Neurodevelopmental, nutritional, and clinical outcomes of infants and children with end-stage liver disease awaiting liver transplantation

by

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Abstract

Background/Aim: Patients with pediatric end-stage liver disease (ESLD) are at increased vulnerability for neurodevelopmental delay (NDD), due to exposure to risk factors such as malnutrition, hyperammonemia, and environmental deprivation. We hypothesized that NDD would be prevalent in infants and children awaiting LTx, particularly in the motor skills domain, and that NDD would be associated with pre-LTx malnutrition and adverse pre- and post-LTx clinical outcomes. **Methods:** A secondary analysis of previously collected data from a retrospective study in infants and children (31M/36F) who attended the Pediatric LTx Clinic at the Stollery Children's Hospital (2009-2019) was conducted. The study encompassed six timepoints: LTx assessment, time of LTx, Intensive Care Unit (ICU) discharge, hospital discharge, 6-month follow-up and 12-month follow-up. NDD was assessed at LTx assessment using the Vineland Adaptive Behaviour Scales [motor skill, socialization, communication, adaptive behavior composite (ABC) scores]. The cohort was categorized as having an adequate adaptive level if the had an ABC score ≥85, and as having an inadequate adaptive level if their ABC score <85. Nutritional data (nutritional status, route of nutritional delivery and intake) was collected at LTx assessment. Nutritional status was determined per the Subjective Global Nutritional Assessment (SGNA) tool, the McLaren criteria for wasting (based on percent ideal body weight, %IBW, and the World Health Organization's criteria for stunting (height for age z-score <-2). Growth parameters (daily weight/height gain, weight/height velocity SDS) were collected at all timepoints. Clinical outcomes [encephalopathy, hepato-pulmonary syndrome, hepato-renal syndrome, varices, presence of ascites, infections (total, fungal, bacterial, viral), and hospital visits (type/duration/frequency) were collected pre-LTx. Post-LTx outcomes included ICU/total hospital length of stay (LOS), ventilation dependency, mortality, infection/rejection (type, frequency), and

major complications (vascular, biliary, others). **Results:** Neurodevelopment was predominantly characterized as adequate or low average. Seventy-two percent lacked age-appropriate gross motor skills. A below median motor skills score was associated with increased rates of pre-LTx encephalopathy (trend, p=0.15), post-LTx ICU LOS (trend, p=0.06), and ventilator dependency (p=0.05). SGNA was found to be the strongest predictor of neurodevelopmental outcomes, followed by age (p<0.05). Malnutrition was prevalent (36% moderately malnourished, 55% severely malnourished) in the cohort when classified using the SGNA, but not McLaren and WHO criteria. When aggregating neurodevelopmental and nutritional status, the following phenotypes were identified (prevalence of malnutrition differed when using the 3 definitions): adequate adaptive level ± malnutrition [SGNA: 9% (well nourished) and 57% (malnourished); McLaren criteria: 54% (well nourished) and 11% (malnourished); WHO criteria: 53% (well nourished) and 9% (malnourished)] and inadequate adaptive level ± malnutrition [SGNA: 0% (well nourished) and 34% (malnourished); McLaren criteria: 28% (well nourished) and 8% (malnourished); WHO criteria: 33% (well nourished) and 5% (malnourished)]. An adequate adaptive level ± malnutrition (per any definition) was associated with improved growth parameters [daily weight/height gain (g/day and mm/day) and height velocity SDS] in the 6- and 12-month follow-ups (p<0.05). A lower percentage of those with an inadequate adaptive level ± malnutrition had higher rates of participants achieving age-appropriate weight gain post-LTx (p<0.05). **Conclusions**: Pediatric patients with ESLD have high rates of NDD, particularly in the motor skill domain. Worse scores (overall and domain-specific) are associated with adverse nutritional and clinical outcomes. Nevertheless, evidence shows that malnutrition secondary to ESLD plays a major role in neurodevelopment. NDD must be considered when developing intervention strategies pre- and post-LTx to achieve optimal outcomes and health-related quality of life in this population.

Preface

This thesis is a secondary data analysis of previously collected data from a study entitled "Myopenia in children with end-StAge liver disease awaiting Liver Transplantation (SALT-2)". Dr. Diana Mager is the PI of this study. The primary research project was approved by the Human Ethics Board, University of Alberta (Pro0078499).

This thesis is an original work by Andrea Razcón Echeagaray. The following information summarizes people's responsibilities within this project: Andrea Razcón Echeagaray MSc (cand): data extraction and validation, secondary statistical data analysis/interpretation, and thesis writing under the supervision of Dr. Diana R. Mager. Kaya Persad MSc candidate and Dr. Kerry Wong MD FRCPC: neurodevelopmental and clinical data collection, entry, and extraction, and data auditing. Poh Hwa Ooi RD MSc, Amber Hager RD MSc (cand), and Maryah Robinson-Jackson RKines MSc: clinical and neurodevelopmental, and data collection/entry/extraction/validation/ and data auditing. Yinxuan Li: clinical data audit (bloodwork data). Vera C. Mazurak PhD: on supervisory committee, thesis review and approval. Diana R. Mager RD MSc PhD (PI): study design, data collection, data audit, data analysis and interpretation, thesis review/approval, and supervision of all trainees working on the project. Funding for the primary research project was supported by the Vitamin Fund Graduate Student Award, University of Alberta (awarded to Poh Hwa Ooi). Andrea Razcon Echeagaray was supported by Consejo Nacional de Ciencia y Tecnología (CONACYT) (2021-2022).

Dedication

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Gracias por siempre creer en mí, motivarme, y apoyarme en el proceso de cumplir mis sueños y metas. Gracias por no dejar que el miedo a que su niña se vaya lejos los domine, y por alentarme a convertirme en una persona valiente e independiente. Gracias a ustedes soy quien soy hoy, y esa es una medalla de honor que cargo con todo el orgullo del mundo. Los quiero muchísimo.

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List of Abbreviations (alphabetical order)

ADP; Air-Displacement Plethysmography

BDNF; Brain-derived Neurotrophic Factor

BSID; Bayley Scales of Infant and Toddler Development

BIA; Bioimpedance Analysis

BMI; Body Mass Index

BCAA; Branched Chain Amino Acids

CC; Calf Circumference

CF; Cystic Fibrosis

DXA; Dual-energy X-ray Absorptiometry

ESLD; End-Stage Liver Disease

FM; Fat Mass

FFM; Fat-Free Mass

GH; Growth Hormone

HW; Hydrostatic Weighing

HRQoL; Health-Related Quality of Life

IBW; Ideal Body Weight

ICU; Intensive Care Unit

IGF-1; Insulin-Like Growth Factor 1

INR; International Normalized Ratio

IQ; Intellectual Quotient

IQR: Interquartile Range

LC-PUFAs; Long Chain Polyunsaturated Fatty Acids

LTx; Liver Transplantation

LOS; Length of Stay

MAMA; Mid-Arm Muscle Area

MRI; Magnetic Resonance Imaging

mTOR; Mechanistic Target of Rapamycin Kinase Complex 1

MUAC; Mid-Upper Arm Circumference

MSEL; Mullen Scales of Early Learning

MSUD: Maple Syrup Urine Disease

NDD; Neurodevelopmental Delay

PI3K; Phosphatidylinositol 3-Kinase

AKT; Protein Kinase B

STRONGkids; Screening Tool for Risk on Nutritional Status and Growth

STAMP; Screening Tool for the Assessment of Malnutrition in Pediatrics

SD; Standard Deviation

SE: Standard Error

SGNA; Subjective Global Nutritional Assessment

SMM: Skeletal Muscle Mass

VABS; Vineland Adaptive Behavior Scales

WC; Waist Circumference

WPPSI; Wechsler Preschool and Primary Scale of Intelligence

WISC; Weschler Intelligence Scale for Children

WHO; World Health Organization

List of Appendices

Publications not related to MSc thesis

- Picard K, **Razcon-Echeagaray A**, Griffiths M, Mager DR, Richard C. Currently Available Handouts for Low Phosphorus Diets in Chronic Kidney Disease Continue to Restrict Plant Proteins and Minimally Processed Dairy Products. *J Ren Nutr. 2022;* S1051-2276(22)00071-1. Advance online publication. doi: 10.1053/j.jrn.2022.04.002 .

Abstract presentations related to thesis

- Hager A, **Razcon-Echeagaray A**, Wong K, Noga M, Snyder T, Gilmour SM, Mager DR. Is myopenia associated with adverse neurodevelopmental and clinical outcomes in infants and children with end-stage liver (ESLD) disease undergoing live transplantation? Submitted to Canadian Society of Transplantation. Accepted for poster presentation (virtual). September 24, 2021.

- **Razcón Echeagaray A**, Hager A, Snyder T, Mazurak V, Gilmour SM, Mager DR. Neurodevelopmental and clinical outcomes of infants and children with end-stage liver disease awaiting liver transplantation. Submitted to Alberta Transplant Institute Research Day. Accepted for oral presentation (virtual). June 21, 2022

Abstract presentations not related to thesis (AFNS 602)

- **Razcón Echeagaray A**, Hager A, Robert C, Snyder T, Yap J, Gilmour S, Mager DR. Effect of Branched Chain Amino Acid Supplementation on Neurodevelopmental Outcomes in Infants with Cholestatic Liver Disease Undergoing Assessment for Liver Transplantation. Pediatric Research Day, University of Alberta, Edmonton, Alberta, Canada. Accepted for poster presentation (virtual). April 28, 2021.

Chapter 1: Literature Review

1.1 Introduction

From the in-utero period to five years of life is considered a critical period of neurodevelopment.[1] During this time, any insult or absence of key experiences for the developing nervous system may permanently impact brain function and architecture, as well as future behaviour and cognition.[2] Pediatric liver disease is an insult that can contribute to neurodevelopmental delay (NDD) in infants and children.[3] Several factors associated to it may contribute to risk of less than optimal neurodevelopment. These factors include malnutrition secondary to malabsorption, digestion impairment, increased energy expenditure, hyperammonemia, chronic inflammation, and dysregulation of muscle protein synthesis and proteolysis.[4,5] Furthermore, in end-stage liver disease (ESLD) in children and adults, liver transplantation (LTx) is a therapeutic approach.[6] Use of anesthetics and immunosuppressive medications (e.g. corticosteroids and calcineurin inhibitors), prolonged hospital stays, and ventilation dependency are known risk factors for NDD.[3,7] Pediatric patients with ESLD commonly undergo LTx before 2 years of age, increasing their vulnerability during a period of rapid neurodevelopment, and potentially decreasing their long-term health-related quality of life (HRQoL).[8,9] A broad perspective of this "vicious" cycle can be observed in **Figure 1.1.** This review explores the literature focused on what is known about neurodevelopmental and clinical outcomes related to malnutrition and other hallmarks seen in pediatric ESLD. It also examines relevant methods to detect malnutrition and NDD in this population, aiding in the development of strategies aimed to improve their outcomes before and after LTx.

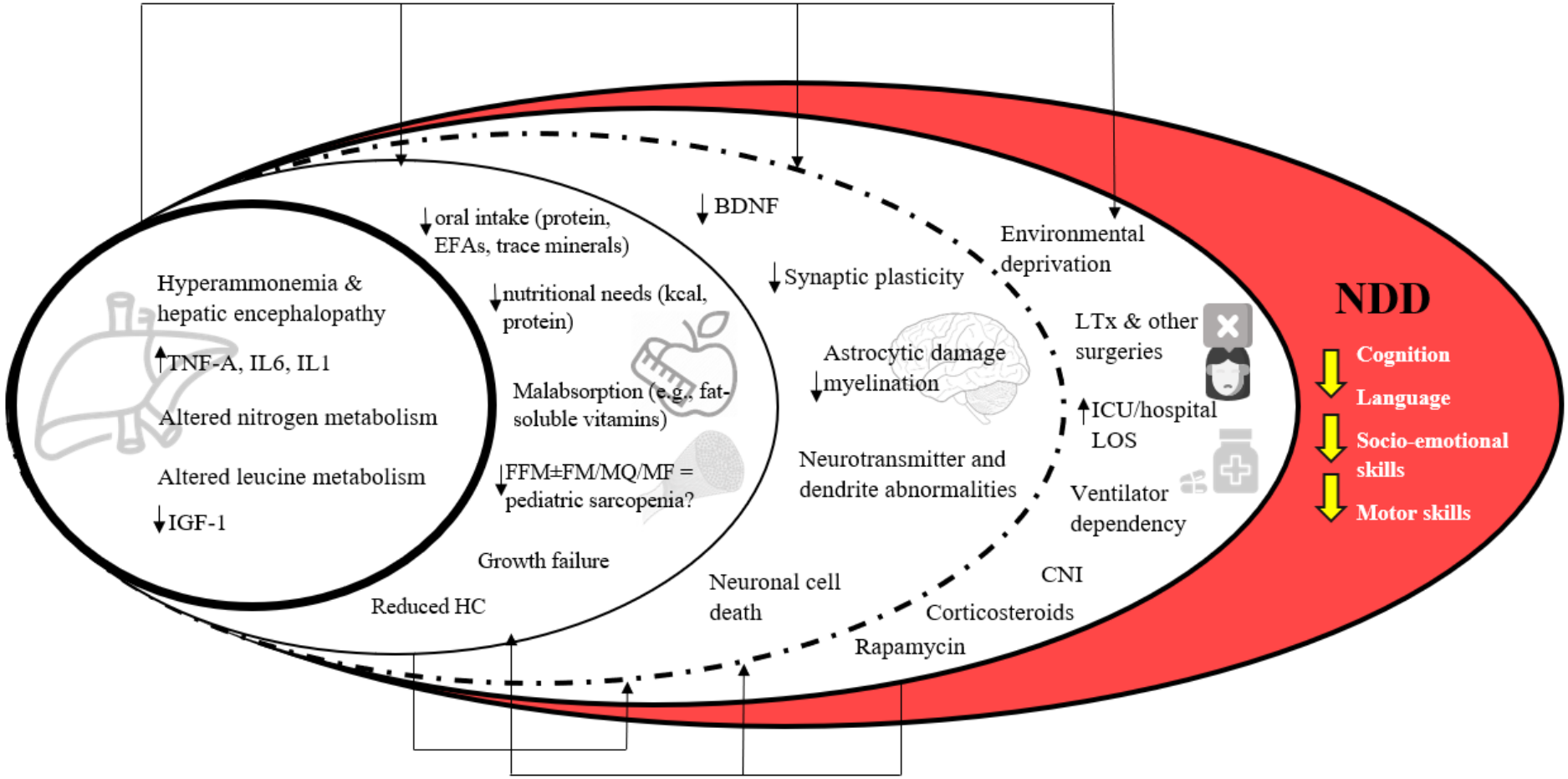


Figure 1.1. A disease-induced vicious cycle: Impact of ESLD-specific metabolic derangements, malnutrition, and external factors on neurodevelopment (and consequent delay), and how they all magnify each other. Dotted lines encircling neurodevelopmental factors indicate that further research is warranted to elucidate causes and effects. BDNF: Brain-derived Neurotrophic Factor, CNI: Calcineurin Inhibitor, EFAs: Essential Fatty Acids, FM: Fat Mass, FFM: Fat Free Mass, HC: Head Circumference, IGF-1: Insulin-like Growth Factor-1, IL6: Interleukin 6, IL1: Interleukin 1, kcal: Kilocalories, MQ: Muscle Quality, MF: Muscle Function, NDD: Neurodevelopmental Delay, TNF-α: Tumor Necrosis Factor Alpha, LTx: Liver Transplantation

1.2 Mechanisms and Prevalence of Malnutrition in Pediatric Liver Disease

Malnutrition is present in 60-80% of children with advanced liver disease, and it has a broad range of etiological factors.[10] Reduced calorie and nutrient intake can be attributed to dysregulation of satiety and appetite (mediated by ghrelin and leptin), a distorted sense of taste and smell, and malabsorption.[4] The latter can be caused by cholestasis, portal hypertension, drug-related diarrhea, and other factors.[4] Decreased oral intake can result in deficiencies of essential nutrients, such as protein, essential amino acids and fatty acids, fat-soluble vitamins (A, D, E, K), and trace minerals (Ca, Mg, Fe, Zn).[4,11] The increased caloric and protein needs of this patient population exacerbates their poor nutritional status.[10] Furthermore, impaired gluconeogenesis and reduced hepatic glycogen stores forces the body to become reliant on protein as an energy source.[11] This can disrupt abnormal plasma amino acid profiles, particularly branched chain amino acids (leucine, valine, isoleucine; BCAAs), which are mainly metabolized in the muscle and are associated with wasting.[11] Malnutrition can manifest in several ways, including growth failure or stunting (reduced growth rate for age, or height-for-age z-score <-2 standard deviations below normative data) and changes in body composition (decrease in muscle mass with or without decrease in adipose tissue), all of which may not resolve even after successful LTx.[6,12] Adding to this, malnutrition has been associated with cellular and hormonal signaling issues. In chronic liver disease specifically, there are known alterations in growth hormone (GH) and insulin-like growth factor 1 (IGF-1).[11] While the mechanisms remain to be elucidated, as liver disease progresses, GH resistance develops, resulting in high GH and low IGF-1 serum levels, contributing to growth failure.[11] Decreased IGF-1 levels are also responsible for suppressed muscle growth and/or muscle breakdown.[4] IGF-1 activates the mechanistic target of rapamycin kinase complex 1 (mTOR) after a chain-reaction where phosphatidylinositol 3-kinase (PI3K) and protein kinase B (AKT) are also

involved.[4] The PI3K/mTOR/AKT pathway is critical in muscle protein anabolism, and with low IGF-1, proteolysis is increased, leading to decreased muscle mass or myopenia. In murine animal models, malnutrition alters cell numbers and migrations, myelination, synaptogenesis, hippocampal formation, and neurotransmission.[13] In humans, malnutrition has been associated with dendritic spine abnormalities, short apical dendrites, and altered neurotransmitter function and response.[3] A reduced head circumference, which reflects brain growth and neurodevelopment, can also be a consequence of malnutrition at an early age.[14] Furthermore, a malnourished child has less energy, curiosity and/or awareness to interact with their environment, which negatively impacts achievement of cognitive, language, socio-emotional, and motor milestones.[13]

1.3 Assessing Malnutrition in Pediatrics

1.3.1 Methods Used to Measure Body Composition and Growth in Children

During growth, there are substantial changes in the distribution, structure, and relative quantities of lean mass and fat mass (FM).[15] Inadequate nutrient and energy intake, disease, as well as all other etiological factors for malnutrition, can result in irreversible alterations of organ and tissue architecture and function.[15] Children with ESLD may present excess adiposity and/or muscle mass deficits that are masked by ascites and edema.[16] This brings upon the risk of being defined as "well-nourished" or "low malnutrition risk" by weight or body mass index (BMI).[16] This highlights the need for body composition assessments in order to determine actual disruptions of normal growth and malnutrition. Body composition methods are typically categorized depending on the number of body compartments they assess.[17] The most commonly used methods, such as anthropometry and bioimpedance analysis (BIA), study the body based on the two-compartment model.[17] In this, body composition is divided into FM and fat-free mass (FFM).[17] Dual-energy X-ray Absorptiometry (DXA), on the other hand, is based on the three-compartment

model, dividing the body into FM, lean soft tissue mass, and bone mass.[17] A description of these and other methods is presented in **Appendix 1.**

As per the Waterlow criteria[18], pediatric malnutrition has been historically assessed with anthropometric measures and determined by deficits in weight for age (underweight), length/height for age (stunting), or weight for length/height (wasting).[19] Additional measures are weight for length and/or low BMI, as well as low mid-upper arm circumference (MUAC).[19] All of these have been associated with lower survival rates, but more specific body composition measurements are necessary.[19] This has been done to define adult malnutrition, relying on objective assessments like the quantification of FFM and FM.[19] In pediatrics, more detailed body composition techniques (e.g. CT/MRI) have been increasingly used to assess FFM and FM alterations secondary to malnutrition and chronic disease.[5,20,21] With a decreased dietary intake, there may be insufficient amino acids available for muscle synthesis, resulting in muscle mass depletion and reliance on skeletal muscle to provide amino acids for metabolic pathways.[22] Nevertheless, non-muscle components of FFM may also be impacted, as limited evidence shows that malnutrition leads to reductions in thymus size (decreasing immune function), or kidney and cardiac volumes.[22] FM provides metabolic precursors and energy for multiple body functions, many of which have high metabolic costs. Adipose tissue also secretes leptin, and low levels of leptin in children with severe acute malnutrition predict mortality.[22] It is also crucial to detect small changes in each compartment over time, as it has been shown that they relate to patient outcomes.[5] Ooi *et al.*[21] found that both deficits of subcutaneous adipose tissue and skeletal muscle mass occur in children with ESLD, and these changes were associated with gross motor delay, reduced energy intake, and increased hospitalizations and infections.

1.3.2 Pediatric Malnutrition Risk Screening and Nutritional Assessment Tools

Early identification of malnutrition (or risk for malnutrition) could allow for a timely intervention, which may limit complications associated with an impaired nutritional status (e.g., NDD).[23] To determine risk for malnutrition and nutritional status, there are two types of tools available: Screening tools and assessment tools (**Appendix 2A and 2B**).[22] The gold standard for nutritional assessment in pediatrics is the Subjective Global Nutritional Assessment (SGNA).[24,25] This tool was created to determine the nutritional status of children with known risk for malnutrition (e.g., hospitalized and/or chronically ill children).[26] By detecting malnourished individuals in whom nutrition-associated morbidities are likely to occur, nutrition intervention may follow. Two of most commonly used screening tools are the Screening Tool for the Assessment of Malnutrition in Pediatrics (STAMP) and the Screening Tool for Risk on Nutritional Status and Growth (STRONGkids). Data collection is based on medical history, physical examination, anthropometric measurements, dietary intake, body composition, functional tests, and biological parameters.[27] With that, screening and assessment tools rely on dynamic parameters, rather than static ones, including recent weight loss, food intake, and disease severity.[22] Nutrition screening tools detect potential or existing malnutrition risk factors, with the aim of identifying those who should be referred to a dietitian for further assessment.[22,23] Nutritional assessment tools are performed on those patients determined to be at nutritional risk by a screening tool, and the data collection process is more detailed than with screening tools.[22] Indeed, when a patient is at risk of malnutrition, investigations are started to determine inadequate intake, reduced absorption, excessive losses, impaired utilization or increased requirements.[27] The ultimate goal of the assessment is to give way to the creation of a short or long-term nutritional care plan in order to improve the patient's overall condition.

1.4 Neurodevelopmental Outcomes Associated with Malnutrition and Liver Disease in Pediatric Populations

1.4.1 Neurodevelopment

Neurodevelopment is the process of development of the central nervous system that occurs predominantly from in-utero to 5 years of age (considered a critical period of neurodevelopment) but is known to continue until late adolescence (**Figure 1.2**).[1,13,28,29] It comprises specific domains that, in orchestration, will allow a child to reach full competence in daily, social, academic, and personal life: Cognition, language, and motor skills.[1,30-32] Socio-emotional and adaptive functioning (also known as daily living skills) are included in several neurodevelopmental assessments. Data on these domains are based on parental/caregiver reports, which are influenced by multiple factors (e.g., parental literacy, cultural expectations).[32,33] Moreover, delays in adaptive behavior are typically secondary to delays in the "main" neurodevelopmental domains, which explains why not all authors include it in the definition.[32] Brain structure/architecture may also be considered a domain in specific cases, such as fetal alcoholic syndrome research.[34]

The cognitive domain encompasses memory, visual-spatial construction, attention, and executive functioning.[35] Memory is the ability to store and classify stimuli for retrieval under different conditions.[35] It is divided into working memory (also called short-term memory) and long-term memory. Working memory is the temporary storage of information (verbal and/or visual) for brief periods, whereas long-term memory consists of information retention for prolonged periods of time.[35] Visual-spatial construction is the ability to manipulate and arrange objects and position them in relation to each other and space.[35] Attention is the ability to selectively focus on specific stimuli while simultaneously ignoring irrelevant information in the environment.[35]

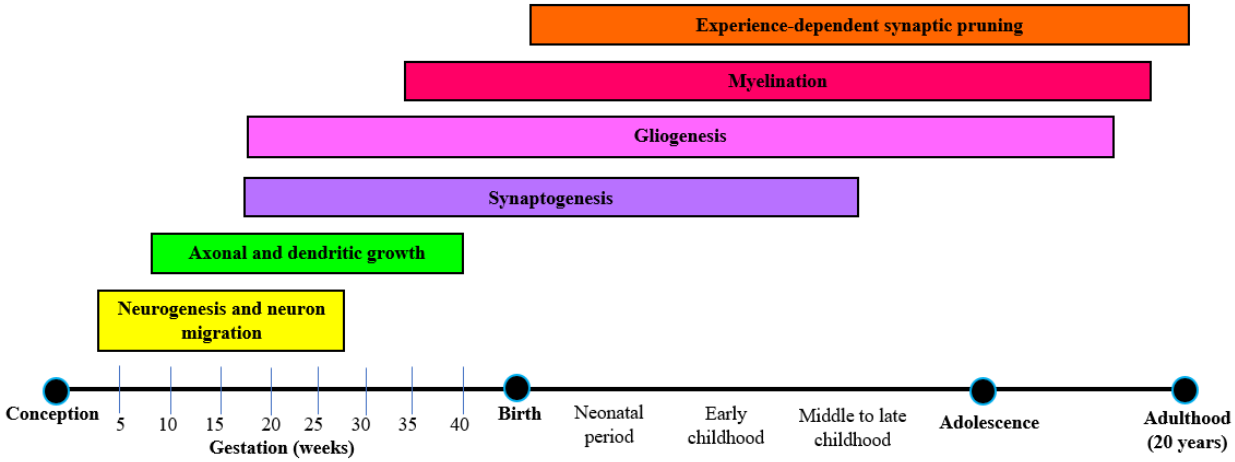


Figure 1.2. Neurodevelopmental growth from the in-utero period to 20 years of age. The pre-natal period to 5 years of age is considered a critical period of neurodevelopment. A neuron consists of a head, long axons and branching dendrites that connect through synapses with other cells. During neurogenesis, neurons are born and then migrate to their final position in the brain. Once there, axons and dendrites grow in each neuron. Afterwards, synaptogenesis may take place in order to create a connected neural system. Gliogenesis consists of the growth and proliferation of glial cells (astrocytes and oligodendrocytes), which will later help support synaptic formation, plasticity, and myelination. The latter is the process in which oligodendrocytes produce myelin around axons to increase the speed of signal transmission within neurons. All these connections continue to be refined by multiple lived experiences and environmental stimulation after birth and through adult life. Adapted from Allswede & Cannon[28], Paraschivescu[36], and Alberts *et al*.[29]

Lastly, executive function is the overlap of other domains to achieve cognitive flexibility, planning, organizing, problem solving, and goal setting. The language domain comprises the expression and reception of oral and written messages (receptive and expressive language).[32,35] Motor development includes the acquisition of observable, reflexive or voluntary goal-directed movements that require upright posture, mobility, and manipulation.[37,38] They are divided in fine motor skills, which involve small muscles to make small and precise movements, and gross motor skills, which are movements that involve large muscles.[32,38]

The pattern and timing of acquisition of neurodevelopmental milestones is similar in healthy children, assuming they are surrounded by an optimal environment (e.g., physical/nutritional/metabolic/mental health, family/community context, sensorial and cognitive stimulation).[2] Additionally, the development of one skill influences that of others (**Appendix**

3).[13,32] For example, motor skill development enables environmental exploration and social interaction, which helps further language development, in turn promoting cognitive growth.[32,37] During the first years of life, there is rapid brain growth, with the brain reaching 80% of its adult weight (**Figure 1.2**).[32] This is reflected in neurodevelopmental gains in gross and fine motor skills (e.g. rolling, standing, walking and self-feeding, pincer grasp, drawing lines, respectively), language abilities, and problem solving.[32] The development of the frontal lobes is significant during the first two years of life, and after a period of decreased velocity, regains momentum between 7 – 9 and 15 years of age.[32] As the child grows older, development of the basal ganglia, amygdala, and hippocampus also occurs.[32] The optimal growth of brain regions is fundamental for acquisition of higher cognitive functions such as abstract thought, working memory, concentration, all of which are crucial for adaptive functioning.[32,39] Although the plasticity of the young brain enables the development of resilience to stress, it is also highly vulnerable to biological and environmental injury (e.g. liver disease and emotional neglect, respectively).[2,40]

In a healthy developing brain, neurons have the ability to strengthen synapse connections, as well as form new ones, when exposed to extrinsic stimuli (e.g. cognitive stimulation).[40] These mechanisms allow for neuroplasticity which leads to adaptive behaviour establishing in a child, but in the context of an injury to the brain, they may be permanently altered or destroyed.[40] Without a doubt, this period of life is considered critical for optimal brain health.[40] By identifying known risk factors for NDD, it is possible to achieve early identification of any specific delays in neurocognitive development.

1.4.2 Mechanisms of Neurodevelopmental Delay in Malnutrition and Pediatric Liver Disease

When it comes to liver disease, there are specific mechanisms that affect brain structure/development and consequent neurodevelopment (**Figure 1.3**). Indeed, with liver

dysfunction there is an inadequate breakdown of ammonia, fatty acids, bilirubin and phenols, all of which are toxic to the brain.[6] Increased serum levels of these substances damage the blood-brain barrier, impacting the biochemical composition of the central nervous system, consequentially leading to glial cell inflammation, cortical atrophy, and demyelination.[6] Other brain components that can be affected are the ventricles, the hippocampus, and the cerebellum.[41] Ammonia, in particular, is an important neurotoxin associated with cognitive delay.[42] With an impaired liver, ammonia accumulates in systemic circulation, eventually crossing the blood-brain barrier and damaging astrocytes, bringing about disturbances in memory, attention, and executive function.[42] Hyperammonemia also increases myostatin expression, consequently promoting muscle autophagy and exacerbating muscle impairments that may ultimately contribute to motor development delay.[4] Pruritus secondary to elevated bile salt levels may lead increased discomfort (e.g., skin itchiness) and this contributes to distractibility, irritability and even sleep deprivation in the affected child.[6] As a consequence, a child's socio-emotional and daily living skills may be negatively impacted, as they refrain from participating in developmentally enriching activities.[6]

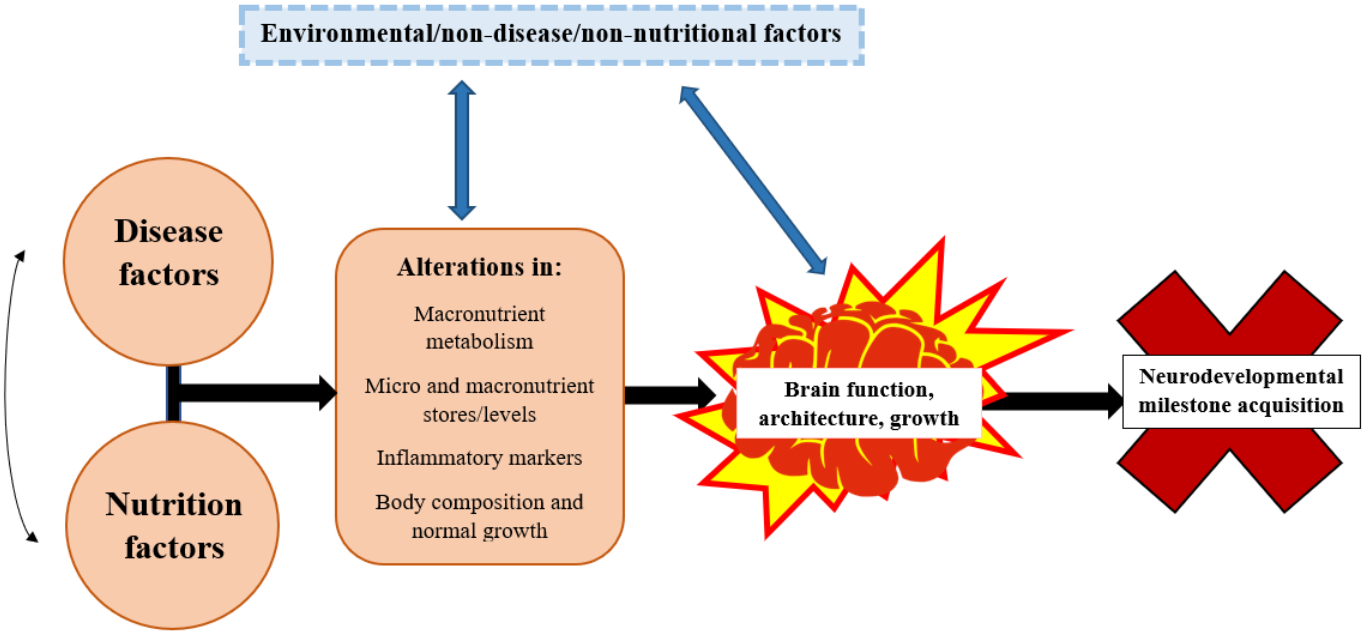


Figure 1.3 Mechanisms of neurodevelopmental delay in pediatric liver disease. Disease and nutrition factors simultaneously affect each other, leading to metabolic, inflammatory and tissue alterations. These, along with environmental/non-disease/non-nutritional factors, impact brain function, architecture, and neurodevelopmental growth, resulting in delay in neurodevelopmental milestone acquisition or regression of previously attained skills

Abdominal muscle strength and balance are fundamental in gross motor development, which are adversely affected in liver disease.[43] Even before LTx, a child may go through several abdominal surgeries. Along with the presence of ascites and an enlarged liver, this contributes to the development of low muscle mass and muscle function, which impede the emergence of motor milestones.[6,44] Furthermore, frequent hospitalizations are common before and after LTx, potentially adding to motor competence impairment (as the child is bedridden) and decreased social, cognitive and language stimulation.[42,44,45] These events often coincide with moderate-severe malnutrition, common in this clinical population, resulting in exacerbation of neurodevelopmental injury, as the brain is deprived of key nutrients (e.g., carbohydrates, essential fatty acids, vitamin E, vitamin D) (**Figure 1.3**).

While LTx will eventually restore nutritional status, reduce ascites, and promote milestone achievement, neurodevelopment can be further compromised by post-LTx medication with neurotoxic potential, like corticosteroids and calcineurin inhibitors.[45] This is because of their potential toxicity to the hippocampus, an important brain structure for learning and memory.[42] Physical fatigue in the first three years post-LTx has been reported in adults that underwent LTx.[45] Similarly, post-LTx children have low rates of participation in organized physical activity in the first year after the procedure which can be secondary to self-reported fatigue, decreased muscle strength and aerobic capacity.[45,46] Reduced participation in physical activities may produce or worsen motor delay, potentially affecting functional outcomes in social and academic settings. Even more so, higher physical capacity has been associated with better mental health outcomes, further impacting HRQoL of children with an impaired motor competence.[44-46] Post-LTx ventilator dependency is a potential source of NDD, as evidence shows that adults that required prolonged mechanical ventilation experienced memory, attention, and processing speed dysfunction after weaning (persisting up until 6 years post-ICU discharge).[47] A proposed mechanism for this is lung injury secondary to excessive lung stretch induced by the mechanical ventilator.[47] This, in turn, may produce an inflammatory response, with inflammatory mediators crossing the blood-brain barrier.[47] Nevertheless, the exact mechanisms for ventilator-induced cognitive dysfunction remain to be elucidated.

An insult to the developing brain may also come in the form of lack of parental-child interaction (induced by prolonged hospitalization, lack of skin-to-skin contact, among other factors), compromising the bond formed in the first years of life. Parental stress from the child's medical needs (e.g., emotional stress, socio-economic strain, marital issues) may also lead several opportunities for NDD.[48] These may include strain in family relationships (e.g., patient to sibling,

parent to relative) and focusing on technical information from the healthcare providers while neglecting psychosocial factors associated with the child (e.g., play time, giving the child the opportunity to socialize and make friends). Ultimately, these may potentially affect a child by limiting social interactions and environmental exploration, leading to altered socio-emotional skills and delayed language development.

Malnutrition can result in permanent damage to brain structures/function that in turn affect neurodevelopment, and its effects may be different depending on the specific nutrient (**Appendix 4**). Nutrients that are typically reported to be deficient in this clinical population include protein, essential fatty acids, fat-soluble vitamins (A, D, E, K), iron, zinc, selenium, and magnesium.[11] In malnourished human and animal models (murine and non-human primates) that are otherwise healthy, malnutrition impacts hippocampal formation, the main brain region associated with spatial learning and memory.[49] In murine models, malnutrition from birth to lactation has been associated to decreased playful social behavior, which is crucial for milestone acquisition in human infants and children.[49] Total free amino acid concentration of the developing brain is higher than the adult brain, and protein deficiency during the critical period of neurodevelopment may cause permanent nervous system damage.[49,50] This includes neuron growth alterations, reduced number of neurons, dendritic arborization, synapses, and decreased brain mass.[49] While not all amino acids can act as neurotransmitters, each one has a role in brain development.[50] Hence, specific amino acid deficiency can cause a direct or indirect insult to the developing brain. Those with liver disease are at increased vulnerability, given their elevated protein needs mixed with reduced oral intake and malabsorption.

Essential fatty acids are fundamental functional components of the brain, taking part in gene expression, neuronal membranes, membrane fluidity modulation, consequentially affecting

receptor and enzyme activities, and ion channels.[13] Essential fatty acids also promote neuronal and dendritic spine growth, synaptic membrane synthesis, which overall impacts signal processing and neurotransmission.[13]

Twenty to 35% of children with chronic liver disease developing fat-soluble vitamin deficiency.[10,51] From a neurodevelopmental perspective, vitamin E and vitamin D are of particular interest, as more than 50% of patients with cholestatic liver disease develop these single-vitamin deficiencies.[51] As evidenced by animal studies, vitamin D is considered a neuro-protector and important antioxidant for the brain, also taking part in neuronal differentiation and apoptosis downregulation in the hippocampus.[52,53] By regulating calcium and phosphate metabolism, vitamin D also has a pivotal role in the development and maintenance of the musculoskeletal system.[53-55] Impaired bone and muscle health (skeletal weakness and/or deformity, muscle weakness, paresthesia, altered muscle cell contraction) secondary to vitamin D deficiency has been associated with delayed gross motor skills in children (e.g. inability to sit and stand independently, delayed walking, difficulty climbing stairs and running).[55-58] Vitamin E has also been found to have a role in neuroinflammation, as its deficiency causes increased expression of inflammatory-related genes in the brain of murine models.[59-61] The most prominent role of vitamin E is cell membrane protection.[62-64] Considering this, axonal degeneration, and loss of myelination secondary to vitamin E deficiency has been associated with skeletal myopathy, retinopathy, ataxia, and hyporeflexia, all of which can worsen neurodevelopmental outcomes.[60-63] Fat-soluble vitamin supplementation for children with liver disease is routinely provided due to the high risk for deficiency in children in the pre-LTx period.

1.4.3 Neurodevelopmental and Neurocognitive Assessment in Pediatrics

The long-term disease effects and impacts on HRQoL are more evident as LTx has improved survival outcomes in pediatric ESLD patients.[13] Cognitive development is considered to be a predictive factor of academic and work achievement.[13,65] Higher education is linked to important health determinants, including better jobs, higher socioeconomic status, access to better healthcare, improved self-esteem, better nutrition and overall better health behaviours and lifestyle.[13,66] Early detection of NDD is urgent to optimize outcomes, hence the importance of knowing and understanding the numerous tools available for infants, children, and youth. Neurodevelopmental assessments focus on achievement of developmental milestones, and typically affect infants and young children, from birth to five years of age.[67] The most commonly used tests for this, particularly in pediatric liver disease, are the Bayley Scales of Infant and Toddler Development (BSID) and the Vineland Adaptive Behavior Scales (VABS).[43] The Alberta Infant Motor Scales (AIMS), the Peabody Developmental Motor Scales (PDMS), and the Bruininks-Oserestky Test of Motor Proficiency (BOT) are also neurodevelopmental tools but focus solely on motor skills.[68] Recent evidence has shown that motor delays detected by the BOT and the PDMS are associated with malnutrition (represented as low height for age or low FFM) in chronic illness, such as intestinal failure with prolonged parental nutrition and ESLD.[44,69] The AIMS and PDMS have also evidenced motor deficits in malnourished infants and children healthy (protein-energy malnutrition and iron deficiency anemia) that are otherwise healthy.[70-72] Low FFM contributes to an altered muscle function, and both derangements to musculoskeletal health are an important component of malnutrition and sarcopenia in chronic disease.[5] The latter has been largely evidenced in adults, but these tools may be potentially useful to close this gap in diseased pediatric populations. Indeed, motor skills assessment tools can be considered as potential measures of

muscle function in the evaluation of pediatric sarcopenia in patients <6 years of age.[73] This is particularly true for the PDMS and the BOT, as they include assessments of strength, a key component of sarcopenia.[74] Cognitive assessment can begin from three years of age and aspects that condition a successful academic performance are typically studied.[67] This includes memory, attention, reading and math skills, non-verbal learning, etc. The most popular neurocognitive tests are the Wechsler Preschool and Primary Scale of Intelligence (WPPSI) and the Weschler Intelligence Scale for Children (WISC).[42,43] **Table 1.1** shows a summary of the range of neurodevelopmental and neurocognitive tools available for sick and healthy children. Overall, the goal of these assessments is to ensure that a child is developing as expected for their age or detect delay.[66] In children considered to be at high risk for NDD, formal and periodic neurodevelopmental/neurocognitive assessments are recommended to allow for early intervention and include it in their medical care.[67] With this, appropriate therapies may be established, and the child's future adaptive functioning may be enhanced.

Table 1.1. Common neurodevelopmental and neurocognitive assessments in pediatrics

Neurodevelopmental test	Ages	Assessed domains	Data collection method	Diagnostic criteria
Multiple-domain assessments				
Denver Developmental Screening Test – 2nd Edition (Denver – II)[75]	0 – 6 years	Motor (fine and gross), language (expressive and receptive, speech clarity), personal-social	Direct elicitation/observation and parental/caregiver report	Caution: Item completed 75 – 90% but failed Developmental delay: Item completed 90% but failed
Bayley Scales of Infant and Toddler Development – 4th Edition (BSID – IV)[76,77]	16 days – 42 months	Cognitive (visual preference, attention, memory, sensorimotor, exploration and manipulation, concept formation); language (receptive and expressive), motor (fine and gross); social – emotional (communicating needs, self-regulation); adaptive behavior (listening and understanding, talking, caring for self, relating to others, playing)	Direct elicitation/observation and parent/caregiver report	Developmental delay: <25th percentile or <2 SD
Bayley Infant Neurodevelopmental Screen (BINS)[78]	3 – 24 months	Neurological functions (muscle tone, movement, asymmetries), motor (fine and gross), language (expressive and receptive), cognition (object permanence, problem solving, visual, goal-directedness)	Direct elicitation/observation	Developmental delay: Low, moderate, high risk Neurological impairment
Child Development Inventory[79]	15 months – 6 years	Motor (fine and gross), language (expressive and receptive), social, self-help, general development	Parental/caregiver report	No score, all items are reviewed to determine "presence" or "absence"
Mullen Scales of Early Learning[80]	0 - 5 years 6 months	Motor (fine and gross), cognitive (visual organization), language (expressive and receptive)	Direct elicitation/observation	Developmental delay: <25th percentile or <2 SD
McCarthy's Scales of Children's Abilities (MSCA)[81]	2 years 6 months – 8 years 6 months	Cognitive, Memory, verbal, perceptual-performance, quantitative, motor (fine and gross),	Direct elicitation/observation	Developmental delay: score<79 (Score mean: 100, SD: 16)
The Battelle Developmental Inventory	0 – 7 years 11 months	Language (expressive, receptive), motor (gross, fine, perceptual), cognitive (attention and memory, perception and	Direct elicitation/observation and parental/caregiver reports	Developmental delay: <2 SD below the mean for each domain

– Third Edition (BDI – 3)[82]		concept, reasoning, and academic skills), socio-emotional (adult interaction, peer interaction, self-concept, and social role), adaptive (self-care, personal responsibility)		
Vineland Adaptive Behavior Scales – 3rd Edition (VABS – 3)[83,84]	0 – 90 years (interview and parent/caregiver form) 3 – 21 years (teacher form)	Communication (receptive, expressive), motor (gross, fine), socialization (interpersonal relationships, play and leisure, coping skills), daily living skills (personal, domestic, community)	Parental/caregiver or teacher report	Developmental delay: Moderately low adaptive level: ABC score= 71 – 85 Low adaptive level: ABC score= 20 – 70
Parent's Evaluations of Developmental Status: Developmental Milestones (PEDS: DM)[85]	0 – 95 months	Motor (fine, gross), language (receptive, expressive), self-help, academics, social-emotional	Direct elicitation/observation and parental/caregiver report	Developmental delay: <16th percentile in each domain
Capute Scales: Cognitive Adaptive Test/Clinical Linguistic Auditory Milestone Scale (CAT/CLAMS)[86]	0 – 36 months	Language (receptive, expressive), visual motor	Parental/caregiver report and observation	Developmental delay: Developmental quotient < 70-75%
Early Learning Accomplishment Profile (E-LAP)[87]	0 – 36 months	Motor (fine and gross), cognitive (attention, sensorimotor, visual preference), language (receptive and expressive), self-help, socio-emotional	Parental/caregiver report and direct observation	Developmental age is calculated based on domain scores
Hawaii Early Learning Profile (HELP)[88]	0 – 6 years	Motor (fine and gross), cognition (learning, play, problem-solving, attention), language (expressive and receptive), socio-emotional, self-help (adaptive behaviors)	Direct elicitation/observation	Developmental age is calculated based on domain scores
The Gesell Developmental	2 years 6 months – 9 years	Cognitive (visual-spatial, numbers, problem-solving) , language (expressive	Direct elicitation/observation and parental/caregiver report	Requires clinical interpretation.

Observation – Revised (GDO – R)[89]		and receptive), motor (fine and gross), socio-emotional , adaptive		Developmental age is provided. Performance is also categorized as "age-appropriate", "emerging" or "concern".
Warner Initial Development Evaluation of Adaptive and Functional Skills (WIDEA-FS)[90]	0 – 37 months	Motor (gross), communication, social cognition, self-care (feeding, drinking, diaper awareness)	Parental/caregiver report	Scores range 50 – 200 points. Assessment is repeated until the child reaches maximum score to determine "skill has been attained".
Domain-specific assessments				
Wide Range of Visual Motor Abilities (WRVMA)[91]	3 – 17 years	Motor (fine motor, visual-motor, visual-spatial)	Direct elicitation/observation	Motor developmental delay: <16th percentile in the composite score (mean: 100, SD: 15)
Behaviour Rating Inventory of Executive Function (BRIEF)[92]	5 – 18 years	Cognitive (executive function)	Parental/caregiver/teacher report, interview	Developmental delay: score >65
Bruininks-Oserestky Test of Motor Proficiency – 2ND Edition (BOT – 2)[93]	4 – 21 years	Motor (fine and gross)	Direct elicitation/observation	Developmental delay: <2 SD below mean for test items (but clinical reasoning is advised)
Alberta Infant Motor Scale (AIMS)[94]	0 – 18 months	Motor (fine and gross)	Observation	Motor developmental delay: <10th percentile at 4 months and <5th percentile at 8 months of age. Percentile ranks for other ages determine delay.
Peabody Developmental Motor Scales – 2nd Edition (PDMS – 2)[95]	0 – 5 years	Motor (fine and gross)	Direct elicitation/observation	Age-equivalents and percentile ranks are provided, but interpretation requires

				clinical reasoning (mean: 100, SD: 15).
Movement Assessment Battery for Children – 2nd Edition (MABC – 2)[96]	3 – 16 years	Motor (fine and gross)	Direct elicitation/observation	Motor developmental delay: ≤5th percentile
Stanford-Binet Intelligence Scales – 5th Edition (SB5)[97]	2 – 85 years	Cognitive (fluid reasoning, knowledge, quantitative reasoning, visual-spatial processing, working memory)	Direct elicitation	Developmental delay: Low average IQ: 80 – 89 Borderline impaired or delayed IQ: 70 – 79 Mildly impaired or delayed IQ: 55 – 69 Moderately impaired or delayed IQ: 40 – 54
Wechsler Preschool and Primary Scale of Intelligence – Fifth Edition (WPPSI – V)[98]	2 years 6 months – 3 years 11 months and 4 years – 7 years 7 months	2:6 – 3:11 years version: Cognitive (verbal comprehension, visual-spatial, working memory) 4:0 – 7:7 years version: Cognitive (verbal comprehension, visual-spatial, fluid reasoning, working memory, processing speed, cognitive proficiency)	Direct elicitation	IQ score 80 - 89 = Low IQ, 70 – 79= borderline, <70 extremely low IQ A low score in a domain is determined to be a "weakness" (mean: 100, SD: 15).
Weschler Intelligence Scale for Children (WISC)[99]	6 – 16 years	Cognitive (verbal comprehension, visual-spatial, fluid reasoning, working memory, processing speed)	Direct elicitation	IQ score 80 - 89 = Low IQ, 70 – 79= borderline, <70 extremely low IQ A low score in a domain is determined to be a "weakness" (mean: 100, SD: 15).

ABC: Adaptive Behaviour Composite, IQ: Intelligence Quotient, SD: Standard Deviation

The definition for NDD includes a 25% delay in functioning when compared to age-matched peers, and 1.5 – 2.0 standard deviations (SD) below the population mean.[1,33,66] NDD can also be established if performance corresponded to a lower age than the child's chronological age.[33] Failing to achieve age-expected neurodevelopmental milestones in two or more domains in individuals that are 5 years of age or younger is defined as global developmental delay.[32] Tools are chosen based on the individual child's needs, weaknesses and strengths, hence there is no gold-standard method for neurodevelopmental or neurocognitive assessment.[33] When all neurodevelopmental areas need to be evaluated, multiple-domain tests are warranted. In cases where only a particular area is of concern, specific-domain assessments are to be used. The BSID, VABS, AIMS, PDSM, BOT, WPPSI and WISC are standardized norm-referenced tests with precisely defined administration criteria and comparable scores, making them commonly used tools for pediatric clinical populations.[33,43,68,76,83,94,95,98-100] The VABS and the BSID-IV are especially useful in outpatient and inpatient settings, as they allow scoring through parental reports. This means that the child does not need to be awake, moving, or even compliant in order to categorize their neurodevelopment.

The BSID is a test of neurodevelopmental functioning that targets young children aged 1-42 months at risk for impairments.[77] It assesses the following neurodevelopmental domains: cognition, language, motor skills, socio-emotional functioning, and adaptive behavior.[77] Data is collected through direct elicitation and observation, and the revised version, BSID-IV, also includes parental/caregiver questionnaire.[74,77] In the BSID-III, scores were 0 (no credit) or 1 (credit), but in the BSID-IV, scoring is polytomous (0,1,2).[74,77] While the BSID is standardized, this was mostly based on a North American population, which is limiting.[77] Furthermore, the full assessment is time-consuming (30-70 minutes, depending on the age of the child), and requires

appropriate training.[74,77] The BSID has been validated against the WPPSI-III and the Peabody Developmental Motor Scales-2nd Edition.[74,77]

The VABS is a neurodevelopmental assessment tool that focuses on measures of adaptive behavior by examining social, communication, motor, and daily living skills.[83] It comes in interview (respondent is a professional that can report on the patient's performance), parent/caregiver (answers are provided through a rating scale), and teacher form (answers provided using a questionnaire), all of them targeting individuals aged 0-90 years.[74,83] The parent/caregiver respond the test when assessing infants and young children, especially if they are sick, as it facilitates data collection without soliciting any performance from the child.[74] The validity of the VABS has been established against the BSID-III.[74] While the normative sample was updated in 2016, the population base is from the United States, which may be a limitation (ethnically), but includes people diagnosed with NDD.[74]

The AIMS is a test of motor skills for infants from birth until the attainment of independent walking (0-18 months).[94] It assesses infant movement in prone, supine, sitting and standing positions, taking 20-30 minutes to complete. Each position or item is scored as "observed" or "not observed" by the administrator, who must be knowledgeable in normal infant motor development.[74] A higher score indicates higher motor maturity. The AIMS was standardized 1990-1992 with a sample of Canadian infants, which means that the normative data is outdated, raising concerns around its use.[74] This tool has established validity with the PDMS and BSID-II, its predictive validity to establish motor delay is considered "good".[74] The AIMS has been used in the assessment of motor outcomes in malnourished infants and children without underlying diseases, and infants born pre-term and very low birth weight.[70,101,102] Protein-energy malnutrition, prematurity and a very low birth weight were associated with delayed motor milestone acquisition.

In contrast, a study found that a high BMI and weight-for-age in children aged <2 years was associated with motor impairment (assessed with the AIMS).[103] Malnutrition and motor deficits have been established in pre- and post-LTx pediatric patients, but evidence is scarce.[44,104] The use of this tool in ESLD remains to be explored further in the context of malnutrition.[44]

The PDMS is a test similar to the AIMS, but targeting children aged 0-5 years.[68] It assesses fine motor skills through grasping and visual-motor integration tasks (fine motor quotient, FMQ), and gross motor skills through reflexes, stationary movement, locomotion, and object manipulation (gross motor quotient, GMQ).[74] FMQ and GMQ scores are combined to obtain a total motor quotient (TMQ), and all subtest scores are combined to obtain a developmental quotient score.[74] Performance is scored in a 3-point scale (0, 1, 2), with the uniqueness that credit is given towards incomplete skills (score of 1), giving recognition of the presence of a skill that is yet to be mastered.[68] Validity has been established with the BSID.[74] The PDMS has been used to analyze the effects of pre/post-natal malnutrition (particularly iron-deficiency anemia)[71,72,105,106] and environmental factors (prematurity, in-utero exposure to cocaine and/or tobacco, poverty)[107-110] on motor development of otherwise healthy children. Exposure to one or all of the aforementioned factors were consistently associated with impaired gross and/or fine motor skills. Little to no evidence of its applicability on pediatric ESLD patients is available.[44]

The BOT, another test of motor development, is targeted for individuals aged 4-21 years. Unlike the previous tools, the BOT is designed to assess children and youth with typical development or known moderate motor deficits (all other tools target children with known motor/developmental impairments and/or at high risk for those).[74] Furthermore, the BOT requires a relatively short assessment time (15-60 minutes).[74] Gross motor skills are assessed through tests of bilateral coordination, balance, running speed and agility, upper limb coordination, and

strength.[68,74] Fine motor skills are scored through tests of fine motor precision, fine motor integration, and manual dexterity. All tasks' scores lead to composite scores in four motor areas: Fine manual control, manual coordination, body coordination, and strength and agility.[74] These comprise the total motor composite score. The BOT was standardized based on a sample of children and youth from the United States and validated against the PDMS.[68,74] There is an abundance of evidence available of associations between chronic and acute malnutrition in healthy and motor skills delay established by the BOT. These include: early childhood stunting and delayed fine motor skills[111], moderate-severe stunting and overall delayed motor skills (low total BOT score)[112-114], early childhood iron deficiency anemia and later motor delay (low total BOT score).[115-117] Low BOT scores have been linked to decreased lean body mass and decreased handgrip strength in children post oncological treatment.[118]

The WISC and the WPPSI are neurocognitive tests that measure general intellectual functioning by assessing verbal comprehension, perceptual reasoning, working memory, and processing speed.[98,99] The WISC was designed for children and youth aged 6-16 years.[99] The WPPSI is aimed at two specific age groups: infants and young children aged 2 and a half years to 3 years 11 months and children aged 4 years to 7 years 7 months.[98] The younger age group is only assessed on verbal comprehension, visual-spatial skills and working memory. The older patients undergo tests of verbal comprehension, visual-spatial skills, fluid reasoning, working memory, processing speed, and cognitive proficiency.[98] Data is collected through direct elicitation for the WISC and the WPPSI. While both are time-consuming (60-90 minutes), recent revisions have aimed to reduced testing time.[119] The WPPSI was normed based on data of North American children. Nonetheless, the standardization process is considered "excellent" given the inclusion of different ethnicities (including minorities), geographic regions, ages, parental education,

intellectual disabilities, etc.[119,120] The standardization of the WISC also included special population (gifted/talented children, mild and moderate cognitive impairment, etc.), but the ethnicity inclusion was not as diverse. Additionally, there is a specific Canadian version available for the WISC.[121] Another potential downside for both tools is that the normative data is based on children whose primary language is English. The WISC and the WPPSI require training and its results must be interpreted by knowledgeable and experienced clinicians.[120] The WPPSI-IV correlated favorable with the WISC-IV.[120] In turn, the WISC-V correlated with the Kaufman Assessment Battery for Children-Second Edition and the Kaufman Test of Educational Achievement, Third Edition.[122]

There are multiple data sources a clinician may rely on while administering neurodevelopmental and neurocognitive tests, including interviews, direct elicitation/observation, parental/caregiver and teacher reports, self-reports, and background questionnaires.[33] Each method must be chosen according to the child's developmental stage.[33] In infants and young children, parental/caregiver reports are important sources. Nevertheless, these reports may be biased sources of information, as they are hugely impacted by their cultural expectations, literacy, and comprehension.[33] Although direct observation is a rich source of data given that the clinician may witness and interpret performance, a child's interaction with an unfamiliar environment may be negatively impacted.[33] This can lead to lack of response in certain areas of the assessment or non-representative scores.[33]

Limitations or weaknesses of the different tests include duration and required training. As all tests have a different duration, it is possible that they may require a lengthy session to obtain all required information. This is problematic because the child may lose interest and refuse to continue participating.[33] Another important barrier is inadequate training of pediatric health

providers. While there are tests that require relatively little training, other instruments must be administered by extensively trained and experienced clinicians, such as the BSID.[33,77]

1.4.4 Implications of Neurodevelopmental Assessment in Pediatric Liver Disease

Malnutrition, body composition, environmental factors, the disease itself, and NDD are interrelated, wherein each element impacts the others. In this context, children are less likely to attain age-expected neurodevelopmental milestones (**Appendix 3**). For this reason, there may be difficulties in determining the source of the delay. Realistically, it is an orchestration of all factors, as rarely there is a single insult to neurodevelopment at a time (**Figure 1.4**), but the problem relies in score and performance interpretation. For instance, a child with ESLD pre-LTx with an altered body composition secondary to malnutrition and metabolic disturbances may perform poorly on motor skill assessments. This may be attributed to the status of their muscle mass, but ascites/increased abdominal girth, pain, irritability, or enteral tubes and intravenous lines can also be responsible.[5,6] Delayed socio-emotional functioning may be attributed to dysfunctional parenting skills or lack of environmental stimulation during hospitalization.[123,124] It can also be a direct result of malnutrition at an early age and a lack of language development that impedes the child to adequately express themself.[67,124] Cognitive delays are usually associated with episodes of hepatic encephalopathy or use of powerful anesthetics post-LTx but may in fact be due to chronic malnutrition or prolonged ventilation.[47,125] Malnutrition may also be secondary to medication use.[126] For instance, neomycin, an antibiotic, may cause excessive fecal losses of fat, electrolytes, and nutrient malabsorption.[126]

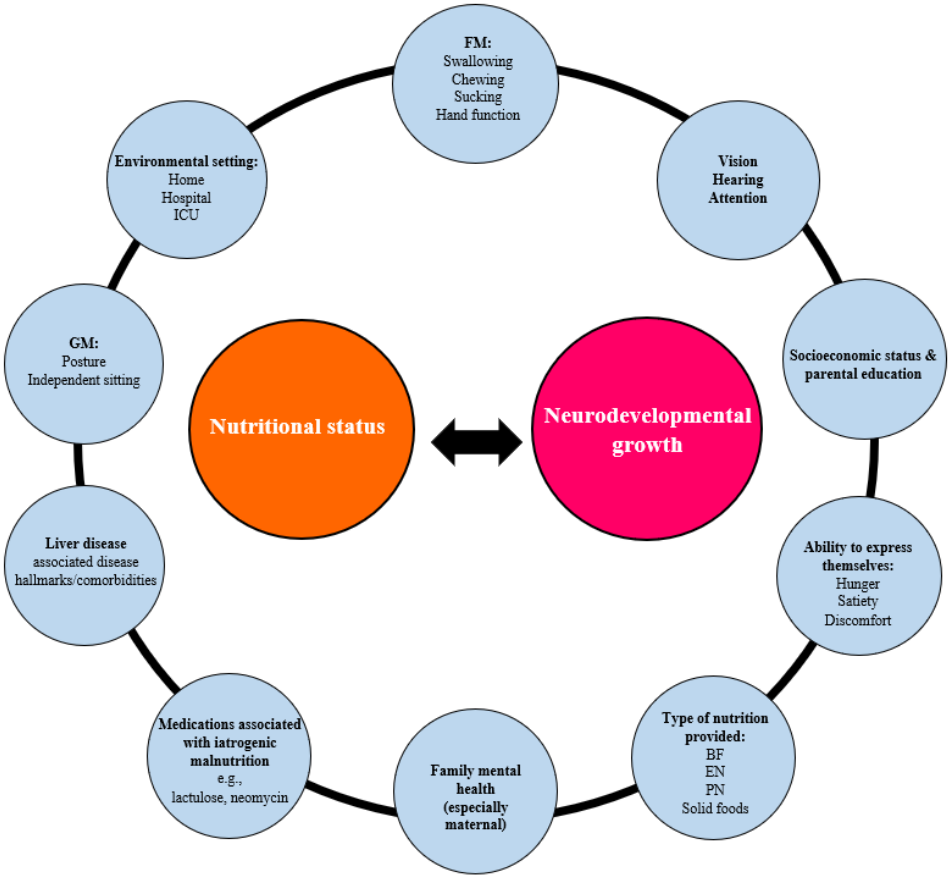


Figure 1.4. Implications of neurodevelopmental assessment in pediatric liver disease. Nutritional status and neurodevelopmental growth (milestone acquisition) impact each other, while simultenously being influenced themselves by other factors that surround the child. These complicate the intepretation of scores and determination of the specific impact each factor has on nutritional risk and neurodevelopmental delay. Adapted from Gladstone *et al*.[67]

Lactulose, typically used for hepatic encephalopathy, may lead to sensation of fullness (decreasing appetite and/or intake), diarrhea and vomiting (both increasing loss of water and electrolytes).[127] NDD itself may impact feeding behavior, as a child's swallowing and self-feeding skills may be altered, potentially exacerbating malnutrition or nutritional risk.[67] Children that are reliant on enteral nutrition, particularly gastric feeding, may have increased risk of gastroesophageal reflux and aspiration pneumonia.[128] This can further delay the exposure to solid foods, impacting their oral motor skills and with that worsening any underlying malnutrition. There

may also be household issues, such as low socioeconomic status or altered family mental health.[129] Additionally, parental reports of performance may result in overestimation of actual neurodevelopment by reporting less severe outcomes.[100] It is for these reasons that interpretation and extrapolation of results is complicated in this context.

1.4.5 Neurodevelopmental and Clinical Outcomes Associated with Malnutrition and Pediatric Liver Disease Pre- and Post-LTx

Pediatric ESLD patients have more NDDs across all domains compared to healthy well-nourished peers.[43] NDDs are common prior to LTx but can persist years after the procedure.[3] The most used tools were BSID[7,125,130,131], VABS[21,125], WPPSI[9,125,132,133], WISC[9,125,132-134], and the Mullen Scales of Early Learning (MSEL).[135,136] The most common pre-LTx NDD is in the motor skills domain, particularly gross motor.[21,44,45,130,131,135,136]. Post-LTx outcomes include overall NDD, cognitive delay (working memory, IQ, processing speed), increased use of special education services, and no improvement in neurodevelopmental scores 3- months and 1-year after LTx. Delays in expressive and receptive language, as well as in socio-emotional skills, have also been reported in patients that underwent LTx between 0-5 years of life, with persistence 1-12 years after the intervention.[43] The most reported predictors/risk factors for neurodevelopmental/neurocognitive delay included pre-LTx growth retardation, weight, disease duration and severity, low albumin, age at LTx and Kasai, and hospital length of stay (LOS). Malnutrition, International Normalized Ratio (INR), hyperbilirubinemia, and elevated serum calcineurin inhibitor levels (particularly 6-months post-LTx) were also highlighted. A summary of studies that evaluate the aforementioned outcomes can be observed in **Table 1.2**.

Table 1.2. Neurodevelopmental outcomes associated with pediatric liver disease

Author	LD etiology	Age of study population	Period of recorded outcome	Assessment tool	Type of assessment	Predictors/risk factors	ND/NC outcomes
Almaas *et al*.[45]	CLD	4-12 years	Post-LTx	M-ABC	Neurodevelopmental	Renal function (total M-ABC score)	Impaired manual dexterity, ball skills, balance, overall low M-ABC score compared to healthy reference group. No changes in M-ABC score 1- or 4-years post-LTx.
Caudle *et al*.[135]	BA	3-20 months	Pre-LTx	MSEL	Neurodevelopmental	Age at Kasai (receptive language), growth (expressive language), INR (gross motor skills), female sex with high C-bilirubin levels	Males and females: Gross motor and expressive language delay Females: Weaker visual reception skills
Caudle *et al*.[136]	BA	7.8±3.9 months	Pre-LTx	MSEL	Neurodevelopmental	INR (fine and gross motor skills), growth (expressive language), age at Kasai (receptive language)	Gross motor and language skills delay
Gilmour *et al*.[125]	BA, A1AD, NHT, AS	1-2 years	Post-LTx	BSID-II, VABS, WPPSI-R, WISC-III, WIAT	Neurodevelopmental, neurocognitive	Pre-LTx growth retardation and hyperammonemia (IQ), elevated calcineurin inhibitor levels (verbal skills)	27% delayed/borderline delayed 46% normal cognition
Gilmour *et al*.[137]	BA, CLD, FLD, etc.	6-18 years	Post-LTx	SAAPS	Academic performance survey with cognitive component	Cyclosporine and non-calcineurin inhibitor immunosuppressant regime use 6-months post-LTx, CMV infection 6 months post-	34% receiving special education, 20% repeated a grade, 33% >10 days of school absence

						LTx and a history of special education pre-LTx (increased special education use)	
Leung *et al*.[132]	AS, PFIC, A1AD	3-17 years	Pre-LTx	WPPSI-III, WISC-IV	Neurocognitive	Malnutrition, liver disease severity, sociodemographic factors	AS: Increased risk for cognitive delay (working memory and processing speed)
Ng *et al*.[130]	BA	1-2 years	Pre-LTx	BSID-II, BSID-III	Neurodevelopmental	Ascites, low weight z-score (motor impairment), unsuccessful hepatoportoenterostomy (motor, cognitive, language delay)	2-5 times higher incidence of motor, cognitive, and language delays during the first 2 years of life
Ooi *et al.*[21]	ESLD	0.4-7.4 years	Post-LTx	VABS-II	Neurodevelopmental	N/A	26% ABC scores <1 SD (mild developmental delay) 67% children with low SATI (±myopenia) had gross motor delay vs 8% with normal SATI
Patterson *et al*.[44]	CLD	Median (IQR): Pre-LT 7 (4-11) months 1-year post-LT 24 (18-32) months	Pre- and Post-LTx	AIMS, PDMS-2	Neurodevelopmental	Height z-score (gross motor skills pre- and post-LT), days on LTx waitlist (fine motor skills)	Pre-LTx: 76% risk/gross motor delay Pre-LTx delay increased risk of post-LTx motor delay (>1 SD below mean on gross motor score) Locomotion skills and object manipulation were particularly affected
Sorensen *et al*.[9]	BA, ALF, CLD,	5-7 years	Post-LTx	WPPSI-III, BBCS,	Neurocognitive	N/A	Delayed IQ, executive function, math and reading

	UCD, WD, A1AD, NH, etc.			WRAT-IV, BRIEF			skills; 4% cognitive delay vs 2% in normative data
Stewart *et al*.[7]	BA	3.5-61 months	Post-LTx	BSID, SBSI, MCDI	Neurodevelopmental, neurocognitive	Infants: height, weight (mental and motor development); head circumference and serum vitamin E levels (mental development) Children: serum bilirubin and albumin levels (overall development)	Children: better mental development than motor skills. Other results were not significant.
Squires *et al*.[133]	BA	3-12 years	Pre- LTx	WPPSI-III, WISC-IV	Neurocognitive	Parent education, male sex, high total bilirubin, and high GGT	No neurocognitive delay overall
Talcott *et al*.[134]	CLD, ALF	Median (SIQR): CLD: 15.5 (11.2) months ALF: 44 (34.9) months	Post-LTx	WISC-IV, WASI	Neurocognitive	Disease duration and growth failure pre-LTx (cognition)	CLD: lower intellectual ability; ALF: normal scores
Wayman *et al*.[131]	BA	Pre-LT: 5-20 months Post-LT: 17-32 months	Pre- and Post-LTx	BSID	Neurodevelopmental	Pre-LTx: weight <5th percentile; at LTx: low albumin, age (<6 months, higher risk); post-LTx: total hospital LOS	Pre-LTx: low-average mental development; psychomotor development 1 SD below norm data. 3-months post-LTx: mental and psychomotor development <1 SD 1-year post-LTx: mental and psychomotor

							development back to pre-LTx levels; 35% with neurodevelopmental delay; 70% gross motor delay.

SD: Standard Deviation; IQR: Interquartile Range; SIQR: Semi-interquartile range; ND: Neurodevelopmental; NC: Neurocognitive; LTx: Liver Transplantation; LD: Liver Disease; BA: Biliary Atresia; CLD: Cholestatic Liver Disease; ALF: Acute Liver Failure; UCD: Urea Cycle Defect; PFIC: Progressive Familial Intrahepatic Cholestasis; FLD: Fulminant Liver Disease; WD: Wilson's Disease; A1AD: Alpha-1-antitryipsin Deficiency; NH: Neonatal Hemochromatosis; NHT: Neonatal Hepatitis; AS: Alagille's Syndrome; MSEL: Mullen Scales of Early Learning; WPPSI-III: Wechsler Preschool and Primary Scale of Intelligence, Third Edition; WISC-IV: Wechsler Intelligence Scale for Children, Fourth Edition; WASI: Wechsler Abbreviated Scale of Intelligence; BBCS: Bracken Basic Concept Scale; WRAT-IV: Wide Range Achievement Test, 4th Edition; BRIEF: Behavior Rating Inventory of Executive Function; SAAPS: School Attendance and Academic Performance Survey; SBSI: Stanford Binet Scales of Intelligence; M-ABC: Movement Assessment Battery for Children; MCDI: Minnesota Child Development Inventory; N/A: Not Assessed; N/R: Not Reported

Regarding other clinical populations, one study compared neurocognitive outcomes of pediatric post-LTx patients and children with cystic fibrosis (CF).[8] They found that the post-LTx group had lower language scores (particularly receptive language), but academic achievement and visual-spatial performance did not differ when compared to the CF group. Children with intestinal failure have been found to present language and gross motor skill delay when compared to reference data.[138] Pediatric patients that underwent renal transplant before 30 months of age showed an improvement in head circumference and cognitive scores after the procedure.[124] Another study found that cardiac transplant patients (1 month – 8 years of age) showed delayed cognition, language, and fine motor skills.[139] Adult survivors of pediatric solid organ transplantation (liver, cardiac, renal) have been reported to have mainly lower physical and social functioning, academic achievement and employment rate.[140-142] In a cohort of adult survivors of pediatric LTx, 32% suffered from an affected neurodevelopmental domain 20 years post-LTx.[143] Anxiety and depression were present in 19% of the cohort, whilst 13% had learning disabilities. In contrast, malnourished infants and children, without an underlying disease, have lower IQ scores, attention deficits, and poor academic performance, all of which persisted up to 45 years of age.[129] Adults with a history of malnutrition during infancy and childhood were found to have offspring with increased cognitive and attention deficits, although the offspring did not experience malnutrition themselves.[129] Another study found that children aged 6-30 months with severe acute malnutrition had significant NDD, particularly in motor skills.[144] One study reported that healthy children that were stunted at 12 months of age but later recovered improved their academic achievement. However, this remained lower than that of children who were never malnourished.[145] Breastfeeding duration is a modifiable factor to improve cognition and overall neurodevelopment.[145]. This practice remains to be explored in pediatric ESLD patients, as they

commonly require specialized formulas (orally or enterally) and parenteral nutrition in order to meet their nutritional requirements. In pre-term infants, providing breast milk via enteral feeding has been trialed successfully.[146-148] Unfortunately, fat losses due to adherence to the feeding tube may be a concern, as fat is a key component of breast milk and has a prominent role in the overall development of a child.[146,147]

One of the limitations of studies evaluating neurodevelopmental outcomes in infants and children post-LTx is the lack of serial evaluations/follow-up. Several studies assess neurodevelopment at liver transplant assessment (pre-LTx) or at a single timepoint post-LTx. Evaluation at different times before and after LTx may allow understanding of the trajectory of neurodevelopment, which is known to wax and wane in healthy children. Serial evaluations, in turn, would enable prediction of outcomes and the establishment of the ideal timing of intervention to reach the most benefit for the patient. Comparisons with healthy age-matched peers are done in order to understand the extent to which ESLD and related comorbidities affect normal child development.[149] Nevertheless, clinical normative data should be explored in order to understand neurodevelopmental outcomes in terms of expectations for infants and children with ESLD.

1.5 Conclusions

This review highlights the multiple risk factors for the surge and progression of NDD that surround patients with pediatric liver disease. While the understanding of the specific impact of each factor remains to be broadly understood, they have been associated with negative outcomes before and after LTx. Ultimately, they may result in a suboptimal HRQoL and persist until adulthood. Malnutrition in particular is an important determinant of neurodevelopment and growth in healthy and diseased populations. Once a child has suffered from malnutrition, nutritional interventions may not be enough to reverse the impact on neurodevelopment. Therefore, a more

detailed exploration and assessment of pediatric ESLD patients pre- and post-LTx is necessary. It should include serial evaluations of neurodevelopment across time, adequacy of nutritional intake and nutritional risk, and parental/environment influences, and even the presence of any protective factors specific to this diseased population. This will allow broader understanding of onset, progression, and persistence of NDD in pediatric ESLD patients. Considering the timing of neurodevelopmental growth, milestone acquisition and malnutrition onset, it may be possible to create an intervention that precedes any major insult to the brain. It is for this reason that the present thesis aims to assess and comprehend associations between neurodevelopment and nutritional risk pre-LTx with clinical outcomes post-LTx. This will highlight the needs for neurodevelopmental prehabilitation before the procedure to improve short- and long-term outcomes.

Chapter 2: Research Plan

2.1 Study Rationale

Neurodevelopment is the process of development of the central nervous system that occurs from in-utero to 5 years of age, and encompasses the acquisition and maturity of cognition, language, motor skills, and socio-emotional skills.[1,29-31] Neurodevelopmental delay (NDD) is defined as a performance in one or more of the domains that corresponds to a lower age than the child's chronological age.[33] It can also be established by a test score 1.5 – 2 standard deviations below age-matched normative data.[30,31,33] In healthy children, NDD may be caused by insults to the developing brain, such as malnutrition, chronic organ failure, and prolonged hospitalization.[2,49] Sixty to eighty percent of pediatric end-stage liver disease (ESLD) patients will develop malnutrition.[10] Liver transplantation (LTx) is commonly the only therapeutic approach to ESLD and may occur during the child's first 1000 days of life (a critical period of neurodevelopment).[1,3,6] Considering the associated metabolic derangements of the disease (e.g., hyperammonemia), and the environmental deprivation that comes with lengthy and repeated hospitalizations, this population is at increased vulnerability for NDD.[3,7,8,45] Evidence shows that malnutrition before LTx can lead to pediatric sarcopenia, in turn increasing post-LTx hospitalization length of stay, infection risk, and mortality.[12,21,150-157] Furthermore, growth retardation, malnutrition, hyperammonemia, and NDD pre-LTx are considered risk factors for post-LTx NDD.[125,130-132,134] Post-LTx, prolonged intensive care unit and hospital stay, increased infection rates, and use of non-calcineurin inhibitor-based immunosuppressant regimes (e.g. sirolimus) have also been reported as predictors of adverse neurodevelopmental outcomes.[125,131,132,137] These may lead to increased need of special education services, poor academic outcomes, a future low socioeconomic

status, less job opportunities, and suboptimal health behaviour.[13,66,137] As a result, the patient's overall health-related quality of life (HRQoL) may be heavily impacted for their lifetime.

LTx has improved survival rates in pediatric ESLD patients, but research must now focus on long-term outcomes. While undergoing LTx may help ameliorate some co-morbid conditions (e.g., metabolic derangements) pre-LTx, in the post-LTx period conditions such as NDD may persist.[44,45,131] How long this delay may last remains to be established in this clinical population, particularly in those that were also malnourished at time of LTx assessment. Studies that focus on NDD in the pre-LTx period do not focus on the impact malnutrition may plan in the persistence of NDD and/or the impact this may have on long term clinical outcomes. This thesis addresses the associations between neurodevelopment, nutrition, and clinical outcomes in infants and children with ESLD pre- and post-LTx. The knowledge gained will help understand the prevalence of NDD in pediatric ESLD patients pre-LTx, the associations between NDD and malnutrition, and how both impact post-LTx clinical outcomes. These findings will enable development of more detailed pre- and post-LTx rehabilitation strategies to improve NDD and malnutrition pre-LTx, potentially impacting clinical outcomes, and long term HRQoL. Additionally, it will open the door for future research that focuses on serial evaluations of neurodevelopment to understand fluctuations and persistence of pre-LTx NDD.

2.2 Objectives and Hypotheses

Chapter 3 of this thesis is a secondary analysis of the study entitled "Myopenia in children with end-StAge liver disease awaiting Liver Transplantation (SALT-2)"(Pro0078499), which includes an evaluation of neurodevelopment in infants and children with ESLD at time of LTx assessment and its associations with nutrition and clinical outcomes pre- and post-LTx (**Table 2.1**).

2.2.1 Study Objectives (presented in Chapter 3)

- **Objective 1:** To evaluate the prevalence of NDD in infants and children with ESLD at time of LTx assessment using the Vineland Adaptive Behaviour Scales, 2nd Edition (VABS-II).
    - **Hypothesis 1:** Infants and children with ESLD undergoing LTx will present NDD, particularly in motor skills [defined as a VABS-II ABC and domain-specific score ≤85 or ≥1 SD below normative data (age-matched healthy subjects)].
- **Objective 2:** To evaluate the associations between NDD in infants and children with ESLD with clinical outcomes [hospitalization, growth, medical complications (such as infection, rejection, biliary, vascular, others), and mortality] in the pre- and post-LTx periods (intensive care unit discharge, hospital discharge, 6- and 12-month follow-ups).
    - **Hypothesis 2:** NDD in infants and children with ESLD at time of LTx assessment will be associated with longer post-LTx hospital and intensive care unit length of stay, reduced growth, higher prevalence of medical complications, and increased mortality rate.
- **Objective 3:** To evaluate the associations between NDD in infants and children with ESLD with pre-LTx nutritional status [determined by Subjective Global Nutrition Assessment category (SGNA), percentage of ideal body weight (%IBW, McLaren criteria for wasting), and height for age z-scores (WHO criteria for stunting)], between NDD and post-LTx growth markers (daily weigh/height gain, weight/height velocity SDS), and between neurodevelopmental status with/without presence of malnutrition with post-LTx clinical outcomes.
    - **Hypothesis 3-A:** NDD in infants and children with ESLD will be associated with higher rates of malnutrition, as determined by SGNA classification of

moderate/severely malnourished (pre-LTx), %IBW 75-90% (moderate malnutrition/wasting) or <75% (severe malnutrition/wasting) (pre-LTx), and height for age z-score <-2 (WHO definition for stunting) (pre-LTx and at the 12-month follow-up).

- **Hypothesis 3-B**: Daily weight/height gain will also be suboptimal and lower than age-expected gained grams or mm/day. Weight/height velocity SDS will not be age appropriate. Those malnourished and with NDD pre-LTx will have adverse clinical outcomes post-LTx.

Table 2.1 Study primary/secondary outcomes and hypotheses

Outcome		Hypothesis	Tool
Primary	NDD prevalence (ABC and domain-specific standard score ≤85)	High NDD prevalence, especially in motor skills	<u>VABS-II</u> • NDD: ABC and domain specific score ≤85
Secondary	Pre/post-LTx clinical outcomes	NDD will be associated with ICU/total hospital LOS, reduced growth, increased medical complications and mortality rate.	Medical history in electronic medical charts
	Pre-LTx nutritional status and post-LTx growth markers	NDD will be associated with high rates of malnutrition, suboptimal daily weight/height gain and weight/height velocity SDS, and those with NDD + malnutrition will have adverse post-LTx clinical outcomes.	<u>Nutritional status</u> **SGNA** • Well nourished • Moderately malnourished • Severely malnourished **McLaren criteria** for wasting (%IBW) • Moderate malnutrition: 75-90% • Severe malnutrition: <75% **WHO criteria** for stunting: • Stunted: height for age z-score <-2 • Not stunted: height for age z-score>-2 z-score <u>Growth parameters</u> • Daily weight/height gain (g/day and mm/day) • Weight/height velocity SDS

ABC: Adaptive Behaviour Composite; IBW: Ideal Body Weight; ICU: Intensive Care Unit; LOS: Length of Stay; LTx: Liver Transplantation; NDD: Neurodevelopmental delay; SGNA: Subjective Global Nutritional Assessment; VABS: Vineland Adaptive Behaviour Scales; WHO: World Health Organization

Chapter 3: Neurodevelopmental, Nutritional, and Clinical Outcomes of Infants and Children with End-Stage Liver Disease Awaiting Liver Transplantation

3.1 Introduction

Infants and children with end-stage liver disease (ESLD) pre and post liver transplantation (LTx) are at risk for brain injury and neurodevelopmental delay (NDD).[3,5,134,136,158] Many predictors of neurodevelopmental status have been studied, including hospital and Intensive Care Unit (ICU) length of stay (LOS), medication use and nutritional deprivation.[3,7,125,136,137] Liver disease and malnutrition alter muscle protein synthesis and degradation, while muscle loss promotes metabolic alterations, synergistically amplifying the neurodevelopmental insult.[159] Sixty to 80% of pediatric ESLD patients develop malnutrition secondary to diseased-induced hypermetabolism, reduced intake, and an altered nutrient absorption and metabolism.[10,11,14,160] Furthermore, 20 to 35% of these children develop fat-soluble vitamin deficiency (A, D, E, K), although other micronutrients such as iron and zinc may be of concern.[10,11,51] Myopenia [skeletal muscle mass (SMM) loss], for which malnutrition has been proposed an etiological factor, occurs in 20-40% patients pre-LTx. [12,21,152,154,161] This is relevant because both malnutrition and an altered body composition have been linked to impairment in motor skills development and overall adverse clinical outcomes post-LTx in children.[12,21,152,154,161] These, in turn, may adversely affect long-term health-related quality of life (HRQoL), impacting all areas of adult daily life, such as academic, work, and personal performance.

In ESLD, NDD may be secondary to cyclical interactions between liver disease-related complications (e.g., metabolic derangements like hyperammonemia), protein-energy malnutrition, and environmental deprivation. For instance, as a neurotoxin, hyperammonemia can lead to astrocytic damage and swelling, white-matter damage, neuronal loss, myelination deficiencies, and

even neuronal cell death.[162,163] Loss of cholinergic neurons, in particular, can greatly compromise cognitive development[42,162,163]. As a myotoxin, ammonia has been shown to upregulate myostatin, a muscle growth and differentiation inhibitor, in avian and murine animal models [164,165]. Effects of myostatin upregulation included increased muscle cell mitochondrial dysfunction, autophagy and decreased satellite cell activation and differentiation, and muscle contractility, which may lead to significant SMM dysfunction and wasting.[164,165] In humans, these changes can adversely impact fine and gross motor skill development, which in turn can alter cognitive function.[166-168] Malnutrition in early childhood can further impair cognition and motor development.[3,7] In humans, it has been associated with dendritic spine abnormalities, short apical dendrites, fewer spines and impaired neurotransmitter function and responsiveness[3,7,134] With the increased caloric and protein needs typically seen in ESLD patients, oral intake may be insufficient to meet the child's nutritional need. Thus, the child may become reliant of on enteral feeding.[11,169] This can compromise their swallowing reflexes, chewing function, tongue control, which can also potentially impact their communication skills, as these movements form the basis for sound production.[170,171] In infants, the parent-child bonding experience (e.g., sensory stimulation via breast/oral feeding, hugging, reading aloud, play time) may become compromised with nutritional rehabilitation and prolonged hospitalization, potentially impacting the cognitive, socialization, socio-emotional domain.[172-175] Prolonged ventilation, increased ICU LOS, use of anesthetics and immunosuppressive therapy, and even chemotherapy agents (in hepatoblastoma patients) all impose an insult to the developing brain and can bring upon delay in all neurodevelopmental domains.[3,125,130,137]

Malnutrition, NDD, and clinical outcomes in infants and children with ESLD are in a cycle wherein each element impacts the other and must all be considered to understand their context.

Unfortunately, there is little evidence about the effect that the presence of NDD and malnutrition impact post-LTx outcomes in infants and children with ESLD. The study objective was to determine the prevalence of NDD [using the Vineland Adaptive Behavior Scales-2nd Edition (VABS) at LTx assessment] in a cohort of infants and children with ESLD awaiting LTx (primary outcome of interest), and to determine whether any associations exist between NDD and nutrition and clinical outcomes pre- and post-LTx (secondary outcomes of interest). We hypothesized that NDD would be prevalent at LTx assessment in infants and children with ESLD (particularly in the motor skills domain) and associated with pre-LTx malnutrition and pre- and post-LTx adverse clinical outcomes.

3.2 Methods

This is a secondary analysis of previously collected data from a retrospective cohort study that was conducted in infants and children with ESLD who underwent LTx assessment at the Pediatric Liver Transplant Clinic, Stollery Children's Hospital in Edmonton, Alberta (2006-2019). Infants and children aged 1 month to 18 years with diagnosed ESLD and with available neurodevelopmental test scores were included. Charts screened for this analysis (n=72) were excluded if children were >18 years of age, did not have available neurodevelopmental assessment scores (VABS) and had presence of a known syndrome or risk factor for NDD other than liver disease [e.g., pre-term birth (≤35 weeks gestations), Simpson-Golabi-Behmel syndrome]. This left a total of 67 patients for review in this analysis. Data were collected and extracted through the electronic medical record (ConnectCare and Organ Transplant Tracking Record files), and paper medical chart review. Recovered information encompassed six timepoints: LTx assessment, LTx admission including day of surgery [LTx wait time, median(IQR): 0.25 (0.2 – 0.4) years] and immediate post-operative ICU and hospital stay until time of first discharge after LTx [total LTx

admission, median(IQR): 43 (28 – 74) days], 6- and 12-month follow-ups (FU) (**Figure 3.1)**. This study was approved by the Health Research Ethics Board at the University of Alberta (Pro00078499).

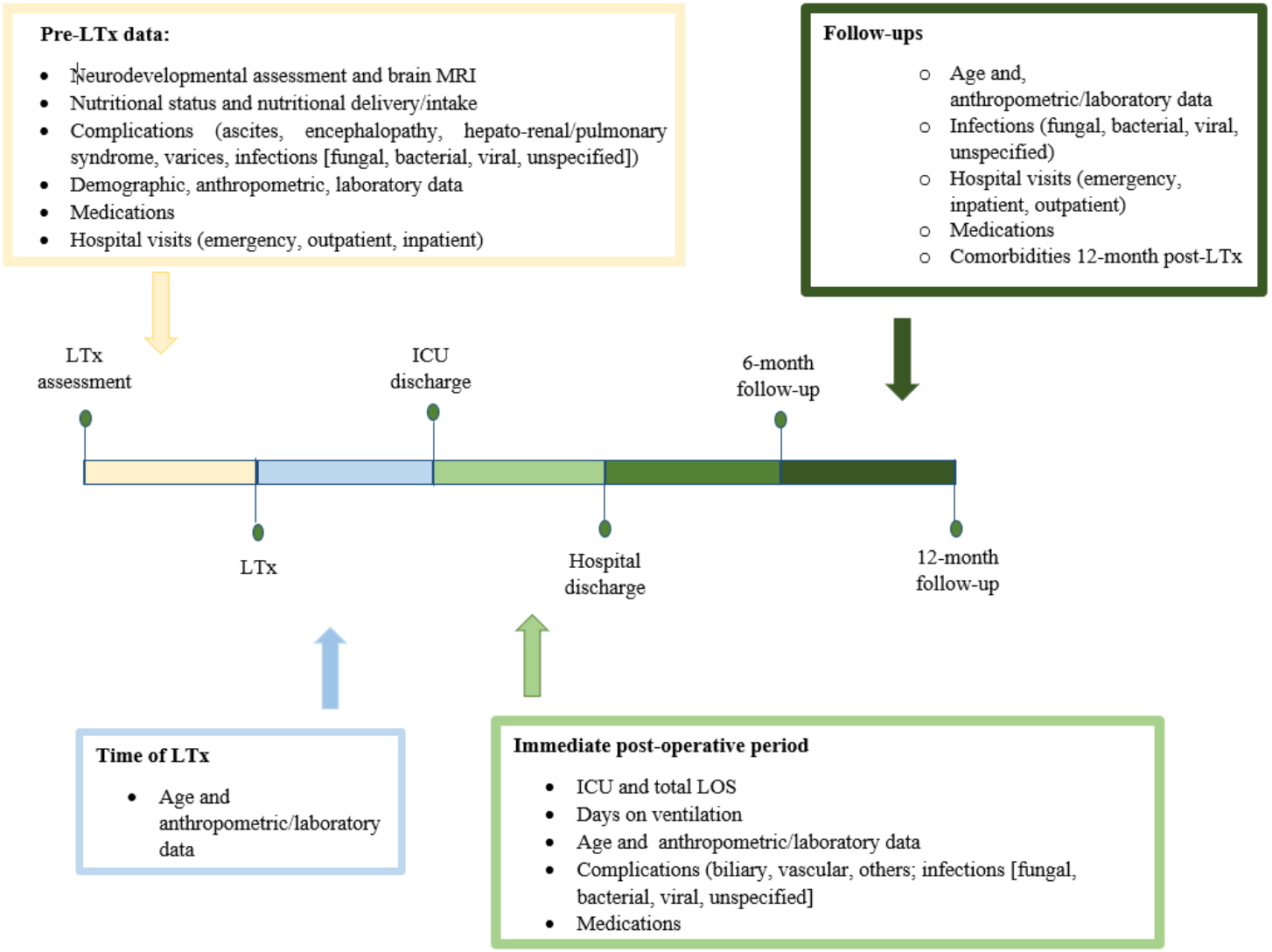


Figure 3.1 Data collection at the different timepoints. Median time length between timepoints: LTx assessment and LTx: 90 (72 – 144) days, LTx and ICU DC: 11 (6 – 26) days, ICU DC and Hospital DC: 43 (28 – 74) days; 6-month and 12-month FU occurred 6 months and 12-months post-LTx, respectively ICU: Intensive Care Unit; LOS: Length of Stay; LTx: Liver Transplant; MRI: Magnetic Resonance Imaging

3.2.1 Demographic, Anthropometric, and Laboratory Data:

3.2.1.1 Demographic Data

Demographic data, such as age, sex, liver disease etiology, caregiver socioeconomic status (SES, established as per the Blishen Index[176], a tool used to establish a socioeconomic score based on income, education, and occupational prestige associated with specific occupations), as well as paternal and maternal educational achievement [post-secondary education completion (yes/no)] were collected at time of LTx assessment. Based on the participant's age, they were categorized as being within the first 1000 days of life if they were ≤2 years per the United Nations (this period spans from conception to the second year of life and encompasses a critical period of neurodevelopment).[177-182]

Other relevant caregiver information included immigration status [family originated from a country other than Canada (yes/no), consanguinity (parents are related as second cousins or closer (yes/no)], and social concerns (e.g., history of drug addiction, teenage pregnancy, child neglect). Disease severity score [Pediatric End-Stage Liver Disease (PELD) score] were recorded at LTx assessment and LTx. PELD was calculated according to the United Network for Organ Sharing and Organ Procurement and Transplantation Network for all patients <12 years of age, as shown below.[183] The equation relies on serum levels of total bilirubin, albumin, international normalized ratio (INR), whether the patient is aged <1 year and whether there is growth failure. PELD scores can be negative and positive numbers and a higher score correlates to an increased disease severity (e.g., PELD score of 32 indicates a higher disease severity than a score of -11).[183]

PELD score= 0.436 [if age is <1 year] – 0.687 x log e [Albumin in g/dL] + 0.480 x log e (Total bilirubin in mg/dL) + 1.857 x log e [International Normalized Ratio] + 0.667 [if growth failure is present (weight and/or height<-2 standard deviations)]

When patients obtained a negative PELD score, it was adjusted to 0 for ease of interpretation as per McDiarmid[184].

3.2.1.2 Anthropometric Data

Weight, height/length, head circumference and body mass index (BMI) were collected at all timepoints (LTx assessment, LTx, ICU and hospital discharge, 6-month and 12-month FUs) and converted to z-scores according to the World Health Organization standards, using the Canadian Pediatric Endocrine Guideline[185]. Height/recumbent length (cm) and weight (kg) were measured to the nearest 0.1 kg/cm by trained personnel using standard procedures in the clinical setting.[21] Weight was measured with a Health-o-meter® Professional Digital Scale (Illinois, USA). Height was measured using a SECA® stadiometer (model 416), and recumbent length was measured with a SECA® infantometer (model 264).[21] Head circumference was measured using a Gulick non-stretch anthropometric tape measure.

3.2.1.3 Laboratory Data

Laboratory parameters included liver function markers [aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase (GGT), total bilirubin, serum albumin, INR, Partial Thromboplastin Time (PTT)], and ammonia. C-reactive protein, white blood cell count, hemoglobin, platelet count, 25-hydroxy vitamin D, alpha-tocopherol (vitamin E), urea, creatinine, sodium, and tacrolimus/sirolimus/everolimus levels were also recorded. Of note, fat-soluble vitamin serum levels (A, D, E, K) were not consistently available for the cohort across timepoints. For these reasons and considering their relevance from a neurodevelopmental perspective, only 25-hydroxy vitamin D and alpha-tocopherol were collected and reported when available. Bloodwork was collected from the medical record at all timepoints. Clinical bloodwork

were processed by the Core Laboratory at Alberta Health Services using validated methodologies.[12,21]

3.2.1.4 Medication Use

Data of relevant medication taken (type) at all timepoints were collected from the medical record, such as immunosuppressive therapy (e.g., tacrolimus, corticosteroids), antibiotics, anticoagulants, vitamin supplements (single preparation vitamin A, D, E, and K supplements, multivitamins), and ursodiol. For reference, recommended fat-soluble vitamin supplementation dosages in this clinical population are as follows: 1000 IU (300µg)/kg/day (vitamin A as retinol), 2000 IU/day (vitamin D as cholecalciferol; final dose is reliant on serum 25-hydroxyvitamin d levels), 50-200 IU/kg/day (vitamin E as alpha-tocopherol)/25 IU/kg/day (vitamin E as alpha-tocopheryl) and 2-5 mg/day (vitamin K as phytonadione or menaquinone).[51] The typical dose of ursodiol for infants and children with ESLD is 10-30/mg/kg/day.[186] A description of the typical medications used at all timepoints can be seen in **Appendix 5**.

3.2.2 Neurodevelopmental Assessment:

Neurodevelopment was assessed at LTx assessment using the Vineland Adaptive Behavior Scales-2nd Edition (VABS, 2005 Version, Pearson Clinical Assessment, San Antonio, TX)[84]. This is a multi-domain parent-report measure of four individual developmental domains: Communication, Daily Living Skills, Socialization and Motor Skills (gross and fine). The sum of the individual domain scores results in the Adaptive Behaviour Composite score (ABC), which determined if the patient's overall neurodevelopment was adequate (normal, moderately high, or high) or inadequate (low or moderately low). Data from the composite and individual domains were recorded as standard score (referred to as "score(s)" throughout the rest of this thesis), standard deviation (SD), percentile, and age-appropriate motor function (yes/no). For reference,

the VABS has a reported mean standard score and SD of 100±15.[83,84] Participants were categorized in the adequate adaptive level group if they had a normal to high ABC score and in the inadequate adaptive level group if the had moderately low to low ABC score (cut-offs for each category can be seen in **Table 3.1)**. The cohort was also categorized based on the group's median score for specific domains: a) motor skills score [median(IQR): 83 (74 – 95)], b) communication score [median(IQR): 100 (89 – 109)], c) daily living skills score [median(IQR): 88 (82 – 97)], and d) socialization score [median(IQR): 97 (87 – 100)]. This resulted in additional subgroups: a) motor skills score ≥83 and motor skills score <83, b) communication score ≥100 and communication score <100, c) daily living skills score ≥88 and daily living skills score <88, and d) socialization score ≥97 and socialization score <97.

Table 3.1. Vineland Adaptive Level Scales, cut-offs for Adaptive Level categories

Adaptive Level	ABC standard scores	Group allocation for this thesis
High	130 – 140	**Adequate adaptive level group**
Moderately high	115 – 129	
Adequate	86 – 114	
Moderately low	71 – 85	**Inadequate adaptive level group**
Low	20 – 70	

Categorization per Sparrow *et al.*[83,187]. These cut-offs can be applied to ABC score and/or specific subdomains. Based on this, the cohort was categorized as having an adequate adaptive level if they had an adequate to high ABC score, and as having an inadequate adaptive level is they had a moderately low to low ABC score. ABC: Adaptive Behaviour Composite. *Vineland Adaptive Behavior Scales Third Edition Copyright © 2016 NCS Pearson, Inc. Reproduced with permission. All rights reserved*

Nine participants had additional cognitive assessment scores available, where elements such as memory, visuospatial skills, and intellectual quotient were scored (**Table 3.2;** results for these can be seen in **Appendix 6**). Six participants were assessed with the Mental Development Index of the Bayley Scales of Infant Development – 3rd Edition (BSID, 2005 Version, Pearson Clinical Assessment, San Antonio, TX)[77], and n=3 with the Wechsler Preschool and Primary Scale of Intelligence – 3rd Edition (WPPSI, 2002 Version, Psychological Corporation, San Antonio,

TX).[188] Results from brain MRI reports performed at LTx assessment were also collected from the medical record for all participants and scored as normal/abnormal based on radiologist assessment.

Table 3.2 Participants with available cognitive assessment scores and its specific components

BSID- Mental Development Index (n=6)	WPPSI (n=3)
Cognitive components	
Exploration and manipulation Object relatedness Concept formation Memory Habituation Visual acuity Visual preference	Verbal comprehension Fluid reasoning Visuo-spatial skills Working memory Processing speed

The cognitive component of the BSID, called Mental Development Index, was applied to n=6 participants. The WPPSI full scale was evaluated in n=3 participants. BSID: Bayley Scales of Infant Development; WPPSI: Wechsler Preschool and Primary Scale of Intelligence

3.2.3 Nutritional Status Assessment, Growth, Nutrient Intake Amounts and Route of Administration Data:

3.2.3.1 Nutritional Status Assessment:

Nutritional status was determined at LTx assessment in 3 ways: a) per the Subjective Global Nutritional Assessment[26] (SGNA), b) presence of wasting [per the McLaren criteria based on percentage of ideal body weight (%IBW)][18] and c) presence of stunting (per the World Health Organization's [WHO] criteria).[189] The SGNA is an assessment method based on clinical judgment rather than quantitative measurements to determine nutritional status (measure of malnutrition) of chronically ill and/or hospitalized children (**Appendix 7**). It encompasses a nutrition-focused medical history (linear growth, weight relative to length/height, changes in body weight, and adequacy of dietary intake, gastrointestinal (GI) symptoms, functional impairment, and metabolic stress are assessed) and a nutrition-focused physical exam (loss of subcutaneous fat, muscle wasting, and edema are evaluated). After aggregating all items, a rating of the child's nutritional

status is obtained: Normal/well nourished, moderately malnourished, and severely malnourished. A detailed explanation of the process of conducting the SGNA has been described by Secker *et al*.[26]

McLaren criteria for wasting (a component of the SGNA and based on %IBW), helped categorized nutritional status as follows: >90% IBW (well nourished), 75-90% IBW (moderately malnourished), <75% IBW (severely malnourished).[18] If a participant had height/length for age ≤-2 z-score they were categorized as stunted, and if height/length for age >-2 z-score they were not stunted (per the WHO criteria for stunting).[189]

3.2.3.2 Growth Data

Growth rates were calculated as absolute weight/height gain (g/d or mm/d) for all timepoints. Height and weight velocity SDS (6-month increments) were calculated at the 6 and 12-month FUs according to reference data by Baumgartner *et al*.[190]

3.2.3.3 Route of Nutritional Delivery and Intake Data

Protein and calorie intake, as well as type of intake at LTx assessment were also recorded. Protein intake was collected as g/kg/day and total g/day, and calorie intake as kcal/kg/day and total kcal/day. Type of intake includes enteral nutrition, parenteral nutrition, orally consuming breastmilk/formula/solid foods, or a mix of the mentioned. A detailed description of the different routes of nutritional delivery can be seen in **Appendix 8**. A description of the associations between route of nutritional delivery/intake data with overall neurodevelopment and specific domains (communication, socialization, daily living skills, socialization, and motor skills) is shown in **Appendix 9.**

3.2.4 Pre- and Post-LTx Clinical Outcomes:

All clinical outcomes data were determined by reviewing the patients' electronic medical record. Pre-LTx was considered the period between LTx assessment to the day before LTx. The post-LTx period comprised the first LTx hospital admission (day of LTx, ICU discharge, hospital discharge), and the 6-month and 12-month FUs. Pre-LTx outcomes included complications at time of assessment (ascites, hepato-renal/hepato-pulmonary syndrome, varices, encephalopathy, infection), pre-LTx hospital visits (outpatient, emergency, inpatient [including LOS]). Infection incidence and type was categorized as fungal, bacterial, viral, and unspecified (confirmed infection but no species identified in the medical report). Post-LTx outcomes include immediate post-operative LOS (ICU, total), days on ventilator, infection incidence and type, number and type of post-LTx complications (vascular, biliary, other), graft rejection, re-transplantation, and comorbidities after 12-months post-LTx (**Appendix 10)**.

3.2.5 Statistical Analysis:

Data analysis was completed using the SAS Statistical Software (SAS, Version 9.4; SAS Institute Inc., Cary, NC, USA). Data were expressed as mean±SD for parametric data or median (interquartile range [IQR]) for non-parametric data, unless otherwise specified. The Shapiro-Wilk test was performed to determine normality of the distribution. Non-parametric data were analyzed using the Kruskal-Wallis H test and the Dwass-Steel-Critchlow-Fligner post-hoc pairwise analysis for multiple comparisons. Univariate and multivariate tests were conducted to assess potential relationships between primary outcomes (neurodevelopmental scores/NDD) and secondary outcomes (nutritional status markers and pre- and post-LTx clinical outcomes). Bonferroni post-hoc pairwise analysis for multiple comparisons was conducted for parametric data.

The cohort was divided into the following subgroups for analysis: adequate/inadequate adaptive level (see **Table 3.1)**, above/below median domain specific score (as described previously for motor skills, communication, daily living skills, and socialization), and adequate/inadequate adaptive level ± malnutrition [as defined by SGNA, McLaren (%IBW, marker of wasting), and WHO (stunting)].[18,26,189] Repeated measures of analysis of variance were performed to assess the effects of time on nutritional and clinical outcomes (secondary outcomes). Analysis of covariance was performed to adjust for any variables influencing primary outcomes [e.g., age , PELD scores, family socioeconomic score, alpha-tocopherol, and 25-hydroxy vitamin D serum levels (all were treated as continuous variables)]. Alpha-tocopherol and 25-hydroxy vitamin D were selected specifically amongst other fat-soluble vitamins given their prominent role in neurodevelopment.[52,64,191,192] Additionally, its deficiencies are known to impair motor skills (see **Appendix 4)**, which were hypothesized to be especially impaired in the study population of this thesis.[56,60] Chi-square/Fisher exact tests were used to determine differences in categorical data. A description of continuous and categorical variables used for the present thesis can be seen in **Appendix 11**. A p-value ≤ 0.05 indicated statistical significance.

3.3 Results

3.3.1 Demographic, Anthropometric, and Laboratory Data:

3.3.1.1 Demographic Data

Demographic and anthropometric data across timepoints are presented in **Table 3.3-A**. Our total cohort consisted of n=67 patients (n=36 females, n=31 males). The most prevalent liver disease etiology was biliary atresia (57%). There was a trend towards higher prevalence of biliary atresia in those ≤2 years of age compared to those aged >2 years (61% vs 17%, p=0.14), but no other differences (or trends) where seen between these groups in regard to demographic data

(p>0.05). PELD scores significantly worsened from LTx assessment to time of LTx [median(IQR): LTx assessment: 12 (4 – 18) vs LTx: 17 (7 – 22), p=0.01), indicative of increasing liver disease severity. Overall, 97% of the cohort was within a sensitive period of neurodevelopment (0-5 years of age) and 89% were within the first 1000 days of life (≤2 years of age[177,178,180-182]). Over 90% of the cohort remained within the critical period of neurodevelopment across timepoints. Family SES was 43.7, indicating that families had an average SES overall (similar to Canada's nationwide mean value of 43[125,176]). More than half of the fathers and mothers received post-secondary education (degree completion was not recorded). Five percent and 17% of the study population had presence of consanguinity in their parents and social concerns, respectively. Family SES, age and PELD score did not differ between sexes at any timepoint (p>0.05).

Table 3.3-A. Demographic, anthropometric and growth data in infants and children with ESLD across timepoints

Variables	LTx assessment	LTx	ICU DC	Hospital DC	6-month FU	12-month FU	*p*-value[x]
Sex		31M/36F			19M/24F	18M/21F	1.0
Age, years	0.56[a] (0.39 – 0.85)	0.85[b] (0.65 – 1.19)	0.93[b] (0.72 – 1.35)	1.04[b] (0.8 – 1.46)	1.48[c] (1.09 – 1.88)	2.[d] (1.57 – 2.27)	**<.0001**
Critical period[y], n(%)		65 (97)			41 (95)	36 (97)	0.99
First 1000 days of life, n(%)	60 (89)		58 (87)		33 (77)	20 (54)	**<.0001**
Liver etiology, n(%)		--	--	--	--	--	N/A
- Biliary atresia	38 (57)						
- Other cholestatic/metabolic	21 (31)						
- Acute liver failure	1 (2)						
- Other[v]	7 (10)						
PELD score	12 (4 – 18)	17 (7 – 22)	--	--	--	--	**0.01**
Weight, kg	7[a] (6 – 8.6)	8.3[b] (7.4 – 9.9)	8.4[b] (7.4 – 10.3)	9[b] (7.7 – 10.6)	10.5[c] (9.6 – 12.4)	12.6[d] (11.3 – 14.2)	**<.0001**
Weight z-score*	-0.57±1.3[a,b]	-0.42±1[c]	-0.41±1.3[d]	-0.39±1.2[e]	0.19±1[a]	0.59±1[b,c,d,e]	**<.0001**
Height, cm	65.5[a] (61.6 – 69.5)	69[b] (65.5 – 76.4)	68[b] (65.6 – 76.4)	71.4[b] (68 – 77.8)	77.4[c] (73 – 81.7)	84.9[d] (80 – 87.7)	**<.0001**
Height z-score*	-0.88±1.3	-1.27±1.3	-1.59±1.5	-1.12±1.3	-0.8±1	-0.71±1.1	0.12
HC, cm	41.9±2	43.6±1	--	--	--	--	**0.0002**
HC z-score*	-0.55±1	-0.33±1	--	--	--	--	0.33
Weight gain, g/d	--	11.4 (5 – 15.2)	0 (-23.7 – 22.5)	13.6 (-1.3 – 23.3)	12.1 (6.5 – 17.2)	9.3 (5.6 – 14.4)	0.06

Height gain, mm/d	--	3.12[a] (0.45 – 4.63)	0[b] (0 – 0)	7.3 (0 – 11.9)	3.7[c] (3 – 4.8)	3[c] (2.4 – 3.8)	**0.0002**
% Weight change	--	13.9[a] (4.6 – 30.3)	0[b] (-5.4 – 2.5)	3.8[c] (-0.38 – 9.1)	18.5[d] (8.3 – 27.7)	15.4[e] (8.4 – 27.9)	**<.0001**
% Height change	--	4.6[a] (0.7 – 10.1)	0[b] (0 – 0)	1.5[b] (0 – 3.3)	7.4[c] (4.2 – 9.7)	7.8[d] (5.4 – 9.4)	**<.0001**
Weight velocity SDS**	--	--	--	--	1.1±1.5	1.96±1.7	0.21
Height velocity SDS**	--	--	--	--	0.79 (0.08 – 2.39)	0.01 (-1.16 – 1.15)	0.09

Data presented as mean±SD or median (interquartile range) or percentage (%). [z]*p*-values ≤0.05 are considered statistically significant. Superscripts denote significant differences between timepoints. Chi-square/Fisher's exact test was conducted to analyze categorical data. Repeated measures analysis of variance with post-hoc Bonferroni correction were conducted for parametric data. Kruskall-Wallist H test with Dwass-Steel-Critchlow-Fligner post-hoc pairwise analysis for multiple comparisons were conducted for non-parametric data. *Z-scores were calculated according to the World Health Organization standards, using the Canadian Pediatric Endocrine Guideline.[185] [y]Patients aged 0-5 years are considered to were considered to be within a critical period of neurodevelopment. [z]Based on Blishen index socioeconomic score; Canadian SES is 43.[125,176] Mean family SES was 43.7±13.6. Regarding post-secondary education, 56% (n=23) of the fathers and 52% (n=26) of the mothers achieved it. Twenty-eight percent (n=17) of the parents had immigrant status, 5% (n=3) were in a consanguineous marriage, and 17% (n=10) of the families had social concerns (e.g., domestic violence). [v]Other diagnoses include hepatoblastoma and alpha-1-antitrypsin deficiency. **Calculated by 6-month increments as per Baumgartner *et al*. DC: Discharge; ESLD: End-Stage Liver Disease; FU: Follow-up; HC: Head circumference; ICU: Intensive Care Unit; LTx: Liver Transplant; N/A: Does not apply; PELD: Pediatric End-Stage Liver Disease Score.

3.3.1.2 Anthropometric Data

Weight and weight z-score consistently improved across time, being significantly higher at 12-month FU when compared to values at LTx assessment, LTx, ICU and hospital DC (p<.0001) (**Table 3.3-A**). Even with 51% of the cohort presenting ascites at LTx assessment, there was improvement in in weight/weight z-score during the post hospital DC period (hospital DC, 6-month and 12-month FU). However, the changes in rates of daily weight gain were not significant across time [median IQR: LTx: 11.4 (5 – 15.2) vs ICU DC: 0 (-23.7 – 22.5) vs hospital DC: 13.6 (-1.3 – 23.3) vs 6-month FU: 12.1 (6.5 – 17.2) vs 12-month FU: 9.3 (5.6 – 14.4), p=0.06] . Height and head circumference significantly increased between LTx assessment, LTx, and FU period (p<0.05 for both), but their respective z-score did not significantly change across time (p>0.05). At LTx assessment, LTx, and ICU, and hospital DC males had greater absolute weight (LTx assessment: p=0.03, LTx: p=0.02, ICU DC: p=0.04, Hospital DC: p=0.009) than females, but not height, weight, and height z-scores (p>0.05). Head circumference was also greater in males at LTx assessment (p=0.01). These differences remained when correcting for age (p<0.05). When categorizing the cohort by ≤2 years of age and >2 years of age, older participants had greater weight (p<.0001), weight z-score (p=0.04), and height (p<.0001), but not height z-score, at LTx assessment. By the time of LTx and at hospital DC, weight and height z-scores did not differ (p>0.05). By the 6-month FU, those aged ≤2 years had greater rates of daily weight gain than participants aged >2 years [median(IQR): 3.9 (3.1 – 4.8) vs 1.5 (0.5 – 2.6) g/day, p=0.04). During the 12-month FU, there was a trend towards greater rates of daily height growth in the younger participants (p=0.12). No other anthropometric/growth parameter (e.g., head circumference, weight/height velocity SDS) differed between these groups at the remainder timepoints.

3.3.1.3 Laboratory Data

Laboratory data across timepoints is presented in **Table 3.3-B**. Albumin, ALT, AST, and GGT, which are important markers of liver function, significantly improved across all timepoints ($p<0.0001$). Serum INR and CRP were significantly reduced post-LTx, but only INR normalized ($p<.0001$). Serum ammonia also lowered and normalized in the immediate post-operative period (by the ICU DC, and persisted during hospital DC, 6- and 12-month FUs), but this was not significant ($p=0.18$). Laboratory data did not differ when categorizing the cohort as aged ≤2 years and >2 years ($p>0.05$).

Table 3.3-B. Laboratory data in infants and children with ESLD across timepoints

Variables	LTx assessment	LTx	ICU DC	Hospital DC	6-month FU	12-month FU	*p*-value[x]
AST, IU/L	206[a] (95 – 296)	538[b] (365 – 981)	44.5[c] (33 – 111)	41[c] (33 – 56)	44.5[c,d] (31 – 51)	42.5[c,d] (36 – 55.5)	**<.0001**
ALT, IU/L	149[a] (55 – 240)	446.5[b] (217 – 726)	85[a] (38 – 255)	46[c] (33 – 61)	42[c,d] (25 – 63)	32.5[c,d] (21 – 51)	**<.0001**
GGT, IU/L	197[a] (59 – 539)	59[b] (33.5 – 114)	97[b] (53.5 – 217)	66[b] (27 – 122)	11[c] (5 – 29)	8[c] (5 – 19)	**<.0001**
Albumin, g/L	34±6[a]	33±7[a]	26±5[b]	36±5[a,d]	39±5[d]	39±4.4[d]	**<.0001**
Total bilirubin, μmol/L	194[a] (31 – 277)	99[a] (38 – 199)	22[b] (8 – 57)	10[c] (7 – 13)	7[c,d] (4 – 10)	7[c,d] (4 – 13)	**<.0001**
INR	1.2[a] (1.1 – 1.4)	1.8[b] (1.5 – 2.4)	1.2[a] (1.0 – 1.3)	1.1[a] (1.0 – 1.2)	1.1[a] (1.1 – 1.2)	1.1[a] (1.1 – 1.2)	**<.0001**
PTT, seconds	39.3[a] (35 – 48.5)	57[b] (39 – 105)	47[b] (42 – 74)	44[b,c] (39 – 60)	43[c] (36 – 54)	38[c] (32 – 45)	**<.0001**
Ammonia**	50[a] (40 – 66)	55[a] (39.5 – 70)	39.5[a] (31.5 – 96.5)	35[a] (24.5 – 96.5)	28.9[a] (6.7 – 51)	10[a] (6.4 – 41)	0.18
CRP, mg/L*	5.8[a] (0.9 – 10.2)	29.7[b] (10.4 – 61.6)	35.1[b] (14.6 – 57)	2.8[a,c] (0.9 – 8)	12.5[a,b,c] (0.4 – 57.5)	70.4[a,b,c] (39.3 – 101.5)	**<.0001**

Urea, mmol/L	3[a] (2 – 3.6)	3.9[b] (3.1 – 5.1)	4.2[a,b] (2.4 – 6.8)	4.6[b] (3.5 – 5.9)	4.7[b] (3.5 – 6.4)	5.3[b] (3.6 – 6.2)	**<.0001**
Creatinine, µmol/L	18.3±7[a]	23.9±11.8[b]	19.7±9.5[a,b]	23.1±9.7[b]	25±10.7[b]	22.8±5.7[a,b]	**0.0006**
25-hydroxy vitamin D, nmol/L	22[a] (13.8 – 83)	28[a,b] (14 – 68)	51[a,b] (20 – 159)	78[a,b] (70 – 88)	--	109[b] (82 – 123)	**0.006**
Alpha-tocopherol, mg/L	9[a] (3.1 – 22)	14[a,b] (2 – 54)	24[a,b] (13 – 31)	22[b] (21 – 29)	--	--	**0.04**

Data presented as mean±SD or median (interquartile range) for variables demonstrating parametric and non-parametric distributions, respectively. **p*-values ≤0.05 are considered statistically significant. Superscripts denote statistical difference between timepoints. Repeated measures analysis of variance with post-hoc Bonferroni correction were conducted for parametric data. Kruskall-Wallist H test with Dwass-Steel-Critchlow-Fligner post-hoc pairwise analysis for multiple comparisons were conducted for non-parametric data. *Available for n=2 during the 12-month FU. **Available for n=3 during the 12-month FU. 25-hydroxy-vitamin D and alpha-tocopherol were the only fat-soluble vitamins to be consistently reported in the cohort's medical charts (hence vitamin A and K serum levels were not reported). AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; CRP: C-Reactive Protein; DC: Discharge; ESLD: End-Stage Liver Disease; FU: Follow-up; GGT: γ-glutamyl transferase; ICU: Intensive Care Unit; ; INR: International Normalized Ratio; LTx: Liver Transplant; N/A: Does not apply; PELD: Pediatric End-Stage Liver Disease Score; PTT: Partial Thromboplastin Time. Reference ranges for biochemical data for patients 2 months to 18 years of age: AST (10 – 75 UI/L), ALT (<60 UI/L), GGT (10 – 50 UI/L), serum albumin level (30 – 50 g/L), total bilirubin (<21 µmol/L), INR (0.8 – 1.2), PTT (27 – 39 seconds), ammonia (25 – 55 µmol/L), CRP (0 – 10 mg/L), vitamin D (>75 nmol/L), urea (2 – 7 mmol/L). creatinine (10 – 20 µmol/L), vitamin E (3.8-18.4 mg/L)

Serum creatinine, a renal function marker, increased from LTx assessment to the 6-month FU, lowered by the 12-month FU, but remained outside normal ranges for pediatric populations (p=0.0006). At LTx assessment, 27% and 70% of the cohort presented deficient serum vitamin E and vitamin D levels, respectively. Serum vitamin E and D serum levels significantly increased when comparing values at LTx assessment to hospital DC and 12-month FU, respectively (p=0.04 and p=0.006). Biochemical blood measures were not different between sexes (p>0.05).

3.3.1.4 Medication Use

A graphic description of medication usage (percentage calculated via chi-square analysis) across timepoints can be seen in **Appendix 12-A, B, and C.** Medications that are typically used to treat liver disease complications pre-LTx (e.g., fluid overload), such as diuretics, ursodiol, and lactulose, had a noted decrease in usage (percentage of participants using the medication) from assessment to the 12-month FU (p<.0001). Those that are necessary in the immediate post-LTx period, such as antibiotics, anticoagulation, and steroids had an increased rate of usage (percentage of participants using the medication) from LTx assessment to ICU and hospital DC, lowering again by the follow-ups (p≤0.05). Thirty-four percent had steroid use at any time point (which coincides with the corticosteroid-free protocol implemented at the Stollery Children's Hospital since 2003)[193,194], and immunosuppressive therapy post-LTx consisted of 1-2 medications at a time. Vitamin D (single preparation) was the only supplement that was consistently used by more than half the cohort across all timepoints. Typical dose and frequency in this cohort per their medical charts was 400-2200 IU/daily as cholecalciferol. Vitamin E (single preparation) was used by 44% of the participants at LTx assessment, and this lowered to 2% and 0% by hospital DC and the 12-month FU, respectively (p<.0001). Typical dose and frequency per their medical charts were 100 IU/day as alpha-tocopherol.

3.3.2 Neurodevelopmental Assessment

Neurodevelopmental scores (VABS) at LTx assessment are presented in **Table 3.4**. Global NDD was one of the most prevalent comorbidities post-LTx, with 36% of the cohort presenting delays in >2 neurodevelopmental domains.

83% of the subjects had a normal brain MRI. Overall, 62% of the patients had age-appropriate fine motor skills (p=0.05) whereas 72% did not have age-appropriate gross motor skills (p=0.0005). Most of the participants had either an adequate (63%) or a moderately low adaptive level (26%). Participants that were aged >2 years had higher rates of abnormal brain MRI findings, compared to younger individuals (50% vs 14%, p=0.06), but no other neurodevelopmental outcome differed between age groups (p>0.05).

Table 3.4. Neurodevelopmental scores (Vineland Adaptive Behaviour Scales-2nd Edition) at LTx assessment in infants and children with ESLD

Neurodevelopmental domain score/percentile	Total cohort	Adequate adaptive level	Inadequate adaptive level	p-value[a]
Communication score	100 (89 – 109)	104 (100 – 115)	82 (74 – 100)	**<0.0001**
Communication percentile	50 (23 – 73)	60.5 (50 – 84)	12 (4 – 50)	**<0.0001**
Daily living skills score	89±14.5	94.9±12.2	78.4±12.4	**<0.0001**
Daily living skills percentile	21 (12 – 47)	34 (21 – 50)	6 (3 – 34)	**0.0001**
Socialization score	97 (87 – 100)	100 (94 – 104)	84 (73 – 91)	**<0.0001**
Socialization percentile	42 (27 – 50)	50 (34 – 61)	14 (5 – 34)	**<0.0001**
Motor skills score	84.7±14.6	90.3±13.1	74.9±12	**<0.0001**
Motor skills percentile	13 (5 – 37)	27 (9 – 58)	4 (1 – 13)	**<0.0001**
Age-appropriate Fine Motor Skills? n(%)				
- Yes	38 (62.3)	31 (51)	7 (11)	**0.0007**
- No	23 (37.7)	9 (15)	14 (23)	
Age-appropriate Gross Motor Skills? n(%)				
- Yes	18 (28)	15 (23)	3 (5)	**0.04**
- No	46 (72)	26 (41)	20 (31)	
ABC score	91 (84 – 96)	94 (91 – 101)	81 (69.5 – 84)	**<0.0001**
ABC percentile	26 (14 – 39)	34 (27 – 53)	10 (2 – 14)	**<0.0001**
Adaptive level, n(%)				N/A
- High	0 (0)			
- Moderately high	1 (1)	--	--	
- Adequate	42 (63)			
- Moderately low	17 (26)			
- Low	7 (10)			
Abnormal brain MRI, n(%)	11 (17)	6 (15)	5 (22)	0.50

Data presented as mean±SD or median (interquartile range) for variables demonstrating parametric/non-parametric distributions, or percentage (%). *p*-values ≤0.05 are considered statistically significant. Chi-square/Fisher's exact test was conducted to analyze data. ANOVA and Kruskall-Wallis H test were conducted to assess parametric and non-parametric data, respectively. The high, moderately high, and adequate adaptive level categories were grouped to produce the adequate adaptive level cohort, while the moderately low and low categories comprise the inadequate adaptive level group (per Sparrow *et al.*[71, 72, 170]). Adaptive level category was available for n=67. Age-appropriate fine motor skills (y/n) data were available for n=61/67 and age-appropriate gross motor skills (y/n) data were available for n=64/67. ABC: Adaptive Behaviour Composite; ESLD: End-stage Liver Disease; MRI: Magnetic Resonance Imaging

Motor skills score was the most affected in the study population, with 55% scoring ≤85 or >1 SD below the normative data and 18% scoring ≤70 or >2 SD (**Appendix 13)**. Communication, socialization, and daily living skills overall scores fell within the "adequate neurodevelopment" score category, as their mean or median values were >85. In females, there was a trend towards a higher ABC score (mean±SD: 92.2±13 vs 86.8±12, p=0.08) and socialization score (median [IQR]: 97 [91 – 103] vs 92.5 [84 – 100], p=0.08) when compared to males, but no other neurodevelopmental outcome was associated with sex. No differences were seen in neurodevelopmental outcomes dividing the cohort by liver disease diagnosis (biliary atresia vs other liver diseases), above/below median alpha-tocopherol serum levels at LTx assessment or between those who received corticosteroids at any timepoint (p>0.05).

3.3.2.1 Predictive Variables for Neurodevelopmental Outcomes

An analysis of covariance found the following variables as strong predictors of neurodevelopmental outcomes (**Table 3.5**). Age at LTx assessment was found as a predictor for communication ($R^{2=}$ 0.37, p<0.0001) in opposite ways for the different adaptive level groups: older age at LTx assessment was associated with a greater chance of having higher communication scores in the inadequate adaptive level group, whereas younger patients had higher scores in the adequate adaptive level group. A higher socialization and ABC standard score (but still inadequate) was associated with older age in the inadequate adaptive level group, and vice versa for the adequate adaptive level group (R^2=0.42, p<0.0001 and R^2=0.52, p<0.0001). Regarding the daily living skills and the motor skills domains, age was a predictor of score (R^2=0.33, p<0.0001 and R^2=0.27, p=0.0002), and both groups behaved similarly: older age at LTx assessment was associated with higher daily living skills and motor skills scores.

Table 3.5. Predictive variables for neurodevelopmental outcomes

Outcome variable	Predictive variable*	R^2	*p*-value[a]
Communication score	Age at LTx assessment	0.37	<0.0001
	Family SES	0.25	0.006
Socialization score	Age at LTx assessment	0.42	<0.0001
	PELD	0.41	<0.0001
	Vitamin E serum levels	0.56	<0.0001
Daily Living Skills score	Age at LTx assessment	0.33	<0.0001
	PELD	0.30	0.0001
	Family SES	0.25	0.0002
Motor Skills score	Age at LTx assessment	0.27	0.0002
	PELD	0.27	0.0003
ABC score	Age at LTx assessment	0.52	<0.0001
	PELD	0.59	<0.0001
	Vitamin E serum levels	0.62	<0.0001

*All predictive variables' values were at LTx assessment. The following variables were assessed via an analysis of covariance: Head circumference, height, weight (and their respective z-scores), SGNA score, vitamin E and ammonia serum levels, age, and PELD score. [a]*p*-values ≤0.05 are considered statistically significant. ABC: Adaptive Behaviour Composite.

PELD score at LTx assessment was a predictor of daily living skills (R^2=0.30, p=0.0001), socialization (R^2=0.41, p<0.0001), motor skills scores (R^2=0.27, p=0.0003), and ABC score (R^2=0.59, p<0.0001). For all mentioned domains, a lower PELD score was associated with a higher score in both groups. A higher socioeconomic score was associated with higher communication and daily living skills scores in both groups (R^2=0.25, p=0.006 and R^2=0.36, p=0.0002). Vitamin E serum levels as LTx assessment were a predictor of socialization and ABC score, with a higher serum level being associated with higher scores in both groups (R^2=0.56, p<0.0001 and R^2=0.62, p<0.0001).

A multiple regression analysis, on the other hand, only found age and weight z-score at LTx assessment as predictive variables for specific neurodevelopmental outcomes (**Table 3.6**).

Indeed, a higher weight z-score predicted a higher ABC, daily living skills, and socialization score, while a younger age at assessment predicted a higher ABC and socialization score. No significant predictors were found for the other neurodevelopmental domains.

Table 3.6 Predictive variables of neurodevelopmental outcomes

Outcome variable	Predictive variable*	Slope	Standard error	p-value[a]	R^2
ABC score	Weight z-score	9.83	3.76	0.03	0.46
	Age at LTx assessment	-34.29	11.96	0.02	0.51
Daily Living Skills score	Weight z-score	15.52	4.87	0.01	0.56
Socialization score	Weight z-score	10.45	3.18	0.01	0.57
	Age at LTx assessment	-36.58	9.79	0.006	0.64

*All predictive variables' values were at LTx assessment. The following variables assessed via a multiple regression analysis: Head circumference, height, weight (and their respective z-scores), SGNA score, vitamin E and ammonia serum levels, age, and PELD score. [a]*p*-values ≤0.05 are considered statistically significant. ABC: Adaptive Behaviour Composite.

3.3.3 Nutritional Status Assessment, Growth and Nutrient Intake/Route of Delivery Data:

3.3.3.1 Nutritional Status Assessment

There was a high prevalence of malnutrition (as defined by SGNA scores) in the cohort, as 36% and 55% had an SGNA rating of moderately and severely malnourished, respectively (**Figure 3.2**). Based on SGNA reports, this high prevalence was mostly driven by the following SGNA domains: presence of chronic illness (metabolic stress), suboptimal linear growth (weight and height), and functional impairment.

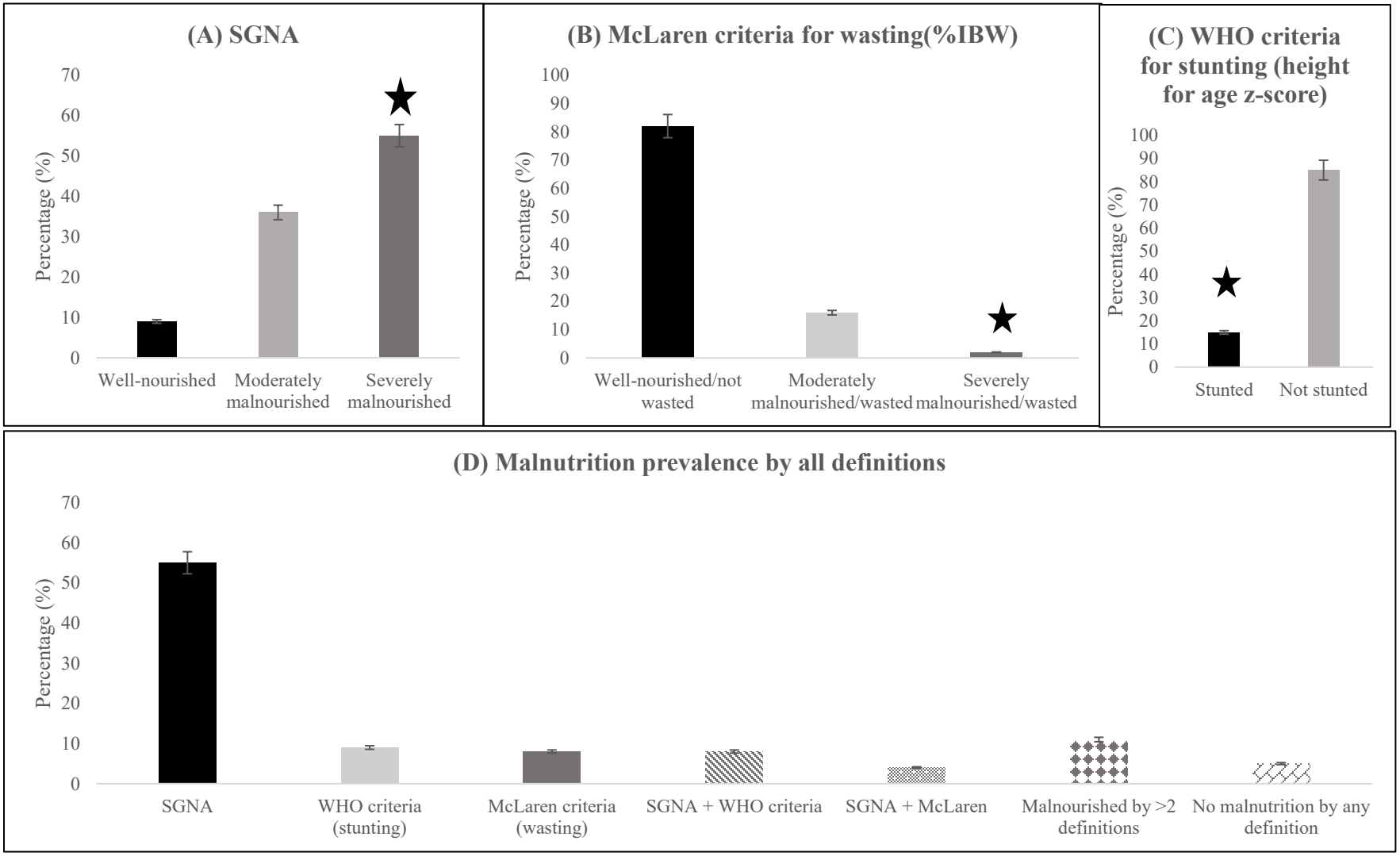


Figure 3.2. Prevalence of malnutrition in a cohort of infants and children with ESLD at LTx assessment, based on three different definitions.(A): The Subjective Global Nutritional Assessment (SGNA)[172] and (B): the McLaren criteria for malnutrition based on %IBW provide three categories for nutritional status: Well-nourished, moderately malnourished, severely malnourished.[173] (C): The WHO criteria for stunting (height z-score≤-2) is based on a dichotomous categorization (yes/no).[174] (D): Prevalence of any malnutrition by SGNA, McLaren criteria, WHO criteria, and a mix of the definitions. Data presented as percentage (%). [a]Chi-square/Fisher's exact test was considered significantly different at $p<0.05$.

Interestingly, when defining malnutrition by %IBW (marker of wasting), 16% were moderately malnourished and 2% severely malnourished; while the definition based on presence of stunting showed a 15% prevalence of malnutrition at time of LTx assessment. Prevalence of wasting at the remaining timepoints was as follows: 3% moderately malnourished/3% severely malnourished (LTx), 12% moderately malnourished/0% severely malnourished (hospital DC), 0% moderately malnourished/0% severely malnourished (6-month FU), and 3% moderately malnourished/0% severely malnourished (12-month FU) (p=0.02). Prevalence of stunting at the remaining timepoints was 35% (LTx), 20% (hospital DC), and 10% (6-month and 12-month FUs) (p<0.05).

When comparing malnutrition prevalence by all three definitions, SGNA illustrated a higher prevalence of malnutrition (moderate/severe) when compared to values obtained via the WHO and McLaren criteria. Eight percent (8%) of the cohort had malnutrition by SGNA + WHO criteria, 4% by SGNA + McLaren criteria, and 5% had no malnutrition by any definition (p<.0001). Malnutrition prevalence as defined by the three definitions did not differ between males and females (p=0.59). There were no differences in prevalence of malnutrition per SGNA, McLaren and WHO criteria between those in the critical period of neurodevelopment (p>0.05).

3.3.3.1.1 Predictive Variables for Neurodevelopmental Outcomes

A lower SGNA score (higher SGNA score reflects a worse nutritional status) score at LTx assessment was associated with a higher ABC (R^2=0.65, p<0.0001), motor skills (R^2=0.36, p=0.0006), and communication score (R^2=0.23, p=0.02) in both adaptive level groups (**Table 3.7)**. In summary, the strongest predictor for neurodevelopmental status (ABC score) at LTx assessment was SGNA score, followed by the previously mentioned factors: PELD score, vitamin E serum levels and age at LTx assessment, respectively.

Table 3.7. Predictive variables for neurodevelopmental outcomes

Outcome variable	Predictive variable*	R^2	*p*-value[a]
Communication score	SGNA	0.23	0.02
Motor Skills score	SGNA	0.36	0.0006
ABC score	SGNA	0.65	<0.0001

*All predictive variables' values were at LTx assessment, using the adaptive level categories (adequate/inadequate) as class. An analysis of covariance was conducted. [a]*p*-values ≤0.05 are considered statistically significant. ABC: Adaptive Behaviour Composite; SGNA: Subjective Global Nutritional Assessment

3.3.3.2 Growth and Ascites Data

There was no weight and height gain from LTx to ICU [median(IQR) time between timepoints: 11 (6 – 26) days), but this significantly improved by hospital DC and the follow-ups for height only (**Table 3.3-A**, *p*=0.0002). The percentage of the cohort suffering from ascites decreased across time, with 56% presenting it at LTx and 38% at ICU DC. This is relevant when interpreting lack of daily weight gain at this timepoints. There was a trend for females to have greater weight velocity SDS at the 6-month FU (mean±SD: 1.77±1 vs 0.16±1.7, *p*=0.07) and they had significantly greater height gain by the 12-month FU (median[IQR]: 3.33[0.7 – 4.8] vs 2.68[2.1 – 3.4] mm/day, *p*=0.05). No other differences were seen in growth data between sexes, and age was not an influencing factor in the statistical differences shown (*p*>0.05). Those who received corticosteroids post-LTx had lower height and height z-score at hospital DC (+corticosteroids vs -corticosteroids): [Height: median(IQR): 69 (67.3 – 71.7) vs 74.3 (68.9 – 78.9) cm, *p*=0.04; height z-score: mean±SD: -1.64±1.4 vs -0.81±1.1, *p*=0.03], the 6-month FU [Height: median(IQR): 73.2 (71.5 – 77.3) vs 78.9 (75.4 – 83.4) cm, *p*=0.03); height z-score: median(IQR): -1.28 (-2.31 to -0.77) vs -0.62 (-1.15 to -0.12), *p*=0.02] and 12-month FU [Height: median(QIR): 81.3 (76 – 86) vs 85.8 (82.1 – 88.2) cm, *p*=0.05; height z-score: mean±SD:-1.44±1 vs -0.40±1, *p*=0.007] when compared to those who never received corticosteroid therapy. Height velocity SDS

at the 6-month FU was also lower amongst those who underwent corticosteroid therapy when compared to those who did not [median(IQR): 0.06 (0.55 – 0.79) vs 1.56 (0.37 – 2.59), p=0.03] These differences were not influenced by age (p>0.05). No differences were seen in weight, weight z-score, daily weight/height gain and weight/height velocity SDS at the remaining timepoints between those who received/did not receive corticosteroid therapy (p>0.05).

3.3.3.3 Nutritional Intake and Route of Delivery Data

The different nutritional delivery routes are described in **Appendix 8.** Most of the cohort was orally fed at LTx assessment via a combination of formula and solid foods (30%), followed by 16% receiving all nutrients through an enteral feeding tube only. Only 6% were exclusively breastfed at this timepoint (all were aged ≤2 years). On average, the cohort receiving oral/infant formula/enteral feeding only was consuming 108±25 kcal/kg/d, with a total of 796±242 kcal/d, and 2.5±1 g of protein/kg/d, with a total average of 18.1±7 g of protein/d (**Table 3.8)**. This resulted in 98% receiving a daily caloric amount that equaled 120% of their basal metabolic rate and 65% meeting the daily protein intake of 2 g/d (for patients aged <2 years), and 1.5 g/d (for those aged >2 years). Only 31% of those aged ≤2 years were meeting the recommended daily caloric intake of 120 kcal/kg. Average daily energy intake (kcal/d) was greater in children aged >2 years (mean±SD: 1282±58 vs 758±207 kcal/d, p<.0001), and average daily protein intake (g/d) did not differ between age groups (p=0.51). However, when expressed as per-kg basis, younger children had greater energy [median(IQR): 106 (97 – 125) vs 84 (77.2 – 85) kcal/kg/d, p=0.05) and protein intakes (median±SD: 2.65±0.9 vs 0.95±0.5 g/kg/d, p=0.003) than their older counterparts. These differences remained significant when adjusting for sex (p<0.05).

Table 3.8 Nutritional intake data of infants and children with ESLD at LTx assessment.

Nutritional intake variable	ESLD cohort 31M/36F
Daily caloric intake per kilogram, kcal/kg/d	108±25
Total daily calories, kcal/day	796±242
Daily grams of protein intake per kilogram, g/kg/d	2.52±1
Total daily protein intake, g/d	18.1±7
Meeting recommended daily caloric intake?*n(%)	
- Yes	12 (31)
- No	27 (69)
Meeting 120% BMR via nutritional intake?**n(%)	
- Yes	41 (98)
- No	1 (2)
Meeting recommended daily protein intake?*** n(%)	
- Yes	28 (67)
- No	14 (33)

Data presented as mean±SD or percentage (%). Nutritional intake data was available for n=42, except for daily caloric intake (available for n=39). *Recommended daily caloric intake for pediatric patients with ESLD aged ≤2 years is 120 kcal/kg/d. **BMR was calculated per the Schofield equation for individuals aged 0-3 years and 3-10 years. ***Daily protein intake recommendations for pediatric ESLD patient are divided by age: <2 years=2 g/kg/d, >2 years= 1.5g/kg/d. BMR: Basal Metabolic Rate; ESLD: End-stage Liver Disease.

3.4.4 Pre- and Post-LTx Clinical Outcomes

Pre- and post-LTx clinical outcomes for the overall cohort are listed in **Table 3.9.** Fifty-one percent (51%) had ascites pre-LTx, increasing to 57% at time of LTx, and lowering to 8% at hospital DC, 2% at the 6-month FU, and 0% at the 12-month FU ($p<0.05$). Regarding other pre-LTx complications, 2% presented hepato-pulmonary syndrome, 9% hepato-renal syndrome, 8% suffered from encephalopathy, and 28% presented esophageal varices. Post-LTx, the cohort had a median ICU LOS of 11 (6 – 26) days, a total hospital LOS of 43 (28 – 74) days, and a ventilator dependency of 5 (2 – 18) days. Graft rejection occurred in 32% of the participants, with only 2% of these being due to primary graft non-function, and 9% underwent a second LTx during the first year post the first LTx.

Table 3.9 Pre- and post-LTx clinical outcomes in infants and children with ESLD

Variable	ESLD cohort
Pre-LTx clinical outcomes	
Ascites, n(%) - Yes - No	 33 (51) 32 (49)
Hepato-pulmonary syndrome, n(%) - Yes - No	 1 (2) 64 (98)
Hepato-renal syndrome, n(%) - Yes - No	 6 (9) 59 (91)
Encephalopathy, n(%) - Yes - No	 5 (8) 60 (92)
Varices, n(%) - Yes - No	 18 (28) 46 (72)
Total pre-LTx infections	1 (0 – 2)
Total pre-LTx bacterial infections	0 (0 – 1)
Total pre-LTx viral infections	0 (0 – 1)
Total pre-LTx fungal infections	0 (0 – 0)
Total pre-LTx undefined infections	0 (0 – 0)
Total pre-LTx inpatient hospital visits	1 (0 – 2)
Inpatient hospital visits length of stay, days	8 (0 – 32)
Total pre-LTx outpatient hospital visits	3 (0 – 7)
Total pre-LTx emergency hospital visits	0 (0 – 1)
Post-LTx clinical outcomes	
Intensive Care Unit length of stay, days	11 (6 – 26)
Total hospital length of stay, days	43 (28 – 74)
Ventilator dependency, days	5 (2 – 18)
Ascites at time of LTx, n(%) - Yes - No	 30 (57) 23 (43)
Ascites at Intensive Care Unit discharge, n(%) - Yes - No	 21 (38) 34 (62)
Ascites at hospital discharge, n(%) - Yes	 4 (8)

- No	47 (92)
Ascites at the 6-month follow-up, n(%) - Yes - No	 1 (2) 40 (98)
Ascites at the 12-month follow-up, n(%) - Yes - No	 0 (0) 34 (100)
Total post-LTx infections	5 (3 – 6)
Total post-LTx bacterial infections	2 (1 – 3)
Total post-LTx viral infections	2 (1 – 3)
Total post-LTx fungal infections	0 (0 – 1)
Total post-LTx undefined infections	0 (0 – 1)
Total post-LTx vascular complications	1 (0 – 2)
Total post-LTx biliary complications	0 (0 – 1)
Total post-LTx other complications	4 (2 – 8)
Total post-LTx complications	5.5 (3 – 11)
Graft rejection, n(%) - Yes - No	 21 (32) 45 (68)
Primary non-function, n(%) - Yes - No	 2 (3) 59 (97)
Re-LTx, n(%) - Yes - No	 6 (9) 59 (91)
Total post-LTx comorbidities	1 (0 – 1)
Total post-LTx inpatient hospital visits	2 (1 – 3)
Inpatient hospital visits length of stay, days	8 (2 – 24)
Total post-LTx outpatient hospital visits	21 (12 – 31)
Total post-LTx emergency hospital visits	1 (0 – 2)

Data presented as median(IQR) or percentage (%). Data availability: pre-LTx clinical data (n=65), except for varices (n=64); post-LTx clinical data: ascites at LTx (n=53), ascites at ICU DC (n=55), ascites at hospital DC (n=61), ascites at 6-month FU (n=41), ascites at 12-month FU (n=34), graft rejection (n=66), primary non-function (n=61), re-LTx (n=65). DC: Discharge, ESLD: End-Stage Liver Disease; FU: Follow-up; LTx: Liver Transplant

Patients aged ≤2 years had greater ICU LOS [median(IQR): 13 (7 – 29) vs 6 (3 – 11) days, p=0.03), total hospital LOS [median(IQR): 43 (32 – 75) vs 26 (22 – 28) days, p=0.008), and

ventilator dependency [median(IQR): 6 (3 – 20) vs 1 (0 – 2) days, p=0.002) than children aged >2 years. No other differences were seen between those aged ≤2 years and >2 years in pre- and post-LTx hospital visits (inpatient, outpatient, emergency), infection rates (bacterial, fungal, viral, undefined), presence of ascites, post-LTx complications and other relevant clinical outcomes (p<0.05). Females had greater rates of ascites at LTx assessment than males (63% vs 37%, p=0.03), but presence of ascites did not differ at the remaining timepoints. Females also had higher total viral infections pre-LTx [median(IQR): 1 (0 – 1) vs 0 (0 – 1), p=0.04], but no other pre-LTx disease complication differ between males and females. Although pre-LTx total inpatient, outpatient and emergency hospital visits did not differ between sexes, females had a greater total length of stay for all inpatient hospitalizations that occurred between LTx assessment and time of LTx than males [median(IQR): 14 (4 – 36.5) vs 3 (0 – 21) days, p=0.03]. A description of pre- and post-LTx reasons for admission can be seen in **Appendix 14**. Ventilator dependency, ICU and total hospital LOS, post-LTx hospital visits, post-LTx complications, and other relevant clinical outcomes did not differ between sexes (p>0.05).

3.3.5 Associations Between Neurodevelopment (Adaptive Level and Specific Domains), Laboratory, Growth, Nutritional Data, and Pre/Post-LTx Clinical Outcomes

3.3.5.1 Laboratory Data

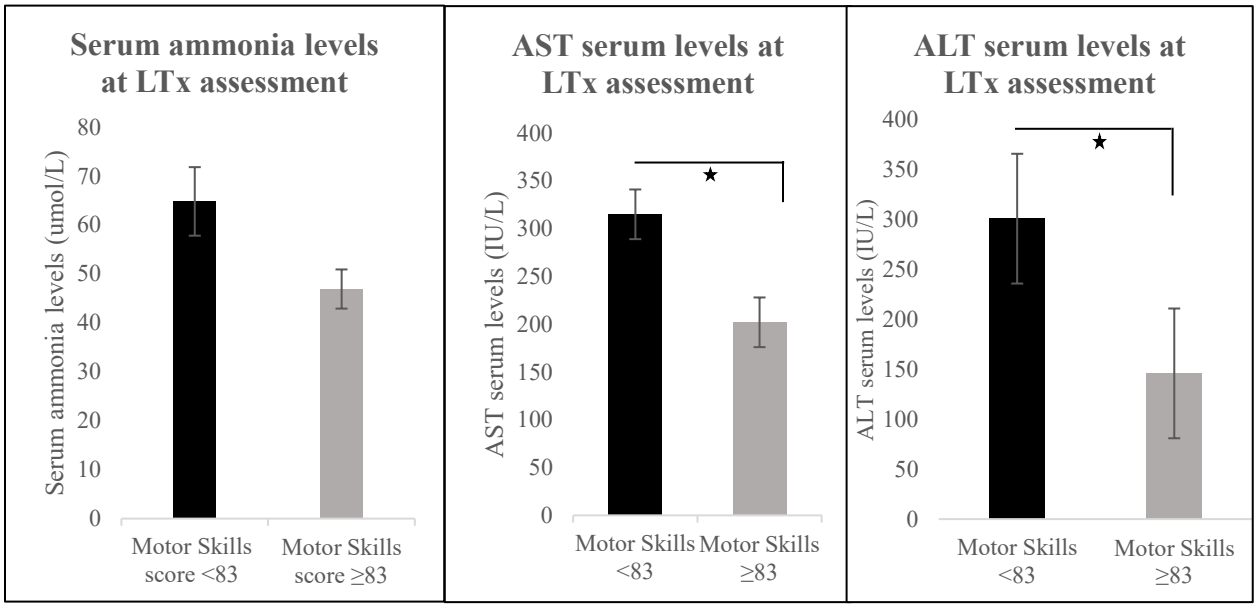
3.3.5.1.1 Associations with Vineland Composite Score Groups (Adaptive Level Groups)

No differences were seen between pre- and post-LTx bloodwork data between the adequate and inadequate adaptive level groups (p>0.05).

3.3.5.1.2 Associations with the Communication, Socialization, Daily Living Skills, and Motor Skills Individual Domains

The different associations observed between laboratory data and specific domains are shown in **Figure 3.3**. When focusing on motor skills score, those with a lower than median motor skill score (median[IQR]: 83 [74 – 95]) trended towards a higher ammonia level [median(IQR): 53 (43 – 89) vs 47 (36 – 55) μmol/L, p=0.06] at LTx assessment, than those with a higher score.

A) MOTOR SKILLS



B) SOCIALIZATION AND C) DAILY LIVING SKILLS

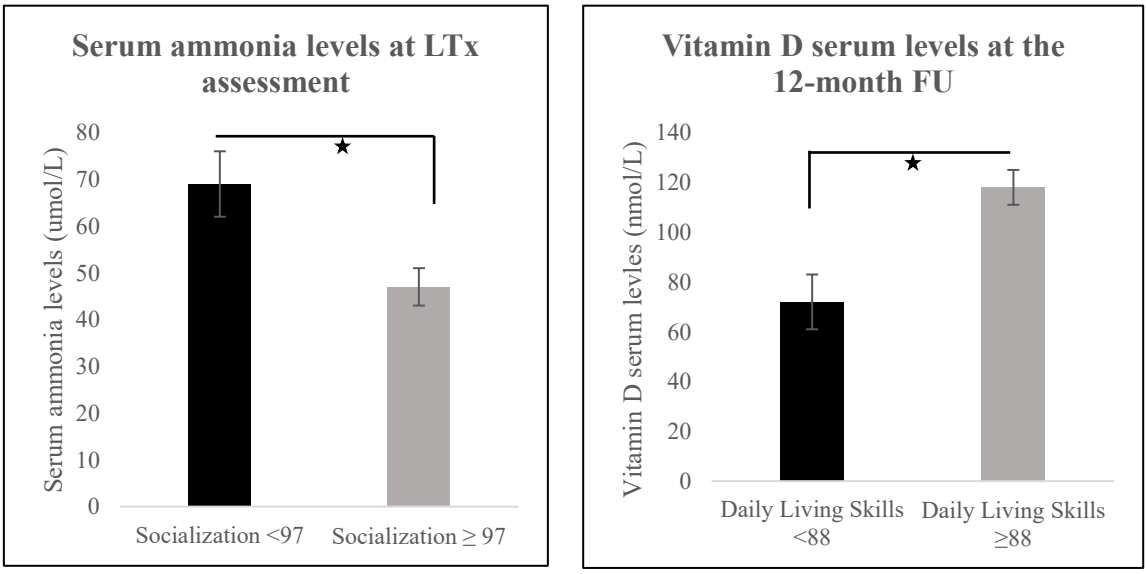


Figure 3.3 Laboratory outcomes associated with specific neurodevelopmental domains (above/below cohort median score. A) A lower than median motor skills score was associated with higher serum ammonia levels (trend) and AST/ALT serum levels. B) A lower than median socialization score was associated with higher serum ammonia levels. C) A lower than median daily living skills score was associated with higher serum vitamin D levels at the 12-month FU. Data is presented as mean±SE.. *Significant at $p \leq 0.05$. AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; FU: Follow-up; LTx: Liver Transplant, SE: Standard Error.

While these values are within the normal pediatric range, those with a lower motor skills score were closer to the upper range of normal.[195] This was also seen in those with a lower than median socialization score [median(IQR: 53 (46 – 95) vs 47 (37 – 57) µmol/L, p=0.01). Pre-LTx, a higher than median motor skills score was also associated with lower AST [median(IQR): 172 (81 – 289) vs 261 (149 – 418) IU/L, p=0.05] and ALT serum levels [median(IQR): 117 (42 – 212) vs 215 (78 – 291) IU/L, p=0.03] and trended towards lower total serum bilirubin [median(IQR): 154 (26 – 251) vs 231 (151 – 353) µmol/L]. No other differences were seen in pre-LTx bloodwork values between above/below than median score in the communication, daily living skills, socialization, and motor skills domains (p>0.05). Post-LTx, a higher daily living skills score was associated with higher vitamin D serum levels during the 12-month FU [median(IQR): 118 (103 – 129) vs 66 (58 – 87) µmol/L, p=0.02). No other differences were seen in post-LTx bloodwork values between above/below than median score in the communication, daily living skills, socialization, and motor skills domains (p>0.05).

3.3.5.2 Growth, and Nutrient Intake/Route of Delivery Data

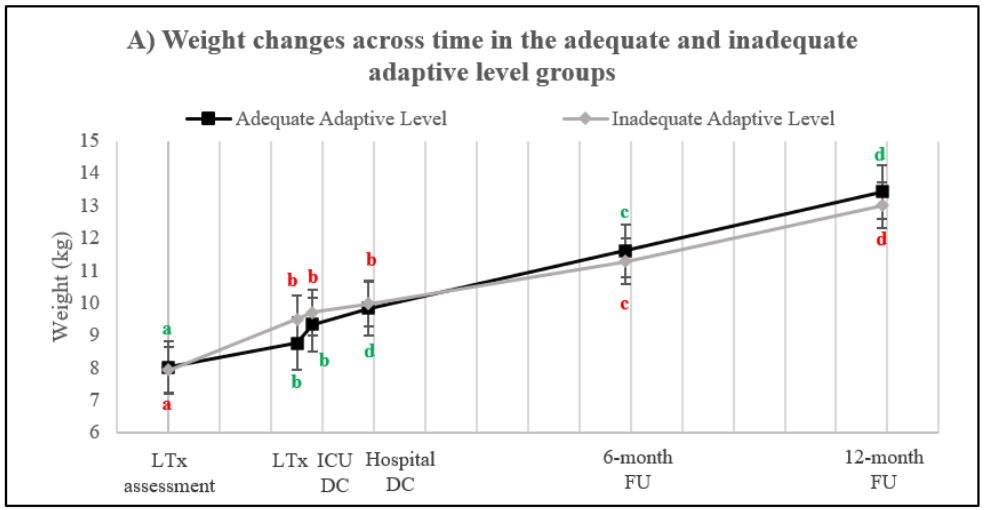
3.3.5.2.1 Growth

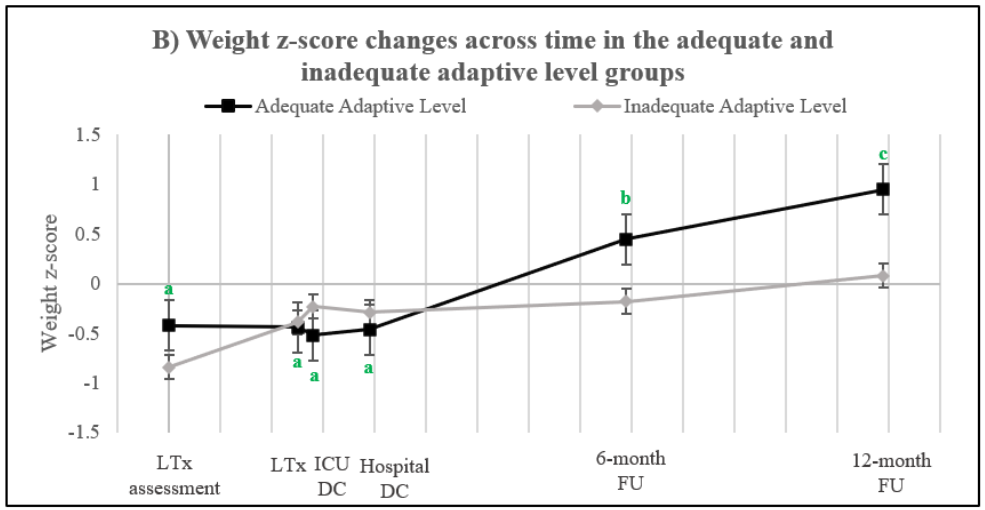
3.5.5.2.1.1 Associations with Vineland Composite Score Groups (Adaptive Level Groups)

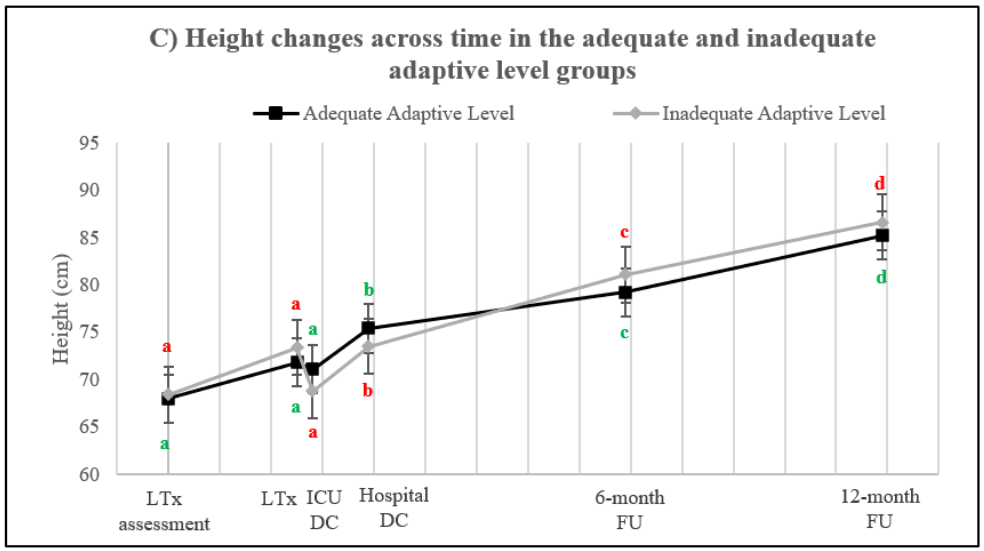
In patients with an adequate adaptive level (adequate Vineland composite scores), weight, weight z-score and height (**Figure 3.4.A**), weight and height growth (g/d and mm/d, respectively, **Figure 3.4.B**) significantly improved from the immediate post-LTx period when compared to the 6- and 12-month FU (p<0.05). Only height and weight, but not their respective z-scores

significantly changed from LTx assessment to the 6- and 12-month FU in those with an inadequate adaptive level.

Infants and children with an adequate adaptive level had greater rates of daily weight gain (g/d) during the 12-month FU than those with an inadequate adaptive level (mean±SD: 11.6±1.2 vs 8.3±1.1 g/d, p=0.03). The adequate adaptive level group had a higher percentage of patients achieving age-appropriate weight-gain during the 6- and 12-month follow-ups, compared to the inadequate adaptive level group (17% vs 6% and 12.5% vs 6%, p=0.0002, **Figure 3.5**). This weight gain was not influenced by ascites at the 6- and 12-month FUs, as few children had post-operative ascites. No differences were seen in head circumference, daily height gain (mm/d), and weight/height velocity SDS between the adequate and inadequate adaptive level groups (p>0.05).







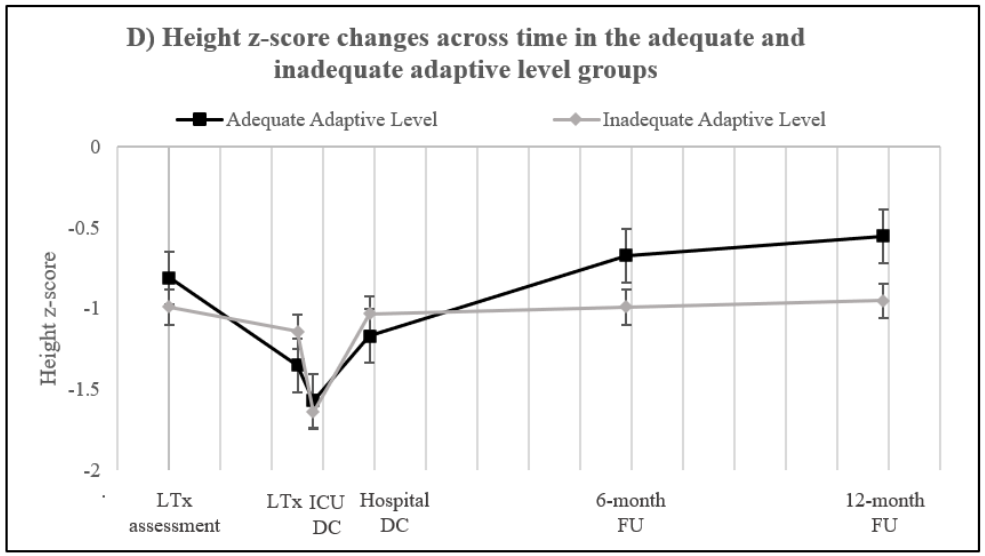
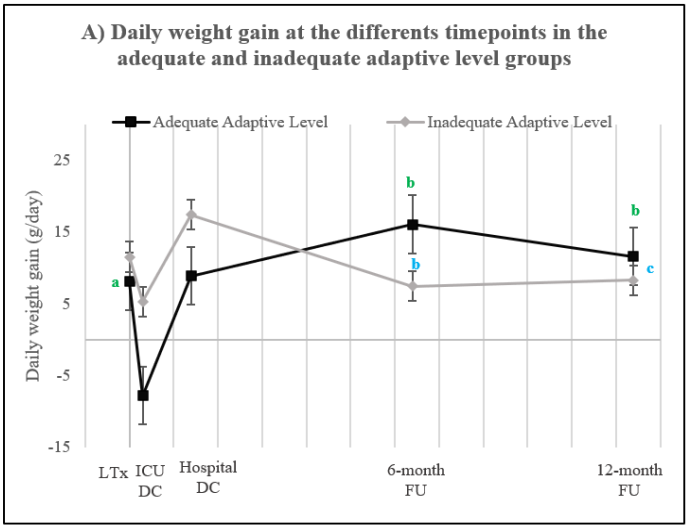
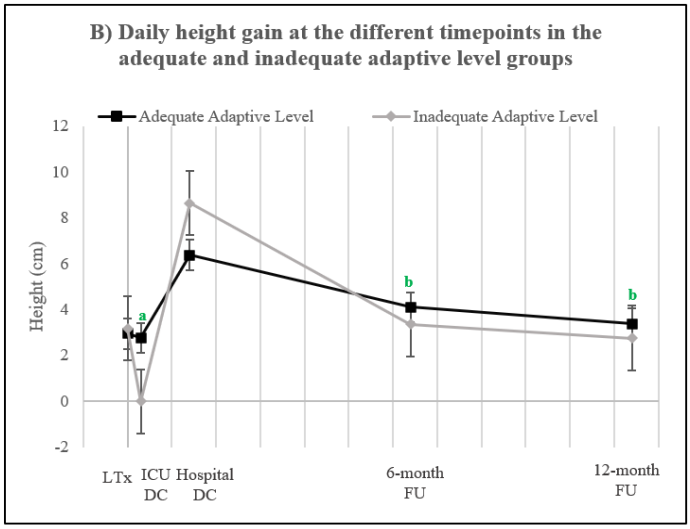
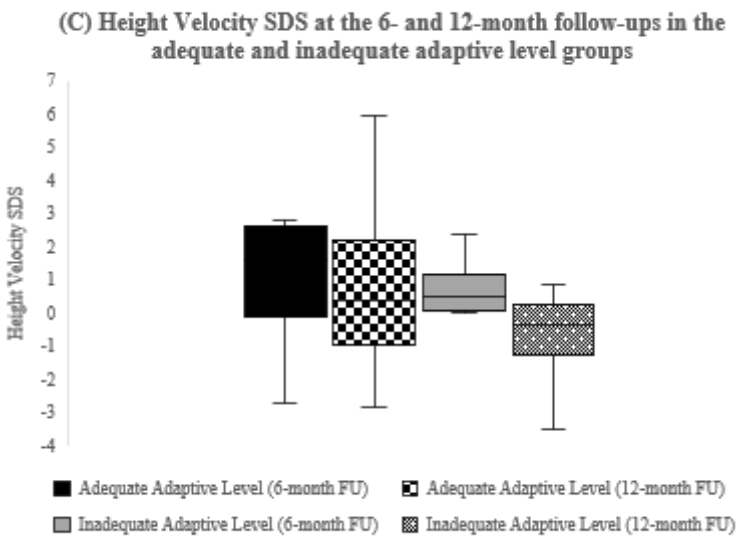


Figure 3.4-A. Anthropometric changes across time in the adequate and inadequate adaptive level groups: (A) weight, (B) weight z-score, (C) height, and (D) height z-score. Data presented as mean±SE. *p*-value≤0.05 is considered significant. Different letter superscripts indicate significant difference between timepoints between/within groups. Median time length between timepoints: LTx assessment and LTx: 90 (72 – 144) days, LTx and ICU DC: 11 (6 – 26) days, ICU DC and Hospital DC: 43 (28 – 74) days; 6-month and 12-month FU occurred 6 months and 12-months post-LTx, respectively. Green superscripts (a, b, c, d) reflect significant changes across time within the adequate adaptive level group. Red superscripts (a, b, c, d) reflect significant changes across time within the inadequate adaptive level group. Weight and weight z-score consistently improved across time in the adequate adaptive level group. In the inadequate adaptive level group there were no changes in weight z-score across time. In both groups, height increased across time, but height z-score did not change significantly. DC: Discharge; FU: Follow-up; ICU: Intensive Care Unit; LTx: Liver Transplantation.







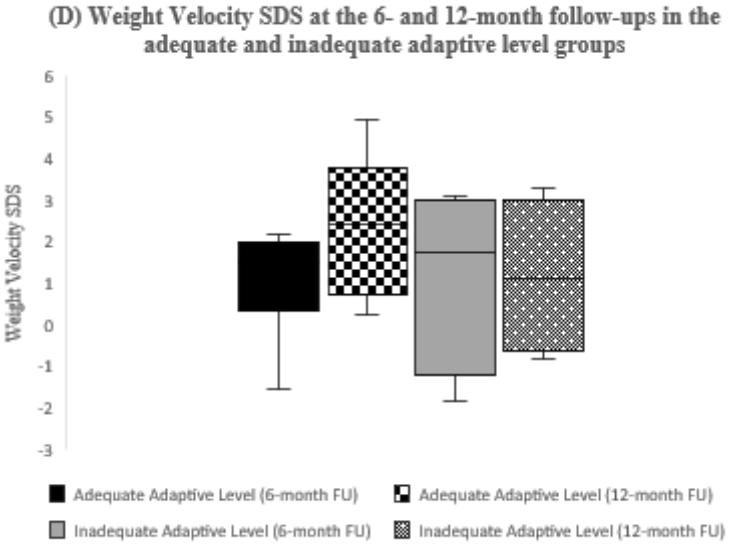


Figure 3.4-B. Anthropometric changes across time in the adequate and inadequate adaptive level groups: (A) daily weight gain, (B) daily height gain, (C) weight velocity SDS (6-month increments), and (C) height velocity SDS (6-month increments). Data presented as mean±SE or median (IQR). *p*-value≤0.05 is considered significant. Different letter superscripts indicate significant difference between timepoints between/within groups. Median time length between timepoints: LTx assessment and LTx: 90 (72 – 144) days, LTx and ICU DC: 11 (6 – 26) days, ICU DC and Hospital DC: 43 (28 – 74) days; 6-month and 12-month FU occurred 6 months and 12-months post-LTx, respectively. Green superscripts (a, b, c, d) reflect significant changes across time within the adequate adaptive level group. Red superscripts (a, b, c, d) reflect significant changes across time within the inadequate adaptive level group. Blue superscripts (b, c) reflect differences between groups. Daily weigh gain increased by the 6- and 12-month follow-ups when compared to that at ICU DC in the adequate adaptive level group only. Their daily weight gain at this timepoints was also greater than that of the inadequate adaptive level group (p=0.03). DC: Discharge; FU: Follow-up; ICU: Intensive Care Unit; LTx: Liver Transplantation.

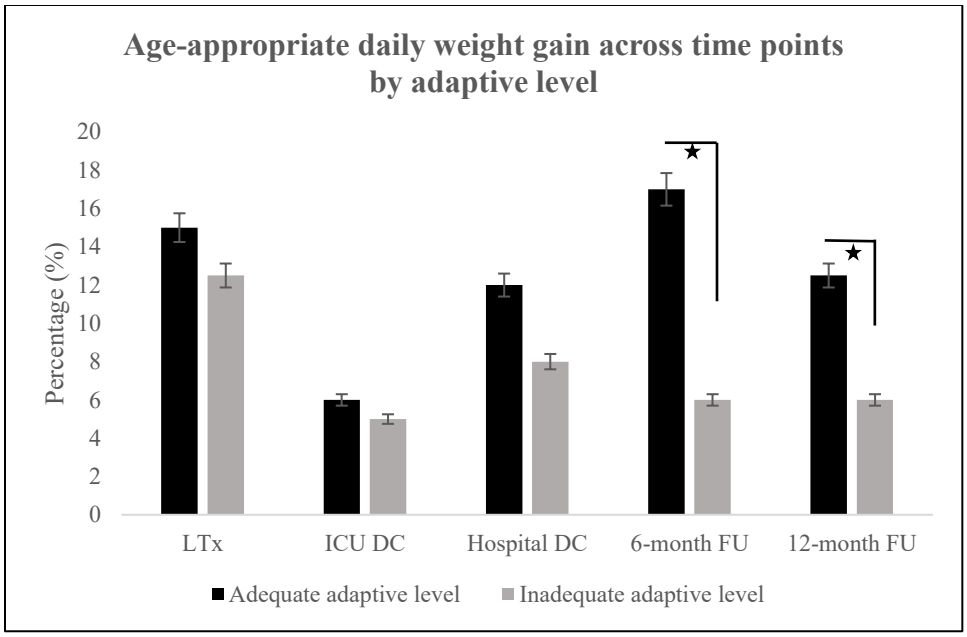


Figure 3.5. Percentage of adequate/inadequate adaptive level groups that achieved age-appropriate daily weight gain at the different timepoints. Data is presented as percentage bars. Data availability differed between timepoints: LTx (adequate, n=38; inadequate, n=21), ICU DC (adequate, n=28; inadequate, n=17), hospital DC (adequate, n=26; inadequate, n=19), 6-month FU (adequate, n=23; inadequate, n=16), 12-month FU (adequate, n=22; inadequate, n=14). Chi-square/Fisher's exact test was conducted to analyze categorical data. *p*-value≤0.05 is considered statistically significant. Median time length between timepoints: LTx and ICU DC: 11 (6 – 26) days, ICU DC and Hospital DC: 43 (28 – 74) days; 6-month and 12-month FU occurred 6 months and 12-months post-LTx, respectively. Age-appropriate daily weight gain was defined per Blenner *et al*.[196]: 26-31 g/day for 0-3 months of age, 17-18 g/day for 3-6 months of age, 12-13 g/day for 6-9 months of age, 9 g/day for 9-12 months of age, 7-9 g/day for 1-3 years of age, 6 g/day for 4-6 years of age. The adequate adaptive level group had a higher percentage of patients achieving age-appropriate weight-gain during the 6- and 12-month follow-ups, compared to the inadequate adaptive level group (17% vs 6% and 12.5% vs 6%, *p*=0.0002).

When the cohort was categorized by adaptive level ± malnutrition per SGNA, McLaren criteria, and WHO criteria (separately), the following was observed: the adequate adaptive level + malnourished group (per SGNA) had greater rates of daily weight gain during the 6-month FU, when compared to the inadequate adaptive level + malnourished (SGNA) counterparts [median(IQR): 16.3 (11.8 – 21.2) vs 5.7 (1.7 – 14.1) g/day, *p*=0.03). At this timepoint, 100% of the adequate adaptive level + malnourished achieved age-appropriate weight gain, compared to only 50% of the inadequate adaptive level + malnourished group (*p*=0.003). Age did not differ between groups at this timepoint. No differences were seen in daily height gain, and weight/height

velocity between these groups (p>0.05). A similar pattern was observed in the adaptive level groups ± malnutrition per McLaren criteria for wasting. The adequate adaptive level + well nourished group had greater rates of daily height gain at the 6-month FU when compared to the inadequate adaptive level + well nourished group (mean±SD: 16.5±7 vs 8.6±8, p=0.03). Despite presence of malnutrition, an adequate adaptive level was associated with achieving age-appropriate weight gain at the 6-month FU, compared to 62% of the inadequate adaptive level + well nourished and 0% of their malnourished counterparts (p=0.001). When combining neurodevelopmental status + malnutrition definition by WHO criteria, significant differences were seen in daily weight gain, daily height gain, and height velocity SD. Indeed, the adequate adaptive level ± malnutrition had greater g/day gained at the 6-month FU when compared to the inadequate adaptive level + well nourished [mean±SD: 14.8±6 (adequate + well nourished) vs 20.4±9 (adequate + malnourished) vs 7.6±8 (inadequate + malnourished), p=0.006). Ninety-four percent of the adequate adaptive level + well nourished and 100% of the malnourished counterpart achieved age-appropriate weight gain at this timepoint as well. In comparison, 53% of the inadequate adaptive level + well nourished and 0% of the malnourished counterpart achieved this. Finally, height growth at the 12-month FU was greater in the adequate adaptive level + malnourished group, compared to the well-nourished group. Specifically, daily height gain (mean±SD: 4.9±2 vs 2.9±1, p=0.02) and height velocity SDS (median±SD: 3.5±2 vs 0.21±2, p=0.03) were greater.

3.4.5.2.2.2 Associations with the Communication, Socialization, Daily Living Skills, and Motor Skills Individual Domains

When focusing on differences based on specific neurodevelopmental domains, those with a lower than median motor skill score (median[IQR]: 83 [74 – 95]) had a greater head

circumference (mean±SD: 42.9±1.6 vs 40.9±1.9 cm, *p*=0.005) at LTx assessment. A higher than median motor skill score was associated with greater daily weight (mean±SD: 11.7±5.7 vs 8.1±4.5 g/day, *p*=0.05) and height gain (mean±SD: 3.5±1.3 vs 2.6±1.1 mm/day, *p*=0.04), as well as height velocity SDS (median[IQR]: 0.70 [-0.3 – 2.63] vs -0.43 [-1.78 – 0.01], *p*=0.02) at the 12-month FU. Weight velocity SDS did not differ between motor skill score groups (6-month FU p=0.30, 12-month FU *p*=0.87). Infants and children with a higher than median socialization score had greater daily weight gain at the 6-month FU (mean±SD: 15.6±8 vs 9.1±7 g/day, *p*=0.02) (**Appendix 15**). During the 12-month FU, this group also had greater daily weight gain (median(IQR): 11.1 (8.9 – 15.1) vs 5.6 (4 – 10.1) g/day, *p*=0.01) , daily height gain (median±SD: 3.8±1.4 vs 2.44±0.7 mm/day, *p*=0.01), and height velocity SDS [median(IQR): 0.99 (-0.69 – 3.09) vs -0.4 (-1.29 to -0.03, *p*=0.01) than those with a lower socialization score. A higher than median daily living skills score was associated with greater weight velocity SDS at the 12-month FU (median±SD: 2.53± 1.6 vs 0.16±0.9, *p*=0.04) (**Appendix 17**). No other differences in anthropometric data (e.g., weight, height) or growth parameters (e.g., weight velocity SDS) were between specific neurodevelopmental domain groups (**Appendix 15, 16, 17**).

3.3.5.3 Nutritional Delivery and Intake

Results for this (associations with Vineland Composite score groups and specific domains) can be seen **in Appendix 9**.

3.3.5.4 Pre- and Post-LTx Clinical Outcomes

3.3.5.4.1 Associations with Vineland Composite Score Groups (Adaptive Level Groups)

The adequate adaptive level group had a lower incidence of post-LTx comorbidities (56% vs 85%, p=0.02). Interestingly, the prevalence of global NDD post-LTx did not differ between adequate vs inadequate adaptive level groups (56% vs 85%, p=0.19).

Thirty-three percent (33%) of the inadequate adaptive level + malnourished group (per WHO criteria for stunting) presented hepato-pulmonary syndrome at LTx assessment, compared to 0% of the rest of the subgroups (p=0.05). There was a trend for those with inadequate adaptive level ± malnutrition (per McLaren criteria for wasting) to present comorbidities during the first year post-LTx when compared to those with an adequate adaptive level ± malnutrition (p=0.11). No other associations were seen in the adequate/inadequate adaptive level ± malnutrition per SGNA, McLaren or WHO criteria when assessing other pre- and post-LTx clinical outcomes (e.g., complications, ICU/total hospital LOS, infections, survival rates, hospital visits).

3.4.4.4.2 Associations with the Communication, Socialization, Daily Living Skills, and Motor Skills Individual Domains

Significant between specific neurodevelopmental domains and pre/post-LTx clinical outcomes can be observed in **Figure 3.6**. There was a trend for those with a higher motor skill to have a lower incidence of pre-LTx encephalopathy (3% vs 15%, p=0.15). Ventilator dependency was longer in those with a lower than median motor skill score (mean±SE: 17.3±5 vs 9.3±2 days, p=0.05). There was also a trend towards longer ICU LOS (median[IQR]: 14 [8 – 32] vs 10 [5 – 26] days, p=0.06). A lower than median daily living skills score was associated with greater post-

LTx total infections (p=0.03) and greater post-LTx fungal infections (p=0.02) (**Appendix 17**). No other differences were between above/below median motor skills, communication, socialization, and daily living skill score groups (p>0.05).

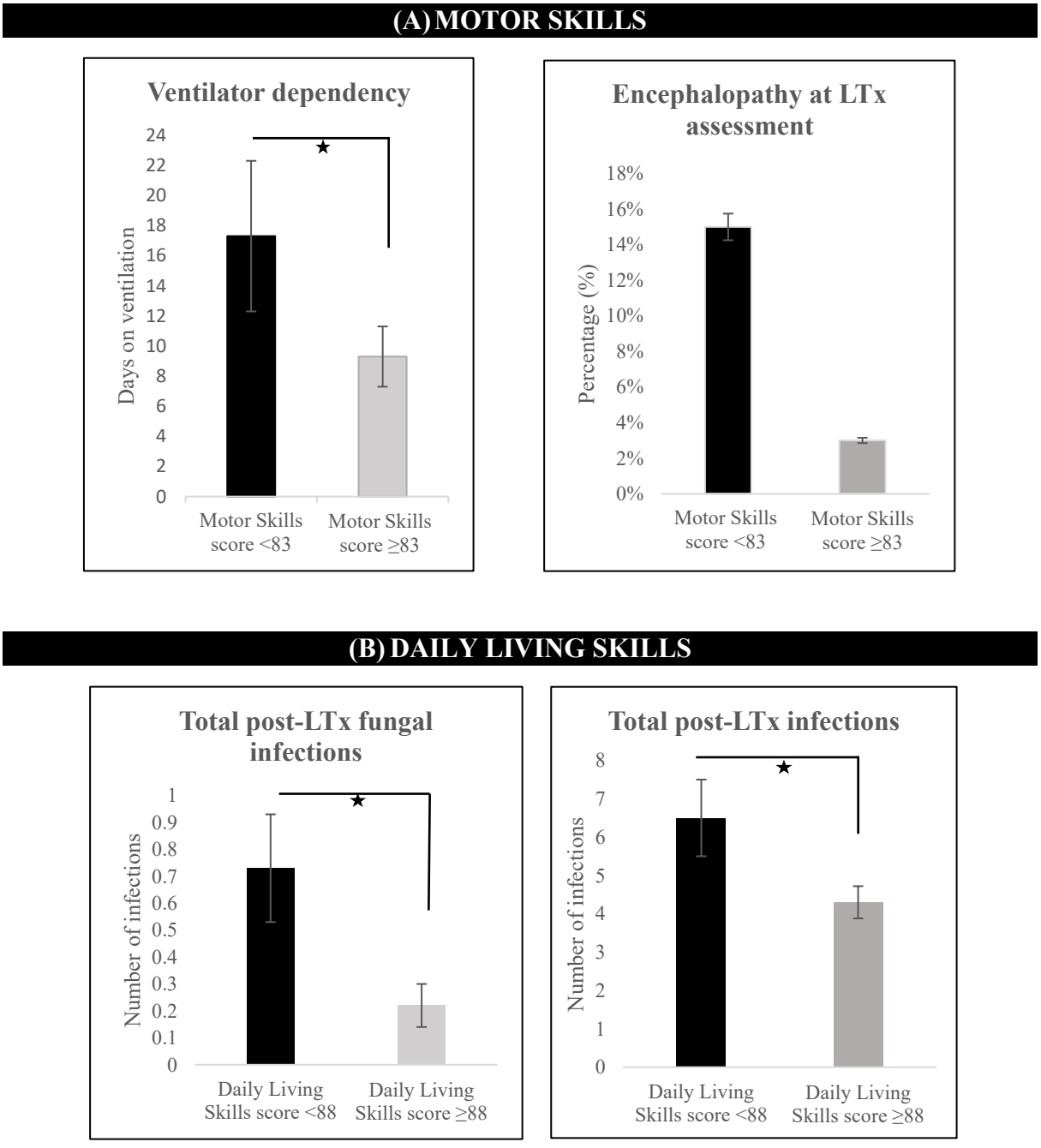


Figure 3.6 Clinical outcomes associated with specific neurodevelopmental domains (above/below cohort median score. A) A lower than median motor skills score was associated with longer ventilator dependency and trended towards significantly higher rate of pre-LTx encephalopathy. B) A lower than median daily living skills score was associated with increased total post-LTx infections and post-LTx fungal infections. Data is presented as mean±SE or percentage bars. Fisher's exact test was used to analyze categorical data. *Significant at $p \leq 0.05$. LTx: Liver Transplant, SE: Standard Error.

3.5 Discussion

To our knowledge, this is the first study to assess the associations between neurodevelopment (per the VABS), nutritional status (determined by SGNA, McLaren and WHO criteria), and clinical outcomes in infants and children with ESLD undergoing LTx. Our main finding is that motor skills was the most affected neurodevelopmental domain in the cohort and was associated with adverse clinical outcomes (prolonged post-LTx ICU LOS and ventilator dependency). A lower score in socialization and daily living skills scores was also associated with lower rates of post-LTx growth (daily weight gain, height weight gain, height velocity SDS) and higher rates of post-LTx infections. Malnutrition was prevalent in the cohort when determined by the SGNA, but not following the McLaren criteria for wasting (%IBW) or WHO criteria for stunting. Furthermore, four distinct neurodevelopmental/nutritional status phenotypes (per SGNA, McLaren criteria, and WHO criteria) were found in the study population, which also correlated with nutritional and clinical outcomes: adequate adaptive level + well nourished, adequate adaptive level + malnourished, inadequate adaptive level + well nourished, inadequate adaptive level + malnourished (**Table 3.10)**.

Table 3.10 Neurodevelopmental/nutritional status phenotypes found in the study population

Neurodevelopmental status*	**Nutritional status****
Adequate adaptive level	Well nourished
	Malnourished
Inadequate adaptive level	Well nourished
	Malnourished

*Defined per the VABS ABC score categorization. **Defined per SGNA, McLaren criteria for wasting and WHO criteria for stunting, separately. By SGNA definition: Adequate adaptive level + well-nourished: n=9, adequate adaptive level + malnourished: n=25, inadequate adaptive level + well nourished: n=0, inadequate + malnourished: n=15.

Those malnourished but with an adequate adaptive level had greater daily weight gain by the 6-month FU and greater height gain at the 12-month FU (when malnutrition was defined by WHO criteria). Regardless of the tool used to define malnutrition, an adequate adaptive level was associated with age-appropriate weight gain post-LTx. Interestingly, an adequate adaptive level did not protect against global NDD 1-year post-LTx. The strongest predictor for neurodevelopmental status was age, followed by SGNA score (driven by metabolic stress, linear growth, and functional impairment according to the dietitian's reports), PELD score, and vitamin E serum levels (LTx assessment). These findings highlight the importance of incorporating a comprehensive toolkit to determine nutritional status pre-LTx, such as the SGNA, along with neurodevelopmental assessment, with a special focus on motor skills. Incorporating knowledge of baseline nutritional and neurodevelopmental status and their pre-habilitation before LTx may be a helpful strategy to achieve an optimal short and long-term HRQoL.

One of our findings was that adaptive level and motor skills, particularly gross motor skills, were predominantly low-average and not age-appropriate, respectively, in the study population. This agrees with findings that, in children with biliary atresia, neurodevelopment before LTx was in the low-average range, and motor skills were below the norm.[3,131,135,136] High rates of motor delay have also been reported in pediatric patients that undergo multivisceral transplant, but LTx-only recipients reportedly have lower rates of motor impairment (96% vs 71%, respectively).[197,198] We also found that motor skills score was associated with adverse clinical outcomes. This relates to a study done in adults with cirrhotic liver disease awaiting LTx that proposed that impaired motor skills (e.g., gait, grip strength, chair stand) are a useful predictor of worse patient outcomes. Indeed, Lai *et al*. established a scoring system in which motor performance can be used to predict mortality and LTx wait list de-listing due to illness.[199]

Motor rehabilitation has been postulated as an appropriate intervention strategy to ameliorate or even prevent motor deterioration in this clinical population, consequentially improving outcomes. It has been argued that physiotherapy aimed to improve motor skills can simultaneously improve cognition, as functional ambulation, for example, requires coordination of the motor and cognitive domains (e.g., memory and visuo-spatial skills with gait).[200] Overall, motor impairment in adults is typically associated with sarcopenia and frailty, but this remains to be further explored in pediatric ESLD.

Liver disease-related malnutrition can also impose a significant insult to the developing brain by limiting availability of nutrients that are fundamental for proper neurodevelopment (e.g., essential amino acids and fatty acids, vitamin E, vitamin D).[11] It is for this known risk of deficiency that patients undergo mandatory vitamin E and D supplementation pre-LTx [recommended doses: 2000 IU/day (vitamin D as cholecalciferol) and 50-200 IU/kg/day (vitamin E as alpha-tocopherol)/25 IU/kg/day (vitamin E as alpha-tocopheryl)].[201-203], but prolonged supplementation of vitamin E post-LTx was not maintained in our study. Early life vitamin E deficiency is associated with long-lasting neurodevelopmental impairments, and serum levels of vitamin E may not necessarily reflect actual cellular uptake and concentration in brain and neural tissues.[204] Neurologic dysfunction manifested as muscle weakness, ataxia, and visual and cognitive impairments were identified in vitamin E-deficient children with cholestatic liver diseases.[205] A promising finding is that prolonged (>1 year) vitamin E supplementation after onset of neurodevelopmental delay can improve neurologic dysfunction and adaptive functioning in healthy children with global NDD and children with liver disease.[206,207] More research is warranted to determine an optimal vitamin E supplementation dose to provide neural protection pre-LTx and allow neural recovery after LTx-induced brain insults (e.g., surgery, anesthetics, inflammatory

state). Along with a higher consumption of vitamin E, there should be an increased intake of long chain polyunsaturated fatty acids (LC-PUFAS), which may not be the case in pediatric ESLD.[204,208] Implementation of a combined approach to nutrition support may help bridge this gap.[209]

Nutrition interventions in this clinical population are based on specialized formulas (e.g., casein hydrolysate formulas) that are consumed orally or enterally.[210] These formulas are rich in nutrients, but are particularly low in LC-PUFAS, as the fat source is medium-chained triglyceride-based oil (MCT oil) due to its ease of absorption.[210] LC-PUFAs have a prominent role in neural membrane maintenance and with prolonged intake they accumulate in the cerebellum, a major driver of motor skills maintenance and learning processes.[211,212] Breastmilk is naturally rich in LC-PUFAs but is not used as an exclusive source of nutrients for children with ESLD as it is not sufficient to meet their elevated nutritional requirements.[6,11,213] Providing breastmilk via enteral feeding has been trialed successfully in neonates in the ICU.[146-148] Unfortunately, fat losses due to adherence to the feeding tube may be a concern, as fat is a key component of breastmilk and has a prominent role in the overall development of a child.[146,147] Given the multiple benefits of breastmilk consumption, clinical trials that assess the efficacy and safety of mixed-feeding with specific ratios depending on the patient's specific needs (e.g., 80% specialized formula, 20% breastmilk) in infants with ESLD are warranted.[209] Alternatively, feedings with formulas enriched with LC-PUFAs (e.g., added fish oil or egg triglycerides) may also be a safe option.[214] Prolonged hospitalization may have also influenced overall study findings related to associations between NDD and the nutritional status of the children in this cohort.

Children who had composite scores indicative of adequate adaptive levels demonstrated significant growth gains post-LTx, regardless of nutritional status at LTx assessment. This was

evidenced by these groups presenting greater daily weight gain and height gain post-LTx. Despite this, weight and height z-scores showed that the groups had not yet reached "catch-up growth", with their weight and height not yet being age-expected. This is unsurprising considering the evidence that shows that malnutrition at an early age may not lead to age-expected recovery after the child's nutritional, health, and environmental conditions improve.[215] Changes in weight have are positively correlated with changes in height (and viceversa) in early childhood; this may explain why the adequate adaptive level + malnourished group (defined by WHO criteria for stunting) consistently had greater weight gain and height gain post-LTx.[215] Gaining weight may reflect recovery from acute malnutrition induced by disease and post-LTx nosocomial infections.[189] Nevertheless, the fact that most of the cohort was in their early childhood could have influenced the rapid growth rate observed. Indeed, accelerated weight and height growth is expected during early childhood; whereas in late childhood these slow down.[190,215] On the other hand, while they seemed to have greater catch-up growth secondary to improvement of their liver disease, evidence shows that it is uncommon for infants and children that suffered from stunting at an early age to fully recover from it.[189,216]

Post-LTx patients will remain immunocompromised indefinitely to avoid graft rejection, and with that acute infections/illness may lead to weight loss (e.g., due to gastrointestinal symptoms and loss of appetite secondary to infection/illness) and recategorization as malnourished. This further complicates the process of reaching a "normal" growth trajectory, as experiencing multiple insults with limited recovery time may cause persistent and exacerbated weight and height deficits.[169,215] This may be an indication that an adequate neurodevelopment may provided greater opportunities of growth recovery post-LTx, even when considering the previously mentioned health risks. Alternatively, an accelerated weight gain at an early age has

been associated with increased risk of cardiovascular disease later in life.[215] The adequate adaptive level + malnourished groups (per McLaren and WHO criteria) had greater weight gain at 6-month FU, which may be a result of a continued nutritional rehabilitation. Given that their height z-scores did not change significantly by this time point, their weight-for-length/height may not be age-appropriate. Most children in our cohort did not receive corticosteroids as immunosuppressive therapy (which coincides with the implementation of corticosteroid-free immunosuppressive therapy at the Stollery Children's Hospital in 2003)[193,194], and there were no neurodevelopmental and demographic (age, sex, liver disease diagnosis) differences between them and those who did receive these medications. However, we found that those who underwent corticosteroid therapy at any timepoint had low height, height z-score, and height velocity SDS post-LTx (hospital DC and 6-month FU). This could be a contributing factor for the lack height z-score changes post-LTx, as corticosteroids can inhibit growth hormone release, insulin-like factor-1 activities, and normal bone development, ultimately impacting growth.[217,218] This coincides with findings by Mager *et al*., where they reported that children on post-LTx corticosteroid therapy had lower weight z-score 1 year post-LTx and height z-score 1 and 4 years post-LTx.[193]

Another contributor for lack of age-appropriate height growth could be nutrient intake (energy and protein) pre-LTx and cessation of nutritional support in the immediate post-LTx period, and consequent worsening of nutritional status. In our cohort, we found that older children (>2 years of age) had lower energy and protein intake (per-kg basis) pre-LTx than those younger than 2 years. Furthermore, Ooi *et al*. reported that post-LTx caloric consumption was lower in patients with myopenia, who also happened to be older than those without myopenia (>2 years of age).[21] This may be due to older participants having a more developed oral function than those aged <2 years, making it "safer" to not start them on nutritional support (pre-LTx) or wean them

from enteral nutrition to an oral diet (post-LTx). Nevertheless, the risk for malnutrition remains, as early satiety and lack of appetite (pre-LTx) and prolonged ventilation and tube feedings (post-LTx), can cause feeding difficulties regardless of the patient's age and oral-motor skills pre-LTx.[219-221] In our cohort, 11% were older than 2 years, making it likely that they also had their nutrition support stopped earlier than their younger counterparts (as both study populations underwent similar clinical practices in the same pediatric hospital). This may increase the risk of suboptimal energy intake, consequentially worsening their nutritional status and further impacting their growth.

Lower scores in the socialization and daily living skills domains were associated with higher serum ammonia levels at LTx assessment and increased post-LTx total and fungal infections, respectively. High levels of serum ammonia, also known as hyperammonemia, can contribute to synaptic dysfunction and neurotransmitter imbalance that lead to memory and attention impairments, for example.[222] Although these are areas of the cognitive domain, they are necessary for age-appropriate development of socialization skills. Indeed, an individual requires memory and attention to retrieve information necessary to interact with another person at an age-appropriate level, form conversations and create connections. This may explain why those with a higher serum ammonia level at LTx assessment had poor performance in the socialization domain. Daily living skills comprise all those abilities required to manage one's needs, such as independent eating, dressing, and personal hygiene. At our study population's age, age-appropriate daily living skills include finger feeding themselves (for the younger participants) and helping in dressing and washing themselves (for the older participants). In reality, due to their disease, most are heavily reliant on enteral or parenteral nutrition, do not engage in playing with food, and may have their parents omitting independent activities. This can lead to malnutrition if, for example, the patient

is weaned from enteral/parenteral nutrition, but their feeding difficulties persist due to them not having engaged with oral stimulation of food.[219,220] This by itself may compromise health by affecting the immune system, which only worsens their already permanent immunocompromised state.

The only anthropometric predictor for neurodevelopmental outcomes pre-LTx was weight z-score, coinciding with findings by Wayman *et al.*[131], where low weight was associated with NDD in children with biliary atresia. A multicentre study found that patients with weight z-scores >2 standard deviations below the 50th percentile at LTx had increased risk of poor cognitive performance than those without growth failure.[43] This may also agree with our finding that SGNA score was also a strong predictor of neurodevelopmental outcomes. While it is now common clinical practice for pediatric ESLD patients to undergo aggressive nutritional rehabilitation, the timing of this and of the LTx may be crucial.[131] Low weight and height secondary to disease effects and malnutrition impair brain development in a critical time, as myelination and glial cell proliferation occur, for example.[3,124,134,136] In our study, head circumference did not differ within and between adaptive level groups and neurodevelopmental/nutritional status combos.

The high prevalence of malnutrition in infants and children with ESLD at LTx assessment indicates the importance of early identification. The SGNA may be one of the best tools to use, as it includes a more comprehensive evaluation of clinical and medical factors (e.g., metabolic stress), in addition to an evaluation of anthropometric data. Indeed, the SGNA includes a specific parameter that the two other tools do not: evaluation of functional impairment. Evaluation of functional impairment is a subjective determination of the extent to which the individual's nutritional status has affected muscle function.[26] While markers of wasting and stunting are certainly important and practical to the diagnosis of malnutrition in children, evaluation of other

factors that impact nutritional status (such as clinical and functional data) are important in the overall assessment of the child.

Age was consistently found to be the strongest predictor of neurodevelopmental outcomes. Younger age at LTx assessment was associated with a higher adaptive level score. This coincides with findings that younger age at LTx is associated lower rates of NDD and better neurodevelopmental prognosis and recovery post-LTx.[197,223-226] Proposed age cut-offs to predict positive neurodevelopmental outcomes include <6 months and <15 months.[131,225] As younger ESLD patients have likely had the disease for a shorter period, in comparison to their older counterparts, disease progression may be lower. Thus, disease effects on neurodevelopment may be less marked in the younger cohort. This is supported by Kaller *et al*.[225,227], Stewart *et al*.[7] and Santos *et al*.[42], as they also found that children with end-stage liver disease who had longer duration of illness presented a higher risk score for cognitive delay. Early childhood (younger age) is also associated with accelerated weight and height growth, which also may be a biological explanation for the improved growth seen in some groups. Younger patients may have greater chances of recovery or resilience due to neuroplasticity, but this is also limited and can be surpassed by multiple neurodevelopmental insults at a time.[42] Wayman *et al*[131]. reported that age of 6 months or younger predicted for lower neurodevelopmental scores, while Krull *et al*.[8] found that younger age was associated with increased language delay. It may be inferred that, unless neurodevelopment and nutrition alike are preserved/rehabilitated before LTx, younger patients with better neurodevelopmental performances will eventually have the same outcomes as their older counterparts. In our cohort, children aged ≤2 years had lower rates of abnormal pre-LTx brain magnetic resonance imaging (MRI) findings than those older than 2 years. This may be attributed to increased neuroplasticity and resilience to neurodevelopmental insult that comes with

younger age.[2,40,228] Nevertheless, the younger group also had a longer ventilator dependency, ICU, and total hospital LOS. With younger age, resilience to surgery, for example, may be lesser than in older and more developed children.[229]

Family SES was a predictor for communication and daily living skills score. This can be attributed to architectural changes on the brain. Brito *et al*. reported that SES has been linked to changes in brain structure, particularly in areas related to memory, executive function, and emotion.[230] Daily living skills, as previously discussed, are skills needed to manage basic needs that eventually lead to independent living, such as eating, ambulating, self-care, and heavily rely on other domains.[83] The same applies to the communication domain, especially memory and executive function.[83] It has been observed that children from low SES households may experience less linguistic, social, and cognitive stimulation from their caregivers and home environment than children from higher SES homes.[230] This has been attributed to the associations observed between SES, time spent with the infant/child, and parental vocabulary, sentence formation, active listening, style of correction and prompting.[231-233] In the context of illness and/or disability, regardless of SES, parents may be less willing to allow the child to engage in activities that stimulate their independency.[234]

This study has several strengths and weaknesses. Neurodevelopmental assessment was conducted with the VABS, a norm-referenced and standardized tool that is commonly used in pediatric clinical populations, particularly in ESLD. However, as the VABS relies on parental reports for data collection and adaptive level category allocation, the results may be heavily influenced by parental education levels and beliefs. Furthermore, children of internationally-born parents may be at added risk of unrepresentative scores, as language may also be a barrier for understanding questions and delivering answers.[100,235-238] Family SES and parental education

levels, consanguinity, and presence of social concerns were included, as all of these factors have been linked to the offspring's neurodevelopment.[230,239-242] Another weakness is that the neurodevelopmental assessment occurred in a single timepoint.

The SGNA serves both as a nutritional screening and assessment tool and is the gold standard to determine nutritional status[26], having been validated in the following pediatric clinical populations: children who underwent major thoracic/abdominal surgery[243], non-surgical hospitalized patients[244,245], children with cancer[246-248], children with cholestatic liver disease[249], and children with developmental disabilities.[250-252] Due to the extensive parameters included in the assessment, these validation studies also reported associations between SGNA scores with clinical outcomes. Indeed, those moderately and severely malnourished (regardless of the clinical population) presented higher rates of infections, increased mortality, hospital LOS, and functional impairment. This allowed increased certainty in the accuracy of the detected associations between nutritional status with neurodevelopmental and clinical outcomes in our study cohort, especially associations with motor skills (due to the functional impairment component of the SGNA). The McLaren criteria for wasting and WHO criteria for stunting are tools that have been used historically in nutritional status assessments. While they are based on anthropometrics only, they have been extensively used to determine associations between wasting and stunting with neurodevelopmental and clinical outcomes in pediatric and adult clinical populations.[111,129,189] Regarding anthropometric changes, the potential collinearity of age, weight, height, and their respective z-scores may have led to less accurate predictive coefficients, as they are highly correlated among themselves (and may have caused redundancy in the model).

The study included a small number of children with a heterogeneous group of liver diseases, with metabolic liver diseases (n=11) and hepatoblastoma (n=4) patients undergoing

chemotherapy (known neurotoxic agents) having a higher risk for NDD, making this a potential confounding variable. Additionally, the clinical course of a metabolic and cancerous liver disease can differ from biliary atresia. A priori power analysis was not conducted before data collection and post-hoc analysis was not done for any of the primary variables, which may have impacted the ability to determine significant differences. There is also a potential of selection bias with perhaps sicker or more neurodevelopmentally delayed children being excluded due to them not having VABS scores available (exclusion criteria).

3.5 Conclusions

Survival of pediatric patients with ESLD that undergo LTx has greatly improved, and the focus must be expanded beyond survival and graft function. Indeed, the long-term impacts of the disease and its effects on several determinants of HQROL, such as cognitive, motor, and language development, daily living skills, mental health, and physical function are now of great interest. Our main findings support this, as infants and children with ESLD presented high prevalence of malnutrition and neurodevelopmental delay, particularly in motor skills. Low motor skills scores were also associated with adverse clinical outcomes such as prolonged ICU LOS and ventilator dependency. NDD with and without malnutrition seem to be associated with worse post-LTx outcomes. Considering that these are modifiable factors, pre-habilitation of nutritional status and neurodevelopmental milestones before LTx may be beneficial to secure optimal clinical outcomes and HRQoL in this population.

Chapter 4: Conclusions and General Discussion

4.1 Introduction

Neurodevelopmental delay (NDD) is defined as performance in one or more neurodevelopmental domains that is lower than expected based on normative data or the patient's chronological age.[33] The neurodevelopmental domains are cognition, language, motor skills, and socio-emotional skills.[1,30-32] Pediatric end-stage liver disease (ESLD) is a major source of insults to the developing brain, such as hyperammonemia, malnutrition, and environmental deprivation secondary to hospitalization.[3,7,8,125] NDD in the context of ESLD is typically studied focusing on one risk factor at a time: Disease-related risk factors (metabolic derangements and hospitalizations) or malnutrition. Nevertheless, NDD is a result of an orchestration of all elements, as well as potential contributor to malnutrition itself. [67] Malnutrition can further worsen disease-related effects, and vice versa.[11,145] It is imperative to consider this when aiming to comprehend NDD in ESLD patients, in order to create the best possible therapeutic interventions. The present thesis examined NDD in a pediatric ESLD cohort to understand its prevalence and its associations with pre-LTx malnutrition, and clinical outcomes before and after LTx. Our main objective (**Objective 1**) was to determine prevalence of NDD, and our hypothesis (**Hypothesis 1**) was that NDD would be present in this cohort, particularly in motor skills. We also aimed to assess associations between NDD and pre/post-LTx clinical outcomes (**Objective 2**). We hypothesized that those with NDD would have a prolonged ventilator dependency and ICU/hospital LOS, more complications, and increased mortality (**Hypothesis 2**). Finally, our goal was to evaluate relationships between NDD and nutritional status (**Objective 3**). We hypothesized that NDD in infants and children with ESLD would be associated with higher rates of malnutrition (**Hypothesis**

3-A) and poor growth post-LTx (**Hypothesis 3-B**). This research plan allowed us to have a broader look at the complex context in which infants and children with ESLD are in before and after LTx.

4.2 Overall Research Findings

In this study, it was demonstrated that infants and children with ESLD awaiting LTx have a high prevalence of pre-LTx NDD (**Hypothesis 1**), with a third of the cohort having a moderately low Adaptive Behaviour Composite (ABC) score (considered an inadequate adaptive level). The most affected neurodevelopmental domain (in which patients scored ≥1 SD below normative data) was motor skills (**Hypothesis 1**). Even if patients had an adequate ABC score, more than half of our population did not have age-appropriate motor skills, particularly gross motor (**Figure 3.9**). Those with a lower than median motor skill score had higher rates of pre-LTx encephalopathy, post-LTx intensive care unit (ICU) length of stay (LOS), and increased ventilator dependency (**Hypothesis 2**). A lower than median daily living skills score was associated with higher infection rates (total and fungal) post-LTx. Furthermore, malnutrition was prevalent in the overall cohort but only when assessing nutritional status with the Subjective Global Nutritional Assessment (SGNA) tool (**Hypothesis 3-A**). Regardless of nutritional status (± malnutrition), those with an inadequate adaptive level had lower rates of weight and height gain 6-months and 12-months post-LTx. This indicates that in our cohort malnutrition was not the defining feature between the associations between NDD and clinical outcomes. (**Hypothesis 3-B**).

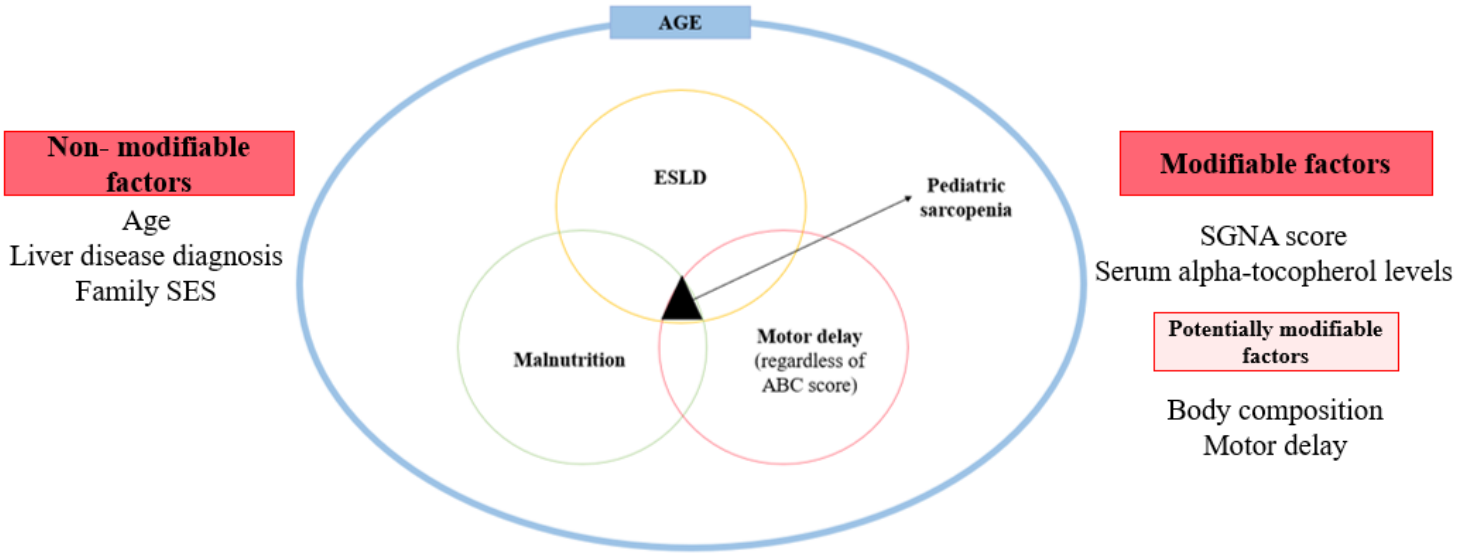


Figure 3.7 The center of the complex enigma that involves pediatric patients with ESLD is pediatric sarcopenia, surrounded by modifiable and non-modifiable factors. Regardless of the individual's overall development (ABC score), motor delay is a predictor of poor clinical outcomes in our study. More research is warranted to determine the modifiability of body composition and motor skills in infants and children with ESLD pre- and post-LTx. ABC: Adaptive Behaviour Composite; ESLD: End-stage Liver Disease.

Previous findings of our group determined that pediatric ESLD patients awaiting LTx have an altered body composition, as they present different phenotypes defined by their muscle mass and/or adipose tissue (myopenia ± low subcutaneous adipose tissue).[21] These may explain the high prevalence of low motor skill scores seen in the study cohort, which in turn may influence the development (or lack thereof) of other neurodevelopmental domains.[13,32] The interaction of factors like ascites, enlarged liver, and invasive LTx surgery can result in a reduction of muscle strength and mobility, consequently affecting proper achievement of gross motor milestones.[6] Myopenia, muscle weakness and low physical performance brought upon by malnutrition and hypermetabolic states may further contribute to motor delay.[5,21] Each individual factor mentioned can potentially explain the association found between a low motor skill score and adverse pre- and post-LTx clinical outcomes, such as increased ICU LOS. Evidence shows that pre-operative malnutrition is associated with post-surgery adverse outcomes, such as increased infection rates, prolonged mechanical ventilation and ICU/total hospital LOS.[253] Malnutrition and its associated effects (e.g.

myopenia) weaken the immune system and resilience to stress.[253] Nonetheless, a rehabilitation intervention is warranted to ameliorate pediatric myopenia and motor skill delay in pediatric ESLD patients, as well as underlying malnutrition.

More than half the cohort was well within the critical and sensitive periods of neurodevelopment (first 1000 days and first 5 years of life, respectively).[1,2] This may also explain the high prevalence of NDD, as their immature brains were subjected to multiple insults early in their lives, many as soon as they were born (e.g., neonatal hyperammonemia-induced seizures in patients with urea cycle disorders). Age was also the strongest predictor for neurodevelopmental status in our study. It has yet to be determined whether age at LTx and at disease/malnutrition onset determines or is protective of NDD. While younger patients may be more vulnerable to insults to their developing brain, older individuals may have had longer periods of suboptimal intake and disease duration, thus more opportunities to affect their neurodevelopment. Those who underwent LTx assessment at a younger age (e.g., soon after birth) may have initiated treatment earlier, controlling metabolic derangements that are known NDD risk factors, such as hyperammonemia. This coincides with previous findings from our research group, where younger children had higher intake of protein and energy and lower rates of myopenia.[21] Alternatively, older patients who likely were malnourished for a longer period and who also had lower protein and energy intake, had higher rates of an altered body composition (myopenia ± low subcutaneous adipose tissue). These findings denote the effects of a prolonged disease duration with older age. More research is warranted to elucidate protective and risk factors of timing of interventions and insults to the developing brain.

A higher percentage of patients with higher than median motor skills score had overall better clinical outcomes pre- and post-LTx. This was also observed when assessing groups by

inadequate/adequate adaptive level. The main takeaway from the present study is that neurodevelopment, rather than malnutrition, was the major driver for associations with pre- and post-LTx clinical outcomes. This may be related more to all the implications that an adequate neurodevelopment can have in the determinants of health in pediatric ESLD. Younger age was a predictor of higher neurodevelopmental scores in our cohort. Thus, those who were younger, who likely had an adequate neurodevelopmental status, did not suffer from a prolonged disease duration (and related effects) like their older counterparts with worse neurodevelopmental performance. Undergoing LTx assessment at a younger age allows for early initiation of nutritional and medical therapies to improve health status in order to tolerate LTx surgery.[11] As nutritional status and ESLD-related metabolic derangements (e.g., hepatic encephalopathy) are known determinants of neurodevelopment, intervention before these worsen can provide greater opportunities to achieve adequate neurodevelopment and optimize post-LTx clinical outcomes.[3,43,49]

These are also determinants of body composition, for example, as malnutrition and ESLD can impact muscle mass and fat mass.[21,152,153,254] Previous findings in our group showcased that older children with ESLD have higher rates of an altered body composition (myopenia ± low subcutaneous fat).[21] Additionally, pre-LTx myopenia has been associated with adverse clinical outcomes post-LTx (e.g., increased infection rates, prolonged hospital stay).[5,21,150,152,153,155] This, paired with the fact that in our cohort older age was a predictor of worse motor performance, may also explain why an adequate neurodevelopment was associated with better outcomes. Nevertheless, our findings that younger age was associated with prolonged ICU, hospital, and ventilator dependency should also be considered, as these can be great sources of NDD secondary to environmental deprivation. These may explain why global NDD at the 12-month FU did not

differ between younger and older patients and between those with an age-appropriate neurodevelopment pre-LTx.

The individual effects malnutrition, NDD, and liver disease on clinical course are complex to determine. Nevertheless, our findings showcase the importance of proper neurodevelopment pre-LTx to ensure optimal clinical outcomes, as well as in-hospital stimulation, especially in those within the critical period of neurodevelopment (first 1000 days of life = ≤2 years of age).[177,180,255] More research is warranted on the efficacy of neurodevelopmental rehabilitation interventions pre-LTx, and their impact on post-LTx clinical outcomes. Additionally, a continued intervention post-LTx should be trialed, regardless of neurodevelopmental status pre-LTx, to ensure achievement of milestones that were missed during the LTx hospitalization period or recovery in the event of regression. The previously mentioned findings do not negate the fact that nutritional interventions pre- and post-LTx in this clinical population can be improved in order to support optimal growth and development.

4.3 Clinical Implications

Nutritional support in infants and children with ESLD pre- and post-LTx can greatly improve to support optimal growth and development. For infants with ESLD within their first 1000 days of life, breastmilk is important in terms of immune protection and neurodevelopment.[2,180,212] However, this should be evaluated in the context of ESLD, as it is imperative that nutrition support allows infants and toddlers to meet their high calorie-protein and micronutrient needs. Breastmilk by itself may fall short in that regard.[10] Research is warranted in exploring the efficacy of a nutritional support intervention based on inclusion of casein hydrolysate formulas along with breastmilk in personalized ratios to ensure tolerance and fulfilment of their unique nutritional requirements.

For older children with ESLD (>2 years), prolongation of nutritional support may be needed. Regardless of their oral-motor function pre-LTx, (which is likely more developed than in younger patients) a more detailed and gradual weaning process is necessary if they were on enteral nutrition before initiating oral feeds.[219,220] This is to ensure that feeding difficulties will not curtail nutritional intake, leading to malnutrition and potentially affecting future growth.[219] Neurodevelopment goes beyond 2 years of life, and it should not be assumed that older children with an adequate oral-motor function pre-LTx, for example, do not suffer from neurodevelopmental delay/regression post-LTx.[173]

During the immediate post-LTx period, the number of days spent in the ICU/hospital, as well as mechanically ventilated, are crucial in regard to neurodevelopment. Environmental deprivation may occur.[124] This can also be disruptive for language and social development, as the activities and noises may impede a child to interact with another patient if they are in a multi-bed hospital room.[255] A prolonged hospital stay poses a higher risk for the development of infections and sepsis, which is particularly worrisome in a transplanted child, as they are permanently immunosuppressed.[2,256]

Current evidence shows that pediatric ESLD patients post-LTx have increased use of special education services, imposing further unique challenges.[137] It is imperative to not only to improve existing intervention programs post-LTx (e.g., by increasing frequency, duration and intensity of sessions), but more so to enhance pre-habilitation during the pre-LTx period, as this may be the most critical for neurodevelopmental preservation.[257,258] This is because any insults to the developing brain at this time may persist and worsen during the post-LTx period.

Neurodevelopmental interventions pre-LTx should focus on milestone achievement to rehabilitate NDD, highlighting all domains, but particularly motor skills given the potential

association between motor delay with myopenia (and related adverse clinical outcomes). Intensive interventions (inpatient, outpatient or home-based 60-minute sessions, 3-5 times a week) are associated with increased rates of neurodevelopmental recovery in children with developmental impairments.[258] These interventions typically target abnormal responses (or lack thereof) in specific domains and focus on leading the child towards an age-appropriate response, until it becomes an automatic reaction.[258]

Post-LTx interventions may be preceded by a more detailed neurodevelopmental assessment, as the child will likely be able to perform tasks in order to determine neurodevelopmental status (regression, worsening of delay, or even improvement). In healthy children, neurodevelopment is known to wax and wane as they grow.[32] Additionally, some true delays only become evident as children face increasing developmental demands (e.g., initiating oral feedings, starting pre-school, etc.).[32] For these reasons, serial evaluations are necessary during the post-LTx period to determine changes in domain-specific or overall delays. The ideal assessment used during the post-LTx period would encompass all domains, is standardized, has normative data available, is valid and sensitive.[32] Use of performance-based neurodevelopmental tools may be necessary to reduce sources of bias and ensure representative scores.

Considering the evidence for motor impairment in this clinical population, motor assessment tools such as the Peabody Developmental Motor Scales (PDMS) and the Bruininks-Oserestky Test of Motor Proficiency (BOT) may be of use. Indeed, both tools include assessments of strength, a key component of sarcopenia. The PDMS has been used to determine motor impairments in children with ESLD, autism spectrum disorder, and those born premature.[44,109,259] The BOT has been used to determine prevalence of motor impairment in pediatric clinical populations, such as children with intestinal failure and alpha-mannosidosis.[69,256,260] It has also

been shown to successfully detect stability or improvements in scores, indicating progressive motor skill acquisition.[260] Evidenced determinants of motor performance in the BOT are weight (healthy children with and without obesity) and lean body mass (children with intestinal failure).[69,261]

4.4 Strengths and Limitations

The strengths of this study include the use of a standardized neurodevelopmental tool, commonly used in ESLD in hospital settings in Canada. The SGNA was used to determine nutritional status, and it is the gold standard in pediatrics. Caregiver factors that are determinant for a child's neurodevelopmental status were included, such as family SES, parental education levels, consanguinity, and presence of social concerns.[114,230,240,242] The statistical limitations of categorical variables must be considered, as a larger sample size is warranted to increase power and explore significance of associations (or lack thereof). Additionally, the reported associations (or lack thereof) seen in the primary and secondary outcomes between groups may be related to insufficient power in our sample size.

Neurodevelopment was assessed at a single time point (LTx assessment), hence the progression, maintenance or improvement of scores were left unexplored. Many infants are very ill during LTx assessment, and thus, neurodevelopmental scores may not be entirely representative. The VABS relies on parental reports, which may be a biased source of information. There were no differences in age, sex, neurodevelopmental outcomes, and SGNA scores seen in our cohort between patients with biliary atresia and those with other liver diseases. Nevertheless, a small portion of our cohort included heterogeneous liver disease diagnoses. This may be a potential confounding variable, as metabolic liver disease and hepatoblastoma patients undergoing chemotherapy are at higher risk for NDD (due to metabolic crisis-induced seizures and neurotoxic

chemotherapy agents, respectively). Additionally, their clinical course pre-LTx differs from that of a patient with biliary atresia, for example. These patients follow a "metabolic diet", which is poor in the specific nutrient that they are unable to metabolize (e.g., branched chain amino acids), and rich in others (e.g., fat and carbohydrates). With that, they may be more likely to develop obesity or high fat mass pre-LTx, whereas this is not typically seen in a patient with biliary atresia. Body composition data was not included, which may have provided a better understanding of the cohort's nutritional status, as well as determining associations between NDD and an altered body composition.

Limited evidence shows that a physiotherapy intervention on neurologically normal children that are at risk of motor delay helped them achieve age-appropriate motor skills.[104] For this reason, future directions include exploring the utility of neurodevelopmental pre-habilitation pre-LTx, particularly gross motor rehabilitation (although rehabilitation of all other domains should also be explored). Pairing the latter with the aggressive nutritional rehabilitation that these patients typically receive before LTx, may allow overall improvement of their overall pre-LTx status, and potentially their post-LTx outcomes. Another opportunity is to study neurodevelopment post-LTx via serial evaluations to understand whether it waxes and wanes and to establish the ideal timing of intervention to reach the highest benefits for the population.

4.5 Conclusions

Early detection of NDD is urgently needed to optimize outcomes, hence the importance of studying neurodevelopment in pediatric liver disease. Pre-habilitation of neurodevelopmental milestones, along with an aggressive nutritional intervention that focuses on increasing SMM before LTx may be beneficial to secure optimal clinical outcomes and HRQoL in this population. Several areas of opportunity were found to further improve the likelihood of optimal outcomes (all

of which can impact long-term HRQoL), such as increasing breastfeeding rates, securing mandatory vitamin E supplementation, and advocating for policy changes and improved models of care for the chronically ill child.

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Appendices

Appendix 1. Body Composition Methods

Body composition methodology	Measured body compartments	Advantages	Limitations
Field methods			
Anthropometry[17,262-265] Skinfolds (SF) - Triceps, biceps, subscapular, suprailiac, abdominal, thigh (quadriceps femoris) Circumferences - Mid-upper arm circumference (MUAC) - Calf circumference (CC) - Waist circumference (WC) Extra: - Mid- arm muscle area (MAMA, calculated using both MUAC and triceps skinfold)	Skinfolds: Total body fat (subcutaneous fat) Circumferences: Muscle mass (CC, MUAC, MAMA), intra-abdominal fat (WC)	- Minimal training required - Simple to perform - Non-invasive - Inexpensive equipment - Practical/accessible in clinical settings (can be performed at patient's bedside).	- Circumferences: May not detect small changes in muscle mass. Peripheral measurements should be included to account for whole body fat distribution. - MUAC and MAMC: Individual variations in humerus diameter are not considered. - Intra- and inter-observer variability. - Considerable expertise and training necessary. - Predictive equations can be a source of error, depending on the study population. - Not sensitive to presence of intramuscular adipose tissue or edema.

Bioelectrical Impedance Analysis (BIA)[5,17,20,157,262-264,266]	Fat mass, fat free mass	- Fast - Minimal training required, observer-independent - Easy to perform - Relatively low-cost - Portable - Minimal participant burden - Can provide measures of muscle quality and function using phase angle.	- Relies on prediction equations - Pediatric reference values are only available for patient 3 years old and above. - Difficult to ensure children are fasted and adequately hydrated - Regression equations have limited suitability and thus validity may vary. - Patient must lie still prior to measurement (difficult in infants and young children) - Sources of error: limb length, physical activity, nutrition status, hydration level (e.g., edema), and electrodes placement - Fat free mass can be overestimated in obese patients and underestimated in patients with normal weight. - Limited sensitivity in evaluating the trunk of the body (less suitable for patients with uneven distribution of fat) - Contraindicated in subjects with a cardiac pacemaker or an implantable cardioverter defibrillator.
Laboratory methods			
Cross-sectional imaging[5,17,20,262,263,267-272] - Computed Tomography (CT) - Magnetic Resonance Imaging (MRI)	Adipose tissue: total, subcutaneous, visceral, intramuscular Muscle mass: total and specific muscles (e.g., psoas, paraspinal,	- Both: Most accurate methods for in vivo quantification of body composition, can differentiate between fat depots and specific muscles.	- CT: Pediatric reference values are available for patients aged 2-20 years, but this is based on a single study that lacked a multi-ethnic population. - CT: Contains highly ionizing radiation.

	abdominal wall muscle areas)	- CT: Fast, accessible, gold standard for VAT determination, - MRI: Does not involve ionizing radiation	- MRI: Long process, costly, limited capacity at most facilities. - CT: A single muscle measurement may not reflect total skeletal muscle mass (e.g., measuring psoas muscle area in pediatrics). - Both: Patient must lie still (difficult in infants and young children), high level of training and expertise needed.
Densitometry[17,262,263 265,273] - Air-displacement plethysmography (ADP) - Hydrostatic weighing (HW)	Fat mass, fat free mass	- ADP: Quick to perform, low participant burden (suitable for pediatric patients), valid method for patients as young as 1 week old. No special training is required for its use. - HW: Gold standard for measurement of body volume	- Both: Costly; limited accessibility; assumes density of fat mass and fat free mass derived from chemical analysis of cadavers of males aged 25 – 48 years (questionable validity). If hydration assumptions are violated (altered hydration status), accuracy decreases. - ADP: Peapod equipment is limited to infant weight <10 kg. Ancillary equipment for the adult ADP allows valid measured for children 2-6 years old, but a gap remains for those aged 6 months to 2 years. - HW: Time-consuming, extensive equipment, high participant burden. Not suitable for small children and subjects unable to hold their breaths under water.
Dual-energy X-ray Absorptiometry (DXA)[17,262,263,274]	Fat mass, lean soft tissue mass, total body mineral	- Observer-independent - Can assess total and regional body composition	- Expensive equipment - Technical expertise necessary - Patient must lie still (difficult in infants and young children)

		- Excellent precision for whole-body measurements - Non-invasive and modest patient cooperability required - Low radiation - Not dependant on assumptions of bone density (as with densitometry) - Relatively short scan time - Sedation of infants and children is typically not necessary	- Limited data in children <2 years of age - Assumes constant hydration of lean soft tissue (it varies with age, gender, and disease) - Sources of error: Variations in measurements depending on machine manufacturer, models, and software upgrades; inaccurate patient positioning, presence of metallic implants, antecedent administration of radioactive tracer to the patient, or the patient being too wide or too tall for the scan field. - Reduced validity of body composition measurements in very lean or highly obese subjects.
Hydrometry (Isotope dilution method)[17,262,265] - Deuterium dilution - Tritiated water - O^{18} labelled water	Total body water (fat free mass)	- High precision and accuracy - Gold standard for determination of total body water	- Costly - Time-consuming - High level of technical expertise required - Hydration factor is assumed to estimate fat free mass and fat mass from TBW (ideally, population-specific factors would be used) - Sources of error: type of fluid measured (blood, saliva, urine), isotopic equilibrium time, correction for dilution space, and the analysis method used to measure isotopic enrichment - Dose spillage is common in infants (accurate dose quantification may be challenging but can be minimized using a syringe).

Ultrasound[17,263,264,274,275]	Adipose tissue: total, subcutaneous, visceral, intramuscular Muscle mass: Area, width, skeletal muscle mass	- Non-invasive - Relatively inexpensive - Less time-consuming than CT/MRI - Portable - Allows muscle mass measurement by assessing an individual muscle (detection of "site-specific" sarcopenia) - Validated method (against CT and MRI)	- Requires lengthy training and high level of expertise - No universal guidelines for measurement of body composition - Patient must lie still (difficult in infants and young children) - Excess transducer pressure and orientation can influence muscle size measurements - Proper landmarking is critical - Hydration and proximity to exercise must be controlled prior to measurement - Preperitoneal fat used as an approximation of visceral fat.
Total body potassium[17,262,276-279]	Body cell mass	- Body cell mass is involved in O2 consumption, CO2 production and energy expenditure (determinants of nutritional status). - Can reflect skeletal muscle mass development (decrease in total body potassium may indicate muscle atrophy). - Independent of changing hydration status (e.g., edema) - Radiation free	- Indirect measurement of muscle mass: Potassium is present in all organs and tissues of the body; skeletal muscle mass to potassium ratio changes with growth. - Patient must lie still (difficult in infants and young children) - Costly - Limited data based on multi-ethnic populations (African American and Hispanic). Prediction models stem from small sample sizes.
In vivo neutron activation analysis[17]	Total Body Nitrogen (fat free mass)	- Quick to perform	- Limited availability - Radiation exposure - Costly

		- Possible to quantify several elements in the body (O, C, Na, Ca, P, H).	

Appendix 2A. Description of target population and components of pediatric malnutrition risk screening tools and nutritional status assessment tools.

Tool	Inpatient[a] or Outpatient/Specialty[b] use?	Description	Components			
			Weight/age, weight/length, height velocity	Weight change/loss	Appetite/dietary intake	Clinical information[1]/Other[2]
SCREENING TOOLS						
Pediatric Malnutrition Screening Tool (PMST)	Inpatient	Modified version of STAMP for hospitalized children age <2 to 17 years; screens for under- and overnutrition	X	--	X	--
Integrated Management of Childhood Illness (IMCI)	Inpatient	Designed by the World Health Organization to be used by health workers in developing countries	--	--	--	X[2]
Pediatric Nutrition Risk Score (PNRS)	Inpatient	Developed for hospitalized children aged >1 month, at risk of acute malnutrition	--	--	X	X[1]
Pediatric Nutrition Screening Tool (PNST)	Inpatient	Developed to improve simplicity of nutrition screening in hospitalized children aged 0-16 years	X	X	X	X[1]
Pediatric Yorkhill Malnutrition Score (PYMS)	Inpatient	Developed for hospitalized children aged >1 year	--	X	X	X[1]
Screening Tool for the Assessment of Malnutrition (STAMP)	Inpatient	Developed for hospitalized children aged 2-17 years; allows for repeated screening	X	--	X	X[1]

Screening Tool for the Assessment of Malnutrition in Pediatrics-Modified (Modified-STAMP)	Outpatient/Specialty	Modified version of STAMP for children in the outpatient setting	X	--	X	X[1]
Screening Tool for Risk of Nutritional status and Growth (STRONGkids)	Inpatient	Developed for hospitalized children to decrease complexity of previously available tools	--	X	X	X[1,2]
Nutrition Risk Screening Tool for Children and Adolescents with Cystic Fibrosis (NRST-CF)	Outpatient/Specialty	Developed for children aged 2-20 years with cystic fibrosis in the inpatient or outpatient setting	X	X	--	X[1]
Nutrition Screening Tool for Childhood Cancer (SCAN)	Outpatient/Specialty	Developed for children with a cancer diagnosis	--	X	X	X[1]
ASSESSMENT TOOLS						
Subjective Global Nutrition Assessment (SGNA)	Inpatient	Developed for chronically ill/hospitalized children to detect malnourishment and risk of malnutrition-related morbidities.	X	X	X	X

Adapted from Becker *et al*.[23] with permission from Elsevier. [a]Tool was designed for patients in an inpatient/hospital setting. [b]Tool was designed to be used in patients in an outpatient setting or specialty clinic setting. [1]Clinical information may include one or more of the following: Visible severe wasting; bipedal edema; medical condition or diagnosis; severity of disease or intensive treatment; gastrointestinal symptoms, pain or other symptoms causing inability to eat; visibly under- or overweight. [2]Other information may include one or more of the following: Dietary behavior, physical activity/sedentary behaviour, pre-existing nutrition intervention, food security, screen time, dietary habits, cooking techniques, meal patterns. X= Component is included in the tool. --= Component is not included in the tool.

Appendix 2B. Scoring and definitions for malnutrition risk and impaired nutritional status in pediatric malnutrition risk screening tools and nutritional status assessment tools.

Tool	Scoring system	Definition for malnutrition risk/impaired nutritional status
SCREENING TOOLS		
Pediatric Malnutrition Screening Tool (PMST)[280]	Clinical diagnosis, estimated nutritional intake, and weight and height percentiles are scored 1-3 points, adding up to a maximum score of 9 points.	Malnutrition risk 0-1 points: Low 2-3 points: Medium 4-9 points: High
Integrated Management of Childhood Illness (IMCI)[281]	Protein-energy malnutrition is identified by presence of visible severe wasting and bipedal edema in a dichotomous manner (yes/no).	If signs are identified, the child is referred for an inpatient nutritional assessment.
Pediatric Nutrition Risk Score (PNRS)[282]	Food intake/ability to eat, ability to retain food due to vomiting and diarrhea, pain, disease severity, and anthropometrics are scored 0-3 points, adding up to a maximum score of 5 points	Risk of nutritional depletion 0 points: Low 1-2 points: Moderate 3-5 points: High
Pediatric Nutrition Screening Tool (PNST)[283]	Weight loss, poor weight gain, decreased intake, and physical examination are scored in a dichotomous manner (yes/no)	If 2 or more questions are answered "yes", the child should be referred for a nutrition assessment
Pediatric Yorkhill Malnutrition Score (PYMS)[284]	BMI, weight loss, dietary intake, and predicted effect of the current condition on nutritional status are score 0-2 points, adding up to a maximum score of 6 points	Malnutrition risk 0 points: Low 1 point: Medium 2-6 points: High
Screening Tool for the Assessment of Malnutrition (STAMP)[285]	Clinical diagnosis, nutritional intake, and weight and height percentiles are scored 0-3, adding to a maximum score of 9	Malnutrition risk 0-2 points: Low 2-3 points: Medium 4-9 points: High
Screening Tool for the Assessment of Malnutrition in Pediatrics-Modified (Modified-STAMP)[286]	Clinical diagnosis, nutritional intake, and weight and height percentiles are scored 0-3, adding to a maximum score of 9	Malnutrition risk 0 points: Low 1-3 points: Medium 4-9 points: High

Screening Tool for Risk of Nutritional status and Growth (STRONGkids)[287]	Subjective clinical assessment, clinical diagnosis, nutritional intake/losses, weight loss, and weight gain are scored 0-2 points, adding up to a maximum score of 5 points	<u>Malnutrition risk</u> 0 points: Low 1-3 points: Medium 4-5 points: High
Nutrition Risk Screening Tool for Children and Adolescents with Cystic Fibrosis (NRST-CF)[288]	Weight gain, height velocity, and BMI percentile are scored 0-2 points, adding up to a maximum score of 6 points.	<u>Malnutrition risk</u> 0-1 points: No risk/low 2-3 points: Moderate 4-6 points: High
Nutrition Screening Tool for Childhood Cancer (SCAN)[289]	Presence of high-risk cancer, undergoing intensive treatment, GI symptoms, weight loss, oral intake, and physical exam are scored 1-2 points, adding up to a maximum score of 12 points.	<u>Malnutrition risk</u> 0-2 points: Not at risk 3-12 points: At risk
ASSESSMENT TOOLS		
Subjective Global Nutrition Assessment (SGNA)[26]	Subjective assessment of appropriateness of height for age, changes in weight, adequacy of dietary intake, GI symptoms, nutrition-related functional capacity, metabolic stress of disease, and physical exam.	<u>Nutritional status:</u> Well nourished Moderately malnourished Severely malnourished

BMI: Body Mass Index; GI: Gastrointestinal

Appendix 3. Neurodevelopmental milestones from birth to 5 years of age

Age	Motor skills	Language	Cognition	Socio-emotional skills
Newborn	Primitive reflexes GM: Moro, Babinski, ATNR, Flexor posture FM: Grasp	Primitive reflexes: Alert to sound, starts to loud sounds, variable cries, root, suck	Prefers high pitched voice, fix & follow slow horizontal arc, prefers contrast, faces, colours	Parent-child bonding, self-regulation/soothing
2 months	GM: Head steady when held, head up 45° in prone position FM: Hands open more frequently, bats at objects	Turns to voice, cooing	Prefers usual caregiver, attends to moderate novelty, eyes follow past midline	Child-parent attachment, social smile
4 months	GM: Sits with support, head up 90° prone (arms out), rolls front to back FM: Palmar grasp, reaches and obtains items, brings objects to midline	Laugh, razz, "ga", squeal	Anticipates routines, purposeful exploration of objects (eyes, hands, mouth)	Turn-taking conversations, explores caregiver's face
6 months	GM: Postural reflexes, tripod sitting position, rolls both ways FM: Raking grasp, transfer objects from hand to hand	Non-specific babble	Stanger anxiety, looks for dropped or partially hidden objects	Expresses emotions (happy, sad, mad), memory lasts ~24 hours
9 months	GM: Changes position from all fours to sitting, sits well with hands free, pulls to stand, creeps on hands and knees FM: Inferior pincer grasp, pokes at objects	Says "mama" and "dada", gestures "bye-bye", "up" (to be held), gestures games ("pattycake")	Object permanence, uncovers toy, "peek-a-boo"	Separation anxiety
12 months	GM: Walks a few steps, wide-based gait FM: Fine pincer (fingertips), voluntary release, throws objects, finger-feeds self small food items (e.g., cheerios)	Learns 1 new word with meaning (besides "mama" and "dada"), inhibits with "no!", responds to own name, 1-step command with gesture	Cause and effect, trial and error, imitates gestures and sounds, uses objects functionally (e.g., rolls toy car)	Explores from secure base, points at wanted items, narrative memory begins

15 months	GM: Walks well FM: Uses spoon, opens top cup, builds towers of 2 blocks	Points to 1 body part, 1-step command with no gestures, 5-word word bank, jargoning	Looks for a hidden/moved object if they saw it being moved, experiments with toys to make them work	Shared attention (points at interesting items to show caregivers), brings toys to caregivers
18 months	GM: Stoops and recovers FM: Carries toys while walking, removes clothing, builds towers with 4 blocks, scribbles with fisted pencil grasp	Points to objects, identifies 3 body parts, 10-to-25-word word bank, embedded jargoning, labels familiar objects	Imitates housework, symbolic play with doll/bear (e.g., gives teddy bear "a drink")	Increased independence, parallel play
2 years	GM: Jumps on two feet, walks up and down stairs using step-to-step or marking time pattern FM: Handedness is established, uses fork, builds tower with 6 blocks, imitates vertical stroke	Follows 2-step commands, 50+ words in their word bank, speech is 50% intelligible, builds and communicates 2-word phrases, uses I/me/you/plurals	Uses new problem-solving strategies without rehearsal, searches for hidden object after multiple displacements	Tests limits, throws tantrums, negativism ("no!") and possessiveness ("mine!"), self-awareness starts developing
3 years	GM: Walks up stairs alternating feet, runs FM: Undresses self, toilet trained, draws circles and +, turns pages of books	Follows 3-step commands, 200-word word bank, speech is 75% intelligible, builds and communicates 3–4-word phrases, uses W questions ("why? where? who?"), states full name, age, sex	Simple time concepts (now, later, a few minutes), identifies shapes, compares two items (e.g., "this one is bigger/smaller"), counts to three.	Separates easily from caregivers, learns to share, empathy, cooperative play, role play
4 years	GM: Hops on one foot, walks downstairs alternating feet FM: Draws crosses, squares, diagonals; cuts shape with scissors, buttons/unbuttons clothing, builds tower of 10 blocks	Builds longer sentences that are 100% intelligible, tells stories, uses past tense	Counts to 4, identifies opposites and up to 4 colours.	Has a preferred friend, creates elaborate fantasy play
5 years	GM: Balances on one foot for 10 seconds, skips, may learn to ride a bicycle FM: Draws a person with 10 body parts, uses tripod pencil grasp,	Vocabulary comprises 5000 words, uses future tense, uses word play/jokes/puns, has phonemic awareness	Counts to 10 accurately, recites the ABC's, recognises some letter, obtains pre-literacy and numeracy skills	Has a group of friends, follows group rules and games with rules

	writes their name, copies letters, increasing independent daily living skills			

ABCs: Alphabet, ATNR: Asymmetrical Tonic Neck Reflex, FM: Fine Motor Skills, GM: Gross Motor Skills, Adapted from Dosman *et al*.[290]

Appendix 4. Key macro and micronutrients for optimal neurodevelopment.

Nutrient	Role in neurodevelopment	Neurodevelopmental domain (s) affected by deficiency
Macronutrients		

Amino acids[49,291-293]	• Precursors of neurotransmitters or neurotransmitters themselves
	  ○ Tryptophan: Substrate for serotonin
	  ○ Phenylalanine: Involved in phenylethylamine production
	  ○ Tyrosine: Substrate for epinephrine and norepinephrine synthesis
	• Precursors for growth factors (BDNF, IGF-1), enzymes, and peptide hormones
	• Neural stem cell proliferation, migration, and differentiation
	• Axonal and dendrite development
	• Gliogenesis
	• Synaptic plasticity
		• Cognitive domain: Memory, learning, numeracy
		• Socio-emotional domain: Emotional regulation
		  ○ Tryptophan deficiency may lead to decreased melatonin levels, associated with circadian malfunctioning; this can increase risk of mood dysregulation.
		  ○ Tyrosine deficiency: increased levels of apathy.

LC-PUFAS[13,49,291,294]	• Core fatty acids in gray matter
	• Visual and pre-frontal cortex development
	• Neuronal membrane structure
	• Neuronal and dendritic spine growth
	• Synaptogenesis
	• Myelination
	• Neurotransmitter synthesis
		• Cognitive domain:
		  ○ Executive function (DHA, ALA)
		  ○ Attention: (DHA)
		  ○ Overall cognitive function: LA, DHA
		• Motor skills domain: Gross motor skills (LA)
		• Language domain (ALA)

Glucose[292,295-297]	• Neuron, oligodendrocyte, and astrocyte metabolism • Modulation of electrophysiologic potential of neurons	• Global (all domains may be affected, as per studies focused on disorders of glucose metabolism)
Micronutrients		
Vitamin A[298,299]	• Neuron differentiation	• Cognitive domain: Memory and learning skills

	• Neural tube development • Synaptic plasticity	
Vitamin D[49,52,192,291,298,300,301]	• Cell proliferation • Induction of nerve growth factors in glial cells and neurons • Neural apoptosis inhibition • Upregulation of serotonin expression	• Cognitive domain: Memory, learning, attention, IQ • Motor skills domain: Gross motor skills • Socio-emotional skills: Emotional regulation (anxiety), maladaptive behaviours
Vitamin E[60-64,302]	• Maintenance of redox balance in the brain • Protection of cell membranes and myelination • Neurotransmitter modulation • Neural stem cell protection	• Motor skills domain: Via development of ataxia, gait/gross motor movements may be impaired • Language domain: Via development of ataxia, expressive and receptive language may be impaired. • Socio-emotional domain: Emotional regulation (anxiety).
Vitamin B1[298,303]	• Energy metabolism in the brain • Conduction of nerve impulses • Neurotransmitter synthesis • Myelination	• Motor skills domain: Gross motor movements • Language domain: Expressive and receptive language
Vitamin B6[298,304]	• Energy metabolism in the brain • Neurotransmitter synthesis • Amino acid synthesis, breakdown, and interconversion	• Cognitive domain: Memory and learning skills
Vitamin B12[13,291,298]	• Myelination • Protection against neuronal degeneration • Neurotransmitter synthesis • Synaptogenesis • Neuronal structure	• Motor skills domain: Gross/fine motor movements may be impaired via hypotonia and involuntary muscle movements. • Socio-emotional domain: Emotional regulation • Cognitive domain: Visuo-spatial skills, memory, IQ

Folate[13,49,298,305]	• Neural stem cell proliferation • Neural tube closure • Neuronal structure • Amino acids interconversion to neurotransmitters • DNA methylation	• Cognitive domain: Executive function; intellectual disability associated with neural tube defects • Language domain: Speech ability • Socio-emotional domain: Emotional regulation • Motor skills domain: Gross motor skill impairment associated with neural tube defects.
Vitamin C[298,306]	• Maintenance of redox balance in the brain • Myelination • Neuronal density and maturation • Neurotransmitter modulation	• Cognitive domain: Visuo-spatial skills
Choline[13,49,298]	• Cell membrane integrity (phospholipids) • Neurotransmitter precursor • Neural stem cell proliferation, differentiation, and migration • Neural tube closure • DNA methylation	• Cognitive domain: Memory, learning skills, visuospatial skills; intellectual disability associated with neural tube defects • Motor skills domain: Gross motor skill impairment associated with neural tube defects.
Iron[13,49,291-293,298]	• Brain energy metabolism • Neurotransmitter synthesis • Myelination • Electrophysiologic potential of neurons	• Cognitive domain: Attention, visuo-spatial skills, executive function. • Motor skills domain: Fine and gross motor skills. • Socio-emotional domain: Emotional regulation (anxious attachments, depression, hesitancy, unhappiness), sociability.
Iodine[13,49,291,292,294,298]	Thyroid hormone-dependent roles: • Neuronal cell differentiation, maturation, and migration • Myelination • Dendrite and axon growth • Neurotransmission	Severe in utero deficiency • Global (all domains are affected): Secondary to cretinism Mild-to-moderate in utero deficiency • Cognitive domain: Executive function Post-natal deficiency

	• Synaptogenesis • Synaptic plasticity	• Cognitive domain: Executive function, visuo-spatial skills, reading comprehension • Motor skills domain: Fine motor skills
Zinc[13,49,291-294,298,307]	• DNA synthesis • Neurogenesis • Neuronal maturation and migration • Synaptogenesis • Synaptic vesicle component • Electrophysiologic potential of neurons • Neurotransmitter modulation	• Cognitive domain: Memory, learning skills, attention • Socio-emotional skills: Emotional regulation (anxiety) • Motor skills domain: Gross and fine motor skills
Copper[293,298]	• Myelination • Maintenance of redox balance in the brain • Neurotransmission • Brain energy metabolism	• Motor skills domain: Gross motor skills

AA: Arachidonic acid; ALA: Alpha-linolenic acid; BDNF: Brain-derived neurotrophic factor; DNA: Deoxyribonucleic acid; DHA: Docosahexaenoic acid; IGF-1: Insulin-like growth factor 1; IQ: Intellectual quotient; LA: Linoleic acid

Appendix 5. Typical medications used the study population

Medication type	Examples
Immunosuppressive therapy	Corticosteroids Prednisone, methylprednisolone, dexamethasone, betamethasone, hydrocortisone Antimetabolites Mycophenolate mofetil Biologic agents (monoclonal antibodies) Basilixumab, Daclizumab Calcineurin inhibitors Tacrolimus Non-calcineurin inhibitor-based therapy Sirolimus, everolimus
Antibiotics	Trimethoprim-sulfamethoxazole, rifampin, metronidazole, vancomycin, cefotaxime, linezolid, imipenem-cilastatin, piperacillin-tazobactam
Anticoagulants	Enoxaparin, dalteparin, tinzaparin, heparin, warfarin, clopidogrel bisulfate, dipyridamole, rivaroxaban
Vitamin supplements	Single preparation vitamin supplement Vitamin A (retinol/retinyl palmitate) Vitamin D (vitamin D3/D2) Vitamin E (Alpha-tocopherol) Vitamin K (vitamin K1/K2) Multivitamin (water or fat-soluble multivitamins)
Ursodiol	Synthetic ursodeoxycholic acid
Diuretics	Spironolactone, furosemide, hydrochlorothiazide
Lactulose	--
Others	Proton pump inhibitors (lansoprazole, pantoprazole, omeprazole), analgesics (morphine), sleep-aids (melatonin), calcium channel blockers (amlodipine), antifungals (nystatin, fluconazole), antihistamine (diphenhydramine), barbiturate

	anticonvulsants/hypnotics (phenobarbital), benzodiazepines (lorazepam) , potassium binders (sodium polystyrene), laxative (MiraLAX), antiemetic/prokinetic (domperidone)

Categorization for immunisuppresive therapy agents was extracted from Anghel et al.

Appendix 6. Neurocognitive scores in n=9 ESLD patients at LTx assessment

Of those with a BSID score available, n=4 had an adequate cognitive level which coincided with an adequate adaptive level per the VABS. One patient had an inadequate cognitive level (moderately low cognitive level) in contrast with an adequate adaptive level per the VABS. On the contrary, one patient had an adequate cognitive level, contrasting with an inadequate adaptive level (moderately low ABC score) per the VABS, reflecting overall overall developmental delay but age-appropriate cognitive development. Regarding those with available WPPSI scores, n=2 had an inadequate cognitive level (moderately low or low cognitive level), which contrasted with an adequate adaptive level per the VABS. One patient had an adequate cognitive level but had unavailable VABS scores.

Cognitive parameter	Value
BSID (n=6)	
Cognitive Composite Score	97.5±7
Cognitive percentile	44.3±16
Cognitive level	
- High	0 (0)
- Moderately high	0 (0)
- Adequate	5 (83)
- Moderately low	1 (17)
- Low	0
WPPSI (n=3)	
Verbal IQ score	86.3±11
Verbal IQ percentile	14 (6 – 45)
Performance IQ score	65.5±9
Performance IQ percentile	2*
Full scale IQ score	77±17
Full scale IQ percentile	4 (1 – 27)
Cognitive level	
- High	0 (0)
- Moderately high	0 (0)
- Adequate	1 (33)
- Moderately low	1 (33)
- Low	1 (33)

Data presented as mean±SD or median (interquartile range) or percentage (%). The BSID and WPPSI are based on a mean±SD standard scores of 100±15 overall and per domain. The BSID cognitive score is based on assessment of memory, concept formation, object relatedness, visual acuity, among other relevant cognitive functions. The WPPSI

verbal IQ measures verbal comprehension (e.g., verbal reasoning, verbal concept formation); the performance IQ measures visuospatial intellectual abilities, and the full scale IQ summarizes overall ability across different cognitive functions. BSID: Bailey Scales of Infant Development; WPPSI: Wechsler Preschool and Primary Scale of Intelligence. LTx: Liver Transplantation; ESLD: End-stage Liver Disease; IQ: Intellectual Quotient.*Available for n=1.

PEDIATRIC SGNA RATING FORM

Consider severity and duration of changes, as well as recent progression when rating each item.

NUTRITION-FOCUSED MEDICAL HISTORY	SGNA SCORE		
	Normal	Moderate	Severe
Appropriateness of Current Height for Age (stunting) a) Height percentile: _____ ☐ ≥ 3rd centile ☐ just below 3rd centile ☐ far below 3rd centile			
b) Appropriate considering mid-parental height[a]?: ☐ yes ☐ no			
c) Serial growth[b]: ☐ following centiles ☐ moving upwards on centiles ☐ moving downwards on centiles (gradually or quickly)			
Appropriateness of Current Weight for Height (wasting) Ideal Body Weight = _____ kg Percent Ideal Body Weight: __ __ __ % ☐ >90% ☐ 75-90% ☐ <75%			
Unintentional Changes in Body Weight a) Serial weight[b]: ☐ following centiles ☐ crossed ≥ 1 centile upwards ☐ crossed ≥ 1 centile downwards			
b) Weight loss: ☐ < 5% usual body weight ☐ 5-10% usual body weight ☐ >10% usual body weight			
c) Change in past 2 weeks: ☐ no change ☐ increased ☐ decreased			
Adequacy of Dietary Intake a) Intake is: ☐ adequate ☐ inadequate - hypocaloric ☐ inadequate - starvation (ie, taking little of anything)			
b) Current intake versus usual: ☐ no change ☐ increased ☐ decreased			
c) Duration of change: ☐ < 2 weeks ☐ ≥ 2 weeks			
Gastrointestinal Symptoms a) ☐ no symptoms ☐ one or more symptoms; not daily ☐ some or all symptoms; daily			
b) Duration of symptoms: ☐ < 2 weeks ☐ ≥ 2 weeks			
Functional Capacity (nutritionally related) a) ☐ no impairment, energetic, able to perform age-appropriate activity ☐ restricted in physically strenuous activity, but able to perform play and/or school activities in a light or sedentary nature; less energy; tired more often ☐ little or no play or activities, confined to bed or chair > 50% of waking time; no energy; sleeps often			
b) Function in past 2 weeks: ☐ no change ☐ increased ☐ decreased			
Metabolic Stress of Disease ☐ no stress ☐ moderate stress ☐ severe stress			

[a]Mid-parental height: Girls: subtract 13 cm from the father's height and average with the mother's height. Boys: add 13 cm to the mother's height and average with the father's height. Thirteen cm is the average difference in height of women and men. For both girls and boys, 8.5 cm on either side of this calculated value (target height) represents the 3rd to 97th percentiles for anticipated adult height. (29)

[b]30% of healthy term infants cross one major percentile and 23% cross two major percentiles during the first 2 years of life, typically towards the 50th percentile rather than away from it. This is normal seeking of the growth channel.

Appendix 7. Pediatric Subjective Global Nutritional Assessment (SGNA). Reproduced from Secker *et al*.[26] with permission from Elsevier.

PHYSICAL EXAM	SGNA SCORE		
	Normal	Moderate	Severe
Loss of subcutaneous fat ☐ no loss in most or all areas ☐ loss in some but not all areas ☐ severe loss in most or all areas			
Muscle Wasting ☐ no wasting in most or all areas ☐ wasting in some but not all areas ☐ severe wasting in most or all areas			
Edema (nutrition-related) ☐ no edema ☐ moderate ☐ severe			

GUIDELINES FOR AGGREGATING ITEMS INTO GLOBAL SCORE

In assigning an overall global score, consider all items in the context of each other. Give the most consideration to changes in weight gain and growth, intake, and physical signs of loss of fat or muscle mass. Use the other items to support or strengthen these ratings. Take recent changes in context with the patient's usual/chronic status. Was the patient starting off in a normal or nutritionally-compromised state?

Normal/Well nourished
This patient is growing and gaining weight normally, has a grossly adequate intake without gastrointestinal symptoms, shows no or few physical signs of wasting, and exhibits normal functional capacity. Normal ratings in most or all categories, or significant, sustained improvement from a questionable or moderately malnourished state. It is possible to rate a patient as well nourished in spite of some reductions in muscle mass, fat stores, weight and intake. This is based on recent improvement in signs that are mild and inconsistent.

Moderately malnourished
This patient has definite signs of a decrease in weight and/or growth, and intake and may or may not have signs of diminished fat stores, muscle mass and functional capacity. This patient is experiencing a downward trend, but started with normal nutritional status. Moderate ratings in most or all categories, with the potential to progress to a severely malnourished state.

Severely malnourished
This patient has progressive malnutrition with a downward trend in most or all categories. There are significant physical signs of malnutrition—loss of fat stores, muscle wasting, weight loss >10%—as well as decreased intake, excessive gastrointestinal losses and/or acute metabolic stress, and definite loss of functional capacity. Severe ratings in most or all categories with little or no sign of improvement.

	Normal	Moderate	Severe
OVERALL SGNA RANKING			

Appendix 7. *(Continued).* Pediatric Subjective Global Nutritional Assessment (SGNA). Reproduced from Secker *et al*.[26] with permission from Elsevier.

Appendix 8. Types of nutritional intake in a cohort of infants and children with end-stage liver disease awaiting liver transplantation.

Type of intake	Description
Enteral nutrition only	Patient's intake is solely through an enteral feeding tube, no oral intake.
Mixed (enteral nutrition + oral intake)	Patient is consuming most of their requirements orally (formula/solid foods/breastmilk), with top-ups via the enteral feeding tube; or is receiving most of their requirements through the feeding tube and grazing throughout the day to maintain oral intake.
Enteral + parenteral nutrition	Patient is receiving daily nutrient requirements through both an enteral feeding tube and parenteral nutrition.
Oral intake (just formula)	Patient is consuming formula orally (through a bottle)
Oral intake (formula + solid food)	Patient is consuming formula orally (through a bottle) and is also eating solid foods (or just starting to)
Oral intake (formula + breastfeeding)	Patient is consuming formula orally (through a bottle) and is also breastfed.
Oral intake (only solid food)	Patient nutrient intake and diet consists of a variety of solid foods, no formula/breastmilk.
Oral intake (exclusively breastfed)	Patient's sole nutrient intake is via breastfeeding

Appendix 9. Associations between route of nutritional delivery/intake data with overall neurodevelopment and specific domains (communication, socialization, daily living skills, socialization, and motor skills)

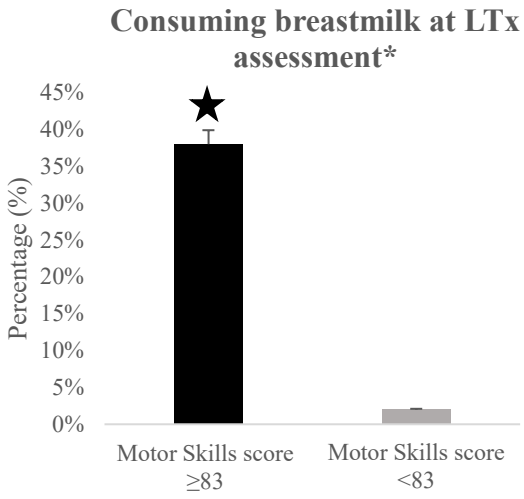
A. Nutritional Delivery and Intake

A-1. Associations with Vineland Composite Score Groups (Adaptive Level Groups)

Albeit not significant, 27% of the adequate adaptive level group was consuming breastmilk at LTx assessment, compared to 12% of the inadequate adaptive level group (p=0.21). No differences between routes of nutritional delivery were observed between the adaptive level ± malnutrition per any definition (p>0.05).

A-2 Associations with the Communication, Socialization, Daily Living Skills, and Motor Skills Individual Domains

A higher percentage of patients with a greater than median motor skill scores were consuming breastmilk (exclusively breastfed or alternated with formula) when compared with the lower motor skill group (38% vs 8%, p=0.02, **Figure 3.9**). These differences were not seen when focusing on the remaining neurodevelopmental domains (**Appendix 12, 13, 14)**. No other nutritional variable differed between the specific domain groups.



Appendix 9-Figure 1. Breastmilk consumption in the above/below than median motor skills score groups. Breastmilk consumption included exclusively breastfed and consumption of breastmilk and formula in different ratios. Data is presented as percentage bars. Fisher's exact test was used to analyze categorical data. **p*-value≤0.05 is considered statistically significant. LTx: Liver Transplantation.

Appendix 10. Post liver transplantation clinical outcomes

Outcome	Description
Immediate postoperative LOS	ICU and total LOS in days during the first LTx hospital admission
Ventilator dependency duration	Days on ventilation after surgery, during the first LTx hospital admission.
Infection incidence and type	Fungal, bacterial, viral, and unspecified (confirmed infection with unspecified species in the medical report) at all timepoints.
Number and type of postoperative complications (during LTx hospital admission, 6-month and 12-month follow-up)	Ascites at all timepoints.
	Vascular complications: portal vein or hepatic artery stenosis, portal vein or hepatic artery thrombosis, inferior vena cava occlusion, portal vein aneurysm, hepatic ischemia.
	Biliary complications: bile leaks, biliary strictures, bile duct necrosis, sludge and stone formation, sphincter of Oddi dysfunction, cholestasis.

	Other: respiratory (pleural effusion, respiratory failure, chylothorax, etc.), gastrointestinal (bowel obstruction, pancreatitis, bowel perforation, etc.), renal (acute renal failure, acute kidney injury, etc.), cardiac (pericardial effusion, cardiac arrest/infarction, etc.), neurological (seizures, tremors, paralysis, etc.), nutrition (feeding problem, failure to thrive).
Graft rejection	Acute or chronic graft rejection.
Re-transplantation	Patient was re-listed for and underwent re-LTx in the 12-months after the first LTx.
Comorbidities that developed and/or persisted after 1-year post-LT	Obesity, cardiac, cancer, renal, gastrointestinal, endocrine, psychological, language delay, motor delay, cognitive delay, global developmental delay, other impairments.

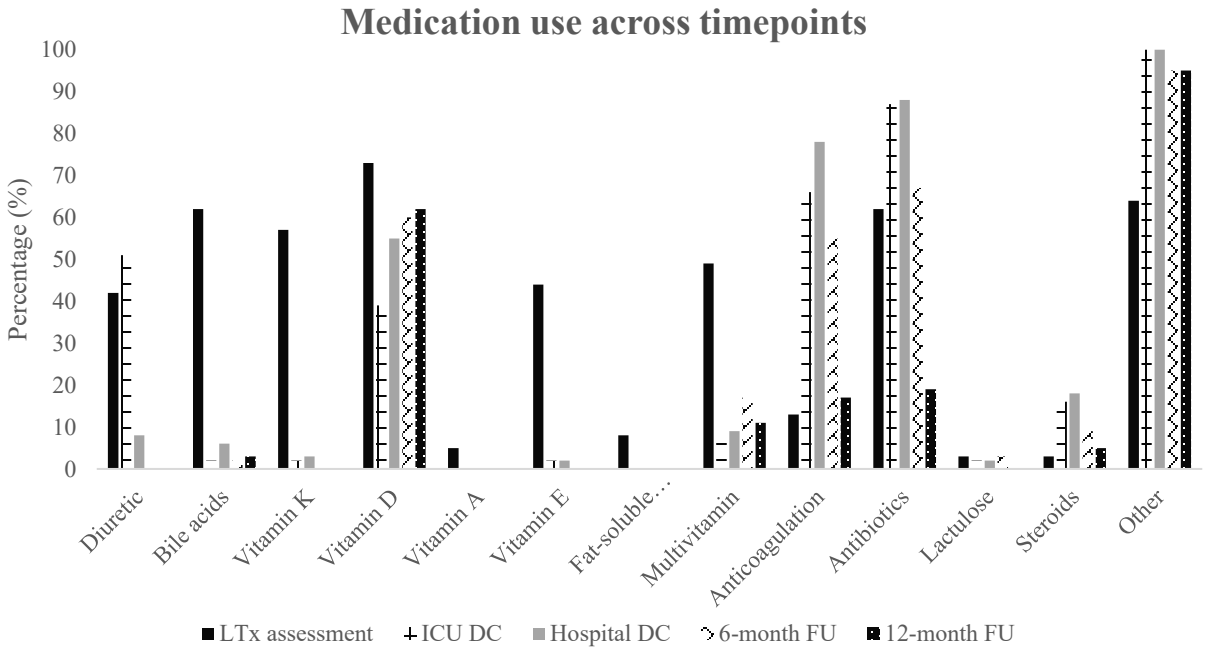
LOS: Length of stay; LTx: Liver Transplantation.

Appendix 11. Continuous and categorical variables used for statistical analysis in the present thesis

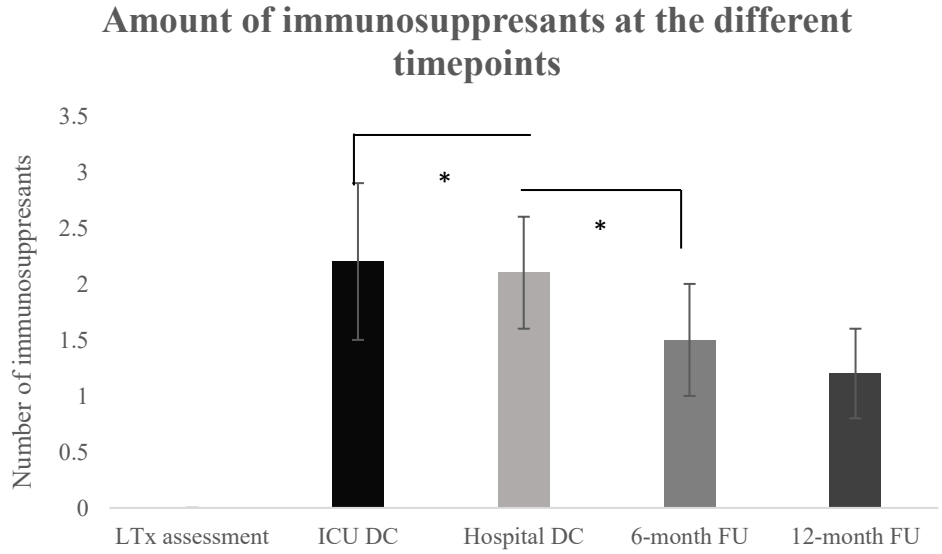
Continuous	Categorical
• Age (years) • PELD score • Family socioeconomic score • Weight • Weight z-score • Height • Height z-score • Head circumference • Head circumference z-score • Daily weight and height gain • Weight and height velocity SDS • Total protein intake and g/kg/day • Total calorie intake and kcal/kg/day • Number of inpatient, outpatient, and emergency hospital visits	• Sex (female/male) • Diagnosis (biliary atresia, other cholestatic/metabolic, acute liver failure, other) • Paternal/maternal achieving higher education (yes/no) • Foreign status (yes/no) • Consanguinity (yes/no) • Social concerns (yes/no) • Survival (yes/no) • Stunting (yes/no) • SGNA (well nourished, moderately malnourished, severely malnourished) • %IBW (well nourished, moderately malnourished, severely malnourished) • Route of nutritional delivery

• All bloodwork (e.g., ALT, AST, urea, ammonia, alpha-tocopherol, albumin) • ICU/total hospital LOS • Ventilation dependency • Infections (total, bacterial, fungal, viral, undefined) • Number of post-LTx major complications (vascular, biliary, other, total) • Total number of comorbidities developed in the 1st year post-LTx • Communication, socialization, daily living skills, motor skills, and ABC scores, SD, and percentiles.	• Achieving age-appropriate weight-gain at timepoint (yes/no) • Achieving age-expected calorie/protein intake (yes/no) • Above/below median alpha-tocopherol serum levels • Medication use (yes/no for all types) • Presence of ascites, encephalopathy, hepato-pulmonary/renal syndrome, varices (yes/no) • Rejection (yes/no) • Re-LTx (yes/no) • Presence of comorbidities (yes/no) • Age-appropriate fine and gross motor skills (yes/no) • Above/below median Communication, socialization, daily living skills, motor skills score • Adequate/inadequate adaptive level • Neurodevelopmental status±malnutrition

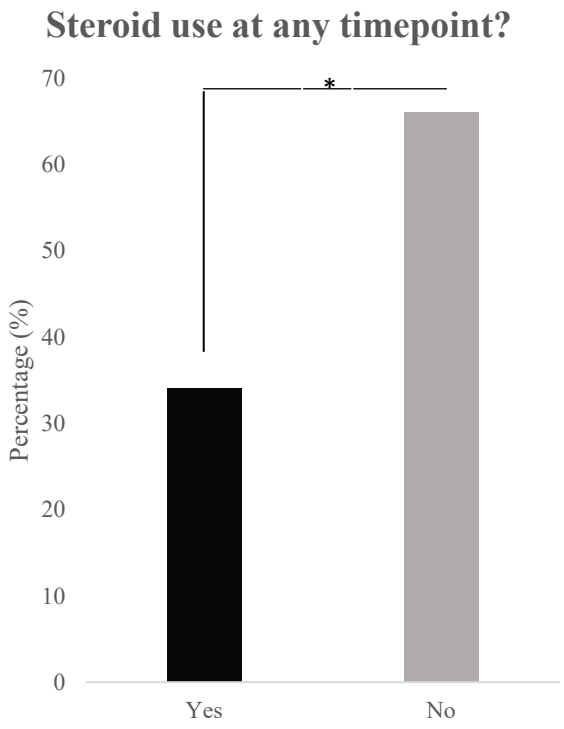
ABC: Adaptive Behaviour Composite; ALT: Alanine aminotransferase, AST: Aspartate aminotransferase; %IBW: Percentage of Ideal Body Weight; ICU: Intensive Care Unit; LOS: Length of Stay; LTx: Liver Transplantation; PELD: Pediatric End-stage Liver Disease; SD: Standard Deviation; SGNA: Subjective Global Nutritional Assessment



Appendix 12-A. Medication usage across timepoints. Data presented as percentage bars. DC: Discharge; FU: Follow-up; ICU: Intensive Care Unit; LTx: Liver Transplant



Appendix 12-B. Amount of immunosuppressant medications used per timepoint. Data presented as percentage bars. *Significant at p≤ 0.05. DC: Discharge; FU: Follow-up; ICU: Intensive Care Unit; LTx: Liver Transplant



Appendix 12-C. Steroid usage in the cohort post-LTx. *Significant at p≤ 0.05. LTx: Liver Transplant

Appendix 13. Frequency of ESLD patients that fell under the different neurodevelopmental score cut-off points.

Domains	≤85 raw score (>1 SD below mean)	≤78 raw score (>1.5 SD below mean)	≤70 raw score (>2 SD below mean)
Communication n(%)	16 (25)	10 (15)	5 (8)
Daily Living Skills n(%)	26 (40)	15 (23)	5 8)
Socialization, n(%)	15 (23)	9 (14)	5 (8)
Motor Skills, n(%)	36 (55)	25 (38)	12 (18)
ABC score, n(%)	24 (36)	10 (15)	7 (11)

Neurodevelopmental assessment (Vineland Adaptive Behaviour Scales-2nd Edition) was performed at liver transplant assessment. Score interpretation: >1 SD below mean=mild delay; >1.5 SD below mean= moderate delay; >2 SD below mean= significant delay. ESLD: End-Stage Liver Disease; SD: Standard Deviation; ABC: Adaptive Behavioural Composite.

Appendix 14. Reasons for inpatient admissions pre- and post-LTx

Pre-LTx	Post-LTx
Worsening liver function	
Failure to thrive/nutritional rehabilitation	Failure to thrive/nutritional rehabilitation/feeding intolerance
Procedures, e.g., chemotherapy, liver biopsy	Procedures, e.g., Broviac cathether/biliary tube removal, liver biopsy
Electrolyte imbalances, e.g., hypomagnesemia	Gastrointestinal issues, e.g., bowel obstruction, diarrhea, vomiting, gastrointestinal bleed
Gastrointestinal issues, e.g., nausea, vomiting, diarrhea, gastrointestinal bleed	Rejection
Infections (viral, bacterial, fungal, undefined)	Infections (viral, bacterial, fungal, undefined)
	Others: Incisional hernia repair, fracture

The pre-LTx period encompasses the time between LTx assessment and time of LTx. The post-LTx period encompasses the time between immediate post-LTx hospital discharge to the 12-month follow-up. LTx: Liver Transplantation

Appendix 15. Summary of outcomes associated with above/below median Communication scores

Variable	**≥ Median Communication score**	**<Median Communication score**	**p-value**
DEMOGRAPHIC DATA (at LTx assessment)			
Sex - Females - Males	 21 (53) 19 (47)	 14 (56) 11 (44)	0.78
Age, years	0.55 (0.41 – 0.79)	0.49 (0.38 – 0.79)	0.83
Diagnosis - Biliary atresia - Other cholestatic/metabolic - Acute liver failure - Other	 23 (58) 12 (30) 1 (2) 4 (10)	 13 (52) 9 (36) 0 (0) 3 (12)	0.92
PELD score	13 (5.5 – 18)	11 (0 – 19)	0.62
Social concerns, n(%)	7 (19)	3 (14)	0.73
Consanguinity, n(%)	0 (0)	3 (14)	**0.05**
NUTRITIONAL STATUS AND ROUTE OF DELIVERY DATA (at LTx assessment)			
SGNA category, n(%) - Well nourished - Moderately malnourished	 4 (15) 8 (30) 15 (56)	 0 (0) 7 (47) 8 (53)	0.32

- Severely malnourished			
%IBW, n(%) - Well nourished - Moderately malnourished - Severely malnourished	 29 (94) 2 (6) 0 (0)	 23 (68) 10 (29) 1 (3)	**0.004**
Stunting - Yes - No	 6 (67) 3 (33)	 34 (61) 22 (39)	1.0
Neurodevelopmental/nutritional status combos (SGNA), n(%) - Adequate adaptive level + well nourished - Adequate adaptive level + malnourished - Inadequate adaptive level + well nourished - Inadequate adaptive level + malnourished	 4 (15) 16 (59) 0 (0) 7 (26)	 0 (0) 8 (53) 0 (0) 7 (47)	0.20
Neurodevelopmental/nutritional status combos (McLaren), n(%) - Adequate adaptive level + well nourished - Adequate adaptive level + malnourished - Inadequate adaptive level + well nourished - Inadequate adaptive level + malnourished	 28 (70) 5 (13) 7 (17) 0 (0)	 7 (28) 2 (8) 11 (44) 5 (20)	**0.0003**
Neurodevelopmental/nutritional status combos (WHO), n(%) - Adequate adaptive level + well nourished - Adequate adaptive level + malnourished - Inadequate adaptive level + well nourished - Inadequate adaptive level + malnourished	 25 (68) 5 (14) 7 (19) 0 (0)	 8 (32) 1 (4) 14 (56) 2 (8)	**0.002**
Exclusively breastfed, n(%)	2 (8)	1 (5)	0.65

Appendix 15. (*Continued*)

Variable	≥ Median Communication score	<Median Communication score	p-value
ANTHROPOMETRIC DATA (at LTx assessment)			
Weight, kg	7.1 (6 – 8.5)	6.6 (5.9 – 8.5)	0.36
Weight z-score	-0.51±1.3	-0.61±1.2	0.76
Height, cm	65.5 (62.2 – 67.7)	65 (61 – 71)	0.39
Height z-score	-0.81±1.3	-0.86±1.2	0.86
Head C, cm	42.1±2.2	40.8±0.7	0.16
Head C z-score	-0.47±1	-0.53±0.80	0.89
PRE-LTx COMPLICATIONS (at LTx assessment)			
Ascites, n(%)	21 (55)	10 (40)	0.24
Hepato-renal syndrome, n(%)	4 (10)	1 (4)	0.64
Hepato-pulmonary syndrome, n(%)	0 (0)	0 (0)	N/A
Encephalopathy, n(%)	2 (5)	2 (8)	1.0
Varices, n(%)	8 (22)	8 (32)	0.36
Total pre-LTx infections	1 (0 – 2)	1 (0 – 3)	0.17
LABORATORY DATA (at LTx assessment)			
AST, IU/L	227 (127 – 301)	168 (75 – 296)	0.21
ALT, IU/L	174 (78 – 238)	104 (48 – 240)	0.26
GGT, IU/L	237 (97 – 664)	112.5 (23 – 341)	0.08
Albumin, g/L	33±5	34±6	0.40
Total bilirubin, µmol/L	205 (75 – 284)	159 (10 – 277)	0.44
INR	1.2 (1.1 – 1.4)	1.2 (1 – 1.4)	0.71

PTT, seconds	38 (34 – 42)	45 (37 – 56)	0.10
Ammonia	53 (40 – 81)	46 (37 – 53)	0.18
CRP, mg/L	6 (1.4 – 13.3)	6 (0.4 – 8.1)	0.51
Urea, mmol/L	3 (2 – 3.5)	3 (2 – 3.9)	0.89
Creatinine, µmol/L	17 (13.5 – 21)	17 (14 – 21)	0.97
Vitamin D, nmol/L	19.5 (11 – 59)	32 (19 – 86)	0.07
Vitamin E, mg/L	7 (3 – 14)	9 (3 – 28)	0.56
POST-LTx OUTCOMES			
ICU LOS, days	13 (6.5 – 31)	10 (7 – 18)	0.39
Ventilator dependency, days	6 (2 – 20)	5 (1 – 10)	0.28
Total hospital LOS, days	44 (32.5 – 77.5)	37 (27 – 59)	0.31
Weight at 6-mo FU, kg	11.7±3.3	10.6±2.1	0.26
Weight z-score at 6-mo FU	0.39±1	0.05±0.9	0.27
Height at 6-mo FU, cm	78.6 (73.1 – 83.4)	74.6 (72.6 – 79.6)	0.19
Height z-score at 6-mo FU	-0.61±1	-1.03±0.9	0.19
Weight velocity SDS at 6-mo FU	0.71 (0.22 – 2.18)	1.89 (1.49 – 2.02)	0.57
Height velocity SDS at 6-mo FU	0.79 (0.03 – 2.2)	0.73 (0.4 – 2.49)	0.65
Daily weight gain at 6-mo FU, g/day	15.1±8	8.7±8.7	**0.03**
Daily height gain at 6-mo FU, mm/day	4±1.2	3.48±1.6	0.26
Weight at 12-mo FU, kg	13.5 (11.6 – 14.4)	11.8 (10.8 – 13.9)	0.10
Weight z-score at 12-mo FU	0.87±1	0.28±0.9	0.08
Height at 12-mo FU, cm	85.3 (82 – 88.5)	83 (76 – 86.8)	0.31
Height z-score at 12-mo FU	-0.41±1	-1.04±1.1	0.09

Appendix 15. (*Continued*)

Variable	≥ Median Communication score	<Median Communication score	p-value
Weight velocity SDS at 12-mo FU	1.35±1.8	2.92±1.1	0.11
Height velocity SDS at 12-mo FU	0.96±2.6	-0.29±1.8	0.13
Daily weight gain at 12-mo FU, g/day	10.49±5.6	9.47±5.5	0.59
Daily height gain at 12-mo FU, mm/day	3.32±1.4	2.88±1.1	0.35
Total post-LTx infections	4.5 (3 – 7)	5 (3 – 6)	0.64
Total post-LTx bacterial infections	2 (1 – 3)	2 (1 – 3)	0.81
Total post-LTx viral infections	1 (1 – 3)	2 (0 – 3)	0.94
Total post-LTx fungal infections	0 (0 – 1)	0 (0 – 1)	0.99
Total post-LTx undefined infections	0 (0 – 1)	0 (0 – 0.5)	0.07
Total bacterial infections (pre/post-LTx)	3 (1 – 4)	2 (1 – 4)	0.55
Total viral infections (pre/post-LTx)	2 (1 – 3)	2 (1 – 4)	0.38
Total fungal infections (pre/post-LTx)	0 (0 – 1)	0 (0 – 0)	0.32
Total undefined infections (pre/post-LTx)	1 (0 – 1)	0 (0 – 1)	0.07
Total post-LTx complications	6 (3 – 13)	5 (3 – 11)	0.53
Deceased, n(%)	3 (10)	5 (15)	0.51

Data presented as mean±SD or median (interquartile range) or percentage (%). *p*-values ≤0.05 are considered statistically significant. Other diagnoses include hepatoblastoma and alpha-1-antitrypsin deficiency. DC: Discharge; FU: Follow-up; ICU: Intensive Care Unit; LOS: Length of Stay; LTx: Liver Transplant

Appendix 16. Summary of outcomes associated with above/below median Socialization scores

Variable	≥ Median Socialization score	<Median Socialization score	p-value
DEMOGRAPHIC DATA (at LTx assessment)			
Sex - Females - Males	 22 (67) 11 (33)	 13 (41) 19 (59)	**0.04**
Age, years	0.42 (0.37 – 0.6)	0.67 (0.5 – 0.92)	**0.01**
Diagnosis - Biliary atresia - Other cholestatic/metabolic - Acute liver failure - Other	 20 (61) 10 (30) 0 (0) 3 (9)	 17 (53) 11 (34) 1 (3) 3 (9)	0.89
PELD score	13 (7 – 16)	11.5 (0 – 19)	0.69
Social concerns, n(%)	4 (15)	6 (20)	0.73
Consanguinity, n(%)	0 (0)	3 (10)	0.24
NUTRITIONAL STATUS AND DELIVERY DATA (at LTx assessment)			
SGNA category, n(%) - Well nourished - Moderately malnourished - Severely malnourished	 3 (13) 6 (26) 14 (61)	 1 (5) 9 (47) 9 (47)	0.38
%IBW, n(%) - Well nourished - Moderately malnourished - Severely malnourished	 25 (76) 7 (21) 1 (3)	 28 (88) 4 (12) 0 (0)	0.37
Stunting - Yes - No	 7 (21) 26 (79)	 3 (9) 29 (91)	0.30
Neurodevelopmental/nutritional status combos (SGNA), n(%) - Adequate adaptive level + well nourished - Adequate adaptive level + malnourished - Inadequate adaptive level + well nourished - Inadequate adaptive level + malnourished	 3 (13) 18 (78) 0 (0) 2 (9)	 1 (5) 6 (32) 0 (0) 12 (63)	**0.0006**
Neurodevelopmental/nutritional status combos (McLaren), n(%)			**<.0001**

- Adequate adaptive level + well nourished	24 (73)	11 (34)	
- Adequate adaptive level + malnourished	6 (18)	1(3)	
- Inadequate adaptive level + well nourished	2 (6)	17 (53)	
- Inadequate adaptive level + malnourished	1 (3)	3 (9)	
Neurodevelopmental/nutritional status combos (WHO), n(%)			**<.0001**
- Adequate adaptive level + well nourished	21 (70)	12 (38)	
- Adequate adaptive level + malnourished	6 (20)	0 (0)	
- Inadequate adaptive level + well nourished	3(10)	17 (53)	
- Inadequate adaptive level + malnourished	0(0)	4 (9)	
Exclusively breastfed, n(%)	3 (12)	0 (0)	0.24
Weight, kg	7.6±3	8.2±2.7	0.45
Weight z-score	-0.52 (-1.38 – 0.15)	-0.55 (-1.39 – 0.39)	0.87
Height, cm	63 (60.6 – 66.3)	66.4 (64.9 – 71.5)	**0.01**
Height z-score	-0.82±1.5	-0.93±1.2	0.76
Head C, cm	41.3±2	43.3±1.6	**0.01**
Head C z-score	-0.59±0.9	-0.53±1.3	0.87
Head C z-score	-0.59±0.9	-0.53±1.3	0.87
PRE-LTx COMPLICATIONS (at LTx assessment)			
Ascites, n(%)	15 (63)	14 (44)	0.41
Hepato-renal syndrome, n(%)	4 (12)	2 (6)	0.67
Hepato-pulmonary syndrome, n(%)	0 (0)	1 (3)	0.49
Encephalopathy, n(%)	1 (3)	4 (13)	0.19
Varices, n(%)	5 (21)	12 (32)	0.36

Appendix 16. (*Continued*)

Variable	≥ Median Socialization score	<Median Socialization score	p-value
Total pre-LTx infections	1 (1 – 2)	1 (0 – 2)	0.24
LABORATORY DATA (at LTx assessment)			
AST, IU/L	176 (100 – 289)	239 (111 – 355)	0.55
ALT, IU/L	128 (60 – 229)	183 (75 – 250)	0.49
GGT, IU/L	221 (97 – 569)	181 (32 – 278)	0.21
Albumin, g/L	34 (29 – 37)	33 (28 – 36)	0.65
Total bilirubin, µmol/L	196 (110 – 251)	195 (9 – 306)	0.98
INR	1.2 (1.1 – 1.5)	1.2 (1.0 – 1.4)	0.31
PTT, seconds	39 (35 – 56)	39 (35 – 45)	0.47
Ammonia	47.5 (37 – 57)	53 (45.5 – 95)	**0.04**
CRP, mg/L	8.1 (5 – 11.8)	1.5 (0.3 - 6.2)	0.09
Urea, mmol/L	3.1 (2.1 – 3.7)	3 (1.7 – 3.5)	0.51
Creatinine, µmol/L	18 (15 – 21)	15 (12 – 19.5)	0.06
Vitamin D, nmol/L	21 (11 – 46)	27 (15 – 83)	0.32
Vitamin E, mg/L	6.3 (3 – 13)	9 (3.1 – 27)	0.43
POST-LTx OUTCOMES			
ICU LOS, days	10 (8 – 29)	12.5 (6.5 – 23)	0.91
Ventilator dependency, days	5 (2 – 10)	5.5 (2.5 – 15.5)	0.93
Total hospital LOS, days	43 (28 – 71)	42.5 (28 – 72)	0.87
Weight at 6-mo FU, kg	11.3±3.6	11.3±1.8	0.98

Appendix 16. (*Continued*)

Variable	≥ Median Socialization score	<Median Socialization score	p-value
Weight z-score at 6-mo FU	0.26±0.9	0.25±1.1	0.99
Height at 6-mo FU, cm	75.4 (72.6 – 79.6)	79 (76.6 – 80.9)	0.22
Height z-score at 6-mo FU	-0.78±0.8	-0.77±1.2	0.98
Weight velocity SDS at 6-mo FU	1.15±1.3	2.33±0.6	0.27
Height velocity SDS at 6-mo FU	0.81 (0.37 – 2.59)	0.62 (0.24 – 1.92)	0.71
Daily weight gain at 6-mo FU, g/day	15.6±8.4	9.1±7.8	**0.02**
Daily height gain at 6-mo FU, mm/day	4.22±1.2	3.48±1.3	0.10
Weight at 12-mo FU, kg	13.6±3.5	13±2.5	0.57
Weight z-score at 12-mo FU	0.80±1	0.37±1	0.22
Height at 12-mo FU, cm	86.5±12.3	85.5±7.7	0.78
Height z-score at 12-mo FU	-0.54±1	-0.86±1.3	0.39
Weight velocity SDS at 12-mo FU	1.98±1.6	1.75±3.6	0.88
Height velocity SDS at 12-mo FU	1.28±2.7	-0.74±1	**0.01**
Daily weight gain at 12-mo FU, g/day	11.1 (8.9 – 15.1)	5.63 (4 – 10.1)	**0.01**
Daily height gain at 12-mo FU, mm/day	3.68±1.4	2.44±0.7	**0.01**
Total post-LTx infections	4 (3 – 6)	5 (3 – 6)	0.80
Total post-LTx bacterial infections	2 (1 – 3)	2 (0 – 3)	0.60
Total post-LTx viral infections	2 (0 – 3)	1 (1 – 3)	0.90
Total post-LTx fungal infections	0 (0 – 1)	0 (0 – 0.5)	0.62
Total post-LTx undefined infections	0 (0 – 1)	0 (0 – 0.5)	0.16

Appendix 16. (*Continued*)

Variable	≥ Median Socialization score	<Median Socialization score	p-value
Total bacterial infections (pre/post-LTx)	2.8±2	2.6±2.4	0.71
Total viral infections (pre/post-LTx)	2 (1 – 3)	2 (1 – 4)	0.71
Total fungal infections (pre/post-LTx)	0 (0 – 1)	0 (0 – 1)	0.50
Total undefined infections (pre/post-LTx)	1 (0 – 1)	0 (0 – 1)	0.21
Total post-LTx complications	5 (2 – 8)	6 (4 – 13.5)	0.16
Deceased, n(%)	4 (12)	4 (13)	1.0

Data presented as mean±SD or median (interquartile range) or percentage (%). *p*-values ≤0.05 are considered statistically significant. Other diagnoses include hepatoblastoma and alpha-1-antitrypsin deficiency. DC: Discharge; FU: Follow-up; ICU: Intensive Care Unit; LOS: Length of Stay; LTx: Liver Transplant

Appendix 17. Summary of outcomes associated with above/below median Daily Living Skills scores

Variable	≥ Median Daily Living Skills score	<Median Daily Living Skills score	p-value
DEMOGRAPHIC DATA (at LTx assessment)			
Sex - Females - Males	 21 (54) 18 (46)	 14 (54) 12 (46)	1.0
Age, years	0.5 (0.34 – 0.97)	0.6 (0.44 – 0.79)	0.30
Diagnosis - Biliary atresia - Other cholestatic/metabolic - Acute liver failure - Other	 19 (49) 13 (33) 1 (3) 6 (15)	 18 (69) 8 (31) 0 (0) 0 (0)	0.09
PELD score	11 (3 – 17)	15 (8 – 19)	0.30
Social concerns, n(%)	3 (10)	7 (27)	0.16
Consanguinity, n(%)	2 (7)	1 (4)	1.0
NUTRITIONAL STATUS AND DELIVERY DATA (at LTx assessment)			
SGNA category, n(%) - Well nourished - Moderately malnourished - Severely malnourished	 3 (11) 9 (33) 15 (56)	 1 (7) 6 (40) 8 (53)	1.0
%IBW, n(%) - Well nourished - Moderately malnourished - Severely malnourished	 33 (85) 6 (15) 0 (0)	 20 (77) 5 (19) 1 (4)	0.37
Stunting - Yes - No	 4 (10) 35 (90)	 6 (23) 20 (77)	0.18
Neurodevelopmental/nutritional status combos (SGNA), n(%) - Adequate adaptive level + well nourished - Adequate adaptive level + malnourished - Inadequate adaptive level + well nourished - Inadequate adaptive level + malnourished	 3 (11) 19 (71) 0 (0) 5 (19)	 1(7) 5 (33) 0 (0) 9 (60)	**0.03**

Neurodevelopmental/nutritional status combos (McLaren), n(%) - Adequate adaptive level + well nourished - Adequate adaptive level + malnourished - Inadequate adaptive level + well nourished - Inadequate adaptive level + malnourished	 28 (72) 5 (13) 6 (15) 0 (0)	 7 (27) 2(8) 13 (50) 4 (15)	**0.0001**
Neurodevelopmental/nutritional status combos (WHO), n(%) - Adequate adaptive level + well nourished - Adequate adaptive level + malnourished - Inadequate adaptive level + well nourished - Inadequate adaptive level + malnourished	 28 (76) 3 (8) 6 (16) 0 (0)	 5 (20) 3 (12) 14 (56) 3 (12)	**<.0001**
Exclusively breastfed, n(%)	2 (7)	1 (5)	1.0
ANTHROPOMETRIC DATA (at LTx assessment)			
Weight, kg	6.9 (6 – 8.8)	7 (6.1 – 8.1)	0.80
Weight z-score	-0.32±1.1	-0.89±1.4	0.08
Height, cm	66 (61 – 68.5)	65.4 (62 – 69.5)	1.0
Height z-score	-0.76±1.3	-1.04±1.4	0.40
Head C, cm	41.6±2.2	42.2±1.8	0.30
Head C z-score	-0.68±1	-0.42±1.1	0.52
PRE-LTx COMPLICATIONS (at LTx assessment)			
Ascites, n(%)	19 (51)	13 (50)	0.91
Hepato-renal syndrome, n(%)	2 (5)	4 (15)	0.22
Hepato-pulmonary syndrome, n(%)	0 (0)	1 (4)	0.41
Encephalopathy, n(%)	3 (8)	2 (8)	1.0
Varices, n(%)	10 (27)	7 (28)	0.93

Appendix 17. (*Continued*)

Variable	≥ Median Daily Living Skills score	<Median Daily Living Skills score	p-value
Total pre-LTx infections	1 (0 – 2)	1 (0 – 2)	0.86
SELECT LABORATORY DATA (at LTx assessment)			
AST, IU/L	174 (93 – 293)	232 (154 – 352)	0.41
ALT, IU/L	128 (45 – 237)	179 (101 – 275)	0.22
GGT, IU/L	181 (74 – 362)	219 (66 – 1360)	0.26
Albumin, g/L	34 (28 – 38)	34 (31 – 36)	0.68
Total bilirubin, µmol/L	183 (26 – 262)	222 (136 – 306)	0.32
INR	1.2 (1.1 – 1.4)	1.1 (1.0 – 1.5)	0.28
PTT, seconds	40 (36 – 49)	38 (34 – 48)	0.44
Ammonia	49 (40 – 57)	53 (41 – 81)	0.51
CRP, mg/L	6.6 (0.9 – 8.5)	5.3 (1.4 – 11.8)	0.84
Urea, mmol/L	2.6 (2 – 3.5)	3.2 (2.7 – 3.8)	0.31
Creatinine, µmol/L	17 (13 – 23)	17 (14 – 19)	0.51
Vitamin D, nmol/L	22 (11 – 90)	22 (17 – 81)	0.90
Vitamin E, mg/L	9.5 (4 – 22)	8 (2 – 27)	0.49
POST-LTx OUTCOMES			
ICU LOS, days	10 (6 – 25)	12.5 (7 – 32)	0.35
Ventilator dependency, days	4 (2 – 18)	6.5 (3 – 19)	0.56
Total hospital LOS, days	43 (27 – 50)	45 (32 – 82)	0.36

Appendix 17. (*Continued*)

Variable	≥ Median Daily Living Skills score	<Median Daily Living Skills score	p-value
Weight at 6-mo FU, kg	11.7±3.5	10.9±1.8	0.38
Weight z-score at 6-mo FU	0.43±0.9	0.01±1.1	0.18
Height at 6-mo FU, cm	75.7 (72.6 – 80.9)	78.8 (76.1 – 81.4)	0.29
Height z-score at 6-mo FU	-0.79±1	-0.76±1	0.91
Weight velocity SDS at 6-mo FU	1.3±1.3	1.7±1.5	0.71
Height velocity SDS at 6-mo FU	1.19 (0.34 – 2.59)	0.59 (0.13 – 1.52)	0.39
Daily weight gain at 6-mo FU, g/day	13.91±8.7	11.01±8.7	0.32
Daily height gain at 6-mo FU, mm/day	3.95±1.4	3.77±1.2	0.70
Weight at 12-mo FU, kg	12.8 (11.3 – 14.4)	12.9 (11.2 – 13.9)	0.90
Weight z-score at 12-mo FU	0.72±1.1	0.47±0.9	0.46
Height at 12-mo FU, cm	84.2 (79.3 – 86.8)	85.8 (82.5 – 88.2)	0.39
Height z-score at 12-mo FU	-0.67±1.2	-0.69±1	0.95
Weight velocity SDS at 12-mo FU	2.53±1.6	0.16±0.9	**0.04**
Height velocity SDS at 12-mo FU	0.75±2.5	-0.04±2	0.35
Daily weight gain at 12-mo FU, g/day	9.64 (5 – 15.1)	9.1 (7.8 – 11.7)	0.95
Daily height gain at 12-mo FU, mm/day	3.2±1.3	2.9±1.3	0.65
Total post-LTx infections	4.3±2.6	6.5±5	**0.03**
Total post-LTx bacterial infections	1 (1 – 3)	2 (0 – 4)	0.43
Total post-LTx viral infections	2 (1 – 3)	1.5 (1 – 3)	0.59
Total post-LTx fungal infections	0 (0 – 0)	0 (0 – 1)	**0.01**

Appendix 17. (*Continued*)

Variable	≥ Median Daily Living Skills score	<Median Daily Living Skills score	p-value
Total post-LTx undefined infections	0 (0 – 1)	0 (0 – 1)	0.60
Total bacterial infections (pre/post-LTx)	2 (1 – 3)	3 (1 – 4.5)	0.30
Total viral infections (pre/post-LTx)	2 (1 – 3)	2 (1 – 4.5)	0.71
Total fungal infections (pre/post-LTx)	0 (0 – 1)	0 (0 – 1)	0.22
Total undefined infections (pre/post-LTx)	0 (0 – 1)	0 (0 – 1)	0.86
Total post-LTx complications	4.5 (2 – 8)	6.5 (5 – 15)	0.08
Deceased, n(%)	5 (13)	3 (12)	1.0

Data presented as mean±SD or median (interquartile range) or percentage (%). *p*-values ≤0.05 are considered statistically significant. Other diagnoses include hepatoblastoma and alpha-1-antitrypsin deficiency. DC: Discharge; FU: Follow-up; ICU: Intensive Care Unit; LOS: Length of Stay; LTx: Liver Transplant