

# One Liver for Four Children: First Clinical Series of Liver Cell Transplantation for Severe Neonatal Urea Cycle Defects

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**Background.** Urea cycle disorders (UCD) have a poor prognosis despite dietary and pharmacologic therapy, especially if the onset of the disease is within the neonatal period. They are promising target diseases for liver cell transplantation (LCT), which may be a less invasive alternative or supplementation to orthotopic liver transplantation.

**Methods.** Cryopreserved hepatocytes were isolated under good manufacturing practice conditions. Four children with severe neonatal UCD (age 1 day–3 years) received multiple intraportal infusions of cryopreserved hepatocytes from that same donor, a 9-day old neonate. Portal vein access was achieved surgically in two children, whereas the umbilical vein was suitable for interventional catheter placement in two neonates. Cell applications were carefully monitored by means of Doppler ultrasound and portal vein pressure.

**Results.** LCT was feasible in all children. No signs of portal vein thrombosis or extrahepatic shunting were observed. All children showed metabolic stabilization during observation periods of 4 to 13 months. One child with prenatally diagnosed ornithine transcarbamylase deficiency died after 4 months from a fatal metabolic decompensation.

**Conclusions.** Given the poor prognosis of UCD with conservative therapy, LCT caused considerable beneficial effects. Periods of hyperammonemia and clinically relevant crises could be reduced during an observation period of up to 13 months. Though cell therapy is not a permanent therapeutic option, bridging to liver transplantation may be substantially improved.

**Keywords:** Hepatocyte transplantation, Cryopreserved hepatocytes, Ornithine transcarbamylase deficiency, Carbamoylphosphate synthase deficiency, Citrullinaemia.

(*Transplantation* 2009;87: 636–641)

The cumulative incidence of all urea cycle disorders (UCD) is estimated to be 1:8000 life births (1). The prognosis of children with UCD has improved substantially as a result of dietary and pharmacologic treatment (2). However, medical

and dietary treatment cannot prevent metabolic decompensation leading to brain damage and early death in the majority of patients with neonatal UCD. Therefore, UCD have been the target for different enzyme replacement strategies. Whereas trials of gene therapy using viral vectors have been discontinued after the death of a patient in 1999 (3), orthotopic liver transplantation (OLT) is now a routine therapy improving long-term outcome (4). However, it requires major surgery with considerable risk for technical complications and perioperative metabolic derangement especially in neonates. Additionally, in most countries there is a lack of suitable donor organs.

As an alternative to OLT, liver cell transplantation (LCT) has been suggested. To our knowledge, five children with UCD have been treated with this innovative method since 1997 (5–9). In most of these cases, at least some improvement was observed. Different treatment protocols were followed regarding the type of liver cells (fresh or cryopreserved), cell dose, route of application, and immunosuppression. Therefore, it is difficult to judge both safety and efficacy.

In this article, we describe the first series of children with neonatal UCD who were treated with cryopreserved human hepatocytes from a single donor. Isolation and pro-

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Received October 28, 2008. Revision requested November 12, 2008.

Accepted November 27, 2008.

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ISSN 0041-1337/09/8705-636

DOI: 10.1097/TP.0b013e318199936a

**TABLE 1.** Patient data related to application of liver cells

	Patient 1	Patient 2	Patient 3	Patient 4
Diagnosis	CPS1-deficiency	Citrullinaemia	OTC deficiency	OTC deficiency
Sex	Male	Female	Male	Male
Age at diagnosis	Day 2	Day 3	Prenatal diagnosis	Day 4
Age at LCT	10 wk	3 [11/12] yrs	6 hr	9 d
Bodyweight at LCT	6 kg	14 kg	3.5 kg	3.9 kg
Donor	HLK024	HLK024	HLK024	HLK024
Mean vitality (%)	74	77	71	64
Vital cells/vital cells per kg	$1.37 \times 10^9 / 0.23 \times 10^9$	$1.46 \times 10^9 / 0.10 \times 10^9$	$0.64 \times 10^9 / 0.18 \times 10^9$	$0.56 \times 10^9 / 0.16 \times 10^9$
Total cells/total cells per kg	$1.87 \times 10^9 / 0.31 \times 10^9$	$1.89 \times 10^9 / 0.14 \times 10^9$	$0.94 \times 10^9 / 0.27 \times 10^9$	$0.87 \times 10^9 / 0.25 \times 10^9$
Applications	6	4	3	2
Portal vein access	Surgical middle colic vein	Surgical middle colic vein	Interventional umbilical vein	Interventional umbilical vein
Immunosuppression	Tacrolimus, methylprednisolone	Basiliximab, tacrolimus, prednisolone	Basiliximab, cyclosporin A, prednisolone	Tacrolimus, methylprednisolone

LCT, liver cell transplantation; CPS, Carbamoylphosphate synthase.

cessing of cells under granulocyte/macrophage progenitors conditions make these hepatocytes eligible as a drug. In three different centers, intraportal liver cell transfusions were applied to four patients under similar conditions making the individual results comparable.

## MATERIALS AND METHODS

### Patients

All of our patients had severe UCD with neonatal onset. Three children presented with hyperammonemic coma and required hemofiltration/hemodialysis. In patient 3, the diagnosis was made prenatally. Relevant clinical parameters and details on LCT are summarized in Table 1.

Patient 1 (carbamoylphosphate synthase 1 deficiency) presented at the second day of life. In the first 2 months, the boy had frequent hyperammonemic crises despite intensive pharmacologic and dietary treatment. Early OLT was declined by the surgeons because of cardiomyopathy which probably resulted from huge glucose supply. The patient was transferred to the metabolic center in Heidelberg for LCT at the age of 3 months. Unfortunately, severe brain damage had already developed as a result of prolonged and frequent hyperammonemic episodes.

In patient 2, citrullinemia was diagnosed at the third day of life. During the next 3 years, the patient experienced frequent hyperammonemic crises resulting in considerable psychomotor delay. Because her metabolic state was unstable despite intensive conservative treatment, OLT or LCT was suggested. The family opted for LCT in Heidelberg as an individual therapeutic trial.

The diagnosis of ornithine carbamylase deficiency in patient 3 was already established prenatally by mutation analysis. The index case was a brother who died in hyperammonemic coma at the age of 3 days despite intensive metabolic emergency therapy. The family opted against termination of pregnancy, and the boy was born at term by cesarean section.

The prognosis of children with prenatally diagnosed UCD is much better than in neonates who are clinically diagnosed but still remains guarded (10). Therefore, LCT was planned before birth. Conservative therapy was started immediately after birth, and LCT was initiated after 6 hr at the Department of Pediatrics in Hannover.

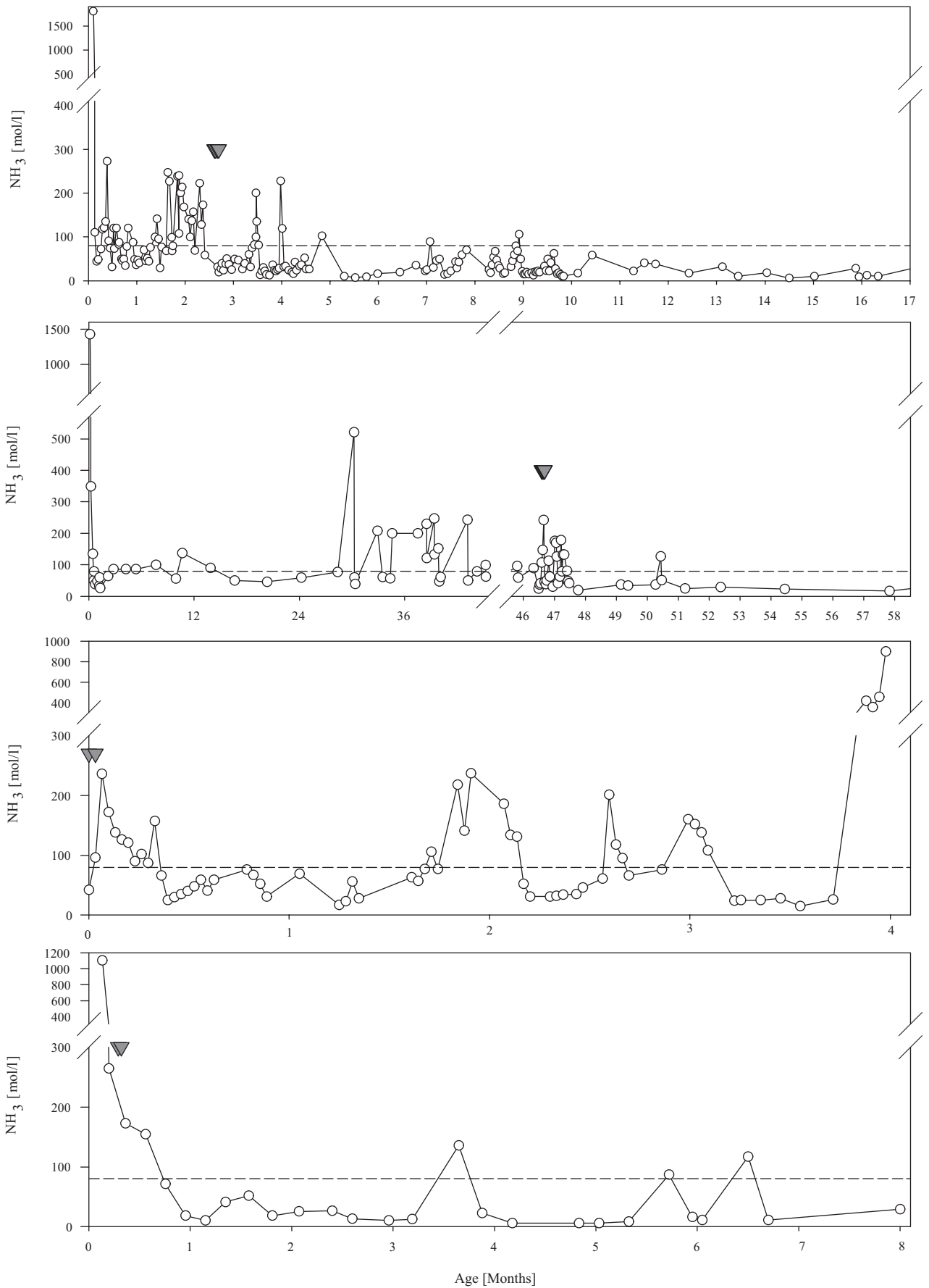
Patient 4 presented on the third day of life, and the diagnosis of ornithine carbamylase deficiency was made soon after the boy had been transferred to the University's children hospital in Padova. Control of ammonia levels proved to be difficult despite intensive pharmacologic treatment and extracorporeal detoxification. He was immediately put on the waiting list for OLT but because of the severe condition, LCT was suggested as an alternative and it was performed at day 8.

### Liver Cell Transplantation

Cryopreserved human hepatocytes manufactured under granulocyte/macrophage progenitors conditions were provided by an industrial partner (Cytonet GmbH & Co KG, Weinheim, Germany). Isolation of cells from livers not appropriate for whole organ transplantation was performed as described before (11). The four patients all received cells from the same donor, a 9-day old neonate. Perfusion of this organ yielded a total of  $20.5 \times 10^9$  hepatocytes, of which a total of  $5.6 \times 10^9$  cells were used for the therapeutic trials described in this article.

In patients 1 and 2, access to the portal vein was achieved by surgical insertion of a Hickman catheter into the middle colic vein. The position of the catheter was verified by intraoperative contrast imaging. In the two neonatal patients, patency of the umbilical vein allowed noninvasive interventional catheter placement in the left portal vein branch. In patient 3, the patent ductus venosus was intermittently blocked by an additional balloon catheter which prevented shunting of infused cells.

Cryopreserved hepatocytes were thawed under controlled conditions immediately before LCT. In the two neo-



natal patients, the dimethyl sulfoxide (DMSO) content of the cell suspensions was reduced from 5% to approximately 0.5% by an additional washing step. The cell suspension was applied manually over 20 to 60 min per session and was gently shaken during the procedure to avoid cell sedimentation. Details on the individual applications are given in Table 1. Vital signs were continuously recorded during and after the applications. Portal vein flow was monitored by Doppler ultrasound and measurement of the portal vein pressure in patient 1. Decreases in the flow velocity or increase in portal venous pressure occasionally prompted discontinuation of the application for 1 to 5 min. Immunosuppression was performed according to each center's guidelines for OLT (Table 1). All patients received steroids that were tapered down according to the individual center's protocols. Tacrolimus was started orally at a dosage of 0.3 mg/kg in two divided doses, the dosage was adapted as required to reach trough levels of 8 to 10 ng/mL. Cyclosporin A was started orally at a dosage of 10 mg/kg in two divided doses, aiming at trough levels of 150 to 200 ng/mL. In patients 2 and 3, 10 mg basiliximab intravenous was given additionally for induction at days 1 and 5.

## RESULTS

### Safety of Cell Application

Portal vein access was uncomplicated in all four patients. No major changes in cardiocirculatory parameters and portal vein flow were observed during cell applications (Table 1). Portal vein pressure in patient 1 increased by approximately 30% after each application and returned to baseline values after 8 to 12 hr.

Ten hours after the fourth liver cell transfusion, patient 2 suddenly became unresponsive and showed compromised pupillary response. Magnetic resonance imaging-angiography was normal, and blood levels of tacrolimus, electrolytes, and glucose. The child recovered after approximately 30 min. The remaining two hepatocyte applications were withheld. No further neurologic symptoms were observed thereafter.

### Efficacy of Liver Cell Transplantation

Plasma ammonia levels and protein intake of the patients are given in Figures 1 and 2, respectively. Glutamine levels were closely paralleled by ammonia levels in all patients.

In patient 1, two short episodes of hyperammonemia because of gastroenteritis and pulsed steroids for suspected rejection were observed shortly after LCT. During the next 11 months, he was admitted twice overnight because of intercurrent infections, but never developed life-threatening metabolic crises. Plasma urea increased after LCT from  $18 \pm 9$  mg/dL to  $25 \pm 8$  mg/dL. Protein intake could be increased 5 months after LCT but was lowered 2 months later because of hyperalimentation and extensive weight gain. The patient was put on the waiting list for OLT.

Ammonia levels in patient 2 normalized after reduction of the high-steroid doses given for immunosuppression and slight modification of the drug therapy. Apart from a brief increase after catheter removal ammonia levels remained

within the normal range. Plasma urea levels increased by approximately 40% ( $8 \pm 1$  vs.  $13 \pm 3$  mg/dL,  $P=0.01$ ). A gastroenteritis caused by norovirus infection 9 months after LCT could be managed without hyperammonemia. Protein intake could be increased 10 months after LCT.

In patient 3, no metabolic crises occurred in the neonatal period, however there was a single seizure at day 10 possibly as a result of hypophosphatemia. Two hyperammonemic episodes occurred during intercurrent infections and could be controlled by conservative therapy. Protein tolerance increased considerably (Fig. 2). Increased levels of urinary orotic acid excretion were found initially (255 mmol/mol creatinine, normal range  $<3.6$ ) and normalized (1.2 mmol/mol creatinine) after LCT. Urea concentration was in the low normal range even during anabolism. Sadly, the boy died despite hemodialysis after a fatal metabolic crisis possibly triggered by norovirus infection in combination with an upper airway tract infection at 4 months of age.

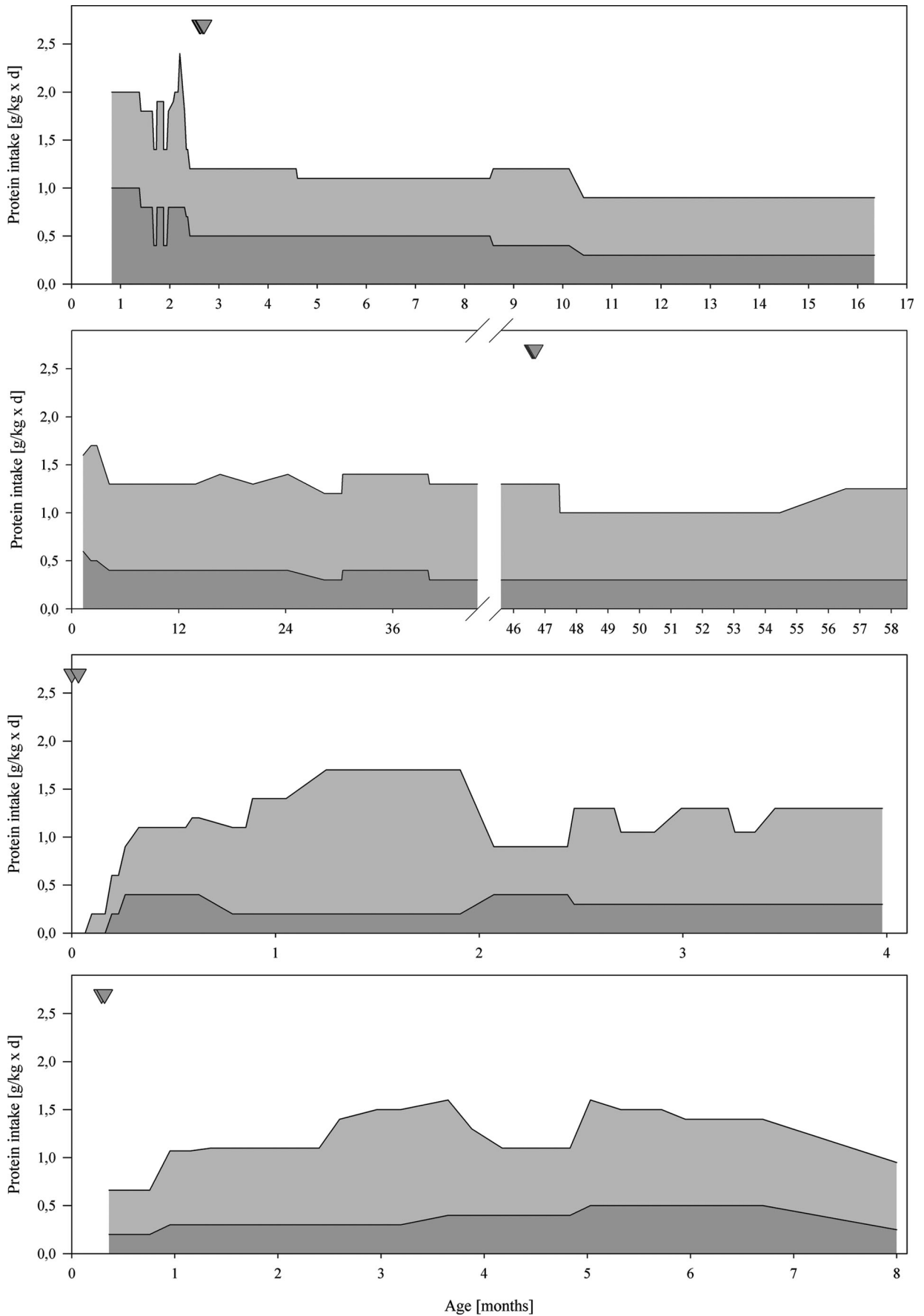
No significant hyperammonemia or clinical deterioration has been observed in patient 4 over a period of 6 months after LCT. Protein intake could be constantly increased (Fig. 2). At 6.5 months of age, a viral infection caused moderate and transient hyperammonemia. Protein intake was reduced thereafter. The patient's neurologic development is improving. High levels of urinary orotic acid ( $>2000$  mmol/mol creatinine, normal range  $<3.6$ ) were observed at the time of diagnosis. At the age of 5 months, an allopurinol challenge showed orotic acid levels within normal ranges. The boy is on the waiting list for OLT.

## DISCUSSION

Although the first two attempts at LCT for UCDs were not successful (5, 6), the three following patients experienced metabolic stabilization and could be bridged to OLT without further neurologic damage (7–9). Although encouraging, the published cases are heterogeneous. In this article, we present the first series of patients who were treated following similar protocols in three centers using cells from the same donor. Thus, results and complications can be directly compared. Moreover, since only about one third of the donor liver cells were used for the treatment of all four children, we could demonstrate the potential of LCT to overcome donor shortage.

No technical problems occurred during catheter placement and liver cell applications. Two patients showed transient hyperammonemia early after cell application accompanied by elevation of liver enzymes. Similar effects have been reported in animal experiments after infusion of albumin macroaggregates into the portal vein (12). It can be speculated that plugging of the sinusoids causes ischemia resulting in minor cell death, proteolysis, and hyperammonemia. Additionally, only approximately 30% of the transplanted cells are expected to engraft (13), the remnant susceptible to cell lysis with subsequent protein load. In one of our patients, we observed a severe neurologic complication that appeared to be related to cell application. The symptoms were suggestive of transient brainstem ischemia as has been described in patients after bone marrow transplantation, and may have been caused by the cryopreservative DMSO (14, 15). Similar adverse reactions have been reported for tacrolimus (16) and other calcineurin inhibitors (17) even with therapeutic blood

**FIGURE 1. (Continued)** Ammonia levels of patients 1 to 4. *Gray triangles* indicate the time points of LCT, the *dashed lines* age-appropriate ammonia levels.



levels. We decided to reduce DMSO content of the infused cell suspension after this episode, although this meant a reduction in the vitality of cells.

Several immunosuppressive regimens have been used in animal experiments and human LCT. In our patients, calcineurin inhibitors and basiliximab were well tolerated. High-dose steroid therapy proved to be difficult probably because of increased protein catabolism (18). Cell rejection after LCT is hard to detect as the amount of engrafted cells is small. We speculate that rejection occurred in patient 3 leading to a fatal metabolic decompensation. In this patient, low cyclosporin A levels were transiently observed during an episode of severe gastroenteritis. Thus, LCT probably warrants continuous and sufficient immunosuppression. Encouraging results of steroid-free immunosuppression protocols in OLT (19) could be a perspective.

Clinical and metabolic stabilization was noted in all patients. Urea production (patients 1 and 2) and excretion of orotic acid (patients 3 and 4) improved in addition to a reduction of frequency and severity of hyperammonemic episodes. However, a definitive follow-up parameter to judge the function of transplanted cells in vivo is lacking. Thus, beneficial effects caused by the transplanted cells cannot be clearly distinguished from those achieved by improved conservative therapy in the two older children. Nevertheless, in contrast to their previous history no clinically relevant crises have been noted in both patients over an observation period of 13 and 11 months, respectively. One has been listed for OLT. In the two neonates, LCT was conducted much earlier with the primary aim of bridging to OLT without neurological damage. It has been working well for patient 4, who is also on the waiting list for OLT. From the tragic course of patient 3, we learned that the time point for loss of cell function is almost impossible to predict.

In conclusion, this study demonstrates that it is technically feasible to develop a pharmaceutical preparation of liver cells and use it in children with severe neonatal UCD. The cryopreserved cell preparations can be used on demand and have the potential to significantly improve long-term outcome by bridging the patients to OLT without further neurological deterioration. As a consequence our series should be extended into a pharmacologic clinical trial.

**FIGURE 2. (Continued)** Total protein intake (lighter gray areas) and supply with essential amino acids (darker gray areas) in patients 1 to 4. Gray triangles indicate the time points of LCT.

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