REVIEW

Perfusion settings and additives in liver normothermic machine perfusion with red blood cells as oxygen carrier. A systematic review of human and porcine perfusion protocols

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SUMMARY

Liver machine perfusion (MP) at normothermic temperature (NMP) is a promising way to preserve and evaluate extended criteria donor livers. Currently, no consensus exists in methodology and perfusion protocols. Here, the authors performed a systematic literature search to identify human and porcine studies reporting on liver NMP with red blood cells. A qualitative synthesis was performed concerning technical aspects of MP, fluid composition, gas supply, and liver positioning. Thirty-seven publications including 11 human and 26 porcine studies were considered for qualitative synthesis. Control mode, pressure, flow, perfusate additives, and targeted blood gas parameters varied across human as well as porcine studies. For future analyses, it is advisable to report flow adjusted to liver weight and exact pressure parameters including mean, systolic, and diastolic pressure. Parenteral nutrition and insulin addition was common. Parenteral nutrition included amino acids and/or glucose without lipids. Taurocholic acid derivatives were used as bile flow promoters. However, short-term human NMP without taurocholic acid derivatives seems to be possible. This finding is relevant due to the lack of clinical grade bile salts. Near physiological oxygen tension in the perfusate is doable by adjusting gas flows, while blood gas parameters regulation needs more detailed description.

Introduction

The increasing shortage of liver grafts forced the transplant community to use marginal grafts. Given the higher vulnerability of these grafts to cold storage, interest in machine perfusion (MP) was reconsidered [1–3].

A major drawback in the field is the differences in reporting [4]. The optimal perfusion protocol is also a matter of debate [3,5–8]. Consequently, the publications on MP have exhibited great discrepancies and the need for a standardized reporting appeared as already observed in other fields of surgery [9]. Recently, a standardized nomenclature and reporting guidelines for MP were proposed. Temperature ranges were defined; hypothermic MP (HMP) 0–12 °C, midthermic MP (MMP) 13–24 °C, subnormothermic MP (SMP) 25–

34 °C and normothermic MP (NMP) 35–38 °C. Technical aspects of MP, perfusion fluid composition, and oxygenation modes are among the checklist parameters for reporting [4].

Currently, many different variants of MP exist, ranging from HMP to NMP. HMP is a simple and effective method to rescue extended criteria grafts [10–13]. However, a lack of functional assessment possibility was acknowledged [6,14]. NMP is an alternative for graft function evaluation. Some recent case series reported the possible evaluation of discarded human liver grafts and successful subsequent transplantation [15,16]. Another possible application of NMP could be the rescue of steatotic livers [17–19]. There is no doubt that NMP requires oxygen carriers to satisfy the oxygen demand. Red blood cells (RBC) as oxygen carrier for NMP are common. The growing number of publications gives enough support to systematically analyze NMP protocols with RBC.

The aim of this review was to perform a systematic search for existing RBC-based NMP protocols. Review questions included technical aspects of MP, perfusion fluid composition, and oxygenation modes as proposed by international experts group [4]. In addition, liver positioning was reported. Although there are some reviews on the topic [8,20,21], a systematic one is missing.

Methods

This systematic review was conducted according to the PRISMA statement [22–24]. A systematic literature search of the databases Cochrane, Embase, Medline, PubMed, and Scopus was conducted on 23rd of May, 2017, including only publications in English. The electronic search strategy was reported in the Table S1. Limited manual search was performed in January 2018. Nonquantitative findings were systematically interpreted, and qualitative synthesis was used to draw conclusions. All human and porcine studies with liver NMP using RBC were included. We excluded studies using artificial oxygen carriers. Results of studies with synthetic hemoglobin-based oxygen carriers (HBOC) were disappointing, even with stopping of the ongoing clinical trials by FDA due to safety reasons [25]. Furthermore, HBOC causes vasoconstriction, contributes to reactive oxygen species release, and interferes with laboratory measurements [25]. Their production price or viral safety is another unsolved issue [25]. We excluded also another artificial oxygen carrier perfluorocarbon. Perfluorocarbon is excreted through the lungs without metabolism

[26]. Thus, keeping the constant perfusate concentration in a system with oxygenator could be a dilemma. In addition, perfluorocarbon causes macrophage activation [26]. HMP, MMP, and SNP protocols [4] were also excluded to ascertain a consistent population and keep the fluency of data interpretation. Furthermore, we did not expect relevant variances, and some review articles are available on the topic [8,10,11,13,27].

Data extraction and collection

Three reviewers (D.E., M.S., and F.L.) independently screened all available abstracts. Full-text articles of selected abstracts were obtained and eligible studies included in the qualitative synthesis. The endpoints of the qualitative synthesis were portal and arterial perfusion parameters, perfusion fluid additives, provided gas supply protocols, and liver positioning.

Results

Literature search

The systematic search identified 1358 abstracts. Of those, 636 were duplicates and excluded during electronic search. Nine studies were added by manual search. Totally 731 abstracts were screened, and 177 articles were found to be eligible for full-text screening. After full-text screening, 140 articles were excluded from further analysis, leaving 37 publications to be included in the qualitative synthesis (Fig. 1). Of those, 11 were human studies and 26 were porcine. Most groups applied almost the same perfusion protocol in different studies with varying perfusion duration and graft characteristics. Perfusion protocols were not changed from pig to human studies in groups with experience in both. Thus, the articles with the most detailed description were chosen, while the remaining papers were excluded as a repetition. This was also the reason to exclude some studies using RBC in SNP [28,29].

Liver blood circulation: control mode, pressure, and flow

Human studies

Perfusion was controlled by setting a pressure limit in both HA and PV in the majority of the studies, while two studies used a flow limit. In one study, the reservoir was kept at a fixed height to maintain the hydrostatic pressure for PV flow without applying any control,
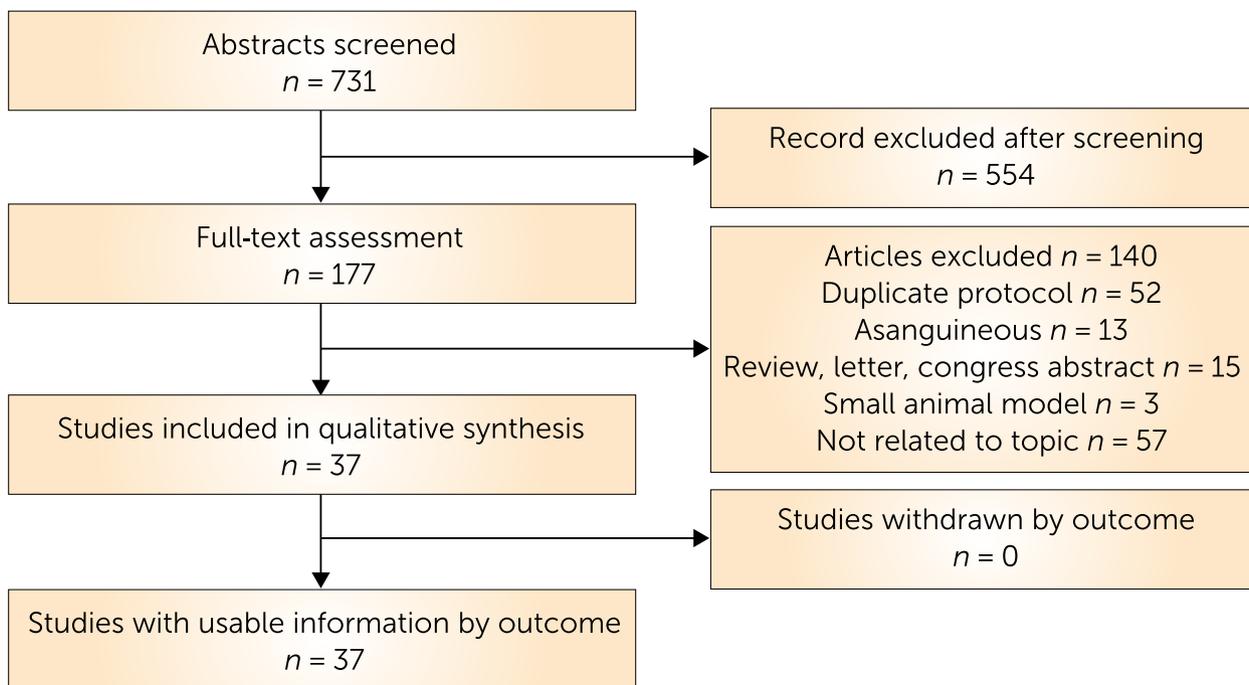
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┌─────────────────────────────────┐
│        Abstracts screened        │
│            n = 731               │
└─────────────────────────────────┘
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              │─────────────────────────────►┌─────────────────────────────────┐
              │                               │  Record excluded after screening │
              │                               │            n = 554               │
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│       Full-text assessment       │           ┌─────────────────────────────────┐
│            n = 177               │           │    Articles excluded n = 140     │
└─────────────────────────────────┘           │    Duplicate protocol n = 52     │
              │─────────────────────────────► │     Asanguineous n = 13          │
              │                               │ Review, letter, congress abstract n = 15 │
              │                               │   Small animal model n = 3       │
              ▼                               │   Not related to topic n = 57    │
┌─────────────────────────────────┐           └─────────────────────────────────┘
│ Studies included in qualitative synthesis │
│            n = 37                │           ┌─────────────────────────────────┐
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              │─────────────────────────────► │            n = 0                 │
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│ Studies with usable information by outcome │
│            n = 37                │
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Figure 1 PRISMA flow diagram.

which lead to mean portal pressure of 17.9 mmHg [30]. Applied pressure in HA varied considerably from 40 to 100 mmHg. Nonpulsatile and pulsatile flow patterns in HA were used in five and three studies, respectively, with centrifugal pump. Two studies used a roller pump, and one study did not specify flow and pump type in HA. In studies with pulsatile flow, only mean pressure was reported while systolic and diastolic ones were missing. Data on HA flow were reported in two manners, either in ml/g liver tissue/min or in ml/min in whole liver. In those studies, where flow was reported in ml/min for the whole liver, flow ranged from 100 to 450 ml/min. The weight of the organ was lacking in most studies. In a study by Mergental with a pressure control, a liver with a weight of 1382 g showed a HA flow of 623 ml/min, higher than the HA flow in the liver weighting 2400 g (491 ml/min) [15]. Portal vein (PV) perfusion was performed by either gravity derived pressure or a pump. The reported PV pressure was higher than physiologic one in some studies. In eight studies, where flow was reported in ml/min for the whole liver, flow ranged from 660 to 1500 ml/min. Again, the liver weight was not reported. In a study by Banan *et al.* [6], the authors reported a liver weight range between 1750 and 5000 g and flow in PV 1400 ± 200. In the study by Mergental, the liver with 1382 g had a PV flow of 1500 ml/min and was higher than the flow in the liver weighting 2400 g

(700 ml/min) [15]. VC was kept open in six studies. Three studies placed a cannula to drain VC. Of those, one study reported the VC pressure of 1.3 mmHg, which was controlled by pump head rotational speed [30].

Porcine studies

Perfusion of HA and PV was controlled by setting either a pressure limit in 10 studies or a flow limit in eight studies. In four studies, the control mode was different between HA and PV. Applied pressure in HA varied considerably from 40 to 120 mmHg. Both nonpulsatile and pulsatile flow patterns in HA were used. HA flow in six studies, reporting the data in ml/g liver tissue/min, ranged from 0.14 to 0.38. In seven studies where flow was reported in ml/min for the whole liver, flow in the HA ranged from 90 to 400 ml/min and the weight of the organ was again mostly lacking. The targeted portal pressure was mostly in physiologic range except 4, where pressure higher than 12 mmHg was reported. PV flow varied considerably across studies as observed in HA flow. Details on the VC outflow were poorly reported and only available in 13 studies. Of those, eight kept the VC open. Studies using a closed system with cannulation of the VC, targeted the VC pressure less than 2 mmHg. However, technical aspects VC pressure control were poorly reported (Table 1).

Bolus and continuous perfusate components

Human studies

Bolus infusions prior to perfusion start can be retrieved in eight studies and grouped into two groups: electrolytes and antibiotics. Bicarbonates were used to correct pH prior to perfusion start in all studies except one by Liu *et al.* [31], where bicarbonates were infused after perfusion start. Another common electrolyte was calcium, being used in six of eight studies. The most used prophylactic antibiotics were substances of the cephalosporin group. Anaerobic and gram-positive bacterial infection prophylaxis with metronidazole and vancomycin, respectively, were used in two studies. Only one study used broad spectrum antibiotic meropenem and antifungal fluconazole (Table 2).

Parenteral nutrition, insulin, and taurocholic acid derivatives (TA) were provided as continuous additives. Parenteral nutrition contained glucose and/or amino acids while avoiding lipids and was continuously infused at a rate ranging from 3.5 to 20 ml/h. Insulin dose also varied considerably from 0 to 200 U/h. Some studies adjusted insulin depending on the perfusate glucose level. TA was used in three studies, while five, including also the transplant studies by Mergental and Watson, did not include TA in the protocol [15,32]. All included studies except one administered prostaglandins continuously, although the type of administered prostaglandins varied.

Porcine studies

Similar to human studies, cephalosporin group was common for bacterial infection prophylaxis. In contrast to human studies, bicarbonates and calcium supplements were less common (Table 3). Parenteral nutrition infusion rate varied and included amino acids and/or glucose without lipids. Inclusion of insulin and TA in perfusion protocols was inconsistent. If insulin was part of the protocol, the applied dose varied from 1 to 125 U/h. In opposite to human studies, prostaglandins were less common and some other vasodilators were explored [33,34].

Regulation of blood gas parameters

Human studies

The provided gas supply to the oxygenator was adjusted depending on the PaO_2 value. $PaCO_2$ was adjusted depending on targeted pH value. The targeted PaO_2 was higher than the physiologic level in almost all human studies. In one human study, the authors compared the post-transplant course between PaO_2 of 621–971 mmHg and PaO_2 153–187 mmHg and reported that avoidance of hyperoxia could prevent vasoplegia and postreperfusion syndrome [32]. The provided gas to the oxygenator either was a fixed mixture of 95% O_2 and 5% CO_2 or regulated separately by the supply of air, O_2 and CO_2 (Table 4).

Porcine studies

Most studies targeted the supraphysiologic PaO_2 similar to human studies. The provided gas to the oxygenator varied and some studies regulated gas flow separately to adjust pH and PaO_2. In four studies, blood in PV was partially oxygenated. Partial oxygenation was created by adding a bypass from the oxygenator to the reservoir [35,36] or the direct mixture of arterial and venous blood in the PV line [37,38].

Liver positioning during MP

Human studies

Most included human studies used a commercially available perfusion machines. In three, authors own developed liver container was described. In these studies, the livers were floating in fluid to avoid compression areas (Table 5).

Porcine studies

In the porcine studies, the authors mostly used a self-made liver container. The preferred type of positioning was to let the liver float in the perfusate or in a physiologic saline solution. In some studies, authors put the liver in a pressure chamber to test the effect of applying an oscillating external pressure on perfusion parameters (Table 5).

Discussion

Ex vivo liver MP is already being used in clinical trials but many relevant aspects are still poorly defined.

Considering perfusion regulation, the question remains unanswered if HA and PV perfusion should be controlled with pressure or flow. Probably, both strategies are applicable, as an increase in flow causes a rise in pressure, and vice versa. However, the optimal pressure and flow in HA and PV to maintain the perfusion

Table 1. Perfusion control mode, pressure, and flow during NMP.

Author	Perfusion control		Pressure mmHG		Flow ml/min		Comment
	Flow	Pressure	HA	PV	HA	PV	
Human studies							
Banan [6]	HA/PV		85	9	450 ± 150	1400 ± 200	
Bellomo [74]	HA/PV		40–60	5–15	100–300	700–1000	
Darin [38]		HA/PV	–	–	–	–	PV control by reservoir height
He [19]		HA/PV	–	–	250–300	800–900	
Karimian [69]		HA/PV	70	11	256 ± 16	748 ± 34	
Liu [31]		HA/PV	60–100	7–10	0.25	0.75	Flow reported as ml/g/min
Mergental [15]		HA/PV	–	–	360–654	700–1500	One liver with different machine
Reiling [61]		HA/PV	70	5–15	300–375	550–750	
Vekemans [75]	PV	HA	80	9	–	1.2	Flow reported as ml/g/min
Vogel [30]		HA	73.6 ± 1.7	17.9 ± 0.7	330 ± 30	986 ± 30	HA and VC control by pump speed. PV without control under gravitational pressure
Watson [32]		HA/PV	60	9	208–349	660–1130	
Porcine studies							
Abouna [76]		HA/PV	–	–	–	–	VC control with gate clamp
Adham [64]	HA	PV	–	–	0.25	0.75	Flow reported as ml/g/min Total hepatic flow control with PV pressure adjustment
Bell [63]	HA/PV		70	5.1	0.2	0.9	Flow reported as ml/g/min
Borie [77]	HA/PV		100–120	15–20	0.11–0.38	0.34–1.15	Flow reported as ml/g/min
Chung [78]		HA/PV	80–100	<10	–	–	
Cimeno [79]		HA/PV	60–80	5–10	–	–	
Echeverri [33]		HA/PV	60	2–4	400	900–1200	
Fondevila [80]		HA/PV	40–60	8	163	745	Flow at perfusion start
He [81]		HA/PV	80–100	8–10	110–240	310–590	
Hickman [68]	HA/PV		–	–	0.14	0.8	Flow reported as ml/g/min
Hoyer [82]	PV	HA	80	–	–	0.8	Flow reported as ml/g/min
Ikeda [83]	HA/PV		–	–	0.33	0.67	PV control by reservoir height Flow reported as ml/g/min
Jablonksi [34]	HA	PV	–	16.2	150–200	–	
Janssen [36]		HA/PV	100	11	–	–	Total flow 1–2 ml/g/min
Mets [60]	HA/PV		<100	<18	95	425	PV/VC control by reservoir height
Nagel [41]		HA/PV	80	8	18	42	Flow reported as ml/100 g/min
Satoh [84]	HA/PV		–	–	0.15	0.75	Flow reported as ml/g/min VC control with pressure gradient
Schoening [48]		HA/PV	58–91	12–23	–	–	PV reservoir 20 cm above the liver
Schön [50]	PV	HA	50–75	3.7–11	150–250	800–1000	
Steffen [73]		HA/PV	80	2–3	–	–	PV control with bubble trap height VC control with reservoir height
Xu [85]	HA/PV		70–80	5–8	300–400	1200–1500	
Zhang [86]	HA/PV	–	–	–	–	–	

Water column converted to mmHg, for pulsatile flow mean arterial pressure was reported.

HA hepatic artery, PV portal vein, VC vena cava.

remains open in this review. Pressure in HA varied considerably between 40 and 120 mmHg and none of included studies provided the reason for applied pressure. To define portal hypertension, hepatic venous pressure gradient (HVPG) is used in clinical practice. HVPG higher than 5 mmHg is defined as portal hypertension and higher than 10 mmHg is termed as clinically significant portal hypertension with increased patient mortality [39]. Pressure target higher than 15 mmHg in PV in some included studies is therefore unusual. No explanations were provided by the authors on the potential reasons. VC target pressure of

Table 2. Baseline perfusate additives prior NMP start.

Author	Antibiotics	Electrolyte	Others
Human studies			
He [19]	Cefoperazone 1.5 g Metronidazol 0.5 g	Bi 30 ml, Ca 10 ml Ma 3 ml	Amino acids 250 ml Extracellular fluid: Succinylated gelatin
Karimian [69]	Metronidazol 0.2 g Cefazolin 0.2 g	Bi 31 Ca 40 ml	Albumin 100 ml, multivitamins insulin 2000 IU, nutrition Extracellular fluid: Fresh frozen plasma
Liu [31]	Vancomycin 0.5 g Cefotoxime 1 g	–	Methyl prednisolone 0.5 g Extracellular fluid: Fresh frozen plasma
Mergental [15]	Vancomycin500 mg Gentamicin 60 mg	Bi 30 ml, Ca 10 ml	Extracellular fluid: Albumin
Reiling [61]	–	Bi, Ca	Extracellular fluid: Albumin
Vekemans [75]	–	Bi	Extracellular fluid: Aqix-RS-I enriched with albumin
Vogel [30]	Cefuroxime 750 mg	Bi, Ca 10 ml	Extracellular fluid: Crystalloid solution, the same group reported also with succinylated gelatin solution
Watson [32]	Meronem/fluconazole	Bi30 ml, Ca, Ma	Amino acids Extracellular fluid:succinylated gelatin or Steen solution
Porcine studies			
Abouna [76]	–	–	Glucose
Borie [77]	Cefamandole 750 mg	–	
Chung [87]	Cefuroxime 750 mg	Bi 40 ml, Ca10 ml	Epoprostenol 40 µg
Echeverri [33]	Cefazolin 1 g Metronidazole 0.5 g	Bi, Ca	Albumin, Amino acids, Vasodilator (BQ-123) and Verapamil in study groups
Gong [88]	–	Ca	
He [81]	Cefuroxime 1.5 g		Glutathione 600 mg Alprostadil10 µg
Hoyer [89]	–	Bi, Ca	D4%W – 40 ml
Satoh [84]	Ampicillin 500 mg	–	Hydroxycortisone 100 mg
Schoening [48]	Tobramycin 40 mg	–	Iloprost
Schön [50]	–	Bi	Fructose, oleat, amino acids, and free radical scavengers
Steffen [73]	–	Bi	Albumin
Xu [85]	Penicillin 40 000 U/l Streptomycin 40 mg/l	–	Insulin (2 U/l), Hydrocortisone (10 mg/l)
Zhang [86]	Cefuroxime 1.5 g		P-E1 10 µg, Glutathione 600 mg

Bi, Bicarbonates, Ca, Calcium, Ma, Magnesium.

<2 mmHg could be explained with prevention of liver congestion. The next perfusion parameter assessed was flow. It is reported that *in vivo* liver flow is 100 ml/min per 100 g liver wet weight [40]. Indeed, some authors applied this reference during perfusion, while others not. The porcine study by Nagel *et al.* [41] controlled the perfusion with pressure in HA and PV and reported total liver flow rate of 60 ml/min per 100 g liver wet weight, which is far below than reference. The human study by Liu *et al.* [31] targeted the reference flow and reported pressure of 60–100 mmHg in HA and 5–10 mmHg in PV. The reasons for such variations were not discussed. The further results in this review also challenge the applicability of the reported *in vivo* reference in *ex vivo* setting. According to this rule, the liver weighting 5 kg in the study by Banan *et al.* [6] should have received 5 l/min blood during NMP. Looking at a

flow rate of 450 ± 150 in HA and 1400 ± 200 in PV in this study, the total flow was most probably not 5 l/min. In the human study by Mergental *et al.* [15], the total flow rate in the liver weighing 1382 g was higher than the flow rate in the liver weighting 2400 g. Of most, uneventful transplant outcome in both cases creates a dilemma in interpretation of clinical relevance of flow rate. Interestingly, flow and pressure questions could not definitively be solved also with the upcoming of cardiopulmonary bypass (CPB) [42]. Applied pressure and flow during CPB varies substantially by institution and is influenced by developed practice paradigms [42,43]. While many institutions maintain the mean arterial pressure higher than 50–60, other data support pressure >70 mmHg. The value 50 mmHg is most probably based on the lower pressure limit of adequate cerebral perfusion autoregulation [42]. In the

Table 3. Continuous perfusate additives during NMP.

Author	Nutrition	Insulin	Bile acid	Prostaglandins	Electrolyte	Other
Human studies						
Banan [6]	PN rate 7 ml/h A 4.25%, G 5%	20 U/h	7 ml/h	Prostacyclin 10 μg/h	–	L-carnitine/exendin-4 in steatotic livers
Bellomo [74]	PN rate 5 ml/h A 3.3%, G 9.7%, L: 3.9%	10 U/h	–	Epoprostenol once	–	
Karimian [69]	–	–	–	–	–	
Liu [31]	PN rate 3.5 ml/h A 5%, G 15%	4 U/h	–	Epoprostenol 6–60 μg/h	Bi o.d.	Multivitamins/trace elements, G
Mergental [15]	–	–	–	Epoprostenol 8 μg/h	–	
Reiling [61]	PN rate 20 ml/h	50–200 U/h	7 ml/h	Prostacyclin 8 μg/h	–	
Vogel [30]	PN rate 15 ml/h	Yes	140 mg/h	Epoprostenol 8 μg/h	–	PN/insulin control
Watson [32]	–	Yes	–	Epoprostenol 2 μg/h	Bi o.d.	
Porcine studies						
Borie [77]	–	4 U/h	–	–	Bi o.d.	
Chung [87]	–	100 U/h	200 mg/h	Epoprostenol 40 μg/h	Bi 40 ml/h	
Echeverri [33]	PN rate 8 ml/h A 4.25% G50W 2–5 ml/h	125 U/h	7 ml/h	P–E1 500 μg/3 h	–	Vasodilator (BQ-123) and Verapamil bolus, Epoprostenol 8 μg/h
Gong [88]	G50W 20 ml/h	–	–	–	–	
Fondevila [80]	–	–	–	–	Bi o.d.	
He [81]	PN rate 10 ml/h A 5%, G 5%	6–10 U/h	–	–	–	Famotidine, 1-benzylimidazole αGPIb
Hoyer [89]	PN rate 4 ml/h G 12 ml/h	1.5 U/h	0.15 mg/h	–	–	
Ikeda [83]	–	–	–	–	Bi o.d.	
Jablonski [34]	–	–	4 pmol/min	–	Bi o.d.	Vasodilators with no or only short effect
Kern [90]	G 8 g/h	8 U/h	–	–	–	
Mets [60]	–	–	–	–	Bi o.d.	
Satoh [84]	G50W	1 U/h	–	P – E1 25 ng/h	–	G > 150 mg/dl
Schoening [48]	–	–	–	Iloprost 2 ng/kg/min	–	
Steffen [73]	–	–	–	–	Yes	
Zhang [86]	PN rate 10 ml/h A 5% G5W 10 ml/h	6–10 U/h	–	–	Yes	G, insulin, pH, electrolyte control

Parenteral nutrition (PN) was reported in rate (ml/h) with concentration (%) of amino acids (A), glucose (G) and lipids (L). Bile acids included taurocholic acid derivatives. Bicarbonates Bi, G50W glucose in water 50%; o.d. on demand.

same context, a flow rate of 2.2–2.5 l/min/m^2 is more common in use [42]. The targeted pressure in HA during NMP seems to be at least 50 mmHg, keeping the upper limit unsolved. The physiologic PV pressure ranges seem to be reasonable. The optimal liver weight adjusted flow is, however, not easy to define. With growing number of publication in the field, this question could be addressed in the future studies. To make future comparisons among studies possible, it is advisable to report flow rates adjusted to liver weight. Furthermore, we probably need to redefine the flow

reference. The reported *in vivo* reference of 100 ml/min per 100 g liver has its origin from early 1970s [40]. Recently reported *in vivo* liver weight adjusted flow rates, measured by modern flow measurement devices in pigs, were far beyond the reference [44].

Another parameter that could have influenced such variation in flow and pressure in *ex vivo* setting is the missing complex *in vivo* regulation mechanisms of intrahepatic blood circulation. In the absence of those physiological control mechanisms in an *ex vivo* setting, resistance in the vascular bed could be substantially

Table 4. Gas supply protocols during NMP.

Author	Target SO$_2$ (%)/PO$_2$ mmHg	PCO$_2$ (mmHg)	Oxygenator gas mixture	Comment
Human studies				
Banan [6]	275–578 mmHg	–	95% O$_2$ and 5% CO$_2$	
Bellomo [74]	HA 60–120 mmHg	–	Air and O$_2$	pO$_2$/pCO$_2$ control with gas flow rate
	PV 20–60 mmHg	–		
Darin [38]	–	–	95% O$_2$ and 5% CO$_2$	Partial oxygenation in PV
Karimian [69]	450 mmHg	–	95% O$_2$ and 5% CO$_2$ 4 l/min	Separate sources of O$_2$ and CO$_2$ is advisable, to adjust the pH
	–	–		
Liu [31]	–	–	O$_2$	pH control with O$_2$ flow rate
Vekemans [75]	300 mmHg	–		
Vogel [30]	–	–	Air and O$_2$ mixture	Automatic control
Watson [32]	HA A: 621–671 mmHg	–	A: O$_2$ and CO$_2$ mixture	Two different oxygen content (A versus B)
	HA B: 153–187 mmHg			
	PV B: 65%–85%	–	B: air and CO$_2$ mixture	pH control with pCO$_2$ flow rate
Porcine studies				
Adham [64]	–	–	A: 76% N$_2$, 19% O$_2$, 5% CO$_2$	High partial oxygen pressure was deleterious to mitochondria
	–	–	B: 95% O$_2$ and 5% CO$_2$	
Bell [63]	–	–	O$_2$ and CO$_2$	pH control with CO$_2$ flow rate
Borie [77]	200–300 mmHg	–	95% O$_2$ and 5% CO$_2$ 1–3 l/min	
Chung [78]	–	–	95% O$_2$ and 5% CO$_2$ 2 l/min	
Cimine [91]	–	–	–	Partial oxygenation in PV
Echeverri [33]	–	–	95% O$_2$ and 5% CO$_2$	
Gong [88]	–	–	O$_2$	pH, PaCO$_2$ adjusted at start
Ikeda [83]	–	–	O$_2$ 1 l/min and CO$_2$	pH control with CO$_2$ flow rate
Jablonski [34]	–	–	95% O$_2$ and 5% CO$_2$	
Janssen [36]	HA 105–150 mmHg	22–45 mmHg	95% O$_2$ and 5% CO$_2$	Partial oxygenation in PV CDI 500, TERUMO for blood gas parameter monitoring
	PV 37–75 mmHg	22–75 mmHg		
He [81]	250–500 mmHg	30–50 mmHg	O$_2$ 0.2 l/min	
Hickman [68]	HA 100%	–	–	
	PV 60–70%			
Hoyer [82]	150–200 mmHg	30–50 mmHg	Air, O$_2$ and CO$_2$	pH control with pCO$_2$ level. Control with gas flow rate
Kern [90]	–	–	O$_2$, and CO$_2$	
Mets [60]	200–250 mmHg	35–40 mmHg	O$_2$, CO$_2$ and air	Control with gas flow rate
Nagel [41]	100%	–	97.5% O$_2$ and 2.5% CO$_2$	
Neuhaus [37]	–	–	–	Partial oxygenation in PV
Satoh [84]	HA 100–150 mmHg	–	30% O$_2$	
	PV 45–60 mmHg	–		
Schoening [48]	350–550 mmHg	–	95% O$_2$ and 5% CO$_2$	Partial oxygenation in PV
Zhang [86]	80–100 mmHg	30–50 mmHg	–	Control with gas flow rate
Abouna [76], Schön [50], Steffen [73], Xu [85]	–	–	95% O$_2$ and 5% CO$_2$	

Table 5. Liver positioning during NMP in self made sets.

Author	Liver position	Comment
Human studies		
Banan [6]	Liver suspended in perfusate	
Bellomo [74]	Liver suspended in net and placed in water bath	
Liu [31]	Liver suspended in perfusate	
Porcine studies		
Adham [64]	Liver suspended in plastic bag	
Abouna [76]	Liver suspended on plastic sling to mimic the diaphragm and subjected to intermittent oscillations by ventilator to stimulate respiratory movement	
Borie [77]	Liver in sterile plastic bag floating in normal saline	
Echeverri [33]	Liver suspended in water bath	
Hoyer [89]	Liver placed in moist and heated chamber	
Jablonski [34]	Liver placed on stainless steel grid, either on the visceral or diaphragm side.	No differences during perfusion between visceral and diaphragm positioning
Jamieson [45]	Liver in intestinal bag and placed onto water-filled balloons within stainless steel bowl	
Janssen [36]	Liver in sterile plastic bags immersed in dialysate. Sealed chamber subjected to intermittent pressure variations by an external air pump (from 0 to 25 cm H_2O).	
Kern [90]	Liver suspended in gauze sling	
Nagel [41]	Liver wrapped in sterile plastic bag and placed on shell to mimic the surface of the diaphragm. Water bath underneath.	
Neuhaus [37]	Liver suspended in perfusate, within organ chamber undergoing external pressure variations	Variation in organ chamber pressure modulated the blood flow in portal vein and vena cava but not in the hepatic artery.
Satoh [84]	Liver floated in a colloid solution	
Schoening [48]	Liver fixed to portal line and floated freely in perfusate	
Steffen [73]	Liver placed in transparent heated organ chamber undergoing external pressure variations	Simulating respiratory movement lead to pressure variation of 25 cm H_2O in the organ chamber
St. Peter [92]	Liver in intestinal bag and suspended in saline solution	
Xu [85]	Liver suspended in perfusate	

altered [29,34]. Prostaglandins were the most commonly used vasodilator as this review shows. They have been shown to increase total blood flow through the liver and oxygen transport capacity in a porcine studies [45,46]. Interestingly, the positive effects of prostaglandins were more prominent in DCD livers [7]. The positive effect of prostaglandins in DCD livers was explained by improved microcirculation and protection of endothelial cells [47], as well as their anti-inflammatory effect [7,48]. The present review could, however, not identify a clear recommendation for the dosage of prostaglandins. Furthermore, the optimal vasodilator for *ex vivo* liver perfusion is yet to be defined [33]. In this context, the interesting study addressing different vasodilators was published recently by Echeverri *et al.* The authors compared BQ123, epoprostenol, and verapamil. The control group had no vasodilators and demonstrated the worst results. Flow in HA during NMP was higher in BQ123 compared with verapamil, epoprostenol, and no vasodilator-treated livers. The positive effect of BQ123 was also expressed with lower peak aspartate aminotransferase levels after transplantation compared to verapamil and epoprostenol. Total bilirubin normalized international ratio and alkaline phosphatase level were lower in the BQ123 and verapamil groups compared to epoprostenol group [33]. Having an approval for human use verapamil could be also an option to test in human studies.

One of the main bolus additive used was antibiotics. Antibiotics were added empirically without microbiologic sampling. Other bolus additives, bicarbonates, and calcium were used in human studies more common

Table 6. Up-to-date practical points for NMP with red blood cells.

NMP aspects	Practical points
Hepatic artery/portal vein perfusion	Target hepatic artery pressure higher than 50 mmHg
	Report mean, systolic and diastolic pressure for pulsatile flow
	Target portal pressure in physiologic range
	Report flow adjusted to liver weight
Bolus and continuous additives	Correct electrolytes prior perfusion start
	Provide parenteral nutrition with amino acids and glucose
	Add hepatotrophic insulin
	Use taurocholic acid derivatives to promote bile flow
	Short term perfusion without bile salts might be possible
	Use vasodilators to modulate arterial resistance
Blood gas parameters	Report gas flow protocol in detail
	Target physiologic PaO_2 level
	Target physiologic pH with adjusting $PaCO_2$ level
	Use separate gas flow to target PaO_2 and $PaCO_2$
	Do metabolic acidosis work up prior bicarbonates use

compared to porcine studies. RBCs for human studies are usually preserved in a citrate-based preservation solution without calcium and have a low pH due to lactate and hence require regular pH and calcium adjustments. Extracellular fluid is also required due to nature of available human RBS for NMP. Fresh-frozen plasma, albumin, gelatins even a crystalloid solution were used in included studies. Of those, albumin is a natural colloid with less side effects compared to synthetic colloids. It possess also antioxidant effect and is a principal binding protein of endogenous and exogenous substances [49]. The price and availability albumin could be considered as disadvantage. In opposite, gelatins are cost-effective and have been used also in clinical setting. However, superiority of one fluid compared to other is yet to be defined. In porcine studies, the blood is usually harvested shortly before perfusion start and all blood parameters are therefore in physiologic range. It could explain why most porcine studies did not include electrolyte correction during perfusion or prior to perfusion start.

Continuous addition of parenteral nutrition was included in most studies. In historical porcine studies, addition of glucose to the perfusate was necessary to achieve optimal bile flow and a low arterial resistance [34]. A positive effect of parenteral nutrition was also observed in a later porcine study by a group from Berlin, Germany [50]. The authors tested short time perfusion with and without adding metabolic support. During short time perfusion of 6 h, the addition of metabolic support substantially reduced the release of cell injury markers and improved the ammonia

metabolism. Electron microscopy also confirmed a positive effect of metabolic support [50]. A recently completed randomized controlled trial, comparing the value of NMP versus cold storage, included insulin and parenteral nutrition without lipids [51]. Furthermore, glucose decline and storage as glycogen is considered as a good sign of liver functionality [6]. In many protocols, insulin was used to correct glucose level, although the insulin dose varied across studies. The addition of insulin most probably takes its origin from a study by Starzl *et al.* [52–54], where insulin was demonstrated to have hepatotrophic effects. The lobe provided with pancreatic hormones from portal blood displayed hypertrophy and hyperplasia, whereas the other lobe supplied with intestinal blood lacking pancreatic hormones became atrophic. In further studies, only insulin was identified to have hepatotrophic effect [55]. Based on these observations, one can conclude that for a successful *ex vivo* liver perfusion, insulin could be essential.

As stated in the results section, most groups applied almost the same continuous additives in human and porcine liver perfusion [30,31,56]. Pig metabolism is five times higher than the human one [57]. Theoretically, to the same dose of parenteral nutrition and insulin during porcine and human liver perfusion, a different response in each is possible. If porcine continuous additives should be adjusted to human setting is unclear. The authors of this review could not find any study which stated that one of the perfusion parameters was changed due to differences between human and pig. During manuscript screening, many porcine studies were excluded, where authors used almost similar

perfusion protocol to explore *ex vivo* perfusion of grafts with steatosis, donated after circulatory death or perfused longer than 24 h. If bolus or continuous additives should be adjusted depending on perfusion duration and graft characteristics remains also unclear.

A continuous bile production is required for the elimination of toxic waste products [58]. A reduced bile secretion leads to intracellular accumulation of those compounds and subsequent liver function deterioration and biliary canaliculi damage [58,59]. TA substitution has been demonstrated to have a positive effect on bile production [60]. Thus, some human and porcine protocols included TA to maintain an adequate bile salt pool [30,34,58,61]. The choice of TA seems to be related to its ideal properties for *ex vivo* perfusion such as sufficient choleretic function and low hemolytic effect [58]. However, the addition of TA is controversially discussed as some authors argue that the concentration of bile salts does not deplete in the first 10 h of NMP [62–64]. Uneventful outcome after transplantation of *ex vivo* perfused human livers without TA in the study by Mergental *et al.* [15] supports the statement that short-term human liver perfusion without TA is possible. These results become even more relevant due to lack of clinical grade bile salts [16].

Blood gas supply and the targeted oxygen values varied considerably. A recent clinical trial published by Watson *et al.* provided some insight into the value of controlling the oxygen supply. The authors transplanted 12 initially discarded human livers after *ex vivo* NMP. Half of the livers were perfused at *supra*-physiological PaO_2, and the other half was perfused at near-physiologic PaO_2. The authors could show a trend toward more severe postreperfusion syndrome and vasoplegia in the patients receiving a liver perfused at supra-physiological $PaO2$ [32]. While the grafts and recipients were very heterogeneous in the study by Watson *et al.*, the same deleterious effect of high $PaO2$ has already been shown by others, where hyperoxia caused severe hepatic reperfusion injury [65]. The negative effect of high oxygen contents during NMP is also supported by evidence during CPB or during resuscitation after cardiac arrest [66,67]. Of note, most of human and porcine studies provided arterialized blood through the PV, while only few provided partially oxygenated blood [32,48,68]. No studies are available exploring advantages, disadvantages, or technical challenges of partial oxygenation compared to arterialized oxygenation in portal blood. A further target of the blood gas supply was $PaCO_2$. CO_2 removal by increasing the gas flow rate leads to alkalosis making possible to use it in controlling pH as shown by included studies. A fixed mixture of

gases, for example, 95% O_2 and 5% CO_2, makes an adjustment of pH and $PaCO_2$ in the perfusion fluid impossible if $PaO2$ is also controlled. Hence, separate sources of O_2, air (N_2) and CO_2 supply are recommended if an individual control of $PaO2$ and $PaCO_2$ or pH is desired [69]. Bicarbonates were used to correct pH alongside $PaCO_2$ adjustment in included studies. However, metabolic acidosis that cannot be corrected by adjusting $PaCO_2$ should urge to search first the causes. If metabolic acidosis related to lack of lactate clearance and poor liver function, bicarbonates would a symptomatic treatment. When bicarbonates are indicated, then it should be administered as slow infusion controlling the amount of produced CO_2 to prevent rapid exacerbation of intracellular acidosis. Further disadvantages of bicarbonates are hyperosmolality due to hypernatremia, volume overload, and pH overcorrection, which could be even more harmful than acidosis [70].

Anatomically, the liver is suspended under the diaphragm and rests on the gastrointestinal organs. Movements of the diaphragm during respiration with consequent intra-abdominal pressure changes may exert alternating negative and positive pressure on the liver vasculature [37]. Some authors were concerned with liver positioning *ex vivo*, where all those parameters are lacking and extensive work on liver positioning was performed [37]. Researchers let the liver float in a container filled with an asanguineous solution to avoid pressure areas. The downside of this technique is that it does not allow to recirculate blood lost from the wound surface [71]. However, the collection of perfusate is not an issue if the liver is floating in its own perfusate [48]. Positioning the liver on its visceral or diaphragm surface did not show any differences [34]. Further *in vivo* studies are also in accordance with this finding and confirmed no influence of gravity in liver perfusion [72]. To imitate intra-abdominal pressure variations due to the respiration, some authors tested the perfusion in a hermetically closed chamber and changing the pressure inside periodically by 25 cm H_2O [37,38,73]. This lead to an oscillation effect on PV and VC flow and homogenous liver perfusion. In contrast, liver perfusion without this oscillation was inhomogeneous, even with thrombosis in peripherial regions. Authors claimed that imitating intra-abdominal pressure variations during *ex vivo* perfusion seems to be beneficial [37].

In summary, this review reported technical aspects of MP, perfusion fluid composition, and oxygenation modes as follow-up of recently published international experts group recommendation for MP [4]. An overview with up-to-date practical points for RBC-based

NMP can be found in Table 6. For future studies, it is advisable to report perfusion protocol more in detail.

Conflict of interest

The authors have declared no conflicts of interest.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Search strategy in Medline, Embase and Cochrane.

REFERENCES

1. Dutkowski P, Linecker M, DeOliveira ML, Mullhaupt B, Clavien PA. Challenges to liver transplantation and strategies to improve outcomes. *Gastroenterology* 2015; **148**: 307.

2. Dutkowski P, Clavien PA. Scorecard and insights from approaches to liver allocation around the world. *Liver Transpl* 2016; **22**(S1): 9.

3. Goldaracena N, Barbas AS, Selzner M. Normothermic and subnormothermic ex-vivo liver perfusion in liver transplantation. *Curr Opin Organ Transplant* 2016; **21**: 315.

4. Karangwa SA, Dutkowski P, Fontes P, et al. Machine perfusion of donor livers for transplantation: a proposal for standardized nomenclature and reporting guidelines. *Am J Transplant* 2016; **16**: 2932.

5. Watson CJE, Kosmoliaptsis V, Randle LV, et al. Preimplant normothermic liver perfusion of a suboptimal liver donated after circulatory death. *Am J Transplant* 2016; **16**: 353.

6. Banan B, Watson R, Xu M, Lin Y, Chapman W. Development of a normothermic extracorporeal liver perfusion system toward improving viability and function of human extended criteria donor livers. *Liver Transpl* 2016; **22**: 979.

7. Liu Q, Nassar A, Farias K, et al. Comparing normothermic machine perfusion preservation with different perfusates on porcine livers from donors after circulatory death. *Am J Transplant* 2016; **16**: 794.

8. Marecki H, Bozorgzadeh A, Porte RJ, Leuvenink HG, Uygun K, Martins PN. Liver ex situ machine perfusion preservation: a review of the methodology and results of large animal studies and clinical trials. *Liver Transpl* 2017; **23**: 679.

9. Lehmann K, Eshmuminov D, Slankamenac K, et al. Where oncologic and surgical complication scoring systems collide: time for a new consensus for CRS/HIPEC. *World J Surg* 2016; **40**: 1075.

10. Dutkowski P, Polak WG, Muiesan P, et al. First comparison of hypothermic oxygenated perfusion versus static cold storage of human donation after cardiac death liver transplants: an international-matched case analysis. *Ann Surg* 2015; **262**: 764; discussion 770–1.

11. 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.

12. Schlegel A, Kron P, Graf R, Dutkowski P, Clavien PA. Warm vs. cold perfusion techniques to rescue rodent liver grafts. *J Hepatol* 2014; **61**: 1267.

13. 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.

14. Perera T, Mergental H, Stephenson B, et al. First human liver transplantation using a marginal allograft resuscitated by normothermic machine perfusion. *Liver Transpl* 2016; **22**: 120.

15. Mergental H, Perera MT, Laing RW, et al. Transplantation of declined liver allografts following normothermic ex-situ evaluation. *Am J Transplant* 2016; **16**: 3235.

16. Watson CJE, Kosmoliaptsis V, Pley C, et al. Observations on the ex situ perfusion of livers for transplantation. *Am J Transplant* 2018; https://doi.org/10.1111/ajt.14687. [Epub ahead of print]

17. McCormack L, Dutkowski P, El-Badry AM, Clavien PA. Liver transplantation using fatty livers: always feasible? *J Hepatol* 2011; **54**: 1055.

18. Nativ NI, Maguire TJ, Yarmush G, et al. Liver defatting: an alternative approach to enable steatotic liver transplantation. *Am J Transplant* 2012; **12**: 3176.

19. 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.

20. Vogel T, Brockmann JG, Friend PJ. Ex-vivo normothermic liver perfusion: an update. *Curr Opin Organ Transplant* 2010; **15**: 167.

21. Kollmann D, Selzner M. Recent advances in the field of warm ex-vivo liver perfusion. *Curr Opin Organ Transplant* 2017; **22**: 555.

22. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Int J Surg* 2010; **8**: 336.

23. Eshmuminov D, Raptis DA, Linecker M, Wirsching A, Lesurtel M, Clavien PA. Meta-analysis of associating liver partition with portal vein ligation and portal vein occlusion for two-stage hepatectomy. *Br J Surg* 2016; **103**: 1768.

24. Eshmuminov D, Schneider MA, Tschuor C, et al. Systematic review and meta-analysis of postoperative pancreatic fistula rates using the updated 2016 International Study Group Pancreatic Fistula definition in patients undergoing pancreatic resection with soft and hard pancreatic texture. *HPB (Oxford)* 2018; pii: S1365-182X(18)30806-2. https://doi.org/10.1016/j.hpb.2018.04.003. [Epub ahead of print]

25. Scholer M, Frietsch T, Jambor C, Knels R. Artificial blood – coming soon or never reaching clinical maturity? *Dtsch Med Wochenschr* 2010; **135**: 575.

26. Riess JG. Perfluorocarbon-based oxygen delivery. *Artif Cells Blood Substit Immobil Biotechnol* 2006; **34**: 567.

27. Schlegel A, Muller X, Dutkowski P. Hypothermic machine preservation of the liver: state of the art. *Curr Transplant Rep* 2018; **5**: 93.

28. Nassar A, Liu Q, Farias K, *et al.* Impact of temperature on porcine liver machine perfusion from donors after cardiac death. *Artif Organs* 2016; **40**: 999.

29. Spetzler VN, Goldaracena N, Echiverri J, *et al.* Subnormothermic ex vivo liver perfusion is a safe alternative to cold static storage for preserving standard criteria grafts. *Liver Transpl* 2016; **22**: 111.

30. Vogel T, Brockmann JG, Quaglia A, *et al.* The 24-hour normothermic machine perfusion of discarded human liver grafts. *Liver Transpl* 2017; **23**: 207.

31. Liu Q, Nassar A, Buccini L, *et al.* Lipid metabolism and functional assessment of discarded human livers with steatosis undergoing 24 hours of normothermic machine perfusion. *Liver Transpl* 2018; **24**: 233.

32. 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.

33. Echeverri J, Goldaracena N, Kaths JM, *et al.* Comparison of BQ123, epoprostenol, and verapamil as vasodilators during normothermic *ex vivo* liver machine perfusion. *Transplantation* 2018; **102**: 601.

34. Jablonski P, Doglas MC, Gordon E, Owen JA, Watts JM. Studies on the isolated perfused pig liver. *Br J Surg* 1971; **58**: 129.

35. Terajima H, Shirakata Y, Yagi T, *et al.* Successful long-term xenoperfusion of the pig liver: continuous administration of prostaglandin E1 and insulin. *Transplantation* 1997; **63**: 507.

36. Janssen MWW, Druckrey-Fiskaaen KT, Omidi L, *et al.* Indocyanine green R15 ratio depends directly on liver perfusion flow rate. *J Hepatobiliary Pancreat Sci* 2010; **17**: 180.

37. Neuhaus P, Blumhardt G. Extracorporeal liver perfusion: applications of an improved model for experimental studies of the liver. *Int J Artif Organs* 1993; **16**: 729.

38. Darin JC. Experiences in *ex vivo* perfusion of the liver. *Am J Surg* 1966; **111**: 870.

39. Abraldes JG, Sarlieve P, Tandon P. Measurement of portal pressure. *Clin Liver Dis* 2014; **18**: 779.

40. Eipel C, Abshagen K, Vollmar B. Regulation of hepatic blood flow: the hepatic arterial buffer response revisited. *World J Gastroenterol* 2010; **16**: 6046.

41. Nagel S, Hegemann O, Groneberg DA, Grosse-Siestrup C. An improved model of isolated hemoperfused porcine livers using pneumatically driven pulsating blood pumps. *Toxicol Pathol* 2005; **33**: 434.

42. Murphy GS, Hessel 2nd EA, Groom RC. Optimal perfusion during cardiopulmonary bypass: an evidence-based approach. *Anesth Analg* 2009; **108**: 1394.

43. Oakes DA, Mangano CT. Cardiopulmonary bypass in 2009: achieving and circulating best practices. *Anesth Analg* 2009; **108**: 1368.

44. Darnis B, Mohkam K, Schmitt Z, *et al.* Subtotal hepatectomy in swine for studying small-for-size syndrome and portal inflow modulation: is it reliable? *HPB (Oxford)* 2015; **17**: 881.

45. Jamieson RW, Zilvetti M, Roy D, *et al.* Hepatic steatosis and normothermic perfusion-preliminary experiments in a porcine model. *Transplantation* 2011; **92**: 289.

46. Wolf S, Schon MR, Krautlein K, *et al.* Effects of epoprostenol and norepinephrine on hemodynamics of the isolated perfused liver. *Transplant Proc* 1998; **30**: 2334.

47. Nassar A, Liu Q, Farias K, *et al.* Role of vasodilation during normothermic machine perfusion of DCD porcine livers. *Int J Artif Organs* 2014; **37**: 165.

48. Schoening WN, Feige I, Schubert T, *et al.* Iloprost donor treatment reduces ischemia-reperfusion injury in an isolated extracorporeal pig liver perfusion model. *Exp Clin Transplant* 2015; **13**: 51.

49. Mitra S, Khandelwal P. Are all colloids same? How to select the right colloid? *Indian J Anaesth* 2009; **53**: 592.

50. Schon MR, Hunt CJ, Pegg DE, Wight DG. The possibility of resuscitating livers after warm ischemic injury. *Transplantation* 1993; **56**: 24.

51. Nasralla D, Coussios CC, Mergental H, *et al.* A randomized trial of normothermic preservation in liver transplantation. *Nature* 2018; **557**: 50.

52. Starzl TE, Porter KA, Kashiwagi N. Portal hepatotrophic factors, diabetes mellitus and acute liver atrophy, hypertrophy and regeneration. *Surg Gynecol Obstet* 1975; **141**: 843.

53. Starzl TE, Terblanche J. Hepatotrophic substances. *Prog Liver Dis* 1979; **6**: 135.

54. Starzl TE, Porter KA, Putnam CW. Insulin, glucagon, and the control of hepatic structure, function, and capacity for regeneration. *Metabolism* 1976; **25** (11 Suppl 1): 1429.

55. Starzl TE, Watanabe K, Porter KA, Putnam CW. Effects of insulin, glucagon, and insuling/glucagon infusions on liver morphology and cell division after complete portacaval shunt in dogs. *Lancet* 1976; **1**: 821.

56. Vogel T, Brockmann JG, Pigott D, *et al.* Successful transplantation of porcine liver grafts following 48-hour normothermic preservation. *PLoS ONE* 2017; **12**: e0188494.

57. Lavallee CM, MacPherson JAR, Zhou M, *et al.* Lipid emulsion formulation of parenteral nutrition affects intestinal microbiota and host responses in neonatal piglets. *JPEN J Parenter Enteral Nutr* 2017; **41**: 1301.

58. Imber CJ, St. Peter SD, De Cenarruzabeitia IL, *et al.* Optimisation of bile production during normothermic preservation of porcine livers. *Am J Transplant* 2002; **2**: 593.

59. Foley DP, Ricciardi R, Traylor AN, *et al.* Effect of hepatic artery flow on bile secretory function after cold ischemia. *Am J Transplant* 2003; **3**: 148.

60. Mets B, Rose-Innes C, Lotz Z, Hickman R, Chalton D. Comparison of *in vivo* and *ex vivo* porcine liver function using the same liver. *J Hepatol* 1993; **17**: 3.

61. Reiling J, Lockwood DSR, Simpson AH, *et al.* Urea production during normothermic machine perfusion: price of success? *Liver Transpl* 2015; **21**: 700.

62. Banan B, Chung H, Xiao Z, *et al.* Normothermic extracorporeal liver perfusion for donation after cardiac death (DCD) livers. *Surgery* 2015; **158**: 1642.

63. Bell R, Shiel AG, Dolan P, Mears DC, Woodman K. The evaluation of the isolated perfused liver as a model for the assessment of liver preservation. *Aust N Z J Surg* 1993; **63**: 44.

64. Adham M, Peyrol S, Chevallier M, *et al.* The isolated perfused porcine liver: assessment of viability during and after six hours of perfusion. *Transpl Int* 1997; **10**: 299.

65. Zinchuk VV, Khodosovsky MN, Maslakov DA. Influence of different oxygen modes on the blood oxygen transport and prooxidant-antioxidant status during hepatic ischemia/reperfusion. *Physiol Res* 2003; **52**: 533.

66. Dell'Anna AM, Lamanna I, Vincent JL, Taccone FS. How much oxygen in adult cardiac arrest? *Crit Care* 2014; **18**: 555.

67. Joachimsson PO, Sjoberg F, Forsman M, Johansson M, Ahn HC, Rutberg H. Adverse effects of hyperoxemia during cardiopulmonary bypass. *J Thorac Cardiovasc Surg* 1996; **112**: 812.

68. Hickman R, Saunders SJ, Simson E, Terblanche J. Perfusion of the isolated pig liver. Functional assessment under control normothermic conditions. *Br J Surg* 1971; **58**: 33.

69. Karimian N, Matton AP, Westerkamp AC, *et al.* Ex situ normothermic machine perfusion of donor livers. *J Vis Exp* 2015; **99**: e52688.

70. Velissaris D, Karamouzos V, Ktenopoulos N, Pierrakos C, Karanikolas M. The use of sodium bicarbonate in the treatment of

acidosis in sepsis: a literature update on a long term debate. *Crit Care Res Pract* 2015; **2015**: 605830.

71. Bellomo R, Marino B, Starkey G, *et al.* Normothermic extracorporeal human liver perfusion following donation after cardiac death. *Crit Care Resusc* 2013; **15**: 78.

72. Lautt WW. *Hepatic Circulation: Physiology and Pathophysiology*. Colloquium Series on Integrated Systems Physiology: From Molecule to Function to Disease. San Rafael, CA: Morgan & Claypool Life Sciences, 2009.

73. Steffen R, Ballmer FT, Luder PJ, Mettler D, Barbier PA. Extracorporeal isolated pig liver perfusion: influence of various blood primes on liver function. *Res Exp Med* 1987; **187**: 265.

74. Bellomo R, Marino B, Starkey G, *et al.* Extended normothermic extracorporeal perfusion of isolated human liver after warm ischaemia: a preliminary report. *Crit Care Resusc* 2014; **16**: 197.

75. Vekemans K, Van Pelt J, Komuta M, *et al.* Attempt to rescue discarded human liver grafts by end ischemic hypothermic oxygenated machine perfusion. *Transpl Proc* 2011; **43**: 3455.

76. Abouna GM, Ashcroft T, Hull C, Hodson A, Kirkley J, Walder DN. The assessment of function of the isolated perfused porcine liver. *Br J Surg* 1969; **56**: 289.

77. Borie DC, Eyraud D, Boleslawski E, *et al.* Functional metabolic characteristics of intact pig livers during prolonged extracorporeal perfusion: potential for a unique biological liver-assist device. *Transplantation* 2001; **72**: 393.

78. Chung WY, Gravante G, Eltweri A, *et al.* The "kidney–liver" multiorgan *ex vivo* perfused model improves the circuit's biochemical milieu during perfusion compared to the "liver–kidney" counterpart. *J Artificial Organs* 2015; **18**: 151.

79. Cimeno A, French BM, Powell JM, *et al.* Synthetic liver function is detectable in transgenic porcine livers perfused with human blood. *Xenotransplantation* 2018; **25**: e12361.

80. Fondevila C, Hessheimer AJ, Maathuis MHJ, *et al.* Superior preservation of DCD livers with continuous normothermic perfusion. *Ann Surg* 2011; **254**: 1000.

81. He X, Ji F, Zhang Z, *et al.* Combined liver-kidney perfusion enhances protective effects of normothermic perfusion on liver grafts from donation after cardiac death. *Liver Transpl* 2018; **24**: 67.

82. Hoyer DP, Paul A, Luer S, Reis H, Efferz P, Minor T. End-ischemic reconditioning of liver allografts: controlling the rewarming. *Liver Transpl* 2016; **22**: 1223.

83. Ikeda T, Yanaga K, Lebeau G, Higashi H, Kakizoe S, Starzl TE. Hemodynamic and biochemical changes during normothermic and hypothermic sanguinous perfusion of the porcine hepatic graft. *Transplantation* 1990; **50**: 564.

84. Satoh S, Terajima H, Yagi T, *et al.* Humoral injury in porcine livers perfused with human whole blood. *Transplantation* 1997; **64**: 1117.

85. Xu H, Berendsen T, Kim K, *et al.* Excorporeal normothermic machine perfusion resuscitates pig DCD livers with extended warm ischemia. *J Surg Res* 2012; **173**: e83–e8.

86. Zhang ZB, Gao W, Shi Y, *et al.* Protective role of normothermic machine perfusion during reduced-size liver transplantation in pigs. *Liver Transpl* 2016; **22**: 968.

87. Chung WY, Eltweri AM, Isherwood J, *et al.* Steps for the autologous *ex vivo* perfused porcine liver-kidney experiment. *J Vis Exp* 2013; **82**: e50567.

88. Gong J, Lao XJ, Wang XM, Long G, Jiang T, Chen S. Preservation of non-heart-beating donor livers in extracorporeal liver perfusion and histidine-trytophan-ketoglutarate solution. *World J Gastroenterol* 2008; **14**: 2338.

89. Hoyer DP, Mathé Z, Gallinat A, *et al.* Controlled oxygenated rewarming of cold stored livers prior to transplantation: first clinical application of a new concept. *Transplantation* 2016; **100**: 147.

90. Kern Jr F, Eiseman B, Normell L. Lipid metabolism in the *ex vivo* perfused liver. *Am J Clin Nutr* 1965; **16**: 116.

91. Cimeno A, Hassanein W, French BM, *et al.* N-glycolylneuraminic acid knockout reduces erythrocyte sequestration and thromboxane elaboration in an *ex vivo* pig-to-human xenoperfusion model. *Xenotransplantation* 2017; **24**: e12339.

92. St. Peter SD, Imber CJ, De Cenarruz-abeitia IL, *et al.* β-galactosidase as a marker of ischemic injury and a mechanism for viability assessment in porcine liver transplantation. *Liver Transpl* 2002; **8**: 21.