

# Anatomical distribution of bronchoalveolar lavage fluid as assessed by digital subtraction radiography

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**ABSTRACT** A digital subtraction imaging technique was used to visualise directly the anatomical distribution of 3 × 60 ml aliquots of saline containing a low concentration of radio-opaque dye, introduced sequentially into a segment of the middle lobe. It was possible to estimate the relative movement of fluid within the segment during the sequential aspiration of each of these aliquots. The first 60 ml aliquot introduced stayed close to the bronchoscope and probably sampled only the proximal airways. With the introduction of cumulative volumes of 120 ml or more, the fluid filled the segment more evenly. Aspiration then moved fluid back from the periphery, implying that the aspirate had also lavaged both distal airways and alveoli.

Bronchoalveolar lavage has become increasingly popular as a diagnostic and research tool over the last 15 years. Its applications have been as diverse as the measurement of mediators after antigen challenge in asthmatic patients<sup>1</sup> and the diagnosis of *Pneumocystis carinii* infection in immunosuppressed patients,<sup>2</sup> although it is most widely used in the assessment and diagnosis of interstitial lung disease.<sup>3</sup> The anatomical distribution of fluid introduced during bronchoalveolar lavage, however, has never been thoroughly investigated and there is wide variation in the way the procedure is performed. The volume instilled by different operators has ranged from 20<sup>4</sup> to 300<sup>5</sup> ml and the site of lavage has varied from first order bronchi<sup>6</sup> to subsegmental bronchi.<sup>7</sup> In this study we have attempted to define the fluid distribution occurring during a standard bronchoalveolar lavage protocol, as routinely used in our practice, and to assess the movement of fluid that occurs at aspiration.

## Methods

Two healthy and fully informed men, aged 28 and 23 years, volunteered to undergo fiberoptic bronchoscopy and bronchoalveolar lavage of one segment of the middle lobe. Premedication with 10 mg papaveretum and 0.6 mg atropine was given intra-

muscularly 30 minutes before bronchoscopy, and 10 mg of diazepam was injected intravenously immediately before the start of the procedure. Over 15 minutes 4 ml of 2% lignocaine were administered by nebuliser (Porta-Neb, Medic-aid) and 1 ml was injected into the trachea via the cricoid membrane, which allowed the procedures to be completed with no more than 2 ml of additional local anaesthetic. Peroral bronchoscopy was then performed with the subject supine and the instrument was wedged into a segment of the middle lobe. The medial segment was chosen for lavage in the first subject (study 1) and the lateral segment in the second (study 2). Both studies were recorded with a computer linked digital radiography system (Picker DAS 211), which exposed each subject to a total of 15 chest radiographs. A baseline image of the right hemithorax was taken in the anteroposterior plane at functional residual capacity (FRC) at the beginning of each study for subtraction from subsequent images. This allowed better low contrast sensitivity. Three successive aliquots of sterile phosphate buffered saline containing a 5% solution of the radio-opaque iodinated dye Iopamidol (Merck) were introduced at 25°C. This dye concentration was selected as a result of a preliminary series of experiments in which test tubes containing 1%, 5%, and 10% iopamidol in 50 ml of phosphate buffered saline were imaged to determine the minimum concentration required for adequate visualisation of contrast. The 5% solution was found to be the lowest concentration yielding adequate images and its osmolarity (307 mmol/l) and viscosity (0.95 cp) closely approximated

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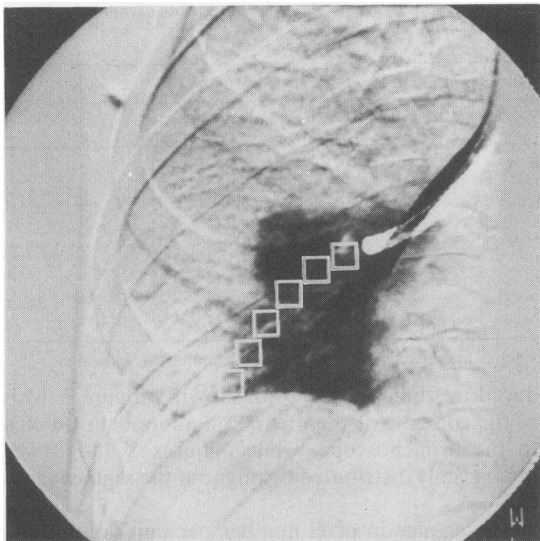


Fig 1 Well defined outline of the medial segment of the middle lobe after the introduction through the bronchoscope of 20 ml of 5% iopamidol in saline. The distribution of the radio-opaque fluid is indicated by the dark areas in the image. The areas of interest are superimposed at approximately 1 cm intervals along the line of the segmental bronchus.

to those of phosphate buffered saline (osmolarity 285 mmol/l, viscosity 0.91 cp) at room temperature.

Each aliquot was introduced at a rate of 1 ml per second by hand held syringe injection, and antero-posterior images were obtained after the following volumes of fluid had been introduced: 5, 10, 20 (fig 1), 30, 40, and 60 ml of the first aliquot and 30 and 60 ml of the second and third. Each aliquot was aspirated immediately after its introduction, at a pressure of 50–100 mm Hg, and the next aliquot was introduced at once. Images were recorded after each aspiration. All images were obtained at functional residual capacity to minimise movement artefact in the subtracted images.

When computer subtraction of the mask from each of the subsequent images was completed, each picture was subjected to the same analysis. Six areas of interest (AOI) containing 400 pixels, each able to assess the amount of dye present, were superimposed on each image at approximately 1 cm intervals along the line of the segmental bronchus, starting from the tip of the bronchoscope (AOI 1) and moving towards the periphery of the lavaged segment (AOI 6). The total quantity of dye in each AOI was measured by the computer, which was calibrated to express the result as a mean pixel number on a scale of 0 (background control) to 128 (value obtained by 5% dye in test tube). Thus although the radiographic image was two

dimensional, dye content was assessed three dimensionally by the computer.

Subtraction of the postaspiration image from the picture immediately preceding it allowed the areas from which fluid had moved during aspiration to be defined for each aliquot by determining the change in pixel number (fig 2).

As the anteroposterior thickness of the segments studied varied considerably with distance from the bronchoscope, an anatomical study was undertaken to determine the anteroposterior dimensions of both medial and lateral segments of the middle lobe. Three normal right lungs freshly removed at necropsy from middle aged men were examined. The middle lobe bronchus was dissected to its bifurcation and the length of each of the segmental bronchi was measured with a fine probe. A long steel needle was passed through the middle lobe in an anteroposterior plane at intervals of 1 cm along the line of each segmental bronchus, and the anteroposterior thickness was directly measured (table 1). The pixel number values for each AOI were then divided by the anteroposterior thickness of the middle lobe at the corresponding point along the segmental bronchus, and are hence expressed per unit volume.

## Results

The total volumes aspirated in studies 1 and 2 were 120 ml and 116 ml (67% and 65% of the fluid

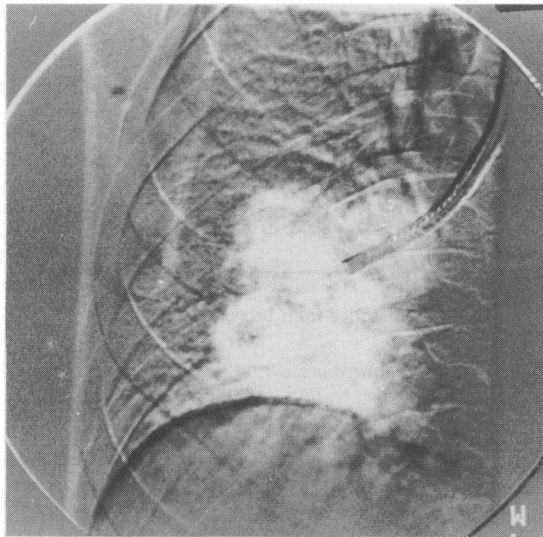


Fig 2 Picture obtained by subtraction of the postaspiration image from the preaspiration image of the third aliquot introduced into the medial segment of the middle lobe. The removal of fluid after aspiration is indicated by the light areas in the image.

Anteroposterior (AP) thickness of medial and lateral segments of middle lobe at 1 cm intervals along the segmental bronchus from the bifurcation of the middle lobe bronchus in three postmortem lungs

Distance from bifurcation (cm)	AP thickness (cm) (median (range))			
	AOI No*	Medial	AOI No*	Lateral
2	1	4.5 (4.0-5.5)	1	4.0 (3.5-4.5)
3	2	5.5 (5.0-6.0)	2	5.0 (5.0-5.5)
4	3	6.0 (5.5-7.0)	3	5.5 (5.5-6.0)
5	4	6.0 (5.5-6.5)	4	6.0 (5.5-6.5)
6	5	4.5 (4.0-4.5)	5	6.0 (5.5-7.0)
7	6	2.5 (2.0-2.5)	6	4.5 (4.5-5.0)
8		1.0 (1.0-1.0)		3.5 (3.0-3.5)
9		0		2.5 (1.5-2.5)
		0		1.0 (1.0-1.0)

\*Areas of interest (AOIs) were placed along the line of the segmental bronchus at convenient points, about 1 cm apart as indicated.

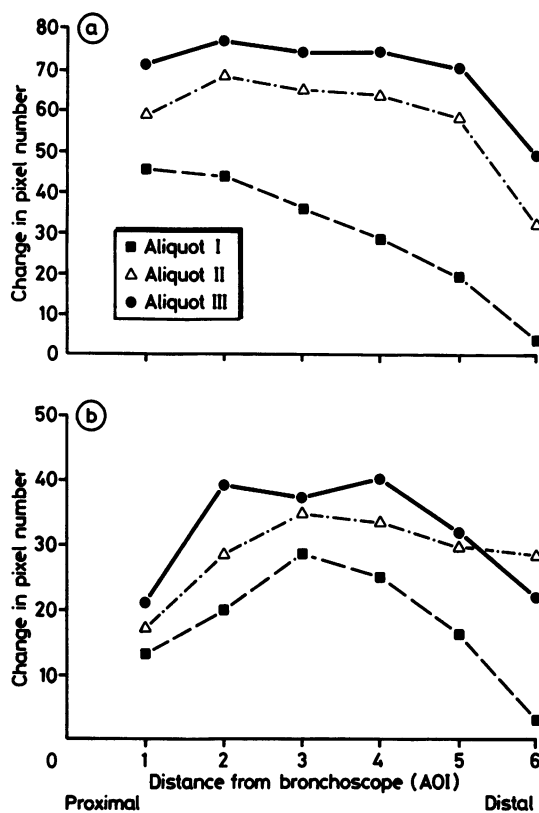


Fig 3 Quantity of dye present, in terms of pixel numbers per unit lung volume, related to distance from the bronchoscope (expressed as area of interest (AOI) for successive aliquots introduced into (a) the medial and (b) the lateral segment of the middle lobe.

installed). These volumes are typical of those obtained under bronchoalveolar lavage conditions in our clinical practice.

The mean pixel number per unit volume for each AOI and the corresponding distance of the AOI from the bronchoscope for each of the three aliquots intro-

duced in studies 1 and 2 are shown in figure 3. Most of the contents of the first 60 ml aliquot stayed close to the bronchoscope, while aliquots 2 and 3 were more evenly distributed throughout the segment studied.

The change in pixel number per unit volume as a result of aspiration for each AOI and the corresponding distance from the bronchoscope for each of the three aspirates in both studies are shown in figure 4. Fluid was aspirated almost exclusively from close to the bronchoscope for the first aliquots but fluid movement occurred throughout the whole segment after introduction of the second and third aliquots. The negative change in pixel values in figure 4a indicates that fluid was still moving towards the periphery of the medial segment during aspiration of the first aliquot in this study. Coughing occurred during aspiration of the third aliquot in study 2, accounting at least in part for the reduction in dye aspirated, as shown in figure 4b.

## Discussion

The possibility that increasing volumes of fluid introduced at bronchoalveolar lavage may lead to perfusion of different anatomical sites has been suggested previously<sup>4</sup> and has been supported by indirect evidence, such as variation in yields of cells<sup>8</sup> and pathogens<sup>9</sup> and alteration in differential cell counts<sup>10</sup> with sequential volume lavage. The anatomical distribution of fluid introduced at bronchoalveolar lavage has not, however, been previously defined by direct visualisation. Since major inferences about disease processes are frequently drawn from bronchoalveolar lavage data, such definition is clearly of great importance. There has been considerable variation in the volume of fluid instilled at BAL<sup>11,12</sup> and in the sites chosen for lavage<sup>13-15</sup> in different centres. We chose to define a protocol for bronchoalveolar lavage of one middle lobe segment, using the sort of volumes typically employed in many of the reported studies.

Although this study does not allow assessment of

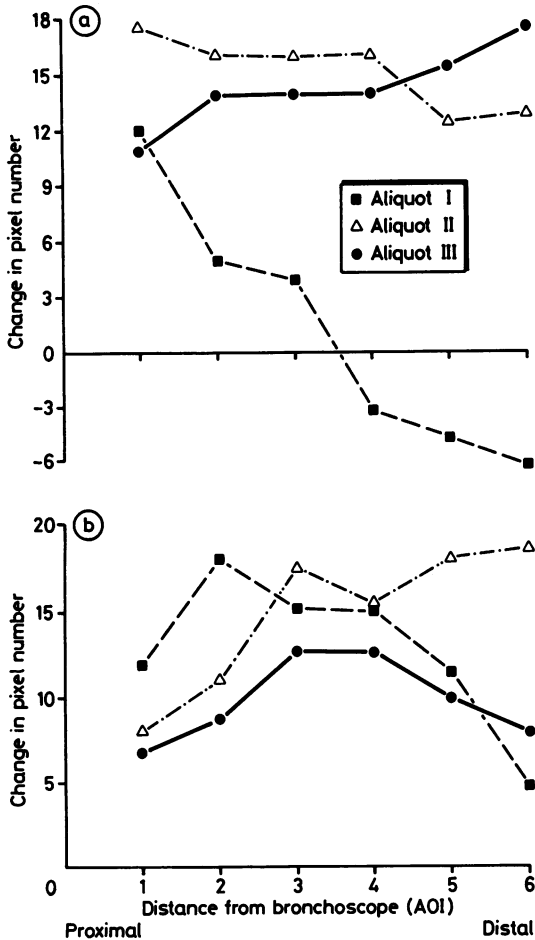


Fig 4 (a) Change in quantity of dye present, in terms of the change in pixel number per unit lung volume, with distance from the bronchoscope after aspiration of successive aliquots introduced into (a) the medial and (b) the lateral segment of the middle lobe. A positive change means that there is less dye in that area after aspiration than before. The negative values for the first aliquot indicate that fluid is still moving towards the periphery of the segment during aspiration.

the evenness of dye distribution in an anteroposterior plane, and is unable to allow for possible flux of fluid into and from the vascular compartment, it offers the first direct evidence of the variation of fluid distribution with volume introduced, and permits an estimate of the areas from which aspirate is returned. We were able, however, to detect only "movement" of fluid from a given area at aspiration, which does not necessarily mean that the fluid was actually aspirated but may indicate that it displaced fluid from other areas.

We have shown that a small volume lavage of 60 ml largely remained near the tip of the bronchoscope, and that aspiration of this introduced volume yielded very little fluid from the lung periphery. Indeed, in one study some of the fluid introduced in the first aliquot was still moving peripherally during the first aspiration. Our study has shown a difference in the uniformity of aspiration of the first aliquot between the medial and the lateral segments, but as only one study was performed in each segment the meaning of this is uncertain. These findings are consistent with the observation that the aspirate of the first 60 ml of introduced fluid has a cell profile suggesting an origin in the airways<sup>10</sup> rather than the alveoli. This volume of instilled fluid may therefore be used most appropriately when airways disease is being investigated.

Although there may be differences in filling patterns between normal and diseased lungs, this study suggests that when a volume of 120 ml or greater is instilled into a single lung segment it appears to perfuse the whole segment. Likewise, aspiration of this volume produces fluid movement from within the whole of the segment. The findings fit well with the observation that the aspirate from this volume of introduced fluid provides a differential cell count more consistent with an alveolar wash.<sup>7</sup> Hence the study of alveolar disease processes may perhaps be best achieved with volumes of at least 120 ml.

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