EJT European Journal of Transplantation

2023;1:47-55
DOI: 10.57603/EJT-008

Review

PERFUSION PROGRAMMES START-UP AND VIABILITY ASSESSMENT: A PRACTICAL GUIDE TO *EX-SITU* NORMOTHERMIC MACHINE PERFUSION IN LIVER TRANSPLANTATION

George Clarke[1-3], Jingwen Mao[2,3], Simon C. Afford[2,3], Hynek Mergental[1-3]

[1] Liver Unit, Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust (UHBFT), Birmingham, United Kingdom; [2] National Institute for Health Research (NIHR), Birmingham Biomedical Research Centre, University of Birmingham and UHBFT, United Kingdom; [3] Centre for Liver and Gastrointestinal Research, Institute of Immunology and Immunotherapy, University of Birmingham, United Kingdom

Summary

Machine perfusion of the liver has been an increasingly adopted approach to overcome the shortcomings of static cold storage, that are most prominent in extended criteria donors. Normothermic machine perfusion (NMP) safety and efficacy were demonstrated in several prospective trials and multiple cohort series. The real-world benefits of this approach are liver functional assessment and extended preservation times. In this review, we aim to provide information regarding the practical aspects of starting an NMP programme and pragmatic guidance towards assessing liver viability for teams new to this field. There are two key areas of focus in assessing liver viability, examining both the hepatocyte and cholangiocyte compartments. Analysis of these areas can predict risk of primary non-function and non-anastomotic biliary strictures, respectively. There is a learning curve in adopting and gaining confidence with the NMP technology. Viability assessment allows transplantation of more marginal livers, but a cautious approach is advised for new centres entering the field.

Key words: machine perfusion, liver transplantation, normothermic, viability assessment

Received: August 28, 2022
Accepted: September 21, 2022

Correspondence

Hynek Mergental
Liver Unit, Queen Elizabeth Hospital Birmingham, University Hospitals Birmingham NHS Foundation Trust, Birmingham, B15 2TH, United Kingdom
Tel.: +44 121 371 4638.
E-mail: Hynek.mergental@uhb.nhs.uk

How to cite this article: Clarke G, Mao J, Afford SC, et al. Perfusion programmes start-up and viability assessment: a practical guide to ex-situ normothermic machine perfusion in liver transplantation. EJT 2023;1:47-55. https://doi.org/10.57603/EJT-008

Abbreviations

CIT: Cold Ischaemia Time; DBD: Donation after Brainstem Death; DCD: Donation after Circulatory Death; HOPE: Hypothermic Oxygenated Machine Perfusion; HMP: Hypothermic Machine Perfusion; NAS: Non-Anastomotic biliary Strictures; NMP: Normothermic Machine Perfusion; PNF: Primary non-Function; SCS: Static Cold Storage; VITTAL: Viability Testing and Transplantation of marginal Livers

INTRODUCTION

Liver perfusion has become increasingly adopted into clinical practice due to its evident abilities to overcome the many shortcomings of static cold storage (SCS) [1-4]. As a consequence of the increasing use of extended criteria donors to meet waitlisting demands, transplant teams are having to assess the optimal

ways to implement the various perfusion technologies, hoping to improve graft quality and transplant outcomes [5]. *Ex-situ* normothermic machine perfusion (NMP) aims to replicate the liver's normal physiological condition and maintain its metabolic activity during preservation [6]. Data collected and the liver's behaviour can then be used to assess its suitability for transplantation [7-9]. Beyond encouraging clinical data, demonstrating a positive impact on liver quality remains challenging. The reassurance provided regarding liver functional activity and the logistical improvements it provides are often considered beneficial enough to establish an NMP programme [2,10-12]. The ability to assess the viability of liver grafts has evolved with the perfusion technologies and looks to improve further as our knowledge and experience grows [8].

This review aims to provide information regarding the practical aspects of starting an NMP programme and pragmatic guidance towards assessing liver viability for teams new to this field.

KEY DECISIONS PRIOR COMMENCING NMP PROGRAMME

Machine perfusion evolution and early pathways to clinical adoption

The introduction of liver perfusion into clinical practice was pioneered by several groups around the world, starting by Guarrera and colleagues in New York using hypothermic (4-10°C) machine perfusion (HMP), Dutkovski in Zurich developing hypothermic oxygenated machine perfusion (HOPE), and Friend in Oxford introducing normothermic (36-38°C) machine perfusion [6,13,14]. Machine perfusion was adopted by several teams very early on through participation in the initial clinical trials, sharing awareness about the new dynamic liver preservation to the wider transplant community [6,15-21]. The teams participating were typically provided with access to the perfusion device, consumables, technical support, and team training that were funded through trials. The developed setup, gained team expertise, and infrastructure facilitated subsequent seamless NMP adoption by those teams [3,22,23]. This historical context explains why some perfusion approaches may be predominant in specific regions [3,23,24].

Contrary to the early adopter's pathway and programmes funding via research resources, the subsequent uptake of the technology has been more challenging [22]. It requires evidence-based decision-making, significant resources, and specialised expertise, all of which have not yet been firmly established [25]. Whilst the regulatory aspects are beyond the scope of this manuscript, it is worth mentioning that the majority of the commercially available devices are CE marked and there are no barriers to their clinical use in Europe. The funding, however, remains the major hurdle to overcome, in particular on the background of the ongoing debate about the real-world clinical efficacy that is aggravated by the lack of supportive health economic data.

DONOR CHARACTERISTICS, TIMING, AND LOCATION OF THE NMP PROCEDURE

Evidence from completed clinical trials is quickly accumulating and evolving and it has become increasingly apparent that certain donor livers benefit from specific perfusion approaches [24,26-30].

It has been widely accepted that liver cooling and rewarming in the context of concomitant ischaemia triggers the irreversible cascade resulting in organ damage. The team in Guangzhou who pioneered ischaemia-free normothermic perseveration demonstrated that if these events can be avoided then even marginal livers typically deemed untransplantable (e.g. donors with 90% macrosteatosis) can be transplanted successfully [31]. Its logistical requirements, however, are currently too challenging for this method to become mainstream [32].

Steatotic organs seem to be most susceptible to damage caused by SCS and benefit most from a normothermic approach with limited cold ischaemia time (CIT). Similarly, in donors after circulatory death (DCD), early intervention seems to be of key importance to minimise risk of developing non-anastomotic biliary strictures (NAS) with normothermic regional perfusion and NMP commenced at the donor centre showing superior stricture outcomes compared to end-ischaemic NMP [26,28,29]. When applied to already cold DCDs, and commenced at the transplant centre, these livers benefited from HOPE [3]. Whilst in the past hypothermic and normothermic perfusion were seen as mutually exclusive and competitive technologies, it has been recognised that these different approaches can be complimentary [5,28,33]. The Groningen team were the first to demonstrate clinical benefits of such combination, treating DCD livers initially with a period of HOPE to minimise the ischaemia-reperfusion injury leading to biliary strictures, followed by functional assessment using NMP [34].

Regarding transplant logistics and extension of the preservation times for long-distance sharing, this aspect used to be dominated by NMP. There is, however, emerging data that HOPE can maintain perfusion for over 12 hours without detriment to the organ [35]. In situations where a liver reaches a transplant centre with excessive CIT causing concern about primary non-function (PNF), the decision about organ usability may be guided by viability assessment by NMP as described later.

Commencing NMP at the donor hospital may be more resource intensive but it follows established organ

preservation and allocation pathways and its superiority over SCS has been demonstrated in several randomised trials [23,24].

In the context of skilled personnel shortage, financial restrictions, and achievable short CITs, commencing perfusion at the transplant centre is often easier in the real-world as it provides access to senior surgeons to prepare the liver for perfusion and implantation, including arterial reconstruction if needed, and the expertise for NMP troubleshooting [22,36]. The back-to-base approach also does not require complex transportable devices and can be applied to selective livers based only on its perceived quality that helps to keep the cost down. As the organs are already cold it provides opportunity to choose the perfusion temperature, though it is still widely accepted that a liver needs to reach normothermia if viability is to be assessed.

LIVER PERFUSION DEVICES

The number of available perfusion devices is increasing, and the expanding market is likely to continue this trend. The issues about the perfusion and temperature are discussed above. The next important aspect to consider is cost. The manufacturers often provide access to the device within loan or lease schemes that are combined with staff training and pump maintenance. The cost of the perfusion disposable kit, blood, fluids, and drugs are therefore the most obvious expense considered when starting a perfusion programme. These details are outside the scope of this review, but in general, transportable NMP devices are more complex and expensive than non-transportable ones, and hypothermic devices are simpler and cheaper than normothermic variations. Device cost often reflects companies' investment into the development, registration, clinical testing, customer support, and the biggest players in the field offer pumps used in large randomised controlled trials.

The impact of device simplicity, or a degree of automatisation and user friendliness in more complex machines is often underestimated. This was less of an issue for teams who pioneered the technology and appreciated the opportunity to amend multiple perfusion parameters. For new teams entering the field now, however, this is a key feature in shortening the personnel learning curve and minimising the risk of user error.

A feature less often highlighted is oxygenator specifications. Although many authors challenge using machines solely to improve transplant logistics, having the option of starting procedures semi-electively in the morning often becomes the most adored perfusion features in newly established programmes. To benefit from this possibility for perfused livers, the device needs to support perfusion lasting a minimum of 12 hours.

THE ORGAN RETRIEVAL, PERFUSION, AND TRANSPLANT TEAMS

Dynamic organ preservation is an emerging subspeciality requiring multi-disciplinary skills. The ability to operate and troubleshoot the device is the key requirement to establishing an NMP programme, that needs to be complemented by surgical expertise in multi-organ retrieval, ability to perform hepatic artery reconstructions, and ability to interpret the perfusion and functional parameters [37]. The stakes are higher in the NMP approach due to the potential risk of losing the organ, albeit this seems to be significantly lower than initially envisaged and reports of graft loss or device malfunction are extremely rare [38].

Although there is a learning curve with the NMP (around 5-10 procedures) it is often quickly overcome by the team's enthusiasm. The real-world challenge it to sustain the programmes by appointing personnel running 24/7 rotas as those initially "hidden costs" might be higher than perfusion consumables. Centres with lower transplant volumes might find this more difficult but for them the need might be less urgent. Multi-organ transplant programmes can pool the perfusion specialists into one team as the skill set is similar. For high-volume teams the justification of the additional manpower and other resources may be easier to justify. Further reading on this topic was well covered by Hunt and colleagues [39].

Transplant professionals are highly motivated individuals and cover commitments often exceeding those in other specialities. In the context of the COVID-19 pandemic, the critical shortage of healthcare workforce and burnout, team resilience and well-being, staff recruitment, and retention are becoming issues needing increasing attention. By extension of the liver preservation times the machine perfusion can significantly improve transplant procedure logistics, making it a semi-elective daytime procedure [2,22]. The novelty of the machine perfusion often provides opportunities for research that can further attract new young colleagues into our speciality. These benefits might be most noticeable in large volume units and whilst difficult to quantify there is evidence demonstrating increased safety for the liver recipients [22,40,41].

VIABILITY ASSESSMENT PROTOCOLS

The ability to monitor liver metabolic activity during NMP provides opportunities to evaluate suitability for transplantation. For this review, we define the viability assessment as an approach directed only to selected livers – in contrast to systematic use in all grafts and applied post-hoc when the ideal time to intervene was lost and the organ reached the transplant team preserved by SCS [27,42,43]. There have been several proposed viability protocols, that

were all derived from small cohorts of perfusions, different type donors, with different key objectives [16,44,45]. To ensure recipients safety, all protocols included multiple measures with favourable readings along those observed physiologically *in-vivo* conditions.

With increasing confidence in the technology and learning from suboptimal outcomes it becomes apparent that strengths and limitations of each protocol should be evaluated in the context of the diagnostic dilemma they are meant to answer. Also, most authors acknowledge that with increasing experience the comfort zone to use suboptimal livers increases and the viability assessment cut off values are being relaxed as described below [44,46].

The functional assessment of donor organs covers two compartments, hepatocytes and cholangiocytes, and these are predominantly responsible for initial liver function and late biliary strictures, respectively.

PRIMARY NON-FUNCTION AND SEVERE GRAFT DYSFUNCTION

The key question – is this liver going to work in the next 7 days? – can be predicted very early on from the consumption of several perfusate substrates, e.g. lactate, glucose, and oxygen or by measurement of synthetic activity e.g. urea, clotting factors, and bile volume. The incidence of PNF in the published NMP series is extremely low. In the first multicentre randomised study comparing NMP commenced in the donor hospital with SCS the authors reported one such event in a liver that did not clear lactate levels below 5 mmol/L [23]. The trial, however, was conducted before any viability criteria were defined and functional assessment taken into the decision making about usability for transplantation.

Lactate clearance

Perfusate lactate clearance is a widely accepted marker of good hepatocyte function. Livers exposed to prolonged SCS, especially steatotic organs, may be slower to recover their function. This difference is demonstrated in Figure 1, showing fast lactate clearance (levels below 2.0 mmol/L within 60-90 minutes in livers put on the device in the donor hospital) in contrast to a slower decline in high-risk livers exposed to CIT around 10 hours (meeting similar lactate levels in 2-4 hours).

The timeframe to assess liver viability is a less defined area which demonstrates the learning curve and increasing confidence with the technology, though this change was facilitated by transition to a device with improved oxygenator specifications allowing longer perfusions. Our initial protocol in pilot discarded liver series assessed viability parameters at 2 hours, it was subsequently validated in the VITTAL trial with assessment at 4 hours and most recently we assess based on readings at 6 hours (Fig. 2).

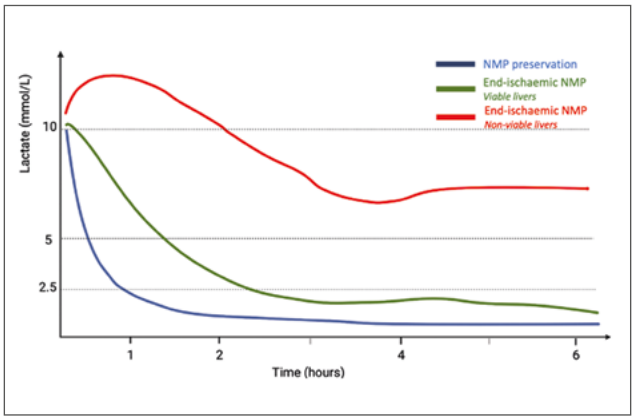

Figure 1. Lactate clearance characteristics. The figure outlines different lactate clearance characteristics according to the NMP approach. The blue curve shows rapid lactate decline typically seen during normothermic preservation, when the NMP procedure is commenced in the donor hospital. For viability assessment following a period of static cold storage, the slope of lactate clearance is usually slower, with most livers meeting the 2.5 mmol/L threshold between 2-4 hours as illustrated by the green curve. The clearance curve of non-viable livers may vary widely, but most livers demonstrate some degree of lactate metabolism and reach levels between 5-10 mmol/L as shown by the red curve (The figure was created in the BioRender.com application).

The rate of lactate clearance (or any other viability marker) is perhaps a good gauge of liver quality and livers meeting the parameters quickly are less likely to develop any degree of graft dysfunction.

Bile production

Presence of good quality bile is convincing and perhaps the most sensitive marker for liver viability, offering the possibility to assess the functioning of both hepatocytes and cholangiocytes. An hourly bile production > 10 mL is the frequently quoted volume anticipated in transplantable livers [45,47]. Bile production is a complex metabolic process and if used beyond simple volume production measurement it provides more accurate information about the organ's overall condition. Importantly, bile composition may help to identify livers at risk of developing late biliary NAS that is of key importance in DCDs. Its role in assessment of DBD livers is of lesser importance, with the main drawbacks being the delayed production and possible failure due to technical complications [10,27,48]. A lag in recovery of the production can be often observed in steatotic livers or organs following extensive CIT with the times being beyond the widely accepted 2-4 hours viability assessment timeframe. To prevent technical problems, bile is best collected by thin tube (8-12 Fr, e.g. paediatric feeding tube, ideally placed under mineral oil to prevent oxidation and changes in bile pH), the cystic duct should be ligated, with the tube

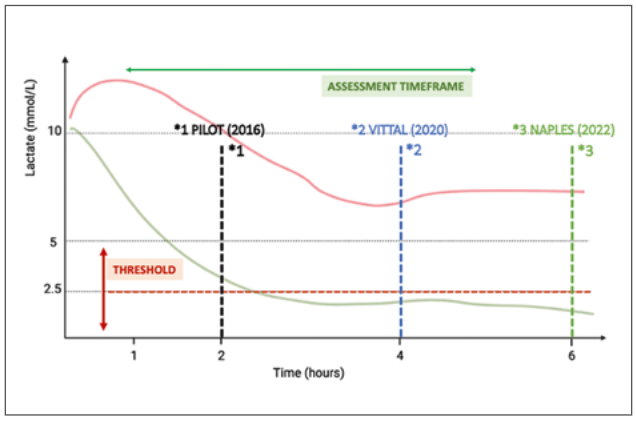

Figure 2. Refinement of the lactate clearance criteria. The initial viability criteria were derived from a small experimental NMP series. The lactate clearance threshold 2.5 mmol/l was reached by all livers that were able to maintain 6-hour perfusion. For our pilot clinical series, the viability was assessed at 2 hours. Along the learning curve and increased confidence with the technology (60 liver transplants using NMP liver) the VITTAL trial assessed the viability within 4 hours. Following analysis of the trial results, the assessment timeframe was extended to 6 hours though applied exclusively to DBD livers. Such extension does have only limited impact on yield of transplantable livers regarding the lactate clearance assessment, however, the time extension allows for more livers to produce bile (The figure was created in the BioRender.com application).

tip positioned well below hepatic ducts confluence, and fixed without obstructing its lumen [1].

Transaminases levels

Contrary to the lactate clearance and bile production, the perfusate release of transaminases provides a snapshot of the damage the liver sustained prior to retrieval, during preservation, and following ischaemia-reperfusion injury. The cut-off values are less well-defined, and this parameter cannot show an improvement over the course of perfusion, however, an exponential rise of the levels is an ominous sign. The Cambridge team reported favourable outcomes if ALT levels were < 6,000 IU/L and this is aligned with our retrospective observations from the VITTAL perfusion cohort [44].

Other parameters

Many other parameters can be measured from the perfusate including urea, clotting factors, C-reactive protein, and albumin [49]. The discriminative power for viable or non-viable livers is, however, lower compared to lactate and therefore most livers deemed viable perform favourably in these markers, and any cut-off values are yet to be defined. Failure to metabolise glucose is a very rare but concerning feature. Perfusate pH is another important parameter to consider, particularly the liver ability to maintain pH within a range of 7.2-7.4 over the course of a perfusion. Its close correlation with the lactate levels, or correction with sodium bicarbonate are other factors to consider when interpreting the results.

The above markers are based on traditional blood gas, bile, and biochemical perfusate analyses. Surprisingly, the new generation of biomarkers based on advanced omics methods are still scarcely reported [49]. Other approaches, for example indocyanine green or methicillin clearance were proposed but not yet reported from clinical series [50-52].

Liver biopsy used to be the gold standard in liver transplantability assessment. With the advancement in NMP viability assessment this situation has changed, however, the perfusion may provide time for more detailed histology evaluation, for example, to assess the pre-perfusion bile duct damage that might contribute to decision to discard DCD livers with worrying features, or to evaluate suspicious extra-hepatic lesions discovered unexpectedly during procurement [53,54].

NON-ANASTOMOTIC BILIARY STRICTURES

Hilar or intra-hepatic biliary strictures are among the most troublesome post-transplant complications, occurring almost exclusively in DCD livers (the incidence in DBD is 1-2%) [55]. There are several markers related with favourable post-transplant outcomes (Tab. I). Whilst the cut-off values may vary between protocols, to minimise risk of NAS DCD livers should produce over 20 mL of alkalotic bile with low glucose and high bicarbonate concentrations within the first 2-4 hours of perfusion.

The Groningen team suggests bile volume ⩾ 10 ml/hr with pH > 7.45, and similarly the Cambridge group reported bile pH > 7.50, glucose level ⩽ 3 mmol/L (respective ⩾ 10 mmol/L lower than the perfusate) as favourable features for the liver longevity [34,44]. The VITTAL trial did not include bile into the transplantability assessment, and some DCD livers developed aggressive features of NAS complications requiring early re-transplantation [27]. Retrospective analyses of collected samples confirmed those were present in livers without bile production, low bile pH or bile bicarbonate. Overall, bile production and bile analysis seem to be of paramount importance to assess biliary integrity to predict DCD livers longevity and avoid NAS [8,48].

REAL-WORLD APPROACH TO THE LIVER VIABILITY TESTING

Even when applied to the most marginal livers widely deemed not suitable for transplantation, a high proportion of those organs will meet some of the viability

Table I. Viability testing parameters.

Established viability testing protocols			
Assessment period	**Birmingham** [1]	**Cambridge** [2]	**Groningen**[3]
	240 minutes	***120-240 minutes****	***150 minutes***
Favourable parameters			
Hepatocyte function	Lactate < 2. 5mmol/L Evidence of bile production Perfusate pH > 7.30	Peak lactate fall ⩾ 4.4 mmol/L/kg/hr Falling glucose beyond 2 hrs < 10 mmol/L ALT < 6,000 UI/L at 2 hrs Perfusate pH > 7.20	Lactate ⩽ 1.7 mmol/L Cumulative bile production > 10 mL Perfusate pH 7.35-7.45
Biliary parameters	No biliary parameters	Biliary pH > 7.50 Bile glucose ⩽ 3 mmol/L (or ⩾ 10 mmol less than perfusate glucose)	Biliary pH > 7.45
Other parameters	Stable arterial flow > 15 0 mL/min Stable portal flow > 500 mL/min Homogeneous perfusion with soft liver consistency		
Pragmatic approach to viability assessment			
	Essential markers	***Desirable markers***	***Other markers***
DBD livers - assessment at 4 hrs	Lactate < 2.5 mmol/L within 4 hrs	Cumulative bile production > 2 5mL ALT < 6,000 UI/L Perfusate pH > 7.20 Falling glucose	Stable vascular flows Homogeneous perfusion with soft liver consistency Biliary pH > 7.50 Bile glucose ⩽ 3 mmol/L (or ⩾ 10 mmol less than perfusate glucose) Bile bicarbonate > 25 mmol/L
DCD livers – assessment at 4-6 hrs	Lactate < 2.5 mmol/L within 4 hours Bile production > 25 mL Bile pH > 7.50 Bile glucose ⩽ 3 mmol/L (or ⩾ 10 mmol less than perfusate glucose) Bile bicarbonate > 25 mmol/L	ALT < 6,000 UI/L Perfusate pH > 7.20 Falling glucose	Stable vascular flows Homogeneous perfusion with soft liver consistency

Abbreviations: ALT: alanine aminotransferase; DBD: donation after brainstem death; DCD: donation after circulatory death; hrs: hours
Note: 1 Mergental et al., 2020; 2 Watson et al., 2017; 3 de Vries 2019; *criteria described as favourable parameters without specification of the timeframe

criteria. For example, in the VITTAL trial, requiring lactate clearance below 2.5 mmol/L within 4 hours, 71% livers met the benchmark and were successfully transplanted with 100% 3-month graft and patient survival [27]. Livers most likely to fail were those with severe macrosteatosis or those exposed to a prolonged CIT. Through our learning with the viability assessment, we extended the assessment period and dropped some markers included earlier in the programme [7,16,27,46]. Indeed, in our series the livers meeting the lactate criteria also uniformly met vascular flows and macroscopic appearance criteria so their diagnostic value is limited. Regarding the bile assessment, our group has successfully transplanted DBD livers meeting the lactate criteria in the absence of bile production [2,27]. We acknowledge, however, that a minority of those organs went on to develop early allograft dysfunction or post-reperfusion syndrome and may not be suitable for all recipients.

The roughly defined cut-off values for some parameters might be re-defined by more sophisticated definitions. For example, the slope of lactate clearance per kg of liver mass per hour can provide more granularity to liver function assessment [44]. In the real-world, however, these are not user friendly and their benefit beyond the initial

1-2 hours would be limited. On this note it is important to mention that all the protocols were developed to ensure patient safety, and hence some safety margins were knowingly included. Teams commencing viability assessment programmes may be overwhelmed by the complexity of decision making and lack of universally accepted transplantability criteria [8]. The starting point is always to improve patient's safety. Cautiously planning to overcome the learning curve by applying more conservative criteria, considering preferably livers of favourable macroscopic appearance exposed to CIT, and selecting lower risk recipient seem to be reasonable starting points prior to proceeding to programme expansion. The initial avoidance of livers considered far beyond a teams' comfort zone may allow faster programmes growth as it prevents hiccups in outcomes and resources wasted from failed perfusions.

ONGOING AUDIT OF NMP PROGRAMMES

Every introduction of new technology needs to be monitored and its outcomes closely evaluated. The NMP programmes are likely to bring multiple benefits for the wider transplant teams, including daytime operating with improved support to deal with any complications, smoothen the re-perfusion phase, and overall improve early post-transplant patients' recovery. Liver transplantation is, however, a highly complex technical procedure whose outcome is clearly influenced by various factors including recipient condition. Occurrence of specific complications, e.g. hepatic artery thrombosis or anastomotic biliary strictures are unlikely to be caused by NMP, as those would have already been exposed in closely scrutinised data from the clinical trials. Technical complication related to the machine use may still appear, for example from a misplaced arterial cannula or biliary drain damaging the structures' lumen above the level of anastomosis. Regular review of the outcomes and critical appraisal of any complications, in particular those unexpected and rarely observed by the programme have an important role for long-term success. In view to excellent outcomes from up-to-date published NMP series the boundaries of the technology use are most likely to come from reflective learning from failures and complications along with the aspects contributing to improved outcomes.

CONCLUSIONS

Machine perfusion is a technology that overcomes the many shortcomings of SCS and is becoming a widely adopted method of liver preservation by transplant teams worldwide. One of the advantages of using NMP is the facilitation of transplanting extended criteria organs with increased confidence in positive outcomes. It is a resource-intensive intervention and the most optimal ways for adoption have yet to be established. Despite its relatively steep learning curve, the setting up of a new NMP brings benefits to both patients and transplant teams alike, and benefits from a multi-disciplinary approach. The NMP approach allows detailed liver functional assessment and objective parameters to determine organ transplantability. The risk of PNF can be predicted from hepatocellular function that is well reflected by perfusate lactate clearance, with other markers including bile volume, perfusate transaminases, or glucose utilisation. Assessment of DCD livers should include analysis of bile composition to exclude organs at increased risk of developing NAS, characterised by a failure to produce alkalotic bile, high glucose, and low bicarbonate concentrations. NMP programmes should monitor outcomes and learn from any complications that might be related to the procedure learning curve.

Acknowledgements

We gratefully acknowledge the generous NMP research support provided by all team members of the Liver Unit at Queen Elizabeth Hospital Birmingham. We also gratefully acknowledge the Welcome Trust for funding that supported the VITTAL trial.

Conflict of interest

The Authors declare no conflict of interest.

Funding

This manuscript did not require any external funding. This paper presents the authors' views and experience gained from research supported by the University Hospitals Birmingham Liver Charities and NIHR Birmingham Biomedical Research Centre at the UHB NHS Foundation Trust and the University of Birmingham. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health. The VITTAL trial was an independent, academic research project funded by the Welcome Trust [200121/Z/15/Z].

Author contributions

HM: conceived the manuscript concept; GC, HM: prepared the manuscript draft; GC, JM: contributed equally to drafting tables, figures, and paper editing; all Authors were involved in the research underpinning the presented data; NMP: expertise, and reviewed the final manuscript version.

Ethical consideration

Not applicable.

References

1 Bruggenwirth IMA, Porte RJ, Martins PN. Bile composition as a diagnostic and prognostic tool in liver transplantation. Liver Transpl 2020;26:1177-1187. https://doi.org/10.1002/lt.25771

2 Hann A, Lembach H, Nutu A, et al. Outcomes of normothermic machine perfusion of liver grafts in repeat liver transplantation (NAPLES initiative). Br J Surg 2022;109:372-380. https://doi.org/10.1093/bjs/znab475

3 van Rijn R, Schurink IJ, de Vries Y, et al. Hypothermic machine perfusion in liver transplantation – a randomized trial. N Engl J Med 2021;384:1391-1401. https://doi.org/10.1056/NEJMoa2031532

4 Martins PN, Buchwald JE, Mergental H, et al. The role of normothermic machine perfusion in liver transplantation. Int J Surg 2020;82S:52-60. https://doi.org/10.1016/j.ijsu.2020.05.026

5 Ghinolfi D, Dondossola D, Rreka E, et al. Sequential use of normothermic regional and ex-situ machine perfusion in donation after circulatory death liver transplant. Liver Transpl 2021;27:385-402. https://doi.org/10.1002/lt.25899

6 Ravikumar R, Jassem W, Mergental H, et al. Liver transplantation after ex-vivo normothermic machine preservation: a phase 1 (First-in-Man) clinical trial. Am J Transplant 2016;16:1779-87. https://doi.org/10.1111/ajt.13708

7 Mergental H, Stephenson BTF, Laing RW, et al. Development of clinical criteria for functional assessment to predict primary nonfunction of high-risk livers using normothermic machine perfusion. Liver Transpl 2018;24:1453-1469. https://doi.org/10.1002/lt.25291

8 Bruggenwirth IMA, van Leeuwen OB, Porte RJ, et al. The emerging role of viability testing during liver machine perfusion. Liver Transpl 2022;28:876-886. https://doi.org/10.1002/lt.26092

9 Watson CJE, Jochmans I. From "Gut Feeling" to objectivity: machine preservation of the liver as a tool to assess organ viability. Curr Transplant Rep 2018;5:72-81. https://doi.org/10.1007/s40472-018-0178-9

10 Reiling J, Butler N, Simpson A, et al. Assessment and transplantation of orphan donor livers – a "back-to-base" approach to normothermic machine perfusion. Liver Transpl 2020;26:1618-1628. https://doi.org/10.1002/lt.25850

11 Watson CJ, Randle LV, Kosmoliaptsis V, et al. 26-hour storage of a declined liver before successful transplantation using ex-vivo normothermic perfusion. Ann Surg 2017;265:e1-e2. https://doi.org/10.1097/SLA.0000000000001834

12 Schlegel A, Porte R, Dutkowski P. Protective mechanisms and current clinical evidence of hypothermic oxygenated machine perfusion (HOPE) in preventing post-transplant cholangiopathy. J Hepatol 2022;76:1330-1347. https://doi.org/10.1016/j.jhep.2022.01.024

13 Guarrera JV, Henry SD, Samstein B, et al. Hypothermic machine preservation in human liver transplantation: the first clinical series. Am J Transplant 2010;10:372-381. https://doi.org/10.1111/j.1600-6143.2009.02932.x

14 Schlegel A, Graf R, Clavien PA, et al. Hypothermic oxygenated perfusion (HOPE) protects from biliary injury in a rodent model of DCD liver transplantation. J Hepatol 2013;59:984-991. https://doi.org/10.1016/j.jhep.2013.06.022

15 van Rijn R, Karimian N, Matton APM, et al. Dual hypothermic oxygenated machine perfusion in liver transplants donated after circulatory death. Br J Surg 2017;104:907-917. https://doi.org/10.1002/bjs.10515

16 Mergental H, Perera MT, Laing RW, et al. Transplantation of declined liver allografts following normothermic ex-situ evaluation. Am J Transplant 2016;16:3235-3245. https://doi.org/10.1111/ajt.13875

17 Watson CJE, Kosmoliaptsis V, Randle LV, et al. Normothermic perfusion in the assessment and preservation of declined livers before transplantation: hyperoxia and vasoplegia-important lessons from the first 12 cases. Transplantation 2017;101:1084-1098. https://doi.org/10.1097/TP.0000000000001661

18 Guarrera JV, Henry SD, Samstein B, et al. Hypothermic machine preservation facilitates successful transplantation of "orphan" extended criteria donor livers. Am J Transplant 2015;15:161-169. https://doi.org/10.1111/ajt.12958

19 Oniscu GC, Randle LV, Muiesan P, et al. In-situ normothermic regional perfusion for controlled donation after circulatory death-the United kingdom experience. Am J Transplant 2014;14:2846-2854. https://doi.org/10.1111/ajt.12927

20 Fondevila C, Hessheimer AJ, Ruiz A, et al. Liver transplant using donors after unexpected cardiac death: novel preservation protocol and acceptance criteria. Am J Transplant 2007;7:1849-1855. https://doi.org/10.1111/j.1600-6143.2007.01846.x

21 Liu Q, Hassan A, Pezzati D, et al. Ex-situ liver machine perfusion: the impact of fresh frozen plasma. Liver Transpl 2020;26:215-226. https://doi.org/10.1002/lt.25668

22 Cardini B, Oberhuber R, Fodor M, et al. Clinical implementation of prolonged liver preservation and monitoring through normothermic machine perfusion in liver transplantation. Transplantation 2020;104:1917-1928. https://doi.org/10.1097/TP.0000000000003296

23 Nasralla D, Coussios CC, Mergental H, et al. A randomized trial of normothermic preservation in liver transplantation. Nature 2018;557:50-56. https://doi.org/10.1038/s41586-018-0047-9

24 Markmann JF, Abouljoud MS, Ghobrial RM, et al. Impact of portable normothermic blood-based machine perfusion on outcomes of liver transplant: the OCS liver PROTECT randomized clinical trial. JAMA surgery 2022;157:189-198. https://doi.org/10.1001/jamasurg.2021.6781

25 Patrono D, Cussa D, Rigo F, et al. Liver machine perfusion survey g. heterogeneous indications and the need for viability assessment: an international survey on the use of machine perfusion in liver transplantation. Artificial organs 2022;46:296-305. https://doi.org/10.1111/aor.14061

26 Mohkam K, Nasralla D, Mergental H, et al. In-situ normothermic regional perfusion versus ex-situ normothermic machine perfusion in liver transplantation from donation after circulatory death. Liver Transpl 2022;Jun 3. https://doi.org/10.1002/lt.26522 [Epub Ahead of Print]

27 Mergental H, Laing RW, Kirkham AJ, et al. Transplantation of discarded livers following viability testing with normothermic machine perfusion. Nat Commun 2020;11:2939. https://doi.org/10.1038/s41467-020-16251-3

28 Gaurav R, Butler AJ, Kosmoliaptsis V, et al. Liver transplantation outcomes from controlled circulatory death donors: static cold storage vs in-situ normothermic regional perfusion vs ex-situ

normothermic machine perfusion. Ann Surg 2022;275:1156-1164. https://doi.org/10.1097/SLA.0000000000005428

29 Roll GR, Quintini C, Reich DJ. In quest of the what, when, and where for machine perfusion dynamic liver preservation: carpe diem! Liver Transpl 2022;Jul 18. https://doi.org/10.1002/lt.26546 [Epub Ahead of Print]

30 Boteon YL, Attard J, Boteon A, et al. Manipulation of lipid metabolism during normothermic machine perfusion: effect of defatting therapies on donor liver functional recovery. Liver Transpl 2019;25:1007-1022. https://doi.org/10.1002/lt.25439

31 He X, Guo Z, Zhao Q, et al. The first case of ischemia-free organ transplantation in humans: a proof of concept. Am J Transplant 2018;18:737-744. https://doi.org/10.1111/ajt.14583

32 Ghinolfi D, Jassem W, Martins PN. Ischemia-free liver transplantation. Is this the right answer to overpass organ shortage and post-liver transplant complications? Lancet Reg Health West Pac 2021;16:100263. https://doi.org/10.1016/j.lanwpc.2021.100263

33 Boteon YL, Laing RW, Schlegel A, et al. Combined hypothermic and normothermic machine perfusion improves functional recovery of extended criteria donor livers. Liver Transpl 2018;24:1699-1715. https://doi.org/10.1002/lt.25315

34 van Leeuwen OB, de Vries Y, Fujiyoshi M, et al. Transplantation of high-risk donor livers after ex-situ resuscitation and assessment using combined hypo- and normothermic machine perfusion: a prospective clinical trial. Ann Surg 2019;270:906-914. https://doi.org/10.1097/SLA.0000000000003540

35 Bruggenwirth IMA, van Leeuwen OB, de Vries Y, et al. Extended hypothermic oxygenated machine perfusion enables ex-situ preservation of porcine livers for up to 24 hours. JHEP Rep 2020;2:100092. https://doi.org/10.1016/j.jhepr.2020.100092

36 Ceresa CDL, Nasralla D, Watson CJE, et al. Transient cold storage prior to normothermic liver perfusion may facilitate adoption of a novel technology. Liver Transpl 2019;25:1503-1513. https://doi.org/10.1002/lt.25584

37 Watson CJ, Harper SJ. Anatomical variation and its management in transplantation. Am J Transplant 2015;15:1459-1471. https://doi.org/10.1111/ajt.13310

38 Bral M, Gala-Lopez B, Bigam D, et al. Preliminary single-center Canadian experience of human normothermic ex-vivo liver perfusion: results of a clinical trial. Am J Transplant 2017;17:1071-1080. (In eng). https://doi.org/10.1111/ajt.14049

39 Hunt F, Johnston CJC, Coutts L, et al. From Haphazard to a sustainable normothermic regional perfusion service: a blueprint for the introduction of novel perfusion technologies. Transpl Int 2022;35:10493. https://doi.org/10.3389/ti.2022.10493

40 Lonze BE, Parsikia A, Feyssa EL, et al. Operative start times and complications after liver transplantation. Am J Transplant 2010;10:1842-1849. (Research Support, Non-U.S. Gov't) (In eng). https://doi.org/10.1111/j.1600-6143.2010.03177.x

41 Lindemann J, Dageforde LA, Brockmeier D, et al. Organ procurement center allows for daytime liver transplantation with less resource utilization: may address burnout, pipeline, and safety for field of transplantation. Am J Transplant 2019;19:1296-1304. https://doi.org/10.1111/ajt.15129

42 Mergental H, Laing RW, Afford SC, et al. Reply to 'Hypothermic machine perfusion before viability testing of previously discarded human livers'. Nat Commun 2021;12:1015. https://doi.org/10.1038/s41467-021-21183-7

43 van Leeuwen OB, de Vries Y, de Meijer VE, et al. Hypothermic machine perfusion before viability testing of previously discarded human livers. Nat Commun 2021;12:1008. https://doi.org/10.1038/s41467-021-21182-8

44 Watson CJE, Kosmoliaptsis V, Pley C, et al. Observations on the ex-situ perfusion of livers for transplantation. Am J Transplant 2018;18:2005-2020. https://doi.org/10.1111/ajt.14687

45 de Vries Y, Matton APM, Nijsten MWN, et al. Pretransplant sequential hypo- and normothermic machine perfusion of suboptimal livers donated after circulatory death using a hemoglobin-based oxygen carrier perfusion solution. Am J Transplant 2019;19:1202-1211. https://doi.org/10.1111/ajt.15228

46 Hann A, Lembach H, Nutu A, et al. Assessment of deceased brain dead donor liver grafts via normothermic machine perfusion: lactate clearance time threshold can be safely extended to 6 hours. Liver Transpl 2022;28:493-496. https://doi.org/10.1002/lt.26317

47 Sutton ME, op den Dries S, Karimian N, et al. Criteria for viability assessment of discarded human donor livers during ex-vivo normothermic machine perfusion. PloS one 2014;9:e110642. https://doi.org/10.1371/journal.pone.0110642

48 Mergental H, Laing RW, Hodson J, et al. Introduction of the concept of diagnostic sensitivity and specificity of normothermic perfusion protocols to assess high-risk donor livers. Liver Transpl 2021. https://dx.doi.org/10.1002/lt.26326 [Epub Ahead of Print]

49 Weissenbacher A, Bogensperger C, Oberhuber R, et al. Perfusate enzymes and platelets indicate early allograft dysfunction after transplantation of normothermically preserved livers. Transplantation 2022;106:792-805. https://doi.org/10.1097/TP.0000000000003857

50 Schreiter T, Sowa JP, Schlattjan M, et al. Human ex-vivo liver model for acetaminophen-induced liver damage. Sci Rep 2016;6:31916. https://doi.org/10.1038/srep31916

51 Lau NS, Ly M, Liu K, et al. Current and potential applications for indocyanine green in liver transplantation. Transplantation 2022;106:1339-1350. https://doi.org/10.1097/TP.0000000000004024

52 Schurink IJ, de Haan JE, Willemse J, et al. A proof of concept study on real-time LiMAx CYP1A2 liver function assessment of donor grafts during normothermic machine perfusion. Sci Rep 2021;11:23444. https://doi.org/10.1038/s41598-021-02641-0

53 Matton APM, de Vries Y, Burlage LC, et al. Biliary bicarbonate, pH, and glucose are suitable biomarkers of biliary viability during ex-situ normothermic machine perfusion of human donor livers. Transplantation 2019;103:1405-1413. https://doi.org/10.1097/TP.0000000000002500

54 op den Dries S, Westerkamp AC, Karimian N, et al. Injury to peribiliary glands and vascular plexus before liver transplantation predicts formation of non-anastomotic biliary strictures. J Hepatol 2014;60:1172-1179. https://doi.org/10.1016/j.jhep.2014.02.010

55 Laing RW, Scalera I, Isaac J, et al. Liver transplantation using grafts from donors after circulatory death: a propensity score-matched study from a single center. Am J Transplant 2016;16:1795-1804. https://doi.org/10.1111/ajt.13699