I-BFM initiative on CSF ALL biomarkers

CSF biobanking SOP

(version 4th May 2021)

Aims/Concept

BASIC: standardised collection, processing and storage of CSF supernatant for soluble biomarker (DNA, RNA, protein, metabolite) research purposes.

OPTIONAL ADDITIONS: cells (cellular nucleic acids, proteins, etc.), extracellular vesicles to store.

CONCEPT: To make collaborations and joined research projects possible. This is much needed due to the rarity of CNS positive cases of paediatric lymphoblastic malignancies. Local or national storage of samples and a central online registry are practical and possible to organize.

Target population

- BASIC: frontline & relapsed (1st, 2nd, etc.) ALL & lymphoblastic lymphoma
- OPTIONS: depending on capacity, sample collection can be narrowed down to relapsed patients and/or CNS positive patients
- Age 0-25 years

Which CSF samples to store

MANDATORY

- From CSF positive frontline cases and from all relapse cases:
  - CSF from all LPs within 3 months from start of treatment.
  - CSF either from first LP during maintenance or from last LP before transplant.
- From all the other patients:
  - initial lumbar puncture at time of diagnosing ALL or relapse,
  - one sample from early induction (d8-15 whenever in the protocol)
  - end of induction,
  - first LP during maintenance or last LP before transplant.
- No LP should be performed specifically for this project. Only to collect CSF from LPs indicated for routine diagnostic or therapeutic reasons.

OPTIONAL

- CSF storage from any other time points when routine LPs are performed.

Sampling and volumes

BASIC / MAIN PROJECT

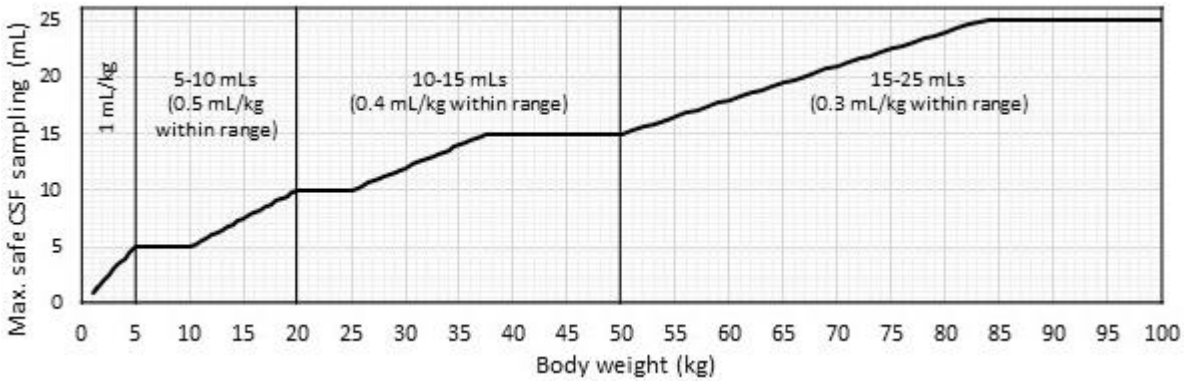
- Take 2 mLs of CSF for research above the volume used for routine diagnostics, aiming for storage of 4 supernatant aliquots 0.5 mL each.

- Use universal containers to collect CSF. Do not add any reagents (e.g. Transfix, TRIzol) to samples aimed for this research project.

OPTIONAL EXTRAs

- More 0.5 mL aliquots to store.
- Larger volume aliquots to store.
- Some published data suggest that it is safe to withdraw a total of
    - 1 mL/kg CSF from infants
    - 5-10 mLs (0.5 mL/kg within this range) CSF from children weighing 5-20 kgs
    - 10-15 mLs (0.4 mL/kg within this range) CSF from children weighing 20-50 kgs
    - 15-25 mLs (0.3 mL/kg within this range) CSF from children weighing 50 kgs and above

See references in Appendix 1 (page 5) of this document to support the safety of these large CSF volume withdrawals. Even larger CSF volumes are routinely lost and replaced during neuro-surgical procedures.
It is difficult to estimate the number of research projects that would use these samples. Especially studies on cells and extracellular vesicles would benefit from larger volumes.



ADDITIONAL POINTS

- Routine diagnostic samples are the highest priority, only extra volumes may be used for research. From small infants it may not be possible to take large enough volumes above that needed for routine diagnostics.
- We recommend using atraumatic (blunt) needles.
- In case of a bloody tap, aim to collect later clearer drops. Numbering the tubes allows laboratory staff to pick the best samples for various purposes.
- Perform LP in lying patient position if taking large volumes.
- Allow passive dripping, do not apply suction.
- There is no agreement on the need of replenishing the CSF space with normal saline after CSF withdrawal, if it poses an infection risk, or if helps preventing post-puncture headache. Apply the local policy.
- Consider the long sampling time when withdrawing large volumes.
- CSF samples should ideally be processed in the lab within 30 mins from sampling; tolerable in 6 hrs. Note: quality of routine CSF tests will also decrease beyond 30 mins.
- Keep samples at +2 to +8 °C until transfer and processing these are not possible immediately.

Local processing of CSF samples

BASIC / MAIN PROJECT

- Use native CSF for banking, before any contamination with reagents, e.g. Transfix, Trizol, etc.
- Keep samples at +2 to +8 °C until the separation of the supernatant from cells if centrifugation is not possible immediately.
- Separate the supernatant from cells ideally within 30 mins, tolerable within 6 hrs
    - Centrifugation at 300 g for 10 mins, no active break.
    - To store 0.5 ml aliquots of the supernatant, ideally in liquid nitrogen, or as cold as possible (-80 °C better than -20 °C, etc).
- If centrifugation and deep freezing are not possible before transfer, the sample will still be accepted. It may still be suitable for DNA and protein research, but probably less so for the analysis of other components.

OPTIONAL

Two extra ways to store research samples – these are only rational/useful in case of CSF samples that may contain leukaemic cells. E.g. diagnostic samples of suspected new or newly relapsing patients, or for early follow up samples of known CNS positive cases.

- Adding TRIzol to the cell pellet (remaining after centrifugation described above) in order to store cellular nucleic acids:
    - after centrifugation, resuspend the cell pellet (which will be invisible to the naked eye) in 800 µl TRIzol reagent and store at -80 °C.

    Trizol will save some RNA (and DNA) but will make the analysis of all other components impossible.
- Storing fixed unstained cytospins (if enough CSF volume, as supernatant aliquots are the priority):
    - Aim for 2 such samples.
    - Encircle the cells on the cytospin by a fat pen before fixation.
    - Pipette 150 µl fresh PFA 4% (cold, stored at 4°C before) on the cells of the cytospins, incubate for 10 mins under a fume hood, gently take away the PFA using a pasteur pipette.
    - Cover the cytopsins with 150 µl PBS 2x for 10 mins, gently rinse the PBS on a lab tissue.
    - After the 2nd time carefully rinse with distilled water using a pasteur pipette or a wash bottle.
    - Dry the slide in a standing position.
    - Store at room temperature in specific slide boxes.

    Immuno-staining and single cell analysis techniques could be used on these cytospin samples later.

Sample transfer

- If transfer is needed to another lab, then do it ideally in as cold circumstances as possible (dry ice, if not possible, then -20 °C or 4 °C etc.)
- Consider freezing samples at the local hospital and ship as a batch every few months to a national centre together in dry ice.

Material request form for clinical departments

See attached. This contains the essential information for proper collection/processing/transport of the CSF samples, also space for data to be noted for each sample. This form accompanies the CSF sample from the procedure room where lumbar puncture is performed to the lab of final banking.

Data and database

Plans are in place to use VIBE. Until then, storing of paper forms are advised, or centres / study groups can store data in Excel files or their own online registries.

Required data on demand (not there in the material request form, usually there in all study registries) for planning research projects:

- Category of relapse (number of relapses, time to relapse, medullary and/or extramedullary involvement at relapse)
- Relapse treatment protocol
- Immunophenotype and genetic features of the patient's leukaemia occurrence

(These items are all included in VIBE, won't be extra task for working groups already using VIBE, and so are consent forms)

Cooperating groups behind this initiative:

- Arja Harila-Saari and colleges, Uppsala University, Sweden
- Christina Halsey and colleges, University of Glasgow, United Kingdom
- Cornelia Eckert and colleges, Charité Universitätsmedizin Berlin, Germany
- Dániel Erdélyi and colleges, Semmelweis University, Budapest, Hungary
- Denis Schewe and colleges, Universitätsklinikum Schleswig – Holstein, Kiel, Germany
- Geertruy Te Kronnie and colleges, University of Padova, Italy
- Janez Jazbec and colleges, Ljubljana University Medical Centre, Slovenia
- Kjeld Schmiegelow and colleges, Rigshospitalet, Copenhagen, Denmark
- Lüder Meyer and colleges, Universitätsklinikum Ulm, Germany
- Manuel Ramirez and colleges, Hospital Universitario Niño Jesús, Madrid, Spain
- Mervi Taskinen and colleges, Helsinki University Central Hospital, Finland
- Shai Izraeli and colleges, Tel Aviv University, Israel
- Wojciech Młynarski and colleges, Medical University of Łódź, Poland

Contact: Dániel Erdélyi at erdelyi.daniel@med.semmelweis-univ.hu

Dániel J. Erdélyi, M.D. Ph.D.
Paediatrician, Haematologist
2nd Department of Paediatrics,
Semmelweis University,
Budapest, Hungary

Appendix 1: literature to support the safety of large CSF sampling volumes:

- Monserrate et al.: Factors associated with the onset and persistence of post–lumbar puncture headache, JAMA Neurol. 2015;72(3):325-332.
- UpToDate Topic 4832 Version 15.0
- Day et al.: Deciphering the factors that influence participation in studies requiring serial lumbar punctures, Alzheimers Dement (Amst). 2020; 12(1): e12003
- Engelborghs et al.: Consensus guidelines for lumbar puncture in patients with neurological diseases. Alzheimers Dement (Amst). 2017 May 18;8:111-126.
- Bonadio: Pediatric lumbar puncture and cerebrospinal fluid analysis. J Emerg Med 2014 Jan;46(1):141-50.
- Suresh et al.: Regional anesthesia, a practice of anesthesia for infants and children, 5th Edn. Philadelphia: Elsevier Saunders, 2013; 835–79
- Rochette et al. Cerebrospinal fluid volume in neonates undergoing spinal anaesthesia: a descriptive magnetic resonance imaging study. Br J Anaesth. 2016 Aug;117(2):214-9.
- Blomquist et al.: Cerebrospinal fluid hydrodynamic studies in children. J Neurol Neurosurg Psychiatry. 1986 May;49(5):536-48.
- Yan WL, Dou ZZ, Chen TM, et al. Impact of supine recumbency duration on the complications after lumbar puncture in children. J Appl Clin Pediatr. 2014;29(12):914–8.
- Ebinger, F. et al. Headache and Backache After Lumbar Puncture in Children and Adolescents: A Prospective Study. Pediatrics. 113: 6 (2004).

	Total CSF volume	CSF production per minute	Precedents for CSF sampling
Young infant	~10 mLs/kg		3 mLs or 1 mL/kg
Toddler	4 mLs/kg in toddlers		up to 20 mLs in 2-16y children; ≥7 mL volumes with no extra AEs
Child	65-150 mLs in 4-13y old children	0.3-1 mL, similar to adults	
Adult	90-160 mLs or 2 mLs/kg	0.4-0.5 mL	30 (-40) mLs; 15-30 mLS w/o AEs

Rationale behind suggested safe sampling:

- <20% of total CSF volume
- below 1.5 hrs CSF reproduction rate