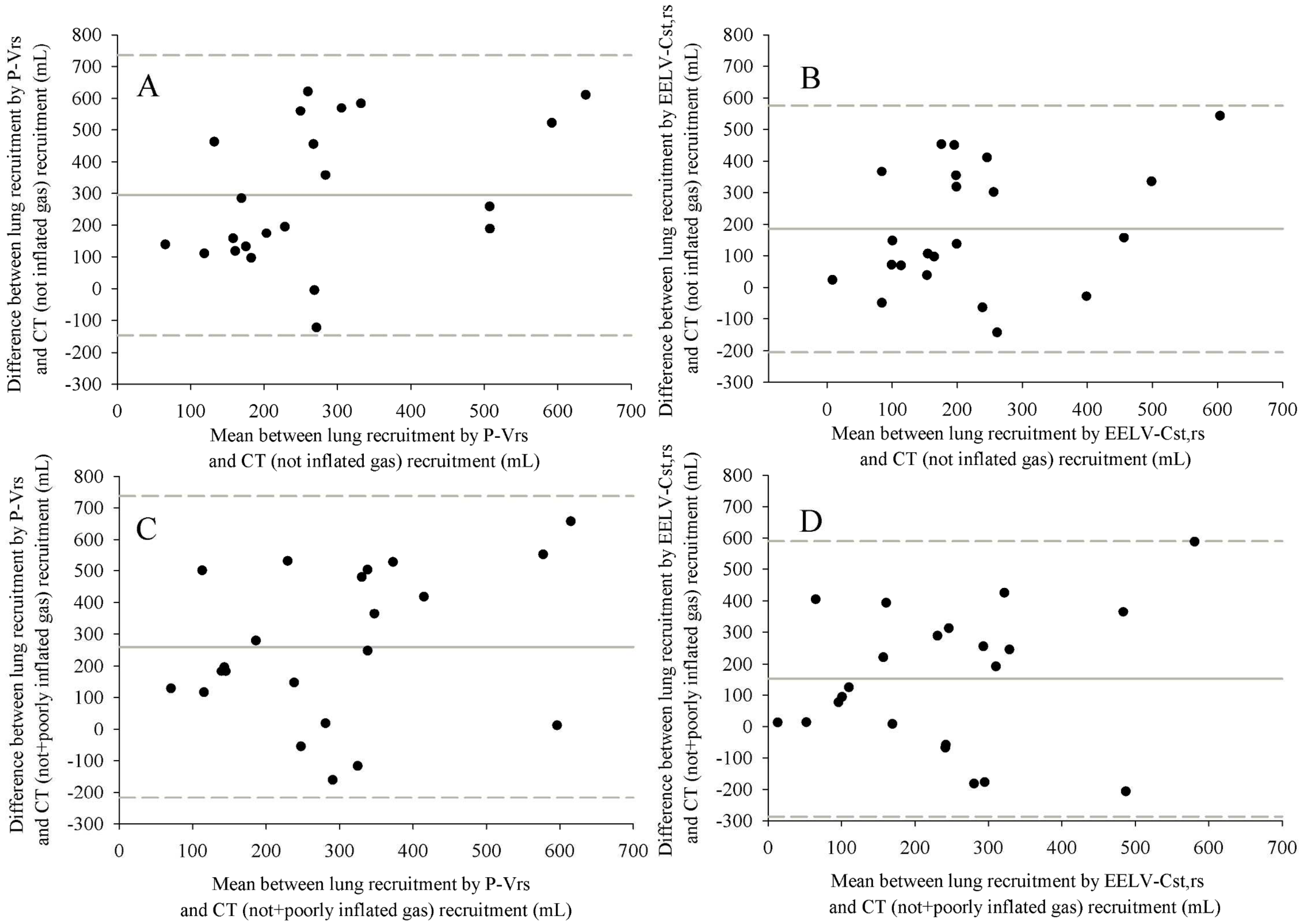
Figure E8. *CT scan-based recruitment (not+poorly-inflated tissue) and severity*



Comparison of Respiratory mechanics-based methds and CT scan-based methods expressed as milliliters of gas. Solid grey lines represent linear regressions. X-axis represents the mean of the two measurements, while Y-axis represents the difference between them. Horizontal grey lines are the mean difference (solid), and at the limits of agreement (mean difference plus and minus 1.96 times the standard deviation of the differences, medium dashed lines).

Panel A: gas associated to recruited tissue (not-inflated) *versus* P-Vrs curve recruitment.

Panel B: gas associated to recruited tissue (not-inflated) *versus* EELV-Cst,rs recruitment.

Panel C: gas associated to recruited tissue (not- + poorly-inflated) *versus* P-Vrs curve recruitment.

Panel D: gas associated to recruited tissue (not- + poorly-inflated) *versus* EELV-Cst,rs recruitment

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Figure E9: *Recruitment as a function of baseline CT scan variables (5 cmH$_2$O PEEP)*

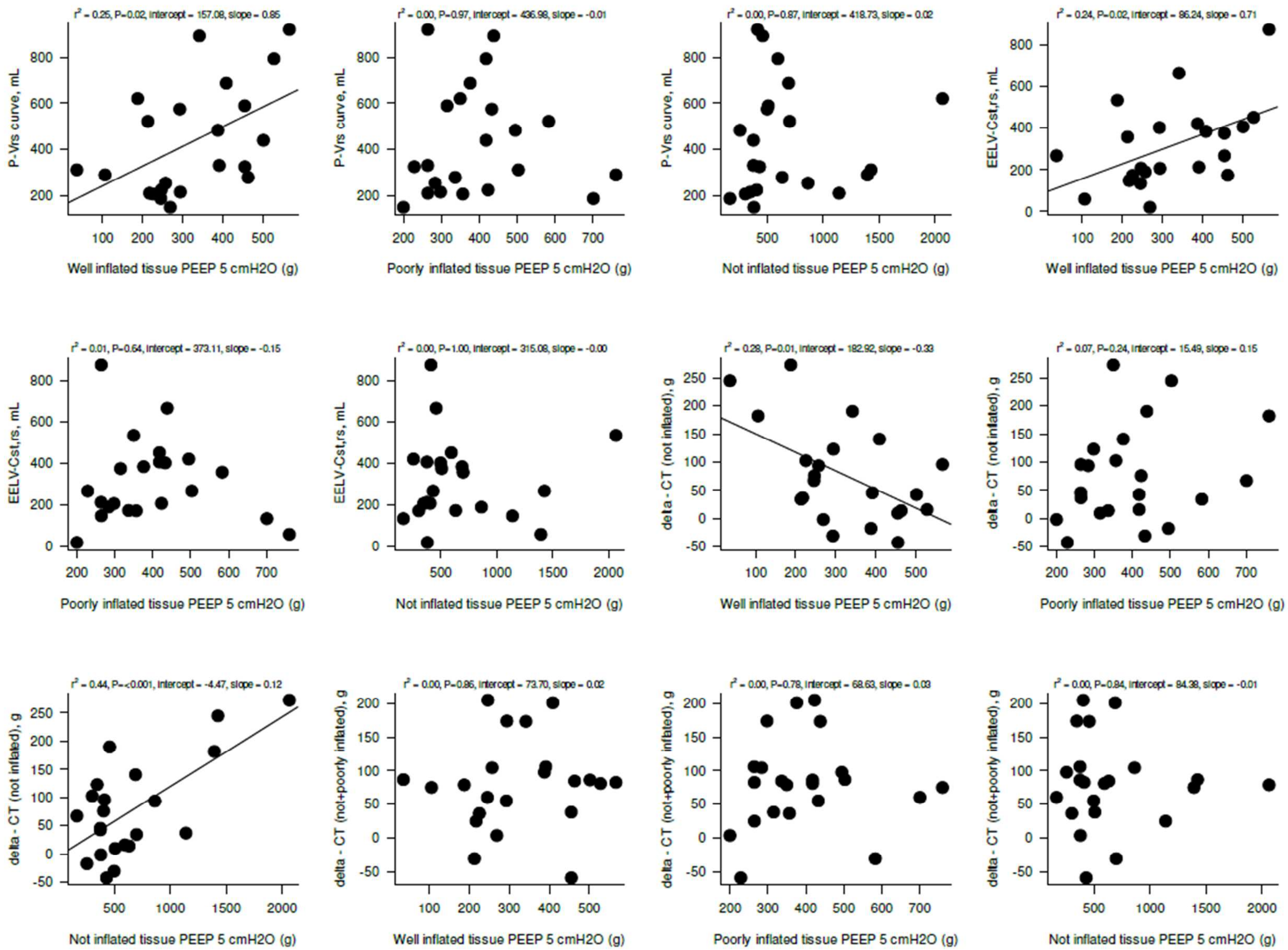

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Figure E10 – ***P-Vrs recruitment (ml) and physiological variables***

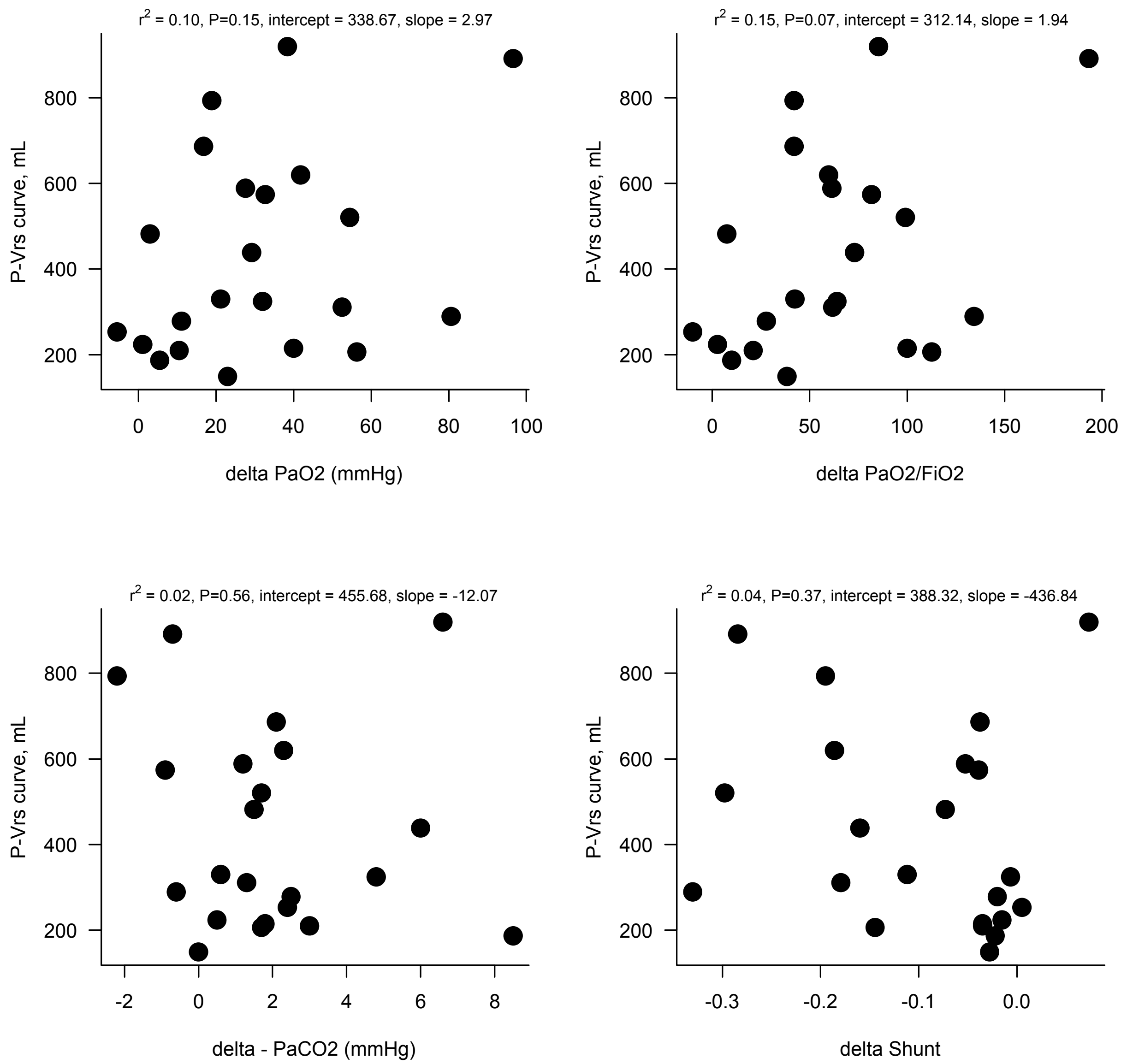

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Figure E11 – *EELV-Cst,rs (ml) recruitment and physiological variables*

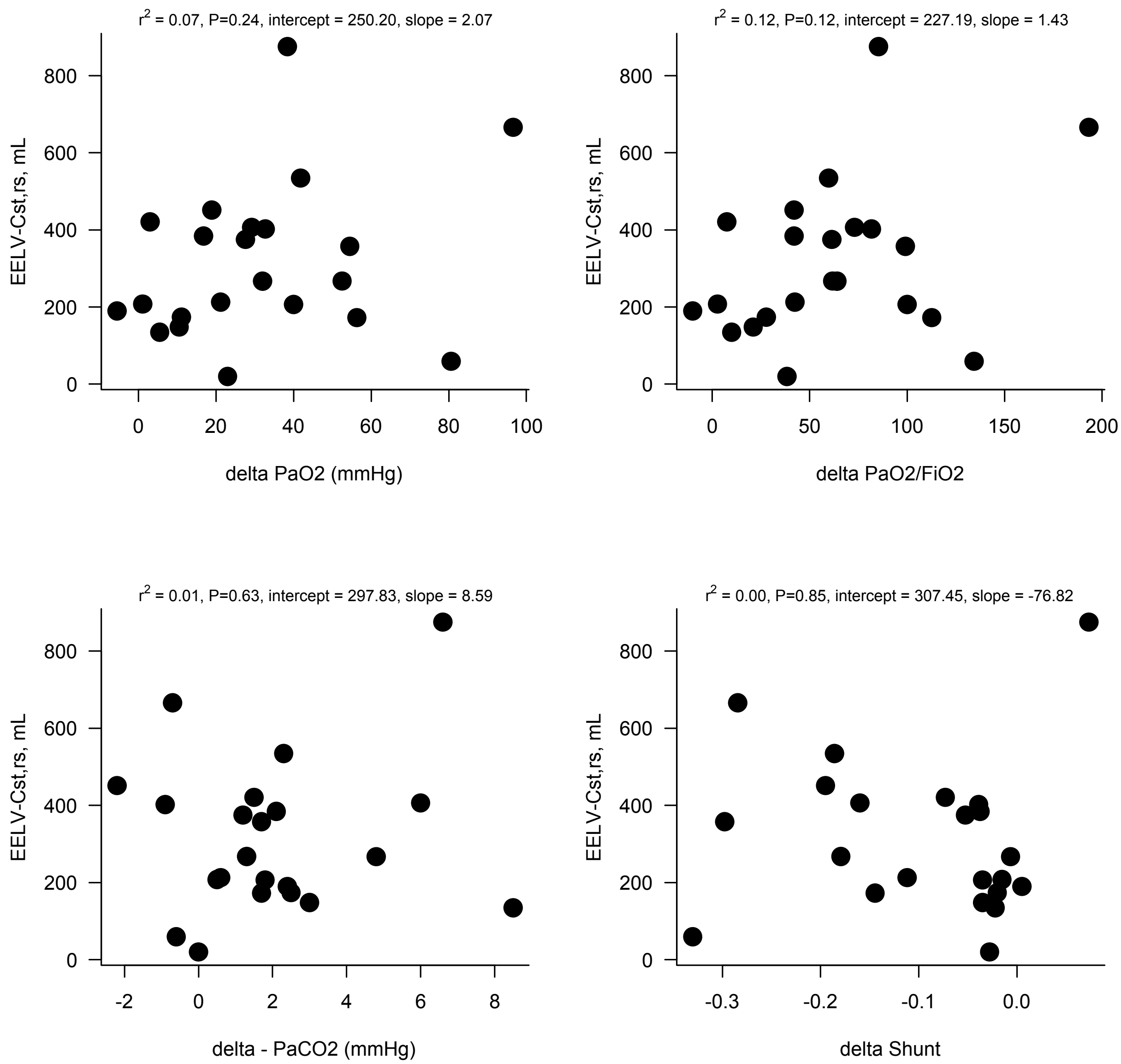

Figure E12 – *CT scan recruitment (threshold -100) and physiological variables*

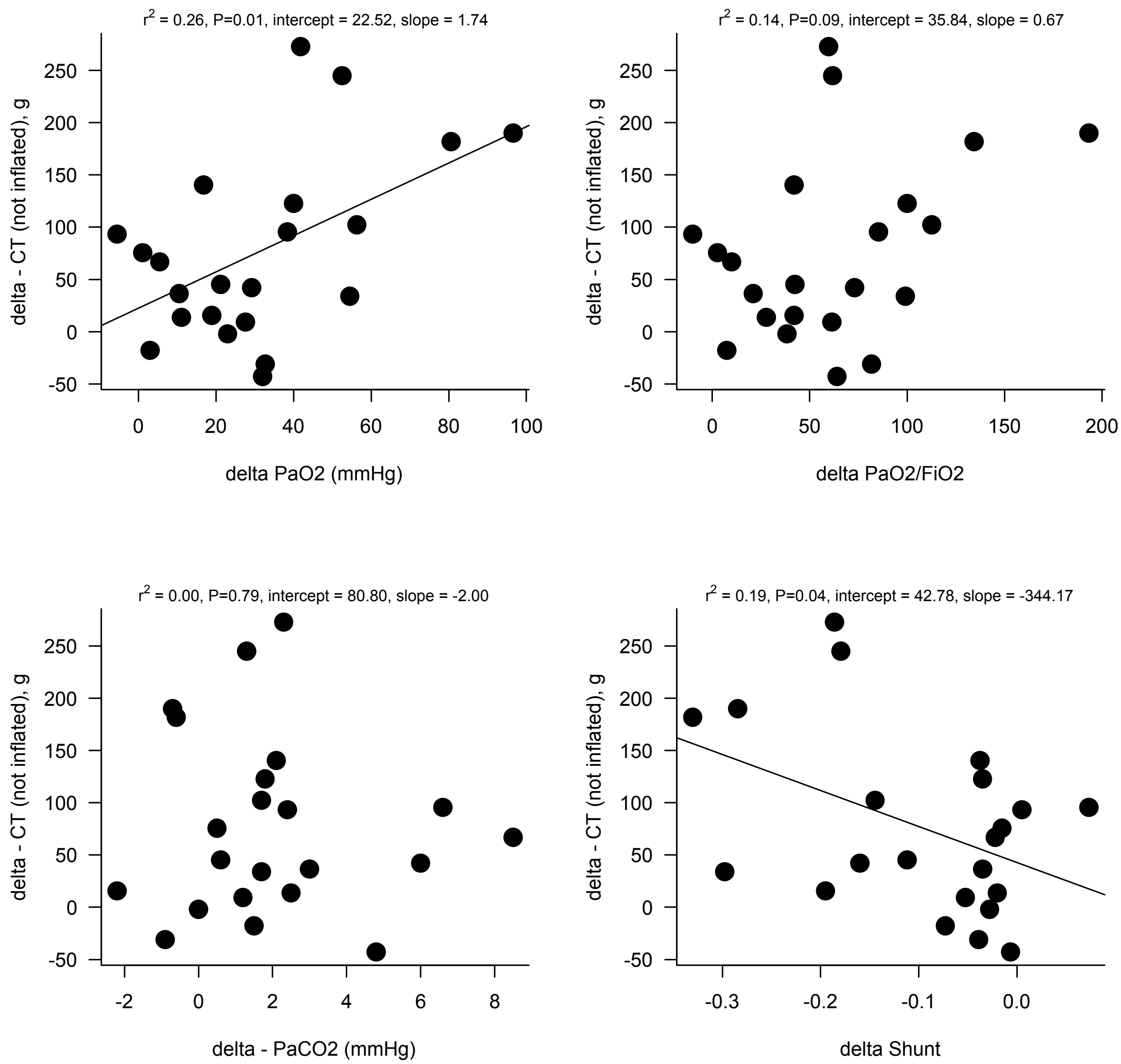

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Figure E13 - *CT scan recruitment (threshold -500) and physiological variables*

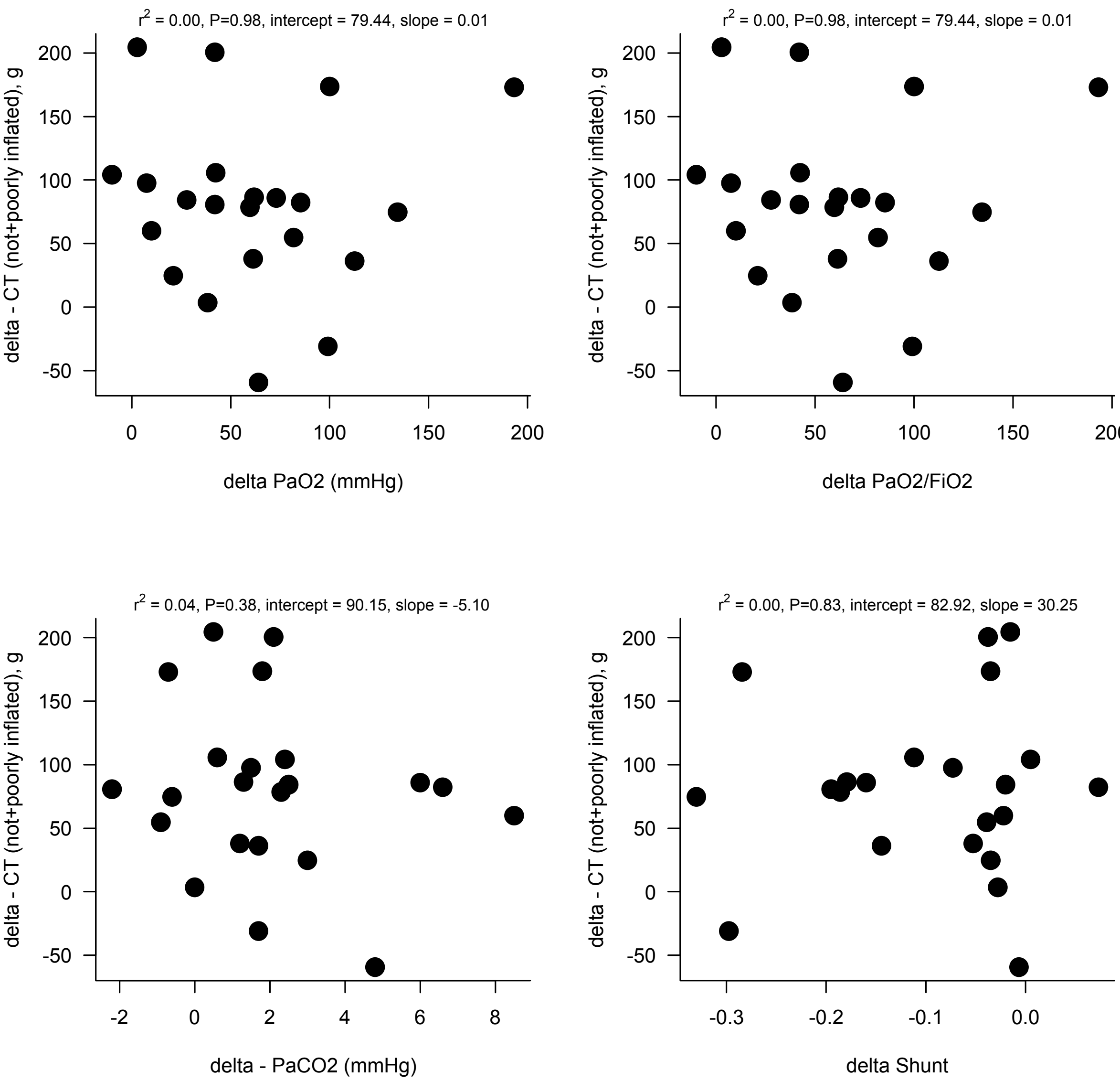
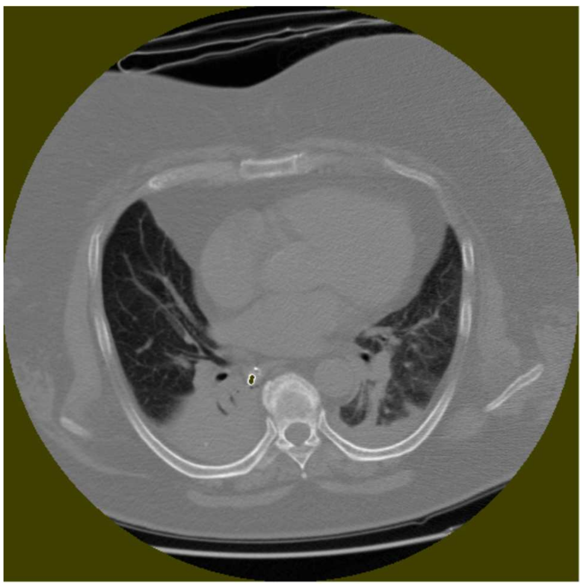


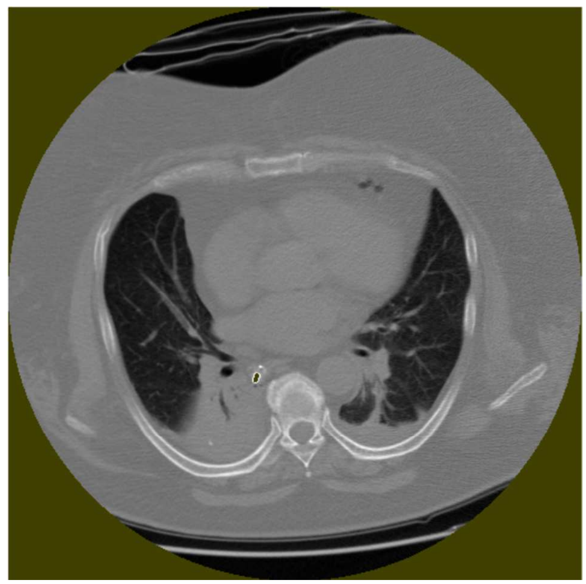
Figure E14 – *Computed tomography and CT scan in a patients with no CT scan recruitment*

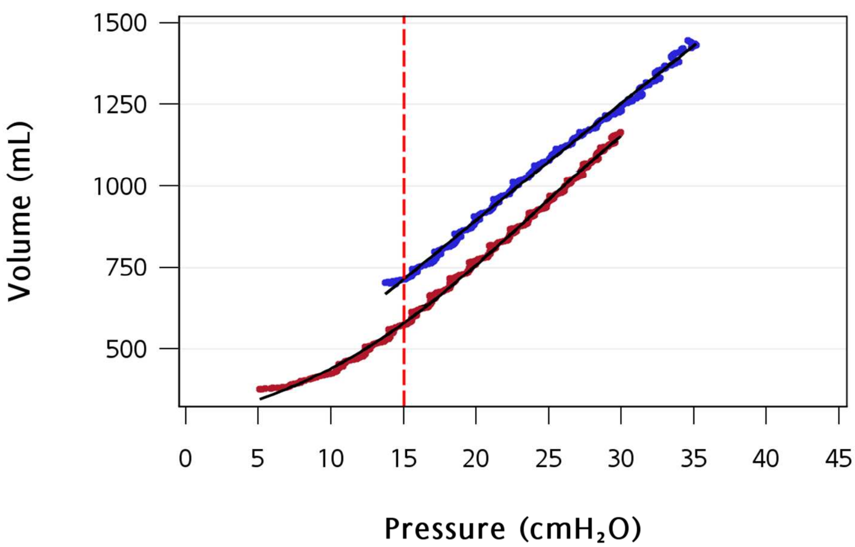
In this patient the amounts of well inflated and poorly inflated tissues remained almost unmodified; no gas entered in the not inflated units (381 g at PEEP 5 cmH_2O and 383 g at PEEP 15 cmH_2O). The gas associated to the well inflated tissue increased from 549 to 879 ml while the gas associated to the poorly inflated tissue remained near-constant (105 ml at PEEP 5 cmH_2O and 91 ml at PEEP 15 cmH_2O). The PV curve method gave a recruitment value of 135 ml.

PEEP 5



PEEP 15





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