REVISION UCD GUIDELINE – AWMF-Leitlinien-Registernummer 027/006

Seit > 5 Jahren nicht aktualisiert, Leitlinie wird zur Zeit überarbeitet

Abbreviations

AA	amino acids
AFC	amniotic fluid cells
AFP	alpha fetoprotein
ARG1	arginase 1
ARG1D	arginase 1 deficiency
ALT	alanine aminotransferase
ASA	argininosuccinic acid
ASL	argininosuccinate lyase
ASLD	argininosuccinate lyase deficiency
ASS	argininosuccinate synthetase
ASSD	argininosuccinate synthetase deficiency
AST	aspartate aminotransferase
BBB	blood brain barrier
BIMDG	British Inherited Metabolic Disease Group
bw	bodyweight
β-OX	β-oxidation defect
CAVHD	continuous arteriovenous hemodialysis
CHO	carbohydrate
CoA	coenzyme A
CPK	creatine phosphokinase
CPS1	carbamoylphosphate synthetase 1
CPS1D	carbamoylphosphate synthetase 1 deficiency
CRRT	continuous renal replacement therapy
CSF	cerebrospinal fluid
CVS	chorionic villi sampling
CVVH	continuous venovenous hemofiltration
CVVHD	continuous veno-venous hemodialysis
DBS	dried blood spot
DNA	deoxyribonucleic acid
EAA	essential amino acids
EDTA	ethylenediaminetetraacetic acid
EFA	essential fatty acids
FAO	fatty acid oxidation
GPB	glycerol phenylbutyrate
HD	hemodialysis
HHH	hyperornithinemia-hyperammonemia-homocitrullinuria
HIHA	hyperinsulinism hyperammonemia
HMG	hydroxy-methyl-glutaryl
HPLC	high performance liquid chromatography
IEM	inborn errors of metabolism
IM	intramuscular
IV	intravenous
IVA	isovaleric acidemia
LCPUFA	longer-chain polyunsaturated fatty acids
LPI	lysinuric protein intolerance
MCAD	medium chain acyl CoA dehydrogenase
MEGDEL	3-methylglutaconic aciduria with deafness, encephalopathy, and Leigh-like syndrome
MMA	methylmalonic aciduria
MLPA	multiplex ligation-dependent probe amplification
MS/MS	ESI-tandemmass spectrometry
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
NAGS	N-acetylglutamate synthase
NAGSD	N-acetylglutamate synthase deficiency
NARP	neuropathy, ataxia and retinitis pigmentosa
NaPBA	sodium phenylbutyrate
NBS	newborn screening
NCGA	N-carbamoyl-L-glutamic acid
NG	nasogastric
NH_3	ammonia
NO	nitric oxide
NOS	nitric oxide synthase
OA	organic acidemia
OAT	ornithine aminotransferase
OLT	orthotopic liver transplantation
OTC	ornitine transcarbamylase
OTCD	ornitine transcarbamylase deficiency
OXPHOS	oxidative phosphorylation
PA	propionic acidemia
PAA	phenylacetic acid
PBA	phenylbutyrate
PC	pyruvate carboxylase
PEPCK	phosphoenolpyruvate carboxykinase
P5CS	pyrroline 5 carboxylate synthase
PD	peritoneal dialysis
PEG-BCT-100	pegylated human recombinant arginase 1
Plt	platelets
PO	per os
RBC	red blood count
RNA	ribonucleic acid
SD	standard deviation
SERAC1	serine active site-containing protein 1
THAN	transient hyperammonemia of the newborn
TMEM70	transmembrane protein 70
TPD	trifunctional protein defect
TPN	total parenteral nutrition
UC	urea cycle
UCD	urea cycle disorder
VLCAD	very long chain acyl CoA dehydrogenase
WBC	white blood count
WHO	world health organization
WPRE	woodchuck hepatitis virus posttranscriptional regulatory element

Introduction

UCDs are inborn errors of metabolism that hamper disposal of the nitrogenous waste (ammonia), also impairing or preventing in most cases arginine synthesis. The cumulative incidence of this group of disorders is uncertain, with figures reported in the range of 1:8.000 to 1:44.000 (Brusilow and Maestri 1996; Dionisi-Vici et al. 2002; Summar et al. 2013; Wilcken 2004). The uncertainty largely stems from the underdiagnosis of fulminant neonatal cases and the lack of reliable and stable biochemical markers of well-establised sensitivity for being used in NBS, particularly for the mitochondrial UCDs. Patient registries are adding to our understanding of the natural course of these diseases (Batshaw et al. 2014; Kölker et al. 2015).

Patients with a complete UC enzyme deficiency often present neonatally, with hyperammonemic coma during the first days of life, with high (25%-90%) mortality despite early and intensive treatment. There has not been much change in the outcome of neonatal patients in the past three decades (Brassier et al. 2015; Burgard et al. 2016; Maestri et al. 1999; Unsinn et al. 2016). Most survivors have severe developmental delay (Bachmann 2003c; Maestri et al. 1999; Msall et al. 1984; Nassogne et al. 2005) and are in high risk of recurrent hyperammonemic crises.

In contrast, late-onset patients, defined as first symptoms after the neonatal period, are attributed to partial deficiencies of UC enzymes and may present symptoms at any age after the neonatal period. Up to 45% of them die prematurely. Their risk of premature death depends on the underlying defect (Harada et al. 2006; Nassogne et al. 2005). Clinical signs in these late-onset chronic patients vary widely, contributing to the frequently long delays before diagnosis is made (Rüegger et al. 2014).

In all the patients with UCDs brain damage correlates with the duration and the severity of acute hyperammonemia (Msall et al. 1984; Nicolaides et al. 2002; Uchino et al. 1998), especially in younger patients possibly because of increased susceptibility of the brain to the deleterious effects of ammonia (Braissant et al. 2013).To improve survival rates and quality of life, UCD patients must be diagnosed as soon as possible (Enns 2008). Ammonia testing, although not yet available in all emergency units (Häberle and Huemer 2015), is the key analysis for detection of UCDs. Therefore, ammonia must be assessed early on in all acutely sick neonates and in all children, adolescents and adults with unexplained neurological symptoms.

In view of the resources required for rapid diagnosis and for efficient timely therapy and intense monitoring of treatment, patients should be transferred to a metabolic centre very early on during the disease course. Experience in the treatment (including extracorporeal detoxification) and diagnosis of IEM with supporting laboratory resources available 24/7 is essential. Additionally, all available plasma and urine samples obtained since initial admission should be forwarded to the centre for timely analysis.

Currently, in addition to this guideline (first published in 2012), there are local protocols for the diagnosis and treatment of UCDs, leading to a high variability of treatment and of drug combinations (Posset et al. 2016).

Aim of this Guideline

The aim of this guideline is to systemise and harmonise the diagnostic pathways and therapy of UCDs in Europe. It is the first revision of a guideline initially published in 2012. It addresses metabolic specialists, pediatricians, dietitians, neonatologists, intensive care specialists, adult physicians, nurses, psychologists and pharmacists involved in the care of UCD patients.

Lysinuric protein intolerance and citrin deficiency are beyond the scope of this guideline because these disorders are extremely rare in most European countries.

Part I: General recommendations

1 CLINICAL DIAGNOSIS – SIGNS AND SYMPTOMS

1.1 *Clinical suspicion of UCD*

Clinical signs and symptoms are non-specific but in most patients neurological symptoms prevail followed by hepatic-gastrointestinal and psychiatric manifestations.
A detailed history should be obtained in all patients with a suspicion of UCD including a drug history. Many patients have a consanguineous background ("are the parents related?") and this along with the family history ("were there any unexplained neonatal deaths in your family?"); past medical ("did s/he suffer from any previous unexplained neurological disorder?") and diet history ("is he/she avoiding high protein foods?") are of particular importance.
Unexpected severe and long-lasting symptoms not responding to standard therapy may direct the suspicion from more common conditions such as neonatal sepsis to UCDs.

1.2 *Acute and chronic presentations*

UCD patients may present with acute or chronic symptoms at any age. Some of the signs and symptoms are common, others are uncommon and few are only described in single patients.
The majority of symptoms are neurological and caused by hyperammonemia induced cerebral edema. The typical neonatal patient will present very similar to a newborn with sepsis. In some cohorts, the majority of UCD patients presented outside the neonatal period (Martin-Hernandez et al. 2014; Summar et al. 2008). Outside the neonatal period, symptoms are largely non-specific.

Table 1 gives an overview of clinical signs and symptoms of acute and chronic manifestations of UCDs (Brusilow and Horwich 2001; Burlina et al. 2001; Gropman et al. 2007; Leonard and Morris 2002; Rüegger et al. 2014; Summar et al. 2008; Trevisson et al. 2007).

Table 1: Clinical signs and symptoms of acute and chronic manifestations of UCDs

Acute presentation	Chronic presentation
• **Altered level of consciousness (from lethargy and somnolence to coma) mimicking encephalitis or drug intoxication** • **Acute encephalopathy (see below)** • **Seizures (mostly in the circumstance of altered level of consciousness)** • Ataxia: mostly in the circumstance of altered level of consciousness • Stroke-like episodes • Transient visual loss • **Vomiting and progressive poor appetite** • Liver failure, coagulopathy (esp. in OTCD and HHH) • **Multiorgan failure** • **Peripheral circulatory failure** • **Psychiatric symptoms (hallucinations, paranoia, mania, emotional or personality changes)** • "Post-partum psychosis" • **In neonates:** **sepsis-like picture, temperature instability, respiratory distress, hyperventilation**	• **Confusion, lethargy, dizziness** • **Headaches, migraine-like, tremor, ataxia, dysarthria** flapping tremor (in adults) • **Learning disabilities, cognitive impairment** • Epilepsy • Chorea, cerebral palsy • Protracted cortical visual loss • Progressive spastic diplegia or quadriplegia (described in ARG1D and HHH syndrome) • **Protein aversion, self-selected low-protein diet** • **(Recurrent) abdominal pain, vomiting** • **Failure to thrive** • **Hepatomegaly, elevated liver enzymes** • **Psychiatric symptoms: hyperactivity, mood alteration, behavioural changes, aggressiveness** • Self-injurious behaviour • *Autism-like symptoms* • **Fragile hair (mainly in ASLD)** • *Dermatitis* • **Episodic character of signs and symptoms** • Specific neuropsychological phenotype in heterozygous OTC females

bold: typical signs and symptoms
standard: uncommon signs and symptoms
italics: signs and symptoms only reported in single patients

Variability of the clinical phenotype

Acute encephalopathy

Different age groups

Postpartum psychosis

Typical signs in specific UCDs

Uncommon clinical presentations

1.3 *Triggers of metabolic crisis*

Besides a high exogenous protein load, any condition causing an increased nitrogen load to the UC may trigger hyperammonemia (Batshaw et al. 2014; McGuire et al. 2013). An endogenous protein load due to catabolism may be caused by:

- Infections
- Fever
- Vomiting
- Gastrointestinal or internal bleeding
- Decreased energy or protein intake (e.g. fasting pre surgery, major weight loss in neonates)
- Catabolism and involution of the uterus during the postpartum period (OTC females)
- Chemotherapy, high-dose glucocorticoids
- Prolonged or intense physical exercise
- Surgery under general anesthesia

Outcome: reliable and early clinical identification of patients

Recommendation #1: We strongly recommend considering a UCD at any age in any acute/intermittent neurological deterioration or psychiatric illness, acute liver failure, suspected intoxication or in the differential diagnosis of neonatal sepsis. Catabolism or protein load may represent triggering factors.
Quality of evidence: moderate

1.4 *Laboratory investigations*

Hyperammonemia is the hallmark of UCDs with mean peak ammonia concentrations > 500 µmol/L in most neonatal patients at presentation (Ah Mew et al. 2013). However, an elevation of plasma ammonia is nonspecific and can only be regarded as a marker for insufficient detoxification of nitrogen (Häberle 2011a). Absence of hyperammonemia makes a UCD very unlikely in symptomatic newborns. In contrast, beyond the newborn period a normal ammonia concentration does not exclude UCDs. The analysis of plasma amino acids and/or urine orotic acid may be especially helpful when samples are taken after recovery from an acute episode.
Ammonia measurement is an emergency procedure because there is a clear correlation between the outcome of a patient and the length of time a patient is hyperammonemic (Bachmann 2003c; Msall et al. 1984; Picca et al. 2001). To shorten the time to diagnosis, an electronic medical record-based warning system was suggested for neonates aged 2–7 days, in which blood gas analysis is ordered without any ammonia studies (Vergano et al. 2013).
For ammonia determination (standard analysis and point of care devices/bedside testing), please see also 10.1
Hyperammonemia is often accompanied by increased plasma glutamine, and hyperglutaminemia may last for some time after normalization of plasma ammonia (Serrano et al. 2011).

Outcome: reliable and early laboratory identification of patients

Recommendation #2: We strongly recommend to determine ammonia in all conditions defined by recommendation #1 as an emergency analysis. Be aware of preanalytical pitfalls.
Quality of evidence: high

If hyperammonemia is confirmed, plasma amino acids, blood or plasma acylcarnitines and urinary organic acids should be analysed urgently. The results should be available within 24 hours but treatment must not be delayed due to pending results.

Outcome: reliable and early laboratory identification of patients

Recommendation #3: If ammonia is elevated, we strongly recommend to immediately take blood samples for analysis of amino acids and acylcarnitines. Then start treatment and collect urine for analysis of organic acids and orotic acid.
Quality of evidence: moderate

2 DIFFERENTIAL DIAGNOSIS

The following Table should direct further confirmatory metabolic investigations.

Table 2: Bedside differential diagnosis of an IEM presenting with hyperammonemia

Parameter	Condition								
	UCDs	Organic acidurias	β-oxidation defects	Carbonic anhydrase Va def.	HMG-CoA lyase def.	HI HA syndrome	Pyruvate carboxylase def. [g]	PEPCK deficiency	TMEM70, SERAC1 def.
Acidosis	+/–	+[e]	+/–	+	+	–	+	+	+
Ketonuria[a]	–	+	absent	+	absent	–	++	+	+
Hypoglycemia[b]	–	+/–	+	+/–	+	+	+	+/–	+/–
↑ Lactic acid[c]	–	+	+/–	+	+/–	–	+	+/–	++
↑ AST & ALT	(+)[d]	–	+	–	+/–	–	+/–	++	–
↑ CPK	–	–	+	–	+/–	–	–	–	–
↑ Uric acid	–	+	+/–	–	+	–	–		++
↓ WBC/RBC/Plt	–	+	–	–	+/–	–	–	–	–
Weight loss	–	+[f]	–	–	+/–	–	+		–

def.: deficiency
[a] In neonates ketonuria (++ - +++) suggests organic aciduria.
[b] Hypoglycemia and hyperammonemia ("pseudo-Reye") can be predominant manifestations of the organic aciduria 3-HMG-CoA-lyase deficiency.
[c] Blood lactate >6 mmol/L, since lower high lactate levels (2-6 mmol/L) may be due to violent crying or to extensive muscle activity.
[d] AST/ALT elevations can be found but are not constant in UCDs.
[e] Can be absent in neonates.
[f] Occurrence only in neonates.
[g] Only type B associated with hyperammonemia but not types A and C.

2.1 *Conditions and genetic disorders other than UCDs presenting with neonatal hyperammonemia*

Most information on the differential diagnoses of neonatal acute hyperammonemia has been collected from expert opinions or reviews (Burton 1998; Clay and Hainline 2007; Ellaway et al. 2002; Leonard and Morris 2002, 2006; Saudubray et al. 2006). In most of these conditions, the clinical status gives initially rise to a suspicion of sepsis. Any secondary impairment of UC function will lead to hyperammonemia and mimic UCDs (Häberle 2013).

Outcomes: reliable and early laboratory identification of patients <u>and</u> improvement of survival

Recommendation #4: As the most common misdiagnosis of early onset UCD patients is neonatal sepsis, we strongly recommend to consider the possibility of a UCD in the differential diagnosis.
Quality of evidence: moderate

<u>Transient hyperammonemia of the newborn</u>

2.2 *Conditions and genetic disorders which can present with late-onset hyperammonemia*

Any condition associated with greatly increased ammonia production and/or with impaired ammonia detoxification can cause hyperammonemia in an individual without a genetic defect in the UC.

Conditions with increased ammonia production or with impaired ammonia detoxification:

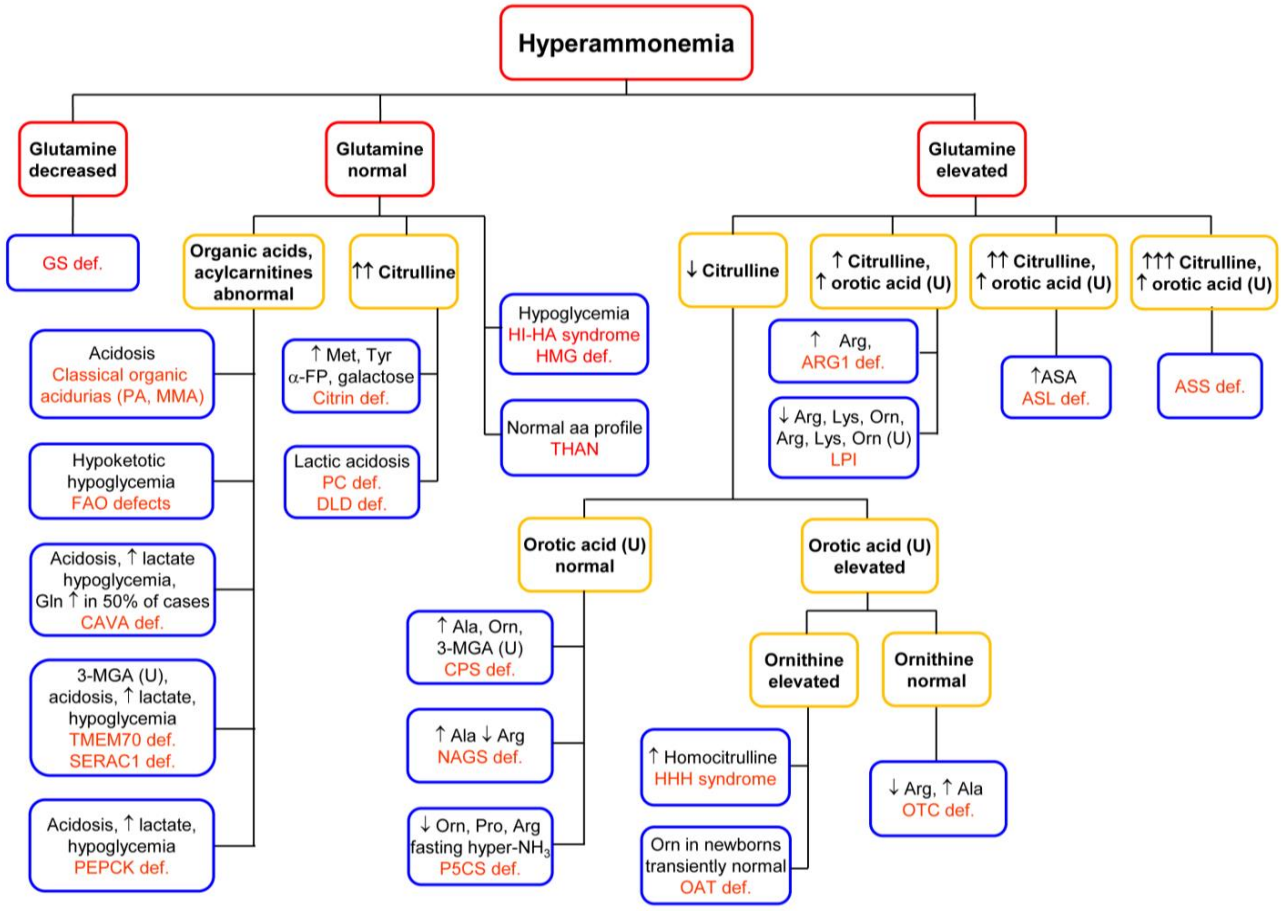
3 BIOCHEMICAL AND ENZYMATIC ANALYSIS

Only half of the UCDs have a specific biochemical pattern. The presence of plasma or urine argininosuccinate is diagnostic for ASLD (unless arginine is strongly elevated, indicating ARG1 deficiency). Likewise, presence of high plasma citrulline is suggestive for ASSD and high urinary orotic acid with low citrulline and low arginine for OTCD.

3.1 *Algorithm for further investigations of hyperammonemia*

In general, the pattern of metabolites is more important than absolute figures. The following algorithm might guide the most concise way to diagnosis.

Figure 1: Diagnostic algorithm for neonatal hyperammonemia



Investigations in plasma if not stated otherwise; U: urine; 3-MGA: 3-methylglutaconic aciduria (Rokicki et al. 2017)

3.2 Enzyme analysis

Enzyme analysis can be used for the confirmation of all UCDs but is not considered the method of choice if genetic testing is available (Gautschi et al. 2014; Kido et al. 2012). An exception to this is ARG1D in which enzyme analysis in red blood cells is simple and reliable (Tomlinson and Westall 1964).
Details on methods and sample requirements are listed in Table 3. Liver tissue should be shock frozen and kept at -80°C until analysis.

Table 3: Overview on methods and required samples for enzyme analysis of UCDs

Disorder	Method	Sample
NAGSD	Stable isotope dilution assay with GCMS	**Liver**[#]
CPS1D	Colorimetric OTC-coupled assay	**Liver**[#]**,** small intestine
OTCD	Colorimetric assay[+]	**Liver**[*#], small intestine[*]
ASSD	Radiometric assay (fibroblasts) ^{14}C-citrulline incorporation (fibroblasts) Colorimetric ASL-arginase-coupled assay (liver)	**Skin fibroblasts**, kidney, liver[#]
ASLD	Colorimetric, arginase-coupled assay (erythrocytes, liver, kidney) ^{14}C-citrulline incorporation (fibroblasts)	**Skin fibroblasts**, Red blood cells[§], liver[#], kidney
ARG1D	Colorimetric assay	**Red blood cells**, liver[#]
HHH syndrome	^{14}C-ornithine incorporation	**Skin fibroblasts**, liver[#]

bold: first choice if analysis in more than one tissue is possible
[+] treatment with carbamylglutamate interferes with some colorimetric assays of citrulline
[#] needle biopsy (>10mg) sufficient either for NAGS assay or for assay of the other five UC enzymes; liver tissue should be snap frozen and kept at -80°C until analysis
[*] reliable in males, but less so in females due to X-mosaicism in all tissues
[§] caution: conflicting results

There are few laboratories in Europe offering enzyme analyses for UCDs. To find a laboratory and for detailed information regarding sample preservation and transport conditions, contact the laboratory or the National Metabolic Society (see list of addresses) or check with Orphanet (http://www.orpha.net) at an early stage.

3.3 Role of liver biopsy or of other tissue samples during work-up for a suspected UCD

4 GENETIC ANALYSIS

4.1 Role of molecular genetic analysis for diagnosis of UCDs

Genetic analysis is the preferred method to establish the diagnosis in disorders in which metabolite profiles are not diagnostic and enzymatic testing is invasive. Genetic testing is further required for genetic counselling and offers the opportunity for prenatal testing. In addition, it can be used for family genotyping.
DNA from peripheral blood cells is usually used but also many other tissues can be source of DNA. In deceased patients or if no other material is available or to test relatives of identified patients, mutation analysis may also be done on DNA from dried blood spots. There are few special situations when RNA must be investigated and these are explained in 11.2 for CPS1D and in 12.2 for OTCD. Mutation analysis should include investigations of known regulatory domains (Heibel et al. 2011).
Apart from these practical and clinical applications, mutation analysis has provided some first insight into genotype-phenotype correlations (Ah Mew and Caldovic 2011; Gao et al. 2003; Häberle et al. 2003a; Sancho-Vaello et al. 2016; Trevisson et al. 2007).

Outcomes: reliable and early laboratory identification of patients and prognostic measures

Recommendation #5: We strongly recommend genetic testing. This will confirm the diagnosis, allow for genetic counselling and in some instances provide information on the disease course. We strongly recommend to preserve DNA, fibroblasts and/or frozen liver tissue in deceased patients with a suspicion of UCD.
Quality of evidence: moderate

Pitfalls and limitations

For single UCDs, different approaches are required; details can be found in the recommendations in Part II of this guideline.

4.2 *Prognostic value of mutation analysis*

4.3 *Role of structure analysis and assessment of pathogenicity of mutations*

5 PRENATAL TESTING

5.1 *Role of prenatal testing in UCDs*

Prenatal testing requires the confirmed diagnosis in an index patient. In following pregnancies, there can be a need for early, fast and safe prenatal testing since most UCDs are considered severe conditions. The diagnosis of an affected fetus allows parents to seek counselling and decide on the further course of the pregnancy in most European countries.
Besides, prenatal testing might be indicated for psychological reasons or to prepare for immediate peri- and postnatal management (Leonard et al. 2008; Maestri et al. 1991; Sniderman King et al. 2005).

Outcome: reliable and early laboratory identification of patients

Recommendation #6: We strongly recommend molecular genetic analysis as the preferred prenatal testing method for all UCDs.
Quality of evidence: high

5.2 *Methods and samples for prenatal testing in UCDs*

Table 4: Recommended analyses and sample requirements

Disorder	Tests recommended
NAGSD	Mutation analysis using DNA from CVS or AFC*
CPS1D	**Mutation analysis using DNA from CVS or AFC** Enzyme analysis, late fetal liver biopsy§
OTCD	**Mutation analysis using DNA from CVS or AFC+** Enzyme analysis, late fetal liver biopsy#,§
ASSD	**Mutation analysis using DNA from CVS or AFC** **Citrulline in amniotic fluid (calculation of ratios)** Enzyme analysis, intact or cultured CVS or cultured AFC
ASLD	**Mutation analysis using DNA from CVS or AFC** **Argininosuccinate and its anhydrides in amniotic fluid** Enzyme analysis, intact or cultured CVS or cultured AFC
ARG1D	**Mutation analysis using DNA from CVS** **Enzyme assay in fetal blood erythrocytes (mid-gestation sampling)**
HHH syndrome	**Mutation analysis using DNA from CVS or AFC** Functional assay in CVS or cultured AFC

bold: first choice
* in case of a request for prenatal testing one should keep in mind that NAGSD is a treatable disorder
§ described in single patients but not widely available and very limited experience
+ In the female fetus the genotype is only able to exclude OTC mutations. Because of the lyonisation it has no predictive value for the resulting phenotype if affected.
# feasible in male, but interpretation not clear in females due to X-mosaicism

6 NEWBORN SCREENING

The current knowledge on NBS for UCDs relies on a small number of publications (Posset et al. 2016; Wilcken et al. 2009).
If NBS for UCDs is implemented, follow-up should be done in specialised metabolic units to avoid delayed or inappropriate treatment.

Current practice of newborn screening for UCDs

6.1 *Newborn screening for mitochondrial UCDs*

There is no routine screening for NAGSD, CPS1D and OTCD. Screening for low citrulline levels alone has a low specificity and probably a low sensitivity at least for late onset OTCD (Cavicchi et al. 2009). Orotic acid can be measured in dried blood spots (D'Apolito et al. 2012; Held et al. 2014; Janzen et al. 2014) but there are no data from larger screening cohorts available.

6.2 *Newborn screening for cytosolic UCDs*

ASSD and ASLD can be detected by elevated citrulline and ASA concentrations in DBS, respectively, with a low false positive rate. There is no information on the false negative rate but it is considered to be zero for the severe deficiency states (Naylor and Chace 1999; Rashed 2001; Sander et al. 2003). A substantial number of mild phenotypes (ASSD and ASLD) may be detected and it is not known how many of them are at risk of decompensation later in life (Barends et al. 2014; Rüegger et al. 2014). ARG1D may be detected by elevated arginine (Jain-Ghai et al. 2011) but the sensitivity and specificity of arginine as a primary NBS parameter is unknown. NBS may have the largest impact on late-onset patients in whom a trend towards an improved outcome was found (Posset et al. 2016).

Outcomes: reliable and early laboratory identification of patients and improvement of survival

Recommendation #7: As patients with UCDs may benefit from early diagnosis, reliable newborn screening is desirable. We recommend to consider newborn screening for ASSD and ASLD. At present, there is not enough data to base a recommendation on newborn screening programs for NAGSD, CPS1D, OTCD, ARG1D and HHH syndrome.
Quality of evidence: moderate

7 MANAGEMENT OF ACUTE HYPERAMMONEMIA

Rationale of diet and ammonia detoxifying drugs in the treatment of UCDs

7.1 *Initial management of acute hyperammonemia*

Before treatment of acute hyperammonemia, the prognosis regarding neurodevelopmental outcome needs to be considered and may influence the decision whether to continue specific treatment or to start palliative care.
The prognosis is considered very poor in patients with any of the following:

1. coma > 3 days
2. significantly elevated intracranial pressure
3. initial or maximal ammonia > 1000 µmol/L (Bachmann 2003c; Picca et al. 2001)

The latter criterion is weaker since not only peak ammonia but also duration of hyperammonemia is important. Single patients with a normal outcome despite initial ammonia > 1000 µmol/L have been reported (De Bie et al. 2011). Peak ammonia levels > 360 µmol/L were markers of poor prognosis (Kido et al. 2012; Nakamura et al. 2014). The decision for palliative care should be made together with metabolic specialists.

Specific therapy in acute symptomatic hyperammonemia must be initiated without delay. It is advised that every pediatric hospital holds the first line medication available and provides a written instruction based on this guideline to avoid time consuming discussions on details of therapy. Diagnosis of the specific defect and the initial medical treatment must proceed simultaneously.

All patients with a hyperammonemic crisis should be transferred to a specialist centre without delay after:

1. stopping protein intake
2. start of i.v. glucose
3. initiation of first line medications as outlined in Table 5
4. collection of plasma and urine for diagnostic purposes without postponing initiation of treatment

Table 5 Levels of hyperammonemia and suggested actions in case of symptomatic patients

Ammonia level (µmol/L)	Action in undiagnosed patient	Action in known UCD patient	Comments
Increased > upper limit of normal	▪ Stop protein intake ▪ Give IV glucose at an appropriate dosage to prevent catabolism (10 mg/kg/min in a neonate) ± insulin[$] ▪ Monitor ammonia blood levels every 3 hours	▪ Stop protein intake ▪ Give IV glucose at an appropriate dosage to prevent catabolism (10 mg/kg/min in a neonate) ± insulin[$] ▪ Monitor ammonia blood levels every 3 hours	▪ Stop protein for max. 24 h ▪ Avoid exchange transfusions as cause of catabolism ▪ Hyperglycemia can be extremely dangerous (hyperosmolarity) ▪ If major hyperglycemia occurs with high lactate (>3 mmol/L) reduce glucose infusion rate rather than increase insulin ▪ Avoid hypotonic solutions ▪ Add sodium and potassium according to the electrolyte results ▪ Take into account the sodium intake if sodium benzoate or sodium PBA are used[§] ▪ L-arginine not to be given in ARG1D ▪ Some concerns of sodium benzoate use in OAs ▪ Avoid repetitive drug boluses ▪ Monitor phosphate levels and supplement early especially during hemodialysis
In addition when >100 and <250 [#]	▪ Start drug treatment with IV L-arginine and sodium benzoate (see Table 6) ▪ Start carbamylglutamate, carnitine, vitamin B_{12}, biotin (see Table 6 and its legend)	▪ Continue drug treatment with L-arginine (plus continue or add L-citrulline for mitochondrial UCDs) and sodium benzoate ± sodium PBA/phenylacetate* (see Table 6), increase dose or give IV ▪ Consider carbohydrate and lipid emulsions per NG tube unless the child is vomiting (enables higher energy intake)	
In addition when 250 to 500	▪ As above ▪ Prepare hemo(dia)filtration if significant encephalopathy and/or early high blood ammonia level or very early onset of disease (day 1 or 2) ▪ Begin hemo(dia)filtration if no rapid drop of ammonia within 3-6 hours	▪ As above, but all drugs per IV ▪ Prepare hemo(dia)filtration if significant encephalopathy and/or early high blood ammonia level or very early onset of disease (day 1 or 2) ▪ Begin hemo(dia)filtration if no rapid drop of ammonia within 3-6 hours	
In addition when 500 to 1000	▪ As above ▪ Start hemo(dia)filtration immediately	▪ As above ▪ Start hemo(dia)filtration as fast as possible	
In addition when >1000	▪ Evaluate whether to continue specific treatment or to start palliative care	▪ Evaluate whether to aim at curative treatment or palliative care	

*If available, an IV equimolar solution of sodium benzoate and sodium phenylacetate can be used: 250 mg/kg as bolus IV/90-120 min, then 250 mg/kg as continuous IV infusion over 24h. The combination of sodium benzoate and sodium phenylacetate is available as a drug, registered by the FDA (available in the EU on Named Patient Basis) and indicated as adjunctive therapy for the treatment of acute hyperammonemia and associated encephalopathy in patients with deficiencies in enzymes of the urea cycle.

[#] This limit of action applies for patients outside the neonatal period; for neonates use >150 and <250.

[$]Monitor blood glucose after 30 min and subsequently every hour, because some neonates are very sensitive to insulin.

[§]1g sodium benzoate and sodium PBA contain 7 mmol Na and 5.4 mmol Na, respectively.

Nota bene: A recent systematic review of clinical and biochemical data from published neonatal onset UCD patients found with the current practice of dialysis no impact on outcome. Authors concluded that "it may be essential for improving outcome to initiate all available treatment options, including dialysis, as early as possible" (Hediger et al. 2018). This paper was not included in the review during the revision of this guideline.

Outcome: improvement of survival

Recommendation #8: We strongly recommend immediate start of measures to reverse endogenous protein catabolism and to promote ammonia detoxification (as detailed in Table 5).
Quality of evidence: moderate

7.2 *Drugs and dosages to be used in acute decompensations of UCDs*

If sodium benzoate, sodium phenylacetate or sodium PBA are given as a bolus, nausea and vomiting are common and, thus, ondansetron (dose 0.15 mg/kg) may be applied in parallel to avoid hyperemesis (Batshaw et al. 2001; MacArthur et al. 2004).

If high doses of benzoate or phenylacetate are used, or if repeated boluses are given, the capacity for conversion into hippurate or phenylacetylglutamine may be exceeded resulting in benzoate or phenylacetate accumulation and toxicity (MacArthur et al. 2004; Praphanphoj et al. 2000).

Regarding dosing in acute episodes, there is no evidence based recommendation available; the following Table is a consensus of the working group of this guideline taking into account the literature (Ahrens et al. 2001; Batshaw et al. 2001; Brusilow and Maestri 1996; Enns et al. 2007; Feillet and Leonard 1998; Leonard and Morris 2002; MacArthur et al. 2004; Summar 2001).

Table 6: Dosages of drugs to be used in acute hyperammonemia and acute decompensations of UCDs

Disorder	Sodium benzoate (to be given IV in glucose 10%)	Sodium PBA/Sodium phenylacetate (to be given IV in glucose 10%)	L-arginine hydrochloride (to be given IV in glucose 10%)	N-carbamylglutamate (only available as oral/enteral drug)
Undiagnosed patient°	250 mg/kg as bolus in 90-120 min, then maintenance 250-500 mg/kg/d[$] > 20 kg bw: 5.5 g/m^2/d	250 mg/kg as bolus in 90-120 min, then maintenance: 250-500mg/kg/d[$]	250(-400) mg/kg (1-2 mmol/kg) as bolus in 90-120 min, then maintenance 250 mg/kg/d (1.2 mmol/kg/d)	100 mg/kg bolus per NG tube then 25-62.5 mg/kg every 6h
NAGSD	same[$]	same[$]	250 mg/kg (1.2 mmol/kg) as bolus in 90-120 min, then maintenance 250 mg/kg/d (1.2 mmol/kg/d)	same
CPS1D & OTCD	same[$]	same[$]	same	-
ASSD	same[$]	same[$]	same	-
ASLD[‡]	same[$]	same[$]	200-400 mg/kg (1-2 mmol/kg) as bolus in 90-120 min, then maintenance 200-400 mg/kg/d (1-2 mmol/kg/d)	-
ARG1D*	same[$]	-	**AVOID**	-
HHH syndrome	same[$]	same[$]	250 mg/kg (1.2 mmol/kg) as bolus in 90-120 min, then maintenance 250 mg/kg/d (1.2 mmol/kg/d)	-

°In undiagnosed patients, use of a combination of the drugs in table 6 seems justified, consider additional use of carnitine 100 mg/kg IV, hydroxycobalamin 1 mg IM/IV, and biotin 10 mg IV/PO
*The risk for acute hyperammonemic decompensation is low in ARG1D
[§]If citrulline is given, there is usually no need for concomitant use of L-arginine
[$]If on hemodialysis/hemodiafiltration doses should be increased to 350 mg/kg/d (maintenance dose)
[‡]In ASLD, L-arginine therapy for acute decompensations might be sufficient for some patients
Maximal daily drug dosages: sodium benzoate 12 g/d, sodium PBA 12 g/d, L-arginine 12 g/day

Cave: The doses indicated in Table 6 can be used at the start of treatment but must be adapted depending on plasma ammonia and amino acids.
Sodium benzoate and sodium PBA/phenylacetate should be given in parallel in severe acute decompensation. In less severe cases, a step-wise approach with initial sodium benzoate and if hyperammonemia persists or worsens, the addition of sodium PBA/phenylacetate can be chosen.

7.3 *Management of a neonate at risk of a UCD at birth*

This section has been adapted from the 'BIMDG Management Protocol of a baby at risk of a urea cycle disorder' (http://www.bimdg.org.uk):

Management during pregnancy following a previous index-patient

7.4 *Criteria to start extracorporeal detoxification*

In neonates and children

Effective medical treatment may avoid the need for hemo-dialysis/filtration but the dialysis team must be informed at the start of medical treatment. Within the first four hours, the response to the medical management should be evaluated. If the response is regarded inadequate, extracorporeal detoxification should be started.

Realize that patients receiving efficient extracorporeal detoxification may still have a poor cognitive prognosis (Kido et al. 2012). Some authors therefore advocate that patients with a "peak ammonia level greater than 180 µmol/l at the onset should receive hemodialysis" (Kido et al. 2012).

Outcomes: improvement of survival and preservation of cognitive function and prevention of neurological disease by use of extracorporeal detoxification

Recommendation #9. Parallel to medical treatment, we strongly recommend to prepare extracorporeal detoxification in patients with severe neurological symptoms induced by hyperammonemia. Start extracorporeal detoxification as soon as possible, unless initial medical treatment has already led to sufficient improvement of ammonia levels and clinical situation.
Quality of evidence: moderate

Nota bene: A recent systematic review of clinical and biochemical data from published neonatal onset UCD patients found with the current practice of dialysis no impact on outcome. Authors concluded that "it may be essential for improving outcome to initiate all available treatment options, including dialysis, as early as possible" (Hediger et al. 2018). This paper was not included in the review during the revision of this guideline.

7.5 Management of acute hyperammonemia in adults

Outcomes: improvement of survival and preservation of cognitive function and prevention of neurological disease by use of extracorporeal detoxification in adults

Recommendation #10. We recommend extracorporeal detoxification be considered as a first line treatment in acute hyperammonemic decompensations in adults.
Quality of evidence: low

7.6 *Methods to be used for extracorporeal detoxification*

HD provides the highest ammonia extraction, as solute clearance is related to dialysate flow rate and blood flow rate, in addition to the surface area of the dialytic membrane. However, after discontinuation of HD, some patients may have an acute relapse of hyperammonemia.
In neonates, it has been shown that ammonia removal may be lower with HD than with CVVHD because of frequent technical and hemodynamic complications related to HD in infants (Sadowski et al. 1994), but, if working, intermittent HD is safe and efficient (Tsai et al. 2014). CVVHD may however be better tolerated, providing a

continuous extraction with excellent ammonia clearance and it can be considered as the first line therapy in small infants (Hiroma et al. 2002; Picca et al. 2001; Schaefer et al. 1999; Spinale et al. 2013).

Outcomes: improvement of survival and preservation of cognitive function and prevention of neurological disease by use of extracorporeal detoxification

Recommendation #11: We recommend hemodiafiltration as the method of choice for ammonia detoxification. We recommend considering peritoneal dialysis, which is less effective for ammonia removal, as a bridging technique when no hemodiafiltration is available and for transfer of patients to a metabolic center. We strongly recommend against performing exchange transfusion to treat hyperammonemia.
Quality of evidence: high

7.7 *Dietary management during acute decompensation*

It is crucial to **stop catabolism by promoting and maintaining anabolism** in any patient with an acute hyperammonemic decompensation. In most, oral feeding will not be feasible during the acute phase because of impaired consciousness and vomiting. A central venous line should be inserted in the severely ill patient, to help maximise energy intake. A glucose infusion should be started as quickly as possible; if hyperglycemia occurs, continuous IV insulin should be given. Administration of lipids (1-2 g/kg/d) can also provide additional energy and help reconstitute anabolism. To avoid protein catabolism and consequent increased ammonia production, the reintroduction of protein/amino acids/EAAs must not be delayed more than 24 hours. Some authors even advocate to consider supplementation with EAAs and BCAAs at the start of acute treatment since plasma concentrations of all measured EAAs were low or low-normal in almost all samples from UCD patients at admission for acute hyperammonemia; supplementation should preferably be given via the enteral way because of the contribution of the splachnic system to protein retention and metabolism (Boneh 2014; Rodney and Boneh 2013). If the patient cannot be fed enterally, IV amino acids should be commenced, increasing daily to the required amount/kg.
If intravenous amino acid mixtures are used, the exact composition needs to be considered to avoid those with high aromatic amino acids and low in BCAAs (Bachmann 2006).
Enteral feeding should be re-started as soon as possible. The composition of the feed will vary dependent on IV therapy and ammonia concentrations. It may initially be protein free; see the emergency regime:

Table 7: Emergency regime for protein-free feeding in infants and children (adapted from (Dixon 2007))

Age	Glucose polymer concentration % CHO	Energy/100ml Kcal	kJ	Suggested daily fluid volume	Feeding frequency
up to 6 m	10	40	167	150 ml/kg	2 to 3 hourly oral/bolus day and night or continuous tube feeds using enteral feeding pump
7-12 m	10-15	48	202	120 ml/kg	
1 y	15	60	250	1200 ml	
2-9 y	20	80	334	*	
>10 y	25	100	418	*	

* For children > 10 kg normal fluid requirements can be calculated as:
11-20 kg: 100 ml/kg for the first 10 kg, plus 50 ml/kg for the next 10 kg
20 kg and above: 100 ml/kg for the first 10 kg, plus 50 ml/kg for the next 10 kg, plus 25 ml/kg thereafter up to a maximum of 2500 ml/day

Newly diagnosed newborns, infants or children

Neonates or infants

Children

Dietary management of intercurrent illness at home in known UCD patients

Outcomes: improvement of survival and improvement of metabolic stability by dietary intervention

Recommendation #12: We strongly recommend for treatment of acute hyperammonemia establishing and maintaining anabolism by providing high-dose glucose ± insulin (plus lipids if a fatty acid oxidation disorder has been excluded). We recommend keeping the period of protein-free nutrition no longer than 24 hours.
Quality of evidence: moderate

8 LONGTERM MANAGEMENT OF UCDS

Longterm treatment of UCDs is challenging for patients and families because of the poor palatability, volume and frequency of diet and drug administrations; all these are serious barriers to adherence (Shchelochkov et al. 2016). Evaluation of the literature on long-term treatment of UCDs shows that the majority of publications on diet and pharmacotherapy are reviews, expert opinions and case reports but only single case control or cohort studies. Recommendations provided in this guideline are therefore mostly grade D and only few are grade C.

8.1 *Principles of diet for long-term treatment of UCDs*

The aims of the long-term treatment are to maintain stable metabolic control, to eliminate chronic complications (Berry and Steiner 2001; Brusilow and Horwich 2001; Leonard and Morris 2002) and achieve normal growth. For most patients, this can only be achieved by a combination of:

- medications which increase waste nitrogen excretion
- a low-protein diet
- supplementation of essential nutrients such as vitamins and minerals
- EAA supplementation
- emergency regimen for treatment of intercurrent illnesses

Low protein diet

Most UCD patients are treated with a protein restricted diet with some variation between European centers (Adam et al. 2013). Controlled data to support protein restriction are scarce but clinical experience and biochemical considerations are in favour of this.

Restricting natural protein requires careful planning and monitoring to meet the individual requirements for normal growth and development and obligatory nitrogen losses, whilst maintaining metabolic stability. Over-restriction must be avoided to prevent malnutrition, essential amino acid deficiency and metabolic instability (Leonard 2001; Singh et al. 2005). To prevent endogenous protein catabolism an adequate intake of energy should be provided (Singh 2007).

The prescribed protein intake is best provided from a combination of low and some high biological value protein foods, to help ensure adequate intakes of all EAAs. The diet can then be provided primarily from normal food with little reliance on alternative energy supplements and manufactured low protein foods. Ideally the daily protein intake should be equally divided between feeds or meals to avoid giving a high protein load and to optimise nitrogen retention.

Outcomes: improvement/maintenance of metabolic stability and preservation of cognitive outcome and prevention of neurological and hepatic complications

Recommendation #13: Deficiencies of caloric and/or essential amino acid and other nutrients can cause metabolic instability and morbidity. We strongly recommend involving a specialist metabolic dietitian to balance nutritional requirements with metabolic stability, following the FAO/WHO/UNU guidelines for protein and energy requirements.
Quality of evidence: moderate

Table 8: Safe levels of protein intake for different age groups according to FAO/WHO/UNU 2007.

Child		**Female**		**Male**	
Age	g/kg bw/day	Age	g/kg bw/day	Age	g/kg bw/day
Months (breast fed infants)		Years		Years	
1	1.77				
2	1.50				
3	1.36				
4	1.24				
6	1.14				
		11	0.90	11	0.91
Years (weaned infants)		12	0.89	12	0.90
0.5	1.31	13	0.88	13	0.90
1.0	1.14	14	0.87	14	0.89
1.5	1.03	15	0.85	15	0.88
2	0.97	16	0.84	16	0.87
3	0.90	17	0.83	17	0.86
4-6	0.86-0.89	18	0.82	18	0.85
7-10	0.91-0.92	> 18	0.83	>18	0.83

EAAs and BCAA supplementation

Outcomes: improvement/maintenance of metabolic stability

Recommendation #14: We recommend supplementation of essential amino acids, especially of branched-chain amino acids, if natural protein tolerance is very low and/or if the patient receives phenylbutyrate.
Quality of evidence: moderate

Energy requirements

Table 9 shows recommended daily intakes of energy for different age groups for the healthy population according to FAO/WHO/UNU 2007 (WHO Technical Report Series 2007).

Age	Energy requirements (kJ/kg)		Energy requirements (kcal/kg)	
Children, Years	Males	Females	Males	Females
0.5	335	340	80.0	81.2
2.5	348	334	83.1	79.8
5.0	315	305	75.2	72.8
10	275	248	65.7	59.2
15	230	193	54.9	46.1
Adults, moderate activity level, 70kg body weight Years				
18-29	183	159	43.7	38.0
30-59	175	148	41.8	35.3
Adults, moderate activity level 50kg body weight Years				
18-29	212	180	50.6	43.0
30-59	212	183	50.6	43.7

Supplementation of vitamins, minerals and trace elements

Supplementation of essential fatty acids

8.2 *Practical aspects of diet for long-term treatment of UCDs*

Outcomes: improvement/maintenance of metabolic stability and preserving quality of life and reducing the burden of disease

Recommendation #15: We recommend individualized dietary management, and parents' and patients' training to strengthen their competence for a life-long dietary treatment.
Quality of evidence: moderate

Low protein feeds for infants
For dietary management during acute metabolic decompensation and re-introduction of protein see 7.7.

Breast feeding

Bottle feeding (see example 1)

Clinical monitoring

Weaning onto a low protein diet

Tube feeding

Some UCD patients have difficulties in achieving an adequate dietary intake and tube feeding becomes essential to prevent metabolic decompensation.

Tube feeding needs to be implemented or considered in patients who have one or a combination of the following problems:

- inability to suck or swallow due to neurological handicap or severe developmental delay
- difficulty with the daily administration of EAAs and drugs with an unpleasant taste
- poor appetite and/or food refusal with resultant inadequate energy intake
- gastrointestinal problems – vomiting, reflux, retching
- emergency management during intercurrent illnesses

Outcomes: improvement/maintenance of metabolic stability and preserving quality of life and reducing the burden of disease

Recommendation #16: We recommend early consideration of tube feeding to ensure nutritional adequacy, administration of medications and supplements, prevention of catabolism and/or maintenance of metabolic stability.
Quality of evidence: low

Low protein diet for children and adolescents

Self selected low protein diets

EAAs supplements

BCAA supplements

EFAs and LCPUFAs

Low protein diet for adults

Adult patients with UCDs need a low protein diet throughout life.

Pregnancy and lactation

Females with UCDs and especially female OTC carriers are in danger of life threatening encephalopathy because of the catabolic state that can be triggered in both the intrapartum and postpartum period. Multidisciplinary management is essential and careful follow-up is necessary, from pre-pregnancy counseling to plan management of the drug treatment and close monitoring of the nutritional status.

There are already some case reports describing the successful management of pregnancies in women with UCDs of which most but no all describe OTC females (Ituk et al. 2012; Kim et al. 2012; Langendonk et al. 2012; Mendez-Figueroa et al. 2010; Worthington et al. 1996). The focus of the dietary management during pregnancy should be to prevent undernutrition of protein. The metabolic situation should be closely monitored; to adjust the diet, the following recommendations during pregnancy and lactation might be helpful (see Table 10).

Table 10: Protein intake during pregnancy and lactation (WHO Technical Report Series 2007)

Pregnancy trimester	Additional safe protein intake (g/day) FAO/WHO/UNU 2007	Additional energy requirement (KJ/day) FAO/WHO/UNU 2007
1	1	375
2	10	1200
3	31	1950
Lactation		
First 6 months	19	2800
After 6 months	13	1925

During labor, neuroaxial anesthesia may reduce catabolism; therefore, early epidural analgesia seems prudent (Ituk et al. 2012).

8.3 *Pharmacotherapy in long-term treatment of UCDs*

Drugs which are routinely used for long-term treatment of UCDs comprise nitrogen scavengers (sodium benzoate, sodium PBA or sodium phenylacetate, glycerol phenylbutyrate), L-arginine, L-citrulline and carbamylglutamate. Some of the medications are available as powder, capsule, tablet or liquid. This might cause practical problems for the patient if no unambiguous prescription is written (Summar 2001; Wilcken 2004).

Outcomes: improvement/maintenance of metabolic stability and preserving quality of life and reducing the burden of disease

Recommendation #17: We suggest providing written drug treatment sheets to parents, pharmacists and persons involved in patient care.
Quality of evidence: low

Efficacy and dosage of nitrogen scavengers

Table 11: Dosages of drugs to be used perorally for long-term treatment of UCDs

Disorder	Sodium benzoate[$]	Sodium PBA[°$], or equivalent dosages of GPB	L-arginine[$] (hydrochloride and/or free base)	L-citrulline[$]	Carbamyl-glutamate[$]
NAGSD	-	-	-	-	10-100 mg/kg/d
CPS1D	up to 250 mg/kg/d*# max. 12 g/d	<20 kg: up to 250 mg/kg/d*# >20 kg: 5 g/m²/d# max. 12 g/d	<20 kg: 100-200* mg/kg/d or: 0.5-1 mmol/kg/d >20 kg: 2.5-6 g/m²/d max. 6 g/d	100-200 mg/kg/d§ max. 6 g/d	-
OTCD	same	same	same	100-200 mg/kg/d§ max. 6 g/d	-
ASSD	same	same	<20 kg: 100-300*# mg/kg/d or: 0.5-1.5 mmol/kg/d >20 kg: 2.5-6 g/m²/d# max. 8 g/d	-	-
ASLD	same	-	<20kg: 100-300*# mg/kg/d or: 0.5-1.5 mmol/kg/d >20kg: 2.5-6 g/m²/d# max. 8 g/d	-	-
ARG1D	same	same	-	-	-
HHH syndrome	same	same	<20 kg: 100-200* mg/kg/d >20 kg: 2.5-6 g/m²/d max. 6 g/d	100-250 mg/kg/d§ max. 6 g/d	-

Legend to Table 11: all medications should be divided into three to four doses daily taken with meals and distributed as far as possible throughout the day.
° sodium PBA was considered of second choice for long-term treatment by most guideline group members. It should be given together with sodium benzoate in patients in which benzoate alone is not enough
* serum/plasma levels of benzoate/PBA and plasma levels of arginine should be monitored
# in some patients higher doses are needed (the US FDA studies consider doses up 450 - 600 mg/kg/day in children weighing less than 20 kg and 9.9 to 13.0 g/m²/d in children weighing more than 20 kg, adolescents and adults), according to expert advice
§ if citrulline is given, there is usually no need for concomitant use of L-arginine
$ 100 mg equal 0.694 mmol sodium benzoate; 0.537 mmol sodium PBA; 0.475 mmol arginine hydrochloride; 0.574 mmol arginine base; 0.571 mmol citrulline; 0.532 mmol carbamylglutamate, respectively

Outcomes: improvement/maintenance of metabolic stability and treatment during pregnancy

Recommendation #18: Nitrogen scavengers are a mainstay of therapy in UCD patients. We recommend to adjust the dose for each patient.
Quality of evidence: moderate

Adverse effects and toxicity of nitrogen scavengers

Nitrogen scavengers are safe at the recommended doses but can be toxic at high doses.

Use of nitrogen scavengers in pregnancy

The use of nitrogen scavenger drugs in pregnancy has not been systematically investigated but there are single anecdotal reports on successful pregnancies on PBA (Batshaw et al. 2001; Ituk et al. 2012; Lamb et al. 2013). According to data available, nitrogen scavenger drugs should be used with caution. Based on their experience and on few anecdotal reports, the working group of this guideline regards sodium benzoate to be the safer choice if medication has to continue.

Outcomes: improvement/maintenance of metabolic stability and treatment during pregnancy

Recommendation #19: Continuation of treatment with nitrogen scavengers is generally necessary in pregnant UCD patients. Based on biochemical mechanisms, we suggest the use of sodium benzoate. There is insufficient evidence for commenting on fetal outcomes after nitrogen scavenger therapy in pregnancy.
Quality of evidence: low

L-arginine and L-citrulline for long-term treatment

Outcomes: improvement/maintenance of metabolic stability and prevention of complications

Recommendation #20: We strongly recommend L-arginine and/or L-citrulline supplementation in UCD patients (may not be required in mild phenotypes, and L-arginine is contraindicated in ARG1D). We recommend monitoring plasma arginine levels in all UCD patients.
Quality of evidence: moderate

N-carbamylglutamate

Carglumic acid (synonymous: N-carbamyl-L-glutamate) is a licensed drug in Europe (and also approved by the FDA in the United States) for the use in primary NAGSD (see also 11.3) (Häberle 2011b, 2012). In addition, its use has been suggested as an emergency medication in neonatal hyperammonemia of unknown etiology and hereby, as a tool for differential diagnosis in unclear neonatal hyperammonemia (Guffon et al. 2005). Although there are no controlled studies on the benefit of carbamylglutamate in neonatal hyperammonemia its use should be considered in severe hyperammonemic decompensations.

Outcomes: improvement of survival and prevention of complications

Recommendation #21: We recommend using N-carbamyl-L-glutamate as the first line medication for treatment of NAGSD and as an emergency drug during acute hyperammonemia of unknown etiology.
Quality of evidence: high

Carnitine for long-term treatment

Antibiotics for treatment of hyperammonemia in UCD patients

8.4 Special situations: vaccinations and surgery & anesthesia

Vaccinations

Outcome: prevention of complications

Recommendation #22: We strongly recommend performing vaccinations following the national schedule.
Quality of evidence: moderate
We suggest antipyretic treatment if temperature exceeds 38°C.
Quality of evidence: low

Ammonia monitoring during surgery & anesthesia

Outcomes: prevention of complications and management during surgery and anesthesia

Recommendation #23: We suggest performing elective surgery in UCD patients in centres with a metabolic expertise and resources including emergency treatment options for hyperammonemia.
Expert opinion

9 LIVER TRANSPLANTATION FOR UCD PATIENTS

Liver transplantation has been performed in all UCDs except NAGSD. Successful OLT allows for normalization of the diet and withdrawal of alternative pathway therapy (Kim et al. 2013; Whitington et al. 1998). The pre-operative management before liver transplantation should rigorously avoid catabolism.

Outcome: improvement of survival and cognitive outcome

Recommendation #24: We recommend to consider liver transplantation in patients with severe UCDs without sufficient response to standard treatment, without severe neurological damage and ideally whilst in a stable metabolic condition.
Quality of evidence: moderate

9.1 *Longterm neurological outcome after liver transplantation*

While liver transplantation is effective in preventing further hyperammonemic decompensations, previously lost neurological milestones have only been regained in single cases because pre-existing brain damage is likely to be irreversible (Kawagishi et al. 2005; Kim et al. 2013). The majority of patients so far remain developmentally delayed but preserve their pre-transplantation neurodevelopmental level (Busuttil et al. 1998; Ensenauer et al. 2005; Fletcher et al. 1999; McBride et al. 2004; Newnham et al. 2008; Perito et al. 2014; Santos Silva et al. 2001; Stevenson et al. 2009).

9.2 *Quality of life after liver transplantation*

Most large pediatric liver transplant programs nowadays reach excellent patient outcome with 95%-100% one-year and 90%-100% 5-year patient survival rates (Bourdeaux et al. 2007; Kim et al. 2013).

9.3 *Indications and ideal age for liver transplantation*

In patients with severe neonatal onset UCDs, especially CPS1D and male neonatal OTCD, liver transplantation may be performed as early as possible. In UCD patients suffering from recurrent metabolic decompensations with need for hospitalizations despite medical therapy or in UCD patients with difficult social circumstances resulting in poor compliance, liver transplantation should be considered before irreversible neurological damage is present.

Patient survival is improved with increased age at transplantation: the 5-year patient survival rate was 88% for children with UCDs who were <2 years old at transplant and 99% for children who were ≥2 years old at transplant (p=0.006; total number of patients: 186) (Perito et al. 2014). If possible, liver transplantation in UCD patients should be performed not before 3 months of age because of higher rates of complications and a lower survival rate if liver transplantation is done below age 3 months and/or below 5 kg bw (Noujaim et al. 2002; Sundaram et al. 2003).

The patient should be fully immunised. Regarding the neurological outcome, patients transplanted before 1 year as opposed to after 1 year might benefit more (McBride et al. 2004).

Outcomes: improvement of survival and cognitive outcome and quality of life

Recommendation #25: In patients with neonatal onset (except NAGSD), we strongly recommend liver transplantation before irreversible neurological damage. Transplantation between 3 and 12 months of age and when body weight exceeds 5 kg is associated with a more favourable outcome.
Quality of evidence: moderate
We strongly recommend considering liver transplantation in patients with severe progressive liver disease and/or with recurrent metabolic decompensations requiring hospitalisations despite standard medical therapy.
Quality of evidence: high

9.4 *Recommended types of donor and transplant*

9.5 *Ethical considerations related to liver transplantation*

The decision on liver transplantation is influenced by medical and ethical considerations. One issue is the dilemma of donors in living related transplantations. Another difficulty is related to the decision in severely handicapped children.

10 MONITORING

Clinical and biochemical monitoring depends on age and metabolic stability of the patient. Infants will need more frequent monitoring and adjustment of their diet and treatment than older stable patients.
In practice, young and severely affected patients should be reviewed at least every 3 months while older or less severely affected patients may only need annual reviews.

Clinical and nutritional monitoring

Biochemical monitoring

Outcomes: achievement of metabolic stability and of normal growth and weight

Recommendation #26: We strongly recommend for all UCD patients regular clinical, biochemical and nutritional monitoring by a multidisciplinary metabolic team following individualised schedules.
Expert opinion

10.1 *Monitoring in plasma*

Ammonia

Ammonia measurements are important both during acute decompensations and long-term follow-up. In particular, fasting ammonia levels correlate strongly and positively with the risk and frequency of hyperammonemic crises, suggesting that UCD patients benefit from tight ammonia control (Lee et al. 2015). Recommendations for the techniques of ammonia collection and measurement have been published (Bachmann 2014; Barsotti 2001). The aim is to keep plasma ammonia levels in the normal range (50 µmol/L is the upper normal limit with the enzymatic method beyond neonatal age) but at least below 80 µmol/L (Feillet and Leonard 1998; Leonard and Morris 2002).

Micromethods for ammonia measurement

Amino acids
Monitoring of plasma amino acids at regular intervals is recommended in order to adapt the dietary and pharmacological treatment to the individual requirements of each patient (Bachmann 2014).

Outcomes: achievement of metabolic stability and of normal growth and weight

Recommendation #27: We recommend for longterm management as target level for ammonia <80 µmol/L, for glutamine <1000 µmol/L, for arginine in the high normal range and for EAAs and BCAAs in the normal range.
Quality of evidence: high

Other parameters

Value of drug levels for monitoring UCD treatment

10.2 *Monitoring in urine*

Spot urine
Presence of ketone bodies in urine can indicate persistent catabolism, may guide adjustment of energy intake and can be easily done at home.

Orotic acid and orotidine

Amino acids

Other parameters

10.3 *Role of neuroimaging*

In UCDs, pattern and extent of brain MRI abnormalities vary depending on the stage of disease and correlate with neurological outcome (Bireley et al. 2012). Improvement of neuroimaging methods can potentially have a critical role in clinical monitoring and treatment (Gropman et al. 2013).

Practical considerations

Outcomes: prognostic markers for acute and long-term management

Recommendation #28: We recommend brain magnetic resonance imaging, if possible together with spectroscopy, in UCD patients, even in the absence of neurological and/or cognitive impairment, as this may help to adjust treatment. Timing should be decided on a case by case evaluation.
Quality of evidence: moderate

10.4 *Psychological aspects in the clinical care for UCD patients*

The cognitive outcome of patients with UCDs depends predominantly on the extent and duration of hyperammonemia (Gropman et al. 2007). Children presenting with neonatal onset have poorer outcome concerning cognitive, adaptive and behavioural functioning.

Outcome: neurological complications

Recommendation #29: We recommend testing for IQ, development and specific strengths/weaknesses in all patients, including those with milder disease or female OTCD heterozygotes. They may develop specific weaknesses in executive functions even if the IQ is normal.
Quality of evidence: moderate

Until recently, only little attention has been given to the psychological status of patients and families affected by inborn errors of metabolism including UCD. Most of the papers addressing this topic are reviews, uncontrolled studies or expert opinions.

Outcomes: quality of life and reducing the burden of disease

Recommendation #30: We recommend including psychological monitoring and counselling as an important component of the care of UCD patients and their families since health-related quality of life, anxiety, stress and psychosocial factors are important outcome parameters.
Quality of evidence: high

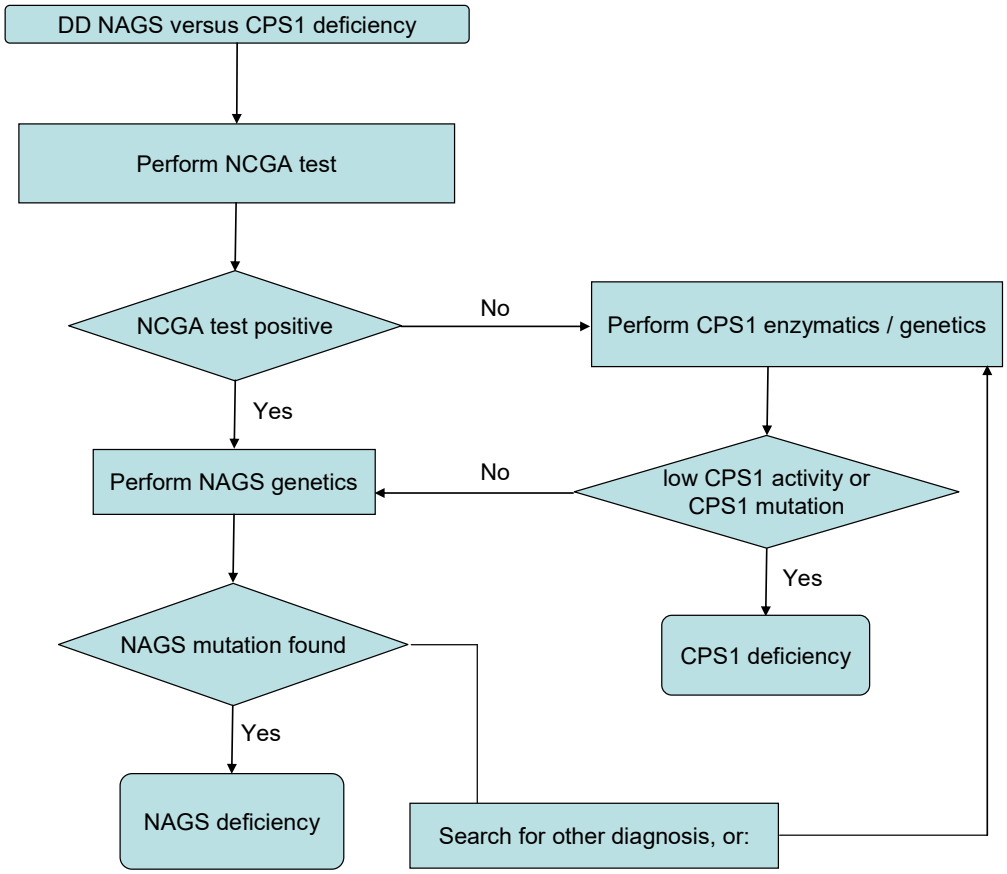
Part II: Detailed recommendations

11 NAGS AND CPS1 DEFICIENCY

Deficiencies of NAGS and CPS1 can not be differentiated on the basis of clinical presentation or standard laboratory investigations. Plasma amino acid profiles are comparable (showing elevated glutamine and often decreased citrulline and arginine). The concentration of urinary orotic acid is not increased. For this reason, the diagnostic work-up is described here together for both disorders.
For the differential diagnosis of neonatal onset deficiencies of NAGS versus CPS1, the so called NCGA test has been suggested (Guffon et al. 2005) but even a negative response to NCGA does not exclude NAGSD (Nordenstrom et al. 2007) while a positive response was also observed in patients with CPS1D (Ah Mew et al. 2010; Kuchler et al. 1996; Williams et al. 2010).

The following algorithm provides a recommendation how to proceed in patients with a biochemical profile suggestive of NAGS- or CPS1 deficiency.

Figure 2:



11.1 *The role of enzyme analysis for diagnosis of NAGS or CPS1 deficiency*

11.2 *Genetic analysis of NAGS and CPS1 deficiency*

Outcome: reliable and early diagnosis of NAGS and CPS1 deficiencies

Recommendation #31: We strongly recommend genetic analysis for the diagnosis of NAGSD and for CPS1D, since NAGS activity assay is not generally available and enzymatic diagnosis of CPS1D requires liver or intestinal mucosa, respectively.
Quality of evidence: high

11.3 *Treatment of NAGS deficiency – special considerations*

Outcome: achieving metabolic stability in NAGS deficiency

Recommendation #32: We strongly recommend monotherapy with carbamylglutamate as the treatment of choice outside acute decompensations in NAGSD.
Quality of evidence: high

11.4 *Treatment of CPS1 deficiency – special considerations*

The role of NCGA was further investigated using recombinant pure human CPS1 (Diez-Fernandez et al. 2013). Hereby, it was shown that human CPS1 expressed in vitro is protected from protease degradation and thermal inactivation by NCGA in the presence of ATP. In another study using pure enzyme, protein misfolding was found in the case of several CPS1 mutants rendering chaperone treatment for CPS1D an interesting alternative (Diez-Fernandez et al. 2014). This possibly points towards a potential pharmacological chaperone effect of NCGA on CPS1 but needs further evaluation.
Treatment using NCGA was reviewed in (Daniotti et al. 2011; Häberle 2012) and was tested in five patients with late-onset CPS1D; in four patients, ureagenesis was improved and one patient showed marked improvement in nitrogen metabolism (Ah Mew et al. 2014).

12 OTC DEFICIENCY

Male OTCD patients manifest in their majority as neonates and belong to the group of UCDs with the highest mortality (60%) during their initial presentation; in female OTCD however, neonatal onset was only 7% in a large meta-analysis (Burgard et al. 2016). A tentative diagnosis of OTCD can generally be made based on clinical history and the biochemical findings (hyperammonemia, increased plasma glutamine and alanine concentrations, low plasma citrulline concentration, and excretion of urine orotic acid). Of note, unlike in all other UCDs, patients often have no consanguineous background. Enzymatic or genetic testing should be performed to confirm the diagnosis and/or plan future prenatal testing.

12.1 *Enzyme analysis for diagnosis of OTC deficiency*

12.2 *Genetic analysis of OTC deficiency*

Outcome: reliable and early diagnosis of OTC deficiency

Recommendation #33: We strongly recommend genetic analysis for diagnosis of OTCD. We recommend determining OTC enzyme activity assay in plasma, liver or intestinal mucosa if genetic analysis fails.
Quality of evidence: high

12.3 *Investigations to diagnose suspected female OTC carriers*

The most appropriate test to ascertain female carrier status is mutation analysis (Tuchman 1992). However, mutation analysis is not always informative. In the following, alternative investigations are discussed:

Allopurinol testing, Protein loading, and/or Pedigree analysis

12.4 *Treatment of OTC deficiency – special considerations*

Treatment of clinical variants of OTC deficiency

Patients with recurrent liver failure or other gastointestinal complications

13 ASS DEFICIENCY (CITRULLINEMIA TYPE 1)

In general, the diagnosis of citrullinemia type 1 is straightforward with strongly elevated plasma citrulline levels and increased urine orotic acid in the absence of plasma argininosuccinate. In these situations, further confirmation might not be required unless future prenatal testing should be prepared.
ASSD can also present with acute liver failure (de Groot et al. 2011; Faghfoury et al. 2011).
There is a single case report on hypertrophic cardiomyopathy and cataracts in an adult patient with neonatal-onset citrullinemia but it remains unclear whether this is a complication of the disease, the long-term drug treatment or simply a coincidental finding (Brunetti-Pierri et al. 2012).

13.1 *Enzyme analysis of ASS deficiency*

13.2 *Genetic analysis of ASS deficiency*

Outcomes: reliable and early diagnosis of ASS deficiency and prenatal testing

Recommendation #34: We strongly recommend genetic analysis for diagnostic confirmation and for prenatal testing in citrullinemia type 1.
Quality of evidence: high

13.3 *Treatment of ASS deficiency – special considerations*

14 ASL DEFICIENCY

ASLD is readily diagnosed by the characteristic metabolite pattern as there is no known differential diagnosis if argininosuccinic acid is found in plasma or urine. However, to estimate the level of residual enzyme activity can still have impact on the management of a patient (Hu et al. 2015b; Kleijer et al. 2002). Likewise, many affected families will ask for later prenatal testing. For these reasons, further investigations should be considered in all patients.
Besides having the greatest proportion of patients with poor cognitive outcome in several studies (Ah Mew et al. 2013; Martin-Hernandez et al. 2014; Rüegger et al. 2014), a unique but poorly understood feature in ASLD concerns the constant intellectual decline in patients who never experienced a hyperammonemic decompensation (Nagamani et al. 2012b). There are even patients known, who were prospectively treated based on positive NBS or family history and still suffered from severe neurological sequelae.
The extended (in comparison to the other urea cycle enzyme defects) phenotype may in part be explained by the fact that ASL is required for systemic nitric oxide (NO) production (Erez et al. 2011b). ASLD patients were reported to develop complications that were possibly related to systemic NO deficiency including arterial hypertension and neurocognitive deficits (Erez et al. 2011a). One patient, when receiving a NO donor (isosorbide dinitrate), benefited from this intervention and showed even improvement in some neuropsychological parameters (Nagamani et al. 2012a). A double blind, randomized, placebo-controlled, crossover study of NO supplementation in ASLD patients assessing endothelial function and blood pressure as primary endpoints is currently recruiting patients (ClinicalTrials.gov: NCT02252770).

14.1 *Enzyme analysis of ASL deficiency*

14.2 *Genetic analysis of ASL deficiency*

Outcomes: reliable and early diagnosis of ASL deficiency <u>and</u> prenatal testing

Recommendation #35: We recommend metabolite analysis for confirmation of ASLD since presence of ASA in high concentrations in plasma or urine is diagnostic.
Quality of evidence: high
We strongly recommend genetic confirmation for family counselling and as method of choice for prenatal testing.
Quality of evidence: high

14.3 *Treatment of ASL-deficiency – special considerations*

Outcomes: achieving metabolic stability in ASL deficiency <u>and</u> cognitive and hepatic outcome

Recommendation #36: We recommend against high-dose L-arginine supplementation in ASLD because of neurological and hepatic complications. We recommend using L-arginine for long-term management at the same dosages as for other UCDs in combination with nitrogen scavenges and protein restriction.
Quality of evidence: moderate

<u>Monitoring of patients with ASL deficiency</u>

Particular attention should be given to monitoring blood pressure levels as arterial hypertension was more commonly observed in ASLD (Brunetti-Pierri et al. 2009; Erez et al. 2011a).

15 ARGINASE DEFICIENCY

ARG1D markedly differs from other UCDs because it usually does not present during the neonatal period and first symptoms occur between 2 and 4 years of age (Crombez and Cederbaum 2005; Scaglia and Lee 2006; Schlune et al. 2015). The main symptoms are progressive spastic paraplegia and often only during acute hyperammonemic episodes, hepatomegaly; seizures can also be the presenting symptom. Hyperammonemia is less frequent than in other UCDs but patients can have neonatal and/or recurrent hyperammonemic crises (Jain-Ghai et al. 2011; Scholl-Bürgi et al. 2008; Zhang et al. 2012).
ARG1D results in increase of plasma arginine in all patients but the levels may only be slightly elevated under treatment (Cederbaum et al. 1982). Therefore, normal or slightly increased arginine plasma concentrations do not exclude ARG1D. It is recommended to confirm the diagnosis by enzymatic or genetic analyses in every new patient. Urine orotic acid levels are often elevated.

15.1 *Enzyme analysis for arginase deficiency*

15.2 *Genetic analysis of arginase deficiency*

15.3 *Treatment of Arginase deficiency – special considerations*

Outcomes: achieving metabolic stability in ARG1 deficiency and prevention of neurological complications and burden of dietary treatment

Recommendation #37: We recommend following standard UCD dietary and medical (without the use of L-arginine) treatment in ARG1D. We suggest adherence to a strict protein restriction to reduce plasma arginine levels to as low as possible aiming for the upper reference range.
Quality of evidence: moderate

16 HHH SYNDROME

HHH syndrome is due to a deficient ornithine transporter (ORNT1) of the mitochondrial membrane encoded by the *SLC25A15* gene (reviewed in (Martinelli et al. 2015). There is a typical metabolic profile comprising the name-giving triad with urine homocitrulline as specific marker allowing for diagnosis on the basis of just biochemical analysis (Palmieri 2008). However, if confirmation by another method is required or if prenatal testing is planned, functional analysis or genetic testing can be applied.

16.1 *Functional analysis of ORNT1*

16.2 *Genetic analysis of the SLC25A15 gene*

16.3 *Treatment of HHH syndrome –special considerations*

Outcome: achieving metabolic stability in HHH syndrome

Recommendation #38: We recommend low-protein diet and citrulline or arginine supplementation in HHH syndrome. The impact of these measures on pyramidal dysfunction is unclear.
Quality of evidence: moderate

17 FUTURE DEVELOPMENTS

17.1 *Perspectives of molecular genetics in UCDs*

17.2 *Role of specific neuroprotection by drugs and hypothermia*

17.3 *Cell therapies*

17.4 *Gene therapy*

17.5 *Enzyme replacement therapy*

17.6 *Experimental therapy and novel approaches*

18 REFERENCES

Part I: General recommendations

1. CLINICAL DIAGNOSIS – SIGNS AND SYMPTOMS

1.1 Clinical suspicion of UCD

Clinical signs and symptoms are non-specific but in most patients neurological symptoms prevail followed by hepatic-gastrointestinal and psychiatric manifestations.
A detailed history should be obtained in all patients with a suspicion of UCD including a drug history. Many patients have a consanguineous background ("are the parents related?") and this along with the family history ("were there any unexplained neonatal deaths in your family?"); past medical ("did s/he suffer from any previous unexplained neurological disorder?") and diet history ("is he/she avoiding high protein foods?") are of particular importance.
Unexpected severe and long-lasting symptoms not responding to standard therapy may direct the suspicion from more common conditions such as neonatal sepsis to UCDs.

1.2 Acute and chronic presentations

UCD patients may present with acute or chronic symptoms at any age. Some of the signs and symptoms are common, others are uncommon and few are only described in single patients.
The majority of symptoms are neurological and caused by hyperammonemia induced cerebral edema. The typical neonatal patient will present very similar to a newborn with sepsis. In some cohorts, the majority of UCD patients presented outside the neonatal period (Martin-Hernandez et al. 2014; Summar et al. 2008). Outside the neonatal period, symptoms are largely non-specific.

Table 1 gives an overview of clinical signs and symptoms of acute and chronic manifestations of UCDs (Brusilow and Horwich 2001; Burlina et al. 2001; Gropman et al. 2007; Leonard and Morris 2002; Rüegger et al. 2014; Summar et al. 2008; Trevisson et al. 2007).

Table 1: Clinical signs and symptoms of acute and chronic manifestations of UCDs

Acute presentation	Chronic presentation
• **Altered level of consciousness (from lethargy and somnolence to coma) mimicking encephalitis or drug intoxication** • **Acute encephalopathy (see below)** • **Seizures (mostly in the circumstance of altered level of consciousness)** • Ataxia: mostly in the circumstance of altered level of consciousness • Stroke-like episodes • Transient visual loss • **Vomiting and progressive poor appetite** • Liver failure, coagulopathy (esp. in OTCD and HHH) • **Multiorgan failure** • **Peripheral circulatory failure** • **Psychiatric symptoms (hallucinations, paranoia, mania, emotional or personality changes)** • "Post-partum psychosis" • **In neonates:** **sepsis-like picture, temperature instability, respiratory distress, hyperventilation**	• **Confusion, lethargy, dizziness** • **Headaches, migraine-like, tremor, ataxia, dysarthria** flapping tremor (in adults) • **Learning disabilities, cognitive impairment** • Epilepsy • Chorea, cerebral palsy • Protracted cortical visual loss • Progressive spastic diplegia or quadriplegia (described in ARG1D and HHH syndrome) • **Protein aversion, self-selected low-protein diet** • **(Recurrent) abdominal pain, vomiting** • **Failure to thrive** • **Hepatomegaly, elevated liver enzymes** • **Psychiatric symptoms: hyperactivity, mood alteration, behavioural changes, aggressiveness** • Self-injurious behaviour • *Autism-like symptoms* • **Fragile hair (mainly in ASLD)** • *Dermatitis* • **Episodic character of signs and symptoms** • Specific neuropsychological phenotype in heterozygous OTC females

bold: typical signs and symptoms
standard: uncommon signs and symptoms
italics: signs and symptoms only reported in single patients

Variability of the clinical phenotype

There is broad variability of the clinical phenotype in each disorder (maybe due to different genotypes and the extent of present and previous crises) with mild courses described for almost all UCDs (Caldovic et al. 2005; Ficicioglu et al. 2009; Häberle et al. 2003a; Kurokawa et al. 2007).
Within families, severe neonatal forms tend to recur with a similar course in most UCD subtypes. In contrast, in OTCD, mild as well as severe courses affecting both sexes in single families are described (Ahrens et al. 1996; Ausems et al. 1997). Especially heterozygous females with OTCD may present with a wide variety of clinical presentations probably depending on X-chromosomal lyonisation (Enns 2008; Gyato et al. 2004). Even within one family affected by CPS1D, the clinical phenotype varied markedly (Klaus et al. 2009).
Some UCD patients present with episodic symptoms that can resolve without any or with non-specific therapeutic interventions.

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Acute encephalopathy

The list of differential diagnoses of acute encephalopathy is long and depends on the age of the patient.
However, the suspicion of a UCD should rise immediately in patients with acute or chronic encephalopathy at any age (Cartagena et al. 2013). The presence of another acute disease or trauma does not rule out a UCD which might have been unmasked by the acute incident.

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Different age groups

Newborns typically present with acute neurological distress or a sepsis-like picture; in a cohort of 103 subjects with neonatal-onset UCDs, 88% presented clinically by age 7 days (Ah Mew et al. 2013).

Outside the newborn period, all signs and symptoms of acute or chronic presentations occur (see Table 1). Beside poor appetite, vomiting and loss of consciousness which is common to all ages, newborns tend to show seizures more frequently. In contrast, extrapyramidal symptoms occur predominantly after 4 years of age. Spasticity and pyramidal symptoms in the diseases with high arginine (ARG1D; HHH syndrome) also develop during childhood or later. Psychotic symptoms often occur around puberty.

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Postpartum psychosis

In females with the suspicion of postpartum psychosis, UCDs have to be considered as differential diagnosis (Enns et al. 2005; Häberle et al. 2010). This is especially common for OTCD but is also reported for ASSD and for CPS1D (Fassier et al. 2011). Plasma ammonia and amino acids should be analysed in all women with acute unexplained neurological symptoms in the postpartum period.

Typical signs in specific UCDs

Abnormalities of the hair shaft leading to **fragile hair** (often but not entirely correctly described as trichorrhexis nodosa) are a frequent finding in patients with ASLD (Brusilow and Horwich 2001; Kvedar et al. 1991; Patel and Unis 1985; Smith et al. 2005). ARG1D and HHH syndrome may present as **progressive spastic diplegia** without obvious hyperammonemic episodes (Cowley et al. 1998; Crombez and Cederbaum 2005; Salvi et al. 2001).

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Uncommon clinical presentations

Stroke-like episodes or “metabolic strokes” have been described in patients with deficiencies of CPS1, OTC, ASS and HHH syndrome and they are clinically unspecific (Al-Hassnan et al. 2008; Choi et al. 2006; Christodoulou et al. 1993; Keegan et al. 2003; Nicolaides et al. 2002; Sperl et al. 1997). A “metabolic stroke“ may be only detected by diffusion MRI studies (see also 10.3) and the affected area is not explained by vascularisation. The lesions may be reversible particularly if appropriate and early treatment is introduced.

Chorea was the presenting sign in a female heterozygous for OTCD (Wiltshire et al. 2000). **Cerebral palsy** has been described in patients with OTCD and ARG1D (Scheuerle et al. 1993; Singh et al. 2005) without a history of hyperammonemia or cerebral edema. Different forms of **epilepsy** were described in patients with ASLD and other UCDs (Grioni et al. 2014; Grioni et al. 2011).

Episodic **transient visual loss** was the first symptom of OTCD in a 32-year-old male (Snebold et al. 1987) and **protracted cortical visual loss** was reported in a 5-year-old girl (Anderson and Brodsky 2010) but these symptoms might be more common than apparent from the literature.

Autism-like symptoms were described at the time of disease manifestation in a girl with OTCD (Gorker and Tuzun 2005) and in a boy with CPS1D (Serrano et al. 2009) but again, this might be more common than apparent from the literature.

Dermatitis was reported in patients with OTCD and ASLD (Kleijer et al. 2002; Pascual et al. 2007), however, it was probably the result of protein malnutrition.

Particularly after puberty and in adult patients, acute, chronic or episodic **psychiatric symptoms** can be the only suggestion of a UCD (Sedel et al. 2007). Non specific behavioural problems have been also described in late onset forms during childhood (Serrano et al. 2009).

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1.3. Triggers of metabolic crisis

Besides a high exogenous protein load, any condition causing an increased nitrogen load to the UC may trigger hyperammonemia (Batshaw et al. 2014; McGuire et al. 2013). An endogenous protein load due to catabolism may be caused by:

- Infections
- Fever
- Vomiting
- Gastrointestinal or internal bleeding
- Decreased energy or protein intake (e.g. fasting pre surgery, major weight loss in neonates)

- Catabolism and involution of the uterus during the postpartum period (OTC females)
- Chemotherapy, high-dose glucocorticoids
- Prolonged or intense physical exercise
- Surgery under general anesthesia

In some late onset patients an unusual protein load (e.g. a barbecue, parenteral nutrition) caused nausea and even coma (for adult onset UCDs, see for instance (Summar et al. 2005)).
In addition, there are some drugs associated with an increased risk of hyperammonemia. Valproate and L-asparaginase/pegaspargase are the most important but topiramate, carbamazepine, phenobarbitone, phenytoine, primidone, furosemide, hydrochlorothiazide, salicylates have also been associated with hyperammonemic decompensation, although the causative mechanisms have not always been established.

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Outcome: reliable and early clinical identification of patients

Recommendation #1: We strongly recommend considering a UCD at any age in any acute/intermittent neurological deterioration or psychiatric illness, acute liver failure, suspected intoxication or in the differential diagnosis of neonatal sepsis. Catabolism or protein load may represent triggering factors.
Quality of evidence: moderate

1.4 Laboratory investigations

Hyperammonemia is the hallmark of UCDs with mean peak ammonia concentrations > 500 µmol/L in most neonatal patients at presentation (Ah Mew et al. 2013). However, an elevation of plasma ammonia is nonspecific and can only be regarded as a marker for insufficient detoxification of nitrogen (Häberle 2011a). Absence of hyperammonemia makes a UCD very unlikely in symptomatic newborns. In contrast, beyond the newborn period a normal ammonia concentration does not exclude UCDs. The analysis of plasma amino acids and/or urine orotic acid may be especially helpful when samples are taken after recovery from an acute episode.
Ammonia measurement is an emergency procedure because there is a clear correlation between the outcome of a patient and the length of time a patient is hyperammonemic (Bachmann 2003c; Msall et al. 1984; Picca et al. 2001). To shorten the time to diagnosis, an electronic medical record-based warning system was suggested for neonates aged 2–7 days, in which blood gas analysis is ordered without any ammonia studies (Vergano et al. 2013).
For ammonia determination (standard analysis and point of care devices/bedside testing), please see also 10.1
Hyperammonemia is often accompanied by increased plasma glutamine, and hyperglutaminemia may last for some time after normalization of plasma ammonia (Serrano et al. 2011).

Outcome: reliable and early laboratory identification of patients

Recommendation #2: We strongly recommend to determine ammonia in all conditions defined by recommendation #1 as an emergency analysis. Be aware of preanalytical pitfalls.
Quality of evidence: high

If hyperammonemia is confirmed, plasma amino acids, blood or plasma acylcarnitines and urinary organic acids should be analysed urgently. The results should be available within 24 hours but treatment must not be delayed due to pending results.

Outcome: reliable and early laboratory identification of patients

Recommendation #3: If ammonia is elevated, we strongly recommend to immediately take blood samples for analysis of amino acids and acylcarnitines. Then start treatment and collect urine for analysis of organic acids and orotic acid.
Quality of evidence: moderate

Since most methods of organic acid analysis are not reliable for the quantification of orotic acid, it is recommended to perform a specific HPLC based measurement of urinary orotic acid.

In addition, it is recommended to keep an aliquot of each plasma, serum, urine and if available, CSF at -20° C for further analysis and anticoagulated full blood for DNA isolation, especially if the acute episode is fatal (Leonard and Morris 2002; Marin-Valencia et al. 2010).

About 50% of patients with an acute presentation initially showed **respiratory alkalosis** in one study (Nassogne et al. 2005) but this was not seen in 91 patients in a recent report (Martin-Hernandez et al. 2014). Still, as respiratory alkalosis is rarely caused by any other disorder in a newborn it should prompt immediate measurement of ammonia. Any advanced metabolic decompensation may result in mixed respiratory and metabolic acidosis and therefore the acid-base status is of limited use in the diagnosis of UCDs (Bachmann and Colombo 1988).
Acute liver failure has been reported as the presenting sign in patients with OTCD, ASSD and HHH syndrome (de Groot et al. 2011; Faghfoury et al. 2011; Fecarotta et al. 2006; Ito et al. 2004; Mhanni et al. 2008; Teufel et al. 2011).
In HHH syndrome, **coagulation disturbance**, especially factor VII and X deficiencies, is a frequent finding (Dionisi Vici et al. 1987).

Urea cycle flux studies using stable isotopes
In vivo use of stable isotopes monitored by mass spectrometry was reported to measure the activity of the complete urea cycle in humans by using different labeled substances (Caldovic et al. 2004; Lee et al. 2000; Tuchman et al. 2008a; Yudkoff et al. 2010; Yudkoff et al. 1996; Yudkoff et al. 1998). Modification of the method even allowed to distinguish asymptomatic carriers of UCDs from controls (Lee et al. 2000). Further, stable isotopes confirmed the poor activation of CPS1 in NAGSD (Caldovic et al. 2004; Tuchman et al. 2008a) and as well the potential benefit of NCGA treatment in some partial CPS1D patients (Ah Mew et al. 2014).

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2. DIFFERENTIAL DIAGNOSIS

The following Table should direct further confirmatory metabolic investigations.

Table 2: Bedside differential diagnosis of an IEM presenting with hyperammonemia

Parameter	Condition								
	UCDs	Organic acidurias	β-oxidation defects	Carbonic anhydrase Va def.	HMG-CoA lyase def.	HI HA syndrome	Pyruvate carboxylase def. [g]	PEPCK deficiency	TMEM70, SERAC1 def.
Acidosis	+/–	+[e]	+/–	+	+	–	+	+	+
Ketonuria[a]	–	+	absent	+	absent	–	++	+	+
Hypoglycemia[b]	–	+/–	+	+/–	+	+	+	+/–	+/–
↑ Lactic acid[c]	–	+	+/–	+	+/–	–	+	+/–	++
↑ AST & ALT	(+)[d]	–	+	–	+/–	–	+/–	++	–
↑ CPK	–	–	+	–	+/–	–	–	–	–
↑ Uric acid	–	+	+/–	–	+	–	–		++
↓ WBC/RBC/Plt	–	+	–	–	+/–	–	–	–	–
Weight loss	–	+[f]	–	–	+/–	–	+		–

def.: deficiency
[a] In neonates ketonuria (++ - +++) suggests organic aciduria.
[b] Hypoglycemia and hyperammonemia ("pseudo-Reye") can be predominant manifestations of the organic aciduria 3-HMG-CoA-lyase deficiency.
[c] Blood lactate >6 mmol/L, since lower high lactate levels (2-6 mmol/L) may be due to violent crying or to extensive muscle activity.
[d] AST/ALT elevations can be found but are not constant in UCDs.
[e] Can be absent in neonates.
[f] Occurrence only in neonates.
[g] Only type B associated with hyperammonemia but not types A and C.

2.1. Conditions and genetic disorders other than UCDs presenting with neonatal hyperammonemia

Most information on the differential diagnoses of neonatal acute hyperammonemia has been collected from expert opinions or reviews (Burton 1998; Clay and Hainline 2007; Ellaway et al. 2002; Leonard and Morris 2002, 2006; Saudubray et al. 2006). In most of these conditions, the clinical status gives initially rise to a suspicion of sepsis. Any secondary impairment of UC function will lead to hyperammonemia and mimic UCDs (Häberle 2013).

Examples are:

- Inborn errors of metabolism
  - Organic acidurias (MMA, PA, HMG CoA lyase deficiency, IVA)
  - Fatty acid oxidation defects
  - Carbonic anhydrase Va deficiency (Diez-Fernandez et al. 2016; van Karnebeek et al. 2014)
  - Pyruvate carboxylase deficiency
  - Citrin deficiency (citrullinemia type 2)
  - HIHA syndrome (rare in neonates)
  - Lysinuric protein intolerance
  - OAT deficiency (only in neonates)
  - mitochondrial OXPHOS defects
  - phosphoenolpyruvate carboxykinase (PEPCK) deficiency (Santra et al. 2016)
- Liver shunting, transient hyperammonemia of the newborn (see below)
- Liver failure of any cause (drugs, infections)
- Drugs interfering with the UC

◄ back to short version

Outcomes: reliable and early laboratory identification of patients and improvement of survival

Recommendation #4: As the most common misdiagnosis of early onset UCD patients is neonatal sepsis, we strongly recommend to consider the possibility of a UCD in the differential diagnosis.
Quality of evidence: moderate

Transient hyperammonemia of the newborn

The term THAN refers to a condition described in premature infants (and sometimes also in term newborns) and is often associated with severe systemic disease or respiratory distress syndrome. The cause of THAN is not clear but there is speculation that it may be due to shunting of blood via the open ductus venosus with the high portal vein levels of ammonia (up to 300 µM) thus escaping clearance in the hepatic UC (Ballard et al. 1978; Hudak et al. 1985; Tuchman and Georgieff 1992). In this condition ammonia may be greatly increased, whilst glutamine is usually in the reference range resulting in a plasma ratio of glutamine/ammonia < 1.6 (Bachmann 2003a). Therapy of asymptomatic patients might not be necessary; in symptomatic patients, extracorporeal detoxification and drug therapy has been described but the benefit from this is not clear (Hudak et al. 1985; Tuchman and Georgieff 1992).

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2.2 Conditions and genetic disorders which can present with late-onset hyperammonemia

Any condition associated with greatly increased ammonia production and/or with impaired ammonia detoxification can cause hyperammonemia in an individual without a genetic defect in the UC.

Conditions with increased ammonia production or with impaired ammonia detoxification:

- Increased catabolism
- Infection, in particular intestinal overgrowth, genito-urinary tract infections with urease or arginine deiminase expressing bacteria (e.g. Proteus mirabilis, Klebsiella species, Mycoplasma hominis) (Kenzaka et al. 2015), uretero-sigmoid anastomosis

- Chemotherapy (particularly asparaginase or pegaspargase), bone marrow transplantation, multiple myeloma
- Steroid therapy
- Protein load by TPN, severe exercise, starvation, gastrointestinal bleeding
- Seizures, trauma, hemorrhage
- HIHA syndrome
- Distal renal tubular acidosis (Hsu et al. 2015)
- Transurethral prostate resection syndrome (caused by glycine solution used during the procedure)

Conditions with impaired ammonia detoxification:

- Other inborn errors of metabolism (see list in 2.1)
- Acute liver failure (e.g. in infections, ethanol combined with paracetamol)
- Exogenous intoxication (e.g. amanita phalloides, drugs)
- Chronic liver failure
- Porto-systemic shunting (surgery or vascular malformations, or open ductus venosus Arantii)
- Drugs (interfering with the UC; e.g. valproic acid, carbamazepine)
- “Reye syndrome”

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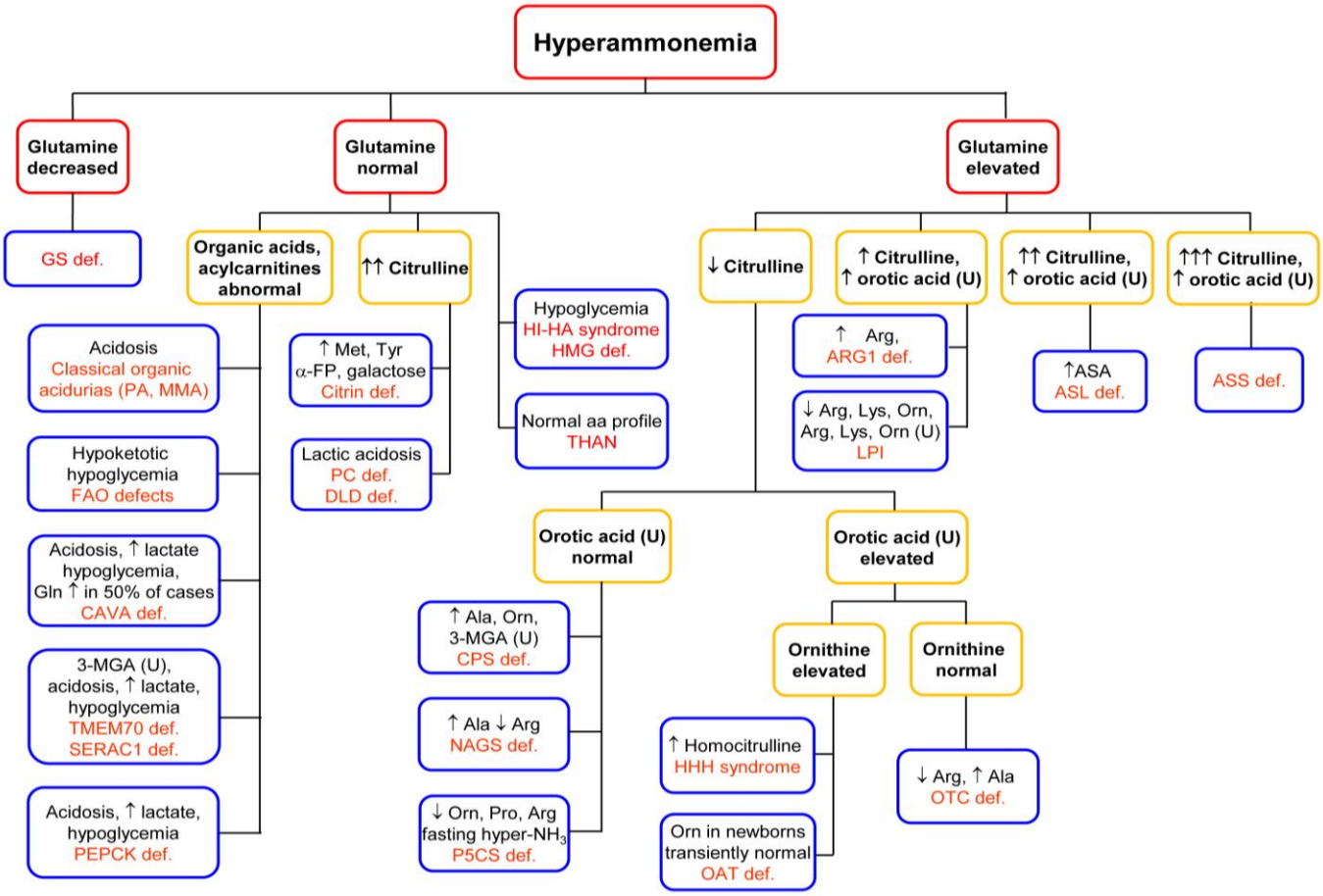
3. BIOCHEMICAL AND ENZYMATIC ANALYSIS

Only half of the UCDs have a specific biochemical pattern. The presence of plasma or urine argininosuccinate is diagnostic for ASLD (unless arginine is strongly elevated, indicating ARG1 deficiency). Likewise, presence of high plasma citrulline is suggestive for ASSD and high urinary orotic acid with low citrulline and low arginine for OTCD.

3.1. Algorithm for further investigations of hyperammonemia

In general, the pattern of metabolites is more important than absolute figures. The following algorithm might guide the most concise way to diagnosis.

Figure 1: Diagnostic algorithm for neonatal hyperammonemia



17.7

Investigations in plasma if not stated otherwise; U: ***urine***

3.2 Enzyme analysis

Enzyme analysis can be used for the confirmation of all UCDs but is not considered the method of choice if genetic testing is available (Gautschi et al. 2014; Kido et al. 2012). An exception to this is ARG1D in which enzyme analysis in red blood cells is simple and reliable (Tomlinson and Westall 1964).

Details on methods and sample requirements are listed in Table 3. Liver tissue should be shock frozen and kept at -80°C until analysis.

The determination of the respective enzyme activity can confirm all UCDs (Brown and Cohen 1959; Nuzum CT and Snodgrass PJ 1976) including NAGSD (Colombo et al. 1982; Tuchman and Holzknecht 1990) and allows confirmation of the diagnosis. Some kinetic enzyme variants exist that are not detected in common assays at substrate saturation and in the case of OTCD small biopsies may give normal results both in symptomatic and asymptomatic female carriers because of the possible mosaic expression in the liver (Tuchman 1992). The request for enzyme assays, especially in those disorders where liver or intestinal biopsy has to be performed (CPS1, OTC, NAGS), has dwindled progressively as molecular genetic diagnosis has become available for every disorder of the UC.

When evaluating enzyme activity results it has to be remembered that low protein diets decrease the activity of UCD enzymes (Aebi H 1976) which can lead to false interpretation of the low values. Some enzymes, especially CPS1, are less stable than other UCD enzymes, so it is important to get information on the sample history; specifically, ask for length and degree of protein restriction and whether the biopsy was taken from a vital liver or post mortem. In the latter case, time span until sampling after death needs to be documented (Nuzum CT and Snodgrass PJ 1976).

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Table 3: Overview on methods and required samples for enzyme analysis of UCDs

Disorder	Method	Sample
NAGSD	Stable isotope dilution assay with GCMS	**Liver**[#]
CPS1D	Colorimetric OTC-coupled assay	**Liver**[#]**,** small intestine
OTCD	Colorimetric assay[+]	**Liver**[*#], small intestine[*]
ASSD	Radiometric assay (fibroblasts) ^{14}C-citrulline incorporation (fibroblasts) Colorimetric ASL-arginase-coupled assay (liver)	**Skin fibroblasts**, kidney, liver[#]
ASLD	Colorimetric, arginase-coupled assay (erythrocytes, liver, kidney) ^{14}C-citrulline incorporation (fibroblasts)	**Skin fibroblasts**, Red blood cells[§], liver[#], kidney
ARG1D	Colorimetric assay	**Red blood cells**, liver[#]
HHH syndrome	^{14}C-ornithine incorporation	**Skin fibroblasts**, liver[#]

bold: first choice if analysis in more than one tissue is possible
[+] treatment with carbamylglutamate interferes with some colorimetric assays of citrulline
[#] needle biopsy (>10mg) sufficient either for NAGS assay or for assay of the other five UC enzymes; liver tissue should be snap frozen and kept at -80°C until analysis
[*] reliable in males, but less so in females due to X-mosaicism in all tissues
[§] caution: conflicting results

The **Pro and Contra considerations** regarding enzyme analysis for UCDs are summarized here:

Pro	Contra
- to distinguish between deficiencies of NAGS and CPS1 - if the genetic diagnosis fails - for genotype-phenotype correlation studies - in retrospective studies in necropsy tissue - in experimental settings (gene therapy, hepatocyte infusions) - to ascertain the absence of a UCD in donor livers	- kinetic enzyme variants exist that are not detected in common assays at substrate saturation - in OTCD, small biopsies may give normal results in female carriers and patients because of the possible mosaic expression in the liver - low protein diets decrease the activity of UCD enzymes which can lead to false low values - liver or intestinal biopsy has to be performed (NAGS, CPS1, OTC)

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There are few laboratories in Europe offering enzyme analyses for UCDs. To find a laboratory and for detailed information regarding sample preservation and transport conditions, contact the laboratory or the National Metabolic Society (see list of addresses) or check with Orphanet (http://www.orpha.net) at an early stage.

3.3. Role of liver biopsy or of other tissue samples during work-up for a suspected UCD

Liver biopsy and subsequent assays of enzyme activities can establish the diagnosis in all UCDs from a single needle biopsy specimen of liver tissue (Nuzum CT and Snodgrass PJ 1976) including NAGSD (Colombo et al. 1982). Fibroblasts can be utilized for indirect assays of ASS and ASL activities (Kleijer et al. 2002; Kleijer et al. 1984) and for the diagnosis of the HHH syndrome (Shih et al. 1982). Red blood cells are the preferred source for assaying ARG1 (Tomlinson and Westall 1964). The intestinal mucosa can be used for assaying CPS1 and OTC. Most of these enzymes can be quantified prenatally, either in fetal liver biopsies (CPS1, OTC), amniotic cells or chorionic villus cells (ASS, ASL, HHH) and umbilical cord fetal erythrocytes (ARG1). However, liver and intestinal biopsies are invasive procedures, the results of activity assays are more variable than mutation identification and prenatal mutation tracking is simpler than enzyme activity assays in fetal tissues. Therefore, enzyme assays, particularly in liver biopsies, are recommended only if genetic testing fails or if urgent need for confirmation of the diagnosis cannot await the result of genetic testing.
In patients who died without confirmed diagnosis, preservation of a skin biopsy for fibroblast cultures and of liver tissue (immediate deep freezing required) immediately post-mortem for later enzyme studies should be considered. It should be noted that low activities are also found when in the final days the protein supply was insufficient.

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4. GENETIC ANALYSIS

4.1. Role of molecular genetic analysis for diagnosis of UCDs

Genetic analysis is the preferred method to establish the diagnosis in disorders in which metabolite profiles are not diagnostic and enzymatic testing is invasive. Genetic testing is further required for genetic counselling and offers the opportunity for prenatal testing. In addition, it can be used for family genotyping.
DNA from peripheral blood cells is usually used but also many other tissues can be source of DNA. In deceased patients or if no other material is available or to test relatives of identified patients, mutation analysis may also be done on DNA from dried blood spots. There are few special situations when RNA must be investigated and these are explained in 11.2 for CPS1D and in 12.2 for OTCD. . Mutation analysis should include investigations of known regulatory domains (Heibel et al. 2011).
Apart from these practical and clinical applications, mutation analysis has provided some first insight into genotype-phenotype correlations (Ah Mew and Caldovic 2011; Gao et al. 2003; Häberle et al. 2003a; Sancho-Vaello et al. 2016; Trevisson et al. 2007).

Outcomes: reliable and early laboratory identification of patients and prognostic measures

Recommendation #5: We strongly recommend genetic testing. This will confirm the diagnosis, allow for genetic counselling and in some instances provide information on the disease course. We strongly recommend to preserve DNA, fibroblasts and/or frozen liver tissue in deceased patients with a suspicion of UCD.
Quality of evidence: moderate

Next-generation sequencing is emerging also in the field of UCDs but has not yet entirely replaced Sanger sequencing (Amstutz et al. 2011).
In the future, genetic analysis might become a prerequisite for planning of therapies, i.e. the use of chaperones or nonsense-read-through drugs. Also, disease associated genotypes or haplotypes might help to improve the understanding of the phenotypic variation in UCDs. See also chapter on future therapies (chapter 17).

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Pitfalls and limitations
Mutation analysis does not always provide a definite diagnosis. Information in detection rates are lacking for most UCDs but this problem is most relevant in OTCD, where mutation analysis by sequencing of coding exons including flanking intronic regions detects a disease-relevant mutation only in about 80% of patients (Yamaguchi et al. 2006). To improve genetic diagnostics, additional methods such as array-comparative genomic hybridization (CGH), RNA-based sequencing or MLPA need to be applied (Cohen et al. 2012; Engel et al. 2008; Shchelochkov et al. 2009; Wang et al. 2011b).
In RNA based diagnostics of ASLD, a high frequency of splice variants has been demonstrated, both in patients and controls (Linnebank et al. 2000).

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For single UCDs, different approaches are required; details can be found in the recommendations in Part II of this guideline.

4.2. Prognostic value of mutation analysis

With current knowledge, the genotype may allow a general statement about the severity of the course of an individual. A null allele in a male OTC patient or null mutations on both alleles in CPS1D predict a severe clinical course with a high risk of recurrent hyperammonemic crises.
Some mutations in the *OTC*, *ASS*, and *ASL* genes are known to be associated with variant clinical courses (Gao et al. 2003; Häberle et al. 2003a; Kleijer et al. 2002; Numata et al. 2008; Trevisson et al. 2007; Yamaguchi et al. 2006). However they do not exclude an unfavourable outcome if the patient is exposed to crises due to exogeneous causes, especially during the first 3 years of life or at puberty.
Future studies are needed to substantially improve the prognostic value of mutation data.

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4.3. Role of structure analysis and assessment of pathogenicity of mutations

The 3-D structures of human OTC, ASS, ASL, arginase and of CPS1 have been determined (see Protein Databank, PDB, http://www.pdb.org/pdb/home/home.do). Structures of bacterial Nags (see also the PDB) may serve as approximative models for the human proteins. The structure of the product of the *SLC25A15* gene (the gene that is mutated in the HHH syndrome) has been modelled on the basis of the experimentally determined structure of another transporter protein. Therefore, for all UC enzymes the effects of mutations can be evaluated on the basis of the corresponding protein structures, as it has already been done in a number of reports (Ash et al. 1998; Berning et al. 2008; Pekkala et al. 2010; Shi et al. 1998; Tessa et al. 2009; Trevisson et al. 2007; Yefimenko et al. 2005). Exploitation of structural information can help predict the functional impact of at least some mutations.

In vitro expression studies of the human UCD enzymes or their bacterial or yeast homologous proteins provide a direct approach for testing the effects of clinical mutations, as has been exemplified with several UCDs (Berning et al. 2008; Doimo et al. 2012; Engel et al. 2012; Pekkala et al. 2010; Tessa et al. 2009; Yefimenko et al. 2005). However, since theses analyses are currently not available for most of the patients' mutations they can not be recommended as standard procedures.

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5. PRENATAL TESTING

5.1. Role of prenatal testing in UCDs

Prenatal testing requires the confirmed diagnosis in an index patient. In following pregnancies, there can be a need for early, fast and safe prenatal testing since most UCDs are considered severe conditions. The diagnosis of an affected fetus allows parents to seek counselling and decide on the further course of the pregnancy in most European countries.
Besides, prenatal testing might be indicated for psychological reasons or to prepare for immediate peri- and postnatal management (Leonard et al. 2008; Maestri et al. 1991; Sniderman King et al. 2005).
Any prenatal testing should only take place after genetic counselling taking into consideration the individual religious, personal and cultural background. Since counselling always addresses the specific aspects of the respective disorder, there is a need for the collaboration of geneticists and metabolic specialists. Genetic counselling is especially challenging in female OTC carriers since the course of disease can not be precisely predicted.

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Outcome: reliable and early laboratory identification of patients

Recommendation #6: We strongly recommend molecular genetic analysis as the preferred prenatal testing method for all UCDs.
Quality of evidence: high

5.2. Methods and samples for prenatal testing in UCDs

First reports on prenatal testing in UCD date back to the 1970s and both metabolite studies as well as enzymatic investigations were performed in single cases and case series (Fleisher et al. 1979; Jacoby et al. 1981; Kleijer et al. 1984; Spector et al. 1980). It was confirmed that many UCD enzymes' activities were measurable already at early fetal stages (Colombo and Richterich 1968; Oyanagi et al. 1980; Raiha and Suihkonen 1968). Tests were done in AFC or CVS which are both accessible with less risk to the fetus than a fetal liver biopsy or tissue collection by fetoscopy. A small number of publications report on both the general feasibility of enzymatic prenatal testing in all UCDs except NAGSD and a variety of possible pitfalls and technical difficulties (Fensom et al. 1980; Jacoby et al. 1981; Kamoun et al. 1995; Oyanagi et al. 1980; Vimal et al. 1984; Yoshino et al. 1997). In the past decades, genetic testing of all UCDs also in the prenatal setting has been established and used routinely (Häberle and Koch 2004).

If the mutation in the index-patient is not known, or if only one disease allele could be identified, prenatal testing can be done using a late fetal liver biopsy or informative haplotypes (including parental haplotypes). However,

haplotype-based diagnostic can only be considered safe if the diagnosis in the index-patient is out of question (Sniderman King et al. 2005).

Metabolite studies can be done in ASSD and ASLD using amniotic fluid amino acid analysis (Mandell et al. 1996). Excellent sensitivity and specificity have been reported in ASSD but it is important to use amniotic fluid from carrier mothers when setting up a normal range (Chadefaux-Vekemans et al. 2002; Miller et al. 2014). Combining metabolite assay and enzyme function allows ruling out false negative results of each method. For example, an increased ASA in amniotic fluid confirms that low or absent enzyme activity in AFC or CVS is not due to preanalytical factors and thus rules out false positive results.

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Table 4: Recommended analyses and sample requirements

Disorder	Tests recommended
NAGSD	Mutation analysis using DNA from CVS or AFC*
CPS1D	**Mutation analysis using DNA from CVS or** AFC Enzyme analysis, late fetal liver biopsy§
OTCD	**Mutation analysis using DNA from CVS or** AFC+ Enzyme analysis, late fetal liver biopsy#,§
ASSD	**Mutation analysis using DNA from CVS or AFC** **Citrulline in amniotic** fluid (calculation of ratios) Enzyme analysis, intact or cultured CVS or cultured AFC
ASLD	**Mutation analysis using DNA from CVS or AFC** **Argininosuccinate and its anhydrides in amniotic** fluid Enzyme analysis, intact or cultured CVS or cultured AFC
ARG1D	**Mutation analysis using DNA from CVS** **Enzyme assay in fetal blood erythrocytes (mid-gestation sampling)**
HHH syndrome	**Mutation analysis using DNA from CVS or AFC** Functional assay in CVS or cultured AFC

bold: first choice
* in case of a request for prenatal testing one should keep in mind that NAGSD is a treatable disorder
§ described in single patients but not widely available and very limited experience
+ In the female fetus the genotype is only able to exclude OTC mutations. Because of the lyonisation it has no predictive value for the resulting phenotype if affected.
# feasible in male, but interpretation not clear in females due to X-mosaicism

6. NEWBORN SCREENING

The current knowledge on NBS for UCDs relies on a small number of publications (Posset et al. 2016; Wilcken et al. 2009).
If NBS for UCDs is implemented, follow-up should be done in specialised metabolic units to avoid delayed or inappropriate treatment.

Current practice of newborn screening for UCDs

There are screening programmes for the cytosolic enzyme deficiencies (ASSD, ASLD, and ARG1D) in some countries, including most US states, Taiwan and Australia. However, the benefit of NBS for UCDs is still controversial and subject to evaluation. In Austria, for instance, screening for ASLD was abandoned in 2000 because of a high rate of positive newborns who remained asymptomatic and who were considered to be mild variants.

Approximately 50% of all patients affected by UCDs show severe symptoms during the first days of life often even before the samples for newborn screening are taken. These patients would most probably not benefit, even if the diagnosis is suspected or confirmed early, because the outcome is extremely poor in this patient group (Bachmann 2003b; Krivitzky et al. 2009; Wilcken et al. 2009).

Accurate detection of glutamine and ornithine by NBS based on tandem mass spectrometry poses major problems because of their instability in blood spots (glutamine) or interference by production of ornithine by red cell arginase while blood dries on the card.

In HHH syndrome, patients with neonatal onset can have normal levels of ornithine and may be missed by NBS (Sokoro et al. 2010). In certain populations with an incidence as high as one affected child for approximately every 1,500 individuals, screening for founder mutations may be feasible (Sokoro et al. 2010).

Besides technical issues, a major concern is the delay in time between sampling and obtaining the result (transport to centre, weekends without analysis) and due to time lost until the first therapeutic measures are started. Thus, patients with an early manifestation (e.g. day 1 or 2) would not benefit from NBS.

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6.1. Newborn screening for mitochondrial UCDs

There is no routine screening for NAGSD, CPS1D and OTCD. Screening for low citrulline levels alone has a low specificity and probably a low sensitivity at least for late onset OTCD (Cavicchi et al. 2009). Orotic acid can be measured in dried blood spots (D'Apolito et al. 2012; Held et al. 2014; Janzen et al. 2014) but there are no data from larger screening cohorts available.

At the Mayo Clinic, Rochester MN, a pilot study is being conducted collecting NBS data or blood spots of proven OTC/CPS1/NAGS patients trying to find an algorithm starting from low citrulline as primary marker followed by 'a second tier calculation' using glutamine (which is determined as one peak together with glutamate and pyroglutamate) and arginine. The final results of this collaborative project might change this recommendation (Rinaldo P., personal communication).

A major concern is the identification of females with low citrulline levels in whom exclusion or confirmation of OTCD and decision on the need for treatment could be extremely difficult. In addition, there are other conditions resulting in hypocitrullinemia, such as NARP mutations, deficiency of pyrroline-5-carboxylate synthase, and Pearson syndrome (Baumgartner et al. 2005; Rabier et al. 1998).

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6.2. Newborn screening for cytosolic UCDs

ASSD and ASLD can be detected by elevated citrulline and ASA concentrations in DBS, respectively, with a low false positive rate. There is no information on the false negative rate but it is considered to be zero for the severe deficiency states (Naylor and Chace 1999; Rashed 2001; Sander et al. 2003). A substantial number of mild phenotypes (ASSD and ASLD) may be detected and it is not known how many of them are at risk of decompensation later in life (Barends et al. 2014; Rüegger et al. 2014). ARG1D may be detected by elevated arginine (Jain-Ghai et al. 2011) but the sensitivity and specificity of arginine as a primary NBS parameter is unknown. NBS may have the largest impact on late-onset patients in whom a trend towards an improved outcome was found (Posset et al. 2016).

As mentioned above many of the affected children will be symptomatic before NBS blood sampling is performed. Although patients with an early clinical presentation will not benefit from NBS those patients with a later clinical presentation might well do so.
In ASSD, newborns with mild variants but no need for dietary therapy are known to be detected and overtreatment must be avoided. Nevertheless, in single patients with so called "mild" citrullinemia fatal decompensations have been reported. Some of these patients may benefit from emergency measures during catabolic situations (Berning et al. 2008; Gao et al. 2003).
The sensitivity and specificity of elevated arginine as primary target in NBS for ARG1D is not known. Arginine levels may be within the normal range in the first days of life.

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Outcomes: reliable and early laboratory identification of patients and improvement of survival

Recommendation #7: As patients with UCDs may benefit from early diagnosis, reliable newborn screening is desirable. We recommend to consider newborn screening for ASSD and ASLD. At present, there is not enough data to base a recommendation on newborn screening programs for NAGSD, CPS1D, OTCD, ARG1D and HHH syndrome.
Quality of evidence: moderate

7. MANAGEMENT OF ACUTE HYPERAMMONEMIA

Rationale of diet and ammonia detoxifying drugs in the treatment of UCDs

In humans, dietary protein consumption generally exceeds the basic requirement for growth and maintenance of health (Fulgoni 2008; WHO Technical Report Series 2007). Protein that is in excess of physiological requirement is catabolised and an important by-product of this process is ammonia, which is highly toxic. The main physiological function of the UC is to convert ammonia into a relatively non-toxic and easily excretable compound, urea. Urea production and excretion are linearly related to the intake of dietary protein (Young et al. 2000). Under normal circumstances, the UC has enormous reserve capacity, enabling humans to tolerate very large protein loads; studies have shown that normal adults can tolerate a protein intake as high as 10 g/kg/day without suffering from hyperammonemia (Rudman et al. 1973).

In the presence of a UCD, urea synthesis is severely reduced. The clinical severity of a given UCD correlates with the degree of reduction in endogenous urea synthesis. An increased dietary protein intake (above minimum requirements) in these patients overwhelms the capacity of the UC and results in the accumulation of waste nitrogen as ammonia, glutamine and alanine. Conversely, a reduction in excess dietary protein intake reduces the load on the UC and promotes metabolic stability and this forms the basis for dietary management of the UCDs. Theoretically, a decrease in protein intake of 0.1 g/kg/day results in a reduction in waste nitrogen of 16 mg/kg/day.

Alternative pathway therapy has become the mainstay of drug treatment of the UCDs since the 1980s (Batshaw et al. 2001). The drugs sodium benzoate and sodium PBA undergo enzyme mediated conjugation in the liver with the amino acids glycine and glutamine respectively, to form the non-toxic and easily excretable compounds hippurate and phenylacetylglutamine. In patients with UCDs, the resultant removal of glycine and glutamine from the amino acid pool has the net effect of providing an alternative means of ammonia disposal (hence the name “alternative pathway”) and of decreasing the load on the UC.

The rationale for using L-arginine in citrullinemia and argininosuccinic aciduria is to increase the production of citrulline and argininosuccinic acid; both compounds are excretable in the urine and provide alternative means of ammonia removal in these conditions (Batshaw et al. 2001). For the proximal UCDs, L-arginine or L-citrulline help increase the flux through the UC and help increase urea synthesis (Lee et al. 2000); the replacement of these amino acids is also needed because their formation is hampered by the enzyme defect (resulting in L-arginine becoming an essential amino acid) and thus the UC is not sufficiently primed.

Nitrogen scavengers used for treatment of acute neonatal decompensations (Bachmann 2003c; Batshaw et al. 2001) and of episodes of acute hyperammonemia outside the neonatal period (Enns et al. 2007) have been shown to improve mortality. However, this has to some extent been at the cost of quality of life of surviving patients (Bachmann 2003c).

It has been shown that prospective treatment including diet improves neonatal metabolic control, neurological prognosis and survival in comparison to previous familial cases (Maestri et al. 1991).

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7.1. Initial management of acute hyperammonemia

Before treatment of acute hyperammonemia, the prognosis regarding neurodevelopmental outcome needs to be considered and may influence the decision whether to continue specific treatment or to start palliative care.
The prognosis is considered very poor in patients with any of the following:

1. coma > 3 days
2. significantly elevated intracranial pressure
3. initial or maximal ammonia > 1000 µmol/L (Bachmann 2003c; Picca et al. 2001)

The latter criterion is weaker since not only peak ammonia but also duration of hyperammonemia is important. Single patients with a normal outcome despite initial ammonia > 1000 µmol/L have been reported (De Bie et al. 2011). Peak ammonia levels > 360 µmol/L were markers of poor prognosis (Kido et al. 2012; Nakamura et al. 2014). The decision for palliative care should be made together with metabolic specialists.

Specific therapy in acute symptomatic hyperammonemia must be initiated without delay. It is advised that every pediatric hospital holds the first line medication available and provides a written instruction based on this guideline to avoid time consuming discussions on details of therapy. Diagnosis of the specific defect and the initial medical treatment must proceed simultaneously.

1. All patients with a hyperammonemic crisis should be transferred to a specialist centre without delay after:
2. stopping protein intake

3. start of i.v. glucose
4. initiation of first line medications as outlined in Table 5
5. collection of plasma and urine for diagnostic purposes without postponing initiation of treatment
6.

7. Table 5 Levels of hyperammonemia and suggested actions in case of symptomatic patients

Ammonia level (µmol/L)	Action in undiagnosed patient	Action in known UCD patient	Comments
Increased > upper limit of normal	▪ Stop protein intake ▪ Give IV glucose at an appropriate dosage to prevent catabolism (10 mg/kg/min in a neonate) ± insulin$ ▪ Monitor ammonia blood levels every 3 hours	▪ Stop protein intake ▪ Give IV glucose at an appropriate dosage to prevent catabolism (10 mg/kg/min in a neonate) ± insulin$ ▪ Monitor ammonia blood levels every 3 hours	▪ Stop protein for max. 24 h ▪ Avoid exchange transfusions as cause of catabolism ▪ Hyperglycemia can be extremely dangerous (hyperosmolarity) ▪ If major hyperglycemia occurs with high lactate (>3 mmol/L) reduce glucose infusion rate rather than increase insulin ▪ Avoid hypotonic solutions ▪ Add sodium and potassium according to the electrolyte results ▪ Take into account the sodium intake if sodium benzoate or sodium PBA are used§ ▪ L-arginine not to be given in ARG1D ▪ Some concerns of sodium benzoate use in OAs ▪ Avoid repetitive drug boluses ▪ Monitor phosphate levels and supplement early especially during hemodialysis
In addition when >100 and <250 #	▪ Start drug treatment with IV L-arginine and sodium benzoate (see Table 6) ▪ Start carbamylglutamate, carnitine, vitamin B12, biotin (see Table 6 and its legend)	▪ Continue drug treatment with L-arginine (plus continue or add L-citrulline for mitochondrial UCDs) and sodium benzoate ± sodium PBA/phenylacetate* (see Table 6), increase dose or give IV ▪ Consider carbohydrate and lipid emulsions per NG tube unless the child is vomiting (enables higher energy intake)	
In addition when 250 to 500	▪ As above ▪ Prepare hemo(dia)filtration if significant encephalopathy and/or early high blood ammonia level or very early onset of disease (day 1 or 2) ▪ Begin hemo(dia)filtration if no rapid drop of ammonia within 3-6 hours	▪ As above, but all drugs per IV ▪ Prepare hemo(dia)filtration if significant encephalopathy and/or early high blood ammonia level or very early onset of disease (day 1 or 2) ▪ Begin hemo(dia)filtration if no rapid drop of ammonia within 3-6 hours	
In addition when 500 to 1000	▪ As above ▪ Start hemo(dia)filtration immediately	▪ As above ▪ Start hemo(dia)filtration as fast as possible	
In addition when >1000	▪ Evaluate whether to continue specific treatment or to start palliative care	▪ Evaluate whether to aim at curative treatment or palliative care	

*If available, an IV equimolar solution of sodium benzoate and sodium phenylacetate can be used: 250 mg/kg as bolus IV/90-120 min, then 250 mg/kg as continuous IV infusion over 24h. The combination of sodium benzoate and sodium phenylacetate is available as a drug, registered by the FDA (available in the EU on Named Patient Basis) and indicated as adjunctive therapy for the treatment of acute hyperammonemia and associated encephalopathy in patients with deficiencies in enzymes of the urea cycle.

This limit of action applies for patients outside the neonatal period; for neonates use >150 and <250.

$Monitor blood glucose after 30 min and subsequently every hour, because some neonates are very sensitive to insulin.

§1g sodium benzoate and sodium PBA contain 7 mmol Na and 5.4 mmol Na, respectively.

Nota **bene:** A recent systematic review of clinical and biochemical data from published neonatal onset UCD patients found with the current practice of dialysis no impact on outcome. Authors concluded that “it may be essential for improving outcome to initiate all available treatment options, including dialysis, as early as possible” (Hediger et al. 2018). This paper was not included in the review during the revision of this guideline.

Outcome: improvement of survival

Recommendation #8: We strongly recommend immediate start of measures to reverse endogenous protein catabolism and to promote ammonia detoxification (as detailed in Table 5).
Quality of evidence: moderate

Clinical prognostic indicators
The most relevant indicators for prognosis seem to be total coma duration (Msall et al. 1984; Picca et al. 2001) and peak ammonia levels (Enns et al. 2007; Schaefer et al. 1999; Uchino et al. 1998). Coma duration longer than 30 hours before dialysis adversely affects the outcome (Picca et al. 2001). The importance of the speed of detoxification on short-term outcome is not well understood (Schaefer et al. 1999) and the outcome is probably influenced by other factors. As an indication for improved treatment, survival rates of patients have in some studies improved over the last decades likely due to improved awareness and increased use of liver transplantation (Kido et al. 2012); however, a large meta-analysis including 24 reports did not find improvement of survival during the last three decades (Burgard et al. 2016).
Although levels of peak ammonia were significantly higher in proximal than in distal UCDs, neurocognitive outcome did not differ in the 2 groups (Ah Mew et al. 2013).

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7.2. Drugs and dosages to be used in acute decompensations of UCDs

If sodium benzoate, sodium phenylacetate or sodium PBA are given as a bolus, nausea and vomiting are common and, thus, ondansetron (dose 0.15 mg/kg) may be applied in parallel to avoid hyperemesis (Batshaw et al. 2001; MacArthur et al. 2004).

If high doses of benzoate or phenylacetate are used, or if repeated boluses are given, the capacity for conversion into hippurate or phenylacetylglutamine may be exceeded resulting in benzoate or phenylacetate accumulation and toxicity (MacArthur et al. 2004; Praphanphoj et al. 2000).

Regarding dosing in acute episodes, there is no evidence based recommendation available; the following Table is a consensus of the working group of this guideline taking into account the literature (Ahrens et al. 2001; Batshaw et al. 2001; Brusilow and Maestri 1996; Enns et al. 2007; Feillet and Leonard 1998; Leonard and Morris 2002; MacArthur et al. 2004; Summar 2001).

Table 6: Dosages of drugs to be used in acute hyperammonemia and acute decompensations of UCDs

Disorder	Sodium benzoate (to be given IV in glucose 10%)	Sodium PBA/Sodium phenylacetate (to be given IV in glucose 10%)	L-arginine hydrochloride (to be given IV in glucose 10%)	N-carbamylglutamate (only available as oral/enteral drug)
Undiagnosed patient°	250 mg/kg as bolus in 90-120 min, then maintenance 250-500 mg/kg/d[$] > 20 kg bw: 5.5 g/m^2/d	250 mg/kg as bolus in 90-120 min, then maintenance: 250-500mg/kg/d[$]	250(-400) mg/kg (1-2 mmol/kg) as bolus in 90-120 min, then maintenance 250 mg/kg/d (1.2 mmol/kg/d)	100 mg/kg bolus per NG tube then 25-62.5 mg/kg every 6h
NAGSD	same[$]	same[$]	250 mg/kg (1.2 mmol/kg) as bolus in 90-120 min, then maintenance 250 mg/kg/d (1.2 mmol/kg/d)	same
CPS1D & OTCD	same[$]	same[$]	same	-
ASSD	same[$]	same[$]	same	-
ASLD[‡]	same[$]	same[$]	200-400 mg/kg (1-2 mmol/kg) as bolus in 90-120 min, then maintenance 200-400 mg/kg/d (1-2 mmol/kg/d)	-
ARG1D*	same[$]	-	**AVOID**	-
HHH syndrome	same[$]	same[$]	250 mg/kg (1.2 mmol/kg) as bolus in 90-120 min, then maintenance 250 mg/kg/d (1.2 mmol/kg/d)	-

°In undiagnosed patients, use of a combination of the drugs in table 6 seems justified, consider additional use of carnitine 100 mg/kg IV, hydroxycobalamin 1 mg IM/IV, and biotin 10 mg IV/PO
*The risk for acute hyperammonemic decompensation is low in ARG1D
[§]If citrulline is given, there is usually no need for concomitant use of L-arginine
[$]If on hemodialysis/hemodiafiltration doses should be increased to 350 mg/kg/d (maintenance dose)
[‡]In ASLD, L-arginine therapy for acute decompensations might be sufficient for some patients
Maximal daily drug dosages: sodium benzoate 12 g/d, sodium PBA 12 g/d, L-arginine 12 g/day

Cave: The doses indicated in Table 6 can be used at the start of treatment but must be adapted depending on plasma ammonia and amino acids.
Sodium benzoate and sodium PBA/phenylacetate should be given in parallel in severe acute decompensation. In less severe cases, a step-wise approach with initial sodium benzoate and if hyperammonemia persists or worsens, the addition of sodium PBA/phenylacetate can be chosen.

7.3. Management of a neonate at risk of a UCD at birth

This section has been adapted from the 'BIMDG Management Protocol of a baby at risk of a urea cycle disorder' (http://www.bimdg.org.uk):

Management during pregnancy following a previous index-patient

A careful history is essential and should be reviewed by a specialist. In particular:

- When did the previous child become ill, in the neonatal period or later?
- Is the likely diagnosis known?
- Is the father identical to the one in the previous pregnancy? (in recessive disease)

If a previous sibling became ill shortly after birth:

- Consider prenatal testing even at a late stage if not done yet.
- Consider transferring the mother before birth to a centre with all facilities for managing an affected baby.
- Consider delivering the baby by caesarian section as this minimizes the metabolic stress of birth and the timing of the delivery is known.
- Consider giving sodium benzoate to the mother from 38 weeks gestation to make the management easier; however, this is experimental and only reported in a single paper (Das et al. 2009).
- Consider applying phenobarbitone for a few days before delivery in order to induce the fetal conjugating enzymes already in utero (Leonard et al. 2008). Albeit experimental, this could be helpful to ensure maximal efficacy of benzoate and PBA after birth and also to avoid toxicity.

In the third trimester, obtain supplies of medicines used in UCDs. Plan a protein free feeding regimen, for temporary use. Ideally, a protein free infant formula with added vitamin and minerals should be used. If mother wishes to breast feed, have appropriate support for her to express breast milk until breast feeding can be commenced.

Inform the clinical biochemistry laboratory about the impending birth, as it is essential that results are available quickly.

The management will depend on the course in the index patient

- **If the previous sibling became ill during the neonatal period: Go to section A**
- **If the previous sibling became ill after the neonatal period: Go to section B**

Important note: Whilst females with OTCD usually have mild disease some with unfavorable lyonisation will have severe disease. As it is not possible to predict the phenotype, females with a severe mutation should be managed as directed in section A until the phenotype is obvious.

Section A: For neonates at risk of becoming ill in the newborn period

- After birth, rapid transfer of the baby to the neonatal unit.
- Start 10% glucose IV at 4 ml/kg bw/h (glucose 6.6 mg/kg/min) within 30 min of birth.
- If the baby is fine at 4 h offer a protein free feed (PO or by NG tube).
- Reduce glucose infusion if feeding is tolerated.
- Give sodium benzoate 50 mg/kg and L-arginine hydrochloride solution 50 mg/kg PO. Continue the same dose 6 hourly thereafter until the diagnosis is known or a change is advised.

- At 6 hours of age measure plasma ammonia:
  - Plasma ammonia < 80 µmol/L: monitor at 6 hourly intervals. Continue protein free feeds approximately 4 hourly. Stop the glucose infusion if ammonia remains <80 µmol/L at 24 h of age and introduce normal infant formula or breast feeding.
  - Plasma ammonia 80 – 150 µmol/L: repeat in 4 h and if it stays at this concentration, monitor at 6 hourly intervals. Continue protein free feeds and glucose infusion.
  - Plasma ammonia > 150 µmol/l or if the baby becomes unwell: repeat immediately, stop feeds and see Table 5 for actions to be taken.

- In addition to plasma ammonia, measure plasma amino acids (quantitatively) urgently at

approximately 12 h of age regardless of the plasma ammonia concentration. If the diagnosis is to be made by molecular genetics send blood sample. Cord blood should not be used because of the possibility of maternal contamination.

Section B. If the previous sibling became ill after the newborn period
- If the birth is complicated (birth asphyxia, etc.) start a glucose infusion as soon as possible
- Start normal milk feeds (breast milk or infant formula); however, these should not exceed 2 g of natural protein/kg bw/day (i.e. corresponding to a maximum of 450 ml of infant formula = 130 ml/kg, 1.7g protein/kg for a 3.5 kg baby)
- At 24 h of age measure plasma ammonia and amino acids (quantitatively)
  - Plasma ammonia < 60 µmol/L: repeat in 24 h.
  - Plasma ammonia 60 – 150 µmol/L: repeat in 12 h.
  - Plasma ammonia > 150 µmol/L or if the baby becomes unwell: repeat immediately and see Table 5 for actions to be taken.
- At any later stage after 24 hours of life:
  - Plasma ammonia < 80 µmol/L at 48 h: continue to offer milk feeds and observe.
  - Plasma ammonia is 80 – 150 µmol/L and the baby is well: repeat at 12 hourly intervals. Request results of plasma amino acids. Change feeds to protein-free formula.
  - Plasma ammonia is > 150 µmol/L or if the baby becomes unwell: repeat immediately and see Table 5 for actions to be taken.

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7.4. Criteria to start extracorporeal detoxification

In neonates and children

Effective medical treatment may avoid the need for hemo-dialysis/filtration but the dialysis team must be informed at the start of medical treatment. Within the first four hours, the response to the medical management should be evaluated. If the response is regarded inadequate, extracorporeal detoxification should be started.

This time window is usually needed to alert the dialysis team and prepare vascular access (Enns et al. 2007; Picca et al. 2008). Some authors suggested a blood ammonia level exceeding 400 µmol/L (Spinale et al. 2013) or of 500 µmol/L (Prietsch et al. 2002) as the limit to immediately start extracorporeal detoxification. However, there are serious concerns to define rigid thresholds because there is a major difference between a rapidly rising plasma ammonia concentration and one that slowly reached the same value.

Extracorporeal detoxification in neonates (but also in infants and children) requires collaboration with an experienced team.

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Realize that patients receiving efficient extracorporeal detoxification may still have a poor cognitive prognosis (Kido et al. 2012). Some authors therefore advocate that patients with a “peak ammonia level greater than 180 µmol/l at the onset should receive hemodialysis” (Kido et al. 2012).

Outcomes: improvement of survival and preservation of cognitive function and prevention of neurological disease by use of extracorporeal detoxification

Recommendation #9. Parallel to medical treatment, we strongly recommend to prepare extracorporeal detoxification in patients with severe neurological symptoms induced by hyperammonemia. Start extracorporeal detoxification as soon as possible, unless initial medical treatment has already led to sufficient improvement of ammonia levels and clinical situation.
Quality of evidence: moderate

Nota bene: A recent systematic review of clinical and biochemical data from published neonatal onset UCD patients found with the current practice of dialysis no impact on outcome. Authors concluded that “it may be essential for improving outcome to initiate all available treatment options, including dialysis, as early as possible” (Hediger et al. 2018). This paper was not included in the review during the revision of this guideline.

17.8 Management of acute hyperammonemia in adults

Adult patients, as children, should have an emergency guideline, immediate on-call access to telephone advice from an adult metabolic physician, and a pack of iv medication (benzoate, PAA/PBA, arginine) at home, which they bring to their nearest hospital in the event of illness or signs of encephalopathy. As such the majority arrive at hospital with no-mild-moderate encephalopathy, and prompt iv treatment and dealing with the underlying precipitant should stabilize the patient. Treatment in known patients is therefore instituted early and extracorporeal detoxification hopefully rarely required.
In adults, hemodialysis or continuous veno-venous hemofiltration is considered as a first line treatment in severe acute decompensations. Since it is readily available in most intensive care units and can be started quickly, it may be recommended even if the diagnosis is not yet certain. Moreover, the risk of such a procedure is much lower than in children and it should be started at the site of the first presentation before transfer to a specialised center.
Since intracranial hypertension and cerebral edema appear earlier in adults than in children, extracorporeal detoxification should be considered if ammonia concentration exceeds 200 µmol/L. The decision to start extracorporeal detoxification should not be based on the ammonia level alone but this threshold should be adjusted according to co-morbidities, availability of and tolerance to medicines.

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Outcomes: improvement of survival and preservation of cognitive function and prevention of neurological disease by use of extracorporeal detoxification in adults

Recommendation #10. We recommend extracorporeal detoxification be considered as a first line treatment in acute hyperammonemic decompensations in adults.
Quality of evidence: low

7.6. Methods to be used for extracorporeal detoxification

HD provides the highest ammonia extraction, as solute clearance is related to dialysate flow rate and blood flow rate, in addition to the surface area of the dialytic membrane. However, after discontinuation of HD, some patients may have an acute relapse of hyperammonemia.
In neonates, it has been shown that ammonia removal may be lower with HD than with CVVHD because of frequent technical and hemodynamic complications related to HD in infants (Sadowski et al. 1994), but, if working, intermittent HD is safe and efficient (Tsai et al. 2014). CVVHD may however be better tolerated, providing a continuous extraction with excellent ammonia clearance and it can be considered as the first line therapy in small infants (Hiroma et al. 2002; Picca et al. 2001; Schaefer et al. 1999; Spinale et al. 2013).
In the earliest experiences, hyperammonemic patients were treated with peritoneal dialysis (PD) and exchange transfusion. PD was shown to be of superior efficacy compared either to exchange transfusions, which produces only transient ammonia removal, or to pharmacological treatment alone (Batshaw and Brusilow 1980). Since PD provides however slower ammonia clearance, extracorporeal therapies are preferred in these patients in order to achieve faster plasma ammonia removal. After the introduction of techniques and devices suitable for very small children, hemodialysis (HD), hemodiafiltration (HDF) or forms of continuous renal replacement therapy (CRRT), e.g. continuous veno-venous hemofiltration (CVVH), continuous arteriovenous hemodialysis (CAVHD) and continuous veno-venous hemodialysis (CVVHD), have been claimed to provide maximal efficiency in ammonia removal when compared with PD.

Technical considerations
Once the decision for dialysis is made, the use of CVVHD equipped with a high-precision dialysate delivery system and with high dialysate flow is recommended (Picca et al. 2001). Adequate catheter performance (blood flow limited by small neonatal catheters diameter) and dialysate flow in CVVHD are essential to provide optimal ammonia clearance in these patients (Schaefer et al. 1999).
Likewise, a constant hemodynamic monitoring must be provided.

Typical CRRT prescription includes:

Access should be percutaneous: a 5 F, 7.5 cm dual-lumen catheter is placed in the right internal jugular, umbilical or femoral vein (Hackbarth et al. 2007). If possible, a 7 F cather should be used (Spinale et al. 2013).

1. Modality: CVVHD is performed with a CRRT machine equipped with neonatal blood lines and filter with adequate surface (depending on body surface of the patient).
2. Priming: to avoid chilling, the circuit is preprimed with warm albumine or warmed packed red blood cells and saline (50% proportion).

3. CRRT variables: the blood flow rate is dependent on the diameter of the catheter and should be between 10 to 50 ml/kg bw/min.
4. The dialysis flow rate must be appropriate and adapted to the expected clearance provided by the filter and the blood flow.
5. K^+ must be systematically added to the dialysis solution.
6. Infusion: depending on the dialysis solution used, a continuous infusion of D-fructose-1,6-diphosphate (100 mg/kg/d) might be required to compensate for phosphate loss.
7. Arginine, citrulline and drugs are also extracted by these procedures and should be controlled and replaced.
8. CRRT should continue until the ammonia level is 100-200 µmol/L (Picca et al. 2008; Spinale et al. 2013).

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Outcomes: improvement of survival and preservation of cognitive function and prevention of neurological disease by use of extracorporeal detoxification

Recommendation #11: We recommend hemodiafiltration as the method of choice for ammonia detoxification. We recommend considering peritoneal dialysis, which is less effective for ammonia removal, as a bridging technique when no hemodiafiltration is available and for transfer of patients to a metabolic center. We strongly recommend against performing exchange transfusion to treat hyperammonemia.
Quality of evidence: high

7.7. Dietary management during acute decompensation

It is crucial to **stop catabolism by promoting and maintaining anabolism** in any patient with an acute hyperammonemic decompensation. In most, oral feeding will not be feasible during the acute phase because of impaired consciousness and vomiting. A central venous line should be inserted in the severely ill patient, to help maximise energy intake. A glucose infusion should be started as quickly as possible; if hyperglycemia occurs, continuous IV insulin should be given. Administration of lipids (1-2 g/kg/d) can also provide additional energy and help reconstitute anabolism. To avoid protein catabolism and consequent increased ammonia production, the reintroduction of protein/amino acids/EAAs must not be delayed more than 24 hours. Some authors even advocate to consider supplementation with EAAs and BCAAs at the start of acute treatment since plasma concentrations of all measured EAAs were low or low-normal in almost all samples from UCD patients at admission for acute hyperammonemia; supplementation should preferably be given via the enteral way because of the contribution of the splachnic system to protein retention and metabolism (Boneh 2014; Rodney and Boneh 2013). If the patient cannot be fed enterally, IV amino acids should be commenced, increasing daily to the required amount/kg.
If intravenous amino acid mixtures are used, the exact composition needs to be considered to avoid those with high aromatic amino acids and low in BCAAs (Bachmann 2006).
Enteral feeding should be re-started as soon as possible. The composition of the feed will vary dependent on IV therapy and ammonia concentrations. It may initially be protein free; see the emergency regime:

Table 7: Emergency regime for protein-free feeding in infants and children (adapted from (Dixon 2007))

Age	Glucose polymer concentration % CHO	Energy/100ml Kcal	kJ	Suggested daily fluid volume	Feeding frequency
up to 6 m	10	40	167	150 ml/kg	2 to 3 hourly oral/bolus day and night or continuous tube feeds using enteral feeding pump
7-12 m	10-15	48	202	120 ml/kg	
1 y	15	60	250	1200 ml	
2-9 y	20	80	334	*	
>10 y	25	100	418	*	

* For children > 10 kg normal fluid requirements can be calculated as:
11-20 kg: 100 ml/kg for the first 10 kg, plus 50 ml/kg for the next 10 kg
20 kg and above: 100 ml/kg for the first 10 kg, plus 50 ml/kg for the next 10 kg, plus 25 ml/kg thereafter up to a maximum of 2500 ml/day

During the changeover period continuous NG feeding may overcome some of the difficulties with feed tolerance. As IV fluids are decreased, enteral fluids should be increased accordingly to ensure an adequate energy, electrolyte (taking into account sodium from nitrogen scavengers) and nutrient intake at all times. It is important to take account of any amino acids from parenteral nutrition when changing to enteral feeds so the combined intake

does not exceed the prescribed daily amount of protein. The EAA products for UCD can be used instead of or in combination with natural protein to help reduce the total nitrogen load, if ammonia increases with protein re-introduction. Levels of ammonia should guide the re-introduction of protein but this must not be delayed. In practice, protein is usually re-introduced over 2 – 4 days. Some centres may also analyse amino acid profiles daily to adjust dietary management. Energy intake should be maximised to around 120% of age adjusted requirements (Table 10) for about 2 weeks to promote anabolism. If fluids are restricted, extra energy (as glucose polymer and or fat emulsion) can be added to the feeds to prevent catabolism. When concentrating feeds, care needs to be taken to avoid osmotic diarrhoea; thus, the osmolarity (mainly due to the mono- or dimers of calories and EAA mixtures) should be below 500 mOsm/L in neonates and infants and below 600-700 mOsm/L in older children (but 400 mOsm/L in malabsorption states).

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Newly diagnosed newborns, infants or children

The method of feeding will vary: the sickest patients will initially need to be fed parenterally, then by continuous NG tube feeds with progression to frequent bolus, then oral feeds (6/8 times per day) or diet as they recover. After the initial 24-48 hour protein free stabilization period, unless plasma ammonia is still markedly elevated (i.e. >200 µmol/L, but the entire clinical situation should be taken into account), enteral feeds should be started. IV fluids should be weaned off as enteral feeds are gradually increased to tolerance. A protein free feed or 10 to 20% glucose polymer (depending on age) may be given first to assess feed tolerance. Protein is introduced once the plasma ammonia falls to < 100 µmol/L. As a guide protein can be increased daily by 0.25-0.5 g protein/kg up to the individual tolerance over a period of 2 to 4 days with daily controls of ammonia and amino acids. Each child's protein requirement needs to be individually determined, the 'safe level of protein intake for age' (Table 8) can be used as a guide but lower amounts can still be adequate for many patients. Protein reintroduction may vary depending on ammonia concentrations, metabolic state and feed tolerance. Drugs can be changed to oral preparations once enteral feeds are tolerated.

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Neonates or infants

Protein is provided from breast milk or infant formula and combined with a protein free infant formula to give an adequate energy, nutrient and fluid intake for age. During the initial introduction of protein it is simpler and more accurate to use expressed breast milk. If a mother wishes to breast feed, she should be supported because even in severe UCDs some breast feeding will be feasible, once the metabolic state stabilises. To help establish breast feeding in neonates and mother's milk supply it is important to allow the baby to suckle. Once the infant is on the full protein allowance and more stable, breast feeding should be established without delay.
Go to 8.2 for more practical details on low protein infant feeding.

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Children

A limited volume of a standard pediatric enteral feed can be used to provide protein. Additional energy, fluid and vitamin and mineral supplements will be needed to provide recommended intakes for age. These can be added to the feed or given separately. Once the child is more alert the low protein feeds can be changed to a low protein diet. If the child has suffered a severe neurological insult, long term tube feeding may be necessary.
Go to 8.2 for more practical details on low protein feeds and diet.

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Dietary management of intercurrent illness at home in known UCD patients

Home-management of intercurrent illness e.g. in mild febrile illness is possible but under medical supervision and only if the clinical condition (i.e. neurological status) is stable and ammonia levels are not increased. Patients are at risk of protein catabolism with rapid accumulation of ammonia and glutamine due to infection, loss of appetite and poor oral intake. To help prevent catabolism an 'emergency regimen' (Table 7) should be implemented: the usual protein and EAA intakes are stopped temporarily and frequent drinks/feeds or tube feeds of glucose polymer are given day and night (Dixon 2007; Dixon and Leonard 1992). Glucose polymer concentrations and volume depend on the child's age (Table 7). Some children will not drink glucose polymer so it can be flavoured with squash/cordial to improve palatability. The usual doses of medicines should be continued. If the feeds or drugs are not tolerated or

if the patient is not responding or has diarrhoea and vomiting patients should be admitted to hospital for IV therapy (Table 5). The protein should be stopped for the minimum time possible (maximum 24-48 hours). As the patient recovers the usual protein and EAA intake should be reintroduced over 1 to 3 days as clinically indicated. The underlying cause of the infection should be treated as normal.
Parents/carers need detailed written advice on the emergency regimen (oral and IV) and have supplies of glucose polymer for use at home, at school and for holidays. If a hospital admission is necessary parents/carers should be advised to always take their written emergency regimen instructions (oral and IV) and glucose polymer.

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Outcomes: improvement of survival and improvement of metabolic stability by dietary intervention

Recommendation #12: We strongly recommend for treatment of acute hyperammonemia establishing and maintaining anabolism by providing high-dose glucose ± insulin (plus lipids if a fatty acid oxidation disorder has been excluded). We recommend keeping the period of protein-free nutrition no longer than 24 hours.
Quality of evidence: moderate

8. LONGTERM MANAGEMENT OF UCDS

Longterm treatment of UCDs is challenging for patients and families because of the poor palatability, volume and frequency of diet and drug administrations; all these are serious barriers to adherence (Shchelochkov et al. 2016).
Evaluation of the literature on long-term treatment of UCDs shows that the majority of publications on diet and pharmacotherapy are reviews, expert opinions and case reports but only single case control or cohort studies. Recommendations provided in this guideline are therefore mostly grade D and only few are grade C.

8.1 Principles of diet for long-term treatment of UCDs

The aims of the long-term treatment are to maintain stable metabolic control, to eliminate chronic complications (Berry and Steiner 2001; Brusilow and Horwich 2001; Leonard and Morris 2002) and achieve normal growth. For most patients, this can only be achieved by a combination of:

- medications which increase waste nitrogen excretion
- a low-protein diet
- supplementation of essential nutrients such as vitamins and minerals
- EAA supplementation
- emergency regimen for treatment of intercurrent illnesses

Parents/caregivers need continued support to manage the low protein diet and periods of illness. They need written instructions with contact details for the metabolic and local hospital teams. Nurseries and schools also need informed guidance and visits by a clinical nurse specialist or metabolic dietitian is recommended. Local hospitals need up to date written instructions on management during illness.

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Low protein diet
Most UCD patients are treated with a protein restricted diet with some variation between European centers (Adam et al. 2013). Controlled data to support protein restriction are scarce but clinical experience and biochemical considerations are in favour of this.

Restricting natural protein requires careful planning and monitoring to meet the individual requirements for normal growth and development and obligatory nitrogen losses, whilst maintaining metabolic stability. Over-restriction must be avoided to prevent malnutrition, essential amino acid deficiency and metabolic instability (Leonard 2001; Singh et al. 2005). To prevent endogenous protein catabolism an adequate intake of energy should be provided (Singh 2007).

The prescribed protein intake is best provided from a combination of low and some high biological value protein foods, to help ensure adequate intakes of all EAAs. The diet can then be provided primarily from normal food with little reliance on alternative energy supplements and manufactured low protein foods. Ideally the daily protein intake should be equally divided between feeds or meals to avoid giving a high protein load and to optimise nitrogen retention.

The FAO/WHO/UNU 2007 (http://whqlibdoc.who.int/trs/WHO_TRS_935_eng.pdf) have set **'safe levels of protein intake'** for infants, children and adults (see Table 8) and these can be used to guide protein prescription for all patients. These have been calculated as the mean requirement +2 SD in order to meet or exceed the requirements of healthy individuals. Accordingly, for many patients a protein intake below the 'safe levels' can still be adequate (Dixon 2007). Protein requirements per kg/bw decrease with age and this needs to be taken into account when planning a low protein diet.

In UCDs protein requirements and protein tolerance will vary depending on the disorder, its severity, growth velocity, age, frequency of infections and dose of nitrogen scavengers. Regular review and monitoring is therefore essential to assess the changing needs of the patient. The 'safe level of protein intake' (see Table 8) can be used as a guide but this needs to be titrated against individual tolerance and biochemical control to decide upon the final daily protein prescription. Metabolic control may be easier to achieve in early infancy compared to older children as a higher protein intake per kg/bw is tolerated due to higher growth velocity (Lee et al. 2005; Leonard and Morris 2002; WHO Technical Report Series 2007).

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Outcomes: improvement/maintenance of metabolic stability and preservation of cognitive outcome and prevention of neurological and hepatic complications

Recommendation #13: Deficiencies of caloric and/or essential amino acid and other nutrients can cause metabolic instability and morbidity. We strongly recommend involving a specialist metabolic dietitian to balance nutritional requirements with metabolic stability, following the FAO/WHO/UNU guidelines for protein and energy requirements.
Quality of evidence: moderate

Table 8: Safe levels of protein intake for different age groups according to FAO/WHO/UNU 2007.

Child Age	g/kg bw/day	**Female** Age	g/kg bw/day	**Male** Age	g/kg bw/day
Months (breast fed infants)		Years		Years	
1	1.77				
2	1.50				
3	1.36				
4	1.24				
6	1.14				
		11	0.90	11	0.91
Years (weaned infants)		12	0.89	12	0.90
0.5	1.31	13	0.88	13	0.90
1.0	1.14	14	0.87	14	0.89
1.5	1.03	15	0.85	15	0.88
2	0.97	16	0.84	16	0.87
3	0.90	17	0.83	17	0.86
4-6	0.86-0.89	18	0.82	18	0.85
7-10	0.91-0.92	> 18	0.83	>18	0.83

EAAs and BCAA supplementation

For some patients an EAA or BCAA supplement is prescribed as part of the total daily protein intake. Use and doses of EAAs supplements varies markedly between centres. In a large cross-sectional study from 41 European Metabolic centers, EAAs supplements were prescribed in 38% of 464 patients with dome variation between

disorders (74% of ARG1D patients were taking EAAs supplements) (Adam et al. 2013) and generally provide between 20-30% of total protein intake, with up to 50% in ARG1D (Adam et al. 2013; Adam et al. 2012).
Although BCAA supplementation is suggested (Bachmann 2006; Scaglia 2010), experience of supplementation with BCAA appears limited (Adam et al. 2012) and there are no controlled studies related to their use or comparing them with EAAs. They may be useful in patients receiving high doses of sodium PBA and in ARG1D.
The rationales for use of EAAs include:

1. To decrease the load on the UC by diverting nitrogen to non-essential amino acid synthesis (Leonard 2001). However, this concept has never been proven in clinical studies.
2. To ensure adequate intake of EAAs for growth in a low protein diet and further reduce the total nitrogen load by replacing natural food with EAAs (Singh 2007).

The rationales for use of BCAA include:

1. To prevent or treat branched chain amino acids depletion caused by high dose sodium PBA (Scaglia et al. 2004).
2. To reduce, by competition at the BBB, the uptake of the neurotransmitter precursors tryptophan and phenylalanine/tyrosine (Bachmann 2002).

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Outcomes: improvement/maintenance of metabolic stability

Recommendation #14: We recommend supplementation of essential amino acids, especially of branched-chain amino acids, if natural protein tolerance is very low and/or if the patient receives phenylbutyrate.
Quality of evidence: moderate

Energy requirements

Provision of an adequate energy intake is essential to maintain metabolic control and achieve normal growth. Too low an energy intake will cause poor growth and an increase in plasma ammonia and glutamine concentrations due to endogenous protein catabolism. An overnight fast can cause early morning catabolism in some patients.
There are recommended daily energy intakes for infants, children and adults (see below Table 9). However, each patient's growth needs to be individually monitored as energy demands do vary. Gastrostomy feeding is recommended for children with long term feeding issues to provide an adequate energy and nutrient intake.
Adequate energy intake is provided by a combination of the protein containing foods and naturally very low protein foods (fruit, some vegetables, fats and oils, pure sugars). If these do not provide adequate energy, additional can be provided from specially manufactured very low protein foods such as pasta, bread, flour, biscuits and/or energy supplements such as glucose polymer and fat emulsions. Some EAA supplements will also provide energy.

Still some children may be at risk of a low energy intake because:

- of particular dietary preferences
- of poor appetite and anorexia particularly during periods of metabolic instability
- neurological handicap or severe developmental delay with associated mechanical feeding problems

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Table 9 shows recommended daily intakes of energy for different age groups for the healthy population according to FAO/WHO/UNU 2007 (WHO Technical Report Series 2007).

Age	Energy requirements (kJ/kg)		Energy requirements (kcal/kg)	
	Males	Females	Males	Females
Children, Years				
0.5	335	340	80.0	81.2
2.5	348	334	83.1	79.8
5.0	315	305	75.2	72.8
10	275	248	65.7	59.2
15	230	193	54.9	46.1
Adults, moderate activity level, 70kg body weight Years				
18-29	183	159	43.7	38.0
30-59	175	148	41.8	35.3
Adults, moderate activity level 50kg body weight Years				
18-29	212	180	50.6	43.0
30-59	212	183	50.6	43.7

Supplementation of vitamins, minerals and trace elements

As a result of the low protein diet, patients are at risk of vitamin and mineral deficiencies in particular from iron, zinc, copper, calcium and cobalamin (Dixon 2007). Supplementation of vitamins and minerals is necessary and need to be individualised according to gender, age and diet of the patient.

Those on infant formula or tube feeds are also at risk of inadequate vitamin and mineral intakes. Some EAA supplements will contribute to vitamin and mineral intakes and this needs to be taken into account when assessing overall intake and need for supplementation.

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Supplementation of essential fatty acids

Essential fatty acids (EFAs) and their longer-chain polyunsaturated derivatives (LCPUFAs) are required for normal growth and cognitive development. Patients with UCDs on a very low protein diet or receiving most of protein as EAAs mixtures are at risk of EFAs deficiency (Sanjurjo et al. 1997; Vlaardingerbroek et al. 2006) and supplementation may be warranted.

Clinical Monitoring

For clinical and nutritional monitoring, (see 10)

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8.2. Practical aspects of diet for long-term treatment of UCDs

Outcomes: improvement/maintenance of metabolic stability and preserving quality of life and reducing the burden of disease

Recommendation #15: We recommend individualized dietary management, and parents' and patients' training to strengthen their competence for a life-long dietary treatment.
Quality of evidence: moderate

Low protein feeds for infants

For dietary management during acute metabolic decompensation and re-introduction of protein see 7.7.

Breast feeding

Exclusive demand breast feeding has been reported in infants with UCD (Dixon et al. 2000; Huner et al. 2005). Since protein concentration and volumes of breast feeds vary, plasma ammonia, glutamine and alanine concentrations need to be monitored closely to help determine protein tolerance and amount of breast feeding (see also section 10). If demand breast feeding provides too much protein, the amount can be limited by giving a prescribed amount of manufactured protein free infant formula prior to all or some of the breast feeds.

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Bottle feeding (see example 1)

In bottle fed infants the prescribed protein intake should be supplied as normal infant formula and divided between feeds. The safe level of protein intake for age can be used as a guide but this may not be tolerated by all patients (as described in section 8.1) Some of the protein may need to be replaced by EAAs in less stable patients. After each protein feed the infant should be offered a specially manufactured protein free infant formula (otherwise nutritionally complete feed) to provide an adequate nutrient and fluid intake for age. If this is not available a protein free modular feed can be made but this is much more complex for parents (see example 1). Alternatively both feeds (protein containing and protein free) can be mixed together but the total feed volume would then need to be consumed to provide all the protein. Infants on a more generous protein intake may just need additional energy supplement added to the protein containing feed.

Example 1

Feed 1
A measured volume of whey based infant formula to provide the natural protein requirement.
Administration: divided evenly and given at 3 to 4 hourly intervals, 6 to 8 times per day.

Feed 2
Manufactured protein free formula or modular protein free formula.
Administration: give after each of the protein feeds.

Modular protein free infant formula:
(per 100 ml, the nutrient composition should be similar to normal infant formula but protein free)
Ingredients:
glucose polymer 7-10g CHO per 100 ml
fat emulsion 3.6 – 5 g fat per 100 ml
*vitamin and mineral supplement
*sodium and potassium supplement (consider sodium from nitrogen scavengers)
EFA may be needed if the protein feed does not provide sufficient

* taking into account the amount already provided by the infant formula or breast milk

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Clinical monitoring

After discharge from hospital the baby's weight should initially be monitored 1-2 weekly to assess the need for any changes to the feeding regimen.

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Weaning onto a low protein diet

Time and mode of weaning should be the same as for healthy infants. Continuing a late night feed/snack is beneficial to prevent catabolism associated with a prolonged overnight fast.
The weaning period can be difficult because the growth velocity slows and protein requirements per kg are lower (see Table 8); this can result in a more unstable situation than in early infancy. For this reason, regular contact to the metabolic dietitian is important during this period.
To start, protein free foods such as fruits, low protein vegetables, low protein manufactured foods (cereals) are best suited because if they are not well accepted the daily protein intake is not affected (Dixon 2007).

Once these are being taken the protein provided from infant formula or breast milk is gradually replaced by protein containing solids either from home cooked foods such as potato, vegetables or cereals or commercial baby foods. This can be done using an exchange system:

e.g. Bottle fed: one gram of protein from infant feed is replaced by one gram of protein from food.

For breast fed babies the actual daily intake of protein is not known. Therefore it is best to gradually decrease the number of daily breast feeds and replace with protein from food (taking into account that each breast feed is likely to provide more than one gram of protein).
The energy intake provided by protein foods can vary greatly. It is essential to ensure an adequate intake of energy is given from these and lower protein foods. A vitamin and mineral supplement should be added as less breast milk/infant formula is given because low protein foods do not provide such a good source of some of these. Parents/carers need detailed guidance and instruction on how to make this change from feeds to solids to ensure energy and nutrient intake is always adequate.
They need written information on:

- suitable protein exchange foods with food weight to provide 1g of protein
- very low protein foods allowed freely (natural and commercial foods)
- how to calculate protein content of manufactured foods
- specially manufactured low protein foods
- suitable finger foods

To make it easier for parents it is best to structure this information over several teaching sessions as the child progresses with weaning.

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Tube feeding

Some UCD patients have difficulties in achieving an adequate dietary intake and tube feeding becomes essential to prevent metabolic decompensation.
Tube feeding needs to be implemented or considered in patients who have one or a combination of the following problems:

- inability to suck or swallow due to neurological handicap or severe developmental delay
- difficulty with the daily administration of EAAs and drugs with an unpleasant taste
- poor appetite and/or food refusal with resultant inadequate energy intake
- gastrointestinal problems – vomiting, reflux, retching
- emergency management during intercurrent illnesses

Outcomes: improvement/maintenance of metabolic stability and preserving quality of life and reducing the burden of disease

Recommendation #16: We recommend early consideration of tube feeding to ensure nutritional adequacy, administration of medications and supplements, prevention of catabolism and/or maintenance of metabolic stability.
Quality of evidence: low

Some patients with UCDs have constantly high levels of plasma glutamine. This may be a factor contributing to poor appetite because increased plasma glutamine and ammonia can increase brain tryptophan uptake with resulting increase in serotonin synthesis and satiety (Bachmann 2002).

For patients who need long-term tube feeding, a gastrostomy should be considered. However, nasogastric tubes can also be used for the long-term management and particularly for acute episodes both in hospitals and at home. In acute episodes, use of nasogastric tubes will expedite the transfer from parenteral to enteral nutrition. Nasogastric tubes might also be considered if families refuse insertion of a gastrostomy. However, although gastrostomy requires general anesthesia the working group of this guideline recommends considering use of gastrostomy in patients with severe UCDs although there are no controlled studies on this and some patients might become overly dependent on tube feeding (Singh 2009). In a cohort (n=175) from the UK, enteral feeding tubes were used by 25% for feeds and 3% for medications (Adam et al. 2012); similarly, across 464 patients from 41 European Metabolic centers, 18% were prescribed tube feeds (Adam et al. 2013).

The tube feed should provide the prescribed natural protein intake and normal requirements for all other nutrients. The energy intake can be based on normal requirements for age but will need to be decreased in physically handicapped or overweight children.

Example 2: tube feed for a child – ingredients/composition:

- an age appropriate paediatric enteral feed to provide the natural protein
- EAAs/BCAA supplements as prescribed
- glucose polymer and fat emulsion to provide additional energy*
- vitamin and mineral supplements*
- electrolytes (sodium and potassium)*
- fluids according to age

* taking into account the amount which is already provided by the paediatric enteral feed

If the paediatric feed does not contain fibre than an additional source may need to be added to ensure normal bowel habits. The method and timing of feed administration can vary (bolus, continuous, during the day and night). It should be based on feed tolerance and should take account of the child's daily routine. Smaller, more frequent feeds spread over 24 hours are best for children who have problems with vomiting and retching.
It is important to continue offering food and fluids orally unless the child has an unsafe swallow.

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Low protein diet for children and adolescents

Throughout childhood, the low-protein diet needs to be continually adjusted to provide an adequate protein intake. This needs to take account of biochemical results, growth velocity and clinical status (see section 10, Monitoring)
In addition, an adequate intake of energy and all other nutrients must be ensured. There may be periods of poorer appetite and strategies to cope with inadequate intakes need to be used,
e.g:

- if all the protein is not consumed then it can be replaced with milk and glucose polymer
- if energy intake is inadequate, then energy supplements such as glucose polymer or fat emulsions can be added to drinks or fluids. It is important to prescribe a daily amount of these.

Adolescent patients are sometimes more difficult to manage because of: age-related variable compliance, appetite increase and due to the hormonal changes that might contribute to metabolic instability. During puberty the requirements for some minerals change markedly e.g. calcium and iron. The vitamin and mineral supplement may need to be changed to provide for this.

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Self selected low protein diets

Children who present later often have a protein aversion, are already on a self selected low protein diet and eat only a limited range of foods.
Altering a self selected low protein diet can be very difficult. This style of diet is invariably deficient in certain vitamins and minerals such as B_{12}, iron, calcium. The diet needs to be regularly assessed and appropriate vitamin and mineral supplements prescribed.

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EAAs supplements

A variety of EAAs supplements, suitable for patients with UCD of different age groups are available.
Examples are: pure EAAs supplements; flavoured EAAs supplements with carbohydrates (some with added vitamins and minerals) and specialised protein modified infant formulas. The EAA composition of these does vary. Some authors recommend EAAs supplements should be rich in BCAA and not high in tryptophan, phenylalanine and tyrosine (Bachmann 2006).
In practice, pure amino acid supplements are not always well tolerated because of their unpleasant taste, although some of the newer products are much more palatable. Some products are pre-flavoured to improve palatibilty, flavour sachets are available from some nutritional companies, alternatively milk shake flavourings (powder or syrups) or fruit squashes can be added. The daily EAAs intake is best given with natural protein and divided between feeds or meals, thus avoiding giving a protein load.

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BCAA supplements

BCAA supplements can be given as either single amino acids or as a complete supplement. However there is limited published experience with this.

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EFAs and LCPUFAs

Patients on a low protein diet are at risk of EFAs and LCPUFAs deficiencies.
Infants may receive adequate intakes if they are on more generous natural protein intakes and infant formulas supplemented with EFAs and LCPUFAs. Oils which are rich in polyunsaturated fatty acids (e.g. walnut oil, rapeseed oil, sunflower oil) are recommended to improve EFAs intake in older children on low protein diets.

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Low protein diet for adults

Adult patients with UCDs need a low protein diet throughout life.
Regular dietary assessments remain necessary to ensure an adequate protein intake is consumed and that vitamin and mineral supplements are still being taken. A particularly difficult situation might arise in women who are diagnosed post partum and may have protein aversion. Since these women may represent variant forms of UCDs, the dietary regime must be highly individualised and it might be practical to start adjustment of their diet according to safe levels of protein intake.

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Pregnancy and lactation

Females with UCDs and especially female OTC carriers are in danger of life threatening encephalopathy because of the catabolic state that can be triggered in both the intrapartum and postpartum period. Multidisciplinary management is essential and careful follow-up is necessary, from pre-pregnancy counseling to plan management of the drug treatment and close monitoring of the nutritional status.
There are already some case reports describing the successful management of pregnancies in women with UCDs of which most but no all describe OTC females (Ituk et al. 2012; Kim et al. 2012; Langendonk et al. 2012; Mendez-Figueroa et al. 2010; Worthington et al. 1996). The focus of the dietary management during pregnancy should be to prevent undernutrition of protein. The metabolic situation should be closely monitored; to adjust the diet, the following recommendations during pregnancy and lactation might be helpful (see Table 10).

Table 10: Protein intake during pregnancy and lactation (WHO Technical Report Series 2007)

Pregnancy trimester	Additional safe protein intake (g/day) FAO/WHO/UNU 2007	Additional energy requirement (KJ/day) FAO/WHO/UNU 2007
1	1	375
2	10	1200
3	31	1950
Lactation		
First 6 months	19	2800
After 6 months	13	1925

During labor, neuroaxial anesthesia may reduce catabolism; therefore, early epidural analgesia seems prudent (Ituk et al. 2012).

During first trimester, episodes of nausea and vomiting may precipitate dehydration and catabolism and thus an increase of ammonia levels. Careful monitoring of fetal growth is mandatory as there is a risk of growth retardation due to maternal protein restriction. Usually the antenatal period remains uncomplicated because of the increased

nitrogen demands of the placenta, uterus and fetus. It has also been suggested that the liver of an unaffected fetus could detoxify maternal ammonia.
Treatment goals are reduction of excess dietary nitrogen with nitrogen scavengers. Sodium benzoate and sodium phenylbutyrate, used during few pregnancies, did not yet produce teratogenic effects on the fetus. Nevertheless they must be used with caution as there is only limited manufacturers' data on their use in pregnancy. Supplementation of arginine or citrulline is necessary. During acute episodes of hyperammonemia, protein intake should be interrupted for a limited time only (24 hours) and 10% dextrose can be provided. Essential amino acids depletion can trigger protein catabolism; therefore, the transition to enteral feeding is recommended as soon as possible. If hyperglycemia occurs because of high amounts of dextrose infusion, application of insulin is recommended. If the clinical condition deteriorates and the ammonia level increases, hemodialysis or continuous hemofiltration should be commenced.
A written procedure for the management at delivery and the post-partum period is highly recommended accessible to involved obstetricians, metabolic specialists, anesthetists, dietitians and midwifes (Lamb et al. 2013).

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8.3. Pharmacotherapy in long-term treatment of UCDs

Drugs which are routinely used for long-term treatment of UCDs comprise nitrogen scavengers (sodium benzoate, sodium PBA or sodium phenylacetate, glycerol phenylbutyrate), L-arginine, L-citrulline and carbamylglutamate.
Some of the medications are available as powder, capsule, tablet or liquid. This might cause practical problems for the patient if no unambiguous prescription is written (Summar 2001; Wilcken 2004).

Outcomes: improvement/maintenance of metabolic stability and preserving quality of life and reducing the burden of disease

Recommendation #17: We suggest providing written drug treatment sheets to parents, pharmacists and persons involved in patient care.
Quality of evidence: low

Efficacy and dosage of nitrogen scavengers
Sodium PBA is available as a licensed drug in many countries in Europe and has been reported to reduce the risk of hyperammonemic episodes more efficiently than sodium benzoate (Maestri et al. 1996; Maestri et al. 1991) but there are also studies that question this advantage (Comte et al. 2002). Sodium benzoate is not a registered drug but has been used for decades for the treatment of UCDs (and other conditions). There are no consistent recommendations and it should be underlined that the majority of patients treated in the US receive sodium or glycerol PBA alone, whereas most centres in Europe consider sodium benzoate as the first line medication. The majority of guideline group members were in favour of sodium benzoate because of long-term experience, less side effects and safety concerns. The lower maximum doses of PBA in comparison to those recommended in the US are based on safety concerns and on the common practice of combination therapy of PBA and benzoate.
A novel chemical esterified form of PBA, glycerol phenylbutyrate (GPB), has been introduced into the clinic with FDA and EMA approval, after non-inferiority to NaPBA was shown (Lichter-Konecki et al. 2011). GPB avoids sodium intake and is a tasteless liquid. It showed in a phase 3, randomized, double-blind, crossover trial proper metabolic control and might exhibit more favorable pharmacokinetics than NaPB (Berry et al. 2014; Diaz et al. 2013; Mokhtarani et al. 2012; Smith et al. 2013). With recommended dosing, GPB results in phenylacetic acid plasma levels in the desired range in the majority of patients (Mokhtarani et al. 2013). This new drug formulation offers the chance to increase the quality of life in patients by favoring intake.
A slow-release more taste-friendly formulation of NaPBA in form of taste-masked granules has recently been introduced and approved by the EMA. In a switch-over trial, safety and efficacy endpoints were met and palatability was judged superior to other NaPBA formulations (Guffon et al. 2012; Kibleur et al. 2014).
New galenic forms of sodium benzoate that are more taste-friendly have been developed (Combescot et al. 2015; Eckert et al. 2014) but are not yet available as licensed drugs.

To increase the effect of nitrogen scavenger drugs, a 4 times daily dosage, linked to meals, is recommended (Ahrens et al. 2001; Wilcken 2004) but there are also data that advocate the use of PBA between meals (Kasumov et al. 2004). In the case of GPB, three equally divided daily dosages are recommended. To reduce catabolism during the night, the last meal before the night sleep (= fasting period) should contain a relevant proportion, e.g. 25%, of the daily energy, natural protein, EAAs, citrulline and/or arginine intake.

There is no well-defined recommendation on drug dosages for UCD patients; the following table is a consensus of the working group of this guideline taking into account the literature (Batshaw et al. 2001; Brusilow and Maestri 1996; Feillet and Leonard 1998; MacArthur et al. 2004).

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Table 11: Dosages of drugs to be used perorally for long-term treatment of UCDs

Disorder	Sodium benzoate[$]	Sodium PBA[°$], or equivalent dosages of GPB	L-arginine[$] (hydrochloride and/or free base)	L-citrulline[$]	Carbamyl-glutamate[$]
NAGSD	-	-	-	-	10-100 mg/kg/d
CPS1D	up to 250 mg/kg/d*# max. 12 g/d	<20 kg: up to 250 mg/kg/d*# >20 kg: 5 g/m²/d# max. 12 g/d	<20 kg: 100-200* mg/kg/d or: 0.5-1 mmol/kg/d >20 kg: 2.5-6 g/m²/d max. 6 g/d	100-200 mg/kg/d§ max. 6 g/d	-
OTCD	same	same	same	100-200 mg/kg/d§ max. 6 g/d	-
ASSD	same	same	<20 kg: 100-300*# mg/kg/d or: 0.5-1.5 mmol/kg/d >20 kg: 2.5-6 g/m²/d# max. 8 g/d	-	-
ASLD	same	-	<20kg: 100-300*# mg/kg/d or: 0.5-1.5 mmol/kg/d >20kg: 2.5-6 g/m²/d# max. 8 g/d	-	-
ARG1D	same	same	-	-	-
HHH syndrome	same	same	<20 kg: 100-200* mg/kg/d >20 kg: 2.5-6 g/m²/d max. 6 g/d	100-250 mg/kg/d§ max. 6 g/d	-

Legend to Table 11: all medications should be divided into three to four doses daily taken with meals and distributed as far as possible throughout the day.
° sodium PBA was considered of second choice for long-term treatment by most guideline group members. It should be given together with sodium benzoate in patients in which benzoate alone is not enough
* serum/plasma levels of benzoate/PBA and plasma levels of arginine should be monitored
# in some patients higher doses are needed (the US FDA studies consider doses up 450 - 600 mg/kg/day in children weighing less than 20 kg and 9.9 to 13.0 g/m²/d in children weighing more than 20 kg, adolescents and adults), according to expert advice
§ if citrulline is given, there is usually no need for concomitant use of L-arginine
$ 100 mg equal 0.694 mmol sodium benzoate; 0.537 mmol sodium PBA; 0.475 mmol arginine hydrochloride; 0.574 mmol arginine base; 0.571 mmol citrulline; 0.532 mmol carbamylglutamate, respectively

Outcomes: improvement/maintenance of metabolic stability and treatment during pregnancy

Recommendation #18: Nitrogen scavengers are a mainstay of therapy in UCD patients. We recommend to adjust the dose for each patient.
Quality of evidence: moderate

Adverse effects and toxicity of nitrogen scavengers

Nitrogen scavengers are safe at the recommended doses but can be toxic at high doses.

Possible toxicity of sodium benzoate:
Sodium benzoate did lead to low concentrations of plasma glycine (<130 µmol/L) in rare instances and might also impair mitochondrial function due to acetyl-CoA depletion (Griffith et al. 1989; Tremblay and Qureshi 1993). Thus, plasma glycine concentrations should not be < 100 µmol/L. Benzoate plasma levels > 2 mmol/L are toxic.

Possible toxicity of sodium PBA or sodium phenylacetate:
Sodium PBA is, in addition to its role as nitrogen scavenger, an inhibitor of histone 1,2 deacetylation, DNA methylation, protein isoprenylation, and of estrogen-dependent breast-cell responses (Gore et al. 1997; Monneret 2005; Sawatsri et al. 2001). Although the clinical relevance of these inhibitions is not yet clear, these additional effects of PBA are a source of serious concern for the long-term use of this compound.

Adverse effects of sodium PBA:

During long-term treatment with sodium PBA, about 25% of postpubertal females have been affected by amenorrhea or menstrual dysfunction according to a study by Ucyclid (Batshaw et al. 2001). It is not clear if this adverse effect is reversible. Less frequently, decreased appetite, taste disturbances, or disagreeable body odour are reported.
Sodium PBA causes depletion of BCAAs (Batshaw et al. 2014) and it was therefore discussed that this increases the risk of endogenous protein catabolism (Burrage et al. 2014; Scaglia 2010; Scaglia et al. 2004; Tuchman et al. 2008b), which was however not shown in a study addressing whole-body protein metabolism (Marini et al. 2011).

Adverse effects of sodium benzoate and sodium PBA:
If granules, tablets, or undiluted liquid preparations are used, mucositis and gastritis can occur. To avoid mucosal damage, nitrogen scavenger drugs should be taken together with meals and plenty of fluid. Hypokalemia can be the result of increased renal loss and this can occur after bolus doses but also during long-term treatment.
In theory, depletion of acetyl-CoA might impair UC function by a reduced production of N-acetylglutamate and might lead to cellular energetic dysfunction.
Low albumin values found in sodium PBA treated patients are most likely due to diminished protein synthesis caused by the low availability of leucine for glutamine synthesis.
In some patients, metabolic acidosis has been observed especially on a high dose of L-arginine, sodium benzoate and sodium PBA.

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Use of nitrogen scavengers in pregnancy
The use of nitrogen scavenger drugs in pregnancy has not been systematically investigated but there are single anecdotal reports on successful pregnancies on PBA (Batshaw et al. 2001; Ituk et al. 2012; Lamb et al. 2013). According to data available, nitrogen scavenger drugs should be used with caution. Based on their experience and on few anecdotal reports, the working group of this guideline regards sodium benzoate to be the safer choice if medication has to continue.

Outcomes: improvement/maintenance of metabolic stability and treatment during pregnancy

Recommendation #19: Continuation of treatment with nitrogen scavengers is generally necessary in pregnant UCD patients. Based on biochemical mechanisms, we suggest the use of sodium benzoate. There is insufficient evidence for commenting on fetal outcomes after nitrogen scavenger therapy in pregnancy.
Quality of evidence: low

L-arginine and L-citrulline for long-term treatment
Arginine becomes an essential amino acid in all UCDs apart from ARG1D. Supplementation of L-arginine is prescribed to avoid arginine deficiency and to reduce the frequency of hyperammonemic episodes (Brusilow 1984; Keskinen et al. 2008; Nagasaka et al. 2006; Wilcken 2004). The aim should be to achieve fasting plasma arginine concentrations of 70 – 120 µmol/L (Wilcken 2004).
The rational for L-arginine and L-citrulline supplementation is, besides avoiding arginine deficiency, to promote urinary excretion of nitrogen-containing intermediary UC products and to lower the overall waste nitrogen burden in UCD patients (Brusilow and Batshaw 1979). Both L-arginine and L-citrulline are metabolised to serve as a vehicle for nitrogen removal via urine excretion of citrulline and argininosuccinate.
In deficiencies of NAGS, CPS1 and OTC, L-citrulline should be used in first place because in theory, it is metabolised together with aspartate and hereby allows excretion of an additional nitrogen molecule. However, there are no studies that compare the efficacy of L-citrulline versus L-arginine; thus, their preferential use will also depend on local availability and costs involved.
L-arginine can be given as arginine hydrochloride or as a 1+1 mixture of the hydrochloride and the base; the base alone is very poorly soluble in water at acceptable pH. For prescription, the respective molecular weights should be noted (see Table 11). Rarely, L-arginine can cause metabolic acidosis.

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Outcomes: improvement/maintenance of metabolic stability and prevention of complications

Recommendation #20: We strongly recommend L-arginine and/or L-citrulline supplementation in UCD patients (may not be required in mild phenotypes, and L-arginine is contraindicated in ARG1D). We recommend monitoring plasma arginine levels in all UCD patients.
Quality of evidence: moderate

N-carbamylglutamate

Carglumic acid (synonymous: N-carbamyl-L-glutamate) is a licensed drug in Europe (and also approved by the FDA in the United States) for the use in primary NAGSD (see also 11.3) (Häberle 2011b, 2012). In addition, its use has been suggested as an emergency medication in neonatal hyperammonemia of unknown etiology and hereby, as a tool for differential diagnosis in unclear neonatal hyperammonemia (Guffon et al. 2005). Although there are no controlled studies on the benefit of carbamylglutamate in neonatal hyperammonemia its use should be considered in severe hyperammonemic decompensations.

N-carbamylglutamate is an analogue of N-acetylglutamate that is taken up enterally and able to enter the mitochondrion. Thus, it can be used for the activation of CPS1 if N-acetylglutamate is deficient (Bachmann et al. 1982; Caldovic et al. 2004; Kim et al. 1972).

N-carbamylglutamate has also been suggested as a treatment for hyperammonemia in organic acidurias (Tuchman et al. 2008a) and the drug is licensed by the EMEA for this indication.

With recommended doses, there are no reports on adverse effects but high doses can cause symptoms similar to those seen in Chinese restaurant syndrome (Bachmann et al. 1982). There are no studies on long-term safety.

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Outcomes: improvement of survival and prevention of complications

Recommendation #21: We recommend using N-carbamyl-L-glutamate as the first line medication for treatment of NAGSD and as an emergency drug during acute hyperammonemia of unknown etiology.
Quality of evidence: high

Carnitine for long-term treatment

The plasma carnitine status of all UCD patients should be monitored to detect and treat secondary carnitine deficiency.

The value of carnitine supplementation for inborn errors of metabolism in general has been reviewed without a clear recommendation for its use (Nasser et al. 2009). It remains unclear whether plasma carnitine levels correlate reliably to intramitochondrial carnitine levels so the relevance of any low plasma carnitine concentration remains controversial.

Benzoate as well as PBA conjugate to carnitine and can cause secondary carnitine deficiency (Feoli-Fonseca et al. 1996; Mayatepek et al. 1991; Ohtani et al. 1988) which may also be related to excess urinary loss (Ohtani et al. 1988) or to low protein and low carnitine diet.

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Antibiotics for treatment of hyperammonemia in UCD patients

Some members of this guideline group have used neomycin or metronidazole to decrease the load of ammoniagenic bacteria in the colon; however, there are only anecdotal reports but no studies and, thus, no recommendation can be made on this.

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8.4 Special situations: vaccinations and surgery & anesthesia

Vaccinations

Since an elevated body temperature is a common side-effect of vaccinations, UCD patients might be at particular risk of metabolic decompensation due to the resulting catabolism. However, during a recent workshop on OTC females (dealing with 318 decompensations in 110 patients), vaccination was only reported in one patient as the assumed trigger of metabolic decompensation (Häberle and Lachman 2010). The same finding was reported from a retrospective investigation from the US longitudinal study (Morgan et al. 2011).

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Outcome: prevention of complications

Recommendation #22: We strongly recommend performing vaccinations following the national schedule.
Quality of evidence: moderate
We suggest antipyretic treatment if temperature exceeds 38°C.
Quality of evidence: low

Ammonia monitoring during surgery & anesthesia

UCD patients have, theoretically, a high risk of acute metabolic decompensation during surgery and general anesthesia (Berry and Steiner 2001).
It is recommended to perform elective surgery only in patients free of intercurrent illnesses and only in centres with a metabolic department including emergency treatment options such as continuous hemofiltration and the possibility of total parenteral nutrition. Preoperative ammonia and amino acid levels should be in the normal range. Starting on the day before surgery, the pharmacological treatment should be switched to IV applications and anabolism be secured by IV 10% glucose administration. UCD patients should be scheduled to be first on the operating list. General anesthesia has been reported to be safe with midazolam, s-ketamine, fentanyl and isoflurane in combination with surgical field infiltration with ropivacaine (Schmidt et al. 2006). Post-surgical close monitoring of the clinical status and of ammonia is required; the shift to oral medications and disconnecting the IV glucose administration should be done only in a stable metabolic situation.

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Outcomes: prevention of complications and management during surgery and anesthesia

Recommendation #23: We suggest performing elective surgery in UCD patients in centres with a metabolic expertise and resources including emergency treatment options for hyperammonemia.
Expert opinion

9 LIVER TRANSPLANTATION FOR UCD PATIENTS

Liver transplantation has been performed in all UCDs except NAGSD. Successful OLT allows for normalization of the diet and withdrawal of alternative pathway therapy (Kim et al. 2013; Whitington et al. 1998). The pre-operative management before liver transplantation should rigorously avoid catabolism.
In patients with HHH syndrome presenting with acute fulminate liver failure, early diagnosis and medical management have avoided the need for urgent liver transplantation (Fecarotta et al. 2006; Mhanni et al. 2008). While there are less than 10 reports on OLT in CPS1D, ASSD, ASLD, and ARG1D, respectively, more than 40 liver transplantations in patients with OTCD can now be found in the literature. The overall published survival of UCD patients after liver transplantation is the same as in patients transplanted for other disorders (Leonard and McKiernan 2004; Morioka et al. 2005a) but single centre experiences are less favourable (personal communication from Paris centre).
The therapeutic consequences of liver transplantation (e.g. immunosuppression) and the long-term outcome can be predicted to some extent while the prognosis for medically treated surviving UCD patients depends often on non predictable factors (severity and frequency of catabolic hyperammonemic crises by intercurrent infectious diseases or emergency surgery, delay of efficient intervention). Thus, unpredictable outcome is muted by liver transplantation to a standard situation leading to a better quality of life and less anxiety on long-term.
However, it should be realized that expression of cytosolic UC enzymes is not restricted to liver and intestine as is the case with the mitochondrial UC enzymes. ASS and ASL play an important role in brain (for recycling citrulline to arginine → creatine synthesis) and kidney (arginine → GAA → creatine). Thus, liver transplantation helps to prevent hyperammonemic crises but other long-term consequences of the deficiency in brain and kidney may not be prevented. Adequate substitution of L-arginine might still be needed in ASSD and ASLD.

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While liver transplantation is effective in preventing further hyperammonemic decompensations, previously lost neurological milestones have only been regained in single cases because pre-existing brain damage is likely to be

irreversible (Kawagishi et al. 2005; Kim et al. 2013). The majority of patients so far remain developmentally delayed but preserve their pre-transplantation neurodevelopmental level (Busuttil et al. 1998; Ensenauer et al. 2005; Fletcher et al. 1999; McBride et al. 2004; Newnham et al. 2008; Perito et al. 2014; Santos Silva et al. 2001; Stevenson et al. 2009).

While liver transplantation is effective in preventing further hyperammonemic decompensations, previously lost neurological milestones have only been regained in single cases because pre-existing brain damage is likely to be irreversible (Kawagishi et al. 2005; Kim et al. 2013). The majority of patients so far remain developmentally delayed but preserve their pre-transplantation neurodevelopmental level (Busuttil et al. 1998; Ensenauer et al. 2005; Fletcher et al. 1999; McBride et al. 2004; Newnham et al. 2008; Perito et al. 2014; Santos Silva et al. 2001; Stevenson et al. 2009).
While liver transplantation is effective in preventing further hyperammonemic decompensations, previously lost neurological milestones have only been regained in single cases because pre-existing brain damage is likely to be irreversible (Kawagishi et al. 2005; Kim et al. 2013). The majority of patients so far remain developmentally delayed but preserve their pre-transplantation neurodevelopmental level (Busuttil et al. 1998; Ensenauer et al. 2005; Fletcher et al. 1999; McBride et al. 2004; Newnham et al. 2008; Perito et al. 2014; Santos Silva et al. 2001; Stevenson et al. 2009).

Outcome: improvement of survival and cognitive outcome

Recommendation #24: We recommend to consider liver transplantation in patients with severe UCDs without sufficient response to standard treatment, without severe neurological damage and ideally whilst in a stable metabolic condition.
Quality of evidence: moderate

9.1. Long-term neurological outcome after liver transplantation

While liver transplantation is effective in preventing further hyperammonemic decompensations, previously lost neurological milestones have only been regained in single cases because pre-existing brain damage is likely to be irreversible (Kawagishi et al. 2005; Kim et al. 2013). The majority of patients so far remain developmentally delayed but preserve their pre-transplantation neurodevelopmental level (Busuttil et al. 1998; Ensenauer et al. 2005; Fletcher et al. 1999; McBride et al. 2004; Newnham et al. 2008; Perito et al. 2014; Santos Silva et al. 2001; Stevenson et al. 2009).
Some authors advocate for early or pre-emptive liver replacement on the basis that most often neurological damage is acquired during acute phases, the latter decompensations being difficult to predict. The threshold for decision-making in favour of transplantation should not be set too high (and too late), to take into account the major recent advances made in either long-term immunosuppressive management or prevention of technical problems and improved outcomes.

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9.2. Quality of life after liver transplantation

Most large pediatric liver transplant programs nowadays reach excellent patient outcome with 95%-100% one-year and 90%-100% 5-year patient survival rates (Bourdeaux et al. 2007; Kim et al. 2013).
Only few studies dedicated to pediatric series are published, most showing that patients investigated have a “good” or “excellent” quality of life 6-121 months post transplant when non-standard scales were used (Alonso et al. 2003; Bucuvalas et al. 2003; Campeau et al. 2010; Midgley et al. 2000; Morioka et al. 2005a). There is no data currently available on quality of life over 10 years after transplant.

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9.3. Indications and ideal age for liver transplantation

In patients with severe neonatal onset UCDs, especially CPS1D and male neonatal OTCD, liver transplantation may be performed as early as possible. In UCD patients suffering from recurrent metabolic decompensations with need for hospitalizations despite medical therapy or in UCD patients with difficult social circumstances resulting in poor compliance, liver transplantation should be considered before irreversible neurological damage is present.

A similar approach is considered by some teams for selected children with cytosolic UCDs. The latter are often older in age or teenagers, with major difficulty to comply with strict diet or presenting severe collateral effects on growth, school attendance, psychological situation and familial or social integration, respectively.
Among OTC variants there is (in addition to the neonatal period) a second lethal peak between 12 and 15 years of age (Bachmann 1992). Therefore, liver transplantation should also be considered in heterozygous female OTC patients who are symptomatic during the 2 first years of life.
Some authors suggested analysis of liver OTC enzyme activity as an indicator for the need and timing of liver transplantation (Wakiya et al. 2012) but the results may be less clear in heterozygous OTCD females.

Liver transplantation has been reported in acute encephalopathy and/or acute liver failure but these are high risk situations. No general recommendation can be given at present as these situations require case by case discussion if the patient's situation allows.
Liver transplantation was also reported in late-onset CPS1D patient with good outcome allowing resumption of work (Bates et al. 2011).

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Patient survival is improved with increased age at transplantation: the 5-year patient survival rate was 88% for children with UCDs who were <2 years old at transplant and 99% for children who were ≥2 years old at transplant (p=0.006; total number of patients: 186) (Perito et al. 2014). If possible, liver transplantation in UCD patients should be performed not before 3 months of age because of higher rates of complications and a lower survival rate if liver transplantation is done below age 3 months and/or below 5 kg bw (Noujaim et al. 2002; Sundaram et al. 2003).
The patient should be fully immunised. Regarding the neurological outcome, patients transplanted before 1 year as opposed to after 1 year might benefit more (McBride et al. 2004).

Outcomes: improvement of survival and cognitive outcome and quality of life

Recommendation #25: In patients with neonatal onset (except NAGSD), we strongly recommend liver transplantation before irreversible neurological damage. Transplantation between 3 and 12 months of age and when body weight exceeds 5 kg is associated with a more favourable outcome.
Quality of evidence: moderate
We strongly recommend considering liver transplantation in patients with severe progressive liver disease and/or with recurrent metabolic decompensations requiring hospitalisations despite standard medical therapy.
Quality of evidence: high

9.4. Recommended types of donor and of transplant

OLT is the standard procedure and therefore recommended. Auxiliary liver transplantation has been performed in some patients but was associated with a high rate of complications (Morioka et al. 2005a).
In most cases cadaveric organs were used but results of living related transplantations are comparable albeit burdened by a small risk of morbidity to donors. In living donor transplantation the donor can be screened thoroughly and the graft quality and function might be better than in cadaveric organs. With elective surgery the recipient will be in the best clinical condition and metabolically stable (a condition that is not always obtained when transplant is organized as urgent intervention when a post-mortem donor is available). It is also a way to obtain a graft in a short period of time when usually small-size candidates are waiting for many months on the waiting list for grafts from post-mortem donors (Bourdeaux et al. 2007).
In living related transplantation, heterozygosity seems not to be a problem and even asymptomatic OTC heterozygotes have been successful donors while symptomatic heterozygous donors should not be considered (Kasahara et al. 2014; Morioka et al. 2005b; Nagasaka et al. 2001; Wakiya et al. 2011). However, experience is small and heterozygous OTC females carry in addition an increased risk of morbidity and should therefore be considered as donors only with utmost caution.

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9.5. Ethical considerations related to liver transplantation

The decision on liver transplantation is influenced by medical and ethical considerations. One issue is the dilemma of donors in living related transplantations. Another difficulty is related to the decision in severely handicapped children.

10. MONITORING

Clinical and biochemical monitoring depends on age and metabolic stability of the patient. Infants will need more frequent monitoring and adjustment of their diet and treatment than older stable patients.
In practice, young and severely affected patients should be reviewed at least every 3 months while older or less severely affected patients may only need annual reviews.

Clinical and nutritional monitoring
Clinical monitoring should include regular follow-up of growth, head circumference and clinical examination of skin (looking specifically for signs of protein deficiency such as skin rashes) and hair (looking for thin, sparse hair or hair loss) during childhood, puberty and adolescence. Also, ultrasound of the liver for liver size and structure assessment (especially in defects of OTC, ASS, ASL, and ARG1 and in HHH syndrome) should be done. Furthermore, neurological examination and regular neurocognitive development assessments by a child psychologist should be performed at regular intervals depending on the clinical situation of the patient.
Children on low protein diets are at risk of protein, nutrient and energy deficiencies. Regular dietary assessments are therefore essential, either by recall or in the case of suspected nutritional problems a 3 or 5 day detailed diet history and supplement/drug history prior to the clinic visit is recommended. It is important to check compliance with EAAs, vitamin and mineral supplements.
The diet needs to be regularly adjusted for age and growth taking into account the biochemical results (see below).

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Biochemical monitoring
The aim is to assess if dietary treatment and drug dosages are adequate in view of the needs of the individual patient which change with age.
Interpretation of any result should always take into account not only concentrations of ammonia, plasma amino acids and urine orotic acid but in addition anthropometric data, dietary intake and frequency of use of the emergency regimen for illness and compliance with diet and medicines.

Studies of bone density
Every patient on a low protein diet may have an increased risk for osteoporosis and should be monitored accordingly. However, there are no studies of this in UCDs.

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Outcomes: achievement of metabolic stability and of normal growth and weight

Recommendation #26: We strongly recommend for all UCD patients regular clinical, biochemical and nutritional monitoring by a multidisciplinary metabolic team following individualised schedules.
Expert opinion

10.1. Monitoring in plasma

Ammonia
Ammonia measurements are important both during acute decompensations and long-term follow-up. In particular, fasting ammonia levels correlate strongly and positively with the risk and frequency of hyperammonemic crises, suggesting that UCD patients benefit from tight ammonia control (Lee et al. 2015). Recommendations for the techniques of ammonia collection and measurement have been published (Bachmann 2014; Barsotti 2001). The aim is to keep plasma ammonia levels in the normal range (50 µmol/L is the upper normal limit with the enzymatic method beyond neonatal age) but at least below 80 µmol/L (Feillet and Leonard 1998; Leonard and Morris 2002).
Use of venous blood samples is not exact but still helpful whilst capillary blood samples can produce a false-high result by contamination with intracellular fluid and perspiration. Another concern is related to false high ammonia values due to « difficult » sampling, use of tourniquet, crying or struggling.
In some centres, an ammonia profile is routinely evaluated during a 24 hours hospital stay, e.g. before and after several meals including after an overnight fast, but the benefit of this approach needs further evaluation.

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Micromethods for ammonia measurement

Bed-side ammonia measurements require only small amounts of whole blood (20-100 µl, depending on the method used) and yield a rapid result within 5 minutes. For the reasons mentioned below, bed-side methods are recommended only to be used in hospital allowing for a rapid result. The use of bed-side methods by parents or families based on capillary blood sampling is not recommended because of the inaccuracy of the method.
Another limitation can be the upper limit of linearity which is around 280 µmol/L (in case of Ammonia Checker II) (de Keijzer et al. 1997). New devices are however in development that may, at least partly, address the current unsolved issues (Ayyub et al. 2015).
Importantly, all UCD patients should be seen at a hospital if hyperammonemia is suspected and parents should be taught to recognise hyperammonemia clinically at the earliest stage.

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Amino acids

Monitoring of plasma amino acids at regular intervals is recommended in order to adapt the dietary and pharmacological treatment to the individual requirements of each patient (Bachmann 2014).
In UCD patients, glutamine levels at the upper limit of reference and low BCAA levels are a frequent finding (Bachmann 2008). Glutamine as part of the amino acids profile is helpful for assessing the waste nitrogen load in UCD patients although fasting glutamine was found to correlate weakly with daily ammonia exposure and was in one study not a significant predictor of hyperammonemic crisis (Lee et al. 2015). In addition, amino acid profiles are required to monitor levels of UC substrates given to patients (citrulline and/or arginine).
In treated patients it has been shown that, presumably due to prolonged catabolism during the night, plasma glutamine levels are higher early in the morning than during the day (Brusilow 1991). Thus, time of sampling is important and it is recommended to draw blood preprandially with a documented time delay after the end of the last meal of at least 3-4 hours (Bachmann 2014). As a minimal requirement, sampling should always be done at the same time to allow comparison with previous results (Bachmann 2008; Duran 2008).
The aim is to keep plasma EAA levels within the normal range and plasma glutamine levels in the normal range but at least below 1000 µmol/L (Ahrens et al. 2001; Feillet and Leonard 1998; Leonard and Morris 2002; Maestri et al. 1992). Likewise, levels of plasma arginine should be in the upper normal range.
However, this aim is quite « arbitrary » and there's no firm evidence that patients with glutamine levels slightly above 1000 µmol/L are doing worse than those with levels below 1000 µmol/L. It should be kept in mind, that plasma glutamine is not directly influencing the brain glutamine concentration, because the latter is synthesized mainly within the brain. See also Neuroradiology in chapter 10.3.
Plasma amino acids are also used to monitor nutritional status. If EAAs or BCAAs are low, a small increase in natural protein or EAAs supplement may be necessary. This decision should be made taking into account any other factors (such as inadequate protein intake, growth spurt, following illness etc.) which may have resulted in low levels. Nitrogen scavengers may also need to be adjusted at the same time.

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Outcomes: achievement of metabolic stability and of normal growth and weight

Recommendation #27: We recommend for longterm management as target level for ammonia <80 µmol/L, for glutamine <1000 µmol/L, for arginine in the high normal range and for EAAs and BCAAs in the normal range.
Quality of evidence: high

Other parameters

Assessment of vitamins, minerals, trace elements, carnitine, ferritin, cholesterol, and triglycerides in plasma should be considered at regular intervals depending on the degree of protein restriction.
Creatine should be assessed especially in patients with OTCD, ASSD, and HHH syndrome, as in these disorders low creatine concentrations were found along with other changes in creatine metabolism (Boenzi et al. 2012) but the practical consequences of a found low creatine are unclear.
Plasma urea concentrations depend on the arginine supplements (or citrulline) as well as arginine produced by cellular protein catabolism. These also depend on the tubular urine flow rate; therefore urea is not a useful parameter in UCD patients.
Further disease related tests are mentioned in Part II under detailed recommendations.

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Value of drug levels for monitoring UCD treatment

Measurement of drug plasma levels of nitrogen scavenging agents (i.e. sodium benzoate, sodium or glycerol PBA/phenylacetate) is currently under evaluation in studies and is only available in some metabolic centres. It may be helpful to measure drug levels in patients receiving high dosages or repeated boluses (MacArthur et al. 2004).

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10.2. Monitoring in urine

Spot urine

Presence of ketone bodies in urine can indicate persistent catabolism, may guide adjustment of energy intake and can be easily done at home.

24h urine collections are laborious and error prone (missed portions or inclusion of two morning urines). Instead, the second voiding in the morning should be used because creatinine concentrations are close to the daily mean.

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Orotic acid and orotidine

The value of orotic acid and orotidine measurements in urine is controversial. If performed, it might serve as indicator of long-term metabolic stability but there is no firm scientific basis for this, as yet.

In patients with OTCD and HHH syndrome, an increasing orotic acid may indicate carbamylphosphate accumulation and hereby whether there is excessive ammonia load or if the citrulline substitution does not suffice.

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Amino acids

Quantitative amino acid analysis in urine is not recommended for monitoring.

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Other parameters

Measuring of hippurate in urine is a method to assess compliance in patients receiving benzoate.

Urinary phenyacetylglutamine (PAGN) was shown in patients treated with GPB to be a useful clinical biomarker for determining the dose and for monitoring (Lichter-Konecki et al. 2011; Mokhtarani et al. 2012).

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10.3. Role of neuroimaging

In UCDs, pattern and extent of brain MRI abnormalities vary depending on the stage of disease and correlate with neurological outcome (Bireley et al. 2012). Improvement of neuroimaging methods can potentially have a critical role in clinical monitoring and treatment (Gropman et al. 2013).

Brain MRI may have prognostic value in neonatal UCDs (Bireley et al. 2012; Gropman 2010; Gunz et al. 2013). Early and intensive management of hyperammonemia resulted in a single case in improved MRI findings in the acute setting (Ruder et al. 2014). The role of follow up imaging is still unclear in the literature.

In acute metabolic derangement in children, small and large areas of abnormal signal intensity are seen in the brain, most often involving both cortex and underlying white matter, giving them an **infarct-like aspect** (with restricted diffusion). Lesions are often multiple and asymmetrical (Majoie et al. 2004; Takanashi et al. 2003). In addition, “neuroimaging can provide information about the timing, extent, reversibility and possible mechanism of neural injury in a non-invasive manner and can be used as an adjunctive measure to predict clinical and neurocognitive outcome” (Gropman 2010). Different distinct patterns may exist that correlate with outcome: A central and focal pattern of involvement limited to the basal ganglia, perirolandic regions, and internal capsule may

predict a better outcome; diffuse involvement of the cerebral cortex, internal capsule, basal ganglia, and variably thalami and brain stem may predict worse outcome (Bireley et al. 2012; Gunz et al. 2013).
Some authors mentioned that the cerebellum and the occipital lobes are relatively spared. In some cases a single hemisphere is totally involved. However, in other patients almost the whole brain is affected. Some conditions leading to acute hyperammonemia such as citrullinemia or OTCD display rather similar MRI patterns.
However, more data are needed to understand the predictive value of neuroimaging after acute hyperammonemia, but it can be expected that MR imaging and spectroscopy will ultimately be useful for monitoring and evaluation of neuroprotective strategies (Braissant et al. 2013).
In chronic hyperammonemia, defective myelination and **progressive cerebral atrophy** are seen. Some patients have some non-specific punctate white matter hyperintensities.

Necker-Enfants Malades experience: In neonates, neuroimaging shows severe brain swelling. MRI shows **diffuse cerebral edema** and may demonstrate involvement of the basal ganglia with a high intensity signal in the caudate nucleus, putamen, and/or globus pallidus on T2-weighted images. The deep sulci of the insular and perirolandic region may also display T1 shortening (Takanashi et al. 2003). MRS could contribute by showing highly elevated glutamine levels (Choi and Yoo 2001; Connelly et al. 1993) and this is even helpful in detecting subtle changes in OTC females (Gropman et al. 2008; Gropman et al. 2010). The basal ganglia are often prominently involved as well, but the thalamus, brainstem, the occipital region and the cerebellum tend to be relatively spared. After a few months, surprisingly, a very moderate residual hypersignal on insula and rolandic region may be seen.

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Practical considerations

1. MRI should be performed systematically during each episode of coma or stroke-like event between day 1 and day 4 (in order to have a good interpretation of the diffusion apparent coefficient).
2. MRI should be performed in the follow-up, at least in patients not requiring anesthesia: each 2 years in order to correlate the motor and language development, cognitive scores and the brain imaging results.
3. MRI sequences required: Diffusion tensor imaging, axial T2 and FLAIR, sagittal and axial T1, and MRS.

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Outcomes: prognostic markers for acute and long-term management

Recommendation #28: We recommend brain magnetic resonance imaging, if possible together with spectroscopy, in UCD patients, even in the absence of neurological and/or cognitive impairment, as this may help to adjust treatment. Timing should be decided on a case by case evaluation.
Quality of evidence: moderate

10.4. Psychological aspects in the clinical care for UCD patients

The cognitive outcome of patients with UCDs depends predominantly on the extent and duration of hyperammonemia (Gropman et al. 2007). Children presenting with neonatal onset have poorer outcome concerning cognitive, adaptive and behavioural functioning.
In 26 survivors of neonatal hyperammonemic coma, mean intelligence quotient (IQ) was 43 and 79% of patients presented with mental retardation (Gropman and Batshaw 2004). No surviving UCD patient with an initial plasma ammonia level >300 µmol/L or a peak ammonia level > 480 µmol/L had normal psychomotor development (Bachmann 2003c).
Basic intelligence testing was within the range of moderate or severe intellectual disability in 50% of 33 patients with neonatal onset compared to 25% of 59 patients with late onset of disease. Analysis of the different UCD revealed that 33% of the patients with ASSD had an average or above average IQ compared to 40% in ASLD and 66% in OTCD (81% of the OTC patients presented with a late disease onset) (Krivitzky et al. 2009). Nevertheless even in patients with late onset OTC and normal IQ, deficits in executive function and motor planning and execution have been shown (Drogari and Leonard 1988).
The relationship between peak blood ammonia levels during the first hyperammonemic crisis and brain involvement in 151 neonatal patients seems to be linear: the absence of mental retardation and changes in brain images was recorded in 64% of patients with ammonia < 180 µmol/L in contrast to 8% of patients with initial ammonia concentrations > 360 µmol/L (Kido et al. 2012).

Behavioural and emotional problems were increased in 52 patients with UCD from the European Registry and Network of Intoxication type metabolic diseases (E-IMD) registry and highly associated with intellectual functioning (Jamiolkowski et al 2016).

A specific neurocognitive pattern has been described in heterozygous OTC females with average IQ results. They presented with significant strengths in verbal intelligence, verbal learning, verbal memory and reading in contrast to weaknesses in fine motor dexterity/speed (significant) and non-verbal intelligence, visual memory, attention/executive skills and mathematics (non-significant). These results have been interpreted as reflections of a selective vulnerability of the white matter. Clinically asymptomatic outperformed symptomatic OTC heterozygotes and higher residual activity of urea synthesis as measured by stable isotope studies predicted better performance. Neither the allopurinol loading test results nor the mutation type correlated with neuropsychological performance (Gyato et al. 2004). In addition to examining IQ and development it seems crucial to adress these specific patterns of neuropsychological performance in UCD patients (Krivitzky et al. 2009).

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Outcome: neurological complications

Recommendation #29: We recommend testing for IQ, development and specific strengths/weaknesses in all patients, including those with milder disease or female OTCD heterozygotes. They may develop specific weaknesses in executive functions even if the IQ is normal.
Quality of evidence: moderate

Until recently, only little attention has been given to the psychological status of patients and families affected by inborn errors of metabolism including UCD. Most of the papers addressing this topic are reviews, uncontrolled studies or expert opinions**).**

Studies investigating health-related quality of life (HrQoL), psychological adjustment and adaptive functioning in UCDs are sparse and their comparability is limited due to the incoherence of methodological approaches and assessment instruments (Zeltner et al. 2014).
The general concept that parents of chronically ill children have low HrQoL (Hatzmann et al. 2008) must be considered in UCD patients/families because of the chronicity and disease-imposed stresses, perhaps amplified by possible low awareness-related delays in diagnosis and treatment (Cederbaum et al. 2001; Jamiolkowski et al. 2016). In children with movement disorders caused by inborn errors of metabolism, overall HrQoL was significantly reduced. Impaired adaptive functioning and severe movement disorder aggravated this effect (Eggink et al. 2014). A cross-sectional study including 10 children and 14 parents with intoxication-type inborn errors including UCD revealed lower “general well being “ and less leisure activities when compared to leukemia survivors. Parents physical QoL was lower compared to the general population and dietary constraints were a major issue (Fabre et al. 2013). In patient and caregiver focus groups, dietary restrictions and stigmatization / social exclusion were the factors considered most relevant for HrQuoL. Parents and patients stated a need for comprehensive information on disease and treatment mechanisms (Zeltner et al. 2017b).
Gastrostomy may in some cases improve parental QoL (Hatzmann et al. 2009). In a mixed sample of 82 adult metabolic patients including 7 UCD patients, patients requiring dietary treatment experienced impaired QoL e.g. in the physical dimension and with regards to independence (Cazzorla et al. 2012). In contrast, a normal HrQoL in the patients from the E-IMD registry, which was not correlated with intellectual or behavioural / emotional problems, was found (Jamiolkowski et al. 2016). However, this study relied on generic instruments for HrQoL measurement. Only very recently has a disease-specific assessment tool for HrQoL in UCDs and other intoxication type metabolic diseases been developed (Zeltner et al. 2017a), which may be a promising option to study aspects specifically relevant to patients with UCDs and their caregivers.

Although data are sparse at present, the recommendation for chronically ill patients and their families (Bosch et al. 2004; Brumm et al. 2010; Feillet et al. 2010) of routine monitoring of emotional, behavioral and psychosocial parameters should be applied to UCD patients to identify and prevent psychosocial maladjustment (Varni et al. 2005). Psychologists should be involved in patient care from the very moment of diagnosis to facilitate successful coping (Feillet et al. 2010) and to assess cognitive and psychological functions.

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Outcomes: quality of life and reducing the burden of disease

Recommendation #30: We recommend including psychological monitoring and counselling as an important component of the care of UCD patients and their families since health-related quality of life, anxiety, stress and psychosocial factors are important outcome parameters.
Quality of evidence: high

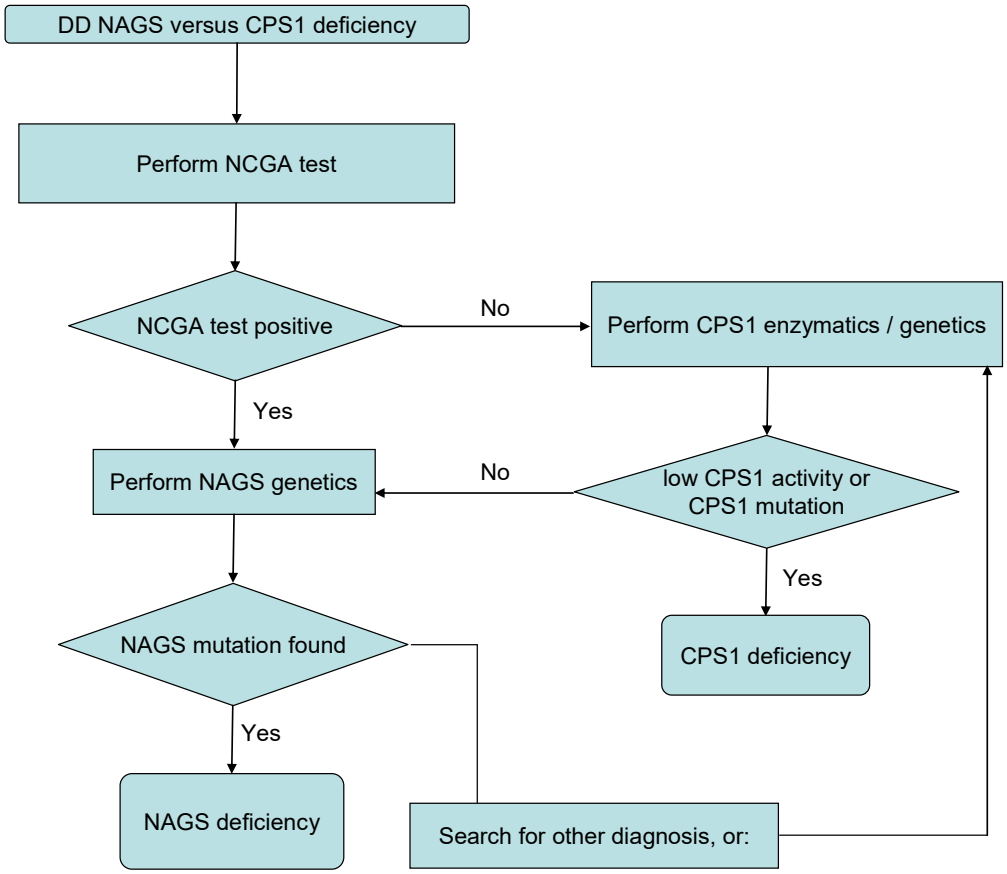
Part II: Detailed recommendations

11. NAGS AND CPS1 DEFICIENCY

Deficiencies of NAGS and CPS1 can not be differentiated on the basis of clinical presentation or standard laboratory investigations. Plasma amino acid profiles are comparable (showing elevated glutamine and often decreased citrulline and arginine). The concentration of urinary orotic acid is not increased. For this reason, the diagnostic work-up is described here together for both disorders.
For the differential diagnosis of neonatal onset deficiencies of NAGS versus CPS1, the so called NCGA test has been suggested (Guffon et al. 2005) but even a negative response to NCGA does not exclude NAGSD (Nordenstrom et al. 2007) while a positive response was also observed in patients with CPS1D (Ah Mew et al. 2010; Kuchler et al. 1996; Williams et al. 2010).

The following algorithm provides a recommendation how to proceed in patients with a biochemical profile suggestive of NAGS- or CPS1 deficiency.

Figure 2:



11.1. The role of enzyme analysis for diagnosis of NAGS or CPS1 deficiency

NAGS enzyme analysis requires at least 10 mg liver tissue (see Table 3).
In the past, a radiometric assay was used (Colombo et al. 1982) but in recent years a stable isotope-based assay became standard (Tuchman and Holzknecht 1990). Enzyme assays have been quoted as unreliable (Heckmann et al. 2005), although generally they appear to have yielded correct diagnoses. In some cases of NAGSD, a decrease in CPS1 levels was mimicking partial CPS1D.

NAGS activity (and the activity of other UC enzymes) is dependent on protein intake, which must be taken into account in the interpretation. Furthermore NAGS (and CPS1) activity decreases rapidly if the liver sample is not immediately frozen and kept frozen during the transport.

Colorimetric CPS1 enzyme analysis normally requires tissue from liver and is based on coupling with OTC (Nuzum CT and Snodgrass PJ 1976). Although the mucosa from the small intestine can also be used, the colorimetric method is at its limit of sensitivity with small mucosal samples, particularly when the CPS1 level is decreased (see Table 3). High-sensitivity radiometric analyses also exist that would be desirable for mucosal samples (Tuchman et al. 1989).
CPS1 liver levels below the normal range have been reported in some patients demonstrated genetically to have NAGSD (Caldovic et al. 2003; Caldovic et al. 2005) or the HIHA syndrome (Ihara et al. 2005). Thus, genetic confirmation must be sought when making a diagnosis of CPS1D on the basis of enzyme assay, particularly if there is substantial residual CPS1 activity in the liver (see also Table 3).

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11.2. Genetic analysis of NAGS and CPS1 deficiency

For suspicion of NAGSD, the main purpose of genetic analysis is to get a rapid and definite diagnosis to start or to continue specific therapy. Genetic analysis can be performed within a few days and is practically non-invasive and more specific than enzyme analysis (Heckmann et al. 2005). It is therefore recommended as the method of choice if NAGSD is suspected (Caldovic et al. 2007; Sancho-Vaello et al. 2016). Mutation analysis for NAGSD should include the known enhancer of the *NAGS* gene (Heibel et al. 2011).

For suspicion of CPS1D, molecular genetic analysis is recommended to establish or confirm the diagnosis unless there is a need for a rapid diagnosis which would require enzyme analysis (Häberle et al. 2011). In some labs, mutation analysis is performed on RNA from cultured fibroblasts (Häberle et al. 2003b) or from lymphocytes after special preanalytics (Kretz et al. 2012).

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Outcome: reliable and early diagnosis of NAGS and CPS1 deficiencies

Recommendation #31: We strongly recommend genetic analysis for the diagnosis of NAGSD and for CPS1D, since NAGS activity assay is not generally available and enzymatic diagnosis of CPS1D requires liver or intestinal mucosa, respectively.
Quality of evidence: high

11.3. Treatment of NAGS deficiency – special considerations

Primary NAGSD should be treated with N-carbamyl-L-glutamic acid (carbamylglutamate), which is a licensed drug in Europe and in the US (Bachmann et al. 1982; Guffon et al. 1995; Morris et al. 1998; Reigstad et al. 2017; Van Leynseele et al. 2014). Most patients on NCGA do, except in acute illness, not need additional drugs or a low protein diet (Cartagena et al. 2013).
There is currently no parenteral preparation of NCGA. The recommended oral/enteral loading dose of 100-250 mg/kg is followed by an initial daily maintenance dose of 100-200 mg/kg divided in (three to) four doses (based on a T ½ = 5-6 hours). The long-term daily maintenance dose should be adjusted to the minimum need according to ammonia levels and can be as low as 10-15 mg/kg bw (Belanger-Quintana et al. 2003; Gessler et al. 2010).
In one patient, a favourable long-term outcome was reported (Gessler et al. 2010) but there exists only a few long term data (Guffon et al. 2011) (however, the members of the working group for this guideline know of at least 20 NAGS deficient patients who are under treatment with carbamylglutamate and do have a good outcome).
In the acute phase, a single NAGSD patient was reported with no response to NCGA (Nordenstrom et al. 2007).
In a novel biochemically salvageable mouse model for NAGSD, which recapitulates the clinical phenotype of the disease, the combined treatment of NCGA and L-citrulline rescued NAGS knockout pups. Survival rate of Nags($^{-/-}$) mice after i.p. injections with NCGA and L-citrulline during the newborn period and p.o. later was 85%, allowing for normal development, apparent health, and reproduction (Senkevitch et al. 2012). Thus, L-citrulline may be a useful adjunct therapy to NCGA in NAGSD.

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Outcome: achieving metabolic stability in NAGS deficiency

Recommendation #32: We strongly recommend monotherapy with carbamylglutamate as the treatment of choice outside acute decompensations in NAGSD.
Quality of evidence: high

11.4. Treatment of CPS1 deficiency – special considerations

The role of NCGA was further investigated using recombinant pure human CPS1 (Diez-Fernandez et al. 2013). Hereby, it was shown that human CPS1 expressed in vitro is protected from protease degradation and thermal inactivation by NCGA in the presence of ATP. In another study using pure enzyme, protein misfolding was found in the case of several CPS1 mutants rendering chaperone treatment for CPS1D an interesting alternative (Diez-Fernandez et al. 2014). This possibly points towards a potential pharmacological chaperone effect of NCGA on CPS1 but needs further evaluation.
Treatment using NCGA was reviewed in (Daniotti et al. 2011; Häberle 2012) and was tested in five patients with late-onset CPS1D; in four patients, ureagenesis was improved and one patient showed marked improvement in nitrogen metabolism (Ah Mew et al. 2014).

12. OTC DEFICIENCY

Male OTCD patients manifest in their majority as neonates and belong to the group of UCDs with the highest mortality (60%) during their initial presentation; in female OTCD however, neonatal onset was only 7% in a large meta-analysis (Burgard et al. 2016). A tentative diagnosis of OTCD can generally be made based on clinical history and the biochemical findings (hyperammonemia, increased plasma glutamine and alanine concentrations, low plasma citrulline concentration, and excretion of urine orotic acid). Of note, unlike in all other UCDs, patients often have no consanguineous background. Enzymatic or genetic testing should be performed to confirm the diagnosis and/or plan future prenatal testing.

12.1. Enzyme analysis for diagnosis of OTC deficiency

OTC enzyme analysis requires liver tissue or small intestine (see Table 3) (Tuchman 1992), or, as recently described using an LC-MS/MS assay, plasma OTC activity can detect hemizygous males and the majority of symptomatic heterozygous females (Krijt et al. 2017). In males, liver or plasma enzyme activity analysis (Krijt et al. 2017; Nuzum CT and Snodgrass PJ 1976) is diagnostic in most cases and should be performed when no mutation is detected. In females enzyme analysis in small liver biopsies is unreliable due to random X-inactivation which can result in a mosaic distribution of residual enzyme activity preventing clear interpretation of the results: the finding of normal activity does not exclude deficiency. However, a very low activity is diagnostic.

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12.2. Genetic analysis of OTC deficiency

In OTCD, an X-linked disorder, mutation analysis can establish the diagnosis. However, detection rate in DNA-based mutation analysis for OTC is only 80%-90% (Caldovic et al. 2015; Yamaguchi et al. 2006). Alternative methods have been described to overcome this diagnostic difficulty (Engel et al. 2008; Shchelochkov et al. 2009). Compared to enzyme analysis in liver tissue, genetic testing is non-invasive and is therefore the method of choice.
Regarding genotype-phenotype correlation, there is increasing knowledge and a number of mutations are known to be associated with late onset of disease but for the majority of mutations this information is lacking (Brassier et al. 2015; Caldovic et al. 2015; Numata et al. 2008; Yamaguchi et al. 2006).

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Outcome: reliable and early diagnosis of OTC deficiency

Recommendation #33: We strongly recommend genetic analysis for diagnosis of OTCD. We recommend determining OTC enzyme activity assay in plasma, liver or intestinal mucosa if genetic analysis fails.
Quality of evidence: high

12.3. Investigations to diagnose suspected female OTC carriers

The most appropriate test to ascertain female carrier status is mutation analysis (Tuchman 1992). However, mutation analysis is not always informative. In the following, alternative investigations are discussed:

Allopurinol testing, Protein loading, and/or Pedigree analysis

Allopurinol testing
This test is safe and easy to perform and might add information in a suspected female OTC when interpreted with caution.
This test relies on the inhibition of orotidine-monophosphate decarboxylase, an enzyme of pyrimidine synthesis, by oxypurinol ribonucleotide, a metabolite of allopurinol (Hauser et al. 1990). Accumulated (mitochondrial) carbamoylphosphate will spill over to the cytosol and enter this pathway resulting in an increase of orotidine and orotic acid in urine of OTC deficient patients. Although the test was initially reported to be both 95% sensitive and 100% specific (Hauser et al. 1990; Maestri et al. 1998) there are concerns towards false-negative as well as false-positive results (Grunewald et al. 2004). Some other conditions, such as ASSD, ARG1D, lysinuric protein intolerance, and HHH syndrome have also been described to result in positive allopurinol testing (Tuchman 1990).
For children, age-related reference values have been developed but not for infants below 6 months (Burlina et al. 1992). More recently, interpretation of the test result based on normalization to the allopurinol dose has been suggested, but again there are only scarce data on newborns and young infants (Riudor et al. 2000).

Protein loading
Protein-loading tests were found to be non-specific, unpleasant, and potentially dangerous because they can lead to symptomatic hyperammonemia; thus, protein loading tests are not recommended (Brusilow and Horwich 2001). A modified protocol with shorter period of urine collection yielded an improved sensitivity by reference to the change in orotic acid excretion, i.e. the ratio of excretions 2-4 h/0-2 h (Potter et al. 2001).
Nevertheless, protein loading of patients suspected to suffer from any UCD can be dangerous and should not be done.
Pedigree analysis
In case of a positive family history, a woman may be identified as carrier by pedigree analysis.

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12.4. Treatment of OTC deficiency – special considerations

L-citrulline can be used instead of L-arginine for treatment of OTCD. This allows for an additional incorporation of a nitrogen molecule used for argininosuccinate formation. However, L-citrulline is not available as IV drug.

Treatment of clinical variants of OTC deficiency
Mild forms in adults (males or females) may only require preventive measures in emergency situations and no (or very moderate) restriction of dietary protein.
Other patients with variant OTCD may have acute decompensations with coma at any age and need long-term treatment and diet. However, the clinical signs may be very mild in such patients, even if ammonia is already elevated and glutamine levels are constantly above 1200 µmol/l. Thus, ammonia should also be monitored twice a year in these patients.

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Patients with recurrent liver failure
Some patients with OTCD may experience metabolic decompensations presenting with liver dysfunction including coagulopathy (Batshaw et al. 2014; Gallagher et al. 2014; Ihara et al. 2013; Laemmle et al. 2016) or fulminant hepatitis often associated to hyperammonemic encephalopathy (Mustafa and Clarke 2006).

In acute liver failure, UCDs in general and OTCD in particular should be considered as differential diagnosis. Patients should be managed in a centre that has the capacity for an emergency liver transplantation even though most of them may recover with metabolic management (Teufel et al. 2011).
Three OTCD patients were reported with recurrent pancreatitis; of these, one patient had an additional mutation in a gene associated with hereditary pancreatitis but it remained unclear how recurrent pancreatitis relates to OTCD (Prada et al. 2012).

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13. ASS DEFICIENCY (CITRULLINEMIA TYPE 1)

In general, the diagnosis of citrullinemia type 1 is straightforward with strongly elevated plasma citrulline levels and increased urine orotic acid in the absence of plasma argininosuccinate. In these situations, further confirmation might not be required unless future prenatal testing should be prepared.
ASSD can also present with acute liver failure (de Groot et al. 2011; Faghfoury et al. 2011).
There is a single case report on hypertrophic cardiomyopathy and cataracts in an adult patient with neonatal-onset citrullinemia but it remains unclear whether this is a complication of the disease, the long-term drug treatment or simply a coincidental finding (Brunetti-Pierri et al. 2012).

13.1. Enzyme analysis of ASS deficiency

Enzyme analysis for citrullinemia type 1 can be done using liver tissue or cultured fibroblasts (see also Table 3).
In the liver, enzyme analysis of ASSD is done with an ASL and arginase-coupled colorimetric assay (Nuzum CT and Snodgrass PJ 1976). ASL is no longer commercially available and thus older assays based on coupling with the endogenous ASL present in the homogenate (Brown and Cohen 1959) have to be used unless the laboratory can prepare its own ASL for supplementation. Because of this and of the less invasive sample procurement, indirect radiometric assays monitoring in cultured fibroblasts the incorporation into protein of radioactivity from ^{14}C-citrulline are more popular (Kleijer et al. 1984). However, since the plasma amino acid profile is diagnostic in most patients, confirmation of the diagnosis is only needed as basis for future prenatal testing (Engel et al. 2009).

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13.2. Genetic analysis of ASS deficiency

In ASSD, genetic analysis is recommended if confirmation of the diagnosis is required, for instance for future prenatal diagnosis. Rarely and only if biochemical results are not clear might it be performed to distinguish from citrullinemia type 2 (Citrin deficiency). In addition, certain genotypes are associated with a mild variant of citrullinemia type 1 allowing for decisions on the need of dietary therapy (Engel et al. 2009; Gao et al. 2003; Häberle et al. 2003a).

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Outcomes: reliable and early diagnosis of ASS deficiency and prenatal testing

Recommendation #34: We strongly recommend genetic analysis for diagnostic confirmation and for prenatal testing in citrullinemia type 1.
Quality of evidence: high

13.3. Treatment of ASS deficiency – special considerations

There are patients with mild citrullinemia of whom many were detected by newborn screening and never developed clinical symptoms (Häberle et al. 2003a). However, in single patients even fatal hyperammonemia occurred in severe catabolic circumstances (Berning et al. 2008; Enns et al. 2005; Gao et al. 2003). Thus, these patients might not need diet or drug therapy but should nevertheless be followed in metabolic centres and have an emergency protocol.

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14. ASL DEFICIENCY

ASLD is readily diagnosed by the characteristic metabolite pattern as there is no known differential diagnosis if argininosuccinic acid is found in plasma or urine. However, to estimate the level of residual enzyme activity can still have impact on the management of a patient (Hu et al. 2015b; Kleijer et al. 2002). Likewise, many affected families will ask for later prenatal testing. For these reasons, further investigations should be considered in all patients.
Besides having the greatest proportion of patients with poor cognitive outcome in several studies (Ah Mew et al. 2013; Martin-Hernandez et al. 2014; Rüegger et al. 2014), a unique but poorly understood feature in ASLD concerns the constant intellectual decline in patients who never experienced a hyperammonemic decompensation (Nagamani et al. 2012b). There are even patients known, who were prospectively treated based on positive NBS or family history and still suffered from severe neurological sequelae.
The extended (in comparison to the other urea cycle enzyme defects) phenotype may in part be explained by the fact that ASL is required for systemic nitric oxide (NO) production (Erez et al. 2011b). ASLD patients were reported to develop complications that were possibly related to systemic NO deficiency including arterial hypertension and neurocognitive deficits (Erez et al. 2011a). One patient, when receiving a NO donor (isosorbide dinitrate), benefited from this intervention and showed even improvement in some neuropsychological parameters (Nagamani et al. 2012a). A double blind, randomized, placebo-controlled, crossover study of NO supplementation in ASLD patients assessing endothelial function and blood pressure as primary endpoints is currently recruiting patients (ClinicalTrials.gov: NCT02252770).

14.1. Enzyme analysis of ASL deficiency

Enzyme analysis of ASLD can be performed in liver tissue with an arginase-coupled direct enzyme test (Nuzum CT and Snodgrass PJ 1976) and in skin fibroblasts with an indirect test in which the incorporation into protein of radioactivity from ^{14}C-citrulline is monitored (Kleijer et al. 2002). Both direct and indirect tests can be used for confirmation of the diagnosis if this is required. See also Table 3.

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14.2. Genetic analysis of ASL-deficiency

If future prenatal testing is requested, mutation analysis is recommended. Also, there is first evidence that some ASL mutations are associated with variant clinical courses so results of genetic testing can be of prognostic value (Balmer et al. 2014; Kleijer et al. 2002; Trevisson et al. 2007).

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Outcomes: reliable and early diagnosis of ASL deficiency and prenatal testing

Recommendation #35: We recommend metabolite analysis for confirmation of ASLD since presence of ASA in high concentrations in plasma or urine is diagnostic.
Quality of evidence: high
We strongly recommend genetic confirmation for family counselling and as method of choice for prenatal testing.
Quality of evidence: high

14.3. Treatment of ASL-deficiency – special considerations

In ASLD, L-arginine treatment alone might be sufficient (Brusilow and Maestri 1996) but this is currently subject to debate. There are concerns towards toxic side-effects of high dose L-arginine supplementation in ASLD based on hypothetical accumulation of argininosuccinate (Keskinen et al. 2008) which might – due to its nature as a tricarboxylic acid - become trapped within the brain (Kölker et al. 2006). These concerns have been substantiated in a double-blind, placebo-controlled, cross-over study comparing low-dose arginine (100 mg/kg/d) combined with PBA (500 mg/kg/d) with high-dose arginine (500 mg/kg/d) alone (Nagamani et al. 2012c). This trial showed that higher doses of arginine in subjects with ASLD result in increases in AST and ALT.

In addition, guanidinoacetate (van Spronsen et al. 2006), NO production and arginine itself may be increased during high-dose arginine therapy causing harm. Based on these considerations, the working group of this guideline recommends use of L-arginine dosages similar to other UCDs (see table 11). Plasma levels of arginine < 200 µmol/L were regarded to be safe (Berry and Steiner 2001).

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Outcomes: achieving metabolic stability in ASL deficiency and cognitive and hepatic outcome

Recommendation #36: We recommend against high-dose L-arginine supplementation in ASLD because of neurological and hepatic complications. We recommend using L-arginine for long-term management at the same dosages as for other UCDs in combination with nitrogen scavenges and protein restriction.
Quality of evidence: moderate

Monitoring of patients with ASL deficiency

Particular attention should be given to monitoring blood pressure levels as arterial hypertension was more commonly observed in ASLD (Brunetti-Pierri et al. 2009; Erez et al. 2011a).
Some centers monitor argininosuccinic acid in plasma and reduce protein intake if levels increase to 400-500 µmol/L. However, this approach has not yet been evaluated in studies.
In some patients with ASLD, elevated triglycerides were found and this may be associated with marked hepatomegaly (Dionisi-Vici C. and Leonard J., personal communications).
In addition, clotting factors (global tests and/or factor II) and liver enzymes should be regularly monitored to detect early the presence of liver disease.

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15. ARGINASE DEFICIENCY

ARG1D markedly differs from other UCDs because it usually does not present during the neonatal period and first symptoms occur between 2 and 4 years of age (Crombez and Cederbaum 2005; Scaglia and Lee 2006; Schlune et al. 2015). The main symptoms are progressive spastic paraplegia and often only during acute hyperammonemic episodes, hepatomegaly; seizures can also be the presenting symptom. Hyperammonemia is less frequent than in other UCDs but patients can have neonatal and/or recurrent hyperammonemic crises (Jain-Ghai et al. 2011; Scholl-Bürgi et al. 2008; Zhang et al. 2012).
ARG1D results in increase of plasma arginine in all patients but the levels may only be slightly elevated under treatment (Cederbaum et al. 1982). Therefore, normal or slightly increased arginine plasma concentrations do not exclude ARG1D. It is recommended to confirm the diagnosis by enzymatic or genetic analyses in every new patient. Urine orotic acid levels are often elevated.

15.1. Enzyme analysis for arginase deficiency

Activity of arginase can be analysed in red blood cells or in liver tissue. Arginase determination in erythrocytes is straightforward and may be more cost-efficient than genetic analysis (Tomlinson and Westall 1964). It is recommended to perform enzyme measurements using red blood cells if the diagnosis needs to be confirmed. See also Table 3.

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15.2. Genetic analysis of arginase deficiency

Genetic analysis is, as an alternative to enzyme analysis, recommended to confirm the diagnosis and offers in addition, if required, information for future prenatal testing.

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15.3. Treatment of Arginase deficiency – special considerations

Treatment of ARG1D follows the general UCD treatment but patients are less prone to metabolic decompensations and hyperammonemia is less common than in other UCDs (Crombez and Cederbaum 2005; Scaglia and Lee 2006). First reports were on dietary therapy alone (Cederbaum et al. 1982) but later sodium benzoate and sodium phenylacetate were introduced (Qureshi et al. 1984). Besides protein restriction, use of EAA supplements is invariably necessary to reduce the nitrogen load. Adherence to treatment is likely to halt the disease progress (Crombez and Cederbaum 2005) although arginine levels and protein restriction had no significant impact on long-term outcome in a retrospective study of 19 patients (Huemer et al. 2016).
Aim of treatment is to keep plasma arginine concentrations < 200 µmol/L which is extremely difficult to achieve (Schlune et al. 2015). In addition, it is not yet clear whether the dietary management of ARG1D is of any benefit to the patient if there is already spastic diplegia.
In some patients, levels of guanidinoacetate were found to be elevated but the role of guanidine compounds for the pathogenesis of ARG1D is not yet clear (Brosnan and Brosnan 2010; Crombez and Cederbaum 2005; Huemer et al. 2016).

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Outcomes: achieving metabolic stability in ARG1 deficiency and prevention of neurological complications and burden of dietary treatment

Recommendation #37: We recommend following standard UCD dietary and medical (without the use of L-arginine) treatment in ARG1D. We suggest adherence to a strict protein restriction to reduce plasma arginine levels to as low as possible aiming for the upper reference range.
Quality of evidence: moderate

16. HHH SYNDROME

HHH syndrome is due to a deficient ornithine transporter (ORNT1) of the mitochondrial membrane encoded by the *SLC25A15* gene (reviewed in (Martinelli et al. 2015). There is a typical metabolic profile comprising the name-giving triad with urine homocitrulline as specific marker allowing for diagnosis on the basis of just biochemical analysis (Palmieri 2008). However, if confirmation by another method is required or if prenatal testing is planned, functional analysis or genetic testing can be applied.

16.1. Functional analysis of ORNT1

Functional analysis is feasible using an indirect method based on the monitoring of ^{14}C-ornithine incorporation into protein in skin fibroblasts or liver tissue. In clinical practice, this indirect assay using cultured fibroblasts is suitable for confirmation in a patient but not for carrier testing (Shih et al. 1982). Patients having OAT deficiency (gyrate atrophy of the retina) also exhibit decreased ^{14}C-ornithine incorporation. See also Table 3.

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16.2. Genetic analysis of the SLC25A15 gene

Genetic analysis is recommended to confirm the diagnosis and, if required, to prepare future prenatal testing. Heterozygotes can only be detected by mutation analysis because biochemical parameters are normal.

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16.3. Treatment of HHH syndrome –special considerations

Treatment of HHH syndrome follows the same principles as for all UCDs: a low protein diet should be implemented with a supplementation of citrulline or arginine. This treatment has resulted in a stable metabolic situation and avoidance of hyperammonemia but could not prevent the development of spastic paraparesis or of cognitive deterioration (Dionisi Vici et al. 1987; Kim et al. 2012; Salvi et al. 2001).
Secondary creatine deficiency has been found in patients with HHH syndrome (as well as in OTCD and ASSD, but not in ASLD) (Boenzi et al. 2012; Dionisi Vici et al. 1987; Morini et al. 2009); therefore, monitoring plasma creatine concentrations to optimize the dose of arginine substitution should be done (Boenzi et al. 2012). If disturbed creatine metabolism is found, substitution of creatine may be considered but there are no studies in UCD patients reported.

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Outcome: achieving metabolic stability in HHH syndrome

Recommendation #38: We recommend low-protein diet and citrulline or arginine supplementation in HHH syndrome. The impact of these measures on pyramidal dysfunction is unclear.
Quality of evidence: moderate

17. FUTURE DEVELOPMENTS

Several animal models with inducible or hypomorphic phenotypes have been recently developed for some of the UCDs allowing, in contrast to the previous knock-out models that died early, testing of novel therapeutic strategies (Kasten et al. 2013; Perez et al. 2010; Sin et al. 2013).
Recent advances in the treatment of hyperammonemia were reviewed including all the presently existing treatment alternatives and of novel promising alleys (Matoori and Leroux 2015).

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17.1. Perspectives of molecular genetics in UCDs

Further genotyping of patients and correlation to their clinical and biochemical courses will improve the knowledge on the prognostic value of different mutations. Also, studies addressing the consequences of the putative structural changes of the mutant proteins will enhance our understanding as will *in vitro* expression and functional analysis of mutant proteins. Finally, disease- and mutation-tailored pharmacological approaches are needed. This underlines the impact of genetic analysis for future treatment of patients with UCDs.

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17.2. Role of specific neuroprotection

Acute and chronic hyperammonemia can result in “cytotoxic brain edema, cell death, impairment of neurite outgrowth, defects in nerve cell migration, or hypomyelination, in turn leading to brain tissue atrophy, ventricular enlargement, gray or white matter hypodensities and demyelination” (Braissant 2010). Novel neuroprotective strategies are currently subject of research investigations in cell culture and animal studies, including the use of MK-801 and other NMDA receptor antagonists, NOS inhibitors, creatine and acetyl-L-carnitine (Bachmann 2002; Braissant 2010; Braissant et al. 2002; Braissant et al. 2013; Felipo and Butterworth 2002; Hermenegildo et al. 1996).

Hypothermia is thought to reduce brain ammonia concentrations and cerebral blood flow but the exact mechanisms remain to be defined (Jalan and Rose 2004). Hypothermia in acute liver failure was shown to be effective against elevated intracranial pressure but the safety and efficacy of therapeutic hypothermia for neuroprotection still needs to be proven in randomized, controlled clinical trials (Rabinstein 2010).

In UCD patients, use of hypothermia was recently investigated in a pilot study (with historical case control) in seven acutely encephalopathic, hyperammonemic neonates with UCDs and OAs (6 UCDs and 1 OAs) requiring dialysis (Lichter-Konecki et al. 2013). Adjunct whole body hypothermia was applied in addition to standard treatment. Patients were maintained at 33.5 °C ± 1 °C for 72 h, then rewarmed by 0.5 °C every 3 h over 18 h. Adjunct therapeutic hypothermia was feasible and safe. This adds to an older case report on mild systemic hypothermia (rectal temperature of 34°C for 48 hours together with hemofiltration) which resulted in a striking fall in plasma ammonia in a neonatal patient with hyperammonemic coma (Whitelaw et al. 2001).
A non-randomized pilot study (phase 2) to test the safety and feasibility of hypothermia treatment as adjunct therapy to conventional treatment of hyperammonemic encephalopathy in neonates versus conventional treatment study was completed in May 2015 after enrolment of five patients (ClinicalTrials.gov Identifier: NCT01624311), but the results are not yet available.

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17.3. Cell therapies

Hepatocyte transplantation has been suggested in recent years as a therapeutical option also for UCDs (Dhawan et al. 2006; Enns and Millan 2008; Meyburg et al. 2009; Meyburg and Hoffmann 2010). Since 1997, only a small number of patients suffering from OTCD, ASSD, CPS1D or ASLD have been reported in the literature with some of them affected by serious complications (Horslen et al. 2003; Meyburg and Hoffmann 2008; Puppi et al. 2008; Stephenne et al. 2006; Stephenne et al. 2005; Strom et al. 1997). Recently, reviews were published on the first 100 patients receiving human hepatocytes for the treatment of liver diseases (Hansel et al. 2014) and on the experience of hepatocyte transplantation for metabolic liver diseases (Jorns et al. 2012).
Different treatment protocols are followed regarding the type of liver cells and the management: hepatocytes may be derived from a living donor (Enosawa et al. 2014), be fresh or cryopreserved, cell doses, routes of application and immunosuppression may vary (Burlina 2004). Hepatocyte transplantation was considered not only attractive with respect to organ shortage but also because it offers the option to bridge until a later liver transplantation (Meyburg et al. 2009).
As an alternative approach to hepatocytes, stem cells have been suggested and liver derived progenitor cells were reported to have some advantages over stem cells derived from other tissues (Sokal 2011). The track from the first hepatocyte transplantation in 2000 to products issued from stem cell technology, and the start of EMA approved clinical trials was recently reviewed (Sokal 2014). Adult human liver stem cells are available from a commercial company and have been reported to be administered portally to single patients (Sokal et al. 2013), proving restricted engraftment into the liver, conversion into adult hepatocytes with evidence for 3.3-fold increase in cell number with respect to the number of injected cells. A clinical phase 1 trial to “assess the safety and to appraise the efficacy of one cycle of heterologous human adult liver-derived progenitor cells infusions in pediatric patients” was completed in April 2015 (ClinicalTrials.gov Identifier: NCT01765283), but there are no results yet available.
Other alternatives, such as the use of peripheral stem cells from blood or mesenchymal stem cells from the umbilical cord, the use of extracorporeal liver-cell based support devices and organoid production or utilization of fibroblasts as progenitors of hepatocytes offer promising expectations that are however not close to use.

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17.4. Gene therapy

Studies aiming at gene transfer have been carried out on two OTCD animal models and on two citrullinemia animal models (Brunetti-Pierri et al. 2008; Lee et al. 1999; Moscioni et al. 2006; Patejunas et al. 1998). A highly successful animal study has resulted in long-term correction of OTCD by woodchuck hepatitis virus posttranscriptional regulatory element (WPRE)-mediated overexpression using a helper-dependent adenovirus (Brunetti-Pierri et al. 2008).
In humans, one adenoviral study for *OTC* gene therapy did not produce important increases in enzyme activity but caused inflammation and death to one enrolled subject (Raper et al. 2002; Wilson 2009). Another general concern of gene therapy is related to the risk of tumorigenesis (Zhong et al. 2013b). However, two case reports did not detect any Ad vector DNA in either tumor or normal tissue from the two patients who had participated in phase I gene therapy trial for OTCD (Zhong et al. 2013a).
More recently, AAV8-gene therapy of spf/ash mice showed the potential of prevention or treatment of hyperammonemia (Cunningham et al. 2011; Wang et al. 2012a). However, preliminary data suggest that re-

delivery of the vector after early neonatal treatment is likely necessary (Cunningham et al. 2013). Further improvement of the efficacy by optimized self complementary AAV2/8 or the use of transposon vector systems can be expected (Cunningham et al. 2015; Wang et al. 2012b).
The potential of AAV vectors in the developing liver was explored in different ASSD mouse models: lethal neonatal hyperammonemia was prevented by prenatal and early postnatal AAV8 or AAVrh10 vector delivery; however, hyperammonemia subsequently recurred limiting survival to no more than 33 days despite vector readministration unless antivector antibodies acquired in milk from vector-exposed dams were avoided (Kok et al. 2013). Another study with hepatotropic AAV8 vectors injected intraperitoneally in hypomorphic ASSD mice rescued >95% of the mice from lethality and survival was extended beyond 100 days after the single vector dose (Chandler et al. 2013).
For ARG1D, long-term survival and prevention of development of neurological abnormalities and cognitive dysfunction of the juvenile lethal Arg1d mouse with AAV gene therapy was shown (Lee et al. 2013; Lee et al. 2012). Similar encouraging results from this group reported that muscle-directed AAV10 gene therapy of Arg1 deficient mice resulted in a control of plasma arginine levels while animals remained hyperammonemic (Hu et al. 2014). The same authors showed that only minimal hepatic arginase activity (3.3%) is necessary for a sufficient ureagenesis to control plasma ammonia levels (Hu et al. 2015a). Another group showed for treatment of ARG1D, that use of AAV10 resulted in animal recovery in an inducible arginase knock-out mouse model (Ballantyne et al. 2015).
The field was reviewed in (Alexander et al. 2012; McKay et al. 2011; Viecelli and Thöny 2014).

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17.5. Enzyme replacement therapy

ARG1D is the obvious UCD in which enzyme replacement therapy would appear more likely to work since the reaction is merely hydrolytic. Frequent blood transfusion help to almost normalize plasma arginine (Mizutani et al. 1987); however, this does only change arginase activity levels in blood but not in the brain; blood transfusions are only useful for gaining time to install therapy.
Attempts carried out in the past with virus-encoded (Terheggen et al. 1975) or erythrocyte-contained arginase (Mizutani et al. 1987; Sakiyama et al. 1984) appear not to have been highly successful. Pegylated human recombinant arginase 1 (PEG-BCT-100) has been tested in a phase 1 clinical trial for cancer (http://clinicaltrials.gov/ct2/show/NCT00988195). This study was completed in August 2009 but no results have been posted on ClinicalTrials.gov.
Arginase-loaded erythrocytes (argocytes; no mention on the source and preparation of arginase) injected to mice with experimental hyperargininemia led to a rapid decrease in blood arginine concentration within 1 h and the effect persisted for at least 4 h (Kaminsky and Kosenko 2012).

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17.6. Experimental therapy and novel approaches

The **intestine** is an important source of ammonia, and novel approaches to reduce intestinal ammonia by stabilized acidic gels and/or even by administration of carbon nanospheres might offer promise.
Intestinal spherical carbon adsorbent (AST-120) was evaluated for its capacity to lower ammonia levels and to attenuate brain edema in a cirrhotic rat model of liver failure by biliary duct ligation (Bosoi et al. 2011). Administration of 0.1, 1 and 4 g/kg/day by gavage decreased ammonia levels and reduced water and ROS in the brain. Likewise, ammonia administration led to coma, which was prevented by 1g/kg AST-120. In conclusion, this activated carbon can efficiently remove ammonia from the intestine, but large doses are required.
Applying the theory of acid-base balance, acetic acid as water phase in a **water in oil microemulsion** was shown efficacious in removing colonic ammonia in healthy rats (Wang et al. 2011a).

The role of brain edema was challenged by novel data on cerebral acute ammonia toxicity, which was attributed to disrupted K+ buffering, with involvement of Na^+-K^+-$2Cl^-$ cotransporter and therapeutic efficacy of the inhibitor of this transporter and diuretic drug bumetanide. To study ammonia toxicity on neurons and astrocytes, two-photon imaging and electrophysiology was done in awake head-restrained mice showing that ammonia rapidly compromises astrocyte K^+ buffering, increasing extracellular potassium concentration and overactivating the Na^+-K^+-2Cl cotransporter isoform 1 (NKCC1) in neurons (Rangroo Thrane et al. 2013). **Inhibition of NKCC1 with the clinically used diuretic bumetanide** potently suppressed ammonia-induced neurological dysfunction. Astrocyte swelling or brain edema in the acute phase was not observed, calling into question current concepts regarding the

neurotoxic effects of ammonia. Instead, the findings identify failure of potassium buffering in astrocytes as a crucial mechanism in ammonia neurotoxicity and demonstrate the therapeutic potential of blocking this pathway by inhibiting NKCC1.

A protective effect of sildenafil on brain function of hyperammonemic rats through reversion of oxidative stress during hyperammonemia was reported (Arafa and Atteia 2013). However, the exact mechanism and potential clinical applications have to be still investigated.
As an alternative way of peritoneal dialysis, the role of **liposome-supported peritoneal dialysis** for detoxification of drugs and endogenous metabolites including ammonia was evaluated in rats with induced hyperammonemia (Forster et al. 2014).
Recently, mouse **liver repopulation with hepatocytes generated from human fibroblasts** was reported (Zhu et al. 2014) and this could be a technique with potential benefit also for UCD patients. However, this technique currently is experimental.

To explore the role of glutamine synthetase (GS) gene therapy for treating hyperammonemia, a baculovirus **containing the GS gene** was constructed for the in vitro and in vivo treatment of hyperammonemia; gene delivery for overexpressing GS in muscle tissue may be a promising alternative for the treatment of hyperammonemia (Torres-Vega et al. 2015).

Novel pharmaceutical approaches are reviewed in (Häberle and McCandless 2014).

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