Title of the case:

Ornithine Transcarbamylase deficiency: A 1-year-old encephalopathic boy (date: 2020)

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1. Clinical presentation

A 13-month-old boypresented with lethargy after vomiting for several days. Prior to that, he was healthy. His parents mentioned that he was a picky eater. Until three weeks before presentation, he was breastfed. Vegetables were introduced at 5 months of age; he tolerated these and fruits well. However, he refused to eat meat and fish. Several days after starting cow's milk formula, he started vomiting; a week later, their general practitioner prescribed an oral rehydration solution. Two days later, he stopped vomiting, and a normal diet was restarted. Over the next three days, he became lethargic, and in the 24 hours before presentation, he was not able to sit or feed.

2. Medical history

The patient was the second child of non-consanguineous Vietnamese parents. The pregnancy and delivery were unremarkable. His development was age-appropriate (he already started walking).

Family history: The child had a healthy brother who is 2 years older. His father was known to have hepatitis B, and his mother was healthy. There were no known serious diseases in the family.

Pertinent dietary and medication history:
The patient was breastfed since birth but breastfeeding was always troublesome. He would start drinking well but then refused the breast after a few minutes. From 5 months on, he ate solid foods. He ate fruit and vegetables well but refused meat, fish and milk products. He tolerates potatoes and eggs. His growth has always been borderline. Three weeks before presentation, he was switched from breastfeeding to bottle feeding to improve his growth; this led to an increase in protein intake, from 1.0 to 1.7 grams/100 ml. Several days after the start of bottle-feeding, he started vomiting. The general practitioner started an oral rehydration solution (glucose and salt) which improved the situation. After feeding was restarted, he became increasingly lethargic.

3. Results of neonatal screening

Neonatal screening was performed in 2019 in the Netherlands. Screening on urea cycle defects and in particular ornithine transcarbamylase deficiency was not included in the neonatal screening at that time (1).

4. Physical examination

At presentation, The child's weight was 8.2 kg (0.6th percentile) and his height was 77 cm (16th percentile). The child was unresponsive and hypotonic. No spontaneous movements were observed. Eyes were midline with normal oculocephalic reflexes. Other reflexes were increased, with Babinski

reflexes present on both sides. There were no evident signs of dehydration on presentation. His physical examination was otherwise normal.

5. **Differential diagnosis**

What is your differential diagnosis based on the 1-3?

- Infectious
- Neurological
- Electrolyte disorders
- Metabolic disorder/ urea cycle defect, organic aciduria

What would be the next step in diagnostics?

- MRI of the brain
- Blood: blood gas with lactate, electrolytes, blood ammonia, liver and kidney functions, C-reactive protein, and blood count with differentiation.
- Metabolic: Amino acid and acylcarnitine analysis in plasma, organic acid and purine and pyrimidine analysis in urine.

6. Imaging data or other functional tests

Figure 1 shows an impression of the axial T2 weighted MRI of the brain of the patient.

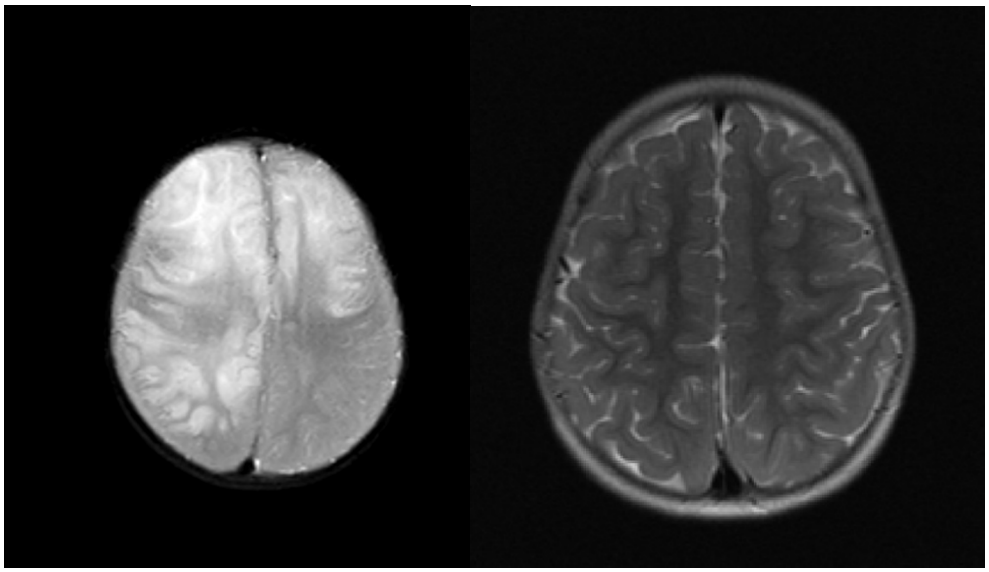

Figure 1. Data on MRI of brain at moment of presentation (13 months)
(Left) MRI of the patient (axial T2 weighted); Diffuse cerebral edema with midline shift.
(Right) normal (T2 weighted) MRI brain of a 2-year old child.
Source of reference: https://radiopaedia.org/cases/normal-brain-mri-2-year-old

7. Clinical chemistry results

Table 1. Clinical chemistry results on day of presentation (13 months)

Analyte	Body fluid	Result	Reference value	Comment
Ammonia	Blood	227	14-43 umol/l	Increased
pH	Blood	7.49	7.35-7.45	Increased
pCO2	Blood	4.2	5.5-6.8 kPa	Decreased
Urea	Blood	2.3	2.5-5.5mmol/l	Decreased
Creatinine	Blood	15	25-70 µmol/l	Decreased
Lactate	Capillary blood	2.9	0.6-2.4 mmol/l	Increased

Reference values, if applicable, are age-based.

The clinical chemistry results indicate a respiratory alkalosis due to a hyperammonemia.

8. Metabolic screening/metabolomics

- Body fluids used were both (heparin) plasma and random urine
- Analysis: amino acid analysis is performed by an electro-spray ionization ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) as described by Waterval et al. Clin. Chim. Acta (2009). The mass spectrometer was used in the multiple reaction monitoring mode (MRM) in the ESI-positive mode. Purines and pyrimidines were analyzed by UHPLC-MS/MS (Waters XEVO TQS).
- Results of screening on the day of clinical presentation are presented in tables 2 and 3.

Table 2. Metabolic profiling of relevant amino acids and related compounds in plasma at the day of clinical presentation (age of patient is 13 months).

Metabolite	Result	Reference value µmol/L	Comment
Glycine	164	115.1 – 404.9	
Alanine	190	136 – 591.9	
Serine	77	56 – 186	
Valine	149	106.1 – 339.9	
Isoleucine	42	31 – 114	
Leucine	72	59 – 215.8	
Threonine	44	45 – 204.8	
Tryptophan	34	22 – 91	
Lysine	489	69.1 – 251.8	Increased
Saccharopine	0	0 – 4	
Pipecolic acid	12	0 – 6	Increased
Histidine	75	42 – 112.9	
Methionine	33	10 – 38	
Glutamine	1130	304 – 749.9	Increased
Glutamic acid	36	22 – 195.9	
Ornithine	15	24 – 167.9	Decreased

Citrulline	5	11 – 46	Decreased
Argininosuccinic acid	0	0 – 0.9	
Arginine	28	16 – 124.9	
Asparagine	33	18 – 84.9	
Aspartic acid	3	0 – 33	
Homocitrulline	1	0 – 13	

Table 3. Metabolic profiling of relevant purine and pyrimidine metabolites in spot urine at the day of clinical presentation (age of patient is 13 months).

Metabolite	Result	Reference value µmol/mmol creatinine	Comment
Dihydro-orotic acid	14	0 – 5	Increased
Orotic acid	1524	0 – 4	Increased
Orotidine	13	0 – 7	Increased
Uracil	657	0 – 36	Increased
Dihydro-uracil	34	0 – 10	Increased
N-carbamyl-ß-alanine	57	0 – 16	Increased
N-carbamyl-ß-AIBA	3	0 – 6	
Pseudo uridine	76	28 – 154	
Uridine	155	0 – 3	Increased
Uric acid	1137	281 – 1579	

Conclusion:
Increase in plasma glutamine and lysine and a decrease in ornithine and citrulline, together with an increase in orotic acid and other pyrimidine de-novo products, indicate a urea cycle defect downstream of carbamoyl phosphate synthetase (CPS 1), most likely an ornithine transcarbamylase deficiency (OTC; OMIM # 311250).

9. Differential diagnosis laboratory

1. Differential diagnosis: defects in urea cycle with increased orotic acid most likely OTCdeficiency (OMIM # 311250)
2. Next approach to diagnostic testing is dedicated sequencing analysis of the OTC gene (OMIM *300461). Enzyme measurement of the enzyme is not indicated since it requires a liver biopsy.

10. Enzyme or genetic diagnosis (or: immunohistochemistry/flowcytometry)
Six days after presentation, genetic results were reported confirming the biochemical diagnosis. Dna was extracted from leukocytes and OTC gene was sequenced. A hemizygous mutation in OTC gene c.829C>T (p.(Arg277Trp) was found. This sequence variant was predicted to be pathogenic according to mutation prediction software and has been described by Finkelstein et al. (1990) as a cause of X-linked ornithine transcarbamylase deficiency (OMIM# 311250).

11. What is final diagnosis?

- OMIM number: 311250
- link with OMIM: https://www.omim.org/entry/311250

- link DDRMD/IEMbase: http://www.iembase.com/disorder/13
- ICIMD classification: Group 1.1 ORPHA number 664

12. Treatment

With only clinical chemistry results?

Before chemistry (ammonia was taken hours after presentation) results were known, treatment with intravenous immunoglobulins (0.4 g/kg/day) and methylprednisolone 30 mg/kg/day was started based on the MRI images. After clinical chemistry results were known (hours after MRI), including ammonia, these were discontinued and a glucose infusion (8 mg/kg/min) with electrolytes was started. The hyperammonemia was treated with sodiumbenzoate as continuous infusion; sodiumbenzoate is an ammonia scavenger which couples with glycine to form hippuric acid. Hippuric acid is excreted in urine.

With metabolic results/enzyme and DNA confirmation

The patient was started on a diet with 0.9 g/kg/day natural protein (age-appropriate protein intake according to WHO standards). Supplementation of both citrulline (enteral) and arginine (parenteral) were provided to optimize urea cycle activity. Glycerolphenylbutyrate was given as ammonia scavenger. His amino acids in plasma were monitored to evaluate the response to treatment. During the acute stage of disease this was done daily; in the stable phase, this was done every 3 months.

13. Clinical course and follow up

After the start of treatment, the child regained consciousness within 3 days and his brain edema resolved within one month without any neurological deficits. The biochemical profile (table 4) normalized as well.

Table 4. Changes in relevant plasma amino acids in patient after start of treatment consisting of ammonia scavengers and supplementation of citrulline and arginine

Amino acid	Result after 3 days*	Result after 5 days	Result after 7 days	Reference value µmol/L
Glutamine	1065	738	484	304-750
Arginine	32	73	243	16-125
Ornithine	26	77	139	24-168
Citrulline	4	12	52	11-46
Argininosuccinate	0	0.3	0.3	0-1
Lysine	265	200	189	69-252
Asparagine	61	58	51	18-85

**days after clinical presentation.*

14.Clinical and biochemical synopsis

What are the take-away points that make this an informative teaching case?

This is a classical urea cycle (figure 1) defect presentation; increase in protein intake led within days to derangement of the urea cycle and an increase in ammonia with respiratory alkalosis because ammonia triggers the central breathing center in the brain. The ammonia rise stimulated the transamination of alpha-ketoglutarate into glutamate and glutamine. This consumption of alpha-

ketoglutarate as intermediate of the TCA cycle hampers energy production (less ATP formation in brain). Brain edema in this OTC patient likely resulted from a rise in glutamate in plasma and CSF (not measured).The increase in glutamate in CSF due to increased ammonia gave brain edema.

The combination of high glutamate with low citrulline suggests N-acetyl glutamine synthase (NAGS), carbamyl phosphate synthetase (CPS1) or an ornithine transcarbamylase (OTC) deficiency, and purine and pyrimidine analysis indicated, in particular the measurement of orotic acid (figure 2).
In this case, all UCD intermediates were low due to insufficient intake of essential amino acids for a longer period of time. This explains even low ornithine which is the substrate for OTC.
Glutamine in CSF could be much higher in CSF (up to 3000-4000 uM) in comparison to glutamine in plasma; that explains brain edema and convulsions (5).

Treatment consisted of the use of ammonia scavengers to quickly reduce the ammonia concentration in blood (in extreme cases dialysis is indicated), a supply of energy substrate to inhibit catabolism and protein breakdown, and supplementation with citrulline and arginine (in OTC deficiency). In this specific case, the patient had age-appropriate protein intake according WHO standards, which he tolerated well.

Correlation between the urea cycle and the de novo pyrimidine synthesis
The urea cycle and de novo pyrimidine synthesis are connected via carbamyl phosphate (CP); an overflow of the urea cycle due to a defect downstream from CP results in the formation of orotic acid and uracil. These metabolites (in urine) in addition to plasma amino acids help to pinpoint the urea cycle defect to a specific enzyme.

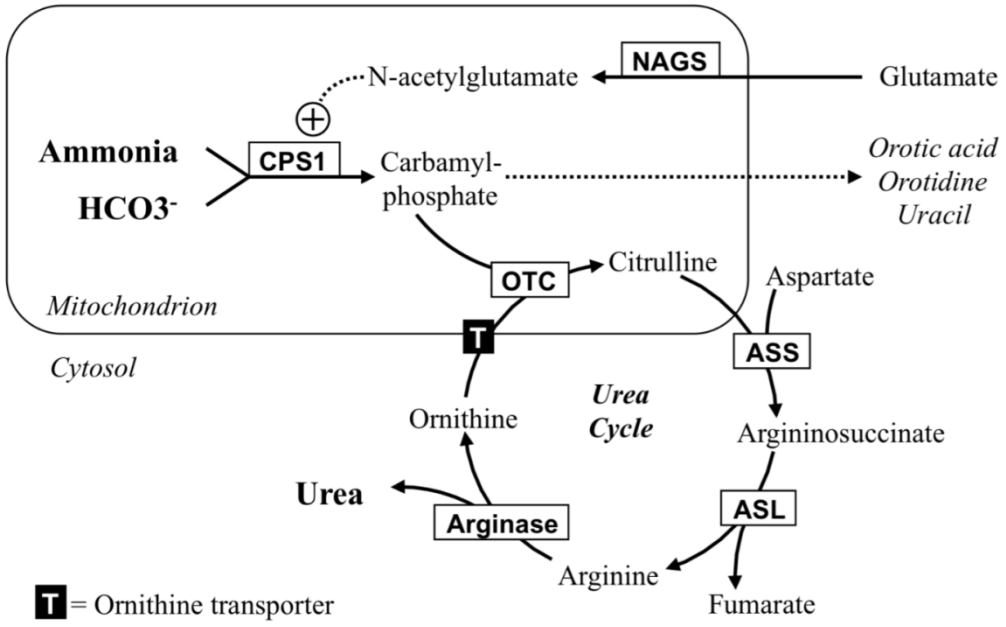

Figure 1. Urea cycle

Abbreviations: CPS1: carbamyl phosphate synthetase 1, OTC: ornithine transcarbamylase, ASS: arginino succinate synthetase, ASL: arginino succinate lyase, NAGS: N-acetyl glutamate synthetase. (Source: Vademecum Metabolicum, electronic version, accessed on 12 June 2021)

In a decision tree, kindly provided by Drs. Schaefers, the hyperammonemia without acidosis, but in this case a respiratory alkalosis, directed us towards amino acids (plasma) and purines and pyrimidines (urine). Low plasma citrulline and normal/elevated ornithine directed towards OTC deficiency which is quite prevalent amongst boys because of the X-linked inheritance.

Figure 2. Diagnostic work-flow in hyperammoniemia

Abbreviations: NAGS: N-acetylglutamae synthetase, P5CS: Δ 1 -pyrroline-5-carboxylate synthetase, CPS: carbamyl phosphate synthetase 1, OTC: ornithine transcarbamylase, ASS: arginino succinate synthetase, ASL: arginino succinate lyase, NAGS: N-acetyl glutamate synthetase, HHH: hyperornithinemia-hyperammonemia-homocitrullinuria., ARG1: argininase, OAT: ornithine aminotransferase, ASL: argininosuccinate lyase, LPI: lysinuric protein intolerance.

15. References

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