Ex Vivo Normothermic Machine Perfusion

15

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Historical Background

Since the first human liver transplant by Thomas Starzl in 1963, liver transplantation (LT) has become the standard therapy for end-stage liver disease, but organ preservation methods have remained largely unchanged. The graft is flushed and cooled with preservation fluid, then stored in an icebox [1]. Static cold storage (SCS), still the gold standard for organ preservation, inevitably produces ischemia/reperfusion injury (IRI) [2, 3]. Fortunately, most grafts can tolerate the injury, but severe IRI can lead to major complications and death.

The detrimental effects of SCS are magnified in marginal organs, and particularly in organs from donors after circulatory death (DCD). The increasing use of marginal organs has spurred interest in improving organ preservation techniques and tools for determining the suitability of marginal organs for transplantation [4, 5].

The concept of machine perfusion (MP) was introduced by Alexis Carrel and Charles Lindberg in 1935 in their work "The Culture of Organs." As early as 1970, Thomas Starzl described the potential benefits of hypothermic oxygenated ex-vivo machine perfusion. He wrote: "After excision of the liver, it can be transplanted immediately or placed in a conservation chamber employing low-flow perfusion, hyperbaric oxygenation, and hypothermia. Using the latter method, the organ can be kept in good conditions for as long as eight hours" [6]. At the time, however, given the reliability of SCS for standard criteria grafts, the logistical and financial challenges of MP led to temporary discontinuation of research on this approach.

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More recently, as technology has advanced and the use of marginal organs has increased, preclinical and clinical studies testing MP have spread from kidney grafts to liver grafts. Currently, several machines are available for clinical use, and ex-situ perfusion of donor livers can be performed at four different temperature ranges: 0–12 °C (hypothermic machine perfusion; HMP); 13–24 °C (mid-thermic machine perfusion; MMP); 25–33 °C (subnormothermic machine perfusion; SMP), and 35–38 °C (normothermic machine perfusion; NMP) [7]. Published reports of MP for liver grafts include results from hundreds of patients worldwide, but several controversies are yet to be solved, including identification of grafts and recipients who might benefit the most from MP.

Rationale

In solid organ transplantation, grafts are exposed to ischemia from the time of cross-clamping in the donor until reperfusion in the recipient. During procurement the graft is abruptly deprived of oxygen, cooled at 4 °C with preservation fluids and slush ice, and its metabolism is slowed but not completely stopped.

Oxygen is essential for cellular activity and production of ATP. As soon as blood flow ceases, the supply of nutrients and oxygen stops. ATP levels rapidly drop, anaerobic metabolism begins, and metabolic waste products accumulate. ATP loss leads to disabling of membrane pumps and membrane integrity damage. This causes edema, influx of calcium, phospholipase activation, inflammation, and cellular death [7, 8].

Furthermore, in an ischemic environment, xanthine dehydrogenase is converted to xanthine oxidase which, during reperfusion in the presence of oxygen, converts accumulated products into free radicals.

When the liver is reperfused, there is massive production of reactive oxygen species (ROS) and cytokines, neutrophil infiltration and impaired hepatic microcirculation leading to inflammation, cellular death (cholangiocytes are most susceptible), and loss of functioning parenchyma. The resulting clinical scenarios can range from silent damage to early graft dysfunction (EGD), primary nonfunction (PNF), and ischemic-type biliary lesions (ITBL) [7, 9–11].

SCS is based on the concept that cooling diminishes cellular metabolism and minimizes ATP depletion. With every 10 °C drop in temperature, metabolism is slowed twofold, but it is never completely stopped, as ATP consumption continues at 1 °C [7].

The ideal method of preservation should mimic the physiological conditions as much as possible in order to reduce IRI-related damage, prolong preservation time, reduce post-LT complications, allow organ viability assessment, and facilitate extended use of marginal organs.

SCS fails to accomplish the majority of these targets.

NMP, on the other hand, perfuses the graft with normothermic, blood-based solutions that recreate the physiological environment, thereby tending to decrease the detrimental effects seen with SCS. NMP should enable prolonged preservation, allow the organ to recover from injuries incurred during retrieval, permit evaluation of organ function before implantation, and minimize IRI injury by reducing the cold ischemic time.

However, the precise mechanism that underlies the beneficial effects of NMP is not completely clear. Probably, normothermic perfusion helps to maintain a healthy endothelium and replenish adenosine triphosphate (ATP). The importance of increasing hepatic ATP in LT has already been demonstrated, with a direct correlation between high hepatic ATP content and good post-transplant outcome [4]. The role of NMP in ATP regeneration has been confirmed in porcine models, where initiation of NMP has been followed by rapid recovery of ATP as well as mitochondrial ATP-ase activity [11]. More recently, human studies have proved histological evidence of glycogen depletion during NMP.

Glycogen preserves hepatocellular integrity and function by supplying glucose for ATP generation. Once glycogen is consumed, ATP depletion ensues, leading to irreversible cell injury and necrosis.

Difference in gene expression between transplanted human NMP and SCS livers has also been shown [4]. When gene expression was compared between pre- and post-reperfusion biopsies, the genes upregulated after NMP were mainly those involved in the control of inflammation. In contrast, the upregulated genes in SCS were mainly those implicated in inflammation, apoptosis, and activation of coagulation [12].

Further, NMP has been shown to reduce injury to liver parenchyma and improve epithelial regeneration in extrahepatic bile ducts, thus preventing the development of ischemic cholangiopathy [13].

Technology

Several NMP circuits have been described that use components developed for cardiopulmonary bypass. Main elements are: a blood reservoir, a pump (some circuits consist of two pumps, one for the portal vein and one for the hepatic artery), an oxygenator, and a heat exchanger. The devices currently used in clinical trials are OrganOx Metra® (OrganOx Ltd., Oxford, UK), Liver Assist®(Organ Assist, Gronigen, the Netherlands), OCS Liver System® (Transmedics, Andover, MA, USA), and the Cleveland NMP circuit (Cleveland Clinic, Cleveland, OH, USA). The machines differ in the type of circuit (closed circuit vs open drainage), type of arterial flow (pulsatile vs continuous), portability vs not, and degrees of automation (regulation of vascular pressures, flows, and blood gases).

Most published clinical trials have tested the OrganOx Metra® device. This machine provides automated pumping, oxygen/air delivery, and heat exchange in order to preserve the perfusate at normal temperature, within physiological ranges for pO_2, pCO_2, pH and at physiological pressures in the vascular hepatic inflow and outflow (hepatic artery pressure from 60 to 75 mmHg; inferior vena cava pressure from 1 to 2 mmHg). Portal flow is continuously measured, but portal pressure is not. The perfusate is pumped out of the inferior vena cava using a centrifugal pump, then heated, and oxygenated. It is subsequently diverted to the hepatic artery through a high-pressure, low-flow system or to the soft-shell reservoir which feeds the portal vein via a low-pressure, high-flow system. Bile production is monitored through duct cannulation. Bile salt, insulin, heparin, and prostacyclin are automatically infused;

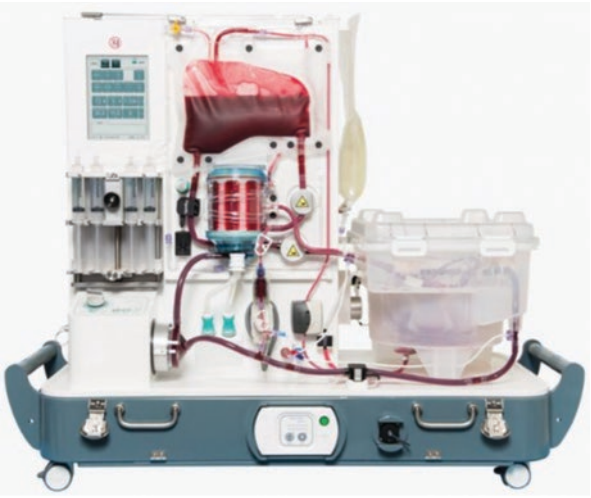

Fig. 15.1 Organ Ox Metra®

glucose and amino acid infusion can be manually regulated. The perfusate comprises 3 units of packed red blood cells cross-matched to the donor, one unit of colloid solution, calcium gluconate, heparin, cefuroxime, and 30 mL of sodium bicarbonate. During priming, the perfusate should reach operating conditions: 37 °C, a pO_2 of 12 kPa, a pCO_2 of 5 kPa, and a pH of 7.35. Acid-base homeostasis is reached by constant blood gas analysis and monitoring and control of pO_2 and pCO_2 levels. Continuous infusions ensure sufficient vasodilatation, protection against coagulation, and an environment with near-physiological metabolic and synthetic liver function (Fig. 15.1).

The Liver Assist® is a pressure-controlled device that provides pulsatile arterial flow and continuous non-pulsatile portal flow via two independent rotary pump circuits. Perfusate is not standardized but is generally made of 3 units of ABO-compatible blood plus a variable quantity of succinylated gelatin. Several other components may be added. Operative conditions are generally set up at 37 °C. Target pressures in the hepatic artery and portal vein are 60 mmHg and 8 mmHg, respectively. Bile can be collected after cannulation of the common bile duct. In the report of a liver recipient who underwent the first ischemia-free organ transplant (IFOT), a Chinese team describes how they manipulated the circuit by adding components to make the connection of the machine to the donor and recipient possible (Fig. 15.2).

OCS Liver System is a device providing pulsatile arterial flow and continuous non-pulsatile venous flow. The perfusate is provided by the company.

The Cleveland NMP circuit initially consisted of two separate pumps, an oxygenator and a heater; it uses a combination of air and oxygen that can be mixed and regulated. Subsequently it was converted to a single pump design where hepatic artery and portal vein flows can be regulated through a C-clamp application to the circuit. Perfusate includes blood and fresh frozen plasma. The only “home-grown” device, the Cleveland NMP is FDA-approved for use in clinical trials but is not yet commercially available. The device is transportable (Fig. 15.3).

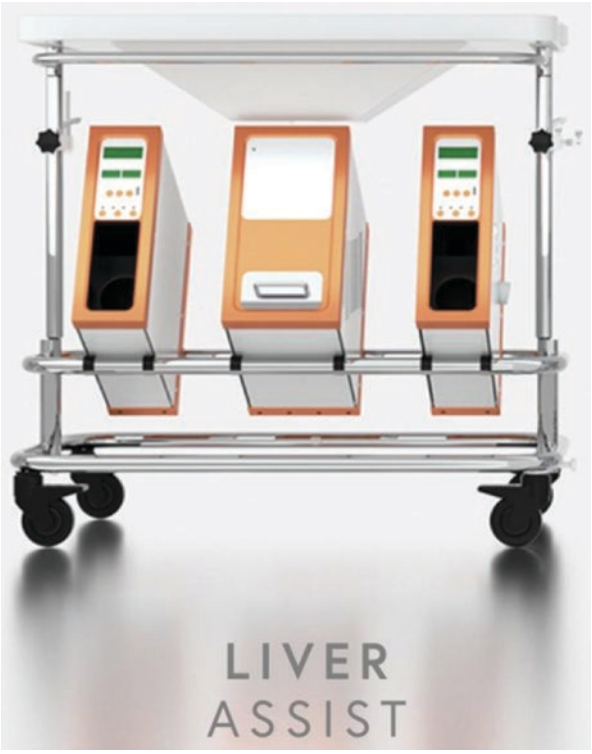

Fig. 15.2 Liver Assist®

Pre-clinical Studies

Pre-clinical studies of NMP were mainly in pigs, which provide appropriate size at reasonable cost. Still, anatomical differences obliged surgeons to alter the LT procedure. Moreover, for immunological reasons, pigs need to be sacrificed early, so late complications such as ischemic-type biliary lesions (ITBL) cannot be studied.

Schon et al. [14] studied the effect of NMP on grafts with an extended warm ischemia time (WIT) of 1 hour. All six animals transplanted with NMP usage survived versus none of the four transplanted after SCS.

Foley at al [15] mimicked an NMP circuit by connecting the liver to an anesthetized pig with an extracorporeal circuit. They found that single perfused livers were completely unable to increase biliary cholesterol in response to bile acid.

Brockmann et al. [16] compared liver transplant outcomes in pigs following either conventional cold preservation or warm preservation. After 20 hours of preservation without warm ischemia, posttransplant survival was improved in NMP livers. With the addition of 40 min of warm ischemia, the differences were even more marked. The authors concluded that organ preservation by warm perfusion, maintaining physiological pressure and flow parameters, enables prolonged preservation and successful transplantation both of normal livers and those with substantial ischemic damage.

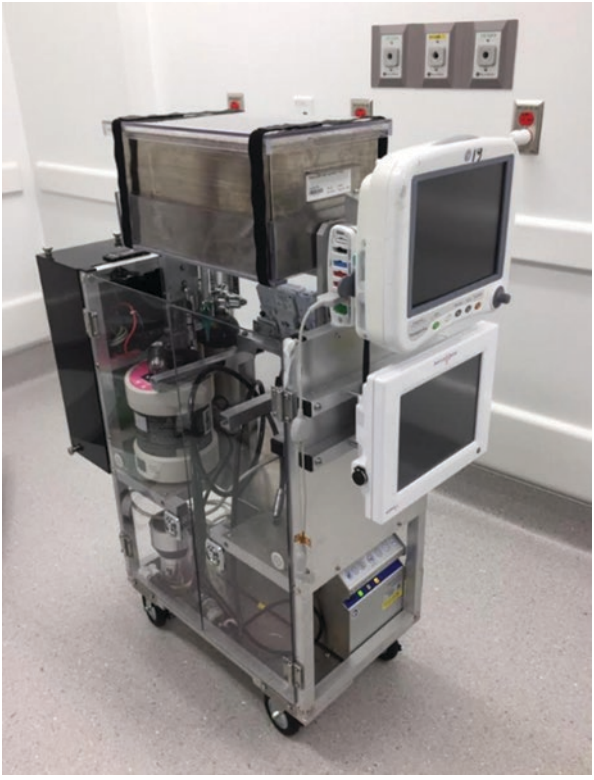

Fig. 15.3 Cleveland NMP

Fondevilla et al. [17] studied the effect of NMP in combination with regional perfusion. Donor pigs underwent 90-min cardiac arrest and were divided into 3 groups. In one group, livers were preserved immediately with cold storage. In the other 2 groups, donors underwent 60-min of normothermic regional perfusion followed by SCS or NMP. Five-day survival was 0 with immediate cold storage, 83% with normothermic regional perfusion+SCS, and 100% in normothermic regional perfusion+NMP. The authors concluded although 60 min recuperative normothermic regional perfusion is better than SCS alone, NMP further improves results and may have a role in preserving DCD livers in the clinical setting.

Boehnert et al. [18] compared cold static with acellular normothermic ex vivo liver perfusion (NEVLP) in a pig model of DCD liver injury. DCD livers (60 min warm ischemia) were cold stored for 4 hours or treated with 4 hours cold storage plus 8 hours NEVLP. Compared to the NEVLP grafts, the cold-stored grafts had higher ALT levels, decreased oxygen extraction, and increased hepatocyte necrosis. Furthermore, in the cold-stored grafts, levels of bilirubin, phospholipids, and bile salts were decreased fivefold, while LDH was sixfold higher and bile duct necrosis was increased. Following transplantation, mean serum AST level was

higher in cold-stored versus NEVLP livers with similar bile production. NEVLP improved hepatic artery perfusion and decreased markers of liver duct injury in DCD grafts.

St. Peter et al. [19] also studied the effect of NMP on DCD pig livers, subjecting grafts to 60 min of in vivo total warm ischaemia before flushing, after which they were preserved for 24 hours either by SCS with the University of Wisconsin (UW) solution or via oxygenated autologous blood perfusion on an extracorporeal circuit. During a 24-hour reperfusion phase, SCS livers showed no evidence of viability, with no bile production or glucose utilization; they also displayed massive necrosis. NMP livers demonstrated recovery of function by synthetic function, substrate utilization, and perfusion hemodynamics.

To address a debate over the possible deleterious effect of brief SCS before NMP, Reddy et al. [20] subjected porcine livers to 60 min of warm ischemia, after which the livers were either cold-preserved in UW solution for 4 hours followed by 20 hours of NMP or preserved with NMP for 24 hours. The NMP group had superior bile production, metabolic activity, and less evidence of hepatocellular damage and sinusoidal endothelial cell dysfunction, leading the authors to conclude that even a short period of cold ischemia significantly compounds the dysfunction of ischemically damaged livers.

Also working with pigs, Liu et al. [13] investigated the effect of NMP on hemodynamics and biliary epithelial regeneration; they reported that it improves biliary regeneration after a major ischemic event and may prevent the development of ischemic cholangiopathy in clinical transplantation. The same group also investigated the role of different perfusates on graft and bile duct viability in NMP porcine DCD livers, concluding that perfusate containing an oxygen carrier is most effective. Specifically, whole-blood perfused livers showed a trend toward better outcomes compared with perfusion with Steen solution plus red blood cells [21].

Clinical Series

Starting in 2016, reports of NMP in clinical scenarios began to emerge. Ravikumar et al. [22] reported the first-in-human phase 1 trial testing safety and feasibility. Twenty patients underwent liver transplantation after NMP. Organs were retrieved using standard techniques, attached to the perfusion device Organox® at the donor hospital, and transported to the implanting center in a functioning state. When NMP livers were matched 1:2 to cold-stored livers, 30-day graft survival was similar (100% NMP vs. 97.5% control, $p = 1.00$). Median peak aspartate aminotransferase (AST) in the first 7 days was significantly lower in the NMP group (417 IU [84–4681]) versus (902 IU [218–8786], $p = 0.03$).

Angelico et al. [23] reported that post-reperfusion syndrome developed in 2 of 12 patients who received cold-stored livers but in none of 6 patients who received NMP livers. The NMP group also had better intraoperative mean arterial pressure at 90 min post-reperfusion, achieved with significantly lower vasopressor requirements and fewer blood products compared with the SCS group.

Mergental et al. investigated the potential of NMP to increase the use of high-risk graftsby, allowing more accurate functional evaluation [24]. Following viability assessment by NMP, five originally rejected livers were transplanted. To be considered viable, livers had to meet the following criteria: the perfusate lactate level had to be less than 2.5 mmol/L or the liver had to produce bile, in combination with at least 2 of the following 3 criteria: (1) perfusate pH greater than 7.30, (2) stable arterial flow of more than 150 mL/min and portal venous flow more than 500 mL/min, and (3) homogeneous graft perfusion with soft consistency of the parenchyma.

Four of the organs had been rejected due to prolonged warm ischemic times in DCDs. The authors reported an uneventful transplant procedure in every recipient, with immediate function in all grafts. Notably, this was the first series to provide specific parameters for graft viability assessment during NMP.

Watson et al. transplanted 12 discarded livers following NMP [25]. The first 6 were perfused at high perfusate oxygen tensions, and the subsequent 6 at near-physiologic oxygen tensions. The authors found that avoidance of hyperoxia during perfusion may prevent postreperfusion syndrome and vasoplegia, and monitoring biliary pH, rather than absolute bile production, may be important in determining the likelihood of posttransplant cholangiopathy. The same group [26] also investigated which parameters could predict graft viability during 47 liver perfusions, of which 22 resulted in transplants. They concluded liver viability during normothermic perfusion can be assessed using a combination of transaminase release, glucose metabolism, lactate clearance, and maintenance of acid-base balance. The evaluation of bile pH may offer a valuable insight into bile duct integrity and risk of post-transplant ischemic cholangiopathy.

Selzner et al. reported the first North American series of LT with NMP using Steen solution in the perfusate [27], concluding that outcomes were comparable to results with SCS. Ten patients who received livers that had been perfused on the Metra device at 37 °C with Steen solution plus 3 units of erythrocytes were compared with a matched historical control group of 30 patients who received SCS grafts. There were no significant differences in aspartate aminotransferase and alanine aminotransferase levels on postoperative days 1–3, graft function by day 7 as assessed by international normalized ratio and bilirubin, duration of intensive care unit stay or hospital length of stay. No graft loss or patient death was observed in either group.

The group from Edmonton [28] reported on 10 grafts preserved with NMP, of which 9 were transplanted. Transplanted NMP grafts were matched 1:3 with transplanted SCS livers. All transplanted livers had good function, similar to controls, and graft survival at 30 days was not statistically different between groups. Intensive care and hospital stays were significantly more prolonged in the NMP group. Authors were criticized because of non-homogenous preservation times that reached

22.5 hours in one case and because NMP was also used as a tool to face logistical problems. Notably authors also reported a graft loss during NMP due to an unnoticed portal vein twisting.

The same group also investigated the effect of transient SCS before NMP [29]. As transportation of the machine to donor's hospital increases costs, prolongs retrieval time, and requires the presence of an experienced surgeon, the authors investigated whether a more practical back-to-base approach after initial SCS would compromise results. They compared outcomes of 26 back-to-base livers and 17 livers procured locally that underwent immediate NMP. The primary outcome measure (safety) was defined as 30-day patient and graft survival. Despite significantly prolonged mean cold ischemia time, the back-to-base livers demonstrated no difference in graft function, incidence of complications, or graft and patient survival.

Ceresa et al. also investigated the safety of a period of SCS before NMP [30], concluding that it was safe. Thirty-one livers were transplanted in the prospective multicenter study. The 30-day graft survival rate was 94%. Median peak serum AST in the first 7 days was 457 U/L, and 4 patients developed early allograft dysfunction (EAD). Postrepefusion syndrome (PRS) was observed in 3 livers. The median duration of initial critical care stay was 3 days, and median hospital stay was 13 days.

The first report of a randomized clinical trial comparing NMP to SCS came from Nasralla et al. [31]. With results from 220 liver transplantations, NMP was associated with a 50% lower level of graft injury as measured by hepatocellular enzyme release. Rates of bile duct complications, graft survival, and patient survival were statistically similar with the two approaches. The authors reported a 50% lower rate of organ discard but did not disclose viability parameters.

Ghinolfi et al. reported another pilot randomized clinical trial [32] on the use of NMP with very old donors. Results did not show any significant difference. This study has a main limitation in the small number of cases so that its results have to be carefully evaluated.

Van Leeuwen et al. investigated a combination of dual hypothermic oxygenated machine perfusion (DHOPE) with NMP in 16 discarded DCD livers [33]. Ex situ NMP (viability assessment phase) was preceded by 1-hour DHOPE (resuscitation phase) and 1 hour of controlled oxygenated rewarming (COR). During the first 2.5hours of NMP, hepatobiliary viability was assessed, using predefined criteria: perfusate lactate <1.7 mmol/L, pH 7.35–7.45, bile production >10mL, and bile pH >7.45. All of the livers cleared lactate and produced sufficient bile volume, but 5 livers were discarded due to low bile pH. The remaining 11 livers (69%) were successfully transplanted, with 100% patient and graft survival at 6 months. The authors concluded that sequential DHOPE-COR-NMP enabled resuscitation and safe selection of initially declined high-risk donor livers, thereby increasing the number of transplantable livers (Table 15.1).

Table 15.1 Clinical studies reporting LT with both DBD/DCD grafts perfused with NMP

Author	N	DBD/DCD	Recipient age	Recipient MELD	Machine	Hospital stay (days)	FU (months)	Graft loss causes	6 months Pt Survival	6 months graft survival	PNF	DGF	PRS	ITBL	Vascular complications
Nasralla et al. [31]	121	87/34	55 (48–62)	13 (10–18)	Organox	15(10–24)	12	2 HAT 1 ITBL 1 non-thrombotic infarction 1 inferior vena cava occlusion 1 PNF	/	/	1	3	15	1	2 HAT 5 hepatic artery stenosis 1 hepatic vein trombosis 1 portal vein trombosis
Mergental et al. [24]	5	1/4	56 (47–66)	10 (7–17)	Organox/ liver assist	10(6–15)	7(6–9)	0	100%	100%	0	0	0	0	0
Bral et al. [28]	9	6/3	54 (43–69)	17 (9–32)	Organox	45(13–114)	6	1 HCV relapse	89%	90%	0	0	0	0	0
Ravikumar et al. [22]	20	16/4	54(33–66)	12 (7–27)	Organox	12 (6–34)	9	1 alcohol relapse	95%	95%	0	0	0	4	0
Selzner et al. [27]	10	8/2	56(45–71)	21 (8–40)	Organox	11(8–17)	3	0	/	/	0	0	0	0	0
Watson et al. [26]	22	6/16		/	Liver assist	/	/	0	94%	75%	1	0	5	4	0
Ghinolfi et al. [32]	10	10/0	57(46–61)	12 (9–16)	Liver assist	12	7–18	1 HAT	100%	90%	0	0	3	3	1 HAT

Author	N	DBD/ DCD	Recipient age	Recipient MELD	Machine	Hospital stay (days)	FU (months)	Graft loss causes	6 months Pt Survival	6 months graft survival	PNF	DGF	PRS	ITBL	Vascular complications
Liu et al. [21]	15	10/5	/	/	Cleveland clinic	17(14–22)	/	1	/	/	0	0	0	1	1 HAT
Angelico et al. [23]	6	4/2	55(34–66)	12 (9–18)	Organox	9(5–14)	/	1	/	/	/	/	/	/	/
Watson et al. [25]	12	3/9	57(46–65)	17 (10–26)	Liver assist	/	9–24	1 PNF	92%	92%	1	0	/	3	/
Bral et al. [29]	43	33/10	57 (40–63) 59 (50–63)	22 (17–24) 25 (21–32)	Organox	16 (12–20) 43 (22–61)	6	Biliary stricture Cholestatic hepatitis	94% 93%	100% 88%	0	11	/	0	0
Ceresa et al. [30]	31	23/8	58 (25–73)	14 (7–24)	Organox	13 (7–31)	12	2 HAT	100%	84%	/	4	3	0	2 HAT
Van Leeuwen et al. [33]	11	11/0	61 (55–66)	14 (13–15)	Liver assist	/	12	0	100	100	0	/	/	1	0

Viability Parameters

Transplant surgeons are called on daily to decide whether or not to use a graft. Historically, the assessment of a liver graft involved a review of the donor's medical history, biochemical and instrumental analysis followed by visual inspection during retrieval and eventually biopsy. Recently some prognostic models have been created to give an estimation of risk of graft failure [34–36]. These models help to reduce uncertainty about graft viability but do not completely eliminate it.

Viability assessment should be directed toward analysis of hepatocellular compartment and cholangiocyte compartment [37].

Hepatocellular Compartment

Liver lobule is divided into 3 zones. Zone 1 is the closest to portal triad and is exposed to higher concentration of oxygen, hormones, and metabolic substrates. Zone 3 hepatocytes include metabolic processes that are less dependent on oxygen.

Gluoconeogenesis from lactate and aminoacids takes part mainly in Zone 1 while glycolysis in Zone 3. Glycogen synthesis from generated glucose happens mainly in Zone 1 while glycogen synthesis from circulating glucose in Zone 3.

During hypothermia, liver faces an oxygen-independent glycogen breakdown [38, 39] that continues during the early phase of NMP.

Shortly after, the high levels of glucose should stimulate glycogenesis, thus causing a glucose fall in the perfusate.

Should the glucose not rise, this could mean there has been a glycogen depletion or panlobular injury [37] (Fig. 15.4).

Lactate metabolism occurs mainly in Zone 1; as this is the last zone to be deprived of oxygen, impaired lactate clearance could mean panlobular injury.

As Zones 2 and 3 do not take part in lactate clearance, their injury cannot be detected by this marker.

Transaminases give an indication of damage but are of a limited function to assess viability.

As the liver has a remarkable regenerative potential, it is unclear what threshold of all of these markers should be adopted to warrantee a complete post-LT functional recovery.

Cholangiocyte Compartment

Ischemic cholangiopathy (IC) is a main concern in LT as its development cannot be predicted.

The possibility to assess bile ducts during NMP is of great interest as it may reduce IC incidence.

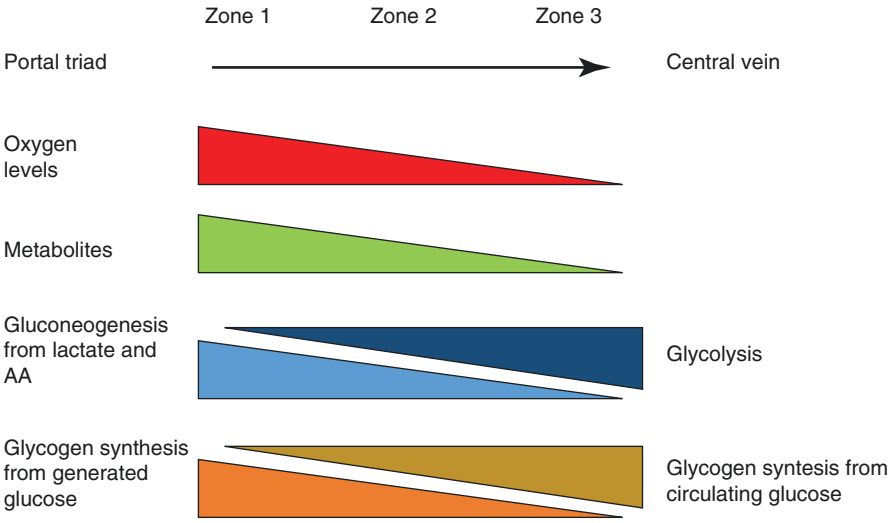

Fig. 15.4 Metabolic zonation of the liver lobule. (Adapted from Watson and Jochmans [37]. With permission from Creative Commoncs Licens 4.0: http://creativecommons.org/licenses/by/4.0/)

Bile normally undergoes deprotonation and glucose removal; deprotonation is achieved by bicarbonate secretion so that an alkali pH should be associated with viable cholangiocytes. Glucose <3 mmol/L should also represent a normal cholangiocyte function.

The amount of bile production has been proposed as a marker of viability. In a preclinical study, Sutton et al. reported their experience with 12 discarded livers. They concluded that bile production can be used as an easily assessable marker of liver graft viability during ex-vivo NMP, given that cumulative production of >30 g of bile during 6 hours NMP was associated with significantly lower release of transaminases and potassium into the perfusate and better hepatobiliary function as reflected by a normalization in glucose and lactate levels and higher secretion of bilirubin; in addition histology showed less signs of venous congestion and hepatocellular necrosis [40]. These findings, although interesting, are not conclusive, as the livers were never transplanted. In other experiences bile production did not appear to be related to post-LT function [25].

Clinically Used Viability Parameters (Table 15.2)

Some authors have proposed some viability parameters in clinical studies.

Table 15.2 Viability parameters proposed in clinical studies

Clinically used viability parameters			
Author/year	# graft	Device	Viability parameters
Mergental et al. [24]	6	Liver assist/ Organox	Lactate <2.5 or bile production plus 2 of the following: pH > 7.3 HA flow >150 ml/min and PV flow >500 ml/min Homogeneous graft perfusion
Watson et al. [25]	12	Liver assist	Lactate Glucose Transaminases pH
Bral et al. [28]	10	Organox	Perfusate biochemistry Need for bicarbonate correction Perfusion flow stability Hourly bile production
Van Leeuwen et al. [33]	11	Liver assist	Perfusate lactate <1.7 mmol/L pH 7.35–7.45 Bile production >10mL Bile pH >7.45

Mergental et al. evaluated 6 discarded grafts, 5 of which were finally transplanted [24]. Lactate had to be <2.5 or bile had to be produced in combination with at least 2 parameters between: pH > 7.3, HA flow >150 ml/min and PV flow>500 ml/min, homogeneous graft perfusion. A graft was not transplanted due to abnormal arterial anatomy, causing a lactate level rising.

Watson et al. reported a series of 12 transplants with livers potentially viable but where the ischemic time would have been unreasonably long or there was uncertainty about the quality based on the subjective evaluation of the retrieving surgeon [25]. Viability was assessed by changes in lactate, glucose, and transaminase concentration as well as on the ability of the liver to maintain pH without supplemental bicarbonate.

Bral et al. reported a series of 10 cases where they evaluated liver perfusion quality by variation in perfusate pH, lactate concentration, vascular stability, and hourly bile production. A graft was discarded due to an unnoticed portal twisting [28].

Van Leeuwen et al. proposed the following parameters to evaluate viability: perfusate lactate <1.7 mmol/L, pH 7.35–7.45, bile production >10mL, and bile pH >7.45 [33].

All of these parameters have been created and proposed based on hypotheses and have never been validated. We are unfortunately far from being sure about what graft will function or not by assessing its function during NMP. Moreover proposed parameters are somewhat restrictive and could result in discarding of livers that could be transplanted without complications.

Ghinolfi et al. [32] reported that 6 of the 10 of the NMP livers they transplanted in their pilot study presented an acidic bile pH. Based on previously proposed data

those grafts should have been discarded but in fact they were successfully transplanted and none of the recipients developed IC.

NMP undoubtedly brings the potential to assess viability of a graft on an objective basis, but more trials are needed to identify optimal markers and their applicability.

DCD and Normothermic Machine Perfusion

The global shortage of organ donors will not be resolved solely by relying on deceased donation following a brain death determination (DBD). Expansion of ECD and particularly deceased donation after circulatory death (DCD) will be needed to address the shortfall of transplantable organs.

In Europe, there are approximately 350,000 cases of cardiopulmonary resuscitation a year (1000 cases per day). Only 40% (400) of such cases are successfully resuscitated to result in a 15% hospital survival and 12% patient survival at the end of 1 year [41]. The remaining 60% that do not recover become a potential for uncontrolled DCD. Two hundred and fifty deaths each day throughout Europe in the ICU becomes an opportunity for controlled DCD at the time of the withdrawal of futile treatment.

DCD liver grafts, due to warm ischemic damage, carry higher risks for delayed graft function (DGF), primary nonfunction (PNF), and biliary complications following transplantation [42]. Because of poor results with DCD liver grafts after conventional cold storage (CS), interest in liver machine preservation was renewed. NMP allows a subjective graft evaluation, and its usage was often directed to assess viability of ECD and particularly of DCD grafts.

Ravikumar et al. reported the first series of 20 NMP perfused livers successfully transplanted. Four were DCD grafts. Results were compared to 40 SCS preserved historical LT [22].

Selzner et al. compared a series of 10 transplanted livers, of which 2 were DCDs, with a historical series of 30 SCS preserved grafts and did not find substantial differences between the two groups [27].

Mergental reported a series of 6 declined livers, 5 of which were deemed transplantable after NMP evaluation. Four were DCDs liver that have been successfully transplanted [24].

Watson et al. reported a series of 12 declined livers successfully transplanted after NMP evaluation; 9 were DCDs, and 8 were alive at 12 months [25].

Bral reported a series of 10 livers (4 DCDs) transplanted after NMP preservation. One out of four DCDs was discarded due to an unnoticed portal vein twisting [28].

Watson reported another series of 47 liver perfusion of which 22 resulted in transplants. Sixteen grafts were from DCDs; 4 IC and 1 PNF have been observed [26].

Bral reported another series in 2018 of 46 NMP livers. Outcomes of back to base livers were compared to grafts perfused at donor's hospital. Ten were DCDs livers [29].

Nasralla reported a large randomized controlled trial on NMP. Thirty-four perfused livers were from DCDs. A specific analisis on DCDs was not reported [31].

Table 15.3 Clinical studies reporting LT with DCDs graft perfused with NMP

	# DCDs	Device	Viability	Median CIT	Median NMP	Recipient median age	Median MELD	PRS	PNF	EAD	IC	Peak ALT
Ravikumar et al. [22]	4	Organox	/	/	570	61 (52–64)	11 (9–12)	/	0	1	0	/
Selzner et al. [27][a]	2	Organox	/	106	480	56	21 (8–40)	/	/	/	/	619 (55–2858)
Mergental et al. [24]	5	Liver assist/ Organox	Lactate <2.5 or bile production plus 2 of the following: pH > 7.3 HA flow >150 ml/min and PV flow >500 ml/min Homogeneous graft perfusion	428	389	56	8 (7–17)	/	0	0	0	1242 (1188–1879)
Watson et al. [25]	9	Liver assist	Lactate Glucose Transaminases Ph	427 min	284	57	17 (10–26)	5	1	/	3	1069 (187–4991)
Bral et al. [28]	4 (3 LT)	Organox		208 (108–284)	735 (196–1347)	62 (62–69)	16 (9–26)	0	0	3	0	/
Watson et al. [26]	16	Liver assist	/	386 (292–448)	/	/	/	5	1	/	4	/
Bral et al. [29][a]	10	Organox	Perfusate biochemistry Need for bicarbonate correction Perfusion flow stability Hourly bile production	4.6 Hours (1–8.4)	9 Hours (3.3–22.4)	58 (40–63)	23 (17–32)	/	0	6	0	437 (252–1536)
Nasrala et al. [31][a]	34	Organox	/	126 (106.5–143)	547.5 (372.5–710.5	55 (48–62)	13 (10–18)	15	1	12	1	/
Ceresa et al. [30][a]	8	Organox	/			58 (25–73)	14 (7–24)	3	0	4	0	/
Van Leeuwen et al. [33]	11	Liver assist	Perfusate lactate <1.7 mmol/L pH 7.35–7.45 Bile production >10mL Bile pH >7.45	/	504	61 (55–66)	14 (13–15)	/	0	/	1	683 (282–757)

[a]Data are cumulative as those on DCDs cannot be extracted

Ceresa reported a series of 31 LT with NMP. Eight were DCDs. A specific analysis on DCDs was not reported [30].

Van Leeuwen reported a series with 11 DCDs that were previously discarded as non-transplantable. Their graft and patient survival was 100% at 12 months [33] (Table 15.3).

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