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Positive Clinical, Neuropsychological, and Metabolic Impact of Liver Transplantation in Patients With Argininosuccinate Lyase Deficiency

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ABSTRACT

Liver transplantation (LTx) is increasingly used in Urea Cycle Defects (UCDs) to prevent recurrent hyperammonemia and related neurological irreversible injury. Among UCDs, argininosuccinate lyase deficiency (ASLD) has a more complex phenotype than other UCDs, with long-term neurocognitive deficits. Therefore, the role of LTx in ASLD is still debated. The impact of LTx on nine patients with early-onset ASLD was assessed through pre- and post-LTx clinical, neuropsychological, MRI and biochemical evaluations. After LTx, no episodes of metabolic decompensations were reported. Neuropsychological evaluations documented significant improvement in cognitive/developmental functioning especially in patients transplanted in early childhood. Improvements were also highlighted in daily living skills and emotional-behavioral problems, with a reduction in attention disturbances and somatic complaints. Movement disorders resolved after LTx in patient transplanted in early childhood. Any patients developed epilepsy with stability of EEG alterations after LTx. A positive effect of LTx on other disease-related outcomes such as growth, diet, medications, hospitalizations, and long-term ASLD-related complications was highlighted. The primary biomarker argininosuccinic acid dramatically reduced in plasma after transplantation with a decreasing trend in CSF at long-term follow-up. Moreover, health-related quality of life improved after LTx, especially when assessed through MetabQoL, a tool designed for intoxication diseases such as ASLD. In conclusion, our study showed a global beneficial impact of LTx in early-onset ASLD patients to avoid episodes of hyperammonemia, and improve neurocognitive outcome, adaptive and behavioral deficits when performed in early childhood with a dramatic benefit in terms of quality of life.

1 | Introduction

Argininosuccinate lyase deficiency (ASLD), also called argininosuccinic aciduria (OMIM 207900), belongs to the urea cycle disorders (UCDs) and is caused by pathogenic variants in *ASL* gene, encoding for argininosuccinate lyase (ASL), the enzyme catalysing the cleavage of argininosuccinic acid (ASA) into arginine and fumarate [1]. ASL also plays a structural function in

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the multiprotein complex channelling arginine to nitric oxide synthase (NOS) for nitric oxide (NO) production [2].

Patients affected by ASLD can manifest with a wide clinical spectrum, ranging from a severe early-onset neonatal hyperammonemic encephalopathy, to a late-onset phenotype characterized by milder episodic hyperammonemia with recurrent vomiting and abnormal feeding behaviour [1]. Long-term complications include chronic liver disease, systemic hypertension, failure to thrive, and neurologic impairment [3]. Biochemically, the disease is characterized by the accumulation of ASA in body fluids, combined with the increase of citrulline, glutamine and orotic acid [1].

Long-term treatment of UCDs aims to decrease ammonia through a protein-restricted diet combined with nitrogen scavengers and, to correct arginine deficiency, by L-arginine (or citrulline) supplementation [1]. In the last years, liver transplantation (LTx) has been increasingly utilized as a therapeutic option for severe UCDs to improve metabolic control and long-term outcome, prevent neurological deterioration, and reduce disease burden [4–6].

Compared to other UCDs, ASLD is a more complex disease, often associated with a poorest neurological outcome as intellectual disabilities, epilepsy, movement and psychiatric disorders, and chronic liver disease [7–11]. For these reasons, the indication of LTx in ASLD is still debated and reports on transplanted cases are mainly focused on the description of surgical-related issues, without systematically addressing the effects of LTx on neurological outcome and on disease-associated metabolic profiles [12–19].

For better understanding the impact of LTx on ASLD, we report on nine early-onset patients, evaluated pre-and post-LTx by assessment of clinical, neurological, neuropsychological outcomes, combined with multimodal brain MRI and MRS studies and disease-associated biomarkers measurements in plasma and CSF.

2 | Patients and Methods

2.1 | Clinical Data

Nine patients with genetically confirmed ASLD were referred to the Metabolic Transplant Team of Bambino Gesù Children's Hospital in Rome. All patients had a history of neonatal hyperammonemia and underwent liver transplantation between April 2012 and March 2023. The study was approved by the institutional Ethical Committee (2119_OPBG_2020), in agreement with the Declaration of Helsinki of 1964 and revised in 2000 and informed consent was obtained from patients or their parents.

Clinical disease course and anthropometric data were investigated before and after LTx. Weight and height are expressed as Z-score, and failure to thrive is considered with weight or height below 5th percentile or Z score < -1.64.

2.2 | Neurological, Neuropsychological, and Quality of Life Assessment

Neurological evaluation and EEG studies were performed before and after LTx. Similarly, patients underwent cognitive/developmental assessment by specific test selected on participant's age [20]. Adaptive behavior was assessed by the Vineland Adaptive Behavior Scales [21] and the Adaptive Behavior Assessment System (2nd Edition) [22]. For both scales, domains scores < 70 indicate a lower adaptive functioning than average. Emotional and behavioral problems were investigated by the Child Behavior Checklist (CBCL) [23].

Health-related quality of life (HrQoL) was assessed by MetabQoL 1.0, a newly developed tool specifically designed for intoxication-type inborn errors of metabolism and by the conventional PedsQL-General Module 4.0 [24]. Three-time points were analyzed (a) before LTx (3.2 months, 0.9–16.8; $n = 9$), (b) 1-year after LTx (1 years, 0.9–1.7; $n = 7$) and (c) last available individual follow-up (7.5 years, 3.2–12.3; $n = 6$).

2.3 | Neuroradiological Studies

Brain MRI studies were performed on a 3 T scanner (Magnetom Skyra, Siemens, Erlangen, Germany) for each patient at three-time points: before LTx, 1-year after LTx and at the last available individual follow-up evaluation. Brain abnormalities were scored by evaluating T2 white matter changes and cortical atrophy. MRI assessments of cortical thickness, diffusion weighted images, neurite morphology [neurite density index (NDI) and orientation dispersion index (ODI)], were studied in two patients (Pt. 1 and 5) and compared with 10 age matched controls [20].

MRI spectroscopy analysis (MRS) was studied in four patients (Pts. 1, 3–5) and compared with 20 age matched controls. Single voxel spectra were recorded at the level of basal ganglia.

2.4 | Laboratory Measurements

Routine clinical chemistry analyses evaluated, pre- and post-LTx, plasma levels of ammonia, transaminases, triglycerides, total cholesterol, and electrolytes. Metabolic analyses, which included determination of amino acids, creatine and guanidinoacetic acid, were performed in plasma and CSF as part of our standard transplant work-up protocol [20].

Amino acids were quantified by HPLC, creatine and guanidinoacetic acid by liquid chromatography tandem-mass spectrometry as previously described [25].

Laboratory values recorded during neonatal hyperammonemia at disease onset and those corresponding to the first 15 days after LTx were not considered for longitudinal evaluations. Plasma samples before LTx were available for amino acids in all patients, for creatine in 8/9 and for GAA in 6/9, while post-LTx samples were determined in 9/9 patients for all determinations. CSF analyses were assessed before and after LTx at specific time points (6 months, 1, 2, 5 and 10 years), samples were collected under general anaesthesia during scheduled procedures as liver biopsy or brain MRI. CSF samples before LTx were available for amino acids in 7/9 patients, for creatine in 6/9 and for GAA in 4/9, while post-LTx samples were determined in 9/9 patients for all determinations.

For correlation studies between plasma and CSF results, data corresponding to simultaneously collected samples were utilized.

2.5 | Statistical Analysis

Continuous data were reported as median and interquartile range, while age of patients and timing of LTx were expressed as median and range (min, max). Wilcoxon and Mann–Whitney for matched pairs and unmatched data were used to compare differences in medians between pre- and post-LTx. Quantile regression with clustered standard errors was used to analyse ASA trends in relation to patients' age and time from transplantation. A Friedman test was used to describe any changes in all neuropsychological evaluations and HrQoL scores at three assessment time-points. Pearson correlation coefficients were calculated to analyse the relations between neuropsychological outcomes, biochemical results, and age at LTx.

Multiple linear regression analyses of continuous and categorical variables (LTx) assessed the influence of clinical and biochemical parameters on neurodevelopmental outcome.

Data and graphical analysis were carried out using Stata 17.1 and GraphPad 8.0.2 (GraphPad Software Inc., San Diego, CA). Statistical significance was set at $p < 0.05$.

3 | Results

Nine neonatal-onset ASLD patients with a median age of 11 years (3.9–24.6) were studied. As shown in Table 1, molecular analysis in our patients identified 9 different homozygous variants in *ASL* gene (NM_000048.4). Baseline clinical features and transplantation-related data are detailed in Table 1. All patients presented in the first week of life with hyperammonemic encephalopathy (ammonia peak 766 μmol/L, 650–1590, n.v. < 100), requiring admission to neonatal intensive care unit and treatment with extracorporeal dialysis in 4/9 patients (Figure 1).

According to current guidelines [1], all patients were treated with protein restricted diet, nitrogen scavengers (i.e., sodium benzoate, sodium phenylbutyrate or glycerol phenyl-butyrate) and L-arginine therapy.

Patients were transplanted at a median age of 7.6 years (1.1–18.9), after a median waiting time of 47 days. In 8/9 patients the main indication for LTx was the prevention of hyperammonemic crises to reduce the risk of long-term complications, while one patient was transplanted for chronic progressive liver disease [8]. Seven out of 9 patients were transplanted from deceased donors with 3 whole livers and 3 partial grafts (left lateral segment) from in situ split liver. The remaining two received a partial graft (left lateral segment) from a living heterozygous parent.

At last available follow-up, patients and graft survival were 100%, with well-functioning graft in all individuals. Transplant-related adverse events and surgical complications included acute rejection (3/9 patients), Epstein–Barr virus and Cytomegalovirus infection (2/9 patients), and biliary stenosis treated with endoscopic retrograde cholangiopancreatography (2/9 patients).

After LTx, all patients discontinued nitrogen scavengers and L-arginine therapy and liberalized the natural protein intake at least up to the WHO recommended daily allowance for age [26], without experiencing novel episodes of hyperammonemia (Table 1).

Before transplantation, 6/9 patients presented with failure to thrive. After LTx, a significant improvement was recorded in height [$\Delta + 1.5$ (0.4–2.0), $p = 0.0039$] and weight [$\Delta + 0.3$ (0.2–1.6), $p = 0.0117$] Z scores (Table 1, Figure S1).

Before transplantation, 4/9 ASA patients required potassium supplementation to correct hypokalaemia that normalized in all subjects at long-term follow-up. At baseline, altered lipid profile with elevated triglycerides and/or cholesterol was identified in 7/9 patients. After LTx, all patients showed a significant reduction of triglycerides (275 vs. 124, n.v. < 100 mg/dL; $p < 0.0001$;) and normalization of cholesterol levels (224 vs. 138, n.v. < 200 mg/dL; $p < 0.0001$).

3.1 | Neurological Outcome

The long-term neurological outcomes are reported in Table S1. Before LTx, EEG alterations were identified in 5/9 patients showing poorly organized background, diffuse/focal slowing activity, and epileptic changes. Following transplantation EEG alterations stabilized and no patient developed epilepsy. Any patients developed epilepsy with stability of EEG alterations after LTx. Movement disorders characterized by fine intention hands tremors, were recorded in 2/9 patients, and resolved after transplantation in one.

3.2 | Neuropsychological Outcomes

Long-term neuropsychological outcomes are reported in Figure 2. Pre-LTx assessment in the four patients transplanted in early childhood [Pts.1–4, median age at LTx 3.1 years (1.1–4.7)] compared to the five later transplanted patients [Pts. 5–9, median age at LTx 8.9 years (7.6–18.9)] showed better DQ/IQ scores (63.5 vs 51). Post-LTx, early transplanted patients showed improvement and stabilization of their intellectual development (post 1-year 70.5, last follow-up 71). Similarly, also later transplanted patients showed stable or mildly improving IQ trends (Figure 2A). The early age at LTx significantly correlated with better developmental/cognitive outcomes ($r = -0.943$, $p = 0.017$), as seen in the two patients transplanted within the first 2 years of life showing normal/improved functioning at medium- (2.8 years., Patient 1) and long-term (12.2 years., Patient 2) follow-up. The evaluation of specific subdomains in the early transplanted patients recorded improving post-LTx trajectories both in verbal (56 vs. 76) and performance (51.5 vs. 77) abilities. Also, late transplanted patients showed improving verbal (58 vs. 65) and stable performance (61 vs. 61) abilities. No differences were recorded between male and female patients for DQ/IQ.

TABLE 1 | Clinical features, molecular analysis, and liver transplantation pre- and post-related data in nine ASLD patients.

Patient (sex)	Genotype ALS (NM_000048.4)	Age at onset (days)	NH3 (μmol/L)	Pre LTx therapy	Liver disease (↑ transaminase/ hepatomegaly)	Age at LTx (years)	Indication for LTx (graft type)	Protein intake (g/kg/die) natural/essential AA		Weight Z score		Height Z score		Follow-up (years)	Post-operative complications
								Pre	Post	Pre	Post	Pre	Post		
Patient 1 male	c.1128C>A (p.Tyr376Ter)	2	536	SB GPB Arg	No/yes	1.1	Poor metabolic control (deceased donor)	1.15/0.2	2.1	−3.11	−0.3	−3.20	0.51	2.8	EBV infection, acute rejection
Patient 2 female	c.1128C>A (p.Tyr376Ter)	3	700	SB Arg	Yes/yes	2.4	Poor metabolic control living donor	1/0.2	1.0	0.66	0.47	−0.50	0.30	12.2	—
Patient 3 female	c.857A>G (p.Gln286Arg)	5	753	SB PBA Arg	Yes/yes	3.9	Poor metabolic control (deceased donor)	0.8/0.2	1.2	0.52	0.82	−0.22	1.4	6.1	Biliary stenosis acute rejection
Patient 4 male	c.857A>G (p.Gln286Arg)	3	766	SB Arg	Yes/yes	4.7	Poor metabolic control living donor	0.8/0.2	1.4	−3.71	−0.52	−4.30	−1.9	8.9	—
Patient 5 female	c.436C>T (p.Arg146Trp)	3	1680	SB GPB Arg	Yes/yes	7.6	Poor metabolic control (deceased donor)	0.9/0.3	1.9	1.47	1.32	0.56	0.98	3.3	EBV, CMV infections, acute rejection
Patient 6 female	c.707G>A (p.Arg236Gln)	7	600	SB GPB Arg	Yes/yes	7.8	Poor metabolic control & chronic liver disease (deceased donor)	1.2	1.8	−1.32	−1.11	−3.2	−1.58	1.2	—
Patient 7 male	c.551_552delCT (p-Ser184*)	3	1500	GPB Arg	Yes/yes	8.9	Poor metabolic control & chronic liver disease living donor	1/0.49	1.9	−2.01	−1.54	−1.45	−1.07	1.4	Biliary stenosis

(Continues)

TABLE 1 | (Continued)

Patient (sex)	Genotype ALS (NM_000048.4)	Age at onset (days)	NH3 (μmol/L)	Pre LTx therapy	Liver disease († transaminase/ hepatomegaly)	Age at LTx (years)	Indication for LTx (graft type)	Protein intake (g/kg/die) natural/essential AA		Weight Z score		Height Z score		Follow-up (years)	Post-operative complications
								Pre	Post	Pre	Post	Pre	Post		
Patient 8 male	c.133 T>A (p.Tyr45Asn)	3	780	SB PBA	Yes/yes	11.9	Chronic liver disease (deceased donor)	1/0.1	1.2	−1.44	−1.21	−3.20	−1.6	12.6	—
Patient 9 male	c.1366C>G (p.Arg456Gly)	3	2930	SB Arg	No/yes	18.9	Poor metabolic control (deceased donor)	0.9/0.3	1.4	−3.11	−2.98	−2.67	−1.21	1.7	—

Abbreviations: Arg: L-arginine; CMV: Cytomegalovirus; EBV: Ebstein bar virus; GPB: Glycerol phenylbutyrate; LTx: liver transplantation; NH3: blood ammonia level (n.v. < 100); SB: Sodium Benzoate.

At pre-LTx evaluation, earlier transplanted patients showed normal/borderline adaptive scores, while the remaining patients had mild to severe disabilities (Figure 2B). Regardless of the age at transplantation, all patients showed a significant improvement in adaptive functioning at last follow-up evaluation (58 vs. 73, $p = 0.0048$), with specific changes in daily living skills (47 vs. 74, $p = 0.005$) (Figure 2B, S2). Moreover, higher scores in communication (73 vs. 63) and socialization domains (85 vs. 66) were recorded in early transplanted individuals.

Baseline CBCL evaluation detected in all patients, borderline to clinical level scores in attention problems, with more relevant behavioral and emotional problems in older patients, especially in anxiety-related symptoms (68 vs. 51, $p = 0.018$) (Figure 2C,D). Post-LTx, significant changes were recorded in total, internalizing and externalizing problems scores (Figure 2C), combined with a reduction of attention problems (67 vs. 59.5, $p = 0.010$) and somatic complaints (63 vs. 50, $p = 0.011$) (Figure 2D). All CBCL domain scores normalized in early transplanted patients, while borderline scores in anxious/depressed, withdrawn and attention problems persisted in those transplanted later (Figure 2D).

3.3 | Health-Related Quality of Life

Baseline MetabQoL evaluations showed impaired quality of life, regardless of patients age and age at LTx (Figure 2E). After LTx, a significant improvement of disease-related issues became evident at the first-year follow-up evaluation, whit a subsequent stabilization over time (Figure 2E). Accordingly, physical (55 vs. 77, $p = 0.031$), mental (55 vs. 79, $p = 0.031$) and social (43 vs. 87, $p = 0.031$) subdomain scores showed significant improvements, as well most of related subscales (i.e., diet, hospitalization, living with disease, emotions with disease, stigma, and severity) (Figure 2F).

The assessment of HrQoL with the generic PedsQL tool also displayed post-LTx improvements (pre-LTx 65 vs. post 1-year 84 vs. last follow-up 86, $p = 0.034$), with meaningful changes in the physical functioning subdomain (66 vs. 90, $p = 0.010$). However, at pre-LTx evaluation only later transplanted patients perceived an impaired quality of life compared to early transplanted ones (60 vs. 82.5), displaying improved post-LTx scores in total (60 vs. 87, $p = 0.029$), physical (62.5 vs. 91, $p = 0.031$) and social (40 vs. 70) scales. Differently, in early transplanted patients PedsQoL detected a satisfactory pre-LTx quality of life, which remained stable at post-LTx evaluations (82.5 vs. 83).

3.4 | Magnetic Resonance Studies

In 7/9 patients, baseline brain MRI displayed T2 hyperintense changes in the deep white matter, mainly in the corona radiata and in periventricular areas, and in 4/9 patients in the superficial white matter of frontal and parietal lobes (Table S2). After LTx, the T2 abnormalities improved in one early transplanted patient (Figure 3), stabilized in five, and partially worsened in one (Table S2). Diffuse and symmetrical cortical atrophic changes detected in 3/9 patients remained stable at post-LTx follow-up (Table S2).

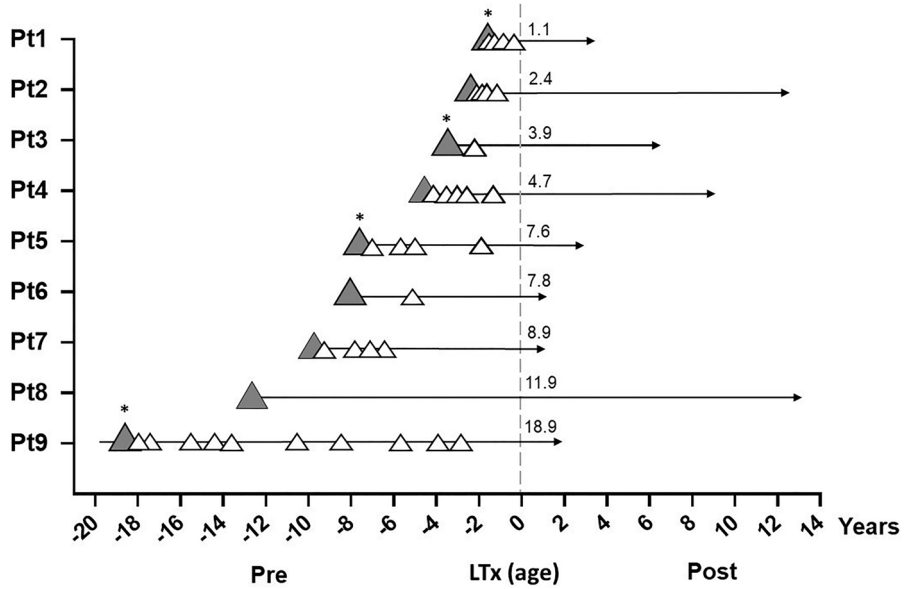

FIGURE 1 | Timeline of hyperammonemic episodes (△), liver transplantation and long-term follow-up in nine ASLD patients. Neonatal hyperammonemia (▲) management required neonatal intensive care unit and 4/9 patients also required dialytic treatment (CVVH *).

In the two patients undergoing brain morphometric study, the uncorrected permutation *p* value maps demonstrated post-LTx an increase of cortical thickness across all brain regions: Δ + 36% in Pt.1 and Δ + 5% in Pt.5, respectively. Consistently, uncorrected p-values maps revealed an NDI increase spreading over the hemispheres in Patient 1 and a stabilization in Patient 5. ODI analysis remained unchanged in both individuals (Figure 4).

As detected by MRS analysis, levels of brain creatine were normal both at pre- and post-LTx evaluation in the four patients studied compared with 20 age matched controls (Table S3).

3.5 | ASA-Related Biomarkers Change in Plasma and CSF

The results of biomarkers analyses in plasma and CFS are reported in Figure 5 and Table S4. After LTx, plasma ammonia levels steadily normalized in all patients, with no relapse of hyperammonemia (Figure 1).

Plasma glutamine, which was intermittently elevated in some patients pre-LTx, showed a significant reduction, with post-LTx values in the normal range in all patients. In CSF, although the median levels did not display significant changes between pre- and post-LTx, we detected a reducing trend towards a safe range. Two patients with elevated pre-LTx glutamine displayed a normalization of its level after LTx.

ASA decreased significantly in plasma and to a lesser extent in CSF. Interestingly, quantile regression analysis of cumulative CSF data showed a decreasing ASA trend with increasing patients' age (coeff. −8.82) and post-LTx follow-up duration (coeff. −1.56) (Table S5). Citrulline levels were significantly reduced after LTx both in plasma and CSF, although remaining above normal levels in both compartments. Plasma arginine, which was supplemented before transplantation, was reduced post-LTx, while remaining within normal range in all patients. In CSF, its concentration was also reduced, with 4/9 patients showing intermittent values below reference range at serial evaluations.

The mildly elevated pre-LTx plasma creatine levels, post-LTx returned to normal in all patients. Different from plasma, pre-LTx CSF creatine showed borderline-low levels in 3/4 tested patients (on arginine supplementation). After LTx, low CSF creatine concentration was recorded in 4/9 patients (without arginine supplementation). Statistical analysis did not display correlations between creatine and its precursor arginine in CSF ($r = 0.10$, $p = 0.717$). The levels of GAA, which were within normal range in both compartments, remained unchanged in plasma and showed reducing values in CSF.

3.6 | Predictors of Neurocognitive Outcomes and Correlations Studies

Multiple linear regression analysis identified ammonia levels at (neonatal) disease onset ($p = 0.0333$) and age at transplantation ($p = 0.0359$) as significant predictors of DQ/IQ outcome, a result further supported by correlation matrix showing that cognitive outcome was negatively associated with ammonia level at disease onset ($r = -0.79$, $p = 0.014$) and with age at transplantation ($r = -0.64$; $p = 0.07$) (Figure 6).

Positive correlations were observed between plasma and CSF arginine ($r = 0.74$; $p = 0.002$), between arginine and citrulline in CSF ($r = 0.70$; $p = 0.003$) and, in pre-LTx samples, between ASA and citrulline in plasma ($r = 0.65$; $p = 0.04$).

4 | Discussion

Liver transplantation is increasingly used in UCDs to prevent recurrence of hyperammonemia and related irreversible

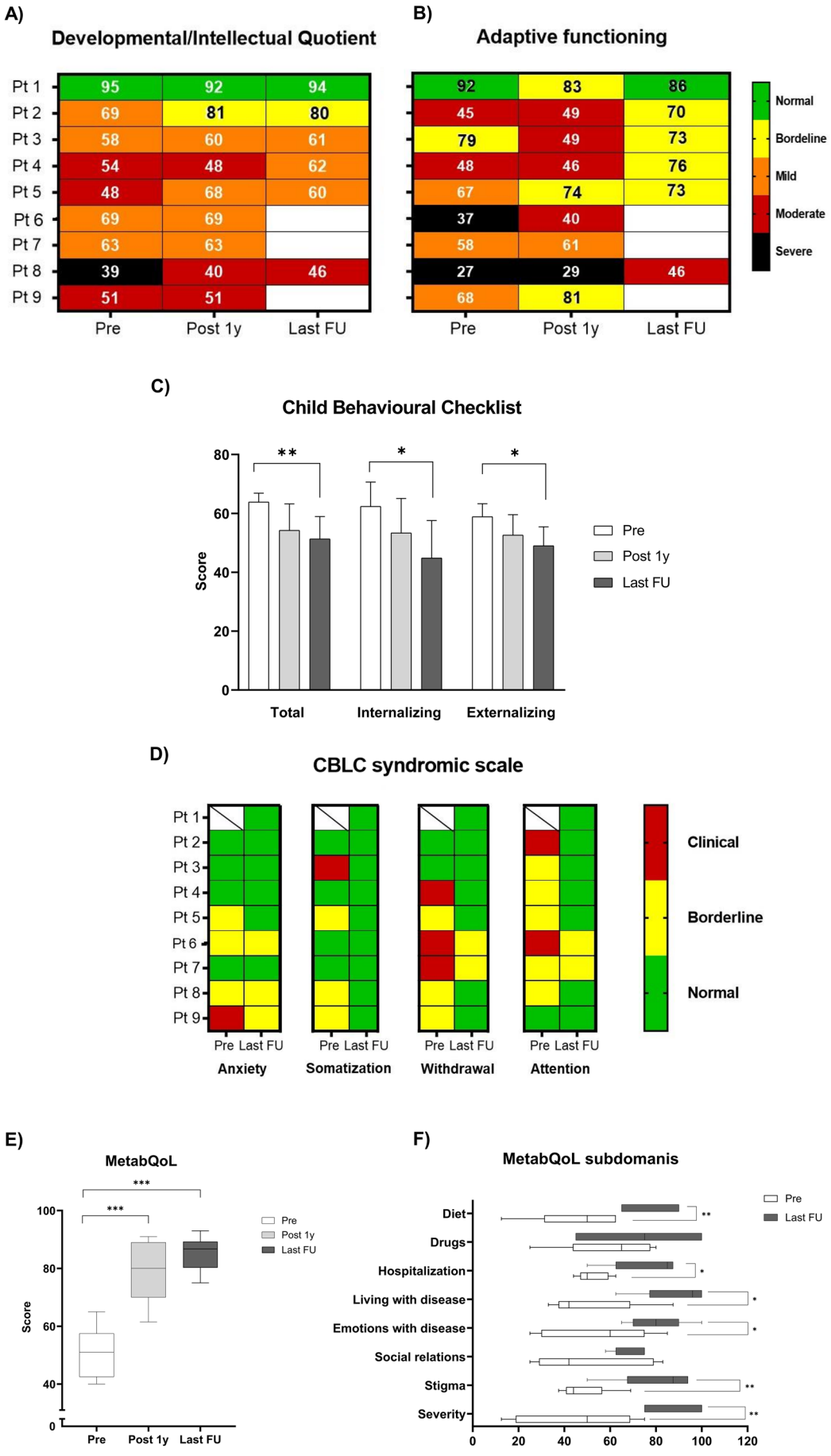

FIGURE 2 | Legend on next page.

neurological injury [1]. As a result of onset modalities and of frequency and intensity of hyperammonemia, UCDs may present with variable degree of intellectual and learning disabilities [27–30]. Differently from other UCD, the incidence of neurocognitive abnormalities in ASLD is higher, despite a lower frequency and duration of hyperammonemic crises, raising the question of what mechanisms, in the so called "ASA paradox" [10], underlie the neurological deterioration in ASLD. The suggested hypotheses include the neurotoxicity of ASA, the formation of guanidino compounds (i.e., GAA and guanidinosuccinic acids), the accumulation of reactive oxygen species, cerebral creatine and agmatine deficiency, an altered metabolism of NO, an abnormal protein nitrosylation and the increased permeability of the blood brain barrier [31, 32]. However, the exact mechanism causing an elective cerebral dysfunction in ASLD still remains to be elucidated and for these reasons the use of LTx is debated, due to a potentially poorest neurological outcome compared to other UCDs [10, 31]. Moreover, only a few

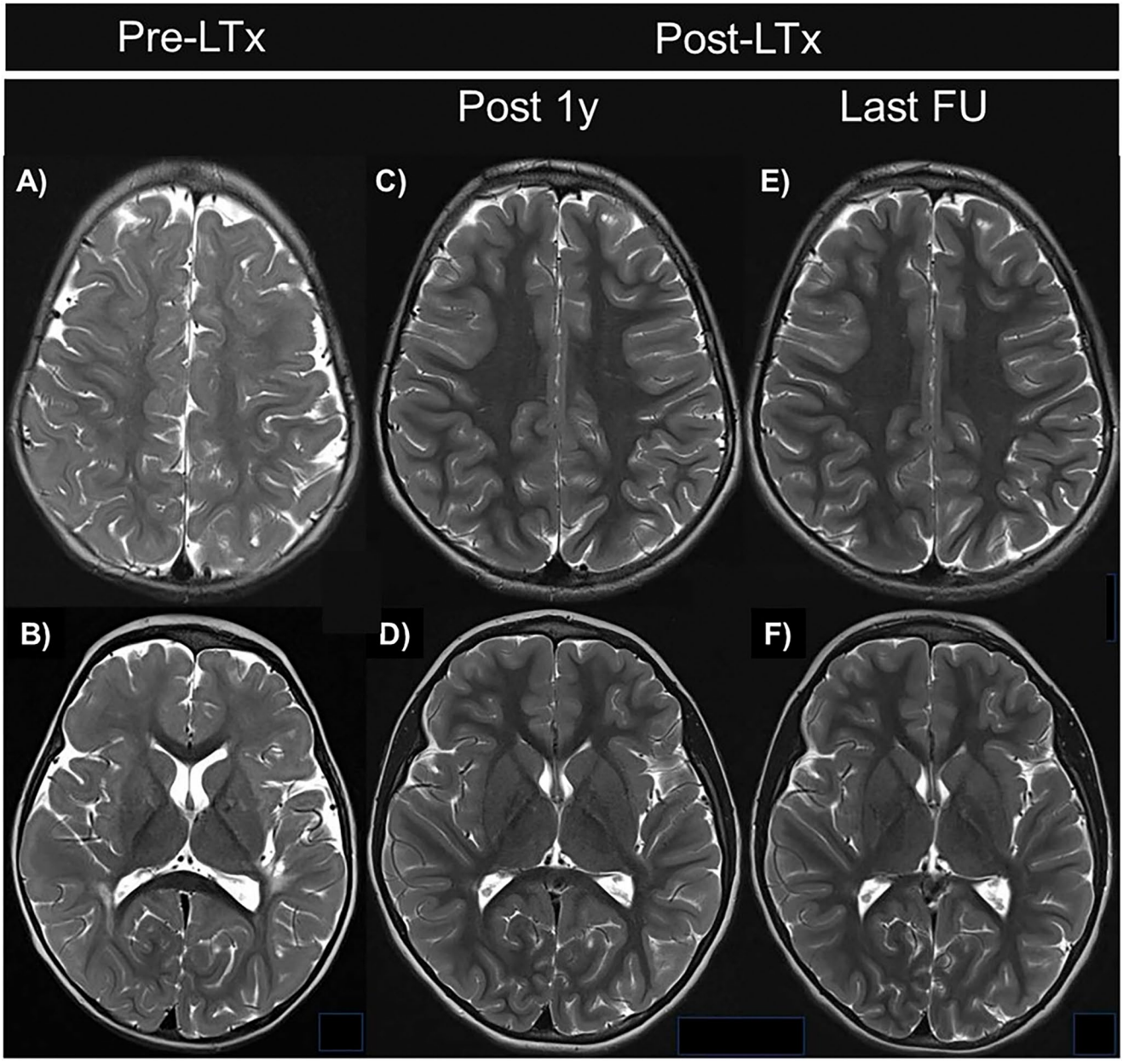

FIGURE 3 | (A–F): T2W brain MRI of Patient 2 pre-LTx (A, B) and after 1 year from LTx (C, D) and at last follow-up (E, F) at centrum semiovale (A, C, E) basal ganglia (B, D, F). The DWM/periventricular hyperintensities in the pre-transplantation MRI (B) disappeared in the post-transplantation MRI (D, F). T2 score improved from 1 to 0.

FIGURE 2 | Neuropsychological outcome in 9 ASLD patients pre- and post-transplantation. (A) Neurodevelopmental and cognitive outcome; (B) Adaptive behaviour assessment; (C–D) Emotional-behavioral outcome; (E–F) Health-related quality of life though MetabQoL and relative subscales. Child Behavioural Checklist and MetabQoL values of each patient are expressed as the median and IQR.

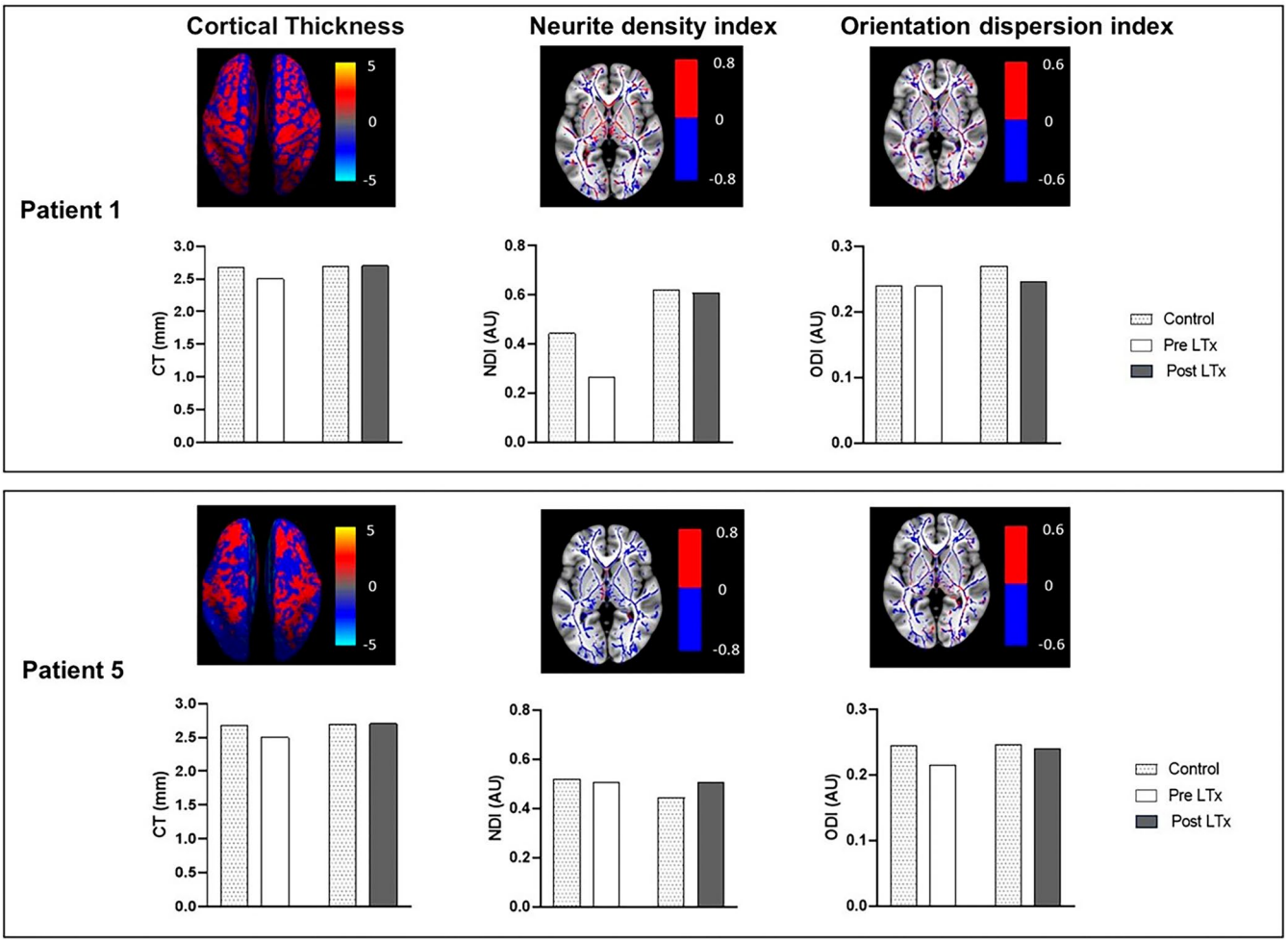

FIGURE 4 | Effect of transplantation on Neurite orientation dispersion density imaging studies (NODDI). Patient 1 and Patient 5 were studied before and after transplantation. Bar plots show the difference in cortical thickness (CT, mm), neurite density index (NDI, Arbitrary unit—AU) and orientation dispersion index (ODI, AU) pre- and post-LTx versus 10 age matched controls.

reports have so far provided details on the post-LTx outcomes (Table 2). Overall, patients did not develop further episodes decompensation, liberalized the protein intake and withdrawn nitrogen scavengers. The non-standardized neuropsychological evaluations, obtained through qualitative clinical and parent reports, showed improving or stabilizing trends [12–18].

In our single center study, by applying a standardized protocol initially developed for methylmalonic aciduria [20] and extended to the assessment of HrQoL, we systematically evaluated the impact of liver transplant in nine patients with neonatal-onset ASLD. All patients were transplanted after a short waiting time on the list, thanks to the adoption in Italy of a national waiting list with priority criteria for patients for metabolic diseases and to the extensive use of the split liver technique and living donor transplantation [33].

At follow-up, no differences in LTx-outcome were recorded between cadaveric and living-related donors.

After LTx, all patients showed sustained metabolic stability, without relapse of hyperammonemia despite withdrawn of nitrogen scavengers, L-arginine therapy, and transition to unrestricted dietary protein intake. Moreover, other characteristic disease-related complications, such as growth failure, hypokalemia, and dyslipidemia improved and/or resolved. As for the commonly reported neurological complications of ASLD [9, 11], no patients developed epilepsy at long-term follow-up, EEG alterations remained stable and movement disorder resolved soon after LTx in one out of two patients.

The longitudinal neuropsychological evaluation demonstrated the efficacy of LTx in ASLD, allowing in early transplanted patients a significant improvement in neurodevelopmental and cognitive functioning and showing that age at LTx, combined with ammonia levels at disease onset, play a major role in determining the long-term neurological outcome.

At baseline assessment, most of patients displayed mild to severe adaptive dysfunctions, another known complication in ASLD [34], that improved after transplantation. Notably, early-transplanted patients showed at follow-up better skills in communication and socialization abilities, improved behavioural, emotional and attention problems, allowing an emotional well-being. Conversely, anxiety traits were persistently recorded in later transplanted patients.

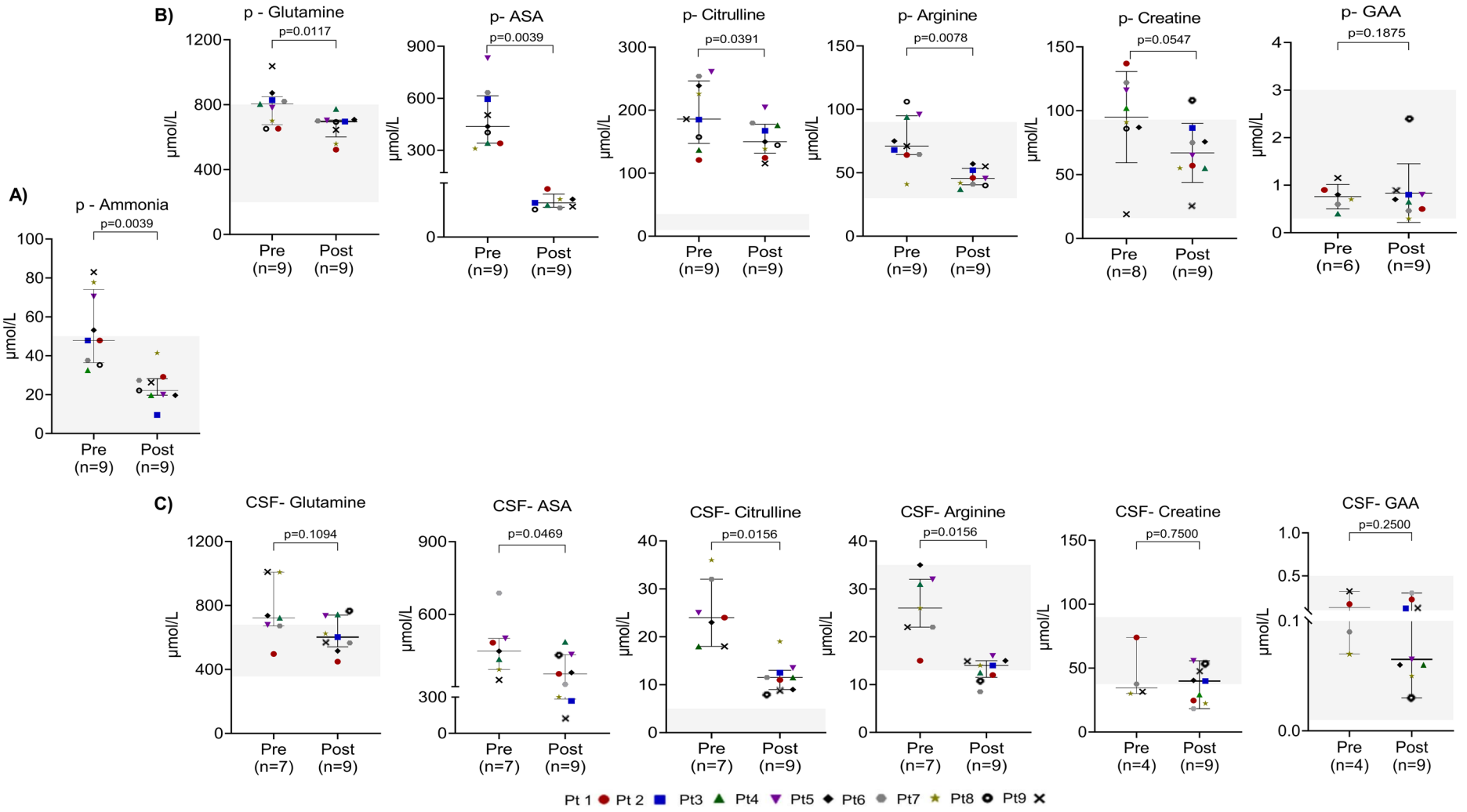

FIGURE 5 | (A–C): Plasma ammonia (A) and ASLD-related biomarkers in plasma (B) and CSF (C) before and after liver transplantation in nine ASLD patients. Arginine values before transplantation were under arginine supplementation. Values of analytes for each patient are expressed as the median and IQR of all values pre- versus post-LTx.

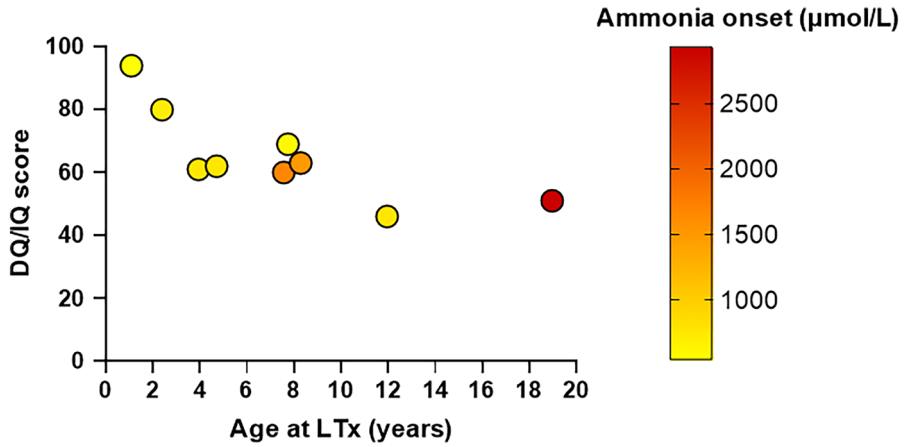

FIGURE 6 | Multiple linear regression analysis identified ammonia levels at (neonatal) disease onset ($p = 0.0333$) and age at transplantation $p = 0.0359$) as significant predictors of DQ/IQ outcome.

These findings highlight that the neurological disease in ASLD patients treated by LTx follows different trajectories when compared with non-transplanted patients [7, 9], as also seen in our 6 additional neonatal-onset patients treated in our center by conventional conservative therapy. Although all of them presented a "non-storming" clinical course, with occasional/sporadic episodes of mild hyperammonemia, 4/6 patients present mild/severe cognitive impairment, with two of them showing epilepsy and severe psychiatric symptoms. Two younger patients, present a borderline cognitive profile. However, in the youngest we detected a rapidly progressive decline of DQ, despite no further hyperammonemic episodes, alongside with the appearance of autistic-like features, which has prompted us to listing for transplantation.

As a consequence of hyperammonemia [35, 36], baseline neuroimaging studies showed in the majority of our patients the characteristic deep and superficial white matter changes, combined with variable degree of cortical atrophy. Serial post-LTx evaluations displayed the stabilization of brain MRI abnormalities. Remarkably, morphometric studies performed in two young ASLD patients demonstrated the increase of cortical thickness and of neurite density, especially in the earlier transplanted subject. Similar to what observed in methylmalonic aciduria these findings demonstrate the potential amelioration of cerebral atrophy in young subjects, in which the plasticity of the brain is still exploitable, underlining the importance of not delay the time of LTx also in ASLD patients [20].

Biochemically, ASA, the primary disease-associated biomarker, showed a significant post-LTx reduction in plasma. Differently, ASA in CSF remained elevated, albeit showing a down warding post-LTx trend. So far, the study of ASA in CSF has been rarely addressed, highlighting the presence of a gradient between CSF and plasma, with a CSF/plasma ratio ranging between 2.3 and 4.8 [37–42]. The high ASA concentration in CSF reflects on one side the expression of ASL deficiency in the CNS [43]. On the other side, similar to what observed in organic acidurias producing dicarboxylic compounds, it is possible that the tricarboxylic compound ASA, could be trapped at blood–brain barrier due to a reduced efflux [44]. In our patients, serial pre LTx evaluations displayed the presence of inter-individual variability in the CSF/plasma ASA ratio, ranging between 0.5 and 2.1 (Figure S3). Interestingly, we found that the concentration of ASA in CSF may change in relation to patients' age and to the temporal distance from LTx, underlining that both genetic and functional determinants play a role in regulating the metabolism of ASA in CNS compartment. Regarding other disease-related biomarkers, the significant arginine reduction in plasma and CSF likely results from the withdrawal of arginine therapy after transplantation, as also supported by correlation studies. Post-LTx, plasma creatine levels remained within the normal range despite withdrawing the supplementation of its precursor arginine. In CSF, creatine was borderline-low in some of our patients after LTx. However, no correlation was detected between creatine and arginine in CSF. Moreover, MRS studies displayed normal creatine concentrations in the brain tissue, both before and after-LTx. These findings highlight the need of further studies focusing on cerebral creatine, raising the question whether ASLD patients should be treated with arginine and/or creatine supplementation after LTx.

The characteristic high citrulline levels were reduced after-LTx in CSF and to lesser extent in plasma, showing a different trend change in comparison to ASA. The persistence of elevated plasma citrulline concentrations after LTx reflects the unchanged dysfunction of ASL in intestinal and renal tissues [14]. Differently, its pronounced reduction in CSF, which may result from the withdrawal of arginine supplementation after LTx, is not of univocal interpretation. To this regard, despite a secondary deficiency in peripheral tissue of the NO synthase enzyme e-NOS in ASLD [2], at CNS level the neuronal isoform nNOS is normally expressed and active in the synthesis of NO and of equimolar amounts of citrulline [45, 46]. It could be hypothesized that the withdrawal of arginine, the substrate for the synthesis of NO through nNOS, may have an impact also on CNS citrulline formation, as supported by the direct correlation between arginine and citrulline in CSF.

HrQoL in transplanted ASLD patients has been so far poorly investigated and never systematically assessed [12, 13, 17]. Our recent study on the impact of LTx in intoxication-type inborn errors of metabolism (i.e., UCDs, organic aciduria, maple syrup urine disease) highlighted a significant improvement in patients' and parents' HrQoL reports, especially when assessed with the specific MetabQoL tool [24]. The present study extends

TABLE 2 | Review of the fifteen ASLD patients reported with at least one pre- and post-LTx assessments.

Reference	Number of patient	Age at LTx (years)	Follow-up (years)	Indication for LTx (graft type)	Post-LTx: therapy/ protein restricted diet	Plasma AA & ASA (μmol/L) pre/post-LTx	Neuropsychological assessment pre/post-LTx	QoL
[12]	1	3.5	5	recurrent HA (deceased donor)	no/no	–	NDD slowing/ progression of NDD	yes
[13]	1	24	2.5	recurrent HA (deceased donor)	no/no	Gln:728 /– Arg:100/– Arg: –/N Cit: –/ ↑	mild ID/improvement vision, language	yes
[14]	1	1.6	4	recurrent HA/ (living donor)	no/no	ASA: 1327/57 Gln: 578/522 Arg: 117/58 Cit: 428/158	–	–
[15]	1	1.6	1.5	recurrent HA/ (living donor)	–/–	–	normal NDD/normal NDD	–
[16]	3	1.6 13.3 1	2.7 3.5 3.5	recurrent HA (Pt2), neurocognitive deficits (Pt6, Pt7) (living (Pt2, Pt7), deceased (Pt6) donor)	no/no	–	mild (Pt2, Pt6) moderate (Pt7) ID/stabilization/improvement	
[17]]	2	2.5 4	5 3.8	recurrent HA (Pt1, Pt2)/ (living (Pt1), deceased (Pt2) donor)	no/no	ASA: 7/– (Pt1), 2.4/–(Pt2) Cit : 220/–(Pt1), 260/– (Pt2)	NDD (Pt1, Pt2)/normal (Pt1), improvement (Pt2)	yes
[18]	6	2–6	5–4.5	recurrent HA (Pt 5),chronic liver disease (Pt 1) (–)	–/no	–	–	–

Abbreviation: AA: aminoacid; Arg: Arginine; ASA: argininosuccinic acid; Cit: citrulline; Cit: citrulline; Gln: glutamine; HA: hyperammonemia; ID: intellectual disability; LTx: liver transplantation; NDD: neurodevelopmental delay; QoL: Quality of Life.

the ASLD patient population and confirms that disease-specific issues were notably compromised pre-LTx, showing significant post-LTx improvements, with positive impact on physical, emotional, and social functioning. Compared to the generic PedsQL tool, MetabQoL resulted to be more sensitive in detecting disease-related aspects, which are particularly relevant in this type of patients. Several factors—such as liberalized dietary regimen, reduced risk of metabolic crises, fewer social limitations, and diminished anxiety about the unpredictable course of the disease—contributed to better exploring the disease burden and, consequently, to evaluate the improving post-LTx changes. MetabQoL, allowed a comprehensive understanding of the positive impact of LTx in ASLD, supporting the need of including the evaluation of quality of live in transplant decision-making protocols for intoxication type metabolic diseases.

In conclusion, our study demonstrated the positive and beneficial impact of LTx in ASLD allowing to reduce disease burden, by avoiding metabolic decompensations, improving/stabilizing neurological and cognitive outcome, and enhancing quality of life. We therefore recommend to considered eligible for early liver transplantation severe forms of ASLD.

Author Contributions

Babara Siri, Diego Martinelli and Carlo Dionisi-Vici designed and coordinated the working group. All authors were involved in conception and design, analysis, and interpretation of data, drafting the article and revising it critically for important intellectual content. Carlo Dionisi-Vici is the guarantor for the article who accepts full responsibility for the work and the conduct of the study, had access to the data, and controlled the decision to publish.

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Ethics Statement

The study was approved by the Bambino Gesù Children's Hospital Ethical Committee (2937 OPBG2022).

Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study. Written informed consent for being included in the study was obtained from all patients or patients' caregivers.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The manuscript has data included as electronic Supporting Information.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.