GLUT1DS and CSF

Prof. dr Michèl A. Willemsen

Radboud university medical center
Department of Pediatric Neurology
Nijmegen, The Netherlands

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Content

- GLUT1DS diagnosis: the central role of CSF analysis
- GLUT1DS: details of CSF results
- Some diagnostic pitfalls
- Some remaining questions on pathophysiologic mechanisms

When to suspect GLUT1DS?

“Hypoglycemia” without hypoglycemia

Early morning movement disorders and seizures

How to diagnose GLUT1DS?

- Phenotype
- CSF plus blood glucose, and CSF lactate
- *SLC2A1* gene

(Glucose transport velocity in erythrocytes; FDG-PET; …)

The importance of the three cornerstones

- Phenotype
- CSF
- *SLC2A1* gene

The importance of the three cornerstones

- Phenotype → broad clnical spectrum
- CSF → sensitive & specific
- *SLC2A1* gene → false negative in 1/10 patients

Different routes to a diagnosis

- Phenotype
- CSF plus blood glucose, and CSF lactate
- *SLC2A1* gene

Classic

- Phenotype
- CSF plus blood glucose, and CSF lactate
- *SLC2A1* gene

Popular ☺

- Phenotype
- CSF plus blood glucose, and CSF lactate
- *SLC2A1* gene

Popular ☹

- Phenotype
- CSF plus blood glucose, and CSF lactate
- *SLC2A1* gene

DNA: 10% false negative
Mutation: disease causing?

Ultra-modern ☺ ☺ ☺

- Phenotype
- CSF plus blood glucose, and CSF lactate
- *SLC2A1* gene

Ultra-modern ☹

- Phenotype
- CSF plus blood glucose, and CSF lactate
- *SLC2A1* gene

Mutation: disease causing?

Incident ☹

- Phenotype
- CSF plus blood glucose, and CSF lactate
- *SLC2A1* gene

Incident ☺

- Phenotype
- CSF plus blood glucose, and CSF lactate
- *SLC2A1* gene

Rule of thumb: follow the classic route!

- Phenotype
- CSF plus blood glucose, and CSF lactate
- *SLC2A1* gene

Rule of thumb: follow the classic route!

- Phenotype
- CSF plus blood glucose, and CSF lactate
- *SLC2A1* gene

CSF analysis:

Simple and cheap
Almost everywhere available
Results on the same day

Reasons not to follow the classic route

- Phenotype
- CSF plus blood glucose, and CSF lactate
- *SLC2A1* gene

Known family (DNA only)
Mild phenotypes (DNA first)
good other candidate genes
no (urgent) reasons for treatment

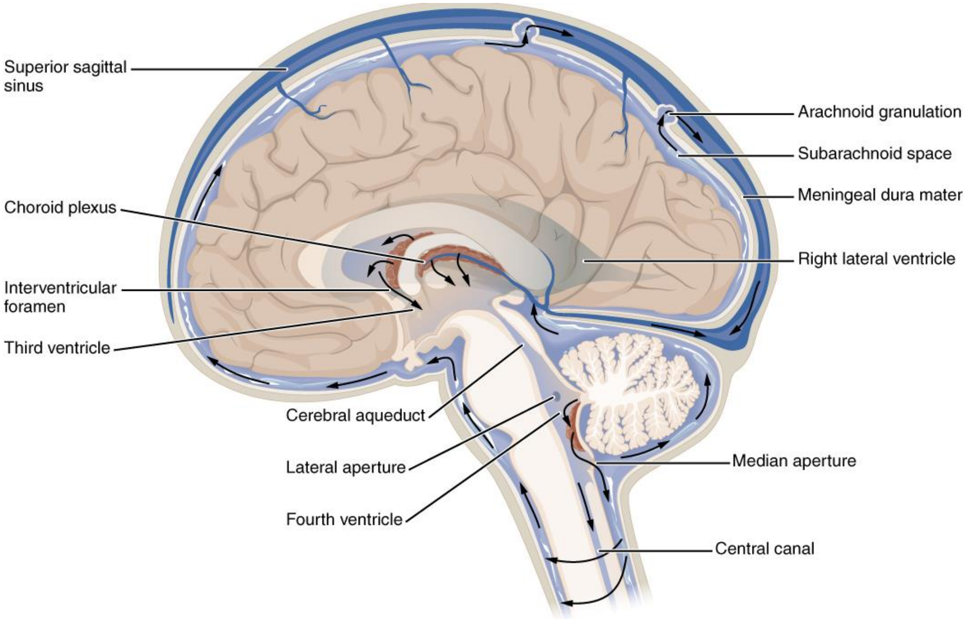
The importance of the three cornerstones

- Phenotype → spectrum
- CSF → sensitive & specific
- *SLC2A1* gene → “false negative in 1/10 patients”

The importance of the three cornerstones

- Phenotype → spectrum
- CSF → sensitive & specific
- *SLC2A1* gene → "false negative in 1/10 patients"

What is the value of CSF analysis in the diagnostic work-up of GLUT1DS?



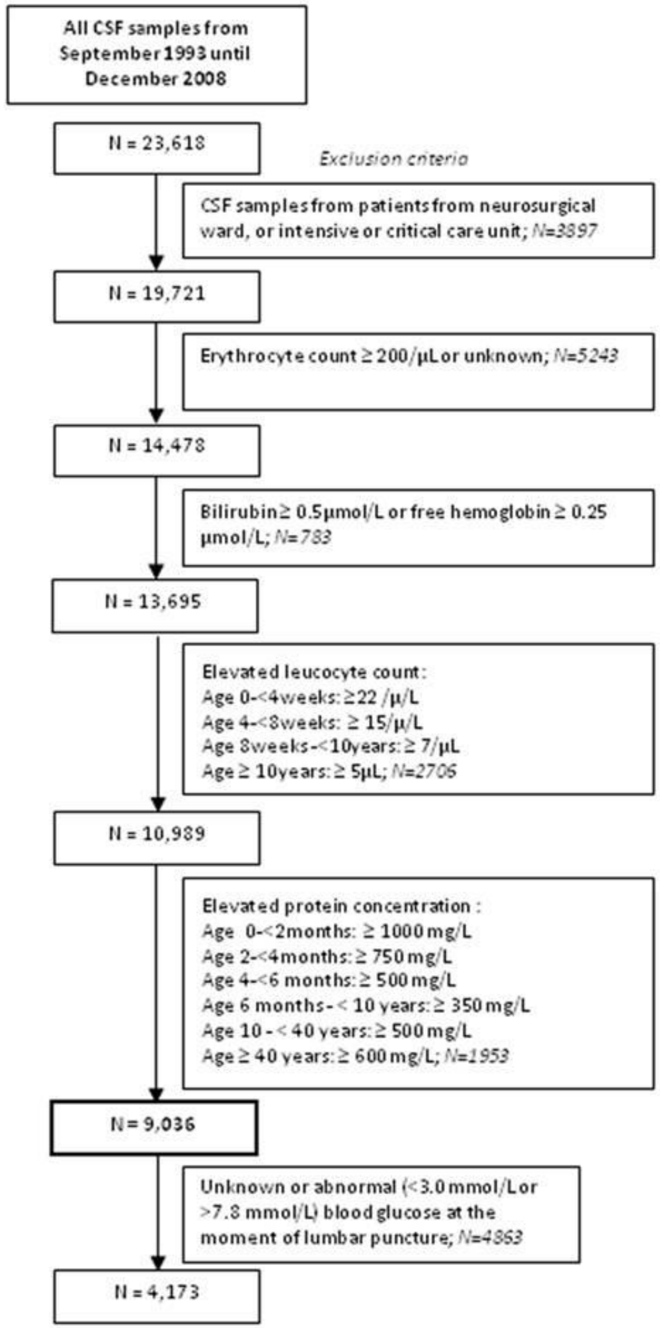
Q1: CSF glucose and lactate reference values?

OPEN ACCESS Freely available online

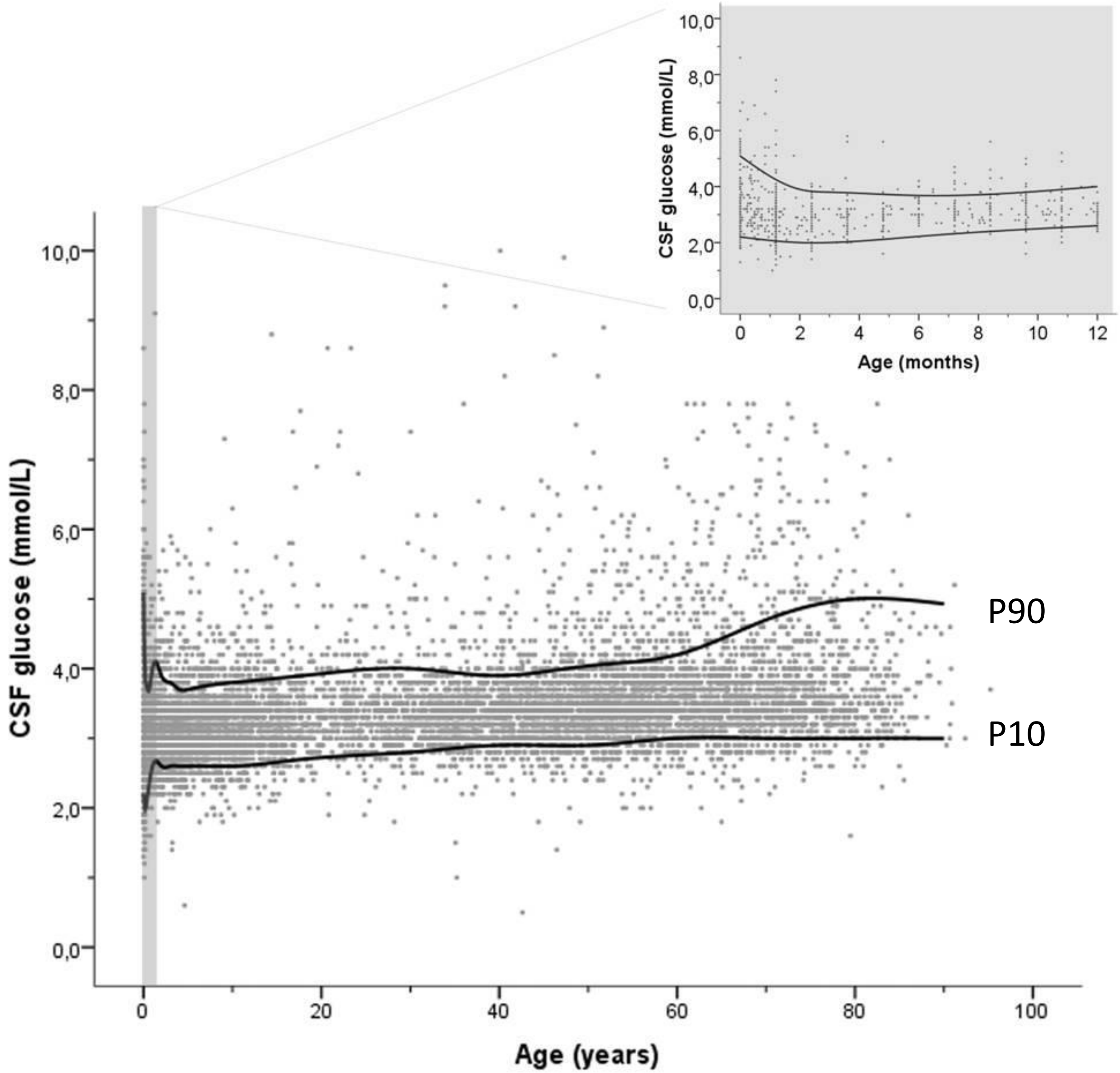
PLoS one

Cerebrospinal Fluid Glucose and Lactate: Age-Specific Reference Values and Implications for Clinical Practice

Wilhelmina G. Leen[1*], Michèl A. Willemsen[2], Ron A. Wevers[3], Marcel M. Verbeek[1,3]


All CSF samples from September 1993 until December 2008
N = 23,618
Exclusion criteria
CSF samples from patients from neurosurgical ward, or intensive or critical care unit; N=3897
N = 19,721
Erythrocyte count ≥ 200/µL or unknown; N=5243
N = 14,478
Bilirubin ≥ 0.5µmol/L or free hemoglobin ≥ 0.25 µmol/L; N=783
N = 13,695
Elevated leucocyte count:
Age 0-<4weeks: ≥22 /µ/L
Age 4-<8weeks: ≥ 15/µ/L
Age 8weeks-<10years: ≥ 7/µL
Age ≥ 10years: ≥ 5µL; N=2706
N = 10,989
Elevated protein concentration :
Age 0-<2months: ≥ 1000 mg/L
Age 2-<4months: ≥ 750 mg/L
Age 4-<6 months: ≥ 500 mg/L
Age 6 months - < 10 years: ≥ 350 mg/L
Age 10 - < 40 years: ≥ 500 mg/L
Age ≥ 40 years: ≥ 600 mg/L; N=1953
N = 9,036
Unknown or abnormal (<3.0 mmol/L or >7.8 mmol/L) blood glucose at the moment of lumbar puncture; N=4863
N = 4,173

CSF age-specific reference values



Leen et al. PLoS ONE 2012

Table 1. Age-specific reference values for CSF glucose, CSF/plasma glucose ratio and CSF lactate.

Age		CSF glucose *mmol/L*	CSF/plasma glucose	CSF lactate *mmol/L*
Neonate	0–4 weeks	1.9–5.6	0.42–1.10 (1.38)*	0.9–2.5 (3.4)‡
Infant	4–8 weeks	1.7–5.6 (5.1)*	0.36–1.20	0.9–2.2
	2–6 months	1.9–4.9 (3.9)*	0.39–1.10	1.0–2.2 (3.3)‡
	6–12 months	2.4–4.9 (4.3)*	0.44–1.05	1.1–2.2
Toddler	1–3 years	2.4–4.2	0.44–0.84	1.0–2.0
Preschool child	3–4 years	2.4–3.8	0.43–0.86	1.0–2.0
School child	4–10 years	2.5–4.0	0.45–0.84	1.1–2.1
(Pre)adolescent	10–18 years	2.6–4.3	0.47–0.83	1.2–2.2
Young adult	18–30 years	2.7–4.4	0.46–0.90	1.2–2.2
Adult	30–50 years	2.8–4.4	0.46–0.88	1.3–2.4
Middle aged	50–60 years	2.8–4.2 (4.8)*	(0.43) 0.48–0.87*	1.3–2.5
Aged	60–80 years	2.9–4.4 (5.6)*	(0.42) 0.46–0.84*	1.4–2.6
	≥80 years	2.9–4.5 (6.1)*	(0.35) 0.42–0.81*	1.4–2.7

Reference values are based on the 5^{th} to 95^{th} percentile values. The subgroups from the original data (see Tables S1,S2,S3) were clustered into age groups which are commonly used in daily clinical practice (as used by MeSH, Pubmed).
*Reference range after exclusion of CSF samples of patients with an unknown or abnormal plasma glucose (<3.0 mmol/L or >7.8 mmol/L) at the moment of lumbar puncture (only represented if >10% different from to the original value). Numbers between brackets represent values without correction for plasma glucose.
‡Reference range after exclusion of CSF samples with CSF lactate >3000 µmol/L (only represented if >10% different from the original value). Number between brackets represents upper limit without exclusion of CSF samples with CSF lactate >3000 µmol/L.

Leen et al. PLoS ONE 2012

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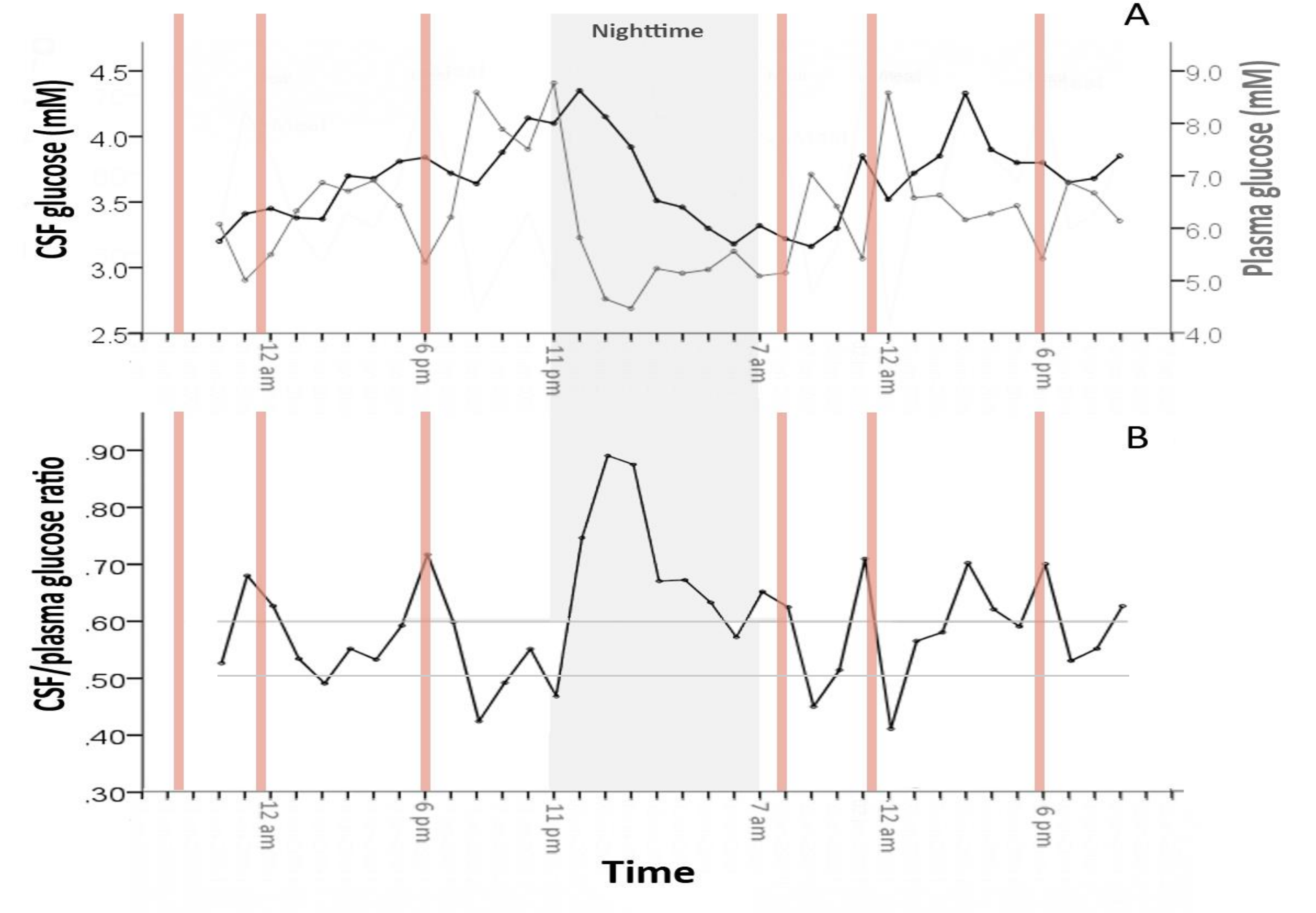
Q1a: Do CSF glucose and lactate fluctuate during the day?

Diurnal fluctuations of CSF glucose and CSF/plasma glucose ratio under physiological conditions in five healthy volunteers



Leen et al. J Cereb Blood Flow Metab. 2016

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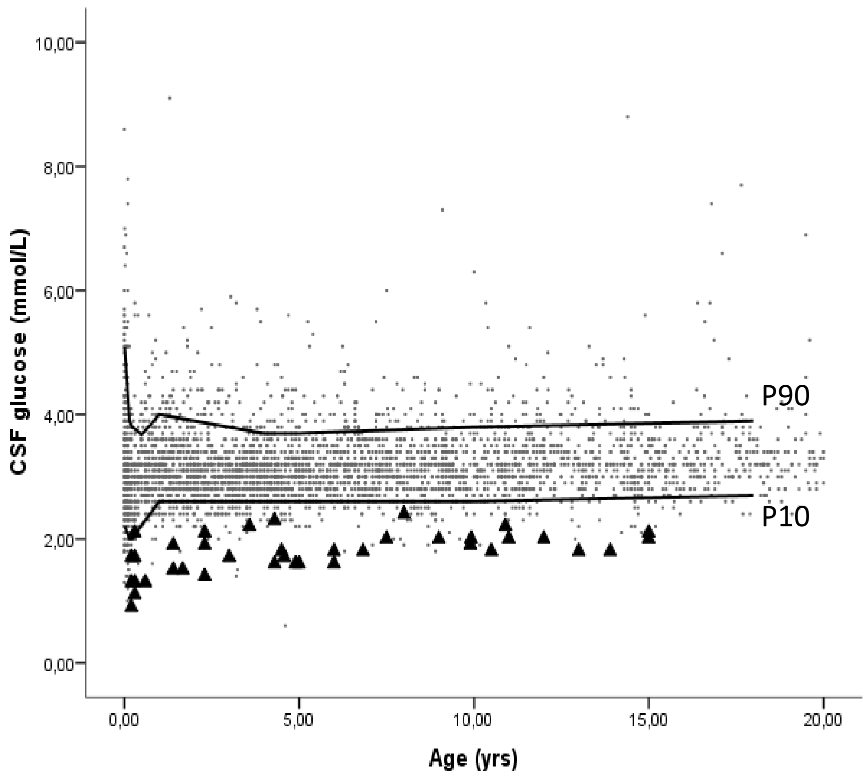
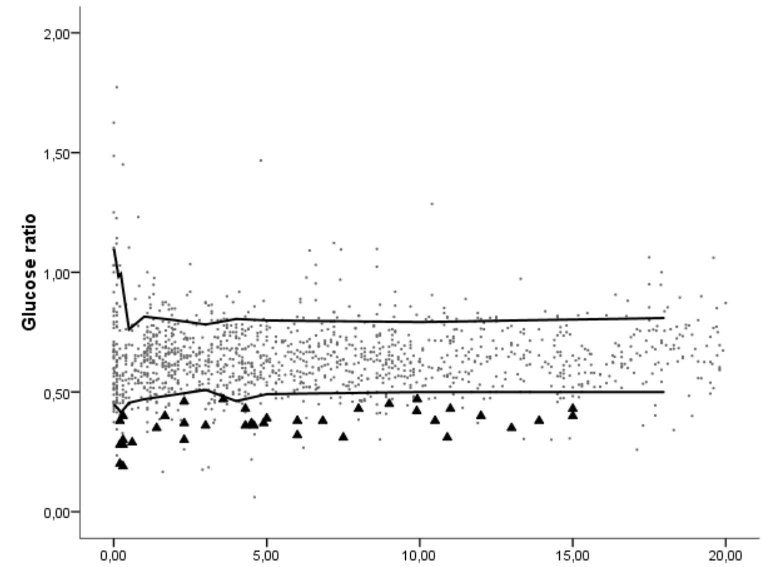
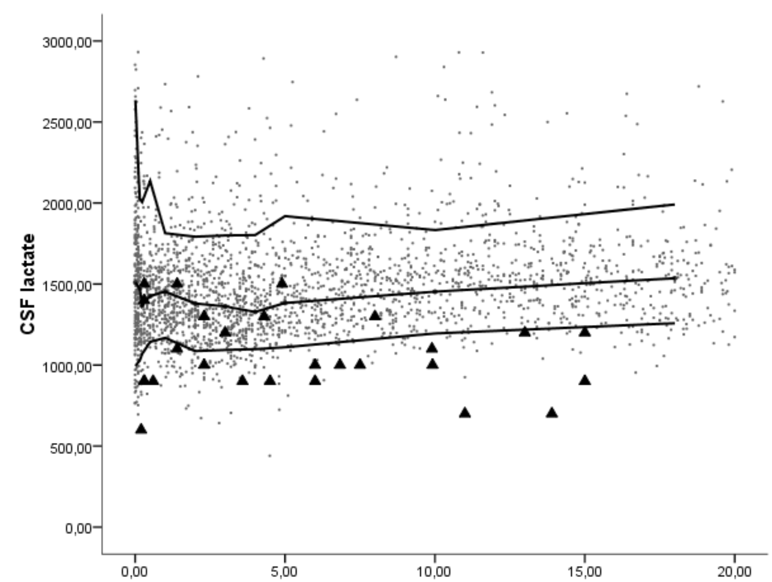
Q2: CSF glucose and lactate values in GLUT1DS?

GLUT1DS *versus* reference values







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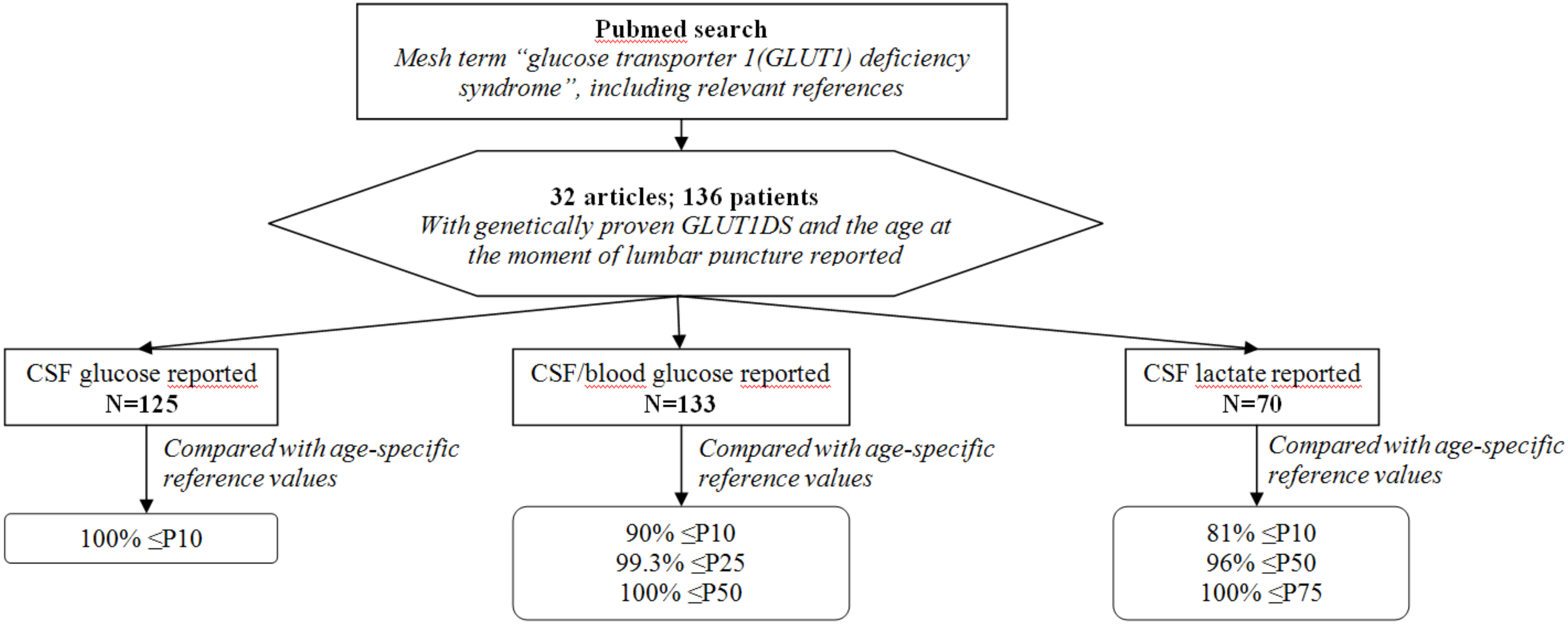
Typical CSF profile of GLUT1DS



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Typical CSF profile of GLUT1DS

CSF glucose ≤ P10

and

CSF/blood glucose ratio ≤ P25 (a)

and

CSF lactate < P90 (b)

(a) The ratio is ≤ P25 in 99% of the cases, but may be P25-P50 in non-classical cases
(b) CSF lactate is ≤ P10 in 81% of the cases, and is always below the P90

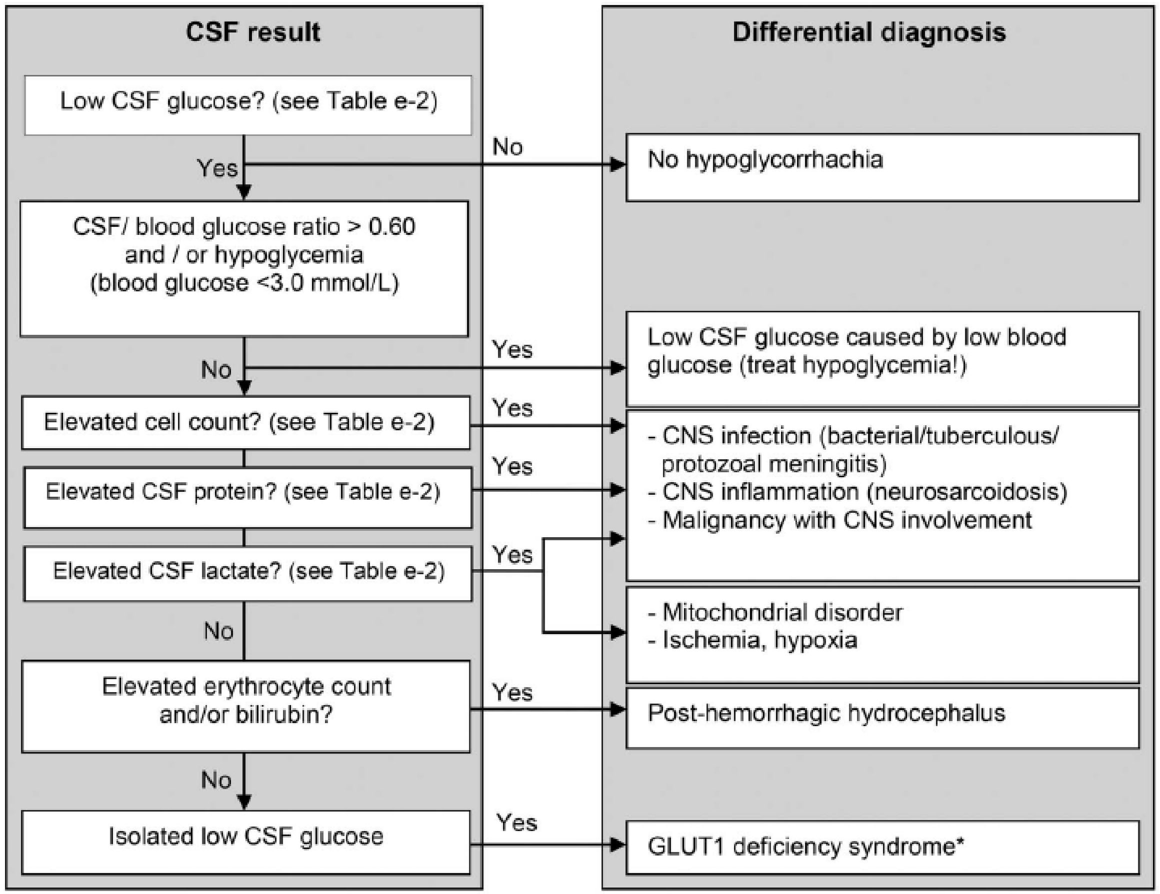
Leen et al. JAMA Neurol 2013

Age		CSF glucose (mmol/L) *P10*	CSF/blood glucose *P25 (P50)*	CSF lactate (mmol/L) *(P10) P90*
Neonate	0-4 weeks	2.2	0.52 (0.67)	(1.0) 2.2
Infant	1-6 months	2.0	0.49 (0.64)	(1.1) 2.1
	6-12 months	2.2	0.54 (0.59)	(1.2) 1.8
Preschool child	1-4 years	2.6	0.58 (0.65)	(1.1) 1.8
School child	4-10 years	2.6	0.56 (0.63)	(1.2) 1.9
(Pre)adolescent	10-18 years	2.7	0.57 (0.64)	(1.2) 2.0
Young adult	18-30 years	2.8	0.60 (0.67)	(1.3) 2.0
Adult	30-50 years	2.9	0.61 (0.67)	(1.4) 2.1
Middle aged	50-60 years	3.0	0.57 (0.67)	(1.4) 2.2
Aged	> 60 years	3.0	0.55 (0.62)	(1.5) 2.4

Leen et al. JAMA Neurol 2013

Q3: low CSF glucose and low CSF lactate values in other disorders?

Figure 1 **Flow chart for the differential diagnosis of a low CSF glucose**



Leen, et al. Neurology 2013

CSF samples with the typical GLUT1DS profile but without GLUT1DS?

Radboudumc CSF database (> 23.000 samples)

9036 samples with normal cell and erythrocyte count and normal protein

41 samples with typical CSF profile

35 samples (0.4%) GLUT1DS negative & **6** samples GLUT1DS positive

Leen et al. JAMA Neurol 2013.

CSF samples with the typical GLUT1DS profile but without GLUT1DS?

Radboudumc CSF database (> 23.000 samples)

9036 samples with normal cell and erythrocyte count and normal protein

41 samples with typical CSF profile

35 samples (0.4%) GLUT1DS negative & **6** samples GLUT1DS positive

n = 5: infection / inflammation
n = 7: no neurological diagnosis
n = 23: miscellaneous, no patients with identical diagnosis

Leen et al. JAMA Neurol 2013.

Q4: technical and methodological pitfalls?

Technical & methodological pitfalls

Patient in steady state: without hypoglycemia!

Technical & methodological pitfalls

Patient in steady state: without hypoglycemia!

Lumbar puncture in steady state: after 4-6 hours fasting!

Technical & methodological pitfalls

Patient in steady state: without hypoglycemia!

Lumbar puncture in steady state: after 4-6 hours fasting!

First things first: blood sample before lumbar puncture!

Technical & methodological pitfalls

Patient in steady state: without hypoglycemia!

Lumbar puncture in steady state: after 4-6 hours fasting!

First things first: blood sample before lumbar puncture!

Don’t use CSF from ventricular system: other reference values!

Technical & methodological pitfalls

Patient in steady state: without hypoglycemia!

Lumbar puncture in steady state: after 4-6 hours fasting!

First things first: blood sample before lumbar puncture!

Don’t use CSF from ventricular system: other reference values!

Order CSF lactate too: it helps in the differential diagnosis!

Technical & methodological pitfalls

Patient in steady state: without hypoglycemia!

Lumbar puncture in steady state: after 4-6 hours fasting!

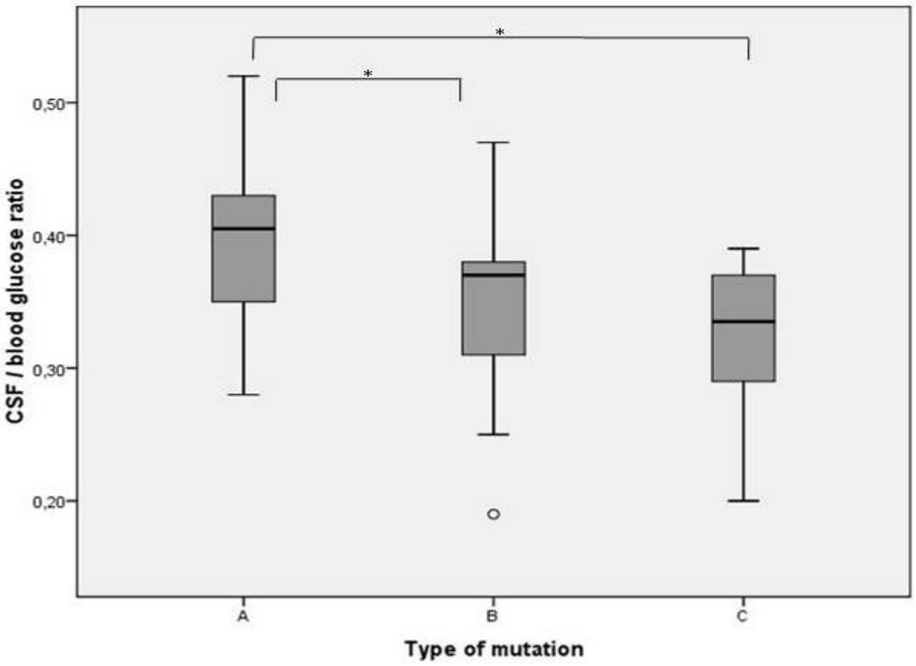
First things first: blood sample before lumbar puncture!

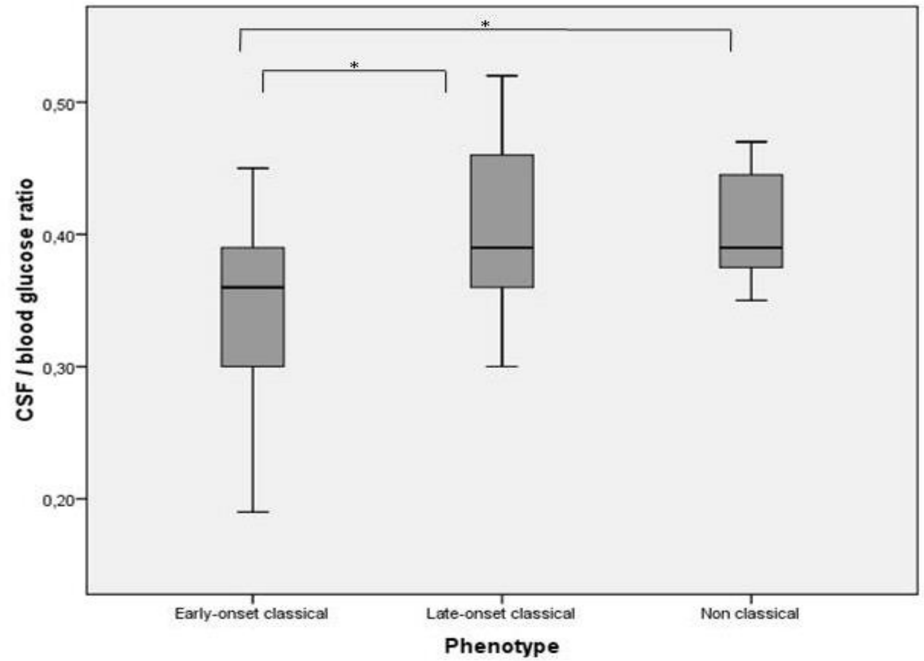
Don’t use CSF from ventricular system: other reference values!

Order CSF lactate too: it helps in the differential diagnosis!

Respect age-specific reference values!

SLC2A1 mutations, but also CSF results may predict the phenotype





Type of mutation

A = missense mutation
B = nonsense, frame shift, splice site, or translation initiation mutation
C = multiple exon deletion

*$P < 0.05$

Leen, et al. BRAIN 2010

Table 4
Relationship between types of SLC2A mutation and the onset age of the first neurological symptoms as well as CSF/blood glucose ratio and mental outcomes.

	Missense mutation	Truncating mutation**	*P* values
N	11	17	
Onset age of the first neurological symptoms (months)	16.27 ± 14.02	4.38 ± 2.64	0.001
CSF/blood glucose ratio	0.388 ± 0.066	0.344 ± 0.037	0.0225
Mental outcomes			
Borderline to Mild	7	3	
Moderate	3	4	0.0166
Severe	1	10	
Epilepsy	11	16	0.6071
Paroxysmal episodes (Complex movement disorders)	7	7	0.2200
Pyramidal sign	5	13	0.1027
Postnatal microcephaly	3/10*	3/15*	0.4553
Short statue	3	6/16*	0.4488
Cyclic vomoting	5	5	0.3205

* A number of cases excluded those without the information.
** It included nonsense mutation, frame shift mutation, splice site mutation and deletion/duplication.

some observations and thoughts

Patient 1 – classic phenotype, very good responder to KD, near normal overall development (2 yrs now)

Onset epileptic seizures at age 3 wks

LP age 7 weeks

CSF glucose 1,2 mM
Blood glucose 4,6 mM
Ratio 0,26

CSF lactaat 0,6 mM

Table 1. Age-specific reference values for CSF glucose, CSF/plasma glucose ratio and CSF lactate.

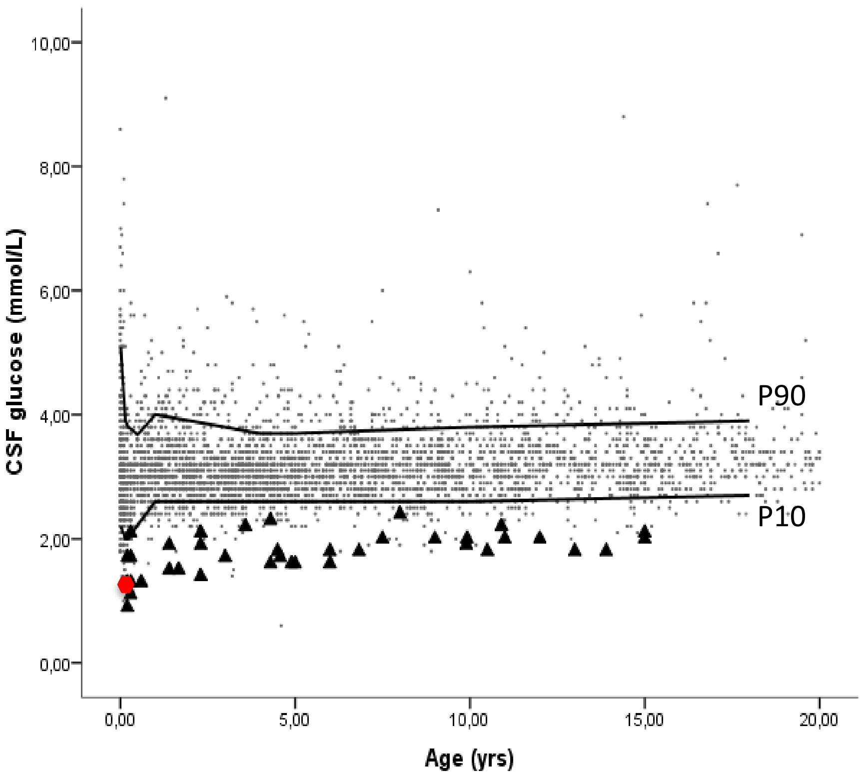
Age		CSF glucose	CSF/plasma glucose	CSF lactate
		mmol/L		*mmol/L*
Neonate	0–4 weeks	1.9–5.6	0.42–1.10 (1.38)*	0.9–2.5 (3.4)‡
Infant	4–8 weeks	1.7–5.6 (5.1)*	0.36–1.20	0.9–2.2
	2–[illegible]	[illegible]	[illegible]	[illegible]
	6–[illegible]			
Toddler	1–[illegible]	1,2	0,26	0,6
Preschool child	3–[illegible]			
School child	4–[illegible]			
(Pre)adolescent	10–18 years	2.6–4.3	0.47–0.83	1.2–2.2
Young adult	18–30 years	2.7–4.4	0.46–0.90	1.2–2.2
Adult	30–50 years	2.8–4.4	0.46–0.88	1.3–2.4
Middle aged	50–60 years	2.8–4.2 (4.8)*	(0.43) 0.48–0.87*	1.3–2.5
Aged	60–80 years	2.9–4.4 (5.6)*	(0.42) 0.46–0.84*	1.4–2.6
	≥80 years	2.9–4.5 (6.1)*	(0.35) 0.42–0.81*	1.4–2.7

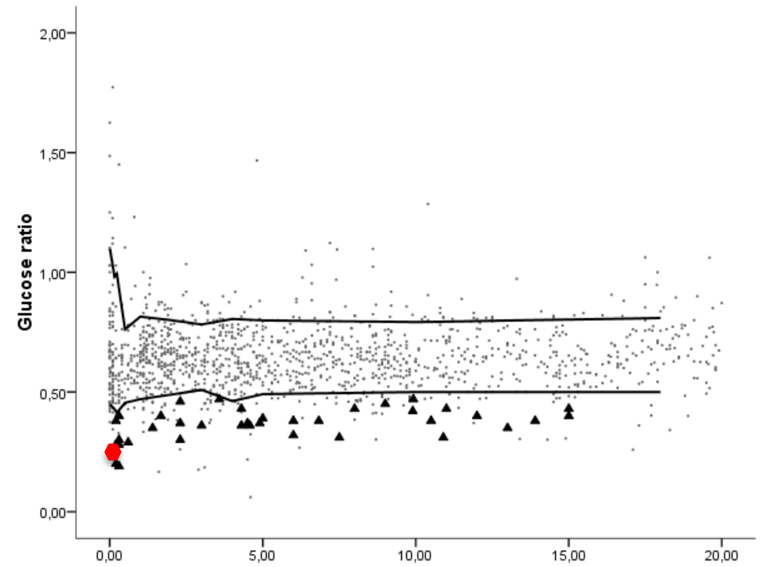
Reference values are based on the 5th to 95th percentile values. The subgroups from the original data (see Tables S1,S2,S3) were clustered into age groups which are commonly used in daily clinical practice (as used by MeSH, Pubmed).

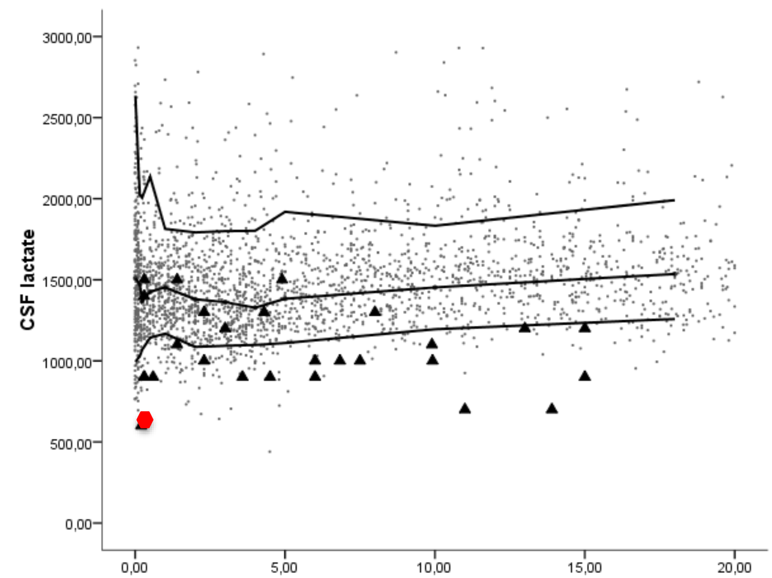
*Reference range after exclusion of CSF samples of patients with an unknown or abnormal plasma glucose (<3.0 mmol/L or >7.8 mmol/L) at the moment of lumbar puncture (only represented if >10% different from to the original value). Numbers between brackets represent values without correction for plasma glucose.

‡Reference range after exclusion of CSF samples with CSF lactate >3000 µmol/L (only represented if >10% different from the original value). Number between brackets represents upper limit without exclusion of CSF samples with CSF lactate >3000 µmol/L.

GLUT1DS *versus* reference values







Patient 2 – mild phenotype

Paroxysmal, non-epileptic headache and vomiting, good response to diet

LP age 16 yrs

CSF glucose 2,6 mM
Blood glucose 4,8 mM
Ratio 0,54

CSF lactate 1,2 mM

Table 1. Age-specific reference values for CSF glucose, CSF/plasma glucose ratio and CSF lactate.

Age		CSF glucose	CSF/plasma glucose	CSF lactate
		mmol/L		*mmol/L*
Neonate	0–4 weeks	1.9–5.6	0.42–1.10 (1.38)*	0.9–2.5 (3.4)‡
Infant	4–8 weeks	1.7–5.6 (5.1)*	0.36–1.20	0.9–2.2
	2–6 months	1.9–4.9 (3.9)*	0.39–1.10	1.0–2.2 (3.3)‡
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(Pre)adolescent	10–18 years	2.6–4.3	0.47–0.83	1.2–2.2
Young adult	18			
Adult	30			
Middle aged	50			
Aged	60			
	≥8			

2,6 **0,54** **1,2**

Reference values are based on the 5th to 95th percentile values. The subgroups from the original data (see Tables S1,S2,S3) were clustered into age groups which are commonly used in daily clinical practice (as used by MeSH, Pubmed).
*Reference range after exclusion of CSF samples of patients with an unknown or abnormal plasma glucose (<3.0 mmol/L or >7.8 mmol/L) at the moment of lumbar puncture (only represented if >10% different from to the original value). Numbers between brackets represent values without correction for plasma glucose.
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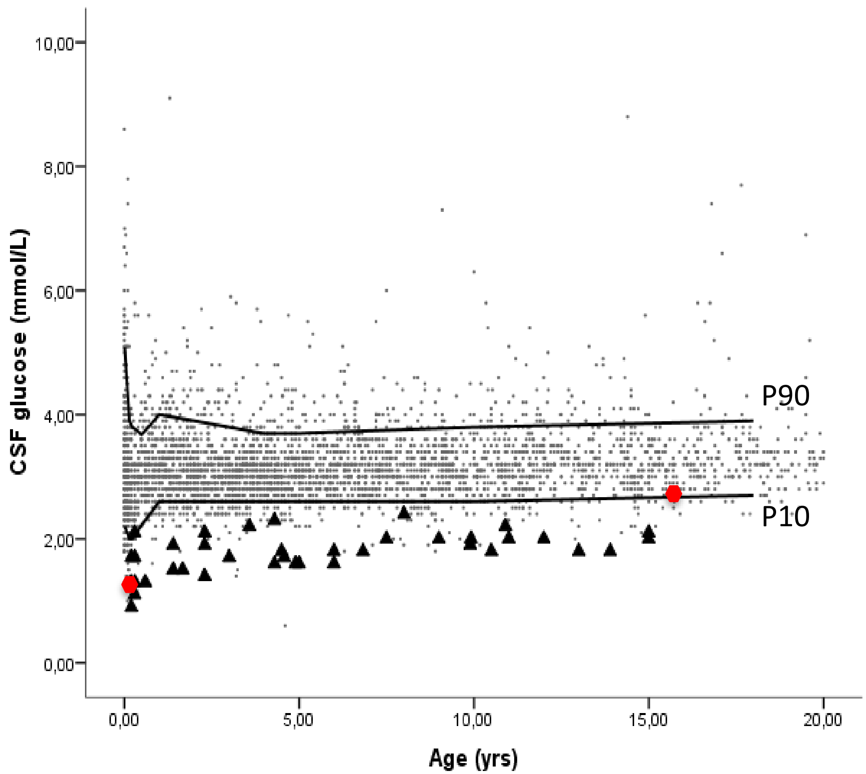
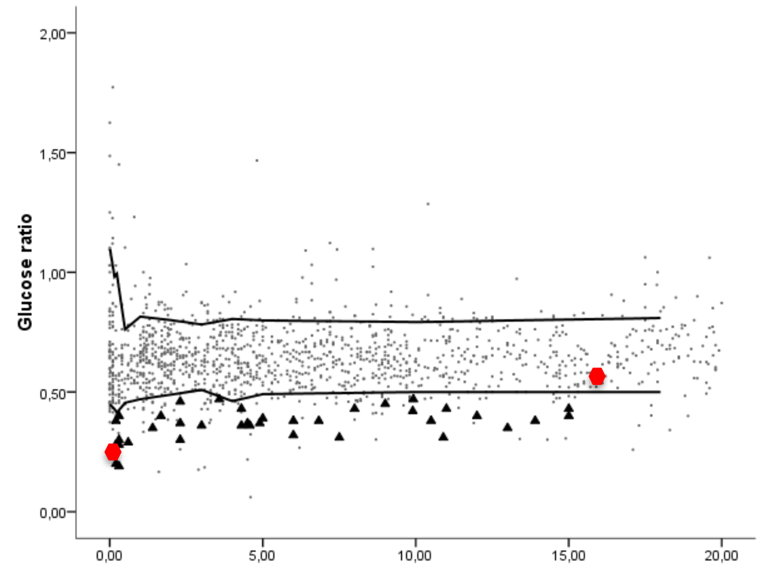
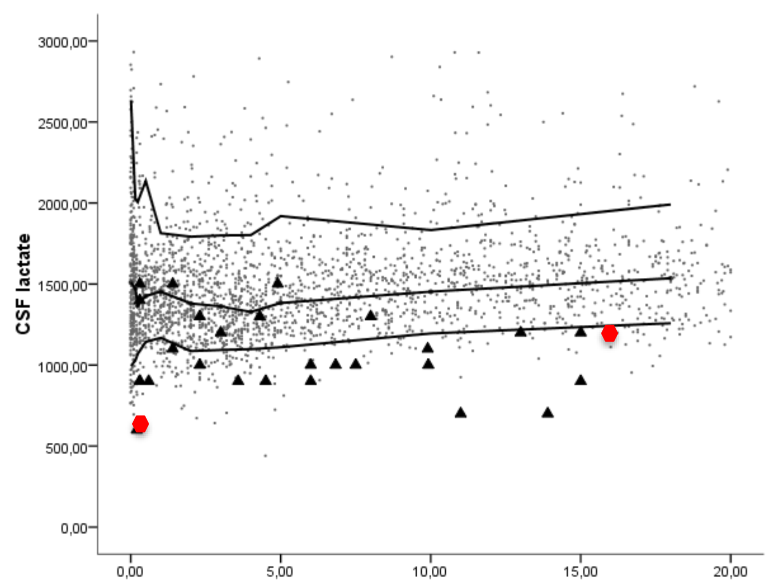
GLUT1DS *versus* reference values

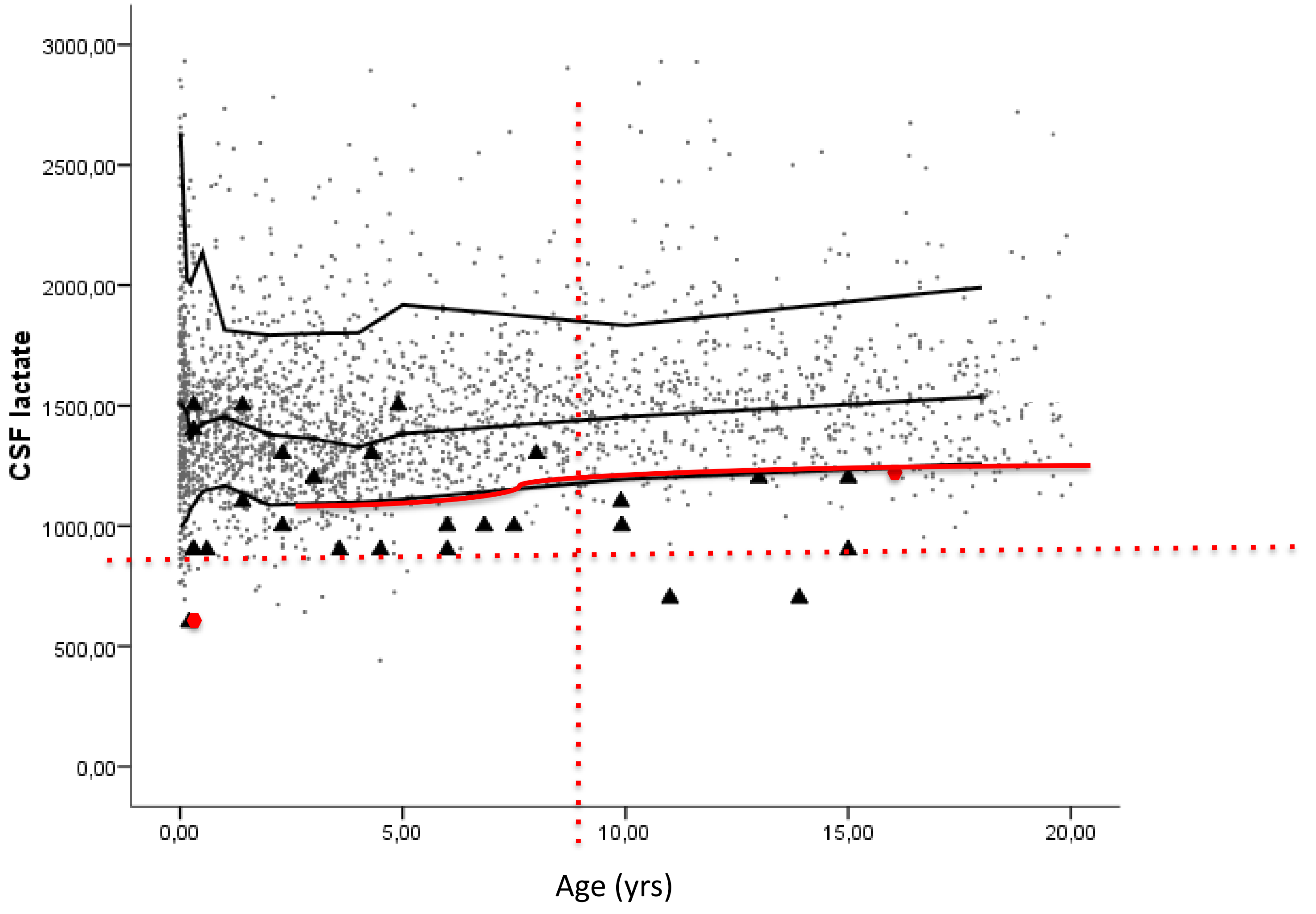






Leen et al. JAMA Neurol 2013

CSF LACTATE: GLUT1DS *versus* reference values



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Hypothesis (not yet studied systematically)

Brain uses lactate as energy source, in health as well as in disease!

This may explain why CSF lactate is low in GLUT1DS and increases on the KD*!

GLUT1DS: the better the brain is able to use lactate, the lower CSF lactate at diagnosis might be, and the milder the phenotype might be!

>>> Novel therapeutic options?

* Klepper, Prostaglandins Leukot Essent Fat Acids 2016
Taher, European Journal of Paediatric Neurology 2015

Table 1 – Some diagnostic and possible therapeutic roles of lactate in inborn errors of metabolism and acquired brain injury.

Lactate production

- Exercise
  - Oxygen demand/delivery mismatch causes rise in plasma lactate
  - Plasma lactate contributes to brain metabolism up to 25%
  - Plasma lactate providing energy for brain metabolism is probably the reason why hypoglycemic symptoms generally don't occur during strenuous exercise
- Glycogen storage disease type 1
  - Enzyme dysfunction causes shift of the pyruvate–lactate equilibrium towards the production of lactate
  - Plasma lactate contributes around half of the energy for brain metabolism
  - Plasma lactate providing energy for brain metabolism is probably the reason GSD1 patients often do not experience hypoglycemic symptoms
- Fructose- 1,6-biphosphatase deficiency
  - High levels of lactate during hypoglycemia may contribute to favorable neurological outcome
- Glucose 1 transporter deficiency syndrome
  - Low CSF lactate is a diagnostic tool
  - Increase in CSF lactate after initiation of the ketogenic diet might be caused by the availability of ketones, which are preferred to lactate as cerebral energy source

Lactate administration

- Diabetes mellitus type 1
  - Human studies: IV lactate resolves hypoglycemic symptoms
  - Animal studies: lactate as adjuvant gives up to 90% reduction of neuronal damage
- Ischemia
  - Animal studies: IV lactate reduces lesion size in ischemia of the brain
- Traumatic brain injury
  - Randomized controlled trials: lactate lowers intracranial pressure and prevents episodes of intracranial pressure after traumatic brain injury

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- Ron Wevers – biochemistry & genetics

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