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Fractional processing of sequential bronchoalveolar lavage fluid from intubated babies

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ABSTRACT: Two groups of intubated newborn babies were studied to determine the clinical effects of interrupted bronchoalveolar lavage (BAL) by suction catheter (S-BAL) and the similarities to adult fibreoptic BAL of fractional processing of sequential lavage fluid (BALF). Both groups were lavaged by two aliquots of 1 ml·kg^{-1}, instilled *via* a blindly placed suction catheter, wedged on two separate insertions through the right main bronchus.

In 14 infants, (sequential lavage group), BALF aliquots were analysed separately. There were no differences in the volumes recovered or total cell counts between the first and second BALF aliquots. Where cell morphology was visible (n=11), the percentage of macrophages, but not the absolute number, increased in the second BALF aliquot ($p<0.01$). BALF urea and epithelial lining fluid volume estimated by urea dilution were similar between the two aliquots (n=8).

In a separate group (blood gas group), vital signs were recorded in 10 infants undergoing S-BAL. At 1 min after lavage there was a rise in mean arterial blood pressure (39 *vs* 49.5 mmHg, $p<0.05$) and a fall in transcutaneous oxygenation (10.6 *vs* 7.5 kPa, $p<0.05$). Recovery was present at 3 min post-S-BAL, but mean blood pressure remained elevated (39 *vs* 45 mmHg, $p<0.05$) and transcutaneous oxygen continued to be lower when compared to baseline values (10.6 *vs* 9.2 kPa, $p<0.05$).

S-BAL of intubated infants appears to sample both the proximal and distal airways and results in changes in vital signs similar to routine non-selective endotracheal suctioning.

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Bronchoalveolar lavage fluid (BALF) can be recovered by "blind" insertion of a suction catheter through the endotracheal tube of a newborn baby and aspiration of instilled saline. This method has provided important information on inflammatory cells [1], proteinase/antiproteinase ratios [2] and mediators [3] during the evolution of neonatal chronic lung disease. However, neonatal bronchoalveolar lavage (BAL) has not been standardized. Instilled saline may be of a fixed volume or adjusted for body weight, injected directly into the endotracheal tube or *via* a wedged suction catheter [1–4]. It is not clear whether any of these methods sample the distal airways.

In fibreoptic bronchoalveolar lavage of adults (F-BAL) inferences about the site of sampling may be drawn from fractional processing of sequential lavage aliquots. Although fibreoptic bronchoscopy of intubated babies is now possible with new ultra-thin devices, these bronchoscopes do not have a suction channel and BAL cannot be performed. Neonatal BALF can, therefore, only be retrieved by some form of "blind" suction. There are no data on sequential analysis of neonatal BALF. In both the normal and diseased adult lung, the first aliquot of BALF samples predominantly bronchial cells. Subsequent aliquots sample more distal airways [5], as long as the lavage volume instilled is adequate [6]. Compared to the first BALF aliquot, fluid recovery, total cell numbers and macrophage differential counts increase in the second or third lavage [7, 8], reflecting the increased proportion of neutrophils in proximal airways [5]. Changes in BALF solutes with sequential F-BAL are less predictable but BALF urea from diluted epithelial lining fluid (ELF) either remains unchanged [9] or tends to increase [10].

We have developed a method of selective "suction bronchoalveolar lavage" (S-BAL) of intubated babies using a wedged catheter passed through the right main bronchus and an instilled volume of 1 ml·kg^{-1}. This is a form of neonatal endotracheal suction, used routinely in our unit. Using suction lavage, we aimed to compare inflammatory cells, urea and estimated ELF volume between the first and second BALF aliquots from intubated babies. Although S-BAL was obtained by an interrupted technique, we hypothesized that changes would be similar to those during sequential F-BAL of adults, if the second lavage

sampled more distal airways. As changes in blood gases and blood pressure have been reported after routine endotracheal suctioning of neonates [11], we measured these parameters during S-BAL in a separate group of intubated infants.

Materials and methods

Subjects

Sequential BALF obtained by suction lavage was obtained from 14 intubated infants (sequential lavage group). All were less than 32 weeks gestation (table 1), 13 were sedated with intravenous pethidine and one paralysed with pancuronium. Diagnoses included respiratory distress syndrome [11] and apnoea [2]. Three infants had received surfactant. S-BAL was performed between 24 h and 10 days of life.

Changes in vital signs during S-BAL were measured in a separate group of 10 infants (blood gas group), all of whom were intubated for respiratory distress syndrome. Infants in this group were less than 32 weeks gestation (table 1) and were lavaged between 48 h and 12 days of life. All of the investigations depended only on procedures which coincided with routine clinical management, which were clinically indicated.

Table 1. – Clinical details of the two groups of intubated babies studied

	Sequential lavage group n=14	Blood gas group n=10
Gestation weeks	28 (25–31)	28 (25–31)
Birth weight g	1005 (700–2110)	1070 (685–2250)
Age at lavage days	4.5 (1–10)	4.5 (2–12)

Data are given as median value and range in parenthesis.

Suction bronchoalveolar lavage

S-BAL was performed in a standard manner by the same operator. The infant's head was turned to the left and a 5 French straight suction catheter loaded with 1 ml·kg^{-1} normal saline was inserted *via* a Luer-stoppered orifice in the right-angled endotracheal tube (ET) adaptor. This permitted partial mechanical ventilation whilst the catheter was *in situ*. The catheter was advanced until resistance was felt and the saline instilled. After two ventilator breaths, the first aliquot of BALF was aspirated into a sputum trap using 60 cmH_2O negative pressure. After withdrawing the catheter, the lavage procedure was repeated. Total lavage time was 30–40 s, with a total instilled volume of 2 ml·kg^{-1}.

Cell analysis

The first and second aliquots of BALF were processed separately. BALF was filtered through a single layer of muslin gauze. The concentration of cells was determined on the filtered BALF by haemocytometer and the total number of inflammatory cells (white cell count (WCC) $\times 10^4$) calculated. Cells were removed by centrifugation at 400×g for 6 min. The resulting supernatant was stored at -80°C. The cell pellet was resuspended in saline and cytospin preparations (Shandon Products Ltd, Cheshire, UK), stained using Diff-Quick (Merz and Dade AG, Düdingen, Switzerland). Differential counts were calculated from 300 cells.

Lavage fluid analysis

The concentration of urea in BALF was measured using a urease method in a Technicon RA-XT discretionary analyser (Technicon Instrument Corp., Basingstoke, UK). At increased sensitivity, a linear dynamic range was achieved over 0.06–6.0 mmol·l^{-1}. BALF samples were analysed for urea as a single batch. Serum urea was measured using the same assay on a blood sample taken for routine clinical management within 6 h of lavage. ELF volume was calculated from the formula:

ELF = (BALF urea/serum urea) × BALF volume (ml)

Changes in vital signs

Before S-BAL the transcutaneous oxygen was calibrated by an arterial gas. Inspired oxygen concentration was increased by 0.1–0.2 (10–20%), if arterial oxygenation was <9 kPa. The inspired oxygen concentration was then kept constant throughout the observation period. Mean arterial blood pressure was measured by a transducer attached to an indwelling arterial catheter. Heart rate, transcutaneous oxygenation ($Ptco_2$) and mean blood pressure were recorded every 30 s for 3 min before S-BAL (baseline period), and at 1 and 3 min post-lavage. The mean value of the 6 pre-lavage observations gave the baseline value.

Postmortem study

At postmortem, a 2.5 kg neonate was intubated and 1 ml·kg^{-1} Omnipaque (Nycomed AS, Oslo, Norway) instilled *via* a 5 French catheter inserted with the infant's head turned to the left. Radiographs were taken just before and 10 s after injection.

Statistical analysis

All values have been expressed as median and range. Wilcoxon's one sample rank sum test was performed on the differences between the two lavage aliquots and on differences between vital signs at the three time points.

Results

Sequential lavage analysis

Cytocentrifuge preparations from three infants were unreadable because of excessive mucus. Seven infants had insufficient supernatant for urea analysis. As lymphocyte differentials were consistently less than 2%, only neutrophils and macrophages were used in calculating differential and total cell counts.

There were no significant differences between percentage BALF recovery, total urea in the sample and calculated ELF volume, between the first and second lavage aliquots (table 2). Total white cell counts were unchanged in the second aliquot. In the subgroup of 11 infants in whom cell morphology was identifiable, the percentage of macrophages increased significantly in the second lavage (p=0.004, table 3).

Table 2. – Total volume and epithelial lining fluid volume recovered in first and second aliquots by suction bronchoalveolar lavage

	n	First BALF aliquot	Second BALF aliquot
Volume instilled ml	14	1.0 (0.7–1.7)	1.0 (0.7–1.7)
BALF recovery %	14	54 (14–43)	59 (36–96)
Total BALF urea µmol	8	0.25 (0.05–0.85)	0.13 (0.07–0.50)
ELF volume ml	8	0.07 (0.01–0.13)	0.05 (0.02–0.06)

Data are given as median value and range in parenthesis. BALF: bronchoalveolar lavage fluid; ELF: epithelial lining fluid. There were no significant differences by Wilcoxon's rank sum test (p=0.11–0.15).

Table 3. – Total cell recovery and differential counts in first and second suction bronchoalveolar fluid aliquots

	n	First BALF aliquot	Second BALF aliquot	p*
Total white cell counts $\times 10^4$	14	18.4 (0.9–423)	10.8 (0.4–336)	0.187
Total white cell counts $\times 10^4$	11†	33.5 (0.9–423)	12.6 (0.4–336)	0.230
Total neutrophil counts $\times 10^4$	11†	21.8 (0.5–102)	1.7 (0.03–140)	0.100
Total macrophage counts $\times 10^4$	11†	7.6 (0–1.6)	7.3 (0.4–1.9)	0.450
Neutrophils %	11†	65 (21–100)	18 (2–80)	0.004
Macrophages %	11†	35 (0–79)	82 (20–98)	0.004

Data are given as median value and range in parenthesis. †: subgroup in which cell morphology was visible; BALF: bronchoalveolar lavage fluid; *: significance by Wilcoxon's rank sum test.

Table 4. – Changes in vital signs of 10 intubated infants during suction bronchoalveolar lavage (S-BAL)

	Pre-S-BAL	Post-S-BAL	
	Baseline	1 min	2 min
Heart rate beats·min^{-1}	149 (129–168)	149 (118–168)	155* (127–170)
Mean blood pressure mmHg	39 (31–58)	49.5* (33–72)	45* (30–68)
Transcutaneous† oxygen kPa	10.6 (9.0–15.0)	7.5* (4.5–8.3)	9.2* (8.4–11.0)

Data are given as median values and range in parenthesis. Difference from baseline values by Wilcoxon's rank sum test (*: p<0.05); †: n=9, one infant excluded for technical reasons.

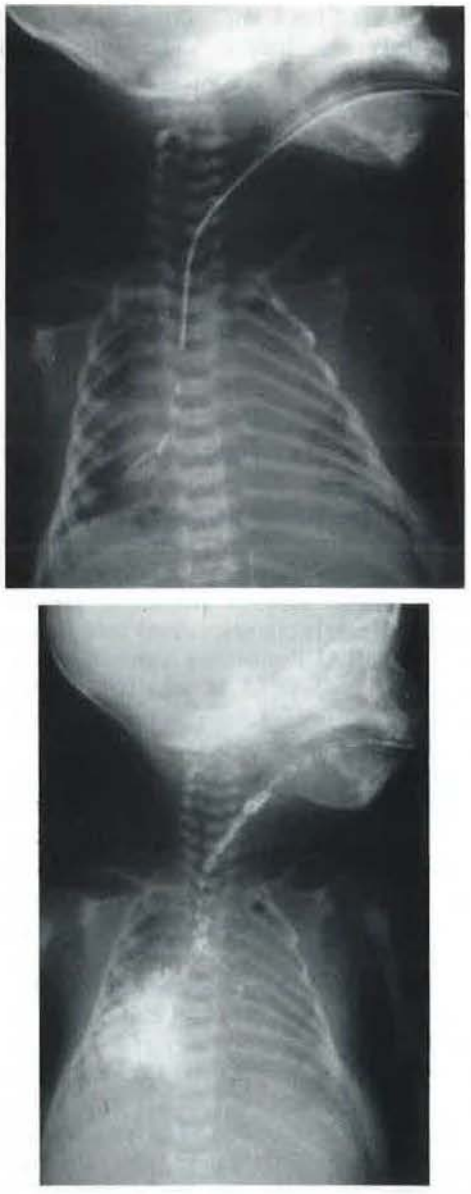

Fig. 1. – a) Radiograph of a 2.5 kg infant intubated at postmortem. With the infant's head turned to the left, a 5 French suction catheter loaded with 1 ml·kg^{-1} Omnipaque has been wedged into a segmental bronchus. b) Radiograph taken approximately 10 s after instillation of 1 ml·kg^{-1} Omnipaque.

There was a corresponding fall in the proportion of neutrophils and a trend for decreased total neutrophil counts in the second BALF aliquot (p=0.10, table 3). The total number of macrophages remained unchanged during sequential lavage.

Vital signs

Heart rate did not change significantly at 1 min post-S-BAL compared to baseline but was elevated at 3 min ($p<0.05$). Mean arterial blood pressure increased at 1 min ($p<0.05$), with incomplete recovery by 3 min post-S-BAL (table 4). Readings of transcutaneous oxygenation ($Ptco_2$) were obtained in nine infants; a technical problem affected the tenth child. $Ptco_2$ fell significantly by 1 min post-lavage ($p<0.05$) and partially recovered by 3 min. Oxygenation fell below 5 kPa in only one infant. The most significant induced fall in $Ptco_2$ occurred in an infant receiving 23% inspired oxygen in whom lavage induced crying.

Postmortem study

The suction catheter passed into the right lower lobe bronchus (fig. 1a) and after injection of 1 $ml \cdot kg^{-1}$ radio-opaque dye, alveolar filling was present in a substantial portion of the basal segments of the right lower lobe (fig. 1b).

Discussion

In this study fractional analysis of sequential BALF obtained by suction lavage (S-BAL) of intubated babies demonstrated significant qualitative changes in cells. The second aliquot contained an increased percentage of macrophages, even though total cell numbers and BALF volume remained constant. Neonatal S-BAL resulted in significant changes in arterial oxygenation and blood pressure with partial recovery by 3 min post-lavage.

The significance of sequential changes during S-BAL depends on the assumption that a "blindly" inserted suction catheter will wedge in the same area on two separate occasions. The exact site of wedging could not be determined for ethical reasons. However, selective placement of straight suction catheters in the right main bronchus using an identical technique occurs in more than 95% of insertions, if the infant's head is turned to the left [12]. During S-BAL, it is possible that the catheter wedged in a different segment of the right bronchus at the second insertion, although a straight catheter should tend to take the same path on each occasion. The postmortem study demonstrated the catheter passing into the right main bronchus and it is likely that this would be the path of least resistance. As the external diameter of the suction catheter was very small (<1.5 mm), it would have wedged in at least a segmental bronchus.

BALF has, in the past, been recovered from adults by wedged catheters [13]. This method has recently been revived in intubated adults, primarily as an aid to bacteriological diagnosis [14]. Fractional processing of BALF from nonfibrescopic BAL has not been reported, although total cell counts and differentials in pooled aliquots are remarkably similar to those from simultaneously performed fibrescopic BAL [15]. It is, therefore, at least theoretically possible that S-BAL and F-BAL of intubated babies would give equivalent results.

In adult F-BAL, the volume of saline instilled is not critical in determining cell differentials [16], as long as the instilled volume of each fraction is >20 ml, and as long as the total volume of instilled fluid is >100 ml [6]. A fixed instilled volume is inappropriate in infants whose weights vary by up to 500%. The 1 $ml \cdot kg^{-1}$ lavage volume for each fraction used in this study should correspond to the 50–70 ml single fraction volume used in adult BAL. It is encouraging that in the postmortem S-BAL, 1 $ml \cdot kg^{-1}$ had the potential to fill the alveolar space. Unfortunately, postmortem analysis does not show the anatomical area sampled by aspiration. Only fractional processing of sequential samples can suggest whether alveolar sampling occurs *in vivo*. In adults, computerized digital subtraction imaging has demonstrated that the first 60 ml of instilled fluid remains in the proximal airway and, although the subsequent fluid shifts are complex, the first aliquot of BALF is mainly "bronchial" [17, 18]. Such an investigation is unethical in sick neonates.

Total inflammatory cell numbers and percentage return did not change between the first and second S-BAL aliquots. Sequential F-BAL in adults using 50–60 ml instilled volumes show an increased fluid recovery and total cell number in the second aliquot [9, 19]. RENNARD *et al.* [5] suggested that changes in cell numbers are due, in part, to alterations in fluid recovery. The constant cell numbers during S-BAL may, therefore, be explained by the similar BALF volumes in the two aliquots.

There is good agreement between neonatal S-BAL and adult F-BAL for the sequential changes in neutrophil and macrophage differentials. RENNARD *et al.* [5] described higher percentages of macrophages in the later "alveolar" lavages compared to "bronchial" samples in a sequential protocol of adult patients. SCHMEKEL and VENGE [9] reported an increase in the total number of macrophages and a decrease in neutrophil numbers in the second 50 ml lavage. However, sequential changes in total neutrophil counts during F-BAL may give less consistent results than differential counts [20]. In this study, analysis of sequential S-BALF demonstrated a change from neutrophil to macrophage predominance in the second aliquot. Direct comparison of S-BAL and F-BAL total neutrophil and macrophage counts is difficult as percentage recovery was not comparable. In addition the wide variability in total cell counts may have obscured any underlying changes, although there was a similar trend to F-BAL for decreased numbers of

neutrophils in the second S-BAL aliquot. It is, therefore, likely that repeated S-BAL in neonates, samples increasingly distal segments.

The urea dilution method for estimating ELF volume has been criticized because urea rapidly shifts from the plasma into the BALF [21]. The trend for increasing total amounts of urea is not usually apparent in the first two F-BAL aliquots [9]. S-BAL is very rapid and should reduce the tendency for urea shifts. Urea was detected in all neonatal S-BAL aliquots where enough supernatant was available. Changes in urea and ELF volume were comparable to adult F-BAL, with no change in total urea in the second aliquot, although the value in correcting for dilution using urea remains unclear.

S-BAL induced significant changes in oxygenation and blood pressure in the small group of infants studied. S-BAL was only performed in place of endotracheal suction, which is a part of the normal nursing care of intubated babies. Non-selective instillation of saline into the endotracheal tube and subsequent aspiration is associated with a fall in oxygenation [11] and a significant rise in mean blood pressure [22] in intubated infants. These changes normally recover by 5 min post-suction [11]. The changes observed with S-BAL were very similar to those reported after non-selective suctioning. Their clinical significance was less apparent. Only one infant became clinically hypoxaemic and, in this subject, there was sufficient capacity to increase her fractional inspired oxygen concentration. No infant became bradycardic and although virtually all increased their arterial blood pressure, the peak level did not cross the proposed "stability boundary" associated with an increased risk of intracranial haemorrhage [23]. The risks of S-BAL are likely to be the same as routine endotracheal suctioning, for example bronchopleural fistula [24]. However, in some cases, selective lavage may have some additional clinical benefit. Saline instilled *via* a suction catheter can be more effective than routine ET suctioning in clearing tenacious secretions [25]. Our clinical practice when performing S-BAL for research is to use it only where inspired oxygenation may be increased and not if significant bradycardia or hypoxia has been recorded during routine suctioning. Where there is a risk that thick secretions could be washed down into peripheral bronchi, "dry" suctioning, without prior instillation of saline, is performed before lavage.

.We have demonstrated that some of the cellular changes during sequential suction bronchoalveolar lavage of neonates are similar to those seen after fractional processing of adult wedged fibreoptic BALF, the second aliquot probably providing a more distal sample of cells and ELF. A sample of pooled first and second S-BAL aliquot should, therefore, reflect cells within both the proximal and distal airways of neonates. Despite the consistent sequential changes in neutrophil differentials, uncertainty remains about whether this technique repeatedly samples exactly the same pulmonary segment. In this respect, S-BAL differs significantly from F-BAL. "Blind" suction lavage of intubated infants offers the opportunity to follow the evolution and resolution of pulmonary inflammation and could be applied to older intubated children.

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