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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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Abbreviations:

CPB	cardiopulmonary bypass
HA	hepatic artery
HBOC	hemoglobin based oxygen carriers
MP	machine perfusion
NSP	normothermic, subnormothermic perfusion
PV	portal vein
RBC	reds blood cells
TA	taurocholic acid and its derivatives
VC	vena cava

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Abstract

Liver machine perfusion 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 machine perfusion, fluid composition, gas supply and liver positioning. 37 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°C–24°C, subnormothermic MP (SMP) 25°C–34°C and normothermic MP (NMP) 35°C–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 analyse 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 supplementary eTable 1. Limited manual search was performed in

January 2018. Non-quantitative 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 are 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 (Figure 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 was 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, which lead to mean portal pressure of 17.9 mmHg.(30) Applied pressure in HA varied considerably from 40 mmHg to 100 mmHg. Nonpulsatile and pulsatile flow-patterns in HA were used in 5 and 3 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 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, the authors reported a liver weight range between 1750-5000g and flow in PV 1400±200.(6) 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 6 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 8 studies. In 4 studies, the control mode was different between HA and PV. Applied pressure in HA varied considerably from 40 mmHg to 120 mmHg. Both nonpulsatile and pulsatile flow-patterns in HA were used. HA flow in 6 studies, reporting the data in ml/g liver tissue/min, ranged from 0.14 to 0.38. In 7 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, 8 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 6 of 8 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.

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 3 studies, while 5, 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. PaCO2 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 in order 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, since 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 remains open in this review. Pressure in HA varied considerably between 40 to 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 less than 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 other not. The porcine study by Nagel et al 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,(41) which is far below than reference. The human study by Liu et al targeted the reference flow and reported pressure of 60 to 100 mmHg in HA and 5 to 10 mmHg in PV.(31) The reasons for such variations were not discussed. The further results in this review, also challenges 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. should have received 5 l/min blood during NMP.(6) 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, the total flow rate in the liver weighing 1382 g was higher than the flow rate in the liver weighting 2400 g.(15) 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 same context, a flow rate of 2.2–2.5 L/min/m2 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 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 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, requires 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 was 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 hours, 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 vs. 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, where insulin was demonstrated to have hepatotrophic effects.(52-54) 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 5 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 possibly. If porcine continuous additives should be adjusted into 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 hours. If bolus or continuous additives should be adjusted depending 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.(59) (58) 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 hours 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 towards more severe post-reperfusion 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 cardiopulmonary bypass or during resuscitation after cardiac arrest.(66, 67) Of note, most of human and porcine studies provided arterialized blood through the portal vein, 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, e.g. 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 PaCO2 or pH is desired.(69) Bicarbonates were used to correct pH alongside PaCO2 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 intraabdominal 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 done.(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 doesn't 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 didn't 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 cmH_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.

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Figure 1 PRISMA flow diagram

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

Water column converted to mmHg, for pulsatile flow mean arterial pressure was reported. HA hepatic artery, PV portal vein, VC vena cava

Author	Perfusion control		Pressure mm HG		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						

Table 2. Baseline perfusate additives prior NMP start

Bicarbonates (Bi), Calcium (Ca), Magnesium (Ma)

Author	Antibiotics	Electrolyte	Others
Human studies			
He(19)	Cefoperazone 1.5 g Metronidazol 0.5 g	Bi 30 ml, Ca10 ml, Ma 3 ml	Amino acids 250 ml Extracellular fluid: Succinylated gelatinor
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	-	Methylprednisolone 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, th same group reported also with succinylated gelatin solution
Watson(32)	Meronem / fluconazole	Bi 30 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, Ca 10 ml	Epoprostenol 40 ug

Echeverri(33)	Cefazolin 1g 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 Alprostadil 10 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, aminoacids 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 ug, Glutathione 600 mg

Table 3. Continuous perfusate additives during NMP

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.

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	7ml/h	Prostacyclin 10 ug/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/ A 5%, G 15%	4 U/h	-	Epoprostenol 6-60 ug/hr	Bi o.d.	Multivitamins/trace elements, G
Mergental(15)	-	-	-	Epoprostenol 8 ug/h	-	
Reiling (61)	PN rate 20 ml/h	50-200 U/h	7ml/h	Prostaciclin 8 ug/h	-	
Vogel(30)	PN rate 15 ml/h	yes	140 mg/h	Epoprostenol 8 ug/h	-	PN / insulin control
Watson(32)	-	yes	-	Epoprostenol 2 ug/h	Bi o.d.	
Porcine studies						
Borie(77)	-	4 U/h	-	-	Bi o.d.	
Chung(87)	-	100 U/h	200 mg/h	Epoprostenol 40 ug/h	Bi 40 ml/h	
Echeverri(33)	PN rate 8 ml/h	125 U/h	7ml/h	P–E1 500 ug/3h	-	Vasodilator (BQ-123) and Verapamil bolus,

	A 4.25% G50W 2-5 ml/h					Epoprostenol 8 ug/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 I. U/h	0.15 mg/h	-	-	
Ikeda(83)	-	-	-	-	Bi o.d.	
Jablonski (34)	-	-	4pmol/min	-	Bi o.d.	Vasodilators with no or only short effect
Kern(90)	G 8g/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

Table 4. Gas supply protocols during NMP

Author	Target SO_2(%) / PO_2 mmHg	Target 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 PV 20-60 mmHg	- -	Air and O_2	pO_2 / pCO_2 control with gas flow rate
Darin(38)	-	-	95% O_2 and 5% CO_2	Partial oxygenation in PV
Karimian(69)	450 mmHg -	- -	95% O_2 and 5% CO_2 4l/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 HA B: 153-187 mmHg PV B: 65% -85%	- -	A: O_2 and CO_2 mixture B: air and CO_2 mixture	Two different oxygen content (A vs. B) pH control with pCO_2 flow rate
Porcine studies				
Adham(64)	- -	- -	A: 76 % N_2, 19% O_2, 5% CO_2 B: 95% O_2 and 5% CO_2	High partial oxygen pressure was deleterious to mitochondria
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)	HA105-150 mmHg PV 37-75 mmHg	22-45 mmHg 22-75 mmHg	95% O_2 and 5% CO_2	Partial oxygenation in PV CDI 500, TERUMO for blood gas parameter monitoring
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)	HA100-150 mmHg PV 45-60 mmHg	- -	30% O_2	
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 cmH20).	
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 cmH_20 in the organ chamber
St. Peter(92)	Liver in intestinal bag and suspended in saline solution	
Xu(85)	Liver suspended in perfusate	

Table 6. Up-to-date practical points for NMP with RBC

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