Abstracts from the Conference

CLINICAL AND BIOCHEMICAL ASPECTS OF PRIMARY AND SECONDARY HYPERAMMONEMIC DISORDERS

JUNE 3, 2022

WARSAW, POLAND

ORGANIZERS
Mossakowski Medical Research Institute, Polish Academy of Sciences
Children's Memorial Health Institute

Conference program

Session I

9.30-9.50 **Biochemical background of ammonia neurotoxicity**
Dr M. Obara-Michlewska, MMRI PAS, Neurotoxicology Department, Warsaw, Poland

9.50-10.10 **Genetic background of hyperammonemias and the diagnostic approach in the era of next-generation sequencing**
Prof. M. Bik-Multanowski, JUMC, Cracow, Poland

10.10-10.30 **The clinical picture of primary hyperammonemias in Poland – an update**
Dr D. Rokicki, IPCZD, Warsaw, Poland

10.30-10.50 **Neuropsychological and cognitive deficits in the course of hyperammonemias**
Dr A. Barczak, MMRI PAS, Warsaw, Poland

10.50-11.10 **Imaging of hyperammonemia-associated brain damage**
Prof. O. Braissant, UNIL, Lausanne, Switzerland

Plenary lecture

11.30-12.30 **Present standards for diagnosis and treatment of congenital hyperammonemia and future prospects**
Prof. J. Häberle, University Children's Hospital Zurich, Switzerland

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12.50-13.10 **Primary hyperammonemias detected by newborn screening in Poland**
Prof. J. Sykut-Cegielska, IMC, Warsaw, Poland

13.10-13.30 **Treatment and therapy of metabolic disorders associated with hyperammonemia**
Dr D. Rokicki, IPCZD, Warsaw, Poland

13.30-13.50 **In search of a common denominator in the course of congenital hyperammonemias**
Dr A. Czarnecka, MMRI PAS, Neurotoxicology Department, Warsaw, Poland

13.50-14.10 **Liver transplantation in primary hyperammonemias**
Prof. I. Jankowska, IPCZD, Warsaw, Poland

14.10-14.30 **Polish experience with liver transplantation and post-transplant outcomes in children with urea cycle disorders**
Dr E. Szymańska, IPCZD, Warsaw, Poland

Preface

The international conference on congenital hyperammonemias, entitled "Clinical and biochemical aspects of primary and secondary hyperammonemic disorders", organized by the Mossakowski Medical Research Institute, Polish Academy of Sciences, and The Children's Memorial Health Institute, was held in the hybrid form on June 3, 2022, in Warsaw, Poland. The conference venue was The Children's Memorial Health Institute in Warsaw, and the lectures were simultaneously broadcast online. The meeting was attended by nearly 65 participants (# 101 registrations).

Due to the exceptional difficulty in diagnosing and treating metabolic diseases associated with hyperammonemia, we recognized the interest and importance in organizing a meeting to present the latest developments in this field. The intention of the conference organizers was to create a translational platform for knowledge exchange and discussion between geneticists, basic research scientists, and physicians of various specialties, including pediatricians, gastroenterologists, and transplantologists. Early diagnosis of hyperammonemia is crucial, as implementing appropriate therapy and proper nutrition minimizes deficits at the central nervous system.

The invited lecturers from Switzerland and Poland exhaustively covered research on the general basic and clinical aspects of a group of inborn diseases caused by a deficiency of one of the enzymes or transporters involved in the hepatic detoxification of nitrogenous waste. The event encompassed the emerging concepts in biochemistry and genetics presented in a clinical context, including modern diagnostic and therapeutic methods.

We were honored to welcome Professor Johannes Häberle from the University Children's Hospital in Zurich, an internationally recognized expert in congenital urea cycle disorders management, who delivered a plenary lecture focused on guidelines of congenital hyperammonemia diagnosis and treatment.

As may be concluded from the content of the abstracts, the first session lectures were devoted to the biochemical and genetic basis of hyperammonemia along with a discussion of available genomic diagnostics methods and the clinical picture of congenital hyperammonemia in pediatric patients in Poland. The second session started with a lecture on modern methods of imaging brain damage caused by ammonia toxicity, followed by therapeutic approaches, including pharmacotherapy, liver transplantation, and gene therapy. The symposium was closed with a short video clip showing the commercial offer of the MMRI PAS research services.

We are most grateful to all who, in various ways, supported the organization of this conference, and to all the participants in this event. The symposium was subsidized and patronized by the Polish Academy of Sciences. Financial support was also provided by Immedica Pharma, Recordati Group, and Nutricia Metabolics Research Fund.

We hope that this scientific event will create future scientific ideas by strengthening and sharing diverse areas for research. There is an urgent need to intense the forces in research targeting specific genes, the influence of environmental factors, and, consequently, the whole genome, epigenome, proteome, and reactome that are future challenges in neurological research. It is our intention to continue the meetings to promote the significance of the early diagnosis of hyperammonemia.

Organizing Committee

Prof. Magdalena Zielińska, Mossakowski Medical Research Institute PAS
Dr Dariusz Rokicki, The Children's Memorial Health Institute
Dr Marta Obara-Michlewska, Mossakowski Medical Research Institute PAS
Dr Anna Czarnecka, Mossakowski Medical Research Institute PAS

LECTURES

Biochemical background of ammonia neurotoxicity

Marta Obara-Michlewska

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Hyperammonemia, an elevated blood ammonia, clinically manifests mostly with neurological dysfunctions, involving impairment in responsiveness and behaviour, deficits in memory and learning or disturbances of the circadian rhythm.

Although ammonia physiologically is necessary for the nitrogen metabolism in the organism, in excess is toxic, mostly to the central nervous system (CNS). The brain is especially vulnerable to hyperammonaemia in the prenatal period of development and in early life, infancy and childhood. The aim of the presentation was to provide background for ammonia pathophysiology and to review how ammonia disturbs the brain metabolism, mitochondrial function, astrocyte volume regulation and neurotransmission.

Ammonia is detoxified in the liver, where in periportal hepatocytes operates the urea cycle, enabling incorporation of ammonia into urea, excreted by the kidneys. Some portion of ammonia is metabolised by the perivenous hepatocytes which synthetize glutamine (Gln) from ammonia and glutamate. Hyperammonemia may be classified into acquired or congenital. The acquired hyperammonemia results from a liver failure due to the e.g. hepatotropic viruses or certain drugs intoxication. The congenital hyperammonemia results from inherited dysfunction of any of the urea cycle enzymes or transporters, or from metabolic errors, causing urea cycle inhibition by toxic metabolites or by substrate deficiencies.

Regardless of the hyperammonemia etiology, ammonia exerts it neurotoxic effects by several overlapping mechanisms. Primarily, ammonia affects function of glial cells in the brain, astrocytes.

First, because astrocytes are a part of the blood brain barrier (BBB) – astrocytic endfeet cover the brain blood vessels in almost 100%. Secondly, astrocytes are almost exclusively endowed in enzyme glutamine synthetase (GS). Since the brain is devoid of the enzymes of the urea cycle, the GS is the only way of ammonia detoxification in the brain. The GS is one of the enzymes of glutamine-glutamate cycle, which enables the fundamental brain function – the excitatory (glutamatergic) and inhibitory (GABAergic) neurotransmission.

As Gln is an osmolyte, its accumulation within astrocytes, occuring upon ammonia-induced upregulation of GS, is thought to underlie the astrocytic swelling and consequent brain edema.

The Trojan horse hypothesis revise this idea and states that it is the Gln entering the astrocytic mitochondria and therein hydrolysed back to ammonia, and thus drives swelling, oxidative-nitrosative stress, mitochondrial permeability transition and respiratory chain failure. Of note, ammonia may directly inhibit the Krebs cycle enzymes. The osmotic imbalance is mechanistically intertwined with the ionic homeostasis. Some research indicate that potassium channels and water channel aquaporin 4 are involved in the pathomechanism of ammonia toxicity.

Further, ammonia contributes to the imbalance of the excitatory and inhibitory neurotransmission. Ammonia downregulates expression astrocytic glutamate transporters and therefore inhibits reuptake of glutamate from the synaptic cleft, contributing to the excitotoxic damage by the N-methyl-D-aspartate (NMDA) receptor overactivation and consequent oxidative stress induction. Moreover, ammonia, by impairment of the NMDA receptors function, inhibits the NMDA-NO-cGMP pathway, what may reflect the disturbed memory formation and cognitive decline, observed in patients suffering from hyperammonemia. The ammonia-induced NMDA receptors signalling impairment was observed not only in neurons but also in astrocytes.

The detailed discussion of other aspects of ammonia neurotoxicity, like interfering with intracellular pH, membrane electrophysiology, GABAergic, serotonergic, noradrenergic neurotransmission or neuroinflammation are beyond the scope of this lecture, that may cover only a portion of the available research.

The most studies that provide data on ammonia neurotoxicity come from different and varying, *in vitro* and *in vivo* rodent studies. The treatment of hyperammonemia requires fast and efficient normalisation of ammonia levels to prevent the damage to the CNS. Secondly, the reason why ammonia accumulates – acquired or congenital – must be identified, to prevent further hyperammonemic decompensations. The understanding of ammonia pathophysiological effects, supported by basic studies, contributes to the efficacy of hyperammonemia treatment and draws attention to the consequences of treatment delay or neglect, especially detrimental for paediatric patients. The traditional view pointed out at reversibility of ammonia-induced impairments, recently however it becomes more certain that neuronal loss and neurological and cognitive decline are permanent and correlate with hyperammonemia severity and duration.

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The clinical picture of primary hyperammonemias in Poland – an update

Dariusz Rokicki

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Urea cycle disorders (UCDs) include eight distinct conditions resulting from eight enzymatic defects. Two of them are caused by transporter dysfunction. Generally, these disorders are inherited autosomal recessive, except for OTC deficiency which has x-linked recessive inheritance and is the most common defect of UCDs.

The frequency of the separate defects varies between below 1 : 2 000 000 (N-acetylglutamate synthase deficiency, Ornithine translocase (ORNT1) deficiency, and citrin deficiency type II) to 1 : 56 500 for ornithine transcarbamylase deficiency (62% of all UCDs). The overall incidence of UCDs is 1 : 35 000. Incidence was calculated as a combination of data from newborn screening programs and ratios of individual conditions from natural history studies (Summer *et al*., 2013). The estimated incidence in Poland is not available as yet.

Assuming that a similar incidence is found in Poland, 163 patients with UCDs were/are expected in the period of the last 15 years. Considering all caveats and limitations, the overall incidence should provide a reliable number of expected patients. These numbers are an approximation, but let us figure out the scale of the problem.

The neonatal course applies to 29% of the patients, which gives us a working number of 47 patients.

The presentation includes the number of patients with diagnosed UCDs from a single centre (Children's Memorial Health Institute, CMHI), the only medical centre in Poland providing comprehensive medical care for UCDs' patients, including extracorporeal detoxification and liver transplantation (LTx). Some included patients are under care in other centres, with our participation past or present. Diagnoses are confirmed genetically or based on biochemical investigations. The limitation of the study is the lack of data from newborn screening.

The numbers of individual detected patients with UCDs are as following:

- OTCD 99 (symptomatic and asymptomatic); 34/99 male (34%) vs. 64/99 female (66%),
- ASLD 9,
- ASS1D (citrullinemia type 1) 5,
- Citrin deficiency 1,
- CPS1D 4,
- NAGSD 2,
- Arginase deficiency and Ornithine translocase (ORNT1) deficiency were not identified in Poland.

Summarizing:

- Probably UCDs are underdiagnosed, especially other than OTCD:
- Diagnosed patients 120,
- Expected number of patients 1990-2021 330 (11 pts/yr),
- Missed 210;
- A highly prevalent number of OTCD is created by two big families members carrying A208T mutation.

Neuropsychological and cognitive deficits in the course of hyperammonemias

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Accumulation of ammonia is the most important contributor to the pathogenesis of hepatic encephalopathy – a neuropsychiatric disorder that accompanies acute or chronic liver damage. It occurs due to impaired metabolism resulting from liver dysfunction and the development of porto-systemic shunting (less common in children).

The central nervous system injury is usually induced by the ammonia's neurotoxic activity, which disturbs brain functioning. Hepatic encephalopathy can cause disabling symptoms but is treatable; therefore, we must identify patients with this condition and use sensitive psychometric tests.

The mildest form of hepatic encephalopathy is described as covert or minimal. It is characterized by subtle motor and cognitive deficits affecting attention, speed of information processing, motor abilities, and coordination.

Its prevalence in adults with chronic liver disease ranges from 30-84%, and in the pediatric population is found in 57% of cases. Cognitive impairment in hepatic encephalopathy mainly affects attention, information processing, and psychomotor skills, causing difficulties in learning and memory, problems with goals' achievement, poor physical and sports performance leading to low grades, decreased self-esteem, and emotional burden, and behavioral problems in children. Adults suffer from insufficient attention, problems in memory, driving a car, and planning a trip. Their basic daily life activities are preserved, but there is a significant impairment of daily functioning, such as social interaction, alertness, emotional behavior, sleep, work, home management, and recreation.

In terms of clinical diagnosis, it is recommended to use the locally established methods, and a minimum of two tests are required to cover the domains of the impairment.

Due to unifying research reports among clinics, the Portosystemic Encephalopathy Syndrome Test is recommended for measuring complex cognitive functions such as attention, accuracy, working speed, and visual orientation. It is a paper-and-pencil test consisting of five subtests: digit-symbol test, line tracing test, serial dotting test, trail-making test A and trail-making test B. The completion takes 15 minutes and requires trained staff, but the test is based on language- and age-adjusted data available in many population.

Imaging of hyperammonemia-associated brain damage

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Ammonium ($NH4^+$) toxicity for central nervous system (CNS), in particular in the field of urea cycle diseases (UCD), can be life threatening and has to be managed as an urgency. Since more than five decades, research advances to better understand the effects of $NH4^+$ on both the developing and the mature brain, as well as to elaborate new treatments or protective strategies to prevent these often irreversible effects on CNS. Both *in vivo*, in the brain of human patients or of animal models, and through the use of *in vitro* (aggregates/neurospheres) or *ex vivo* (brain slices) organotypic cultures of brain cells, various imaging techniques allow to reveal and better understand the hyperammonemia-induced damage to the brain tissue, as well as to test neuroprotective strategies.

Among these, immunohistochemistry and immunofluorescence have allowed to understand how axonal growth is impacted by $NH4^+$ exposure and how creatine is neuroprotective in this context, looking at the axonal marker, phosphorylated medium molecular weight neurofilament (pNFM). Likewise, $NH4^+$-induced damage on astrocytes and oligodendrocytes, and their respective neuroprotection by creatine and ciliary neurotrophic factor (CNTF), could be approached by looking at the markers glial fibrillary acidic protein (GFAP) and myelin basic protein (MBP) or galactocerebroside. $NH4^+$-induced neuronal death by apoptosis through mis-activation of CDK5/p35, and neuroprotection by roscovitine, were also showed by immunohistochemistry against pNFM (neurons/axons) and activated caspase 3 (apoptosis). *In vivo*, MRI demonstrates the hyperammonemia-induced damage to the brain tissue (cell loss, defect of cell migration during development, ventricular dilatation, demyelinization), while magnetic resonance spectroscopy (MRS) allows the non-invasive measure of metabolic disturbances. In particular, ^{1}H-MRS at very high resolution, using magnets of 9.4T or more on the brain of animal models, allows now the *in vivo* measure of more numerous metabolites (including the $NH4^+$-induced increase of brain glutamine, insufficiently balanced by a concomitant decrease of other osmotically active metabolites like creatine, choline taurine and myo-inositol).

Present standards for diagnosis and treatment of congenital hyperammonemia and future prospects

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Several disorders of variable etiology present with hyperammonaemia as the hallmark including inherited as well as in acquired disorders. Hyperammonaemia must therefore be regarded as an unspecific laboratory sign, defined as a plasma ammonia level > 50 µmol/l (and > 100 µmol/l in newborns). Hyperammonaemia should always be regarded as an emergency; possible causes include an increased production of ammonia (e.g. in intestinal bacterial overgrowth, neurogenic bladder) or a diminished detoxification (e.g. in decreased urea cycle flux, blood bypass of the liver or insufficient action of glutamine synthetase). Often, endogenous protein catabolism triggers an increase of ammonia such as during infections or due to any other energy deficit. Detoxification of ammonia takes mainly place in a specific compartment in the liver, namely the periportal hepatocytes, which is the part of the liver lobule with highest urea cycle expression. A defect of any of the involved six enzymes or two transporters and also inhibition of the urea cycle through metabolites or by substrate deficiencies can affect ammonia detoxification.

In addition, various other situations such as organic acidemias, fatty acid oxidation defects, some rare diseases including pyrroline-5-carboxylate synthetase deficiency, the hyperammonaemia-hyperinsulinism syndrome, and the defect of carbonic anhydrase type VA as well as some drugs (e.g. valproic acid, cyclophosphamide) can cause hyperammonaemia. While the aforementioned conditions lead to a secondary impairment of urea cycle function, which might manifest at any age, patients with a primary urea cycle disorder are at risk for developing irreversible hyperammonaemic brain edema during their entire life.

The main prognostic factors are, irrespective of the underlying cause, the duration of the hyperammonaemic coma and the extent of ammonia accumulation.

Thus, early recognition of hyperammonaemia and initiation of specific treatment are of utmost importance. In particular, there is need for a high awareness amongst neonatologists treating sick newborns considered initially as bacterial sepsis cases. As well, neurologists or adult physicians encountering an unusual and unexplained change in the neurological status of a patient should include hyperammonaemia early in their differential diagnosis and work-up.

This lecture briefly discusses the biochemical background of primary and secondary hyperammonaemias, gives an overview on the various underlying disorders including their genetic backgrounds, describes current diagnostic strategies and summarizes the present therapeutic management with a short outlook into future therapies.

Primary hyperammonemias detected by newborn screening in Poland

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In Poland expanded newborn screening (NBS) has been performed since 2014 year. Since then tandem mass spectrometry method has been introduced to detect over twenty inborn errors of metabolism (IEMs); among them also primary hyperammonemias i.e. urea cycle disorders (UCD).

There are several reasons why to screen IEMs such as: their clinical onset may occur at every age, clinical manifestation is heterogenous, disease course may be acute, chronic or insidious, they are "masks" of frequent (acquired) diseases. Sepsis is the commonest misdiagnosis among these last. So the aim of NBS is early (i.e. presymptomatic) detection of IEM followed by introduction of proper medical intervention, what dramatically improves prognosis at patients identified by NBS.

Primary hyperammonemias caused by UCD usually appear as episodes with a risk of acute life threatening events or even sudden death. So NBS is very beneficial in such cases, although interpretation of NBS results using dry blood spot investigated by tandem mass spectrometry method, is not easy. It is considered that such UCD as: argininosuccinate synthase (ASS) deficiency, argininosuccinate lyase (ASL) deficiency and arginase (ARG1) deficiency are mostly identified by NBS. More difficult for detection are: ornithine transcarbamylase (OTC) deficiency (the most frequent UCD), carbamylphosphate synthase 1 (CPS1) deficiency or N-acetylglutamate synthase (NAGS) deficiency. However in Poland during last seven years OTC deficiency was identified in eight cases, ASS deficiency – in five cases and ASL deficiency – in three cases. Such results are due to consider citrulline concentration as a main biomarker (together with important ratios of other amino acids) for UCD detected by NBS. Other parameters such as: arginine and argininosuccinate concentrations in dry blood spot are also of great importance.

Based on NBS results obtained until now, the UCD detection rate in Poland is about 1 : 153 000 births. It should be stressed that early severe clinical manifestation of UCD (when mis- or undiagnosed) results in poor prognosis, so timing of proper diagnosis and treatment is crucial for an outcome. NBS should allow for such quick management, which in case of hyperammonemia is required even before all confirmation tests are completed.

Treatment and therapy of metabolic disorders associated with hyperammonemia

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The pathophysiology of urea cycle defects is complex and not fully understood. Regardless, the increase in ammonia concentration itself seems to be the main pathophysiological factor in UCDs and extremely important in other congenital metabolic diseases associated with hyperammonaemia. On the other hand, the causes of neuropsychiatric disorders in metabolically balanced carriers of the ornithine transcarbamoylase defect with normal ammonia concentration are not explained.

Treatment strategies for UCDs and secondary hyperammonaemia focus on reducing ammonia levels. The main methods are:

- Reduction of protein consumption (low but safe);
- Ammonia scavengers;
- Benzoate sodium:
  - Phenylacetate sodium:
    - Sodium phenylbutyrate,
    - Glycerol phenylbutyrate;
- Arginine and/or citrulline;
- Carglumic acid;
- Extracorporeal detoxification (acute phase);
- Liver or cells transplantation.

Dietary protein restriction is the primary treatment for hyperammonaemia, both during acute exacerbation and chronically. The desired amount of protein in the diet is the minimum safety requirement. In the case of persistent hyperammonaemia, drugs are added to reduce its con-

centration, such as sodium benzoate or the precursors of phenylacetoacetate: sodium phenylbutyrate or glycerol phenylbutyrate. Sodium phenylbutyrate is also used intravenously in acute hyperammonemia. The main advantage of the glycerol form of the drug is its acceptable taste and available form as a solution, which significantly facilitates its use in young children.

In UCDs, arginine is depleted in the body (except for arginase deficiency), and the functioning of the cycle is additionally impaired. Arginine becomes an exogenous amino acid, which requires its supplementation. In some enzymatic defects, it can be replaced by citrulline.

Carglumic acid (carbamoylphosphate analog) is the primary drug used in the deficit of N-acetylglutamate synthase, one of the rarer forms of UCDs, but attempts are also underway to administer the drug in other forms of UCDs. It is also effective in some secondary hyperammonaemia (organic aciduria).

In the case of the inability to achieve metabolic control with diet and drugs, liver transplantation is an available and increasingly widely used therapeutic option. Hepatic cell transplantation has not advanced beyond the clinical trial phase.

Extracorporeal ammonia elimination (hemodialysis, haemodiafiltration), used on an increasing scale, is also effective in acute hyperammonaemia. Peritoneal dialysis has little detoxification potential for ammonia.

Advanced clinical trials are currently underway on gene therapy for OTC deficiency, where the vector is AAV.

In search of a common denominator in the course of congenital hyperammonemias

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Several genetic inborn errors of metabolism, including urea cycle disorders and organic acidemias, result in hyperammonemia. High ammonia levels are neurotoxic, leading to astrocyte swelling, brain edema, coma, and ultimately death. The most susceptible organ is the brain, much more during development than in adulthood. Even though elevated ammonia has been strongly implicated in brain edema formation, evidence of the significance of the correlation between serum ammonia concentration and the severity of neurological impairment is ambiguous. Moreover, assessing a reliable plasma ammonia level is difficult. Measured ammonia concentration is affected by many factors, including the site of blood specimen collection, handling of the specimen, or the analytical method used.

Therefore, our study was focused on finding other peripheral markers common to the course of congenital hyperammonemia. It would also be crucial if this biomarker correlated with the severity of neurological deterioration. Plasma samples were obtained from patients diagnosed and followed at the Children's Memorial Health Institute due to several congenital hyperammonemias (hyperinsulinism-hyperammonemia syndrome; methylmalonic acidaemia; propionic academia; ornithine transcarbamylase deficiency; $n = 11$). Inflammatory cytokines and chemokines, indicators of oxidative stress – 3-nitrotyrosine (3-NT), advanced oxidation protein products (AOPP), glutathione peroxidase (GPx), and systemic biomarker of brain injury, S100 calcium-binding protein B (S100B) were analyzed.

There were no significant changes in the levels of pro-inflammatory cytokines and chemokines that would correlate with ammonia or the type of congenital hyperammonemia. Although in a few recent studies, the determination of 3-NT has helped to identify patients with minimal hepatic encephalopathy, in our study, 3-NT rose markedly in the plasma of some patients but without association with ammonia levels. The study should be repeated on a larger group of patients as measured 3-NT concentrations may have some predictive value. AOPP are typically elevated in patients with renal complications, atherosclerosis, or diabetes mellitus. Glutathione peroxidase (GPx,) is an enzyme that plays an essential role in protecting organisms from oxidative stress-induced damage. Low levels of GPx have been correlated with free radical-related disorders. However, in our patients, there were neither significant increases in AOPP or decreases in GPx, nor a significant correlation between the two parameters. On the other hand, S100B levels showed a linear correlation with plasma ammonia concentration ($p < 0.05$), indicating that measuring this protein, in addition to ammonia, can be valuable in estimating the risk of neurological damage in patients with congenital hyperammonemias. S100B is produced primarily by astrocytes and is widely used as a biomarker of brain injury and blood-brain barrier disruption. S100B is physiologically low or undetectable in serum; elevated serum levels have been detected in several neuropathological conditions. High levels of extracellular S100B stimulate the expression of proinflammatory cytokines and induce apoptosis. It may be promising as a non-invasive diagnostic tool for cases of congenital hyperammonemia since S100B, unlike ammonia, is stable and relatively unaffected by storing, changes in tempera-

ture, freeze-thaw cycles, and hemolysis in the sample. On the other hand, measured serum S100B concentrations are also in part affected by the possible extra-brain sources of S100B. Additionally, S100B at physiological concentration is neurotrophic, and its levels in serum are generally increased in the pediatric population with a growing central nervous system, presenting a significant variation of normal reference levels up to 15 years of age. Therefore, it is essential to establish a reference range in the tested population. Moreover, it is worth considering other peripheral biomarkers of brain injury (such as neuron-specific enolase or ubiquitin C-terminal hydrolase L1).

In conclusion, our preliminary results indicate that it would be recommended to introduce routine S100B measurement in patients with congenital hyperammonemia. It should be confirmed if it can also have predictive value for the progression and severity of underlying neurological impairment during acute decompensation.

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Liver transplantation in primary hyperammonemias

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According to available liver transplantation (LTx) data, UCDs (urea cycle disorders) are the most common indication for liver transplantation among inborn errors of metabolism (IEM) in recent years.

The primary indication for LTx in patients with UCDs is the prevention of progressive neurologic injury resulting from hyperammonemia. The first LTx was performed in OTC (Ornithine transcarbamylase deficiency) in 1989.

Nowadays LTx has been successfully performed in all UCDs except N-acetylglutamate synthase deficiency (NAGSD) (for which exists a curative life-long substitutive therapy). Pediatric long-term outcomes of LTx in children with UCDs are excellent. The 1-year survival rate in large pediatric programs is about 95%,whereas the 5-year survival ~90%, with good quality of life post transplant. The greatest advantages are maintaining or improving a quality of life, no further episodes of hyperammonemia and no longer require dietary protein restriction. LTx has also its drawbacks, like surgical complications post LTx, long-term – mortality risks, side effects of immunosuppression, intake of large amounts of drugs (which requires a very disciplined lifestyle) and other. Generally, LTx will not revert preexisting neurological damage. In guidelines published by Häberle *et al*. in 2019 it is recommended "to consider liver transplantation in patients with severe UCDs without sufficient response to standard treatment, with poor quality of life, without severe neurological damage and ideally while in a stable metabolic condition". As suggested by the authors liver transplantation should be performed in patients with neonatal onset UCD (except for NAGSD) before the onset of irreversible neurological damage.

The authors emphasize that liver transplantation between 3 and 12 months of age and when body weight exceeds 5 kg is associated with more favorable outcomes. Which is why they strongly advocate reconsidering liver transplantation in patients with: severe progressive liver disease, and/or with recurrent metabolic decompensations requiring hospitalizations despite standard medical therapy.

Orthotropic LTx is the standard recommended procedure, but long waiting list duration is associated with risk of hyperamonnemia crisis. Auxiliary LTx was performed in some patients with UCDs but it was associated with a higher complication rate. Living-related donor (LRD) LTx has comparable results to the use of organs from deceased donors. Moreover LRD LTx allows reducing waiting times, as well as donor and organ testing. It permits the transplant to be performed electively when the recipient is in the best clinical and metabolic condition and in the optimal timing after confirmation of the donor phenotype. In Japan, LRD LTx has been performed successfully in multiple cases due to very limited deceased donor availability, including a case using a donor liver heterozygous for X-linked OTC deficiency. In living related LTx, heterozygosity seems not to be a problem and even asymptomatic OTC heterozygotes have been successful donors (after careful enzymatic evaluation) but symptomatic heterozygous donors should not be considered. Decisions on whether or not to perform LTx are influenced by ethical considerations which require an individualized process of decision, in particular when the child is already handicapped or when LRD LTx is considered. Hyperammonemia should be corrected before surgery and perioperative management is focused on nitrogen balance to prevent hyperammonemia (result of an excessive protein breakdown from surgical stress and fasting related catabolism). Presurgical fasting should be minimized (*via* high glucose infusion with additional calories *via* intravenous fat emulsion). Confirming metabolic stabilization with the grafted liver by monitoring of ammonia during and after surgery is very important. Some patients may continue to have low citrulline levels because extrahepatic biosynthesis of citrulline is still deficient but in general in most patients these metabolic aberrations have no clinical impact.

In conclusion: Improvements in both surgical technique and immunosuppression management have resulted in excellent long-term survival after pediatric LTx. LTx should be offered early to patients with severe UCDs, poorly controlled with medical interventions to prevent long term neurological damage.

Polish experience with liver transplantation and post-transplant outcomes in children with urea cycle disorders

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Liver transplantation (LTx) is recommended for various metabolic diseases, including urea cycle disorders (UCDs). The aim of this study was to determine indications and outcomes of LT for UCDs in the tertiary reference Children's Hospital in Warsaw, Poland.

In order to that medical charts of children with UCD who underwent LTx between 2008 and July 2016 were retrospectively reviewed. The following parameters were analyzed: symptoms at time of diagnosis, age at diagnosis, age at transplantation, graft characteristics and survival, postsurgical complications, and biochemical and laboratory results before and after transplantation.

There were 12 patients with UCD who underwent LTx at a mean age of 5 y (0.5-14 y) receiving a total of 14 liver grafts. Four children (33%) received a living donor graft, while 8 (68%) got a deceased donor liver graft. A total number of transplanted organs consisted of 9 (64%) whole-liver grafts and 5 (36%) reduced-size grafts.

The 30-days, 1 yr, 3 yrs and 5 yrs post-transplant patient survival rates were 100%. The 30-days, 1 yr, 3 yrs and 5 yrs graft survival rates were 93% (13/14), 85.7% (12/14), 85.7%, and 78.6% (11/14), respectively.

The median follow-up time after LTx was 7.25 yrs (0.5-14 yrs). The median time from diagnosis to LTx was 3 yrs (0.5-13 yrs), and the average waiting time for a deceased donor graft – 6 months (1-12 months).

Median peak of blood ammonia at presentation was 653 (159-2613) µg/dl (normal < 80 µg/dl), and median peak of blood glutamine was 1273.2 µmol/l (964-3900 µmol/l). There was one episode of hyperammonemia following LTx, but it was not due to UCD.

Six (50%) patients were diagnosed with some degree of developmental delay/neurological impairment before transplantation, which remained stable or slightly improved after transplantation. Patients without developmental delay before transplantation maintained their cognitive abilities at follow-up.

Based on our observation we may conclude that LTx leads to eradication of hyperammonemia, withdrawal of dietary restrictions with low-protein diet, and potentially improved neurocognitive development.