Transplantation of high risk donor livers

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CHAPTER 2

Liver Transplantation in Groningen, The Netherlands: A Single Center Status Report

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Abstract

The liver transplantation program of the University Medical Center Groningen in the Netherlands was started in 1979, making it one of the first programs worldwide. During the past 36 years, a total of 1478 liver transplantations have been performed, 459 of which were in children. One of the first patients transplanted in 1979 is still alive and is one of the longest surviving patients after liver transplantation worldwide. During the last decade, an increasing number of donation after circulatory death (DCD) donor livers have been accepted for transplantation. Over 30% of the livers transplanted in Groningen come from DCD donors. These livers have an increased risk of developing non-anastomotic biliary strictures (NAS). One of the main research topics in Groningen has been the pathogenesis and prevention of NAS. In an attempt to reduce the incidence of NAS after liver transplantation, machine perfusion technology has been developed as an alternative to the traditional method of static cold storage. Researchers of the Groningen liver transplant team were the first in the world to report a method of ex situ normothermic machine perfusion of human donor livers. The efficacy and safety of various types of machine perfusion are currently studied in both animal models and clinical trials. A second line of research in Groningen focuses on alterations in the blood coagulation system in patients with liver disease and undergoing liver transplantation. Groningen researchers were the first to describe a 'rebalanced state' of the coagulation system in patients with liver disease, making them prone to both bleeding and thrombo-embolic complications. Clinicians and researchers at the Groningen liver transplant program will continue to collaborate with a shared focus and the aim to provide innovation and the highest level of care to patients with end stage liver disease.

A brief history of liver transplantation

Pioneers of the field

For approximately 50 years it was thought that the first liver transplantation was reported in 1955 by Welch and colleagues, who performed a series of auxiliary liver transplants in dogs (1). Recently, however, the 1952 work of Vittorio Staudacher came to light (2). He describes an orthotopic liver transplantation (OLT) method performed in dogs, similar to the procedure used in humans today (3). In 1958, surgeons from the Northwestern University in Chicago and Brigham Hospital in Boston conducted liver transplantation experiments in dogs and found that transplantation success hinges on several prerequisites (4,5). First, prevention of ischemic injury was indispensable (4). This was achieved through hypothermic preservation, in this case by flushing the graft with chilled Ringer's lactate. Second, congestive damage to the recipients' splanchnic and systemic venous vasculature was avoided using a venous bypass, yielding improved survival (4,6). During these early stages, developments in kidney transplantation provided a stimulating example to the emerging field of liver transplantation (7). Prior experience with renal transplantation facilitated the use of immunosuppressive agents. For example, treatment with azathioprine before and after liver transplantation together with high doses of prednisone administered at the onset of rejection, increased the overall success of liver transplantation (8). Over the course of 30 years, these developments led to the world-wide implementation of liver transplantation.

The first human liver transplantation

The first human liver transplantation was performed by Thomas Starzl at the University of Colorado in 1963. The operation involving a three-year-old boy, was complicated by an unforeseen degree of adhesions, causing massive – and ultimately fatal – hemorrhage (9). Nevertheless, Starzl and his team performed three more transplantations that same year (9,10). All three cases were complicated by a pulmonary embolism shortly after surgery, which limited follow-up to a maximum of 22 days. It was presumed that antifibrinolytic therapy with ε-aminocaproic acid (EACA), together with the peri-operative administration of cryoprecipitate and blood transfusions resulted in venous thrombosis (9). In addition, the tubing used for the veno-venous bypass activates the coagulation system. By January 1964, three different centers across the world had performed 7 human liver transplantations, all with unsatisfactory results (6, 10, 11), leading to a consensus that the procedure was too technically challenging (12,13). However, as the research focus shifted towards immunosuppression and organ preservation, the introduction of antilymphocyte globulin (ALG) as part of a so-called "triple drug cocktail" (together with azathioprine and prednisone), signified a turning point (14,15). After a series of successes with canine studies, attempts to perform human liver transplantation were resumed in the late sixties. During the summer of 1967, Starzl performed the first successful human liver transplantation on an 18-month old girl in Denver, CO (16). This inspired several other centers across the world. Sir Roy Calne started a human liver transplantation program in Cambridge, UK in 1968 (17). By 1969, Starzl's group in Denver and Calne's group in Cambridge had successfully performed a total of 25 liver transplants (18). Between 1972 and 1979, the university hospitals in Hannover (Germany), Paris (France), and Groningen (The Netherlands) started liver transplantation programs. Together, these academic hospitals laid the foundation for the modern practice of liver transplantation (12,13).

Liver transplantation in Groningen

The first liver transplantation in Groningen took place in 1979 and was performed by surgeons Ruud Krom and Gauke Kootstra, in collaboration with hepatologist Chris Gips. At that time, the survival rate after liver transplantation in other centers was approximately 30% (20,21). Euro-Collins' solution was used as preservation solution (23). Immunosuppression consisted of prednisone and azathioprine. The surgical technique was performed as developed by Starzl and Calne , with the exception of the biliary anastomosis; Krom and colleagues applied an end-to-end choledochocholedochostomy using a T-shaped biliary stent, in preference to the cholecystojejunostomy with Roux-Y reconstruction as described by Starzl (9, 20, 21, 23, 24). This innovation resulted in a significant reduction of biliary complications and is presently the preferred technique worldwide (25). Notably, the Groningen transplant team added selective bowel decontamination to the protocol, which was later adopted by other centers (19,26). Despite elaborate preparation, the first patient to receive a donor liver in Groningen succumbed due to massive intraoperative hemorrhage (24). Yet in the first cohort of 27 procedures, 1- and 2-year survival rates were 60%, and the reported quality of life was excellent (23). The recipient of the first successful liver transplantation performed in Groningen in 1979 is alive today and one of the longest surviving liver transplant recipients worldwide. Based in part on the favorable results reported by the Groningen team, the National Institutes of Health declared liver transplantation an established therapy for end-stage liver disease in 1983 (27).

In the Netherlands, pediatric liver transplantations are only performed in Groningen. The first liver transplantation in a child was performed in 1982 (28). In the years to follow, the liver transplant program in Groningen was refined through scientific research. Since the start of the program, a total of 1478 liver transplantations have been performed throughl January 2016, 459 of which in children (**Figure 1**). From the year 2000 on, 1- and 5-year survival rates for adults and children increased to around 90% (**Figure 2**). These increases were due to improvements in patient selection, organ preservation, surgical technique, and the development of new immunosuppressive regimens (29).

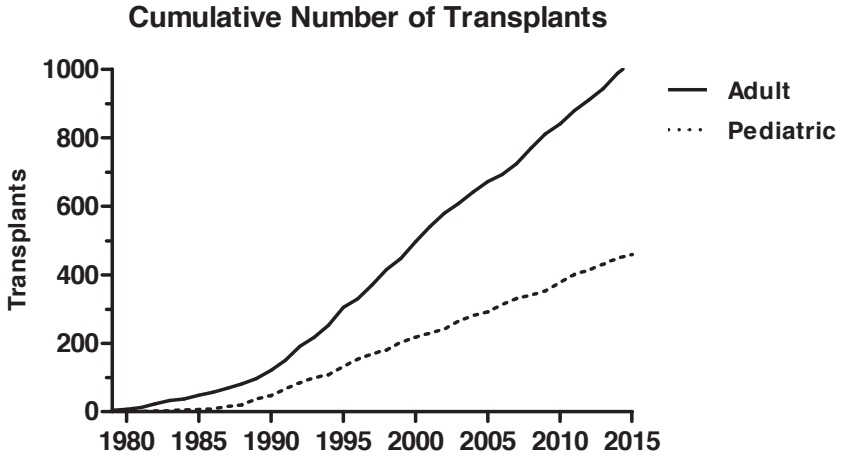

Figure 1. Cumulative number of transplants performed between 1979 and 2015 in Groningen. In 1979 a total of 4 adult liver transplantations were conducted. The first pediatric liver transplantation was performed in 1982. Around 1990, the number of transplantations in both adults and children started to increase due to increasing experience and good results. During the last 36 years, a total of 1019 adult liver transplantations and 459 pediatric liver transplantations have been performed.

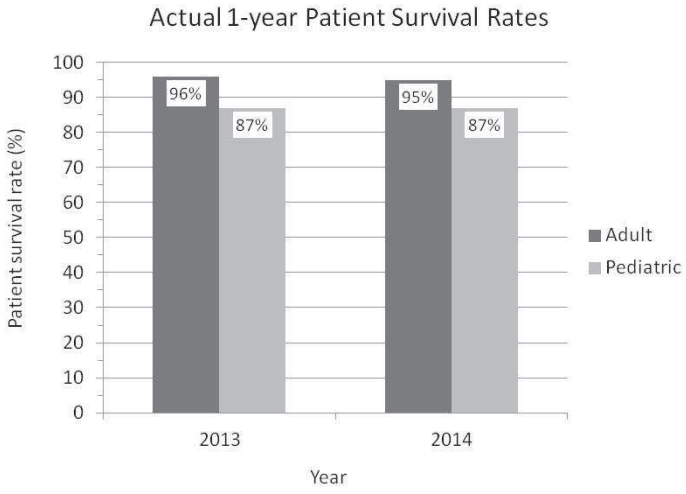

Figure 2. Actual 1-year patient survival rates in adult and pediatric liver transplant recipients transplanted in 2013 and 2014, in Groningen.

Current practice

Indication for transplantation

The most common indications for adult liver transplantation in Groningen are primary sclerosing cholangitis (PSC), post-alcoholic and post-viral cirrhosis (**Figure 3**). The incidence of non-alcoholic steatohepatitis (NASH) is gradually increasing and this type of liver disease is expected to become one of the leading indications for liver transplantation in the Netherlands in the coming years. In children, a liver transplantation is most commonly performed in the setting of congenital disorders (cholestatic or metabolic), pediatric cancer (hepatoblastoma) or acute hepatic failure. In more than 50% of the cases, the leading indication for pediatric liver transplantation is biliary atresia.

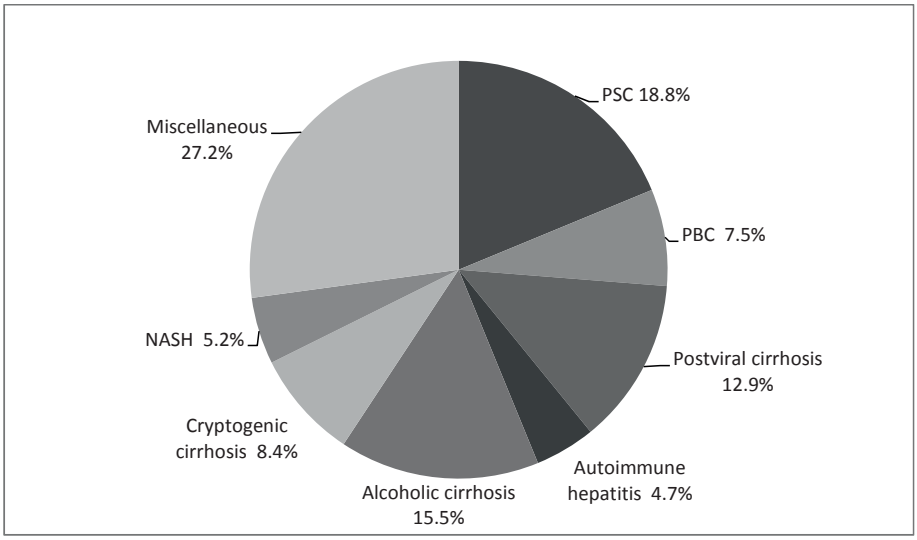

Figure 3. Indications for adult liver transplantation in the time period 2000 - 2014. Primary sclerosing cholangitis, postviral cirrhosis and postalcoholic cirrhosis were the most common indications for transplantation. Abbreviations : PSC, primary sclerosing cholangitis; PBC, primary biliary cholangitis; NASH, non-alcoholic steato-hepatitis.

The Netherlands is one of the countries that belong to the Eurotransplant network for organ sharing. The allocation of donor livers is based on a patient-oriented system. Until December 2006, the Child-Pugh score was used to prioritize patients on the waiting list. Patients with equal Child-Pugh scores were sorted according to the time they had spent on the waiting list. To improve donor liver allocation, the model for end-stage liver disease (MELD) score was introduced in the Eurotransplant region in 2006, leading to an allocation process in which waiting time became less important and mortality on the waiting list decreased.

Immunosuppressive therapy

Protocols for immunosuppressive therapy in the Groningen liver transplant program have gradually changed during the past decades. While the original immunosuppressive protocol in the early 1980s was based on azathioprine and relatively high doses of prednisone, the current protocol is based on a calcineurin inhibitor and a rapid taper of prednisone. While cyclosporin was the only calcineurin inhibitor available between the mid-1980s and mid-1990s, it is currently almost completely replaced by tacrolimus. However, until a few years ago, cyclosporin was still considered the calcineurin inhibitor of choice in patients with an immune-mediated liver disease, such as PSC, primary biliary cholangitis (PBC), or autoimmune hepatitis. Induction therapy has almost universally been part of the immunosuppressive protocol in the Groningen liver transplant program during the past decades. In the 1980s and 1990s this consisted of cyclophosphamide, which was replaced by basiliximab around 1996. Currently, basiliximab is still being used as induction therapy in both adult and pediatric liver transplant recipients.

Intraoperative hemorrhage

For many years, bleeding complications were one of the most frequent causes of morbidity and mortality in liver transplant recipients. The mechanisms underlying this problem, as well as developing strategies to reduce intraoperative blood loss, have been the subject of extensive research in Groningen during the past decades. This has generated a large number of scientific publications and PhD theses. A few important milestones include the identification of hyperfibrinolysis as a critical cause of increased blood loss during liver transplantation, the beneficial effects of the antifibrinolytic drug aprotinin, and identification of the risk of thrombo-embolic complications in liver transplant recipients (30-40). Fundamental research on changes in the hemostatic system in patients with liver disease performed in Groningen has increased the understanding of coagulation abnormalities in patients with liver disease. Whereas the traditional dogma asserted that a diminished coagulation capacity (hypocoagulability) in patients with liver disease was the net result of liver disease-induced changes in hemostasis, it is currently widely accepted that the hemostatic system in these patients are in a so-called 'rebalanced' state, in which both bleeding and thrombotic complications are more likely to occur (41). For example, increased bleeding during liver transplantation is more frequently caused by increased portal venous pressure than by a hypocoagulable state (37). Based on evolving new insights such as these, perioperative blood transfusion requirements have been greatly reduced. Currently, around 25% of all adult first liver transplantations in Groningen are conducted without the need for blood transfusion (33,37).

Shortage of suitable donor livers

The increasing demand for liver transplantation has resulted in a world-wide donor organ shortage. Many efforts have been made to expand the donor pool, such as the acceptance of extended criteria donor (ECD) livers, split- and domino liver transplants, and the introduction of living donor liver transplantation.

Living donor liver transplantation

The Groningen team started a living donor liver transplant program in 2004. Currently, living donor liver transplantations comprise up to 1/3 of all pediatric liver transplantations in Groningen, with 1- and 3- year patient survival rates of 96% and 88%, respectively.

Extended criteria donor livers

In 1968 guidelines defining brain death were published, enabling standardized use of livers derived from brain-dead donors (42). The increasing success rate of liver transplantation, resulted in more indications for liver transplantation, a higher need for donor organs and, subsequently, a shortage. In the late 1990s, the shortage of suitable donor livers for transplantation resulted in a gradual widening of the acceptance criteria for donation. More frequently donor livers were accepted that carried an increased risk for early graft failure or disease transmission. These, so called 'extended criteria donor' (ECD) livers now comprise the majority of donor livers accepted for transplantation in Groningen. ECD livers include steatotic livers, livers donated after controlled circulatory death (DCD), and livers from elderly donors. The first attempts to use DCD livers were made in the 1980s (43-45). In the Netherlands, a national protocol for DCD was implemented in 2001 (46). Since then, the number of DCD liver transplants has gradually increased and now contributes to around 40% of all liver transplants performed. While the number of DCD liver transplants in the Netherlands is still rising, some other countries that belong to the Eurotransplant network (i.e. Germany) do not allow DCD donation (47). The first DCD liver transplant procedure in Groningen was performed in 2004. Whereas overall survival of DCD liver transplant recipients is comparable to that of DBD liver recipients, DCD transplants are associated with an increased rate of biliary complications (**Figure 4**).

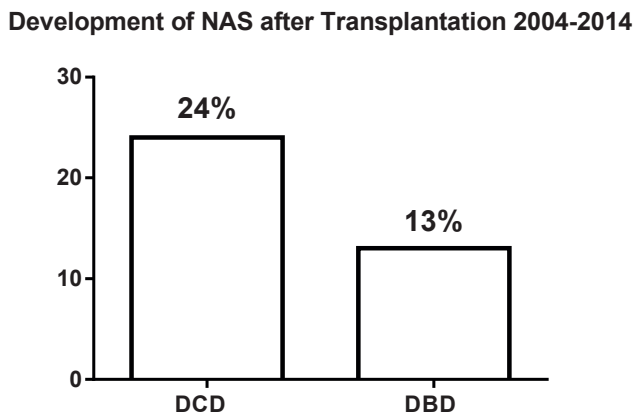

Figure 4. The cumulative incidence of non-anastomotic strictures (NAS) after DBD and DCD liver transplantations performed between 2004 and 2014.

Biliary Complications

Biliary complications occur in 10% to 40% of liver recipients, depending on the definitions used (48-50). Most common are anastomotic bile leakage (2-21%) and anastomotic or non-anastomotic (NAS) bile duct strictures (1-30%) (51). In DCD liver transplantation, NAS occur in up to 30% of cases, with 50% of these resulting in retransplantation or death. The majority of cases of NAS after DCD liver transplantation are observed within 6 months after transplantation. Due to the increasing number of DCD liver transplantations in our center, the pathogenesis and prevention of NAS has become an active area of research with the overall aim to reduce the high rate of biliary complications after this type of liver transplantation. Proposed mechanisms involved in the development of NAS include ischemia-related injury, immune-mediated injury, cytotoxic injury induced by hydrophobic bile salts, and impaired epithelial regeneration (48,52,53) (**Table 1**). The effect of post-transplant bile composition and the role of bile salt toxicity in the development of NAS was extensively studied by the Groningen group, both in experimental animal studies and clinical studies (54,55). It has been demonstrated that bile salts contribute to the development of biliary injury due to the cytotoxic effect on biliary epithelium and other cell types in the bile duct wall. In three independent clinical studies, including one collaborative study from Groningen and Boston (Massachusetts General Hospital) it was recently demonstrated that almost all human donor livers grafts have signs of severe biliary epithelial injury at the time of transplantation (53,56,57). This finding has changed our understanding of the pathogenesis of NAS (52). Although biliary epithelial cell loss is almost universally present in donor livers at the time of transplantation, only a minority (up to 15% in DBD liver transplantation and up to 30% in DCD liver transplantation) develop NAS. This finding suggests that insufficient biliary epithelial regeneration rather than the initial amount of epithelial injury is the mechanism underlying the development of NAS. This hypothesis was confirmed by the identification of histological injury of the peribiliary glands and the peribiliary vascular plexus as independent risk factors for the development of NAS after liver transplantation (52,53,58). The peribiliary glands have been identified as a niche of biliary progenitors cells, which are mobilized after severe biliary epithelial injury (58,59). These findings have made the peribiliary vascular plexus and the peribiliary glands important new targets in our research focusing on the prevention of NAS after liver
transplantation. A much promising method that may enable better preservation of the bile ducts of liver grafts is machine perfusion.

Table 1. Four main mechanisms underlying the development of non-anastomotic biliary strictures (NAS) after liver transplantation.

Ischemia-related injury caused by:
- Warm and cold ischemia associated with organ preservation
- Warm ischemia in donation after cardiac death donors
- Inadequate preservation of the peribiliary capillary plexus
Immune-mediated injury due to:
- ABO-incompatible transplantation
- CMV-infection
- Female organs in male recipients
- Chemokine receptor CCR5-Δ32 polymorphism
Cytotoxic effect of hydrophobic bile salts caused by:
- Inadequate flush out of bile from the bile ducts at organ retrieval
- High biliary bile salt/ phospholipid ratio after transplantation
- Impaired cholehepatic shunt with intracellular accumulation of bile salts in cholangiocytes
- Impairment of the protective HCO_3^- umbrella at the canalicular membrane of cholangiocytes
Impaired biliary epithelial cell regeneration due to:
- Insufficient blood supply to the peribiliary glands

Machine perfusion

First described by Charles Lindbergh and Alexis Carrel in 1935, machine perfusion is an organ preservation technique that involves the *ex situ* perfusion of donor organs using a machine (60). During machine perfusion, an organ is artificially circulated with (a) blood (substitute) provided through the afferent vessel(s). Machine perfusion can be performed in a myriad of ways. Perfusion solution, temperature of the perfusion solution, oxygenation of the perfusion solution, etcetera are all variables in machine perfusion that are being investigated by different research groups including Groningen. The optimal execution of machine perfusion remains to be established and depends on the indication for the perfusion.

A Promising Technique

In general, machine perfusion can serve three different goals: a) improve organ preservation, b) allow functional assessment of a donor organ prior to transplantation, and c) allow longer preservation of donor organs. Due to the increasing use of sub-optimal quality donor organs, such as ECD livers, machine perfusion is currently receiving much attention. It has the potential to provide better preservation of ECD liver grafts, which may contribute to a reduction of graft-related complications after transplantation and may help expand the donor liver pool. Currently, the most widely studied methods of liver machine preservation include hypothermic (4-12 ^{o}C) and normothermic (37 ^{o}C) machine perfusion (61).

Oxygenated hypothermic machine perfusion (HMP) offers the ability to 'resuscitate' DCD livers. In several animal and human studies oxygenated HMP has been shown to increase ATP content in DCD livers, while hepatic ATP content is virtually nil in DCD livers preserved by traditional static cold storage (SCS) only (62,63). Due to a sustained ATP production, oxygenated HMP reduces ischemia-reperfusion injury and consequently bile

duct injury. After a series of experimental animal studies, the technolgy of oxygenated HMP (using a combination of arterial and portal perfusion) was recently tested in a human study in DCD liver transplantation. The results of this first clinical study have shown an excellent outcome with 100% graft and patient survival rates after a complete follow up of one year in all 10 patients included (R. van Rijn, R.J. Porte, et al. unpublished results). These favorable results have led to the initiation of a randomized controlled multicenter trial comparing oxygenated HMP with traditional SCS in DCD liver transplantation. The primary outcome parameter in the trial will be the incidence of NAS at 6 months after transplantation (ClinalTrial.gov identifier NCT02584283)

Normothermic machine perfusion (NMP) is performed at 37 °C, resulting in metabolically active livers, offering the ability to assess donor liver function and viability *ex situ.* By assessing viability of 'sub-optimal' quality livers that would otherwise have been discarded, more livers could be potentially identified as "transplantable". The first study demonstrating the feasibility of NMP of human donor livers was published by researchers from Groningen (64). Current evidence indicates that bile production and a decrease in lactate concentration in the perfusion fluid during NMP are important criteria that can be used to indicate well-functioning and potentially transplantable livers (65). The device that was developed by Groningen-based researchers to enable machine perfusion of human donor livers is now commercially available (www.organ-assist.nl) and allows perfusion at variable temperatures in a range of 10 to 37 ^{o}C (**Figure 5**). Researchers at the Groningen Transplant Center have not only been pioneering in the field of liver machine perfusion, but also in the fields of kidney and lung machine perfusion. This has led to the clinical introduction of machine perfusion technologies in all three types of organ transplantation. To facilitate the clinical application of machine preservation of donor livers, kidneys and lungs, a novel organ perfusion room has been developed. This dedicated facility for donor organ perfusion has been called the Organ Preservation & Resuscitation (OPR) unit and allows simultaneous perfusion of a pair of donor lungs, a liver and two kidneys (**Figure 6**).

Future Research

Ongoing research at the University of Groningen focuses on different approaches to improve our machine perfusion protocols, such as novel perfusion fluids with enhanced oxygen binding capabilities and dynamic temperature machine perfusion. More fundamental work focuses on the precise nature of NAS after transplantation, and how to counter the underlying mechanisms that cause these complications, including insufficient biliary regeneration from the peribiliary glands.

Both fundamental and applied research findings will be required to improve the current practice of ECD and DCD liver transplantation. It is our view that ultimately, an organ will undergo several personalized stages of machine perfusion, subdivided into a primary stabilization phase, a viability testing phase, and a treatment phase. Hypothetically, the organ will first be stabilized with oxygenated HMP, minimizing any ongoing effects of ischemia and replenishing energy stores. Consequently, viability testing can be performed using NMP, which should determine the subsequent treatment regimen depending on the organ's metabolic demands. As technology advances, machine perfusion systems will become increasingly dynamic, so that in the end, each organ will receive a tailor-made machine perfusion treatment.

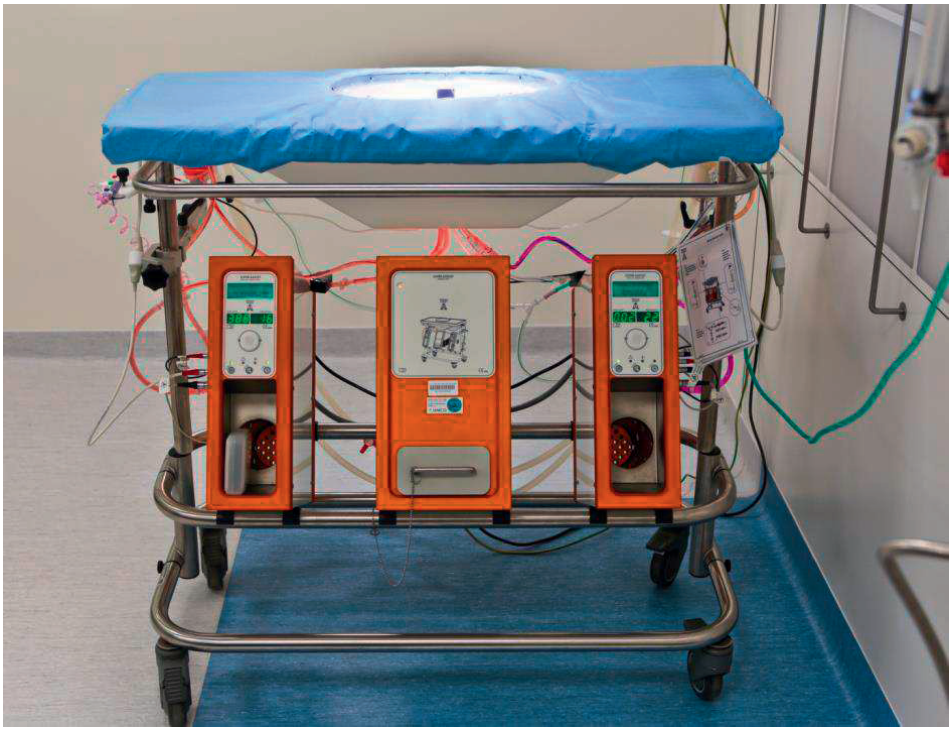

Figure 5. Photo of the Liver Assist, a perfusion device for preservation of human donor liver developed by researchers in Groningen (Organ Assist, Groningen, the Netherlands). The Liver Assist is a pressure-controlled system that allows perfusion of a donor liver via both the portal vein and hepatic artery. The temperature range can be adjusted between 10 and 37 °C.

Figure 6. Photo of the Organ Perfusion & Resuscitation (OPR) unit at the University Medical Center Groningen, the Netherlands. The OPR unit is a central facility within the operation center that allows simultaneous machine perfusion of a donor liver, a pair of donor lungs and two donor kidneys.

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