Proposed Best Practice Checklist

Bronchoscopy and BAL

The role of Bronchoscopy

BAL is usually carried out via a pediatric flexible fiberoptic bronchoscope; in intubated patients BAL is performed after inserting the FFB through the endotracheal tube using a swivel Y connector. Whether the bronchoscopy is performed through an endotracheal tube, a laryngeal mask or facemask, suction should not be used before the bronchoscope has been passed beyond the vocal cords, in order to avoid contamination of bacteriological samples with upper airway flora, as far as possible.

Location of BAL

In case of diffuse disease, the BAL target site should be chosen on the basis of an HRCT performed before the procedure, rather than choosing a traditional BAL site (i.e., the right middle lobe or lingula). In infants it is often easier to perform BAL in the right lower lobe. If BAL and lung biopsy is to be done at the same session, BAL should not be performed in the lung lobe chosen for biopsy.

Amount of fluid and recovery

BAL is carried out using sterile normal saline. BAL volume is adjusted to body weight using 3-4 mL/kg body weight (bw) of normal saline divided into three (to 4) equal fractions in children weighing < 20 kg and 3 mL/kg in 20 mL (or up to 50 ml portions if syringe is available) portions in children weighing > 20 kg. The fluid may be recovered by hand suction using a syringe or by mechanical aspiration into a suction trap. The negative suction pressure should be adjusted to avoid visible airway collapse

Processing BAL Fluid

- part of the first BALF aliquot should be unfiltered and used for microbiological studies, others for cell differential of bronchial fraction

- the following aliquots should be pooled, filtered through 1 or 2 layers sterile gauze to remove mucus (only if a lot of mucus present, which is unlikely in chILD) and used for analysis of cellular and non-cellular components.

o Recovered fluid can be transported at room temperature if the delay between BAL fluid retrieval and delivery to the laboratory is less than 30 minutes. If not it should be kept at 4° C before analysis to optimize cell viability.

o Cytospins are obtained after centrifugation (500 rpm for 10 min).

o For further analysis, the rest of BAL should be centrifuged (2000 rpm for 10 min), giving the **supernatant (**stored at -70°C) and the **cellular sediment (BAL cells)**.

Cryopreservation (BAL cells)

1 Label cryotubes (name, cell count, cell type, freeze date, initials on lid)

2 After passing through the mesh to remove mucus, centrifuge BAL cells for 5 min at 500g and freeze the supernatant in 1.5 ml portions (ideally 2-3 portions).

3 Resuspend in 1-2 ml PBS, then count. Centrifuge again at 500g for 5 minutes, remove supernatant and resuspend cells in 1 ml Bambanker. Then transfer to cryotubes, invert cryotubes 3 times.

Alternative to Bambanker: **medium** composed of 70% RPMI1640, 20% FCS and 10% DMSO (final concentrations).

4 Place cryotubes in a freezing box. Store sample at -80°C (or -20°C until shipping if no -80C).

Endobronchial Biopsy (EBB)

Although the performance of EBB is fully justified as part of bronchoscopy, its role in the investigation of chILD is limited. Specifically, it is not useful for the diagnosis of NEHI, in which condition the increase in Bombesin positive cells is not seen in the proximal airways. However, the presence of non-caseating granulomas may be a pointer to a diagnosis of sarcoidosis.

Material

- Best samples are obtained using a bronchoscope which has a working channel at least 2 mm (smaller instruments ie with a 1.2 mm working channel are much less efficient for biopsy)

- The standard biopsy forceps is a fenestrated cupped forceps

Location

Bronchial biopsies are taken from secondary and tertiary carina under direct vision. It is unwise to perform bilateral invasive procedures

Processing

Four to 6 samples should be taken. One sample should be used for microbiological studies (if clinically relevant); two to four samples are fixed on 4% formaldehyde and embedded in paraffin wax; one to two fresh samples should be kept frozen at -80°C

Transbronchial biopsy (TBB) forceps or cryo

Transbronchial biopsy is useful in suspected lung transplant rejection, but its use in chILD is limited by the sample size. It is most useful in diffuse lung disease with highly specific features, e.g. alveolar

microlithithiasis [1]. There are also significant complications (bleeding, pneumothorax). For most cases of chILD, a surgical biopsy is the investigation of choice.

Material

- Best samples are obtained using a bronchoscope which has a working channel at least 2 mm (smaller instrument ie with a 1.2 mm working channel can be used but are much less efficient)

- The standard biopsies forceps are a fenestrated cupped forceps or an alligator forceps Location

- TBB should be done under fluoroscopy under general anaesthesia

- Samples are always taken on the same side, never bilaterally

Processing

Three to six samples are taken. One sample should be used for microbiological studies; two to four samples are fixed on 4% formaldehyde and embedded in paraffin wax; if surfactant dysfunction disorders are suspected, a sample is placed in glutaraldehyde for EMI. One or two fresh samples can be kept frozen in RNA later at -80°C

References

1. Wallis C, Whitehead B, Malone M, Dinwiddie R. Pulmonary alveolar microlithiasis in childhood: diagnosis by transbronchial biopsy. Pediatr Pulmonol. 1996; 21: 62-4