CSF Investigations in patients with seizures

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Background

- Epileptic seizures common feature in many inherited metabolic disorders
  - particularly those involving cerebral grey matter
- undertake a metabolic work-up of all infants & children with epilepsy in conjunction with additional symptoms
  - impaired early development
  - mental retardation
  - other neurological abnormalities

Basic investigations before considering CSF

Investigation	Urine	plasma
U&E, LFT's, calcium, magnesium		√
Glucose		√
Ammonia		√
Blood gases		√
Biotinidase		√
Lactate		√
Organic acids	√	
Amino acids	√	√
Homocysteine		√
Ketostix	√	
Acylcarnitine profile		√

Some of the disorders detected by the previous list of tests

- Homocystinuria/MTHFR – homocysteine
- Molybdenum co-factor/sulphite oxidase – amino acids (sulphocysteine)
- Canavan disease (aspartoacylase) - organic acids (N-acetylaspartate)
- L-2-hydroxyglutaric/ D-2-hydroxyglutaric aciduria – organic acids
- 4-hydroxybutyric aciduria (SSADH) – urine organic acids
- Malonic aciduria – urine organic acids
- Glutaric aciduria type I & type II – organic acids/acylcarnitines
- Urea Cycle defects – amino acids/ammonia
- Glutathione synthetase deficiency – organic acids (5-oxoproline)
- (?)Pyridoxal-phosphate dependent epilepsy PNPO – organic acids (vanillactic acid)

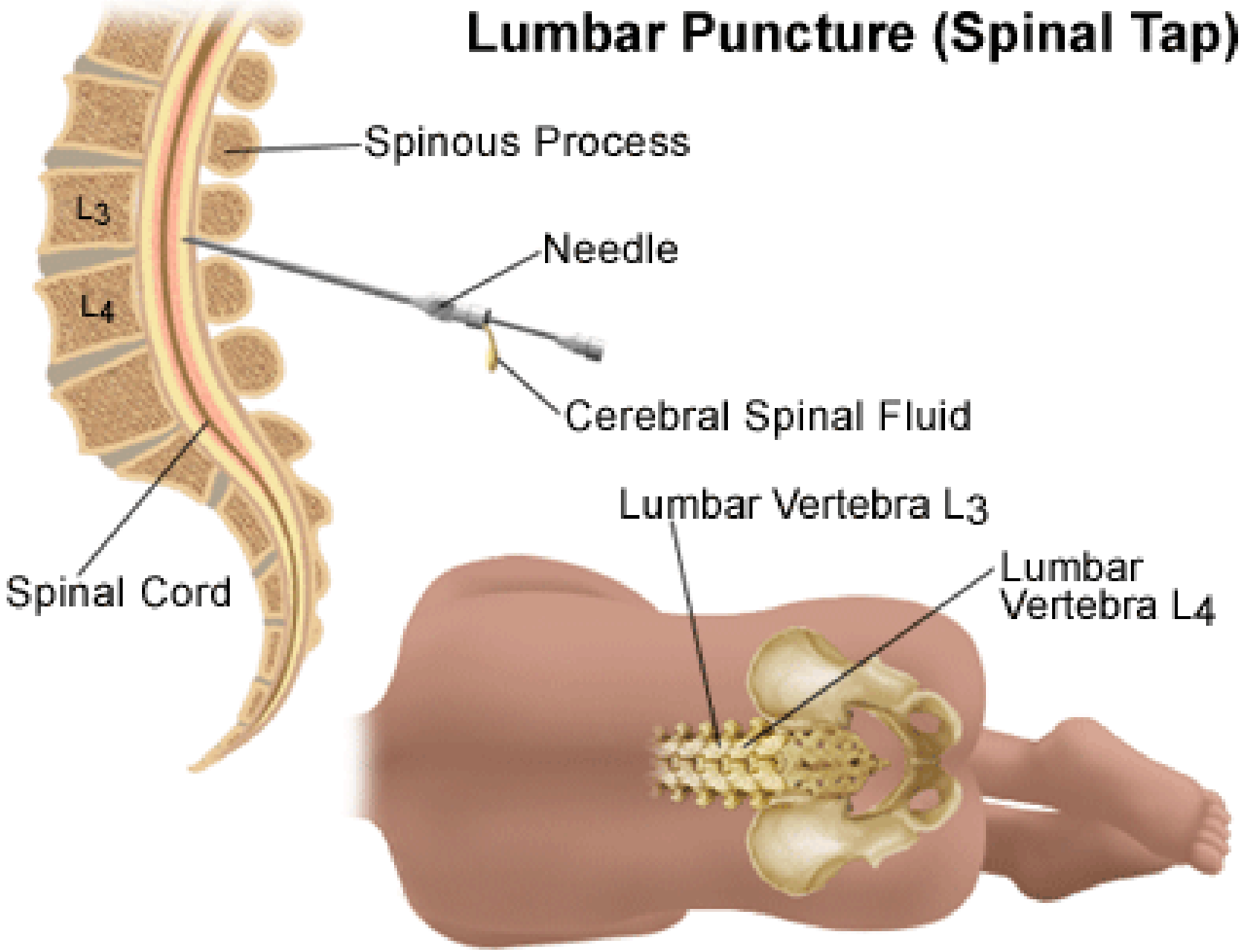
Some investigations require paired samples

	CSF	plasma
glucose	√ (fluoride)	√ (fluoride)
lactate	√ (fluoride)	√ (fluoride)
glycine	√	√
amino acids :- serine, threonine, alanine, glycine proline	√	√

What do we measure in CSF

- glucose
- lactate
- amino acids
  - glycine, serine, alanine, proline, threonine
- pipecolate
- neurotransmitters
- folate /5MTHF
- pterins
    - neopterin, dihydrobiopterin and tetrahydro-biopterin **BH4**

Lumbar Puncture (Spinal Tap)



CSF glucose must be a fluoride sample

- Often requires simultaneous sampling of plasma & CSF
- Take plasma glucose first !!
  - trauma of CSF collection increases plasma glucose
- Often used for exclusion of
  - GLUT1 deficiency – glucose transport protein deficiency
- fasting child plasma 3.0-6.5 mmol/l
  - **CSF glucose 2.8-4.4 mmol/L**
- CSF/plasma glucose ratio (mmol/mmol) 0.65 $\pm$0.1 in normals

CSF glucose

Interpretation

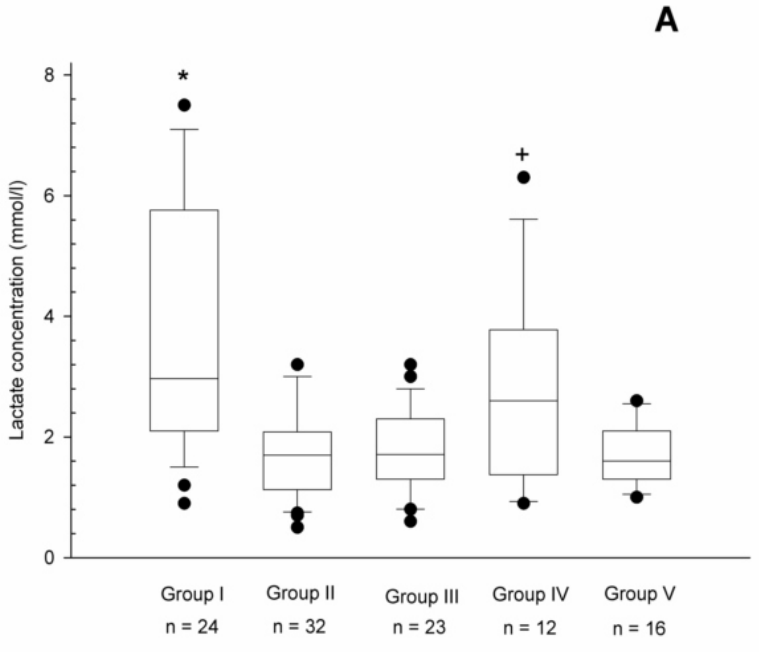
- CSF/plasma glucose ratio (mmol/mmol) <0.6 in GLUT1

- In practice - Leen et al 2010 Brain 133:655-670
- Described 57 patients
- CSF glucose <2.5mmol/l (0.9-2.4)
- Ratio 0.19-0.52 (<0.5 in all but one)
- GLUT1 patients can have a ratio >0.4!
- Normal neonates do sometimes have a ratio of ≤0.4
- May need to repeat assay
- View within clinical context (epilepsy, microcephaly, psychomotor delay)
- Go to mutation analysis of *SLC2A1* gene

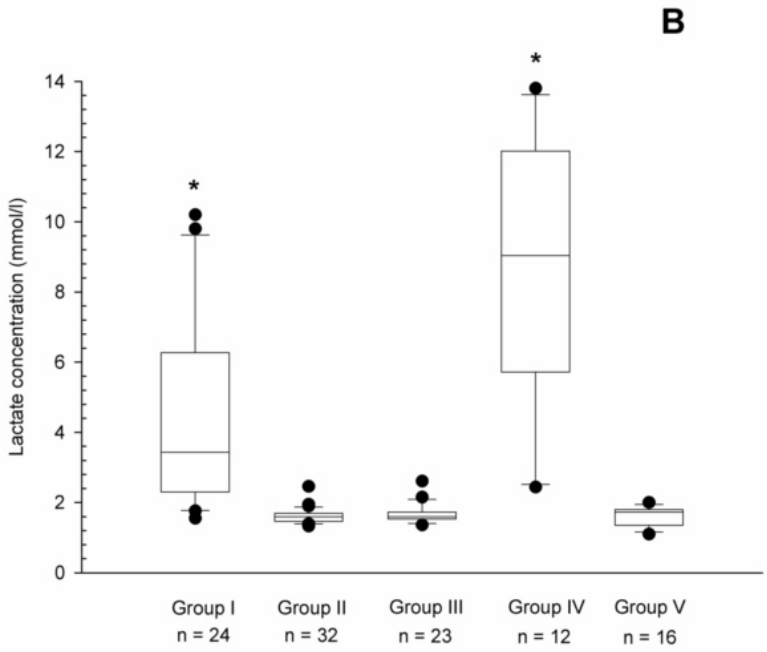
CSF lactate
Fluoride Sample

- Investigation of respiratory chain defects
  - blood staining will increase the CSF lactate

BLOOD (controls <2.3 mmol/L

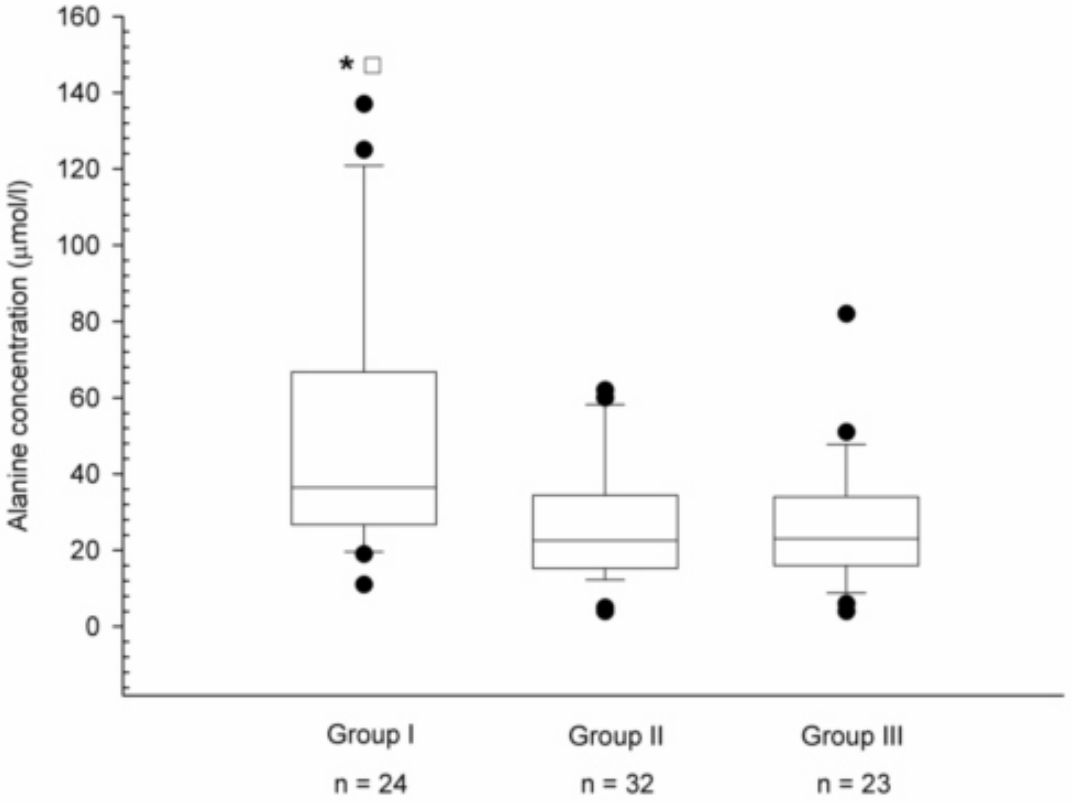


CSF (controls <2.1 mmol/L



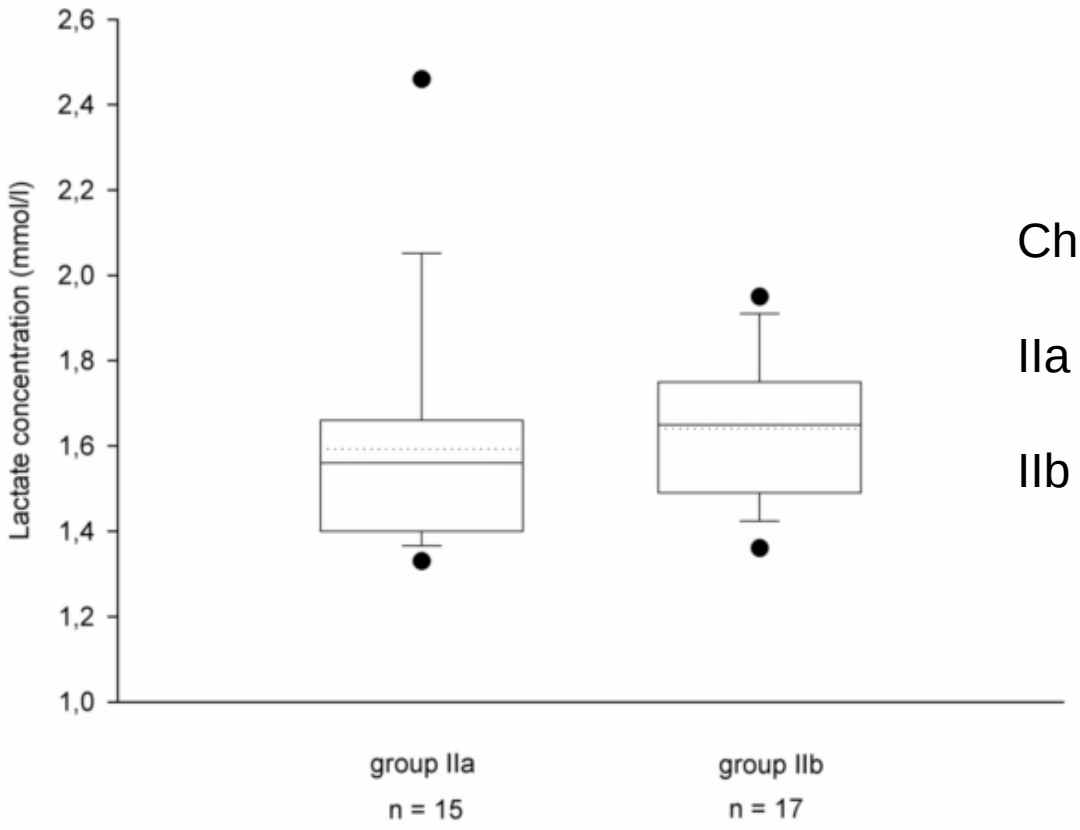
I – Respiratory chain disease - **83% had increased lactate**
II – epilepsy (15 samples 3±0.6 hrs post <3 min seizure) - **3% increased CSF lactate**
III – moderate to severe psychomotor delay - **9% had increased CSF lactate**
IV – bacterial meningitis - **all had increased CSF lactate**
V – acute febrile illness without neuroinfection - **none with increased CSF lactate**

CSF alanine (controls <35 µmol/L)



I – Respiratory chain disease
II – epilepsy (15 samples 3±0.6 hr post seizure)
III – moderate to severe psychomotor delay

CSF lactate (controls <2.1mmol/L)



Children with epilepsy

IIa seizure within 3±0.6 hrs

IIb no recent seizure

CSF lactate/pyruvate ratios

- When & why!!

- In most cases when plasma or CSF lactate are raised so is the L/P ratio
- Measuring lactate on its own is usually enough
- In cases where PDH is a possible diagnosis
  - if CSF lactate is raised
    - CSF L/P ratio is likely be informative
    - Up to ~20 – normal (PDH!)
    - >25 raised (respiratory chain defect)

CSF pyruvate

- Need to collect CSF into an equal volume of perchloric acid (pre-weighed tube)
- Mix & store at -20^{o}C
- From outside laboratories
    - transport on dry ice

Conclusion

In the differential diagnosis of respiratory chain disorders

1. Increased CSF lactate is more reliable than blood lactate
2. Meningitis does significantly increase CSF lactate
3. CSF lactate & alanine are reliable markers even after a brief seizure
4. L/P ratios - use only in differential diagnosis of PDH

CSF amino acids

CSF no preservatives, no blood contamination!

- What do we measure & what is normal?
- CSF Glycine (3-19 µmol/L*)
  - CSF/plasma glycine ratio
- threonine (ref. 12-178 µmol/L)
    - ↑PLP responsive seizures
      - ↓threonine dehydratase
- alanine (ref. 15-60 µmol/L)
- proline (<5 µmol/L) (plasma ref. 66-333 µmol/L)
    - to exclude blood contamination
- serine (35-80 µmol/L)
- ?sulphocysteine (not usually present)

— *Jones, Smith, Henderson. Ann Clin Biochem 2006; 43: 63-66

Differential diagnosis of NKH

- Establish that the patient is “non-ketotic”
  - many organic acidaemias cause “ketotic hyperglycinaemia”
- Causes of non-ketotic hyperglycinaemia
  - valproate reduces hepatic GCS
  - PLP dependent seizures ↓GCS with ↑CSF glycine!
- Requires CSF/plasma glycine ratio
  - Urine organic acids (exclude OA’s)
    - May need to stop valproate

CSF glycine & CSF/plasma glycine ratio

- Plasma glycine
  - age related reference ranges
  - term newborn 56-308 µmol/L
  - NKH 920-1827 µmol/L
  - Atypical 447 µmol/l

- CSF glycine
    - 3-19 µmol/L (97.5 centile Jones et al 2006)
    - 3-10 µmol/L (Sciver)
- neonatal NKH 83-280 µmol/L
- atypical NKH 42, 72 µmol/L

- CSF/plasma glycine ratio
  - normal 0.012-0.04 (usually <0.02)
  - neonatal NKH 0.09-0.25
  - atypical 0.06-0.10

CSF glycine & CSF/plasma glycine ratio in non-ketotic hyperglycinaemia

- Plasma glycine
  - Plasma glycine 988 µmol/L (normal range 56-308)

- CSF glycine
  - 168 µmol/L (ref 3-10 µmol/L)

- CSF/plasma glycine ratio
  - 0.170
  - normal 0.012-0.04 (usually <0.02)

A not uncommon problem

- Preterm Neonate - seizures
- Plasma glycine
  - 1035 µmol/L
  - term newborn 56-308 µmol/L
  - NKH 920-1827 µmol/L
  - Atypical 447 µmol/l

- CSF glycine
- 95 µmol/L
- 3-19 µmol/L (97.5 centile Jones et al 2006) 3-10 µmol/L (Sciver)
- neonatal NKH 83-280 µmol/L
- atypical NKH 42, 72 µmol/L

- CSF/plasma glycine ratio
  - 0.091
  - normal 0.012-0.04 (usually <0.02)
  - neonatal NKH 0.09-0.25
  - atypical 0.06-0.10

- BUT
- CSF proline = 56 µmol/L
- Normal <5µmol/L
- Blood contamination !!!!

CSF Serine

- Low values associated with serine synthesis defects
    - Secondary low 5MTHF
        - Low serine limits one carbon donation to THF
- Blood serine often high after meals
    - Normal plasma 66-333 µmol/L
- Need to take fasting samples
    - both plasma & CSF!

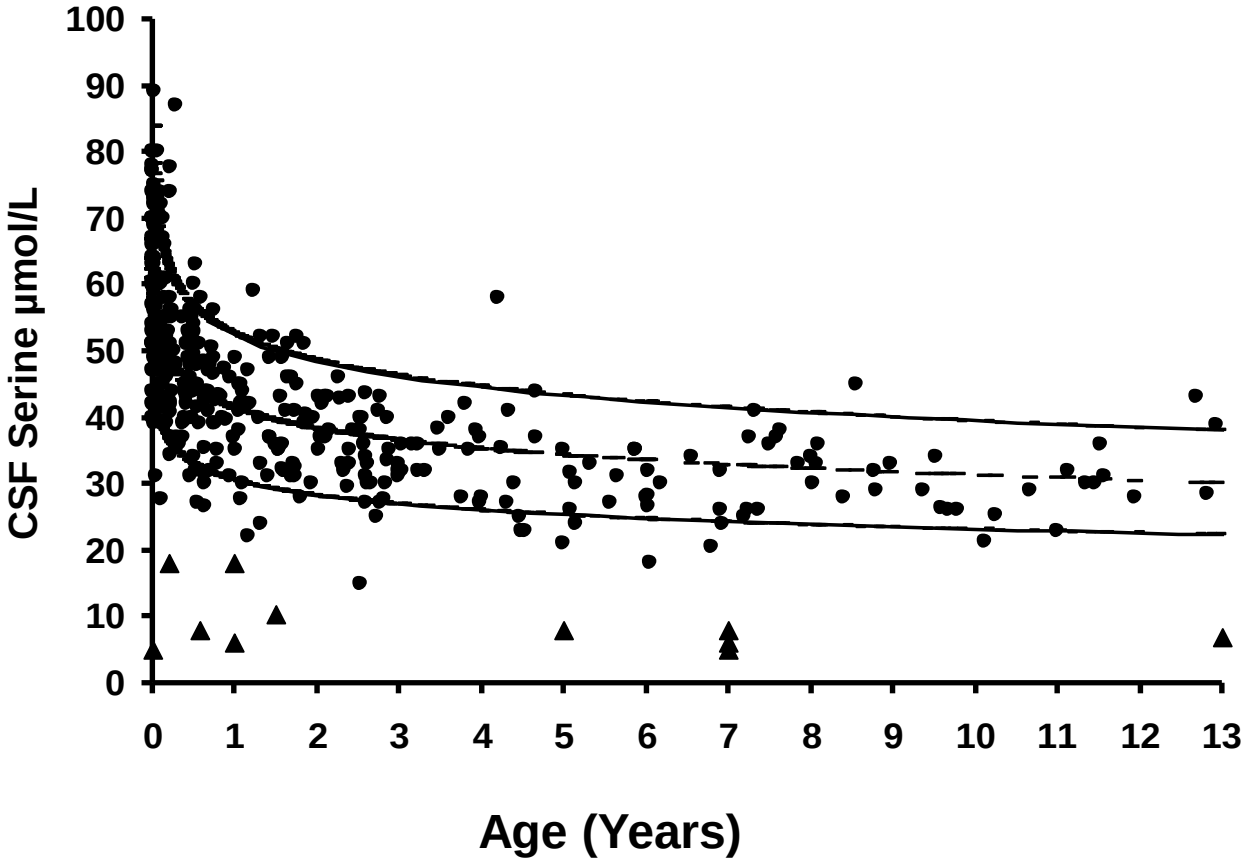
Table 2 Summary of predicted mean CSF serine concentrations and reference intervals for different age groups

(S. Moat et al 2010 Mol Genet Metab)

Age group	Predicted Mean (µmol/L)	Reference Intervals (Mean ±1.96 SD) (µmol/L)
1 week	59	43-74
2 weeks	56	41-70
3 weeks	54	39-68
1 month	52	38-66
2 months	49	36-62
3 months	47	35-60
6 months	44	33-56
9 months	43	31-54
1 year	41	30-52
1.5 years	40	29-50
2 years	38	28-48
3 years	37	27-46
5 years	34	25-43
10 years	31	23-39
15 years	29	22-37
20 years	28	21-35

How low is low!!

Our reference range
35-80 µmol/L



Regression based reference intervals for CSF serine. The upper curve indicates the +1.96SD and the lower line indicates the -1.96SD. The central line represents the mean serine concentration as a function of age. Closed triangles indicate serine concentrations at the time of diagnosis in patients with disorders of serine biosynthesis.

?secondary serine deficiency

J Inherit Metab Dis. 2010 Mar 19.

Fatal cerebral edema associated with serine deficiency in CSF

- Keularts IM, Leroy PL, Rubio-Gozalbo EM, Spaapen LJ, Weber B, Dorland B, de Koning TJ, Verhoeven-Duif NM

- Two young girls with toxic encephalopathy
  - plasma & CSF serine both very low (as low as 3-PGDH)!
    - ?used as gluconeogenic substate

patient 1 Adult

- plasma
  - serine 144 (75-200)
  - glycine 272 (100-450)

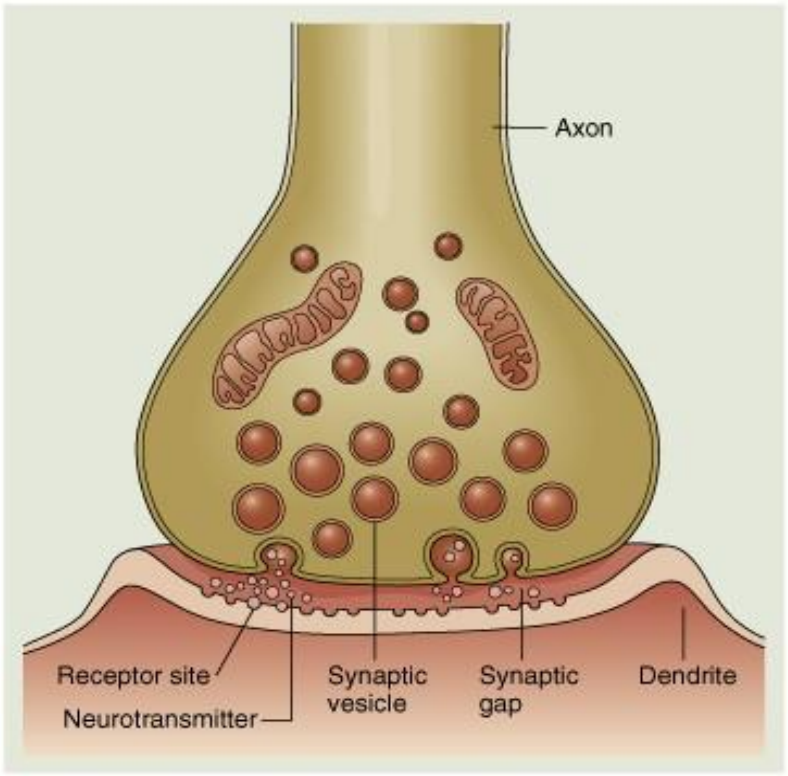
- CSF glycine 9 (3-10)
- CSF serine 25 (35-80)
- Age 20 yrs - range 21-35 (Moat et al 2010)

CSF pipecolate

- Raised in pyridoxine responsive seizures
- CSF most reliable in detecting B6 dependency
- Remains elevated after treatment with B6
- Can do assay on 100µl CSF (plain)

Chemical Neurotransmission

- Neurotransmitters – *Substances that upon release from nerve terminals, act on receptor sites at post-synaptic membranes to produce either excitation or inhibition of the target cell*



CSF Monoamine Metabolites, 5-Methyltetrahydrofolate and Pterins.

To be filled in by requesting clinician/laboratory

Surname: **Forename:**

Hospital: **Hospital No:**

Sex M / F **DOB:**

Specimen date & Time: **Consultant:**

Clinical Details:

Drug therapy: **IMPORTANT!**

PLEASE NOTE the above details are essential to allow for the accurate interpretation of results.

Collection Instructions

- **Tube 1** 0.5 ml for HVA and 5HIAA measurements.
- **Tube 2** 0.5 ml for 5MTHF (folate) determination & pyridoxal phosphate
- **Tube 3** 1.0 ml (contains 1mg of preservative) for pterin (neopterin, dihydrobiopterin and tetrahydro-biopterin).

The 3 CSF samples must be placed in Liquid Nitrogen ***immediately*** after collection (will bubble violently)

Return the liquid nitrogen container with a request form to Clinical Chemistry

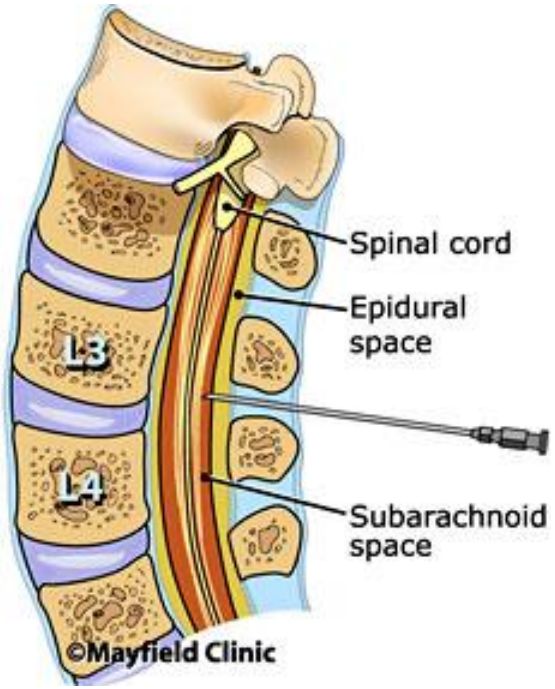
Phone ext 17445 for any queries**.**

Test	Tick if required	Result	Units	Reference Range
HVA*			nmol/l	
5HIAA*			nmol/l	
HVA:5HIAA ratio				1.0-3.7
5MTHF* (folate)			nmol/l	
Neopterin			nmol/l	7-65
Dihydrobiopterin			nmol/l	<0.4-13.9
Tetrahydrobiopterin*			nmol/l	

CSF – Sample Requirements

- *Tube 1* 0.5ml **HVA & 5-HIAA**
- *Tube 2* 0.5ml **5-MTHF**
- *Tube 3* 1.0ml **Pterins**

(DTE/DETAPAC)



Collect at bedside and freeze immediately in liquid N2
Store -70C
Transport on dry ice

Cerebral folate deficiency

J Inher Metab Dis (2010) 33:563-570

Hyland K, Shoffner J, Heales S

Cerebral Folate Deficiency - Neurological syndrome associated with low CSF 5-MTHF and normal peripheral folate.

Therefore need to assess peripheral folate status

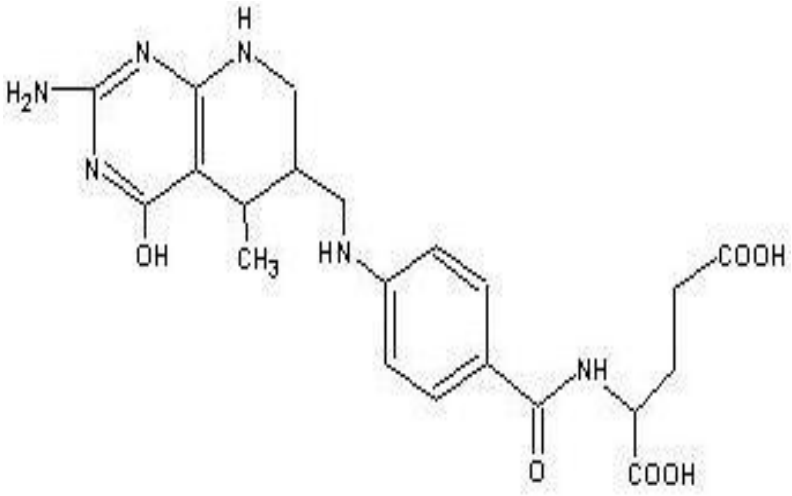
Cerebral Folate Deficiency

- Presentation 4 – 6 months after birth with irritability and sleep disturbance
- Deceleration of head growth (6 – 18 months)
- Psychomotor retardation, sometimes followed by regression.
- Cerebellar ataxia
- Pyramidal tract signs in lower limbs
- Dyskinesis
- Epileptic seizures
- Sub group – autistic features
- Responsive to folinic acid (isovorin L-isomer)
- DO NOT GIVE folic acid (↓CSF 5MTHF)

Cerebral Folate Deficiency

- Production of blocking auto-antibodies against folate receptor.
- ? Produced by exposure to soluble folate binding proteins in human or bovine milk. (Ramekers et al., 2005).
- Milk free diet down regulates folate receptor auto-immunity (Ramekers et al., 2008).
- Blocking auto-antibodies not present in all patients with cerebral folate deficiency.
- Defects in *FOLR1 & FOLR2*

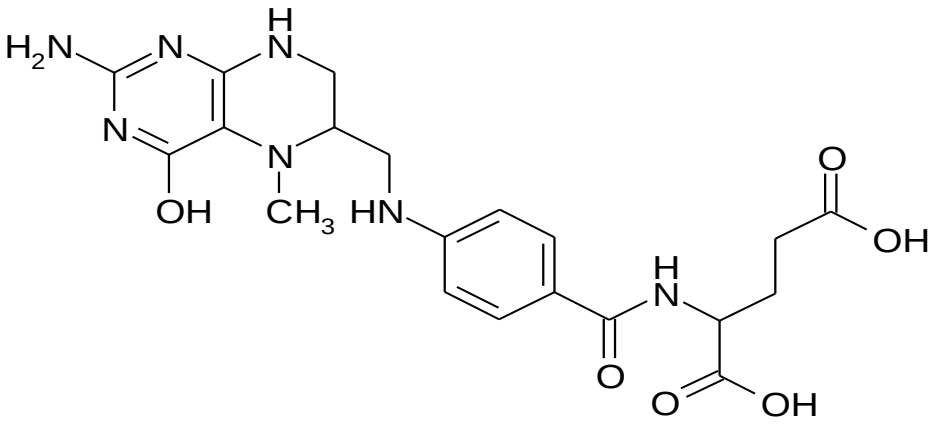
CSF 5-MTHF Deficiency



5-methyl tetrahydrofolate

- DHPR deficiency dihydropteridine reductase
- MTHFR deficiency methyl-tetrahydrofolate reductase
- AADC deficiency **aromatic L-amino acid decarboxylase**
- 3-Phosphoglycerate dehydrogenase def
- Rett syndrome
- Aicardi Goutieres
- Mitochondrial disorders
- L-dopa treatment
- Methotrexate
- Anticonvulsants
- Steroids
- Co-trimoxazole

5-Methyltetrahydrofolate



- CSF deficiency documented in mitochondrial disorders
- 25% of ETC defects associated with CSF 5-MTHF deficiency
- No apparent correlation with magnitude of defect
- Responsive to folinic acid
  - improved neurological function
    - did not halt progression of the disease

Secondary Causes

- Hypoxia
- Neurodegeneration
- Epilepsy
- Gaucher Disease
- Drugs
- Sample Processing

Summary

- Careful clinical evaluation is vital
- Do basic metabolic investigations first
  - This may provide vital clues or even a diagnosis!
- Important to collect appropriate samples e.g. paired plasma & CSF
  - and to process these appropriately
- Use the experts