

Pressure–Time Curve Predicts Minimally Injurious Ventilatory Strategy in an Isolated Rat Lung Model

V. Marco Ranieri, M.D., Haibo Zhang, M.D., Ph.D.,† Luciana Mascia, M.D.,‡ Michael Aubin, R.R.T.,‡ Chang-Yi Lin, M.D.,‡ J. Brendan Mullen, M.D.,§ Salvatore Grasso, M.D.,‡ Matthew Binnie, M.D.,‖ George A. Volgyesi, P. Eng,# Arthur S. Slutsky, M.D.***

Background: **We tested the hypothesis that the pressure–time (P-t) curve during constant flow ventilation can be used to set a noninjurious ventilatory strategy.**

Methods: **In an isolated, nonperfused, lavaged model of acute lung injury, tidal volume and positive end-expiratory pressure were set to obtain: (1) a straight P-t curve (constant compliance, minimal stress); (2) a downward concavity in the P-t curve (increasing compliance, low volume stress); and (3) an upward concavity in the P-t curve (decreasing compliance, high volume stress). The P-t curve was fitted to: $P = a \cdot t^b + c$, where b describes the shape of the curve, $b = 1$ describes a straight P-t curve, $b < 1$ describes a downward concavity, and $b > 1$ describes an upward concavity. After 3 h, lungs were analyzed for histologic evidence of pulmonary damage and lavage concentration of inflammatory mediators. Ventilator-induced lung injury occurred when injury score and cytokine concentrations in the ventilated lungs were higher than those in 10 isolated lavaged rats kept statically inflated for 3 h with an airway pressure of 4 cm H_2O.**

Results: **The threshold value for coefficient b that discriminated best between lungs with and without histologic and inflammatory evidence of ventilator-induced lung injury (receiver–operating characteristic curve) ranged between 0.90–1.10. For such threshold values, the sensitivity of coefficient b to identify noninjurious ventilatory strategy was 1.00. A significant relation ($P < 0.001$) between values of coefficient b and injury score, interleukin-6, and macrophage inflammatory protein–2 was found.**

Conclusions: **The predictive power of coefficient b to predict noninjurious ventilatory strategy in a model of acute lung injury is high. (Key words: Acute lung injury; lung protective strategy; ventilator-induced lung injury.)**

A large number of animal studies have shown that mechanical ventilation can initiate or worsen preexisting lung injury.[1–5] The postulated mechanism responsible for ventilator-induced lung injury (VILI) is that lung damage results from cycling closing and opening of collapsed alveolar units or from pulmonary overdistension.[6] Using an isolated rat model of acute lung injury (ALI), we found that ventilation at low end-expiratory or high end-inspiratory lung volumes worsened the mechanics and morphologic indices of lung injury.[4]

The pressure–volume (P-V) relation of the respiratory system has been studied in animal models and in patients with ALI.[1,4,6–11] The P-V curve is characterized by lower inflection points (LIP) and upper inflection points (UIP).[1,4,6–11] The LIP represents the average critical opening pressure above which alveolar units start to reopen.[8,9] It has been suggested that end-expiratory volume–pressure should be maintained above LIP to avoid cycling end-expiratory alveolar collapse.[6,9] The UIP indicates the volume–pressure values above which stretching and overdistension starts to occur;[6,9] tidal volume should be limited to maintain the end-inspiratory volume–pressure at a level lower than the UIP.[6,9] Recent clinical data suggest that using information from the static P-V relation of the respiratory system may be useful in setting values of positive end-expiratory pressure (PEEP) and tidal volume (V_t) to minimize VILI[10] and improve outcomes.[11] Nevertheless, application of the static P-V curve is limited.[6,9]

We recently found that a downward concavity on the P-t profile corresponded to a static curve with a distinct LIP and a continuous increase in compliance. Conversely, an upward convexity on the P-t profile corresponded to a static P-V curve with a distinct UIP and a continuous reduction in compliance.[12] The current study tested the hypothesis that ventilator settings resulting in a straight P-t profile would minimize the occurrence of VILI in an experimental model of ALI.

Methods

Animal Preparation

Forty-eight male Sprague-Dawley rats (Charles River Laboratories, St. Constant, Québec, Canada) weighing 440 ± 5 g (mean ± SD) were used in accordance with the institutional animal welfare guidelines of the University of Toronto. All animals were anesthetized with intraperitoneal injection of 10 mg/kg of xylazine (Rompun, Bayer Inc., Animal Health Section, Etobicoke, Ontario, Canada) and 50 mg/kg of ketamine (Ketalean; Bimeda-MTC Animal Health Inc., Cambridge, Ontario, Canada). The animals were intubated through a tracheotomy with a sterile angiocatheter (14-gauge Angiocath; Becton Dickens Infusion Therapy Systems Inc., Sandy,

* Associate Professor, Division of Respiratory Medicine, Samuel Lunenfeld Research Institute, Mount Sinai Hospital; and Dipartimento di Chirurgia—Terapia Intensiva, Cattedre Di Anestesiologia e Rianimazione Ospedale S. Chiara. † Research Fellow, Division of Respiratory Medicine, Samuel Lunenfeld Research Institute, Mount Sinai Hospital; and Fellow, Medical Research Council of Canada. § Assistant Professor, Department of Pathology and Laboratory Medicine, ‡ Research Fellow, ‖ Resident, # Technician, ** Professor, Division of Respiratory Medicine, Samuel Lunenfeld Research Institute, Mount Sinai Hospital.

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Address reprint requests to Dr. Ranieri: Dipartimento di Chirurgia—Terapia Intensiva, Cattedre di Anestesiologia e Rianimazione, Università di Pisa, Ospedale S. Chiara, Via Roma 67, 56100 Pisa, Italy. Address electronic mail to: mranieri@teseo.it.

UT). The right internal carotid artery was cannulated (22-gauge Angiocath, Becton Dickens) for blood sampling. The animals were ventilated (Model RV5; Voltek Enterprises Inc., Toronto, Ontario, Canada) and received a V_t of 6–8 ml/kg at a respiratory rate of 25–30 breaths/min with an inspired oxygen fraction (F_{IO_2}) of 1.0. These settings previously have been found to maintain the arterial carbon dioxide tension (Pa_{CO_2}) within the normal range.[4] Pancuronium bromide (Sabex Inc., Boucherville, Québec, Canada) at a dose of 1 mg/kg was injected *via* the dorsal penile vein during anesthesia. The animals were allowed to stabilize for 5 min after the initiation of ventilation. Pilot investigations confirmed that escape behavior did not occur during the entire surgical preparation in the absence of neuromuscular blocking agent.

Lung lavages were performed *via* the intratracheal tube using 25-ml/kg aliquots of normal saline heated to 37°C, as previously described.[4] Lavages were repeated every 8–10 min until the arterial oxygen tension (Pa_{O_2}) was less than 100 mmHg (Model 248; Ciba-Corning Diagnostics Ltd., Essex, UK) while the animal was being ventilated with an F_{IO_2} of 1.0. The animal was then rapidly exsanguinated by dissecting the abdominal aorta through a midline abdominal incision. After opening the chest, the trachea, lungs, and heart were dissected *en bloc* and suspended in a specially constructed plethysmograph (Model PL001, Voltek Enterprises Inc.).

The plethysmograph consists of a double-walled, transparent, plastic box designed to provide constant temperature and humidity for the *ex vivo* lung preparation. The inner box is suspended within the outer box by a cylinder. To maintain a constant temperature in the inner box, a circulating water system is incorporated around the box. Constant humidity was ensured by partially filling the inner box with water. The lungs were suspended in the plethysmograph through the channel of a tightly fitting rubber stopper and connected to the ventilator y-piece. A second channel was connected to a pressure transducer (Digima-Clic ±100 cm H_2O; Special Instruments, Nordlingen, Germany) to monitor changes in pressure (and hence volume). The air temperature of the inner box was kept constant (37°C) by a temperature-controlled heater (model 210; PolyScience, Niles, IL).

A key element of the study was to ensure that tidal inflation occurred with a constant inspiratory flow. The small-animal ventilator used in this study delivers constant inspiratory flow by allowing inspiratory gas to enter the lungs from a high-pressure source (20–50 pounds per square inch) through a high-resistance capillary tube. The flow through the capillary depends on its resistance and on the pressure gradient across the capillary tube. The resistance of the capillary tube is very high compared with the resistance of the respiratory system of the animal being ventilated; thus, the inspiratory flow is relatively independent of changes in respiratory mechanics of the animal and is proportional to the source pressure. The ventilator was connected to the isolated lungs through a small plastic airway connector containing intersecting channels. The compressible volume of the entire breathing circuit was less than 3 ml. V_t, respiratory rate, expiratory time, and the duration of end-expiratory and end-inspiratory occlusions can be set with controls located on the front panel of the ventilator. A water column connected to the expiratory port of the ventilator was used to set PEEP.

Assessment of Respiratory Mechanics

Inspiratory flow was measured through the pressure drop across the capillary tube of the ventilator. This pressure signal was calibrated with the same gas mixture used to ventilate the animal, and the linearity of the pressure transducer was confirmed within the range of flow used in the study.

Airway opening pressure (Pao) was measured proximal to the endotracheal tube with a pressure transducer. Changes in lung volume were estimated by measuring pressure variations inside the plethysmograph calibrated to changes in volume. Transpulmonary pressure (P_L) was calculated as Pao minus the pressure inside the plethysmograph. All variables were displayed and collected (ICU-Lab; KleisTEK Advanced Electronic Systems, Bari, Italy) on a laptop computer equipped with a 12-bit analog–digital acquisition board (DAQ Card 700, National Instrument, Austin, TX) at a sampling rate of 600 Hz. Total PEEP (PEEPt = external PEEP + auto-PEEP) was measured at the end of a 3- to 4-s end-expiratory occlusion. End-inspiratory plateau pressure (Pplat) was measured at the end of a 3- to 4-s end-inspiratory occlusion.

Dynamic Pressure--time Curve during Constant Flow Inflation

During constant flow inflation, the P-t relation on a breath-by-breath basis can be described by a power equation:[12–15]

$$P_L = a \cdot t^b + c$$

where the coefficients a, b, and c are constants. The coefficient a represents the slope of the P-t relation at $t = 1$ s, and the coefficient c is the pressure at $t = 0$. The coefficient b is a dimensionless number that describes the shape of the P-t curve. For values of coefficient $b < 1$, the dynamic P-t curve will present a downward concavity, indicating that compliance increases with time, whereas compliance decreases with time for values of coefficient $b > 1$, producing an upward concavity on the P-t curve. Values of the coefficient $b = 1$ indicate a straight P-t relation and a constant compliance.[7,11,13–15] This interpretation is based on the assumption that re-

$$P_L = a \cdot t^b + c$$

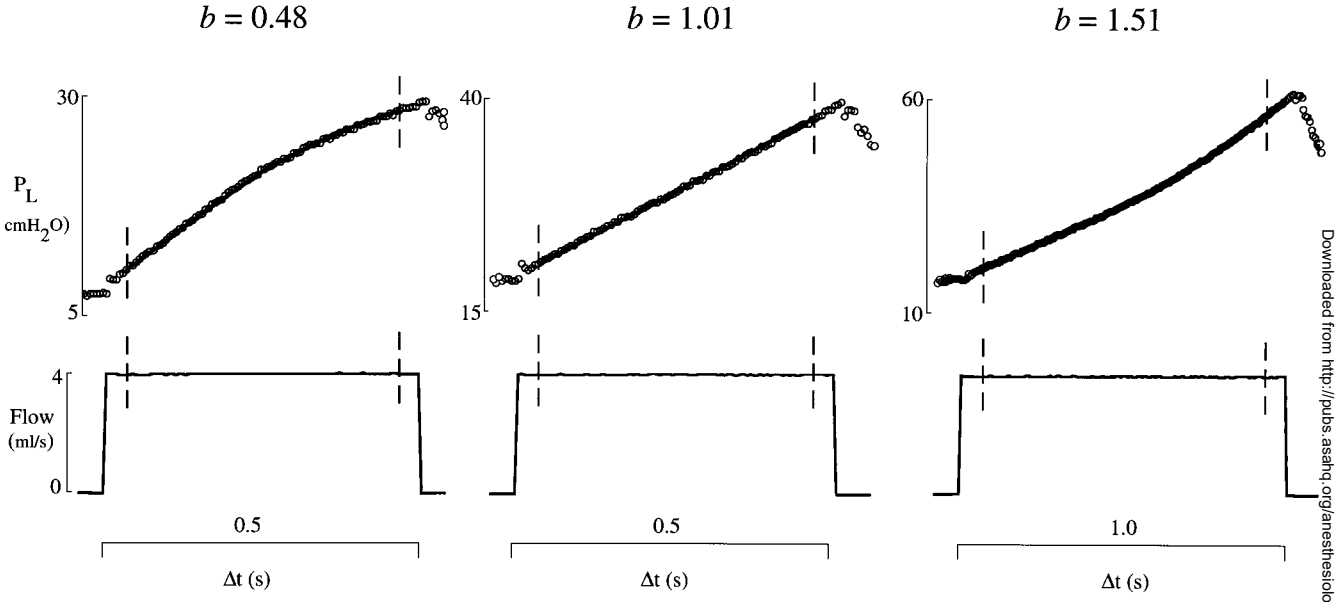

Fig. 1. The conceptual illustration of the dynamic pressure–time (P-t) curve used in the current study. Based on the power equation $P_L = a \cdot t^b + c$, $b = 0.5$ produces a convex P-t curve, indicating continuing recruitment; $b = 1$ produces a straight P-t line, indicating no alveolar continuing recruitment or overdistension; and $b = 1.5$ produces a concave P-t curve, indicating alveolar overdistension. The power equation was applied to the transpulmonary pressure (P_L) signal during a constant inspiratory flow (vertical bars).

sistive and viscoelastic contribution to Pao remain relatively constant over the range of tidal volume.[12–15]

The software identified the beginning and end of each inspiration from the zero crossing points of the flow curve. Inspiratory flow and P_L signals were averaged on a breath-by-breath basis and over a 2- to 3-min period every 5 min. The power equation was then fit to the resulting mean P_L. The curve-fitting procedure was applied to the P_L data points corresponding to the constant part of the mean inspiratory flow. To ensure that the on- and off-flow transients did not skew the results, the curve-fitting procedure included only data points obtained from 50 ms after the beginning of the square wave in inspiratory flow until 50 ms before the end of flow (fig. 1). These values were chosen based on a series of preliminary experiments performed to identify the opening and closing times of the solenoid valves used on the rat ventilator and to verify whether inspiratory flow remained constant in the pressure range used in the current study. Values of coefficients *a*, *b*, and *c* were displayed on the computer screen.

Experimental Protocol

The excised lungs were ventilated with a V_T of 6–8 ml/kg at a respiratory rate of 25–30 breaths/min with an F_{IO_2} of 0.21. PEEP was progressively increased until the P-t relation exhibited a straight line (value of coefficient $b = 0.9–1.1$). Three consecutive "recruiting maneuvers" consisting of an inflation to 40 cm H_2O for 40 s were performed.[4] After the recruitment maneuvers, the PEEP level was increased until the P-t curve showed an upward concavity and a value of coefficient $b > 1.0$ was displayed on the computer. PEEP was then decreased until the P-t curve became straight, with values of coefficient $b = 0.9–1.1$ (fig. 2). The entire procedure to obtain full pulmonary expansion lasted 10–15 min. The lungs were then randomized to one of the following groups:

Minimal stress: Lungs were ventilated to maintain full pulmonary expansion; if necessary, PEEP was adjusted within the 3-h experiment to maintain values of *b* close to 1.00 ($1.1 < b < 0.9$).

Low-volume stress: This condition was obtained by adjusting V_T and PEEP until the P-t curve showed a downward concavity and a value of $b < 1.00$ ($0.8 < b < 0.2$). PEEP was adjusted thereafter to maintain the target values of *b* during the experiment.

High-volume stress: This condition was obtained by increasing the PEEP and V_T levels to obtain an upward concavity of the P-t curve and a value of $b < 1.00$ ($1.8 < b < 1.3$). V_T was then adjusted to maintain the target values of coefficient *b* (fig. 2).

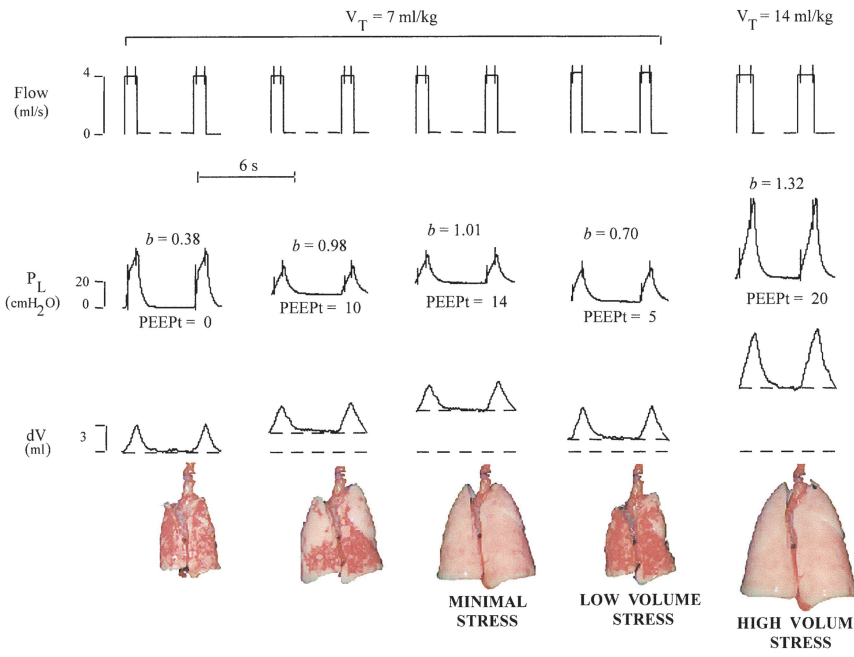

Fig. 2. Representative data record showing physiologic variables (flow, transpulmonary pressure [P_L], and changes in lung volume [ΔV]) and the appearance at end-expiration of the excised lung during the different experimental conditions. The equation $P_L = a \cdot t^b + c$ was applied to the inspiratory P_L during constant flow (vertical bars on flow and P_L). Low volume stress, coefficient b = 0.5, indicates ongoing recruitment; minimal stress, b = 1, indicates no ongoing recruitment or overdistention; and high volume stress, b = 1.5, indicates overdistension. PEEP = positive end-expiratory pressure.

The presence of air leaks was checked during the recruitment maneuver and assessed every 30 min thereafter during experiments. Whenever a leak was found, the lungs were excluded from the study.

At the end of each experiment, the endotracheal catheter was advanced into the right main bronchus, and the corresponding lung was lavaged 3 times with 2-ml aliquots of warm (37°C) normal saline. The bronchoalveolar lavage fluid (BALF) aliquots were pooled and centrifuged (model TJ-6, Beckman Instrumentation, Inc., Palo Alto, CA) at 2,000 rpm for 10 min; the supernatant was frozen at −70°C for the determination of cytokines. The endotracheal catheter was then pulled back in the trachea, and both lungs were immediately fixed by intratracheal instillation of 10% neutral buffered formalin (BDH Inc., Toronto, Ontario, Canada) using a volume equal to one half of the volume at total lung capacity as determined from the initial static P-V curve.[4] After fixation, the nonlavaged lung was isolated and floated in 10% formalin for at least 24 h. The lung was then submitted for histologic analysis.

Assay for Cytokines

Commercial sandwich enzyme-linked immunosorbent assay kits (BioSource International, Inc., Camarillo, CA) were used to determine BALF concentrations of tumor necrosis factor–α (TNF-α; detection limit, 10 pg/ml), interleukin-6 (IL-6; detection limit, 10 pg/ml) and macrophage inflammatory protein–2 (MIP-2; detection limit, 15 pg/ml).[1]

Morphology

A technician using standard histologic techniques processed the formalin-fixed lungs. The nonlavaged lung was serially sectioned (5-μm slices) in a coronal fashion from apex to base, and six random sections were processed for histologic analysis, embedded in paraffin, and stained with hematoxylin and eosin. A pathologist (J.B.M.) who was blinded as to group read the slides. The sections were examined with particular reference to bronchiolar epithelial lesions (necrosis and epithelial sloughing) and hyaline membranes, using a modification of the method of Nilsson *et al.*[4,16] In each lung, the total number of membranous and respiratory bronchioles and alveolar ducts showing hyaline membranes were counted. Membranous bronchioles are conducting airways without cartilage and include terminal bronchioles, which are the most distal generation of membranous bronchioles and the parent generation of respiratory bronchioles. An injury score for each airway type was obtained as the percentage of injured airways of each airway type. In addition, a total airway injury score was obtained for each animal by summarizing the individual airway injury scores and expressing the result as a percentage of the maximal possible score.

Definition of Ventilator-induced Lung Injury

Lungs from the three experimental groups were classified as having VILI if total airway injury score was higher than 26% or BALF concentrations of TNF-α, IL-6, or MIP-2 were higher 190, 2,652, and 665 pg/ml, respectively. These values represent the mean plus 2 SD values[17] of total airway injury score, TNF-α, IL-6, and MIP-2 observed in 10 rats in whom lavaged lungs were kept statically inflated for 3 h with an airway opening pressure of 4 cm H_2O.[1,4]

Table 1. Ventilator Settings and Values of Coefficient *b* of the Dynamic Transpulmonary Pressure–Inspiratory Time Relation Fitted to a Power Equation of the Type $P_L = a \cdot Ti^b + c$*

Experimental Groups	V_T (ml/kg)	RR (breaths/min)	Ti (s)	V_I/Ti (ml/s)	Pplat (cm H_2O)	PEEPt (cm H_2O)	Coefficient *b*
Low-volume stress	7.30 ± 0.57	33.7 ± 1.8	0.50 ± 0.04	3.82 ± 0.36	21.0 ± 3.1	3.9 ± 1.8†	0.46 ± 0.12†
Minimal stress	7.52 ± 0.69	32.6 ± 2.3	0.51 ± 0.05	3.78 ± 0.25	25.5 ± 1.8	14.9 ± 2.1	1.07 ± 0.15
High-volume stress	15.99 ± 0.81†	16.1 ± 1.1†	2.17 ± 0.28†	3.38 ± 0.21	71.6 ± 5.0†	20.6 ± 2.3†	1.49 ± 0.11†

* Values are mean ± SD of the mean. Transpulmonary pressure is calculated as airway opening pressure minus the pressure inside the plethysmograph. † $P <$ 0.001 (analysis of variance and Newman-Keuls test *vs.* minimal stress).

V_T = tidal volume; RR = respiratory rate; Ti = inspiratory time; Pplat = end-inspiratory plateau pressure; PEEPt = total positive end-expiratory pressure (intrinsic PEEP + applied PEEP).

Statistics

All results are expressed as mean ± SD. Regression analysis was performed using the least-squares method. A P value < 0.05 was accepted as significant.

A true-positive result was defined as occurring when coefficient *b* predicted VILI and the total airway injury score and BALF concentrations of TNF-α, IL-6, or MIP-2 were confirmatory (*i.e.,* values were greater than as described previously). A true-negative result was defined as occurring when coefficient *b* predicted the absence of VILI and the total airway injury score or BALF concentrations of TNF-α, IL-6, and MIP-2 were confirmatory. A false-positive result was defined as occurring when coefficient *b* predicted VILI but the total airway injury score or BALF concentrations of TNF-α, IL-6, and MIP-2 BALF were not confirmatory. A false-negative result was defined as occurring when coefficient *b* predicted the absence of VILI but the total airway injury score or TNF-α, IL-6, and MIP-2 BALF concentrations were not confirmatory.

Analysis of the receiver–operating characteristic curve was used to determine the optimal sensitivity and specificity values for coefficient *b* in predicting VILI. The value selected as the threshold for coefficient *b* was the one that resulted in the fewest false-negative classifications. Standard formulas were used to calculate sensitivity, specificity, and positive and negative predictive values.

Results

Eight experiments were excluded because of the development of air leaks following initiation of ventilation (six in the high-volume stress group and two during lung harvest). All excluded experiments were replaced in the randomization, and 10 isolated lungs per group were included in the final data analysis.

There were no significant differences among the three groups in the animal weights, number of lavages required to reach Pao_2 criteria, and values of Pao_2 before the rats were exsanguinated for lung excision (450 ± 6, 440 ± 4, and 420 ± 5 g; 3 ± 1, 3 ± 1 and 4 ± 1 lavages; 74.7 ± 5.2, 67.4 ± 5.7 and 71.6 ± 4.1 mmHg in the low-volume stress, minimal stress, and high-volume stress groups, respectively).

Values of the coefficient *b* in the three groups of experiments after randomization are given in table 1. In the minimal stress group, the P-t relation was linear, indicating that compliance was constant in the V_T range and values of coefficient *b* were between 0.9 and 1.1. This value was obtained by setting V_T levels between 6.2 and 8.1 ml/kg and PEEP levels between 12 and 20 cm H_2O; values of Pplat ranged between 22.4 and 28.7 cm H_2O. In the low-volume stress group, the P-t relation showed a downward concavity, indicating a progressive increase in compliance with increasing volumes. Values of *b* were between 0.2 and 0.8; V_T levels were set between 6.3 and 8.0 ml/kg; PEEP levels were set between 3 and 6 cm H_2O; and Pplat levels were set between 17.4 and 24.4 cm H_2O. In the high-volume stress group, the P-t relation showed an upward concavity, indicating that compliance decreased with V_T; values of *b* were between 1.3 and 1.7; V_T levels were set between 15.8 and 17.1 ml/kg; PEEP levels were set between 18 and 25 cm H_2O; and Pplat levels were set between 68.5 and 76.8 cm H_2O. The correlation coefficient ranged between 0.99 and 1.00.

A significant ($P < 0.0001$) U-shaped relation between individual values of coefficient *b* and total airway injury score ($p = 0.0001$), IL-6 ($p = 0.0025$), and MIP-2 ($p = 0.0019$) was found; the relation between coefficient *b* and TNF-α was not significant (fig. 3). The lowest values of total airway injury score and concentrations of TNF-α, IL-6, and MIP-2 were systematically associated with a straight P-t relation and values of coefficient *b* ranging between 0.9 and 1.1.

The area under the receiver–operating characteristic curve was significantly larger than that of an arbitrary test that would be expected *a priori* to have no discriminatory value (*i.e.,* 0.50) for all markers of VILI, demonstrating that the coefficient *b* was extremely sensitive for predicting noninjurious ventilatory strategies. The threshold value for coefficient *b* that discriminated best between lungs with and without VILI ranged between 0.9–1.1 (fig. 4). For such threshold values, the sensitivity of the coefficient *b* to predict a protective ventilatory

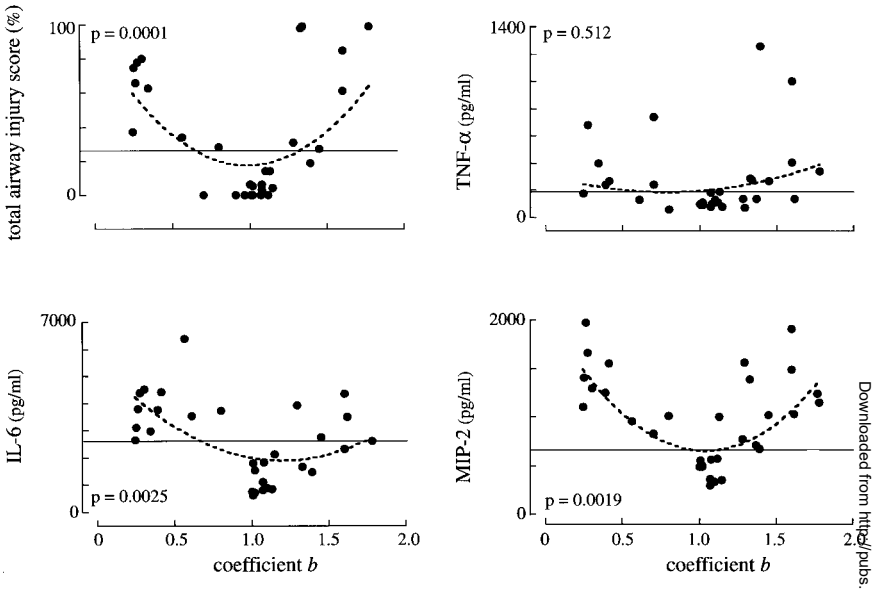

Fig. 3. Individual values of coefficient *b* are plotted against individual values of total airway injury score, tumor necrosis factor–α (TNF-α), interleukin-6 (IL-6), and macrophage inflammatory protein–2 (MIP-2). The horizontal line identifies the mean values of total airway injury score, TNF-α, IL-6, and MIP-2 in nonventilated lavaged lungs. All experiments conducted with ventilator variables leading to a straight pressure–time (P-t) curve (*b* = 1) had values of coefficient *b* below the horizontal line.

strategy was 1.00 for histologic and inflammatory markers of VILI (table 2).

Discussion

The main finding of this study is that the shape of the dynamic P-t profile during constant flow inflation is useful in setting ventilatory parameters to minimize VILI. Using strategies that produced values of the coefficient *b* ranging between 0.90 and 1.10 produced injury that was similar to that observed with no ventilation.

The mechanical factors responsible for VILI are thought to be related to tidal recruitment and derecruitment of previously collapsed alveoli or to pulmonary overdistention.[1,2] The P-V curve of the respiratory system in patients[7–10] as well as in animal models of ALI[1,4,18–20] has a characteristic sigmoid shape, with a LIP corresponding to the pressure–end-expiratory volume required to initiate recruitment of collapsed alveoli and an UIP corresponding to the pressure–end-inspiratory volume at which alveolar overdistension occurs. "Protective" ventilatory approaches have therefore been designed to minimize mechanical injury by using the P-V curve to individualize PEEP (PEEP above the LIP) and V_T (by setting end-inspiratory volume–pressure below the UIP).[10,11] However, using the measurement of the static P-V curve to set "protective ventilatory strategy" has been criticized because of potential harm to patients.[2] In addition, the complexity of measurement and interpretation have precluded its clinical use.[9,21]

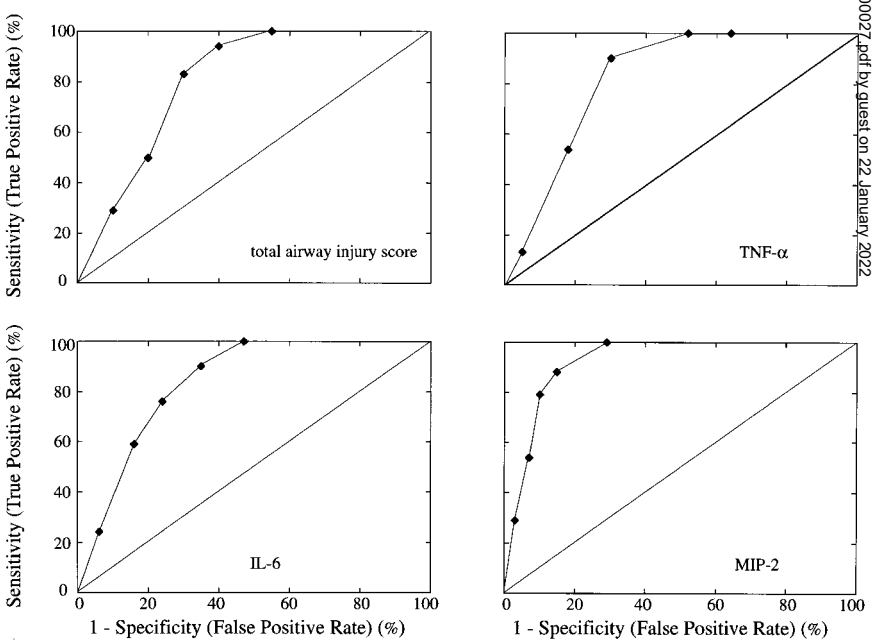

Fig. 4. Receiver–operating characteristic curve for the coefficient *b* generated by plotting the proportion of true-positive results against the proportion of false-positive results for each value of the coefficient *b*. The curve for an arbitrary test that is *a priori* expected to have no discriminatory value is indicated by the line of identity. The accuracy of the coefficient *b* to detect ventilator-induced lung injury is indicated by a receiver–operating characteristic curve that rises rapidly and reaches a plateau. TNF-α = tumor necrosis factor–α; IL-6 = interleukin-6; MIP-2 = macrophage inflammatory protein–2.

Table 2. Accuracy of the Coefficient *b* to Predict Histologic and Inflammatory Evidence of Ventilator-induced Lung Injury

Markers of Ventilator-induced Lung Injury	Sensitivity	Specificity	Positive Predicted Value	Negative Predicted Value
Total airway injury score	1.00	0.45	0.62	1.00
TNF-α	1.00	0.36	0.45	1.00
IL-6	1.00	0.53	0.72	1.00
MIP-2	1.00	0.71	0.86	1.00

TNF-α = tumor necrosis factor-α; IL-6 = interleukin-6; MIP-2 = macrophage inflammatory protein-2.

During constant flow conditions, and if resistances are constant, P_L changes linearly with time when compliance does not change with increasing lung volume. When compliance decreases, P_L is concave upward, and when compliance increases, P_L is concave downward with respect to the time axis.[12–15] Such an analysis of the P-t relation is based on the assumption that during volume-controlled ventilation with a constant flow inflation, the rate of change in pressure is related to the changes in pulmonary compliance.[12–15] Under these circumstances, the P_L profile as function of inspiratory time (t) can be described by a power equation: $P_L = a \cdot t^b + c$. The coefficient *a* is a scaling factor, *c* is the pressure value at t = 0, and the coefficient *b* describes the shape of the P-t curve.

In the current study, the quantification of VILI was based on a morphologic index of pulmonary damage (total airway injury score) and BALF concentrations of inflammatory mediators (TNF-α, IL-6, and MIP-2); values greater than those observed in an identical model of ALI (lung lavage) without mechanical ventilation were identified and used to quantify VILI. We also used the receiver–operating characteristic curve to circumvent the chief problem inherent in the technique of classic decision analysis—namely, dependence on the threshold value that is selected.[17] The areas under the curves were significantly larger than that of an arbitrary test that would be expected *a priori* to have no discriminatory value (*i.e.,* 0.50). The selected threshold value was the one that resulted in the fewest false-negative classifications. This decision was based on the assumption that the disadvantages associated with a false-negative result are higher than those associated with a false-positive result. Choosing as the threshold the value that gives the fewest false classifications (both negative and positive), we have a sensitivity equal to 1.00 for all markers of VILI and a specificity of 0.45, 0.36, 0.53, and 0.71 for total airway injury score, TNF-α, IL-6, and MIP-2, respectively. More importantly, when *b* was between 0.9 and 1.1, the injury was no greater than when the lungs were not ventilated; conversely, strategies that produced values of $b < 0.85$ or $b > 1.15$ did not "guarantee" injury, although there was a significant correlation between values of coefficient *b* and values of total airway injury score, IL-6, and MIP-2 (fig. 3). These results indicate that the use of coefficient *b* in the context of VILI should be seen as the therapeutic target to set protective ventilatory strategy rather than as a monitoring tool to detect VILI. Although values of coefficient *b* different from 1 were in a few cases related with already noninjurious ventilator settings (*i.e.,* low specificity), simple, safe, and inexpensive adjustments of PEEP and V_T leading to a straight P-t curve and to a coefficient *b* equal to 1 resulted in a ventilatory strategy that certainly minimized VILI (*i.e.,* optimal sensitivity).

This analysis requires several assumptions. First, the present study was performed in an *ex vivo* model without a chest wall; a stiff chest wall may influence estimation of the upward–downward concavity on the dynamic P-t curve.[13] Second, in more complex conditions, the P-t curve may be characterized by a sigmoidal shape with an initial downward concavity as a result of alveolar opening, followed by a linear portion, and ending with a final downward concavity as a result of alveolar overdistension. Under these circumstances, it would be best to fit the power equation first to the initial portion of the curve (*i.e.,* to set PEEP) and then to the second portion of the curve (*i.e.,* to set V_T). Third, on a theoretical basis, the time course of applied pressure during constant flow inflation should be characterized by an immediate step change owing to the resistive components, abruptly followed by the progressive increase in pressure reflecting the changes in pulmonary compliance.[14] However, on- and off-flow transients may be the result of pendelluft (*i.e.,* the time required to achieve a steady-state flow to each alveolar unit with different time constants),[22] viscoelasticity,[23,24] and the time required by the ventilator to initiate and stop delivery of constant flow.[12] The first part of the pressure events must therefore be discarded, and only the portion of the P-t relation corresponding to constant flow remains valid. Fourth, a high sampling frequency of the recorded signals is required to achieve an adequate dynamic recording of airway pressure with no phase lag at high frequency. Fifth, resistive and viscoelastic contributions to airway pressure are assumed constant over the range of changes in lung volume. Some of these factors may explain the relatively low specificity (*i.e.,* a relevant number of false-positive results) of the dynamic P-t profile to detect VILI (figs. 3 and 4); it remains to be evaluated whether these assumptions may limit the clinical use of the dynamic P-t curve to set mechanical ventilation.

We previously used two lung overdistension strategies with identical end-inspiratory volumes but different PEEP levels to assess VILI in an isolated rat lung model. We reported that, for a given end-inspiratory volume, the lungs treated with PEEP had significantly lower levels of cytokines than did those treated with zero end-expiratory pressure.[1] This suggests that the use of PEEP may be protective against VILI. In the current study, we confirm and expand our previous observations. Our data show that low PEEP levels (0–6 cm H_2O) yielding a P-t curve with a downward concavity resulted in ongoing recruitment (fig. 2) in association with severe lung damage. This deleterious effect was blunted when a straight P-t curve was obtained by increasing PEEP to as high as 13–20 cm H_2O in the minimal stress group. Our data also demonstrate that high PEEP may be beneficial as long as the P-t profile is linear, with a coefficient b that is close to 1. However, with any additional increases in PEEP and end-expiratory lung volume, an upward concavity in the P-t curve occurred (coefficient $b > 1$) associated with more severe lung damage.[25,26] The types of putative mechanical injury (alveolar opening and closing, downward concavity and coefficient $b < 1$, and alveolar to overdistension, upward concavity and coefficient $b > 1$) were kept distinct by study design. It is interesting to note that the amount of morphologic injury and release of inflammatory mediators in the BALF was similar for both potential mechanisms responsible of VILI[27] as b decreased or increased from 1.

Verbrugge *et al.*[28] recently observed that, in an intact rat model of surfactant deficiency, animals ventilated with high V_T and low PEEP had higher alveolar concentrations of proteins and prostacyclin than animals ventilated with high PEEP and low V_T; no difference was observed in alveolar concentration of TNF-α. In the current study, we confirmed that mechanical ventilation may elicit an inflammatory response, as already described in isolated lungs,[1] isolated and perfused lungs,[29] intact animals,[18] and humans with adult respiratory distress syndrome,[7] although the correlation between TNF-α and coefficient b was not statistically significant. These conflicting data on TNF-α support the concept that only determination of several inflammatory mediators instead of a single cytokine can assess the inflammatory response during different experimental and clinical conditions.[30] Moreover, the isolated lung model may exaggerate the amount of mechanical stress applied to the lung by mechanical ventilation compared with studies performed in intact animals.[28]

This study shows that the shape of the dynamic inspiratory P-t profile during constant flow inflation allows prediction of a ventilatory strategy that minimizes the occurrence of VILI in an isolated lung model of ALI. Modern ventilators are able to deliver excellent square-wave inspiratory flow profiles and are also equipped with monitoring tools that are able to provide online, dynamic P-t curves; however, additional clinical studies are required to confirm the utility of this approach for setting protective ventilatory strategies and minimizing VILI.

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