Best Practice Checklist:

Surgical Lung Biopsy Protocols

Indications

- chILD when the diagnosis is unclear; ideally should be performed prior to commencing treatment, but see below

Relative contra-indications

- Very severe diffuse, endstage lung disease, where an interpretable biopsy may not be obtained
- High risk – advanced respiratory failure, other organ complication, high dose steroid or immunosuppressive therapy
- The child who is close to being ventilated is a particular diagnostic dilemma – the biopsy may mean the child remains ventilated after the procedure, whereas empirical treatment*, if successful*, may mean the child avoids ventilation

Complications

- Those of any surgical procedure
- Pneumothorax, haemorrhage, wound infection, prolonged intubation, pneumonia
- Risk increases with immunosuppressive therapy and prior respiratory insufficiency

Technique (This is not intended to be prescriptive but a guide; we would prefer this technique be used unless there are local circumstances which precludes this).

A) Open lung biopsy

- An experienced surgeon needs only a few minutes operating time
- Incision of 3-4 cm above the area where the biopsy is to be taken, usually lateral dorsal or ventral access
- After opening the pleural space part of the lung is clasped with a clamp and pulled through the thoracotomy opening
- Biopsies from two different lobes are taken, using a stapler

B) VATS

- Single lung ventilation is necessary
- Three trocars, one for the camera and one for the stapler and usually a third one for taking the biopsy, are inserted

HANDLING OF SAMPLES IN CHILDREN WITH SUSPECTED ILD

Referring laboratories should comply with national Accreditation standards and practices, with access to light microscopy and common special stains, immunohistochemistry, genetics services, microbiology and virology services, photographic equipment.

SURGICAL LUNG BIOPSIES

- although biopsy material may be obtained by transbronchial or percutaneous biopsies in theory, surgical lung biopsy is the method of choice
- Biopsy should only be performed in centres capable of processing the biopsy correctly (liaison with the surgical team is essential)

- Ideally, biopsy sites are targeted pre-operatively through HRCT correlation to ensure abnormal areas are sampled.
- Biopsy should yield a sample of at least 10x10x10mm. The tip of the lobe should be avoided.
- To diagnose infection a separate specimen is sent fresh and directly for microbiological study. If tissue is in short supply and a staple line is present, then the tissue attached to the staple line, which would otherwise be redundant, can be used.
- One mm pieces of tissue are taken from different areas and fixed in glutaraldehyde for ultrastructural investigation. Additional tissue should be snap frozen or placed in RNAlater to facilitate additional genetic testing, if possible.

- Ideally pathologist or other experienced person: gently inflates the remaining tissue with formalin *via* a small bore needle, taking care not to over-expand the tissue as this can cause artefact that mimics lymphangiectasia, or wash out alveolar contents such as macrophages
- Otherwise and in most cases, the tissue goes straight into formalin. The specimen will normally be a wedge of subpleural lung, stapled along the surgical margin.
- A description includes its dimensions and any parenchymal or pleural abnormalities. The row of staples is cut off (unless already used). The axis of slicing will depend on the volume of tissue, but ideally aim for sections with the largest possible area.

Staining - outline:

- haematoxylin and eosin (H&E)-stained single section should provide an adequate picture of extent, distribution and nature of any pathology
- stain to highlight collagen/pulmonary vasculature recommended as a routine (e.g. Elastic Van Gieson)
- Depending on H&E findings, further staining may be required, such as a Perls' stain to identify haemosiderosis, and a PAS stain to look for glycogen
- If infection (e.g. TB, Pneumocystis) is suspected, appropriate stains (e.g. Ziehl-Neelsen, Grocott, PAS) will be necessary
- Immunohistochemistry is used at the discretion of the pathologist for identification of tumours and some DPLDs, particularly Langerhans cells (S-100, CD1a)
- A vascular marker such as CD34 is sometimes useful if abnormalities in the vasculature are suspected
- Bombesin staining should be undertaken if NEHI is suspected (this can be done via referral, if required)
- Ultrastructure is infrequently used nowadays in a diagnostic setting, but is of value when assessing inborn errors of metabolism and some surfactant protein gene mutations.

It is therefore worth ensuring assessable tissue is kept in children, even if the decision after reviewing the sections is that analysis is not required.

Report

- should comment on the adequacy of the specimen (e.g. is there sufficient parenchyma if an interstitial lung disease is being investigated), and the nature of any morphological changes. The conclusion should provide a differential diagnosis of the causes of identified histological patterns. There is a proposed classification for paediatric diffuse parenchymal lung disease, which can be used as a template [1].

Handling of Lung tissue - SOPs

There are four principal pathways <u>every</u> lung biopsy should go through.

1. Submit almost all tissue into wax blocks (formalin)
2. taking 2-3 small pieces for electron microscopy which are used to aid diagnosis (glutaraldehyde buffer)
3. if enough is available store some frozen using the protocol below or alternatively fix in solution to conserve RNA and proteins (e.g. RNA*later*® Solution)
4. If tissue amount is small the tissue in the staple line can be sent to microbiology.

1. Formalin fixation for conventional histology (wax block)

<u>Tissue for conventional histology</u> (About 90% of the tissue blocks will be formalin fixed for conventional histology)

- Fix tissue blocks in 4% formalin
- Local pathology will produce wax blocks and slides; the slides are read.
- The wax block will be sent to the Register for further distribution to the reference pathologist
- Alternatively the wax block may be shipped directly to the reference pathologist. Slides are produced, read and then sent together with the wax blocks to the collecting point at the Kids Lung Register.

Long term storage: Storage in formalin is only possible for a short period of time. Therefore embedding into paraffin (= wax block) is essential.

2. Glutaraldehyde fixation for electron microscopy (Epoxid block)

<u>Tissue for electron microscopy</u>

- Three pieces per biopsy site, of about 2x2 mm, are placed in glutaraldehyde buffer (recipe below). The buffer needs to be stored frozen (-20°C) until used.
- Ship to biobank for embedding into epoxide

Long term storage: Material for the electron microscopy cannot be stored in glutaraldehyde and must be embedded in epoxide blocks. Alternatively, one part of the sample can be stored in glutaraldehyde buffer (like the one described above), while the other part is embedded into epoxide (service of the KLR).

<u>Glutaraldehyde fixiation solution for biopsies</u>

Chemicals: Aqua ad injectabilia (Braun, Melsungen, Deutschland); Hepes (Sigma,H3375), paraformaldehyde (Merck), glutaraldehyde (Sigma,G6257).

Protocol:

1. 0,4 M Hepes (238,3 g/mol: 95,32g/L or 9,532g/100ml = 0,4 Mol). Adjust pH to 7,4.
2. Heat 45 ml H_2O at 70°C, add 4 g paraformaldehyde. Add appr. Xxx µl NaOH, or until solution turns clear. After cooling down of the solution the pH needs to be adjusted to pH of 7,4; add 0,4 M Hepes to a final volume of 100ml.
3. Add glutaraldehyde to a final concentration of 0,1% (400µl).

Note: The buffer is stable for 4 weeks at 4°C or 1 year at –20°C, in this case prepare smaller aliquots.

3. (a) RNA*later*® Tissue Collection or (b) direct freezing of the tissue in OCT and liquid nitrogen

(a) Tissue for RNA, DNA and protein analysis

- Use RNA*later*® Solution with ***fresh tissue only***; do not freeze tissues before immersion in RNA*later*® Solution.
- To ensure rapid and reliable stabilization of RNA even in the inner parts of solid tissues, the sample must be cut into slices **less than 0.5 cm thick** before immersion in RNA*later*® Solution. The slices can be any convenient size, provided one dimension of the sample is <0.5 cm.
- Place the fresh tissue in 5–10 volumes of RNA*later*® Solution (approx. 10 µl RNA*later*® per 1 mg tissue)
- Send immediately at ambient temperature to central biobank for further processing and long term storage

(b) Tissue for frozen section processing, immunohistology, RNA analysis, biochemical tests

- Maintain sterile conditions wearing gloves and using surgical scissors or scalpel and forceps.
- Add 1-2 drops of cryomatrix (Tissue Tek O.C.T Compound, Sakura, Ordering No. 4583) into cellulose capsules (Ordering No. 001033-55, Küppers-Primax GmbH, Troisdorf, Germany). Cryomatrix is a medium containing Optimal cutting medium (OCT). Place the tissue samples in the cellulose capsules, cover it with another 3-5 drops of cryomatrix; avoide formation of bubbles.
- Close the capsule and place into liquid nitrogen. After freezing, transfer capsule into a cryovial and store it in liquid nitrogen.
- Transfer from liquid nitrogen (if intermediate storage is necessary place into -80°C freezer) on dry ice and ship to the biobank for long-term storage
- Always process several small pieces (appr. 3 x 3 x 5 mm).
- Long term storage is possible at -70° C or -196° C [2].
- After freezing the tissue into liquid nitrogen, PCR and biochemistry can still be performed. Histopathology will no longer be possible.
- Problems: Artefacts associated with the capsule-freeze technique.

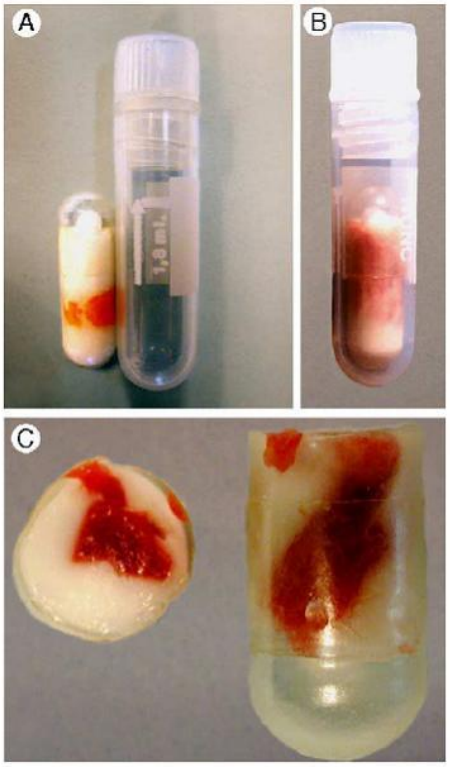

Fig. 2 Capsule-freeze method of tissue storage. A, Cellulose capsule filled with tissue and frozen OCT next to a cryovial. B, The capsule fits easily within the cryovial for convenient storage. C, When needed, slices may be cut from the capsule for histological or other studies. The remaining tissue remains safely embedded within the OCT capsule.

Processing of post-mortem lung biopsies

- also for single organs, e. g. lung and liver.
- post-mortem lung biopsy or autopsy should best be communicated with the parents before the death of the patient. If autopsy is rejected, sometimes it is possible to do a post-mortem biopsy using a truecut needle.
- This may also be a possible solution for people of islamic faith (see [3]).

Autopsy specimen and explanted lungs

All **explanted lungs** from diseased children, as well as unused corresponding donor lung should be immediately processed for the above indicated principal pathways:

(1) each lung should be cut in 1.5 x 1.0 x 0.3 cm sections, put in formalin and processed to paraffin blocks, each labeled uniquely with the location within the lung from which the tissue was obtained and the case number.
(2) Similarly some much smaller pieces of tissue (2x2x2 mm) are conserved in glutaraldehyde buffer.
(3) Importantly 1.5 x 1.0 x 0.3 cm sections should be immediately frozen and stored in liquid nitrogen freezers for subsequent molecular and proteomic studies. Some material may also be put into RNA*later®* Solution and processed as described above; however this is only suitable for smaller amounts of tissue.
(4) Tissue for microbial studies may be obtained.

Autopsy lung sections are prepared for paraffin blocks and frozen sections.

4. Detection of pathogens

Material for microbiology

- Obtain sterile microscope slides for Gram staining of the cut surface of a biopsy. Usually three slides air dried and three fixed with alcohol.
- Additionally small pieces from the edges or left overs can be put in sterile saline for culture and PCR under sterile conditions.
- Close liaison with the microbiology laboratory prior to performing the biopsy is essential.

General note on transportation of the specimens

A) at room temperature (all material for microbiology, fixed for conventional histology, electron microscopy and tissue in RNA*later®* Solution)

B) on-site frozen material must be shipped on dry ice (nitrogen/frozen tissue

1. Deutsch, G.H., et al., *Diffuse lung disease in young children: application of a novel classification scheme.* American journal of respiratory and critical care medicine, 2007. **176**(11): p. 1120-8.
2. Loken, S.D. and D.J. Demetrick, *A novel method for freezing and storing research tissue bank specimens.* Hum Pathol, 2005. **36**(9): p. 977-80.
3. El-Reshaid, W., K. El-Reshaid, and J. Madda, *Postmortem biopsies: the experience in Kuwait.* Med Princ Pract, 2005. **14**(3): p. 173-6