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CALL FOR PAPERS *Bioengineering the Lung: Molecules, Materials, Matrix, Morphology, and Mechanics*

A mathematical model to predict protein wash out kinetics during whole-lung lavage in autoimmune pulmonary alveolar proteinosis

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pulmonary alveolar proteinosis; granulocyte/macrophage colony-stimulating factor autoantibody; whole-lung lavage; protein transfer rate

PULMONARY ALVEOLAR PROTEINOSIS (PAP) is a rare lung disorder in which surfactant-associated phospholipids and proteins abnormally accumulate within alveoli and terminal bronchioles, leading to impaired gas exchange and progressive respiratory failure (6, 33, 40). PAP is classified into three groups based on etiology: autoimmune PAP (aPAP), secondary PAP, and hereditary PAP (6, 17, 40). aPAP is caused by granulocyte/macrophage colony-stimulating factor (GM-CSF) autoantibodies, which prevent surfactant removal by alveolar macrophages (20, 41). aPAP is the most prevalent form of PAP, comprising 90% of all PAP cases (6, 17, 40). Currently, whole-lung lavage (WLL) remains the only standard therapy for aPAP (4, 7, 29). Although WLL improves PAP in about 85–95% of patients, around 15–66% of such patients may require multiple and repeated WLL therapy (1, 4, 37). Removal of the lipoproteinous material by WLL immediately improves both lung volume and ventilation/perfusion ratio, leading to a marked increase in arterial oxygen gas pressure (5, 29, 36). In contrast, the diffusion capacity recovers gradually and incompletely over a 6-mo period (36). In addition, WLL decreases the area of ground-glass opacities but not reticular opacities and inter-

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lobular septal thickening (24). These observations suggest that the efficacy of WLL is not attributable to simple exclusion of accumulated surfactants but rather attributable to the recovery of normal lung structure and function.

GM-CSF autoantibodies and various other proteins (with the exception of large molecules such as IgM) have been reported to transfer the air-blood barrier (2, 9, 39). Both IgG1/albumin and IgG2/albumin ratios of the serum and bronchoalveolar lavage fluid (BALF) are similar, indicating transfer of these proteins (28). IgG most probably migrates by epithelial transcytosis or by paracellular diffusion through the air-blood barrier (13). In the steady state, the air-blood barrier consists of endothelial cells, basement membrane, epithelial cells, and surfactant film (8, 15). Surfactant film reduces leakage of plasma proteins to a minimum (15). In a previous study, disruption of surface tension-lowering properties of surfactant protein B (SP-B) in conditional knockout mice led to constriction of alveolar capillaries that resulted in protein leaks, lung edema, and alterations in alveolar surface area (15). Although no report describes the disruption of air-blood barrier after lung lavage, it is plausible that WLL removes the surfactant film from the alveolar surface followed by leaking plasma proteins.

In the present study, the kinetics of transfer of proteins from the blood and the surfactant to the lavage fluid was examined by measuring their concentrations in aliquots of lavage fluid drained during WLL. For this purpose, we proposed a mathematical model that can account for the transfer of proteins from the blood and the surfactant to the lavage fluid. The transmission coefficients were optimized, and the temporal variations of protein concentrations were simulated. Finally, the proposed model was evaluated by comparison with the measured data. Moreover, we showed the limitations of the present model.

Glossary

A_b	The effective surface area from the blood
A_s	The effective surface area from the surfactant
K_b	The transmission coefficient from the blood
K_s	The transmission coefficient from the surfactant
m_b	The masses of protein in the blood
$m_{in\text{-}out}$	The masses of protein in instilling saline and draining lavage fluid; actually, no protein exists in instilling saline
m_l	The masses of protein in the lavage fluid
m_{out}	The protein mass of drainage
m_s	The masses of protein in the surfactant
R_{cl}	The absorption rate of fluid into the circulation
S_A	The alveolar surface area
V_A	The alveolar volume
V_b	The volume of blood
V_{in}	The fluid volume of instilled saline
V_l	The volume of lavage
$V_{l\text{-}b}$	The fluid volume absorbed into the circulation
V_{out}	The fluid volume of drainage
V_s	The volume of surfactant

MATERIALS AND METHODS

Participants

Nine patients were enrolled in five hospitals in Japan. These hospitals included Tohoku University Hospital, Tokyo Medical University Hachioji Hospital, Aichi Medical University Hospital, Dokkyo Medical University Koshigaya Hospital, and Niigata University Medical and Dental Hospital. Diagnosis of aPAP was performed on the basis of cytological analysis of BALF, pulmonary histopathological findings, or both with high-resolution computed tomography appearance (40). All cases were confirmed to have elevated serum GM-CSF autoantibody levels (21, 41). The institutional review board of each hospital approved the study, and all subjects provided written informed consent. The study protocol was designed according to The Ethical Guideline of Clinical Research by The Japanese Ministry of Health, Labour, and Welfare in 2008.

Data of arterial blood gas analyses and serum markers were collected within 3 days, and pulmonary function tests were within 2 wk prior to WLL.

Procedure of WLL

Seventeen lungs from nine patients with aPAP underwent WLL. We allowed each participating hospital to conduct WLL in accordance with their own procedures. Generally, after administration of general anesthesia, patients were intubated with a double-lumen endotracheal tube to isolate the lungs, after which mechanical ventilation was initiated. After ventilation of the bilateral lungs with 100% oxygen for 5–15 min, saline was instilled into the lavage lung while ventilation of the other lung with 100% oxygen was continued. The instilled saline was then retained for a few minutes and then discharged by gravity into a container until a decrease in outflow was observed. These procedures were then repeated. In each lavage cycle, we prepared a timetable to record the exact time (to the second) of the start of instilling saline, the start of retaining, and the start and end of lavage fluid draining. We measured the volume of drained lavage fluid and used a 10-ml aliquot for further analyses. All samples were stored at −80°C until use.

Measurement of Substance Concentration

The serum and BALF concentration of IgG, GM-CSF autoantibody, transferrin, albumin, β_2-microglobulin, urea, gastrin, and SP-D were measured: IgG were quantified by an ELISA system using Human IgG ELISA Quantitation Set (Bethyl Laboratories, Montgomery, AL) according to the manufacturer's instructions. GM-CSF autoantibody concentrations were measured by an ELISA system as described previously (17). β_2-Microglobulin, gastrin, urea, and SP-D concentrations were measured by latex agglutination immunoassay (LA: LZ test Eiken β_2-M-II; Eiken, Tokyo Japan), radioimmunoassay (gastrin RIA kit II; Fujirebio, Tokyo, Japan), urease-indophenol method (urea nitrogen test; Wako, Tokyo, Japan), and enzyme immunoassay (SP-D kit Yamasa EIA II; Yamasa, Tokyo, Japan), respectively. Serum transferrin and albumin concentrations were measured by turbidimetric immunoassay (TIA; N-Assay TIA Tf-H Nittobo; Nittobo, Tokyo, Japan) and bromocresol purple dye-binding assay (PureAuto A ALB; Kainos, Toyko, Japan), respectively, and those in the BALF were analyzed by LA (N-Assay LA Micro Tf Nittobo) and TIA (AutoWako Microalbumin). These serum samples were collected just before the beginning of WLL.

A Mathematical Kinetic Model to Estimate the Concentration of Proteins in the Lavage Fluid

We postulated that proteins both in the accumulated surfactant material and in the pulmonary capillaries transfer into the lavage fluid. Under such circumstances, the rate of protein transfer to the lavage fluid is assumed to be as follows:

$$\frac{dm_l}{dt} = \frac{dm_s}{dt} + \frac{dm_b}{dt} + \frac{dm_{in-out}}{dt} \quad (1)$$

where the first term in the right-hand side is the transfer rate from surfactant, the second term is the transfer rate from blood, and the

third term is the rate of instilling and drainage. The transfer rate from surfactant dm_s/dt and the transfer rate from blood dm_b/dt are modeled by analogy to the heat transmission model as

$$\frac{dm_s}{dt} = K_S \cdot A_S\left(\frac{m_s}{V_s} - \frac{m_l}{V_l}\right) \quad (2)$$

$$\frac{dm_b}{dt} = K_b \cdot A_b\left(\frac{m_b}{V_b} - \frac{m_l}{V_l}\right) \quad (3)$$

where K_s and K_b are the transmission coefficient from the surfactant and the blood to the lavage fluid, respectively. A_s and A_b are the effective surface area from the surfactant and the blood to the lavage fluid, respectively. The parameters m_l, m_s, and m_b represent the masses of protein in the lavage fluid, surfactant, and blood, respectively. V_l represents the fluid volume in lavage. V_s and V_b represent the fluid volume in surfactant and blood. We assumed that m_b, V_s, and V_b are constant during the WLL.

We calculated the temporal variation of the mass of protein and the volume of fluid in the stages of instilling, retaining, draining, and preparing in each lavage cycle, as described as follows.

Instilling stage. The volume change of protein and lavage fluid in the lung is expressed as:

$$\frac{dm_{in-out}}{dt} = 0 \quad (4)$$

$$\frac{dV_l}{dt} = \frac{dV_{in}}{dt} - \frac{dV_{l-b}}{dt} \quad (5)$$

where V_{in} is the fluid volume of instilled saline, and V_{l-b} is the fluid volume absorbed into the circulation expressed as

$$\frac{dV_{l-b}}{dt} = A_b \cdot R_{cl} \quad (6)$$

where R_{cl} is the absorption rate of fluid into the circulation. The concentration of protein was calculated as the ratio of the mass of protein to the fluid volume calculated from *Eqs. 1–6* according to the procedures described in the following subsection.

Retaining stage. No saline is instilling in the retaining stage, which means

$$\frac{dV_{in}}{dt} = 0 \quad (7)$$

The variation of the mass of protein and the volume of fluid were calculated from *Eqs. 1–7*.

Draining stage. The lavage fluid is drained in this stage, which means

$$\frac{dm_{in-out}}{dt} = -\frac{dm_{out}}{dt} \quad (8)$$

$$\frac{dV_l}{dt} = -\frac{dV_{out}}{dt} - \frac{dV_{l-b}}{dt} \quad (9)$$

where m_{out} and V_{out} are the protein mass and the fluid volume of drainage. The variation of the mass of protein and the volume of fluid were calculated from *Eqs. 1–3* and *6*, *8*, and *9*.

Preparing stage. Substance transfer in this stage may be considered to be similar to that in the retaining stage.

Data Processing and Statistics

Data including patient identity, protein concentrations in the serum or in the BALF of the right and left lungs, vital capacity, the number of cycles, the volume of instilled saline or drained lavage fluid, and time for each lavage stage were entered into a file (Microsoft Excel 2010). Using theoretical equations that solved protein concentrations in the drained lavage fluid (described in RESULTS), we wrote a program using Visual Basic Application to calculate the theoretical concentrations of proteins on the basis of specific variables.

Estimation of the protein concentration in the drained lavage fluid was carried out by numerically integrating differential equations using the following parameters: the volume of instilled saline, drained lavage fluid, time of each stage, the concentration of proteins in the first lavage cycle, effective alveolar and capillary surface area described below, and a given set of transmission coefficients, K_s and K_b. The resulting concentration curve was optimized with actual measurements manually by changing transmission coefficients. The effective areas of alveolar surface and pulmonary capillaries were calculated according to the equations described in Appendix A (10).

Numerical data were evaluated for normal distribution by using Shapiro-Wilk tests. Nonparametric data were analyzed by using Kruskal-Wallis rank sum test. Multiple comparisons were performed through a Bonferroni-adjusted Wilcoxon rank-sum test. All tests were two-sided, and P values <0.05 were considered statistically significant. Data were analyzed by using R-version 2.15.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Demographic and Clinical Findings for Study Subjects

Nine patients with active aPAP were enrolled in this study. Demographic data are shown in Table 1.

The mean age at WLL was 54.3 ± 11.4 yr old, with a male-to-female ratio of 2:1. The duration of the disease from onset was variable, ranging 10–96 mo. Patients showed no evidence of active pulmonary infection. Pulmonary functions and laboratory findings are described in Table 2.

The mean arterial oxygen pressure at room air was 64.0 ± 15.4 mmHg in seven patients and 55.2 and 67.4 mmHg for two patients under nasal oxygen supply. Percentage of vital capacity and percentage of carbon monoxide diffusing capacity were moderately to severely suppressed with 72.1 ± 17.7% and 51.0 ± 21.5%, respectively, whereas forced expiratory volume in 1 s/forced vital capacity was relatively conserved with 85.8 ± 10.9%. The mean serum biomarker levels of Krebs von den Lungen-6, SP-D, and carcinoembryonic antigen were 20,720 ± 13,953 IU/ml, 471 ± 271 ng/ml, and 26.7 ± 22.9 ng/ml, respectively. The mean serum GM-CSF autoantibody levels were 45.8 ± 51.7 μg/ml. These patient characteristics were similar to a past large Japanese cohort with PAP (17).

Timetables and Volume Balance for WLL

As shown in Fig. 1, *A* and *B*, each lavage cycle consisted of four stages: instilling (from the beginning to the end of saline instillation), retaining (from the end of saline instillation until the beginning of drainage), draining (from the beginning until the end of drainage), and preparing (from the end of drainage until the beginning of the next saline instillation). Twelve lungs from seven patients underwent WLL with short-term cycles (210–285 s), whereas five lungs from three patients underwent WLL with long-term cycles (550–634 s) (Table 3). In eight patients, both lungs underwent WLL; however, for one patient, only the right lung underwent lavage. Data for instilled saline volume, discharged lavage fluid volume, and time for each of the stages as defined above are shown in Tables 3 and 4. Lavage was repeated 11 to 29 times (median of 20 cycles) until the lavage fluid appeared clearer (Fig. 1*C*).

Time required for total WLL time ranged from 5,200 to 11,796 s. Instilling, retaining, draining, and preparing time

Table 1. *Demographic data on study subjects who underwent WLL*

Case	Age, yr	Sex	Symptoms*	Onset to WLL†, mo	DSS‡	Smoking Status	Occupational Dust Exposure	Complications
1	45	M	DOE, Occasional Cough	66	2	Ex-Smoker	No	Psoriasis, HT, DL
2	54	F	DOE	43	2	Never	No	HT, DL
3	67	M	Dyspnea, Cough, Hemosputum	10	5	Ex-Smoker	No	Postcerebral Infarction
4	34	M	Dyspnea	42	5	Current Smoker	No	None
5	53	F	Dyspnea, Cough	96	5	Never	No	SSS
6	46	M	None	30	1	Current Smoker	Yes	None
7	66	M	DOE	25	4	Never	No	DM
8	67	F	Dyspnea	36	5	Never	No	None
9	57	M	DOE	28	2	Never	No	None

*Symptoms were recognized as respiratory symptoms. †Onset: time when the 1st respiratory symptom emerged or time of finding an abnormal image that was compatible with pulmonary alveolar proteinosis (PAP). ‡Disease severity score (DSS): defined based on respiratory symptoms and arterial oxygen tension (PaO_2; see Ref. 3). DSS 1: no symptoms and $PaO_2 \geq 70$ mmHg. DSS 2: symptomatic and $PaO_2 \geq 70$ mmHg. DSS 3: 60 mmHg $\leq PaO_2 < 70$ mmHg. DSS 4: 50 mmHg $\leq PaO_2 < 60$ mmHg. DSS 5: $PaO_2 < 50$ mmHg. WLL, whole-lung lavage; DOE, dyspnea on exertion; HT, hypertension; DL, dyslipidemia; SSS, sick sinus syndrome; DM, diabetes mellitus.

ranged 10–225 s, 120–425 s, 52–225 s, and 0–96 s, respectively. In 11 of 17 lungs, the retaining time was designed to be 120 s, but it was variable with 132–425 s in 6 lungs. Time required for other stages varied remarkably as shown in Table 3.

The initial volume of instilled saline ranged within 600–2,300 ml (1,359 ± 435 ml). The initial discharged volume ranged from 150 to 1,282 ml (697 ± 341 ml), with percentage of recovery ranging from 24.3 to 71.4%. The mean volume of instilled saline from the second to the last lavage in each patient varied from 489 to 1,938 ml (893 ± 374 ml). In each cycle, 461–1,896 ml (859 ± 364 ml) of discharged fluid was recovered; the recovery percentage was relatively constant (89.7–100.3%). As a whole, the total volume of saline instilled into each lung was 9,900–28,200 ml, and the total volume of discharged lavage fluid was 8,910–27,000 ml, with total percentage of recovery of 93.4 ± 3.3 (82.1–95.9%).

Simulation of Protein Concentrations in the Drained Lavage Fluid

The theoretical concentrations of IgG, transferrin, albumin, and β_2- microglobulin in the drained lavage fluid were calculated at the end of the draining stage according to the equation described above and by using the procedures detailed in MATERIALS AND METHODS. The theoretical concentrations were plotted on a log scale against time after the beginning of WLL (Fig. 2). The plot for each patient was manually fitted with the protein concentration measured in the drained lavage fluid of each cycle by changing K_s and K_b. Plots for the theoretical concentrations of IgG, transferrin, albumin, and β_2-microglobulin coincide with the measurements (Fig. 2, *A–D*). Data for K_s and K_b are shown in Fig. 3. K_s values for IgG, transferrin, albumin, and β_2-microglobulin ($2.03 \times 10^{-7} \pm 0.902$, $1.95 \times 10^{-7} \pm 0.589$, $1.84 \times 10^{-7} \pm 0.564$, and $1.85 \times 10^{-7} \pm 0.658$, respectively) did not vary among patients (Fig. 3*A*). Importantly, there was no significant difference in K_s values among these four proteins, suggesting that transfer from the surfactant to lavage fluid was independent of molecular weight. However, there was relative variability in K_b among proteins, especially with β_2-microglobulin, which had a K_b that was two orders of magnitude higher than that of the other proteins. K_b values for IgG, transferrin, albumin, and β_2-microglobulin were $4.97 \times 10^{-10} \pm 4.166$, $5.61 \times 10^{-10} \pm 1.990$, $3.82 \times 10^{-10} \pm 1.661$, and $2.28 \times 10^{-8} \pm 0.773$, respectively (Fig. 3*B*). No differences in K_s or K_b values of each protein were found between left and right lungs (data not

Table 2. *Clinical parameters of patients*

	Arterial Blood Gas Analysis			Serum Biomarkers				Pulmonary Function Test			
Case	PaO_2, mmHg	$PaCO_2$, mmHg	A-aDO_2, mmHg	KL-6, IU/ml	SP-D, ng/ml	CEA, ng/ml	GM-Ab, μg/ml	%VC, %	FEV_1/FVC, %	FRC, liters	$\%D_{L,CO}$, %
1	81.3	35.8	24.0	7611	963	6.9	6.8	85.8	82.7	2.51	60.5
2	75.6	39.4	25.1	32070	187	12.5	116.0	81.9	79.43	1.77	38.2
3	67.4*	35.5*	269.0*	46700	518	41.4	25.2	51.4	84.5	2.36	19.2
4	55.2*	38.4*	167.7*	26300	799	49.1	25.0	47.7	78.98	1.52	30.0
5	46.4	34.8	60.1	31093	422	19.8	35.7	47.4	98.1	1.41	ND†
6	72.3	38.8	29.2	8356	236	12.8	35.5	85.9	82.9	2.49	70.7
7	51.2	36.7	52.9	10969	407	73.3	6.7	87.5	109.4	1.71	70.8
8	46.2	38.2	55.1	15700	531	16.5	10.4	77.5	80.3	1.53	ND
9	75.3	39.6	24.9	7684	172	7.9	150.9	83.9	75.8	2.50	67.8

Normal Krebs von den Lungen-6 (KL-6), surfactant protein D (SP-D), carcinoembryonic antigen (CEA), and granulocyte/macrophage colony-stimulating factor autoantibody (GM-Ab) levels were within 500 IU/ml, 110 ng/ml, 5.0 ng/ml, 1.0 μg/ml, respectively. *Nasal oxygen supply. †Diffusing capacity of the lung for carbon monoxide ($D_{L,CO}$) of case 5 was not detected because of low vital capacity (VC). ND, not done. A-aDO_2, alveolar-arterial oxygen difference that was measured; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; FRC, functional residual capacity.

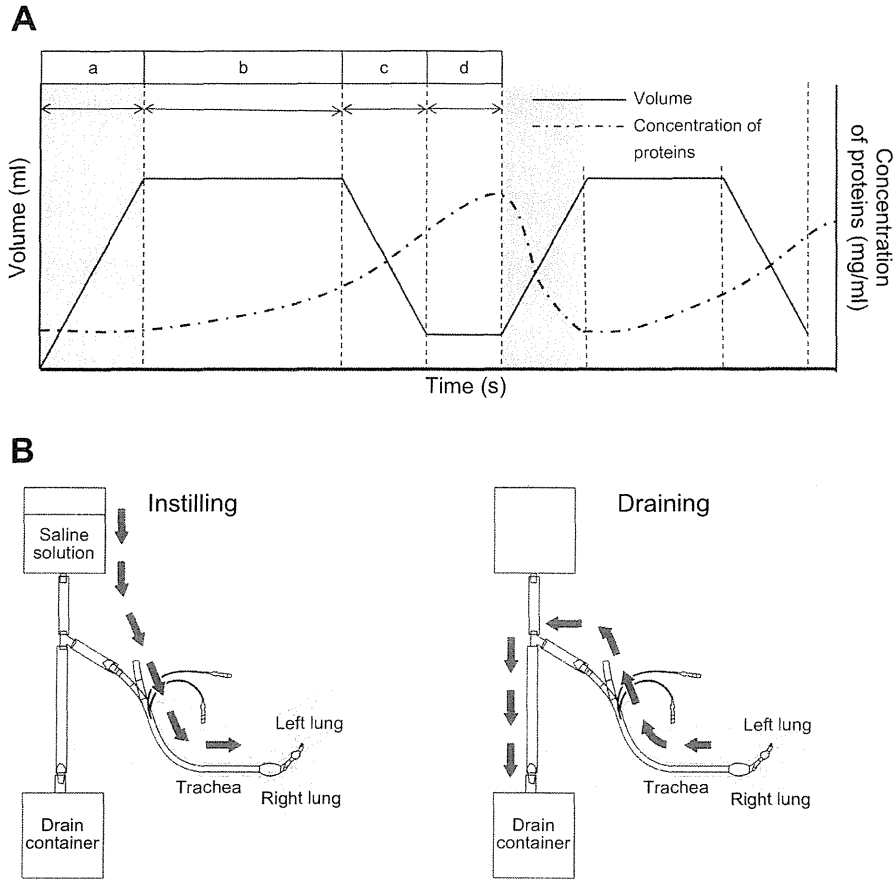

Fig. 1. *A*: conceptual schematic of the time course of the lavage fluid volume in the lung and the concentration of a protein during whole-lung lavage (WLL). Each lavage cycle involved 4 stages: instilling (*a*, from the beginning to the end of saline instillation), retaining (*b*, from the end of saline instillation until the beginning of drainage), draining (*c*, from the beginning to the end of drainage), and preparing (*d*, from the end of drainage to the beginning of the next saline instillation). *B*: schematic of the procedures for instilling (*left*) and draining (*right*) stages. ~0.5 to 2.0 l of saline solution from a bottle was instilled through an endotracheal tube into the lavage lung, retained for a few minutes, and then drained into a draining container. *C*: appearance of the drained fluid obtained from the first to the 20th lavage cycle.

shown). Thus the simulation data confirm the appropriateness of our mathematical model and indicate that the transfer kinetics of proteins into the drained fluid was time dependent.

Durable Effects of the Time on the Lavage Efficiency

To determine the durable effect of each lavage cycle on the slope for the decreasing concentration of each protein in the drained lavage fluid, we evaluated the change in slope of the theoretical curves by varying the duration of the retaining stage in silico. For this purpose, we used the initial data settings in case 4, i.e., the instilling volume of saline; the durations (s) of instillation, retaining, draining, and preparing; and the volume of drained lavage fluid in the first lavage cycle. We found that decreasing curves for the albumin concentration became steeper upon substitution of the shorter time (Fig. 4*A*).

Next, we proceeded to confirm the effects observed in the simulation by using measurements in case 1. We evaluated the rate of declining albumin concentration in the lavage aliquots from a patient who occasionally underwent WLL for the left lung with short-term cycles (120 s, 1–20 cycles) and for the right lung with long-term cycles (540 s, 4–11 cycles). As shown in Fig. 4*B*, the slope of decline for the left lung appeared

Table 3. *Timetable of the stages in each cycle of WLL*

Case	Left/Right	No. of Cycles	Total Time, s	Mean Time ± SD, s*			
				Each Cycle, s†	Stage a, Instilling, s	Stage b, Retaining, s	Stage c, Draining, s	Stage d, Preparing, s
1	L	20	6260	258 ± 15.0	42 ± 3.0	120 ± 1.6	83 ± 42.2	13 ± 18.6
	R‡	11	6895	561 ± 161.7	41 ± 8.1	425 ± 196.2	59 ± 16.4	8 ± 2.6
2	L	20	5200	210 ± 21.9	28 ± 3.0	120 ± 0	52 ± 23.2	13 ± 5.1
	R	20	5450	220 ± 8.3	45 ± 5.9	120 ± 0	52 ± 16.1	8 ± 8.1
3	L	20	5583	237 ± 15.1	36 ± 7.0	120 ± 0	73 ± 18.4	10 ± 2.6
	R	20	6060	236 ± 38.8	35 ± 6.0	120 ± 0	64 ± 26.8	20 ± 16.8
4	L	24	5926	215 ± 19.4	35 ± 4.1	120 ± 0	54 ± 24.7	10 ± 3.3
	R	29	9018	285 ± 17.5	57 ± 3.9	120 ± 0	102 ± 16.8	8 ± 3.3
5	L	20	5230	227 ± 30.6	31 ± 11.9	120 ± 0	76 ± 31.9	11 ± 4.7
	R	20	6480	284 ± 25.3	31 ± 6.2	120 ± 0	121 ± 20.2	19 ± 24.8
6	L	20	5395	266 ± 32.3	10 ± 0	120 ± 0	133 ± 32.0	5 ± 0
	R	20	5680	278 ± 42.2	28 ± 11.2	132 ± 24.6	112 ± 24.8	5 ± 0
7	R	20	6180	283 ± 31.6	52 ± 10.1	120 ± 0	109 ± 41.4	13 ± 11.5
8	L	16	10380	634 ± 264.9	180 ± 32.1	199 ± 28.7	148 ± 50.0	96 ± 250.3
	R	20	11796	550 ± 49.5	188 ± 41.3	200 ± 6.2	153 ± 20.5	16 ± 20.6
9	L	11	6180	553 ± 185.8	146 ± 42.8	193 ± 24.2	174 ± 82.2	40 ± 112.3
	R	13	8280	627 ± 98.1	225 ± 45.2	231 ± 64.1	225 ± 63.3	0 ± 0

*Data are presented as a mean ± SD of time (s) required for 1 lavage cycle. Time for total on each stage of lavage cycle is expressed as a mean ± SD. Instilling time (stage a) is mean time (s) required for instilling saline into the lung. Retaining time (stage b) is mean time (s) applied for retaining saline in the lung. Draining time (stage c) is mean time (s) required for draining lavage fluid to the container. Preparing time (stage d) is mean time (s) required for preparation for the next saline instillation. †Each cycle time is the mean of stage a to d from 2nd to the last lavage. The 1st cycle required 120–1080 s. ‡Time (s) for the 1st 3 cycles ranged within 230–270 s, and that for the 4th to 11th cycles ranged within 625–680 s.

steeper than that for the right lung. The time required to reach 10% of the initial concentration of albumin in the first lavage was 2,730 s for the left lung, whereas it was 4,390 s for the right lung. Notably, both K_S and K_b of the left and right lungs were comparable (1.77×10^{-7} and 4.97×10^{-10} cm/s, respectively, for the left lung; 1.60×10^{-7} and 3.20×10^{-10} cm/s, respectively, for the right lung).

When 1,000 ml of saline was assumed to be instilled into the lung in each cycle, the cumulative amount of albumin drained into the lavage fluid did not differ remarkably within retaining time of 90–570 s (Fig. 4*C*). The curve in short retaining time (90 s) slightly exceeded those in long retaining time (450–570 s) but reversed after 4,000 s. In this setting, the simulation impressed that ~3,200 s (53.3 min) would be required for enough elimination of albumin but that the efficiency of elimination would not significantly change after 5,400 s (90 min).

Effect of Instilled Saline Volume on the Efficiency of WLL

Eqs. 1–3 described in MATERIALS AND METHODS meant that the effect of WLL on elimination of proteins was affected by the instilled saline volume into the lung. As shown in Fig. 4*D*,

Table 4. *Volume balance during WLL*

Case	Left/Right	First Cycle		Second to the Last Cycle			
		Instilled Volume, liters	Drained Volume, liters (recovery %)	Average Instilled Volume, liters	Average Drained Volume, liters (recovery %)	Total Instilled Volume, liters	Total Drained Volume, liters (recovery %)
1	L	1.70	1.05 (61.8)	0.96	0.93 (96.6)	20.0	18.7 (93.7)
	R	1.90	1.10 (57.9)	1.04	1.01 (97.1)	12.3	10.1 (82.1)
2	L	1.40	0.50 (35.7)	0.55	0.51 (91.4)	11.9	10.1 (84.9)
	R	1.50	0.90 (60.0)	0.84	0.81 (96.6)	17.5	16.4 (93.5)
3	L	1.50	1.00 (66.7)	0.85	0.82 (96.3)	16.9	16.6 (93.8)
	R	1.70	0.90 (52.9)	0.88	0.89 (100.3)	18.5	17.8 (95.9)
4	L	0.90	0.37 (41.1)	0.64	0.63 (97.1)	16.5	14.7 (89.3)
	R	1.40	1.00 (71.4)	0.96	0.93 (97.0)	28.2	27.0 (95.7)
5	L	0.60	0.15 (25.0)	0.49	0.46 (94.2)	9.90	8.91 (90.0)
	R	1.00	0.32 (32.0)	0.59	0.56 (95.4)	12.2	11.0 (90.2)
6	L	1.00	0.60 (60.0)	0.55	0.53 (96.6)	11.4	10.7 (93.4)
	R	1.00	0.50 (50.0)	0.63	0.61 (95.8)	13.0	12.0 (92.3)
7	R	1.00	0.50 (50.0)	0.76	0.75 (98.6)	15.5	14.8 (95.5)
8	L	1.10	0.27 (24.3)	0.94	0.85 (89.7)	15.2	13.0 (85.0)
	R	1.30	0.47 (36.0)	0.97	0.95 (98.5)	19.8	18.5 (94.4)
9	L	1.80	0.93 (51.9)	1.58	1.48 (93.8)	17.6	15.7 (89.5)
	R	2.30	1.28 (55.7)	1.94	1.90 (97.8)	25.6	24.0 (94.1)

The instilling volume of the 1st cycle in case 1–7 was determined by the following equations: functional residual capacity (ml) × 0.45 or 0.55 + tidal volume for the left and right lung, respectively. In case 8 and 9, saline was allowed to be instilled into the lung as much as possible from a bottle at 30 cm height from the tracheal tube.