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LIPID ANALYSIS OF THE BRONCHOALVEOLAR FLUID FROM SEASON TWO OF THE BRONCHIOLITIS ENDOTRACHEAL SURFACTANT STUDY (BESS)

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Introduction and aims:

The Bronchiolitis Endotracheal Surfactant Study (BESS) is a national multi-centre phase-2 air-placebo-randomised controlled trial to determine the efficacy and safety of endotracheal surfactant administration (Poractant alfa) in reducing total duration of Mechanical Ventilation by 18 hours or more versus air placebo in the treatment of critically ill infants with bronchiolitis (https://www.bess-trial.org.uk/).

Here we report the blinded data for the sub-mechanistic lipid analysis of bronchoalveolar lavage fluid (BALF) from the season two samples.

Methods:

Fifteen recruitment sites participated for season 2 and a total number of 114 patients were recruited between these sites. Surfactant administration (Poractant alfa) were given three times with 12 hours interval (figure 1).

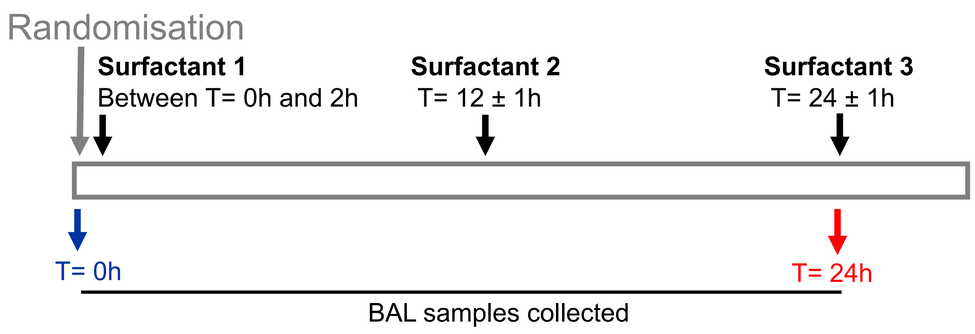

Figure 1. Overview of the surfactant administration and sample acquisition

Aliquots of BALF samples taken at T=0h and 24h were lipid extracted (n=74). An internal standard was added to each sample to enable phospholipid quantification.

Samples were measured by direct infusion electrospray ionisation mass spectrometry using diagnostic MS/MS scans for phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylehanolamine (PE), phosphatidylserine (PS) and sphingomyelin (SM) molecular species.

Results:

All samples contained sufficient lipid to be detected by mass spectrometry, although some samples gave very weak signals probably due to inadequate recovery of alveolar material (**figure 1**).

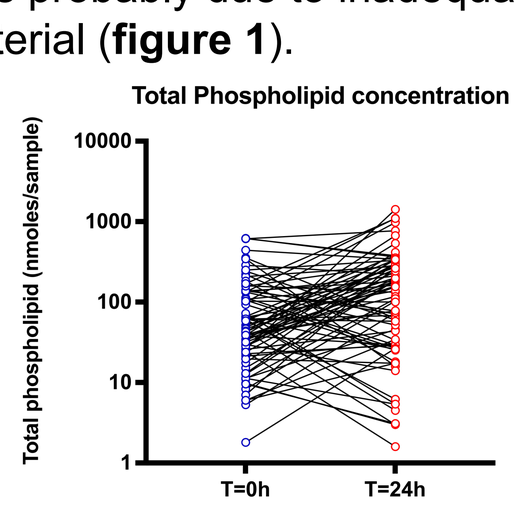

Figure 2. Total amount of phospholipids in T=0h and T=24h samples, n=74.

The percentage PC ranged from 44-86%, while normal surfactant PC is typically about 80% of total phospholipid, a result suggesting a variable contribution of membrane material from inflammatory cells to the total lipid extracts (**figure 3A**).

Different subgroups of phospholipids (SM, PE, PG, PI and PS) were measured in the BALF and varied widely at both T=0 and T=24 hours, which clearly demonstrate that this analysis alone did not discriminate between surfactant and control groups (**figure 3B-F**).

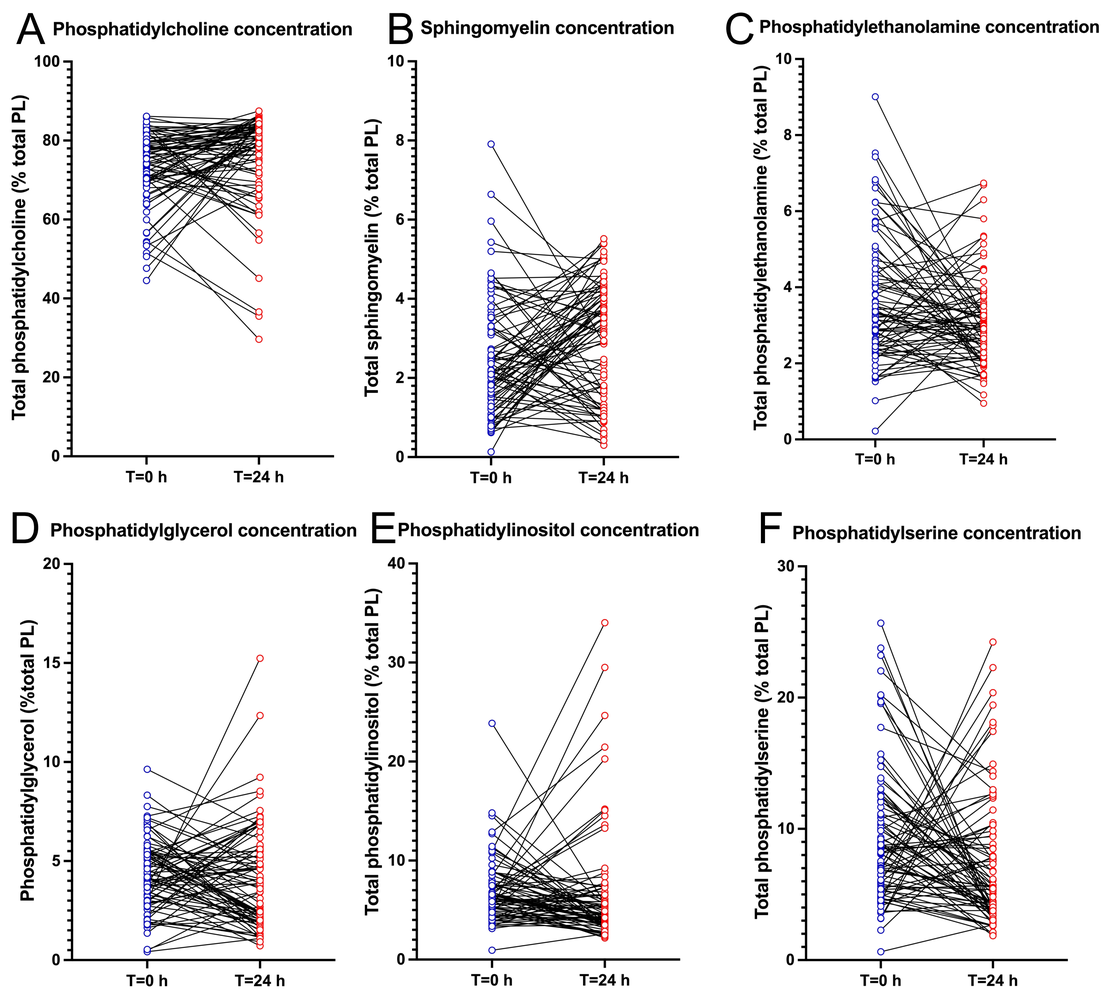

Figure 3. Concentration of different phospholipid classes in paired samples taken at T=0h and T=24h, n=74.

Discussion and conclusion:

- The study showed that the protocols for BALF collection are generally adequate to provide adequate data for analysis.

- There was a tendency for the percentage PC to increase (**figure 3A**) and that of PS to decrease in the T=24h samples (**figure 3F**), which would be consistent with the expected change in the surfactant treatment group.

- The SM distribution at T=24h is potentially diagnostic as there is a clear separation at T=24h for %SM, with this value increasing and decreasing in comparison with T=0h in approximately equal numbers (**figure 3B**).

- Unblinding of the results will enable further analysis of the samples and allow us to evaluate if the generated lipid data can be used as a potential biomarker or for diagnostic purposes.

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BESS website: https://www.bess-trial.org.uk/

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