

# Spectroscopic approach to capillary-alveolar membrane damage induced acute lung injury

Jing Wang PhD<sup>1</sup>, Luis Oppenheimer MD PhD<sup>2</sup>, Paola Fata MD<sup>2</sup>,  
Julio Pintin MD<sup>2</sup>, Ross Stimpson MD<sup>2</sup>, Henry H Mantsch PhD<sup>1</sup>

<sup>1</sup>*Institute for Biodiagnostics, National Research Council of Canada, <sup>2</sup>Department of Surgery, Health Sciences Centre, Winnipeg, Manitoba*

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**BACKGROUND:** Acute (or adult) respiratory distress syndrome (ARDS) is often associated with a high mortality rate in the critical care population. The term acute lung injury (ALI), a primitive phase of ARDS, was introduced by the European and American consensus groups to provide early diagnoses of ARDS. The pathophysiological characterization of ALI/ARDS – an increased pulmonary capillary-alveolar membrane barrier permeability – is generally not included in current intensive care unit diagnosis criteria.

**OBJECTIVES:** To apply the infrared (IR) spectroscopic technique, in combination with the administration of hydroxyethyl starch (HES), to patients with ALI and ARDS.

**PATIENTS AND METHODS:** This retrospective study involved 67 patients from the intensive care unit at the Health Sciences Centre, University of Manitoba, Winnipeg, Manitoba. The methodology was based on the IR spectroscopic determination of HES in patient's bronchial washing fluid. Exaggerated infiltration of HES into the alveolar space was taken as evidence of damage to the pulmonary capillary-alveolar membrane, which in turn provided a diagnosis of ALI/ARDS.

**RESULTS:** The accuracy of determining pulmonary HES leakage in severe lung injury (Partial pressure of arterial oxygen/fraction of inspired oxygen [ $PaO_2/FiO_2$ ] less than 100 mmHg [n=10]), was 100%. The subgroups with  $PaO_2/FiO_2$

between 100 and 200 mmHg (n=23), and  $PaO_2/FiO_2$  between 200 and 300 mmHg (n=22), 56.5% and 77.3%, respectively, showed IR positive for HES leakage.

**CONCLUSIONS:** The proposed IR bronchial washing assay is very sensitive in determining the pulmonary HES leakage in severe lung injury. It is also suitable for evaluating pulmonary leakage at an early phase of the injury, a fact that is particularly important for supportive treatment. The method is advantageous because no radioactive tracers are employed, little sample preparation is required, and it is rapid and minimally invasive, making it convenient to use in the critical care environment.

**Key Words:** *Infrared spectroscopy; Lung injury*

## Utilisation de la spectroscopie infrarouge pour prouver des dommages causés à la membrane alvéolo-capillaire dans une atteinte aiguë du poumon

**HISTORIQUE :** Le syndrome de détresse respiratoire de l'adulte (SDRA) est souvent associé à un taux élevé de mortalité chez les patients nécessitant des soins intensifs. Le terme « atteinte aiguë du poumon » (AAP), une phase précoce du SDRA a été introduit par les groupes de consensus américains et européens pour fournir des diagnostics précoces du SDRA. La caractérisation physiopathologique de l'entité AAP-SDRA – un accroissement de la perméabilité de la

*voir page suivante*

*Correspondence and reprints: Dr HH Mantsch, Spectroscopy Section, Institute for Biodiagnostics, 435 Ellice Avenue, Winnipeg, Manitoba R3B 1Y6. Telephone 204-984-4622, fax 204-984-5472, e-mail Henry.Mantsch@nrc.ca*

membrane alvéolo-capillaire pulmonaire – ne figure pas dans les critères diagnostiques courants des services de soins intensifs.

**OBJECTIFS :** Appliquer la spectroscopie infrarouge (SI) en combinaison avec l'administration d'hydroxyéthylamidon (HEA), à des patients atteints d'une AAP et d'un SDRA.

**PATIENTS ET MÉTHODES :** Cette étude rétrospective portait sur 67 patients du service de soins intensifs du *Health Sciences Centre* de l'Université du Manitoba, à Winnipeg, au Manitoba. La méthodologie a été basée sur la détermination par SI du contenu d'HEA dans le liquide de lavage bronchique des patients. Une infiltration exagérée d'HEA dans l'espace alvéolaire a été considérée comme la preuve d'une atteinte de la membrane alvéolo-capillaire pulmonaire qui, en retour, a fourni un diagnostic d'AAP-SDRA.

**RÉSULTATS :** La précision de la détermination de l'infiltration d'HEA dans l'espace alvéolaire dans le cas d'une atteinte sévère du poumon (pression partielle d'oxygène

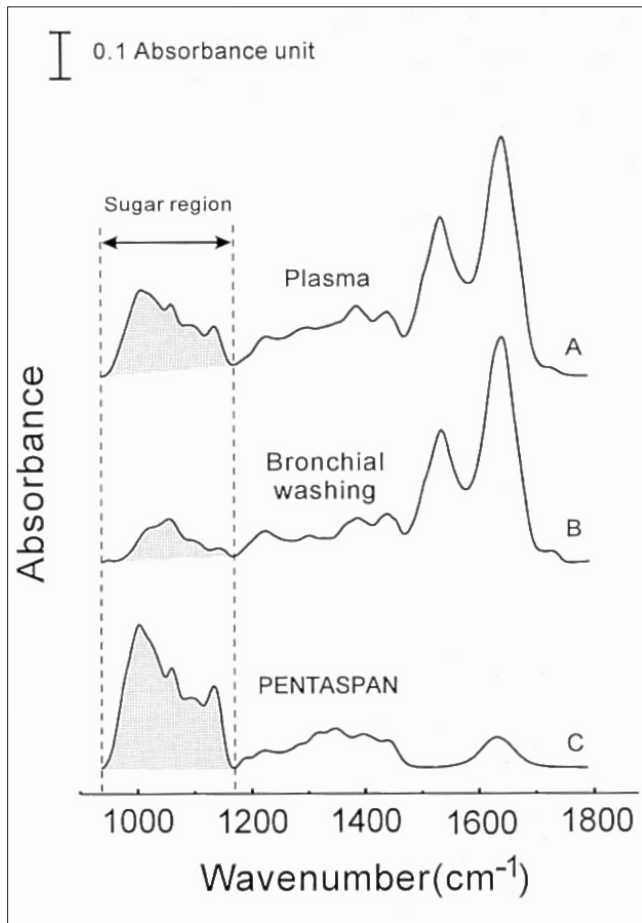
artériel/ concentration d'oxygène inspiré  $[PaO_2/FiO_2]$  inférieure à 100 mmHg [ $n = 10$ ], était de 100 %. Les sous-groupes avec un rapport  $PaO_2/FiO_2$  compris entre 100 et 200 mmHg ( $n = 23$ ), et un rapport  $PaO_2/FiO_2$  compris entre 200 et 300 mmHg ( $n = 22$ ), soit respectivement, 56,5 % et 77,3 %, démontraient une preuve d'infiltration d'HEA à la spectroscopie infrarouge.

**CONCLUSIONS :** La technique proposée pour déterminer par SI le contenu du liquide de lavage bronchique est très sensible pour prouver une infiltration d'HEA dans une atteinte sévère du poumon. Elle est aussi appropriée pour évaluer l'infiltration pulmonaire dans la première phase de l'atteinte, un fait qui est particulièrement important pour appliquer un traitement de soutien. Cette méthode présente l'avantage de ne pas faire appel à des marqueurs radioactifs, nécessite peu de préparation des échantillons, est rapide et peu effractive, ce qui la rend facile d'emploi dans un service de soins intensifs.

The term acute lung injury (ALI) is applied to acute abnormalities of pulmonary function. This condition results from diffuse alveolar damage leading to noncardiogenic pulmonary edema, and is characterized by increased pulmonary vascular permeability because of the breakdown of capillary endothelial and epithelial membrane barriers. Pulmonary edema, hypoxemia and decreased gas exchange function across the alveolar membrane are common clinical features related to this syndrome. The commonly known acute (or adult) respiratory distress syndrome (ARDS) is the severe form of ALI. Despite advances in medical therapy, there is still a high mortality rate due to ARDS, in excess of 50%, in the critical care population. ALI/ARDS diagnosis is largely dependent on the patient's pulmonary function, and is assessed by a lung injury scoring (LIS) system proposed by Murray et al (1). The system includes four parameters: chest x-ray evaluation, hypoxemia score, the respiratory system compliance score, and the optimal positive end-expiratory pressure score. Although the scoring system performs adequately in clinical terms, it provides no direct measure of pulmonary vascular permeability, which is the pathognomonic feature of ALI/ARDS.

Considerable attention has been paid to the determination of lung microvascular permeability in critically ill patients. Despite this, demonstrating the presence of increased capillary permeability remains a challenge. Several methods have been introduced to measure pulmonary capillary permeability in abnormal states. Most methods rely on the use of radioactive isotopes as tracers. Indicator-dilution methods introduce specifically labelled components, typically  $^3H_2O$ ,  $^{14}C$ -urea,  $^{125}I$ -albumin and  $^{51}Cr$  labeled red blood cells, into the circulation (2-4). The time course of these tracers in the blood is then followed in a single pass, requiring rapid and precisely timed arterial blood sampling. Based on partitioning models for the variously labelled components, the permeable surface area for urea and the extravascular volume of lung water (EVLW) can be determined. It is difficult, however, to establish clinically the soundness of these determinations. This is especially true

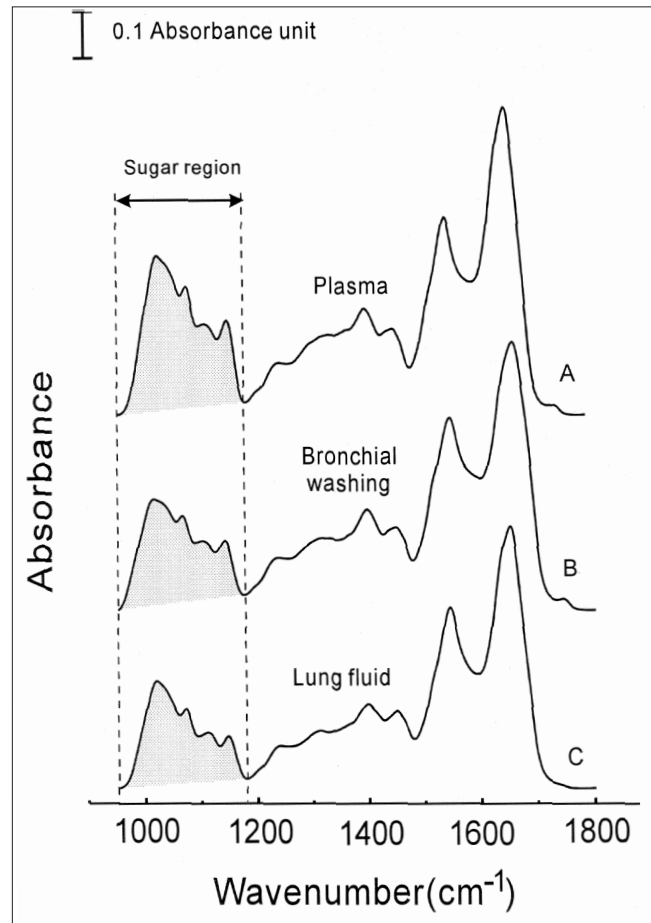
for the critical care population, where vascular tone and tissue permeability characteristics vary considerably. In addition, measuring EVLW by indicator-dilution methods is a costly and tedious bedside procedure. It has a reliability of less than 70% for the best case (5). External pulmonary gamma counting has the advantage of providing a more direct measure of tracer infiltration into the lung (6,7). The technique is, however, cumbersome and thus ill-suited to routine patient monitoring in a critical care environment. Clearly, a better procedure is warranted to diagnose ALI/ARDS effectively, and this procedure should be minimally invasive and cause little trauma to these already critically ill patients. In this paper, we introduce a new approach to detect pulmonary edema induced by increasing capillary-alveolar permeability in ALI. The approach is based on infrared (IR) spectroscopy, a technique used for the clinical diagnosis of diseased tissues and biofluids (8-11), in combination with the administration of hydroxyethyl starch (HES). The IR spectroscopic assessment of pulmonary permeability in ALI/ARDS patients does rely on the determination of PENTASPERAN (Du Pont Pharma, Mississauga, Ontario), which is a low molecular weight HES infiltrating the alveolar space. PENTASPERAN is a substituted glucose polymer with an average molecular weight of 200 to 300 kDa. Clinically, HES is used primarily as a colloid plasma volume expander. In the normal situation, the size of infused HES largely confines these molecules to the intravascular space until they are hydrolyzed by serum alpha-amylases into subunits small enough to be filtered and eliminated by the kidney (12-14). Certain pathological conditions capable of producing an increase in capillary permeability are expected to allow the larger HES macromolecules to leak into the interstitial space, and even enter the alveolar space in severe damage. The detection of HES in patient's lung fluid thus indicates the presence of pulmonary vascular leakage, and HES can be used as an intravascular tracer of increased pulmonary capillary permeability. These HES polymers have a unique IR absorption signature that can be recognized easily and monitored by IR spectroscopy.



**Figure 1** Infrared spectra of plasma (A) and of bronchial washing fluid (B) from a noninjury patient, 4 h after receiving 500 mL of PENTASPAN, and for comparison a spectrum of pure PENTASPAN (C)

## PATIENTS AND METHODS

**Patients and protocol:** Bronchial washing samples were obtained from 67 patients who were admitted to the intensive care unit at the Health Sciences Centre in Winnipeg, Manitoba from July 1995 to April 1996. The patients enrolled in this study were scored according to the LIS. All patients were intubated, put on positive pressure ventilation and received the HES PENTASPAN. The amount of PENTASPAN patients received varied from 150 to 2000 mL. The patient population was divided into two groups: a risk group and an injury group. There were 12 cases in the risk group selected from patients with normal values of hypoxemia score (Partial pressure of arterial oxygen/fraction of inspired oxygen [ $\text{PaO}_2/\text{FiO}_2$ ] greater than 300 mmHg). Based on the clinical impression of the attending physician, they were potentially at risk to develop ALI. The injury group encompassed 55 patients and included ALI as well as ARDS patients. For a case to be diagnosed as ARDS, most, but not all, of the following criteria had to be met within five days of admission to the intensive care unit: hypoxemia score  $\text{PaO}_2/\text{FiO}_2$  less than 200 mmHg with an LIS greater than 2.5; patients with  $\text{PaO}_2/\text{FiO}_2$  over 200 mmHg but with an LIS of

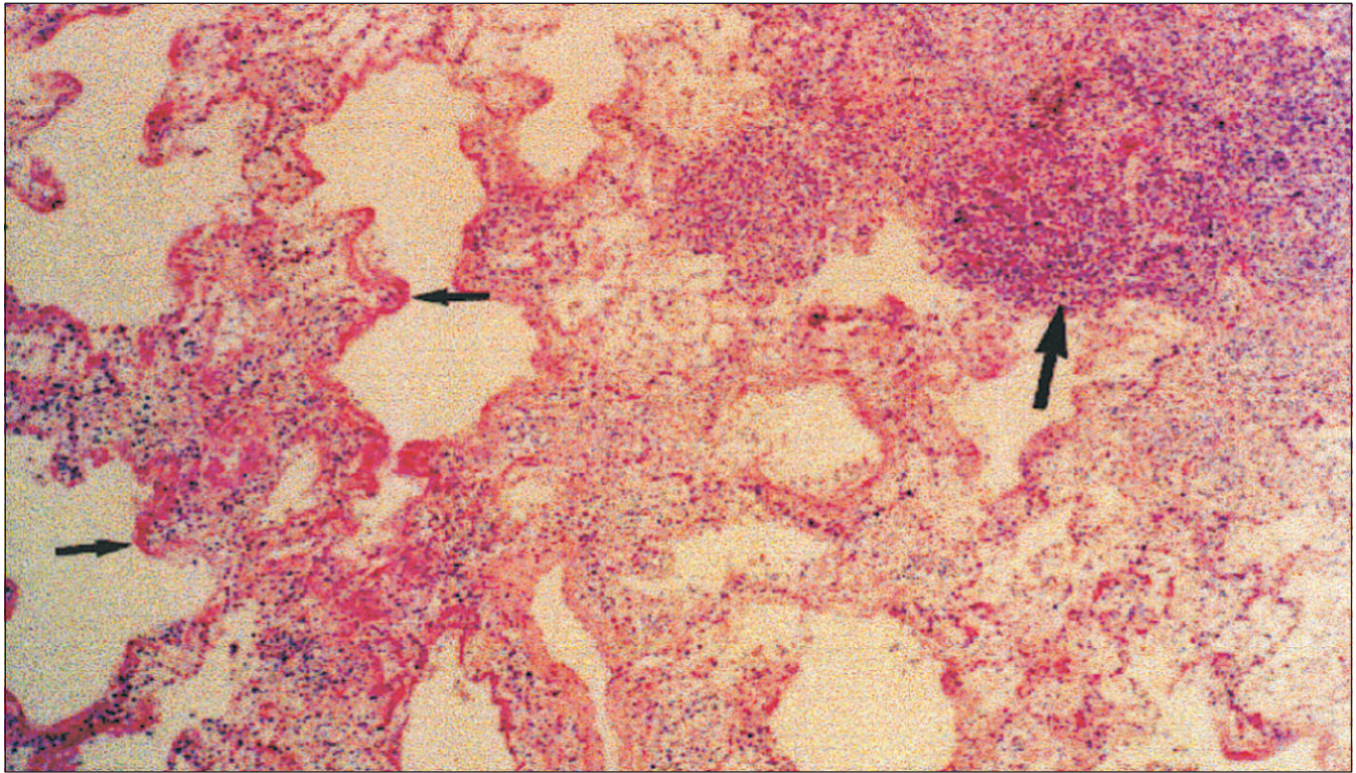


**Figure 2** Infrared spectra of plasma (A) and bronchial washing fluid (B) from an acute (or adult) respiratory distress syndrome (ARDS) patient with extensive PENTASPAN loading, along with the lung fluid spectrum from the same patient (C)

less than 2.5 were also considered as ARDS by clinical decision; bilateral infiltrates seen on anteroposterior chest radiographs; no clinical evidence of increased capillary wedge pressures and pulmonary artery occlusion pressure less than or equal to 18 mmHg (to exclude patients with hydrostatic edema); and underlying disease compatible with ARDS.

Bronchial washing samples were collected by advancing the inline suction catheter through the endotracheal tube until a slight resistance was felt, usually at a distance of 35 to 40 cm. At this distance, the tip of the catheter would be expected to lie in a segmental or subsegmental bronchus. A volume of 10 to 20 mL of normal saline was then flushed down the catheter. After several respiratory cycles, the catheter was aspirated using 50 to 75 mmHg of negative pressure and then withdrawn. Aspirated fluid was collected in a Lukens trap (Sherwood Medical, St Louis, Missouri) with the usual yield approximately 3 to 4 mL. Once obtained, the samples were refrigerated immediately and transported to the Institute for Biodiagnostics, Winnipeg, Manitoba for processing within 24 h. The IR spectrum of the bronchial washing with HES did not change over 24 h. Bronchial washing fluid is the aspiration of small amounts of saline and secretions from large airways. The washing can be performed quickly and easily in an





**Figure 3)** Microscopic section from autopsy lung tissue, showing acute diffuse alveolar damage with hyaline membranes (small arrows) and superimposed bronchopneumonia (large arrow. Leitz Diaplan microscope (Leica, Heidelberg, Germany) (10× magnification)

intubated patient, and no adverse clinical effects of sample collection have been documented. Indeed, the method of bronchial washing is the standard pulmonary cleaning method in many intensive care units.

**Sample preparation and spectroscopic assay:** Bronchial washing samples were homogenized for 2 to 3 mins at 100 rpm and centrifuged at 15,000 rpm for 10 mins. Because of the different amounts of fluid recovered, some specimens had to be diluted with distilled water to adjust the final concentration to the optimal spectroscopic range. An aliquot of 3  $\mu$ l of this bronchial washing was placed on an IR transparent calcium fluoride window, which quickly dried down to form a thin film. This procedure eliminates water, which is a strong IR absorber that dominates the IR spectrum. Spectra were recorded with a FTS-40A (Bio-Rad Laboratories Inc, Cambridge, Massachusetts) IR spectrometer at a resolution of 8  $\text{cm}^{-1}$  over the spectral range of 900 to 4000  $\text{cm}^{-1}$ . Data acquisition time was less than 1 min/spectrum, and the procedure can readily be automated to handle batches of samples.

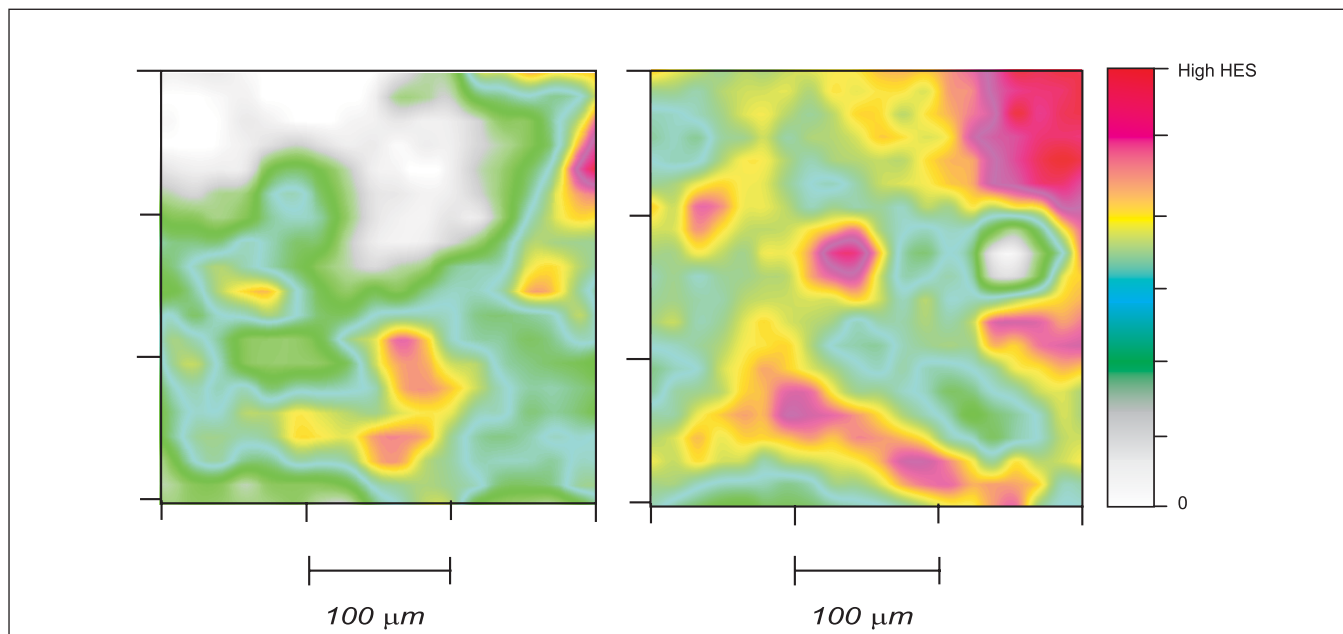
**Data treatment and statistical analysis:** A supervised spectral pattern recognition methodology, linear discriminant analysis, was applied to determine the presence of PENTAS-PAN in the spectrum of the bronchial washing. The methodology had been applied earlier for the classification of synovial fluid spectra to ascertain the nature of the arthritic condition (8). It is based on the determination of discriminatory features in a set of reference spectra, known as the training set. The training set is then used to set up a class model in which the discriminatory features can be used to classify new

unknown objects. The features that are used for spectral classification can be a combination of specific peak frequencies, absorption bandwidth or relative intensities that uniquely characterize the spectral data set in a given class. In this approach, the classification scheme is known a priori and the problem is to devise rules for assigning unclassified individual objects to one of the known classes, ie, predicting the unknowns from the known classes. Because this method was used to determine the HES presence in a patient's bronchial washing fluid, the training set had to represent the two groups of patients, the leak and the nonleak population, and allow the spectral characteristics of each group to be determined. Details regarding the spectral normalization and feature space reduction are given in the Appendix.

## RESULTS AND DISCUSSION

### IR spectroscopic characterization of bronchial washings:

The IR spectra of bronchial washings share many common features with the spectra of other biofluids such as plasma and bronchial alveolar lavage (BAL) fluid. Typical spectra of bronchial washings from noninjury and injured patients are illustrated in Figures 1 and 2. The major absorption bands in these spectra arise from the protein and lipid components in the fluid (9-11). Specific chemical groups in these biomolecules give rise to characteristic absorptions, exemplified by the strong protein bands at 1550 and 1650  $\text{cm}^{-1}$ . The distinct feature of PENTAS-PAN in the region of 980 to 1180  $\text{cm}^{-1}$  (shaded area in Figure 2) is characteristic of starches such as HES and consists of IR bands arising from complex vibra-



**Figure 4** Two-dimensional microspectroscopic images (based on PENTASPAN distribution) of two 10  $\mu\text{m}$  thick lung tissue sections of  $300 \times 300 \mu\text{m}^2$ . The false colour-code ranges from white (no PENTASPAN) through green (traces of PENTASPAN) and yellow (small amounts of PENTASPAN) to red (large amounts of PENTASPAN)

tions of the sugar ring. The spectrum of a patient's bronchial washing with no apparent lung injury, 4 h after receiving 500 mL of PENTASPAN (Figure 1B), showed no significant HES accumulation compared with the matched plasma spectrum (Figure 1A). Figure 2 compares IR spectra of plasma, bronchial washing and lung fluid from an ARDS patient. The spectra of lung fluid and bronchial washing are similar, particularly in the sugar region, suggesting that the determination of HES alveolar leakage can be done equally well by analyzing bronchial washings from ALI/ARDS patients.

The alveolar HES concentration depends on the levels of circulating HES, as well as on the pulmonary capillary permeability and surface area. IR spectra provide a simple means of grossly estimating the leakage of the pulmonary vasculature. In a patient with no apparent lung injury, the alveolar HES concentration differs markedly from the plasma HES loading (Figure 1). In a healthy lung, only a very low molecular weight fraction of infused HES would be expected to diffuse between the alveolar and intravascular space. Rapid elimination of HES by the kidneys leads to only minor steady-state concentrations of a low molecular weight fraction of HES in the alveolar spaces. Increased pulmonary vascular permeability results in higher molecular weight HES fractions gaining access and penetrating into the alveolar space. As a result, the lung fluid and bronchial washing fluid of an ARDS patient show an accumulation of HES (compare Figures 2B and 1B), which is an indicator of increased pulmonary capillary permeability. An increased pulmonary permeability surface area results in faster intravascular-alveolar HES exchange. Thus, the determination of HES in bronchial washing may be of value in evaluating the progression of ALI/ARDS in a clinical setting.

**IR pathology of pulmonary HES leakage:** A demonstration of the pulmonary leakage in an ARDS patient was performed by IR microscopy using autopsy material from a 49-year-old male who presented to the trauma service at the Health Sciences Centre, Winnipeg, Manitoba. The patient was involved in a motor vehicle accident in which a tractor-trailer rolled over him, and he sustained a fractured pelvis with a significant retroperitoneal hematoma. Over the next 48 h, the patient developed clinical signs of ALI/ARDS. He received antibiotics, ventilatory support and continuous venovenous hemodialysis. PENTASPAN was administered for oncotic manipulation. The IR spectra of the bronchial washings, collected 3 h after infusion of PENTASPAN, contained HES.

The patient died four days postinjury. Autopsy histology showed acute diffuse alveolar damage with hyaline membranes and superimposed bronchopneumonia (Figure 3). Frozen autopsy tissue was prepared for IR microscopic examination as  $10 \mu\text{m}$  thin unstained sections. The thin section was placed onto a calcium fluoride window and allowed to dry. A spectrometer equipped with a  $15\times$  objective microscope (Bruker Optik, Karlsruhe, Germany) was used for IR pathological examination. Two randomly selected areas from within the section, each  $300 \times 300 \mu\text{m}^2$ , were investigated by IR microspectroscopy. For each of the selected areas, 100 individual spectra at a resolution of  $4 \text{ cm}^{-1}$ , with each spectrum covering an area of  $30 \times 30 \mu\text{m}^2$ , were recorded. Colour images of the distribution of PENTASPAN within the examined tissue sections are illustrated in Figure 4 and were generated based on the intensity of the PENTASPAN absorption band at  $1030 \text{ cm}^{-1}$ . The false colour code in Figure 4 (right) ranges from white (no PENTASPAN) through green (traces of PENTASPAN) and yellow (small amounts of PENTASPAN) to red (large amounts of PEN-

**TABLE 1**  
**Clinical data and hydroxyethyl starch leakage (HES) determined for the infrared (IR)-bronchial washing assay**

Patient number	HES Leakage	Lung injury score	PaO <sub>2</sub> /FiO <sub>2</sub>	Patient number	HES Leakage	Lung injury score	PaO <sub>2</sub> /FiO <sub>2</sub>
1	+	3.5	84	35	-	1.25	223
2	+	2.75	215	36	+	2.23	132
3	+	3.5	58	37	+	2.5	152
4	+	2	152	38	+	1.75	170
5	+	3.25	74	39	+	1.75	235
6	+	3.25	72	40	+	1	192
7	+	3.25	86	41	+	1.25	295
8	+	3	124	42*	-	0.75	354
9	+	2.5	94	43*	+	0.75	242
10	+	2.75	55	44	+	1.25	214
11	+	0.75	290	45	+	2.5	74
12*	+	0	480	46	+	2.5	96
13	+	0.75	225	47	+	2.5	150
14	+	1	150	48*	+	1.5	310
15	-	1.75	255	49	+	2.25	90
16	+	0.5	283	50*	+	1	366
17	-	1.75	230	51	+	2.25	111
18*	-	0	452	52	-	2.25	160
19	-	2	164	53*	+	0.5	320
20	-	1.75	170	54	+	1	267
21	+	0.33	280	55	+	2	170
22	-	0.33	275	56	+	2.25	242
23	+	1	218	57	+	2.25	147
24	-	1.67	113	58	-	1	268
25*	-	0.5	406	59*	+	0.75	378
26*	-	0.5	320	60*	-	1.25	337
27	-	1.25	155	61	+	0.3	247
28	-	1.5	136	62	-	1.5	170
29*	+	1.75	370	63	+	2.25	242
30	+	1.33	197	64	+	1.5	220
31	-	1.7	180	65	+	1.5	155
32	-	2.67	123	66	+	1.5	292
33	+	1.75	206	67*	+	1	350
34	-	1.3	173				

\*Patients at risk (n=13). + IR positive for leakage (patients with a probability of PENTASPAN leakage greater than or equal to 50%); - IR negative for leakage (patients with a probability of PENTASPAN leakage less than 50%); PaO<sub>2</sub>/FiO<sub>2</sub> Partial pressure of arterial oxygen/fraction of inspired oxygen

**TABLE 2**  
**A 2×2 contingency table comparing clinical Partial pressure of arterial oxygen/fraction of inspired oxygen (PaO<sub>2</sub>/FiO<sub>2</sub>) values with the IR spectroscopic classification**

Group	Injury group (PaO <sub>2</sub> /FiO <sub>2</sub> mmHg)	Risk group (PaO <sub>2</sub> /FiO <sub>2</sub> 300 mmHg)
IR positive for leakage	40	7
IR negative for leakage	15	5

IR Infrared

TASPAN). While both sections contain PENTASPAN with diffuse patterns, the section in Figure 4 (middle) clearly demonstrates a severe tissue injury. The spectroscopic images provide pathological evidence of capillary-alveolar membrane damage and site-to-site variability of PENTASPAN within the injured tissue sections.

**Classification of pulmonary HES leakage in a patient population:** The clinical data and HES leakage determined for the IR-bronchial washing assay are given in Table 1. Using the criteria of the consensus committee from the report of the American-European Consensus Conference on ARDS (15), a comparison can now be made between the clinical



**TABLE 3**  
**PENTASPAN (Du Pont Pharma, Mississauga, Ontario) leakage in the injury population**

Group	0<PaO <sub>2</sub> /FiO <sub>2</sub> <100 (mmHg)	100<PaO <sub>2</sub> /FiO <sub>2</sub> <200 (mmHg)	200<PaO <sub>2</sub> /FiO <sub>2</sub> <300 (mmHg)
Infrared positive for leakage	10	13	17
Infrared negative for leakage	0	10	5

PaO<sub>2</sub>/FiO<sub>2</sub> Partial pressure of arterial oxygen/fraction of inspired oxygen

PaO<sub>2</sub>/FiO<sub>2</sub> values and the HES leakage as determined from the IR spectra. Patients (n=55) with PaO<sub>2</sub>/FiO<sub>2</sub> less than 300 mmHg (which included both ARDS and ALI patients), were placed in the injury group, while patients (n=12) with PaO<sub>2</sub>/FiO<sub>2</sub> greater than 300 mmHg were placed in the risk group. A 2×2 contingency table was set up (Table 2), which compares the clinical PaO<sub>2</sub>/FiO<sub>2</sub> values with the IR spectroscopic classification: 40 of 55 (sensitivity of 72.7%) injured patients showed IR positive for HES leakage. This indicates a strong relationship between the PaO<sub>2</sub>/FiO<sub>2</sub> ratio and the IR results for ALI/ARDS patients, and implies that, in most of the injured patients, IR did detect the HES leakage. To evaluate the leakage associated with the degree of injury, an additional comparison was made using all 55 patients in the injury group. For this comparison, patients were subdivided into three subgroups: a group of patients with severe ARDS who had PaO<sub>2</sub>/FiO<sub>2</sub> ratios of less than 100 mmHg (n=10); a group of ARDS patients with PaO<sub>2</sub>/FiO<sub>2</sub> ratios of 100 to 200 mmHg (n=23); and a group of patients with ALI who had PaO<sub>2</sub>/FiO<sub>2</sub> ratios of 200 to 300 mmHg (n=22) (Table 3). All 10 patients with severe cases of ARDS (PaO<sub>2</sub>/FiO<sub>2</sub> less than 100 mmHg) were found IR-positive for HES leakage by using the IR-bronchial washing method. For the other two subgroups (PaO<sub>2</sub>/FiO<sub>2</sub> between 100 and 200 mmHg and PaO<sub>2</sub>/FiO<sub>2</sub> between 200 and 300 mmHg), a population of 56.5% and 77.3%, respectively, showed IR-positive for HES leakage. These findings suggest that using PaO<sub>2</sub>/FiO<sub>2</sub> to define ALI/ARDS patients is not pathophysiologically specific for the syndrome.

Although the interstitial HES level depends on the HES level in the circulatory system, the appearance of HES in a patient's plasma and bronchial washings exhibits a phase difference (ie, a time course difference). Generally, infused HES takes a very short time to flow into most organs in the body; however, the time for HES to infiltrate the interstitium from capillaries is much longer than that in the circulatory system. In addition, HES in the circulatory system has a much shorter half-life than in the interstitium, because alpha-amylases present in the tissue and in the circulation act at different rates. This implies that measuring plasma HES concentrations and comparing that with fluid from bronchial washing might not truly represent the degree of leakage, especially when the HES concentration in the circulation is lower than in the bronchial washings. To avoid this problem, evaluation of capillary HES leakage should involve an analysis of starch in the bronchial washing fluid without necessarily correlating it with that in plasma. This means that the use of bronchial washing fluid to determine pulmonary permeability yields information on only leakage versus nonleakage. While this qualitative analysis

provides solely a 'leak' or 'nonleak' answer to the problem, it is crucial for providing information on the pathophysiological changes of ALI/ARDS in a clinical setting. Furthermore, the application of pattern recognition techniques for spectral classification may provide a useful clinical index of lung injury.

The IR-bronchial washing assay represents a novel diagnostic methodology to the microvascular injury-induced pulmonary edema. However, the method is only valid over a certain period following HES infusion. Over time, HES is broken down into smaller size molecules, and, therefore, the specificity of the method is inevitably lost. Clinical pharmacokinetic and pharmacodynamic studies showed that, over a 12 h period, the average size of infused PENTASPAN (200/0.5 HES) is approximately 11.28 nm (13), which should still restrict the molecules within the pulmonary capillaries. Table 2 shows that 58% of 'noninjured patients' (seven of 12) in the risk group also had leakage. The significance of identifying a leak in the 'at risk' group can not be underestimated – this would imply aggressively treating these patients to prevent future complications and further lung insult. It is well known that ARDS may take up to 48 h (16) to demonstrate overt clinical features, such as the classic chest x-ray findings. Thus, it is possible that the group at risk may have developed later into the classic ARDS models, while the IR method detected leakage in these patients at an early stage in the course of their disease.

As mentioned, the PaO<sub>2</sub>/FiO<sub>2</sub> ratio assesses the pulmonary gas exchange function rather than addressing the pathophysiological changes in pulmonary vascular permeability. In this sense, the IR technique could provide an additional parameter in the diagnosis of ALI/ARDS. If this technique were to obtain clinical validity in the diagnosis of ARDS and in the identification of at-risk patients, then its application must also be balanced with the clinical picture.

## CONCLUSIONS

The IR-bronchial washing assay method is useful for determining the pulmonary leakage in ARDS, and is particularly sensitive for evaluating the pulmonary leakage in patients who have ALI – the early stage of ARDS. This makes it especially important for supportive treatment. In contrast with other methods used to determine pulmonary microvascular permeability, the IR-based HES assay uses no radioactive tracers and requires little sample preparation. The instrumentation is compact, and the method rapidly provides a measure of pulmonary capillary permeability, which makes it suitable for routine operation in a critical care environment. Nevertheless, the proof-of-concept method described herein is certainly not optimized and would require further development before becoming a practical tool in a clinical setting.

## APPENDIX

**Spectral normalization:** A major problem for the classification of bronchial washing spectra is the large variation in absorption intensities due to the unknown dilution factor involved in sample collection. Therefore, the influence of this unknown dilution factor must be eliminated. One way to address this problem is band-area normalization, which is based on an integration method. First, one has to define an area that remains constant in the spectroscopic region of interest so that every spectrum can be ratioed to this area; thus, a fraction factor is obtained. The 'normalized' spectrum is then obtained by multiplying the original spectrum with this fraction factor. This treatment retains the band shape, but offsets all spectra to a reference value and can be viewed as a mathematical process of correcting the dilution factors.

**Feature space reduction:** A problem when applying linear discriminant analysis to IR spectra is dimensionality. Generally, the spectra to be analyzed consist of many attributes (number of data points) compared with a relatively small number of patients, which form a sparse set in a high-dimension. To circumvent this, an 'attribute selection' methodology for spectral feature selection was used, that reduces the dimension (number of data points) by selecting certain subregions in the spectral range of interest that contain the most diagnostic information for the classification (17).

**Training set:** Two groups, a HES-containing (leak group)

and a HES-free (nonleak group), each consisting of 100 spectra, were used. There were 14 patients in the HES-free group, primarily postoperative and trauma patients with near normal pulmonary function and normal or near normal chest x-rays; these patients were not infused with HES. From the training set, HES-containing spectra were then generated by combining the HES-free spectra with the spectrum of HES, using different mixing factors. The amount of HES in the fluids varied from 0.03 to 3 µg/mL. For cross-validation of the training set, the leave-one-out method was applied, which is commonly used for evaluating the prediction ability of training processes. It is performed by sequentially removing a single spectrum from the training set, then training the classifier on the remaining spectra. Prediction is made for that single test spectrum. This process is repeated for each spectrum in the training set, and the classifier returned to the training set after each test so that the set remains the same size. The purpose of this cross-validation test is to improve the probability that the training set truly represents the full population of the study. The cross-validation showed that the training set had 80% accuracy in predicting HES in the leak group. The misclassified spectra (20%) all had low concentrations of HES (below 0.66 g/mL), representing the prediction limit of the training set. The accuracy of the training set to predict the nonleak population was much higher, at 98%.

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## REFERENCES

- Murray JF, Matthay MA, Luce JM, Flick MR. An expanded definition of the adult respiratory distress syndrome. *Am Rev Respir Dis* 1988;138:720-3.
- Brigham KL, Kariman K, Harris TR, Snapper JR, Bernard GR, Young SJ. Correlation of oxygenation with vascular permeability-surface area but not with lung water in humans with acute respiratory failure and pulmonary edema. *J Clin Invest* 1983;72:339-49.
- Rinaldo JE, Borovetz HS, Mancini MC, Hardesty RL, Griffith BP. Assessment of lung injury in the adult respiratory distress syndrome using multiple indicator dilution curves. *Am Rev Respir Dis* 1986;133:1006-10.
- Harris TR, Bernard GR, Brigham KL. Lung microvascular transport properties measured by multiple indicator dilution method in patients with adult respiratory distress syndrome. *Am Rev Respir Dis* 1990;141:272-80.
- O'Connors MF, Hale JB, Schmidt GA, Wood LDH. Acute hypoxemic respiratory failure. In: Hall JB, Schmidt GA, Wood LDH. *Principles of Critical Care*. New York: McGraw Hill 1998:537-59.
- Basran GS, Byrne AJ, Hardy JG. A noninvasive technique for monitoring lung vascular permeability in man. *Nucl Med Commun* 1985;6:3-10.
- Putensen C, Mutz N, Himmer G. Gamma scintigraphic imaging of lung microvascular permeability in adult respiratory distress syndrome. *Crit Care Med* 1990;18:807-12.
- Eysel HH, Jackson M, Nikulin A, Somorjai RL, Thomson GTD, Mantsch HH. A novel diagnostic test for arthritis: Multivariate analysis of infrared spectra of synovial fluid. *Biospectroscopy* 1997;3:161-7.
- Fabian H, Jackson J, Murphy L, Watson PH, Fichtner I, Mantsch HH. A comparative infrared spectroscopic study of human breast tumors and breast tumor cell. *Biospectroscopy* 1995;1:27-45.
- Liu K-Z, Dembinski TC, Mantsch HH. Rapid determination of fetal lung maturity from infrared spectra of amniotic fluid. *Am J Obstet Gynecol* 1998;178:234-41.
- Schultz CP, Liu KZ, Johnston J, Mantsch HH. Study of chronic lymphocytic leukemia cells by FT-IR spectroscopy and cluster analysis. *Leukemia Res* 1996;20:649-55.
- Mishler JM, Parry ES, Sutherland BA, Bushrod JR. A clinical study of low molecular weight-hydroxyethyl starch, a new plasma expander. *Br J Clin Pharmacol* 1979;7:619-22.
- Ferber HP, Nitschand E, Förster H. Study on hydroxyethyl starch. *Arzneim. Forsch/Drug Res* 1985;35:615-22.
- Quon CY. Clinical pharmacokinetics and pharmacodynamics of colloidal plasma volume expanders. *J Cardiothoracic Anesthesia* 1988;2:13-23.
- Bernard GR, Artigas A, Brigham KL, et al. Report of the American-European Consensus Conference on ARDS: definitions, mechanisms, relevant outcomes and clinical trial coordination. *The Consensus Committee. Intensive Care Med* 1994;20:225-32.
- Marinelli WA, Ingbar DH. Diagnosis and management of acute lung injury. *Clin Chest Med* 1994;15:517-46.
- Nikulin AE, Dolenko B, Bezabeh T, Somorjai RL. Near-optimal region selection for feature space reduction: novel preprocessing methods for classifying MR spectra. *NMR Biomed* 1998;11:209-16.





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