



Evaluation of the Neurodevelopmental Status for Urea Cycle Disorders: Based on Clinical Experience

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ABSTRACT

Aim: Urea cycle disorders (UCD) still have poor neurological outcomes despite early diagnosis and treatment. We aimed to present the neurological outcomes of UCD patients and to determine the main simple and accessible factors affecting these outcomes.

Materials and Methods: This was a descriptive cross-sectional study conducted in two pediatric metabolism centers on 29 patients from 25 unrelated families who were diagnosed and followed with UCD based on clinical presentation, neurological parameters, biochemical measurements, and molecular analysis.

Results: Within the study population, the most common diagnosis was argininosuccinate synthase deficiency in 13 (44.82%) patients, followed by N-acetylglutamate synthase deficiency in five patients (17.24%), ornithine transcarbamylase deficiency in four patients (13.79%), arginase 1 deficiency in three patients (10.34%), carbamoyl phosphate synthase 1 deficiency in three patients (10.34%), and argininosuccinate lyase deficiency in one patient (3.44%). Peak ammonia levels were observed to be significantly higher in those patients with delayed milestones and patients who had Denver II <-2 standard deviation score results ($p=0.032$, $p=0.026$). Effect sizes were large in both groups. Delayed milestones were noted in 17 (94.4%) of the cases with peak ammonia >500 $\mu\text{mol/L}$ ($n=18$). Those patients with abnormal neurological parameters had a significantly higher mean number of hyperammonemic episodes per year. Extracorporeal detoxification was given to eight patients, in combination with therapeutic hypothermia in two patients. Rapid regression was observed in brain edema in those who underwent therapeutic hypothermia.

Conclusion: Our study emphasizes the effect of peak ammonia levels and the frequency of hyperammonemic episodes on neurological outcomes. There were still poor neurocognitive outcomes despite extracorporeal detoxification. This highlights the need to reassess current treatment strategies, including the threshold for starting extracorporeal detoxification if ammonia levels exceed 500 $\mu\text{mol/L}$. The use of therapeutic hypothermia by experienced teams may be promising due to its brain edema-reducing effects.

Keywords: Urea cycle disorders, Hyperammonemia, Citrullinemia, Inborn errors of metabolism, therapeutic hypothermia

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Introduction

The urea cycle is the primary metabolic pathway for nitrogen disposal from the liver. Six enzymes [carbamoyl phosphate synthase 1 (CPS-1, EC 6.3.4.16), ornithine transcarbamylase (OTC, EC 2.1.3.3), argininosuccinate synthase (ASS, EC 6.3.4.5), argininosuccinate lyase (ASL, EC 4.3.2.1), arginase 1 (ARG1, EC 3.5.3.1), N-acetylglutamate synthase (NAGS, EC 2.3.1.1)] and two mitochondrial transporters (aspartate-glutamate carrier and ornithine-citrulline carrier) are coordinated in this pathway (1). OTC, CPS1 and NAGS are mitochondrial enzymes, ASS, ASL, and ARG1 are cytosolic enzymes. Urea cycle disorders (UCD) are inborn metabolism errors and estimated incidence ranges from 1/8,000 to 1/35,000 births (2). UCDs have a highly variably phenotypic spectrum ranging from hyperammonemic encephalopathy, acute liver failure, and spastic paraplegia to asymptomatic. The patients have an increased risk of poor neurological outcome (3). They are often affected by morbidity due to neurocognitive deterioration. Hyperammonemia and its effects on neurological outcome is a common clinical presentation. Disease severity variables such as the age of disease onset, the age of the first hyperammonemic episode (HE), the number of HEs per year, the peak ammonia level, and the plasma amino acid levels affect neurological outcomes (4). However, it is unclear whether the consequences of hyperammonemia alone or brain metabolic changes such as synaptic transmission and glutamine toxicity contribute. Although Zielonka et al. (5) showed a relationship between enzyme activity and neurological outcome in ASSD patients, the correlation of phenotypic severity with genotype or *in vitro* enzyme activity is unclear. In addition, enzyme analysis is not useful in daily practice.

Diagnosis is made by selective metabolic investigation in symptomatic patients, family screening of the index patient, newborn screening, and prenatal testing. The sensitivity of newborn screening in UCD is already low and it is not performed in our country. Guidelines for the diagnosis and management of UCDs have been proposed for the use of single therapeutic tools as well as their combinations (6). UCD guideline recommendations are currently used in our country. However, the UCD guideline is still lacking regarding the impact of adherence to these guidelines on neurological outcomes. Despite the development of extracorporeal detoxification treatments, diet therapy and new ammonia-scavengers, mortality and morbidity in early-onset patients are still poor. In the future, a better understanding of predictive markers is needed in order to

provide more information on phenotypes in neurotoxic metabolites-related diseases.

To the best of our knowledge, there is no single, definitive way to predict the neurodevelopmental status of a patient with UCD. Increasing clinical awareness and neonatal screening programs and the development of new diagnostic and therapeutic proposals are expected to provide better outcomes. Based on our clinical experiences, we aimed to present the neurological outcomes of UCD patients from two metabolic centers and to determine key simple and accessible factors affecting neurological outcomes. We believe that simple predictive factors will help clinicians in practice.

Materials and Methods

Study Design and Data Acquisition

Individuals were included based on a retrospective collection of the data of the ASS deficiency (ASSD), NAGS deficiency (NAGSD), OTC deficiency (OTCD), ARG-1 deficiency (ARGD), CPS-1 deficiency (CPS1D) and ASL deficiency (ASLD) patients who had been diagnosed in two metabolism centers in Turkey. The inclusion criteria were rare biallelic variants in *ASS1*, *NAGS*, *OTC*, *ARG1*, *CPS1* and *ASL* genes classified as likely pathogenic or pathogenic according to the American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology guidelines. All data were retrieved via standardized proformas agreed on by the participating centers.

The follow-up period was every month until 12 months, every 2 months until 6 years of age, and every 3 months thereafter. The clinical variabilities were categorized according to disease onset as early-onset (EO <28 days), late-onset (LO >28 days) and asymptomatic. For phenotyping, the following variables were analyzed within this study: the individual's genetic ancestry, sex, age at last visit, and clinical status. Data on the following clinical and laboratory characteristics were collected: peak ammonia (first clinical presentation), plasma amino acids, the number of HEs per year, the duration of the HEs, extracorporeal detoxification, kidney dysfunction, and hepatocellular dysfunction. We evaluated the neurological status under the headings of delayed milestones, fine and gross motor skills, tone abnormalities, bedridden status, and the Denver II and Wechsler Intelligence Scale for Children-Revised (WISC-R). Denver II was applied to evaluate children aged 0-6 years in terms of development. Patients with a Denver II test result of <-2SDS were considered abnormal, and those with >-2 standard deviation score (SDS) were considered to be within

normal neurological parameters. The validity and reliability of the test was performed by Eratay et al. (7) in Turkey. Patients between 6 and 16 years of age were evaluated using the WISC-R. This test includes verbal and performance subscales. WISC-R was adapted into Turkish, and it has been used widely for years (8). In addition, detailed data on neurological disease, laboratory values, and the clinical features involved were evaluated and recorded according to Human Phenotype Ontology terminology. An asymptomatic female OTCD carrier was also included in this study.

Therapy monitoring was undertaken during follow-up visits which included the evaluation of clinical parameters, laboratory parameters, and dietary consumption records. The "last three-days dietary consumption" records were requested from the patients/parents. Protein, amino acids, energy, and medication intakes were calculated.

Molecular Analyses

All exons and exon-intron junctions of the genes were evaluated by next-generation sequencing methods. Genomic DNA was extracted from peripheral blood samples using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Standardized PCR pools were prepared using a NexteraXT sample preparation kit for next-generation sequencing analysis with the Miseq device (Illumina, Inc.).

Sanger sequencing of the genomic variants identified by exome sequencing or targeted gene sequencing was performed for all patients and their families. Sanger sequencing was used to validate the pathogenic variants within families on the 3500 Genetic Analyzer (Applied Biosystems, Foster City, USA). The sequencing results were analyzed using CLC genomic workbench software. For the clinical interpretation of variants, allele frequency data were obtained from various databases, including gnomAD (<http://gnomad.broadinstitute.org/>) and ExAc (<http://exac.broadinstitute.org/>). The pathogenicity of variants was assessed using in silico prediction tools, such as PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2>), SIFT (<http://sift.jcvi.org>), MutationTaster (<http://www.mutationtaster.org>) and the Human Splicing Foundation (<http://www.umd.be/hsf/>). Variants were classified according to ACMG. All pathogenic variants are described according to the accepted HGVS nomenclature. Nucleotide numbers were derived from complementary DNA sequences.

Statistical Analysis

Statistical analyses of the data were performed using the SPSS software package for Windows software package

(ver.18.0; SPSS Inc., Chicago, IL, USA). As descriptive statistics, numbers, and percentages for categorical variables, mean±SD or median ([minimum (min.)-maximum (max.)] were used for numerical variables. The distribution of data was evaluated using the Shapiro-Wilk test. For numerical comparisons, the Student's t-test or Mann-Whitney U test were used to assess differences between two groups according to the normal distribution of the measured parameters. A significance level of $p < 0.05$ was set to indicate statistical significance. The effect size [Cohen d ($0.20 \leq d < 0.50$: small effect, $0.50 \leq d < 0.80$: medium effect, $d \geq 0.80$: large effect and Rank-Biserial correlation coefficient (≥ 0.50 : large effect)] was calculated for statistically significant results.

This study was performed in accordance with the declaration of Helsinki and was approved by the Clinical Research Ethics Committee of the University of Health Sciences Turkey, Diyarbakir Gaziyaşargil Training and Research Hospital (approval no: 928, date: 05.11.2021).

Results

Demographic and Clinical Findings

A total of 29 UCD patients (17/12, M/F) from 25 different families from two metabolic centers in the southeastern region of Turkey were included in this study. 86.2% of the individuals in our cohort were born to consanguineous parents. Twenty-six (89.65%) patients were diagnosed by selective metabolic investigation after the onset of their symptoms, whereas three (10.34%) were diagnosed by family screening. Within the study population, the most common diagnosis was ASSD in 13 (44.82%) patients, followed by NAGSD in five patients (17.24%), OTCD in four patients (13.79%), ARG1D in three patients (10.34%), CPS1D in three patients (10.34%), and ASLD in one patient (3.44%).

Sixteen (55.17%) patients were EO, 12 (41.37%) patients were LO and one (3.44%) patient was asymptomatic (Female OTC). The mean age at initial symptom was 9.8 ± 9.68 days (median: 4, min.: 1, max.: 28) in the EO group, 21.27 ± 27.07 months (median: 8, min.: 0.3, max.: 85) in the LO group. The mean age at diagnosis was 11.6 ± 11.72 days (median: 4, min.: 2, max.: 38) in EO patients, and 64.97 ± 65.21 months (median: 38.75, min.: 3, max.: 219) in the LO group. The mean age of the patients at last visit was 6.64 ± 8.2 years (median: 4, min.: 0.3, max.: 43 years). The patients' detailed clinical and molecular characteristics are shown in Table I.

The most common initial symptoms were encephalopathy, tachypnea, and seizures in the EO group and delayed milestones, feeding difficulties, and

Table I. Demographic, clinical and molecular characteristics of UCD patients

Family	Patient	Gender	Con.	Diagnosis	Disease onset	CVWHDF	Latest neurodevelopmental status	Gene	Transcript	DNA change	Protein change	Zygoty	Novel	Pathogenicity
F1	P1	M	Yes	ASSD	EO	No	Normal	ASS1	NM_054012.4	c.1085G>T	p.Gly362Val	Homozygous		Pathogenic
F2	P2	F	Yes	ASSD	LO	No	Normal	ASS1	NM_054012.4	c.1085G>T	p.Gly362Val	Homozygous		Pathogenic
F3	P3	M	Yes	ASSD	LO	No	Mild ID	ASS1	NM_054012.4	c.1085G>T	p.Gly362Val	Homozygous		Pathogenic
F4	P4	M	Yes	ASSD	LO	No	Normal	ASS1	NM_054012.4	c.1085G>T	p.Gly362Val	Homozygous		Pathogenic
F5	P5	M	Yes	ASSD	LO	No	Profunda ID, GDD	ASS1	NM_054012.4	c.1085G>T	p.Gly362Val	Homozygous		Pathogenic
F6	P6	M	Yes	ASSD	EO	CVWHDF	Normal	ASS1	NM_054012.4	c.1085G>T	p.Gly362Val	Homozygous		Pathogenic
F6	P7	M	Yes	ASSD	EO	No	Normal	ASS1	NM_054012.4	c.1085G>T	p.Gly362Val	Homozygous		Pathogenic
F7	P8	F	Yes	ASSD	LO	No	Mild ID	ASS1	NM_054012.4	c.1085G>T	p.Gly362Val	Homozygous		Pathogenic
F8	P9	F	Yes	ASSD	EO	CVWHDF	GDD	ASS1	NM_054012.4	c.1168G>A	p.Gly380Arg	Homozygous		Pathogenic
F9	P10	F	Yes	ASSD	EO	CVWHDF	GDD	ASS1	NM_054012.4	c.1168G>A	p.Gly380Arg	Homozygous		Pathogenic
F10	P11	F	Yes	ASSD	EO	CVWHDF	Profunda ID, GDD	ASS1	NM_054012.4	c.1087C>T	p.Arg363Trp	Homozygous		Pathogenic
F11	P12	F	Yes	ASSD	EO	No	Profunda ID, GDD	ASS1	NM_054012.4	c.1087C>T	p.Arg363Trp	Homozygous		Pathogenic
F12	P13	M	No	ASSD	EO	No	Normal	ASS1	NM_054012.4	c.814C>T, c.970+5G>A	p.Arg272Cys, -	Compound heterozygous		Pathogenic/ Pathogenic
F13	P14	F	Yes	NAGSD	EO	No	Normal	NAGS	NM_153006.3	c.1450T>C	p.Trp484Arg	Homozygous		Pathogenic
F14	P15	F	Yes	NAGSD	EO	No	Profunda ID, has ability to walk	NAGS	NM_153006.3	c.1450T>C	p.Trp484Arg	Homozygous		Pathogenic
F15	P16	F	Yes	NAGSD	EO	No	Profunda ID, has ability to walk	NAGS	NM_153006.3	c.1450T>C	p.Trp484Arg	Homozygous		Pathogenic
F16	P17	M	Yes	NAGSD	EO	No	Mild ID	NAGS	NM_153006.3	c.1450T>C	p.Trp484Arg	Homozygous		Pathogenic
F17	P18	M	Yes	NAGSD	EO	CVWHDF	ASD	NAGS	NM_153006.3	c.1450T>C	p.Trp484Arg	Homozygous		Pathogenic
F18	P19	M	Yes	OTCD	LO	No	Profunda ID, GDD	OTC	NM_000531.6	c.172T>C	p.Trp58Arg	Hemizygous	Novel	Likely Pathogenic
F18	P20	M	Yes	OTCD	LO	No	Normal	OTC	NM_000531.6	c.172T>C	p.Trp58Arg	Hemizygous	Novel	Likely Pathogenic
F18	P21	F	No	OTCD	LO	No	Asymptomatic, normal	OTC	NM_000531.6	c.172T>C	p.Trp58Arg	Hemizygous	Novel	Likely Pathogenic
F19	P22	M	No	OTCD	LO	No	Normal	OTC	NM_000531.6	c.774T>A	p.Asn258Lys	Hemizygous		Likely Pathogenic
F20	P23	M	Yes	ARGD	LO	No	Profunda ID, loss of ability to walk	ARG1	NM_000045.4	c.130+1G>A		Homozygous		Pathogenic
F20	P24	F	Yes	ARGD	LO	No	ASD, loss of ability to walk	ARG1	NM_000045.4	c.130+1G>A		Homozygous		Pathogenic

Table I. Continued

Family	Patient	Gender	Con.	Diagnosis	Disease onset	CVVHDF	Latest Neurodevelopmental Status	Gene	Transcript	DNA change	Protein change	Zygoty	Novel	Pathogenicity
F21	P25	F	Yes	ARGD	LO	No	Mild ID, loss of ability to walk	ARG1	NM_000045.4	c.703C>C	p.Gly235Arg	Homozygous		Pathogenic
F22	P26	M	Yes	CPS1D	EO	CVVHDF	Severe ID, GDD	CPS1	NM_001875.4	c.3037_3039del	p.Val1013del	Homozygous		Pathogenic
F23	P27	M	Yes	CPS1D	EO	No	Delayed milestones	CPS1	NM_001875.4	c.3049C>G	p.Pro1017Ala	Homozygous		Likely Pathogenic
F24	P28	M	No	CPS1D	LO	CVVHDF	Delayed milestones	CPS1	NM_001875.4	c.3002G>T; c.3402G>C	p.Arg1001Leu p.Leu1134Phe	Compound heterozygous	Novel/ Novel	VUS/Likely Pathogenic
F25	P29	M	Yes	ASLD	EO	CVVHDF	Mild ID, DD	ASL	NM_000048.4	c.918+5C>A		Homozygous		Pathogenic

M: Male, F: Female, Con: Consanguinity, UCD: Urea cycle disorder, CVVHDF: Continuous venovenous hemodiafiltration, LO: Late-onset, EO: Early-onset, ASLD: Argininosuccinate lyase deficiency, CPS1D: Carbamoyl phosphate synthase I deficiency, ARGD: Arginase I deficiency, OTCD: Ornithine transcarbamylase deficiency, NAGSD: N-acetylglutamate synthase deficiency, ASSD: Argininosuccinate synthase deficiency

gait abnormalities in the LO group. In ASS1D, the most common clinical presentation was hyperammonemic encephalopathy in eight patients with EO and delayed milestones in five patients with LO. All NAGSD patients presented with encephalopathy in the neonatal period. All OTCD patients, except the asymptomatic female OTCD patient, presented with varying degrees of intellectual disability (ID). Two ARG1D patients presented with gait abnormalities; one patient presented with autism spectrum disorder, and gait abnormalities developed in the follow-up. Three CPS1D patients, one of whom was LO (P28), presented with encephalopathy and died during a HE due to ventricular arrhythmia. When the neurological parameters of the patients were evaluated according to their disease onset (EO vs. Late-onset), no significant correlation was found. No significant difference was found between the neurocognitive outcomes of cytosolic UCDs and mitochondrial UCDs. There was no significant difference between the peak ammonia levels and the mean number of HEs per year between these two groups.

Laboratory Findings

The mean peak ammonia level of the EO patients was 1,217.19±595.94 μmol/L (range: 378-2,300), and for the LO patients, it was 419.77±257.19 μmol/L (range: 80-875). Peak ammonia levels were significantly higher in the EO patients (p<0.001). According to the disease groups, the highest peak ammonia levels were seen in NAGSD, CPS1D and ASS1D patients, respectively. There was no significant difference between the mean peak ammonia levels of the cytosolic and mitochondrial UCD group (p=0.89). Peak ammonia levels were observed to be significantly higher in those patients with delayed milestones and those patients who had Denver II <-2SDS results (p=0.032, p=0.026). Effect sizes were large in both groups [Cohen d: -1.04 (confidence interval (CI): -2.10 - 0.09), -1.03 (CI: -2.05 - 0.05); respectively (CI=95th percentile CI)]. Delayed milestones were noted in 17 (94.4%) of those cases with peak ammonia >500 μmol/L (n=18). The comparison of neurological status parameters with clinical and laboratory features is presented in Table II.

Seventy-one HEs were recorded. The mean age at the time of HE was 10.4±7.8 months (two days-16 years). The mean HE per year was significantly higher in those cases with abnormal neurological parameters (p=0.019). Patients with delayed milestones, abnormal gross motor skills, tone abnormalities, and bedridden status had a significantly higher mean number of HEs per year. Also, the effect sizes were large for the abnormal gross motor skills, tone abnormalities and bedridden groups (Rank biserial

correlation coefficients: 0.58, 0.57 and 0.97, respectively). There were abnormal gross motor skills in 11 (84.61%) patients with mean number of HEs per year ≥ 1 and bedridden status in 4 (57.14%) patients with a mean number of HEs per year ≥ 2 .

The mean peak plasma glutamine level was $1,293 \pm 492.4$ $\mu\text{mol/L}$ (N: 123-809, range: 280-2,045). There was no significant relationship between plasma glutamine levels and neurological parameters, peak ammonia, the mean number of HEs per year, or continuous venovenous hemodiafiltration (CVVHDF). The mean initial plasma citrulline level was 875.2 ± 799.6 $\mu\text{mol/L}$ (N: 9-52, range: 224-2,700) in ASSD patients. Peak citrulline levels were

significantly higher in ASSD patients who had abnormal neurological parameters ($p < 0.031$). Although we could not find a significant relationship between the citrulline subgroups and delayed milestones in ASSD patients, delayed milestones occurred in all subjects with a citrulline levels over 500 $\mu\text{mol/L}$. Abnormal fine and gross motor skills and tone abnormalities were observed in all cases with a citrulline level over 1.000 $\mu\text{mol/L}$.

Treatment

In the emergency management, according to the diagnosis groups and ammonia levels, protein intake was stopped, and an appropriate dose of intravenous (IV) glucose support to prevent catabolism, IV sodium benzoate,

Table II. Comparison of neurological status parameters with disease characteristics

		Delayed Milestones		Fine Motor Skills		Gross Motor Skills		Tone Abnormalities	
		No (n=6)	Yes (n=23)	Normal (n=7)	Abnormal (n=22)	Normal (n=16)	Abnormal (n=13)	No (n=15)	Yes (n=14)
Disease Onset	Early Onset	3 (50%)	13 (56.5%)	3 (42.9%)	10 (45.5%)	9 (56.3%)	7 (53.8)	7 (46.7%)	6 (42.9%)
	Late Onset	3 (50%)	10 (43.5%)	4 (57.1%)	12 (54.5%)	7 (43.8%)	6 (46.2)	8 (53.3%)	8 (57.1%)
Disease Group	Cytosolic UCDs (n=17)	3 (50%)	14 (60.9%)	4 (57.1%)	13 (59.1%)	9 (56.3%)	8 (61.5)	6 (40%)	6 (42.9%)
	Mitochondrial UCDs (n=12)	3 (50%)	9 (39.1%)	3 (42.9%)	9 (40.9%)	7 (43.8%)	5 (38.5)	9 (60%)	8 (57.1%)
Peak Ammonia ($\mu\text{mol/L}$)		384.67 ± 318.53	$983.65 \pm 620.38^*$	517.86 ± 374.05	968.5 ± 646	718 ± 423.39	1034.15 ± 778.67	707.87 ± 436.24	1022.43 ± 749.41
Peak Ammonia Levels ($\mu\text{mol/L}$)	50-200	2 (33.3%)	2 (8.7%)	1 (14.3%)	3 (13.6%)	2 (12.5%)	2 (15.4)	2 (13.3%)	2 (14.3%)
	201-500	3 (50%)	4 (17.4%)	4 (57.1%)	3 (13.6%)	4 (25%)	3 (23.1%)	4 (26.7%)	3 (21.4%)
	501-1000	1 (16.7%)	9 (39.1%)*	1 (14.3%)	9 (40.9%)	8 (50%)	2 (15.4%)	7 (46.7%)	3 (21.4%)
	>1001	0	8 (34.8%)*	1 (14.3%)	7 (31.8%)	2 (12.5%)	6 (46.2%)	2 (13.3%)	6 (42.9%)
Mean Number of HEs per Year		0.52 ± 0.52	$1.45 \pm 1.1^*$	0.34 ± 0.18	1.55 ± 1.08	0.75 ± 0.58	$1.91 \pm 1.23^*$	0.73 ± 0.6	$1.84 \pm 1.21^*$
Elevated Glutamine ($\mu\text{mol/L}$)	809-1000	2 (33.3%)	10 (43.5%)	2 (28.6%)	10 (45.5)	6 (37.5%)	6 (46.2%)	5 (33.3%)	7 (50%)
	1001-2000	3 (50%)	13 (56.5%)	4 (57.1%)	12 (54.5)	9 (56.3%)	7 (53.8%)	9 (60%)	7 (50%)
	>2000	1 (16.7%)	0	1 (14.3%)	0	1 (6.3%)	0	1 (6.7%)	0
Elevated Citrulline ($\mu\text{mol/L}$)	52-500	3 (100%)	5 (45.5%)	3 (75%)	5 (50%)	7 (77.8%)	1 (20%)	7 (77.8%)	1 (20%)
	500-1000	0	2 (18.2%)	1 (25%)	1 (10%)	2 (22.2%)	0 (0%)	2 (22.2%)	0
	1001-2000	0	3 (27.3%)	0 (0%)	3 (30%)	0	3 (60%)*	0	3 (60%)*
	>2001	0	1 (9.1%)	0 (0%)	1 (10%)	0	1 (20%)*	0	1 (20%)*
CVVHDF	No	5 (83.3%)	16 (69.9%)	7 (100%)	14 (63.6%)	13 (81.3%)	8 (61.5%)	12 (80%)	9 (64.3%)
	Yes	1 (16.7%)	7 (30.4%)	0	8 (36.4%)	3 (18.8%)	5 (38.5%)	3 (20%)	5 (35.7%)
Length of Stay CVVHDF	0-12 hours	1 (100%)	2 (28.6%)	2 (100%)	1 (20%)	2 (66.7%)	1 (20%)	2 (66.7%)	1 (20%)
	12-24 hours	0	3 (42.9%)	0	2 (40%)	1 (33.3%)	2 (40%)	1 (33.3%)	2 (40%)
	>24 hours	0	2 (28.6%)	0	2 (40%)	0	2 (40%)	0	2 (40%)
Diet Therapy	Protein controlled diet	3 (60%)	5 (21.7%)	2 (33.3%)	6 (27.3%)	5 (33.3%)	3 (23.1%)	4 (28.6%)	4 (28.6%)
	Calculated diet	2 (40%)	18 (78.3%)	4 (66.7%)	16 (72.7%)	10 (66.7%)	10 (76.9%)	10 (71.4%)	10 (71.4%)

Table II. Continued

		Bedridden		Denver II		WISC-R	
		No (n=21)	Yes (n=7)	> -2 SDS (n=8)	< -2 SDS (n=11)	Normal (n=8)	Abnormal (n=11)
Disease Onset	Early Onset	11 (52.4%)	5 (71.4%)	5 (62.5%)	8 (72.7%)	5 (62.5%)	8 (72.7%)
	Late Onset	10 (47.6%)	2 (28.6%)	3 (37.5%)	3 (27.3%)	3 (37.5%)	3 (27.3%)
Disease Group	Cytosolic UCDS (n=17)	12 (57.1%)	5 (71.4%)	3 (37.5%)	7 (63.7%)	3 (37.5%)	7 (63.7%)
	Mitochondrial UCDS (n=12)	9 (42.9%)	2 (28.6%)	5 (62.5%)	4 (36.4%)	5 (62.5%)	4 (36.4%)
Peak Ammonia (µmol/L)		712±465.57	1372.86±796.4		655.88±318.172	1291.73±760.88*	655.88±318.172
Peak Ammonia Levels (µmol/L)	50-200	4 (19%)	0	1 (12.5%)	2 (18.2%)	1 (12.5%)	2 (18.2%)
	201-500	5 (23.8%)	1 (14.3%)	2 (25%)	0	2 (25%)	0
	501-1000	8 (38.1%)	2 (28.6%)	4 (50%)	2 (18.2%)*	4 (50%)	2 (18.2%)*
	>1001	4 (19%)	4 (57.1%)	1 (12.5%)	7 (63.6%)*	1 (12.5%)	7 (63.6%)*
Mean Number of HEs per Year		0.8±0.59	2.85±0.93*		0.738±0.625	1.691±1.288	0.738±0.625
Elevated Glutamine (µmol/L)	809-1000	11 (52.4%)	1 (14.3%)	3 (37.5%)	7 (63.6%)	3 (37.5%)	7 (63.6%)
	1001-2000	9 (42.9%)	6 (85.7%)	4 (62.5%)	4 (36.4%)	4 (62.5%)	4 (36.4%)
	>2000	1 (4.8%)	0	1 (0.12%)	0	1 (0.12%)	0
Elevated Citrulline (µmol/L)	52-500	7 (77.8%)	1 (20%)	3 (100)	3 (33.3%)	3 (100)	3 (33.3%)
	500-1000	2 (22.2%)	0	0	2 (22.2%)	0	2 (22.2%)
	1001-2000	0	3 (60%)*	0	3 (33.3%)	0	3 (33.3%)
	>2001	0	1 (20%)*	0	1 (11.1%)	0	1 (11.1%)
CVVHDF	No	18 (85.7%)	3 (42.9%)*	7 (87.5%)	5 (45.5%)	7 (87.5%)	5 (45.5%)
	Yes	3 (14.3%)	4 (57.1%)	1 (12.5%)	6 (54.5%)	1 (12.5%)	6 (54.5%)
Length of Stay CVVHDF	0-12 hours	2 (66.7%)	1 (25%)	0	2 (33.3%)	0	2 (33.3%)
	12-24 hours	1 (33.3%)	2 (50%)	0	3 (50%)	0	3 (50%)
	>24 hours	0	1 (25%)	1 (100%)	1 (16.7%)	1 (100%)	1 (16.7%)
Diet Therapy	Protein controlled diet	7 (35%)	1 (14.3%)	2 (25%)	3 (27.3%)	2 (25%)	3 (27.3%)
	Calculated diet	13 (65%)	6 (85.7%)	6 (75%)	8 (72.7%)	6 (75%)	8 (72.7%)

*p<0.05, HE: Hyperammonemic episodes, CVVHDF: Continuous venovenose hemodiafiltration, UCD: Urea cycle disorders, WISC-R: Wechsler Intelligence Scale for Children-Revised, SDS: Standard deviation score

IV L-arginine, carbamyl-glutamate, and extracorporeal detoxification treatments were applied. Drug dosages were adjusted in accordance with the current guidelines. In maintenance treatment, a combination of a low protein diet containing essential amino acid supplements, citrulline and/or arginine supplementation, and ammonia scavengers (sodium benzoate, sodium phenylbutyrate, carbamylglutamate) were recommended. The three-day dietary consumption records of the patients were arranged according to the patients' statements, and their natural and synthetic protein intakes were calculated. In accordance with

the recommendations, dietary therapy was applied to all patients except for the female OTCD carrier. The calculated diet (amino acids restricted formula and natural protein restricted diet) was applied to 20 patients (71.42%), and a protein-controlled diet (natural protein intake restricted diet without amino acid restricted formula) was applied to eight patients (28.57%). The compliance rate with the diet, evaluated according to the dietary consumption records, was 96.42%. Thirteen (81.25%) of the EO patients and seven (58.33%) of the LO patients received a calculated diet. The EO patients on the calculated diet had lower natural protein

and higher caloric intake than the LO patients. There was no significant relationship between the diet groups and the neurological parameters ($p=0.648$).

Maintenance metabolic pharmacotherapy was given to 28 patients and a total of five different drugs were used. 71.42% of the patients were given sodium benzoate and 64.28% were given sodium phenylbutyrate (in combination with sodium benzoate), 71.42% were treated with L-arginine, 21.42% with L-citrulline, and 25% with carbamylglutamate. There was no difference in neurocognitive outcomes among ammonia scavengers ($p>0.32$). Intestinal bacterial decontamination with metronidazole or colistin was not used. Carbamylglutamate was used for all NAGSD and CPS1D patients (off-license approval). At the time of the first HE, P26 needed CVVHDF three times, and their ammonia levels could be controlled after carbamylglutamate. We switched to carbamylglutamate because of metabolic acidosis associated with oral sodium benzoate for P28 and their ammonia level was controlled.

Extracorporeal detoxification was initiated in neonates and children with ammonia levels >500 $\mu\text{mol/L}$. Eight patients received CVVHDF in the initial period. Eight patients (27.58%) with ammonia levels >500 $\mu\text{mol/L}$ during their first metabolic crisis received dialysis, and seven of them had encephalopathy before dialysis was initiated. The mean duration of CVVHDF was 11.2 ± 17.4 hours (min.: 4 h, max.: 72 h). The mean pre-dialysis ammonia was $1,410.5\pm 648.42$ $\mu\text{mol/L}$ (min.: 640, max.: 2,045). P26 required intermittent hemodialysis to keep their ammonia levels <200 $\mu\text{mol/L}$. Intermittent hemodialysis was applied for 7 days due to rebound hyperammonemia. After carbamylglutamate, hyperammonemia was under-control. No significant relationship was found between the length of stay in dialysis and pre-dialysis ammonia levels ($p=0.98$). No significant difference was found between the mean duration of dialysis and the disease groups [mitochondrial UCDs ($n=3$, the mean duration CVVHDF: 74.66 ± 44.04 hour) and cytosolic UCDs ($n=5$, the mean duration of CVVHDF: 14.0 ± 5.65 hour)] ($p=0.139$).

ASSD patients P9 and P10 received CVVHDF combination with therapeutic hypothermia (TH) for their first HE. P9 presented with encephalopathy and seizure. There were cerebral edema signs on transfontanelle ultrasound. The initial ammonia level was 2,430 $\mu\text{mol/L}$. Since the ammonia (pre-dialysis ammonia: 2,180 $\mu\text{mol/L}$) level did not decrease adequately with pharmacotherapy, CVVHDF and TH was started. At the 4th hour of CVHDF, ammonia decreased to 740. When ammonia was 380 $\mu\text{mol/L}$, seizure stopped. P10

presented with encephalopathy and their initial ammonia was 2,045 $\mu\text{mol/L}$. CVVHDF and TH were initiated in the second hour of pharmacotherapy because there were cerebral edema signs on transfontanelle ultrasound and pre-dialysis ammonia was 1,800 $\mu\text{mol/L}$. Ammonia decreased to 230 $\mu\text{mol/L}$ at the 12th hour of CVVHDF and TH. CVVHDF was stopped at the 12th hour and TH was stopped at the 24th hour. Significant regression of cerebral edema was observed 4 hours after onset. There was no significant relationship between those who underwent TH during dialysis and those who did not in terms of neurological parameters ($p=0.51$).

Molecular Analyses

Five different variants were detected in the *ASS1* gene, p.Gly362Val (61.5%), p.Gly390Arg (15.3%), p.Arg363Trp (15.3%), p.Arg272Cys (3.8%) and c.970+5G>A (3.8%). Eight patients from seven different families had the p.Gly362Val variant. Three (37.5%) patients with the p.Gly362Val homozygous mutation were EO and five (62.5%) patients were LO. All patients with the p.Gly362Val variant had peak ammonia $<1,000$ $\mu\text{mol/L}$ and initial citrulline $<1,000$ $\mu\text{mol/L}$. One of the three EO patients underwent extracorporeal detoxification for hyperammonemic encephalopathy. All EO patients with p.Gly362Val had normal neurodevelopmental status, however two LO patients had mild ID and one LO patient had profunda ID. In our cohort, P8 and P9 with the p.Gly390Arg variant had EO and peak ammonia $>1,000$ $\mu\text{mol/L}$, HEs per year of 4.5 and 3.1, initial peak citrulline levels of 2,700 $\mu\text{mol/L}$ and 1,804 $\mu\text{mol/L}$, and dialysis durations of 22 and 18 hours. The patients had global developmental delay and bedridden status despite treatment.

The p.Trp484Arg variant was detected in all patients from five different families in the *NAGS* gene. All these patients were the EO phenotype. There was no significant relationship between their peak ammonia levels, dialysis, and neurological parameters. Two variants were detected in the *OTC* gene, the p.Trp58Arg (previously not reported) and p.Asn258Lys from two different families. Two siblings (P19, P20) and their asymptomatic mother (P21) had the p.Trp58Arg variant. In the *CPS1* gene, four different variants (p.Val1013del, p.Gly893Gly, p.Arg1001Leu, and p.Leu1134Phe), two of which were novel, from three different families were detected. While two patients were the EO phenotype, one patient was LO. Two different variants, c.130+1G>A and p.Gly235Arg, were detected in the *ARG1* gene. The c.918+5G>A variant was detected in the *ASL* gene.

Discussion

UCDs still have poor neurological condition despite early diagnosis and treatment (3). The main aim of this study was to address the poor neurodevelopmental outcomes in UCD patients and to investigate contributing factors. The estimated incidence of UCDs ranges from 1/8,000 to 1/35,000 births (2). Although the incidence of UCD in our country is not known exactly, the prevalence was found to be 1:839 in a Turkish pilot study evaluating the general frequency of metabolic disorders (9). Although OTC deficiency is the most common UCD in the world, ASSD is more common in our country (10,11). Consistent with the literature, ASSD (44.82%) was observed most frequently in our cohort.

Patients present with a wide spectrum of clinical severity and findings. The most severe and critical presentation is hyperammonemic encephalopathy resulting in death or severe neurological impairment. EO patients mainly present as hyperammonemic encephalopathy, while LO patients show variable manifestations (3,10,12). In our cohort, 55.17% of the patients were EO and 41.37% were LO. Consistent with the literature, the EO patients presented most frequently with hyperammonemic encephalopathy, and the LO patients with different neurological symptoms in our cohort (1,3). Early diagnosis and treatment are recommended to improve neurodevelopmental outcomes (6). However, despite increasing newborn screenings, improved treatment methods and hemodialysis technology, poor neurological outcomes continue in UCD patients (13). For most EO patients, a positive screening result is not available before the first symptom onset. While neonatal screening for UCD is available in a few regions in Europe, there is still uncertainty about the benefits of NBS for individuals with ASSD and ASLD included in newborn screening programs in the United States (14). Posset et al. (15) reported that the mean ages of initial symptoms of the NBS group and EO group were similar and even earlier in the EO group (3). There is no newborn screening for UCD in our country. Patients were diagnosed during their symptomatic period and by family screening. The mean age at initial symptom was 9.8 days and the mean age at diagnosis was 11.6 days in the EO group. This showed us that the clinical findings in EO patients started early. When the neurological parameters were evaluated according to disease onset (EO/LO), no significant difference was found in our study. There was no significant difference between the peak ammonia levels and the mean number of HEs per year in the EO and LO groups. It remains unclear whether NBS has a beneficial effect

on neurocognitive outcomes, as IQ data have only been reported for a small number of patients in the NBS group in the literature. In some studies, it has been reported that ASSD and ASLD patients diagnosed with NBS have better cognitive outcomes (15). LO UCD carries a significant risk of poor neurologic outcome if not recognized and treated early (16). Due to exposure to neurotoxic metabolites, a long follow-up period is required to determine whether NBS has a lifelong positive effect on neurocognitive outcomes.

Waisbren et al. (3) reported that cytosolic UCDs had poorer neurocognitive outcomes than mitochondrial UCDs. Similarly, Nettesheim et al. (17) and Burgard et al. (18) reported worse neurocognitive outcomes in symptomatic individuals with cytosolic UCDs compared to mitochondrial UCDs, although there were fewer episodes of hyperammonemia, particularly in ASSD and ASLD patients. CPS1D and OTCD tend to have the highest risk of acute neurological damage due to HEs (16,19-21). In our study, no significant difference was found between cytosolic and mitochondrial UCDs in terms of their neurological outcomes.

In previous studies, duration of coma, peak ammonia levels and increased intracranial pressure were accepted as poor prognostic factors (19,22,23). Metabolic elimination of ammonia occurs by conversion to urea in the liver and glutamine by brain and skeletal muscle resulting in swelling of the astrocytes and cytotoxic cerebral edema. Involvement of N-methyl-D-aspartate receptors and neuronal degeneration are seen with hyperammonemia. According to research, mechanisms other than ammonia, such as impaired NO metabolism resulting in oxidative stress in the brain, are thought to contribute to neurological deterioration (3,15). Acute hyperammonemia without cerebral edema is the main pathophysiology which triggers neuronal disinhibition and seizures due to the Na⁺-K⁺-2Cl⁻ cotransporter isoform1 in the neuron. However, the brain damage mechanisms in UCDs are still not fully elucidated. Peak ammonia levels have been associated with poor neurocognitive outcomes in most studies (3,13,15,19,23). In the study of Uchino et al. (23) with 216 UCD patients, the 5-year survival rate was reported as 22% for EO UCD and 41% for LO UCDs. A significant relationship was found between the peak ammonia level during the first HE and neurocognitive outcomes. When peak ammonia levels are <306 µg/dL, there is no serious neurological deficit. However, when this value is over 596 µg/dL, patients either die or have severe neurological defects (23). Bachman reported that none of their patients had normal neurological outcomes when their peak ammonia levels were ≥817 µg/dL (19).

In the Posset et al. (15) study, the baseline ammonia level was associated with impaired neurocognitive outcome in those with mitochondrial UCDS. Waisbren et al. (3) reported a significant relationship between exposure to disease-specific neurotoxic biomarkers such as ammonia, citrulline, and neurocognitive outcomes. Also, there is a relationship between the mean number of HEs per year and poor neurological outcomes (3). Inconsistent with the literature, Msall et al. (20) reported no significant relationship between peak ammonia levels and IQ in 26 patients with neonatal UCDS. However, a significant negative linear correlation was found between stage III or IV hyperammonemia coma duration and 1-year-old IQ levels (20). Consistent with the literature, a significant relationship was found between abnormal neurological parameters and the peak ammonia level and the mean number of HEs per year in our study. The effect sizes of the groups were large. All these studies emphasize the importance of the timely diagnosis and rapid reduction of ammonia levels.

Enzyme activity in ASSD patients correlates with peak ammonia and initial citrulline levels (5). Peak citrulline levels were significantly higher in ASSD patients who had abnormal neurological parameters in our study group. Although we could not find a significant relationship between the citrulline subgroups and delayed milestones in ASSD patients, delayed milestones occurred in all subjects with a citrulline level over 500 $\mu\text{mol/L}$. Abnormal motor skills and tone abnormalities were observed in all cases with a citrulline level over 1.000 $\mu\text{mol/L}$. We think that the reason for not detecting a statistically significant difference is related to the small sizes of the subgroups. Consistent with the literature, we observed a relationship between high citrulline levels and poor neurological outcomes in our study. However, there were patients in our study group who had high citrulline levels but were mentally normal. Plasma citrulline levels can only partially help predict prognosis and neurological outcome, and so it is difficult to give an accurate prognosis. We could not study enzyme levels due to technical limitations. Plasma glutamine is widely used as a biomarker of control in UCD patients (24,25). In addition, plasma glutamine concentrations can be viewed as a predictor of the organism's total nitrogen load. Although diurnal variability is less, it varies with nutritional status and is highest after fasting (26). Levels exceeding 900-1,000 $\mu\text{mol/L}$ are thought to be associated with HEs. Lee et al. (25) reported that glutamine was a weaker predictor than ammonia for HE and had a predictive value when evaluated with concomitant ammonia. However, in our cohort, there was no significant relationship between peak plasma

glutamine levels and peak ammonia, the mean number of HEs per year or neurological parameters. Plasma glutamine levels do not accurately reflect brain levels.

A study in OTCD patients showed that IQ is a valid marker for defining neurocognitive outcomes (27). We used the WISC-R test to determine the neurocognitive status in our patients older than 6 years of age. We evaluated them according to their total IQ scores. However, we could not detect a significant correlation between any clinical or laboratory parameter and their WISC-R scores.

ASS1 deficiency is a urea cycle disorder with an estimated incidence of one in 44,300-250,000 based on the literature (28). ASS1D shows heterogeneous clinical manifestations, such as the EO form with HE, seizures, coma and cerebral edema, the LO form with varying degrees of neurological impairment, and the asymptomatic form. To date, more than a hundred variants in the *ASS1* gene located on chromosome 9q43.11 (#215700) have been reported. In our cohort, five different variants were detected in the *ASS1* gene, p.Gly362Val, p.Gly390Arg, p.Arg363Trp, p.Arg272Cys and c.970+5G>A. The second most common p.Gly362Val variant in Turkey was observed most frequently in our study group. This variant has previously been associated with highly conserved residual enzyme activity and mild or asymptomatic citrullinemia (29,30). In our cohort, five patients had normal neurodevelopmental status and two patients had mild ID. Inconsistent with the literature, P5 with LO had profunda ID and was bedridden. The mean number of HEs of P5 was 3.2 per year and peak ammonia levels were similar to mentally normal ASSD patients. P7 with EO and the p.Gly362Val variant presented with hyperammonemic encephalopathy and underwent CVVHDF and had normal WISC-R scores at the age of eight. The most common variant in the world and in our country is p.Gly390Arg (27). It has been associated with severe clinical form in previous studies. However, Daou et al. (29) reported different clinical presentations with this variant. In our cohort, both patients with this variant had EO, peak ammonia >1.000 $\mu\text{mol/L}$, mean numbers of HEs per year of 4.5 and 3.1, initial citrulline levels of over 1.000 $\mu\text{mol/L}$, and dialysis durations of 22 and 18 hours. Consistent with the literature, both patients had poor neurodevelopmental outcomes (Denver II test <-2 SDS). The p.Arg363Trp variant is associated with EO (28). In our cohort, these patients also had EO, global developmental delay and were bedridden despite early treatment and dialysis. Hypercitrullinemia is used as a biochemical marker for ASS1D, but the genotype-phenotype correlation is not very strong (5).

In our cohort, two siblings had identical genotypes for OTCD. P19 had profunda ID, whereas P20 had normal neurological parameters. Klaus et al. (30) reported two siblings with identical genotypes for CPS1D, one died as a newborn while the other had normal functions at the age of 45 with dietary therapy. While an EO patient who needed CVVHDF had normal development, there was also an adult patient with ID (31). It indicates the absence of a genotype/phenotype relationship in UCD. At present, a precise prediction of neurological outcome is not easy as there is no correlation between genotype, age of onset, and/or phenotype. The reason for this significant difference in neurological presentation is unknown.

A combination of a low protein diet with or without essential amino acid supplements, citrulline and/or arginine, and nitrogen scavengers (sodium benzoate, and sodium glycerol phenylbutyrate) has been suggested for maintenance therapy (6). Lee et al. (25) evaluated the efficacy of therapeutic modalities in UCD patients with the stable isotope protocol. However, sufficient information was not obtained about the prognostic process. The nitrogen removal efficiency of sodium phenylbutyrate is biochemically double compared to sodium benzoate. However, the superiority of each other in terms of disease severity or neurocognitive outcome is not clear in studies (32). Carbamyl acid is licensed for NAGS deficiency. Also, we used it for CPS1D patients off-license. It provided good metabolic control in CPS1D patients who needed recurrent CVVHDF due to resistant hyperammonemia. In our study group, there was no difference in neurocognitive outcome among ammonia-scavengers. These disappointing pharmacotherapeutic data, together with the poor cognitive outcomes in symptomatic UCD patients, suggest an urgent need for new treatments. In some studies, the use of multiple ammonia scavengers resulted in better cognitive outcomes (3). This has been associated with better metabolic control.

CVVHDF is currently the most effective way of ammonia detoxification. In cases of ammonia >500 $\mu\text{mol/L}$ and insufficient reductions in ammonia levels after 4 hours medical treatment, hemodiafiltration is recommended according to the UCD guideline. Based on this, eight patients with ammonia levels >500 $\mu\text{mol/L}$ during their first metabolic crisis received dialysis. Coma is a known predictor of poor outcome and patients with baseline ammonia >360 $\mu\text{mol/L}$ are reported to have a poor prognosis. Kido et al. (33) recommended that patients with a baseline ammonia level above 180 $\mu\text{mol/L}$ should receive hemodialysis. Enns et al. (34) recommended aggressive treatment including

dialysis in neonatal hyperammonemia exceeding 300 $\mu\text{mol/L}$ (34). Spinale et al. (35) recommended extracorporeal detoxification for ammonia level >400 $\mu\text{mol/L}$ in newborns. In our study, we found a significant relationship between poor neurocognitive outcome and initial peak ammonia levels and the mean number of HEs per year. There were still poor neurocognitive outcomes despite extracorporeal detoxification. This highlights the need to reassess current treatment strategies, including the threshold for starting extracorporeal detoxification if ammonia levels exceed 500 $\mu\text{mol/L}$.

It has been shown that TH significantly reduces both morbidity and mortality in perinatal encephalopathy (36-38). Moderate hypothermia has been previously used in adult acute liver failure, and decreases in blood ammonia levels and intracranial pressure have been observed (37). In some studies, TH was used in combination with extracorporeal detoxification in UCD and organic acidemia patients, it was hypothesized that hypothermia may have reduced enzymatic ammonia production and reduced brain edema (38-40). Based on the literature, we also used TH in combination with CVVHDF in two patients with ASSD. When the neurological parameters of CVVHDF and the TH combination with CVVHDF groups were compared, no significant difference was found. However, a significant regression was observed in brain edema four hours after onset in the TH group. The use of TH by experienced teams seems promising due to its neuroprotective effects, ammonia-reducing effects, anti-inflammatory effects, and brain edema-reducing effects. Further studies with large numbers of patients are needed.

Study Limitations

This is a retrospective study and we could not perform enzyme analyses due to technical limitations. Lack of enzyme analysis made it difficult to evaluate the effects of genotype on phenotype. The negative impact of the number of subgroups and the low number of cases on the statistical results cannot be excluded.

Conclusion

There are several reasons why UCDs can have poor neurological outcomes despite early diagnosis. Based on our patient group, which were mostly diagnosed in the symptomatic period, we aimed to examine the parameters which can be easily used by clinicians in estimating disease severity and neurocognitive outcomes. Our study emphasizes the effect of peak ammonia levels and the frequency of HEs on neurological outcomes. This does

not necessarily mean that other interventions are not effective. There were still poor neurocognitive outcomes despite extracorporeal detoxification. This highlights the need to reassess current treatment strategies, including the threshold for starting extracorporeal detoxification if ammonia levels exceed 500 $\mu\text{mol/L}$. The use of TH by experienced teams seems promising due to its brain edema-reducing effects. Further studies with larger numbers of patients are needed. Close monitoring of ammonia levels, rapid and effective intervention, and the prevention of hyperammonemia crises are essential for good neurological outcomes.

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Ethics

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of the University of Health Sciences Turkey, Diyarbakır Gaziyaşargil Training and Research Hospital (approval no: 928, date: 05.11.2021).

Informed Consent: The families of the patients' who participated were informed about this research and signed consent forms.

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Authorship Contributions

Surgical and Medical Practices: A.E.B, E.G., Concept: A.E.B., Design: A.E.B, Data Collection or Processing: A.E.B., E.G., A.T.Ü., H.M.A., İ.T. B.K., M.N.Ö., Analysis or Interpretation: A.E.B., M.K., Literature Search: A.E.B., A.T.Ü., Writing: A.E.B., M.K.

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