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ROLE OF PERFUSION MACHINES IN THE SETTING OF CLINICAL LIVER TRANSPLANTATION: A QUALITATIVE SYSTEMATIC REVIEW

Running title: Perfusion machines and liver transplantation

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Abbreviations:

COR:	controlled oxygenated rewarming
DBD:	deceased brain donor
DCD:	donor after cardiac death
DHOPE:	dual hypothermic oxygenated perfusion
HCC:	hepatocellular cancer
HMP:	hypothermic machine perfusion
HOPE:	hypothermic oxygenated perfusion
ITBL:	ischemic-type biliary lesion
LT:	liver transplantation
MP:	machine perfusion
NOS:	Newcastle-Ottawa Quality Assessment Scale
PRISMA:	Preferred Reporting Items for Systemic Reviews and Meta-Analysis
MELD:	model for end-stage liver disease
NMP:	normothermic machine perfusion

NRP: normothermic regional perfusion
PNF: primary non function

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ABSTRACT

Growing enthusiasm around machine perfusion (MP) in clinical liver transplantation (LT) may be the preamble for standardized practice to expand the donors' pool. The present systematic review investigated all the liver transplantations performed using grafts treated with MP. A systematic review of 309 papers was performed. Eventually, 27 articles were enrolled for the study. A total number of 173 cases was reported. Only 12 cohort studies were identified: the remaining ones were case reports or case series. Hypothermic machine perfusion was performed in 102 (59.0%), normothermic machine perfusion in 65 (37.6%), and controlled oxygenated rewarming in the remaining 6 (3.4%) cases. Donor characteristics, evaluation of graft quality and end-points were not homogeneous among the studies. Overall, post-LT results were excellent, with 1.2 and 4.0% of patients experienced primary non-function and ischemic-type biliary lesions, respectively. Conclusion: no study exists that address the role of MP in selecting liver grafts available for LT. All the published studies mainly focused on the feasibility and safety of this new technology. Further research investigating the selection process of marginal donors is required.

INTRODUCTION

Improvement in ex vivo liver preservation represents one of the main goals in the setting of clinical liver transplantation (LT), aiming at increasing the number of grafts to transplant and improving their quality at the same time.

Simple cold storage [1] has been the mainstay in clinical practice since Geoffrey Collins introduced it in 1969. [2] However, the growing gap between the number of available organs and that of patients awaiting transplants, forced the scientific community to seek new solutions to reduce the waiting list deaths. [3] A more liberal use of the so-called "extended criteria" organs has been proposed in recent times, with the intent of increasing organs for transplantation. This sub-group of grafts includes a miscellaneous of different conditions with the common denominator of being more vulnerable to ischemia, hence carrying an increased risk of post-LT graft dysfunction. [4] Among them, we can surely include donors after cardiac death (DCD), [5] severely steatotic grafts, [6] organs from elderly donors, [7] and livers with a prolonged cold ischemia time (i.e., >12 hours). [8]

Machine perfusion (MP) might represent a way around for utilizing sub-optimal resources minimizing the risk of poor graft function. Initially introduced in the clinical practice for preserving "marginal" kidneys for transplantation, [9] the concept of MP has been progressively translated in the LT setting. After several experimental studies on animal models [10-12] and discarded human livers, [13-15] the first clinical series using MP was eventually reported by Guarrera et al. in 2010. [16]

Since then, multiple experiences have been reported worldwide, fostering high expectations concerning the following implementation of MPs into clinical practice. [11,17-41] However, several obstacles, as the necessity of finding objective MP-related variables to select grafts suitable for LT, still stand.

From this standpoint, a qualitative systematic review was done, identifying all the clinical liver transplants performed using MP grafts. When available, MP-related selective criteria of graft quality and outcome were specifically investigated.

MATERIALS AND METHODS

Search strategy

A systematic search was done about relevant studies focusing on the use of MP in the setting of clinical LT. The search strategy complied with the Preferred Reporting Items for Systemic Reviews and Meta-Analysis (PRISMA) guidelines, as well as PRISMA for abstracts. [42] A search of the electronic databases MEDLINE-PubMed and Cochrane Library was conducted using the following search terms: perfusion machine AND liver transplantation. Studies published before November 30, 2017, were considered.

Screening process

The present qualitative systematic review included a priori search criteria of journal articles among adult (age ≥18 years) human LT recipients. Studies were limited to the English language.

Exclusion criteria were studies lacking sufficient statistical details as well as review articles, nonclinical studies, expert opinions and conference summaries. All the studies coming from the same center were considered and analyzed, due to the possible overlapping of clinical cases reported across the studies. Letters and case reports were also not excluded a priori for the analysis, due to the paucity of reported cases in the literature.

Study Selection

A total of 307 results were observed. Two more papers were added to the research through references. Two reviewers (QL and FM) independently screened the identified studies and their extracted data. In case of disagreement, the paper was discussed by all the authors. After the systematic screening, 27 studies were identified for the systematic review analysis (**Figure 1**).

Quality Assessment

Selected studies were reviewed based on the representativeness of the study population, comparability of cohorts, adequate assessment of outcomes, sufficient length of follow-up, adequacy of follow-up, and source of study funding. The quality of the papers was assessed using the Newcastle-Ottawa Quality Assessment Scale (NOS): studies with scores >6 were defined as high-quality (**Table 1**). [43]

The characteristics coming from each study were collected in four different tables. The following features were collected in **Table 2** (hypothermic experiences) and **Table 3** (sub-normothermic/normothermic experiences): first author's name, reference number, year of publication, country/city, number of patients, type of perfusion protocol used, temperature of MP, type of donor (DCD vs deceased brain donor [DBD]), donor age, donor gender, percentage of graft macro-steatosis, recipient age, recipient gender, patient cause of disease, concomitant presence of hepatocellular cancer (HCC), and model for end-stage liver disease (MELD) score.

The following characteristics were collected in **Table 4** (hypothermic experiences) and **Table 5** (sub-normothermic/normothermic experiences): timing from donor aortic flush to MP start, duration of perfusion on the MP, value of lactates in the perfusate at the beginning and at the end of perfusion, final MP flow in the hepatic artery and portal vein, post-LT transaminases peaks, hospital stay in days, number of graft losses/deaths, number of cases that developed acute kidney injury, primary non-function, and ischemic-type biliary lesions.

RESULTS

The selection process of the articles is explained in **Figure 1**.

As for the selection process according to the PRISMA guidelines, the examined databases provided a total of 309 articles to screen. Forty-two of them were removed after reading titles and abstracts. Of the remaining 267, 240 were not considered eligible for full-text evaluation. Eventually, 27 articles with a total number of 173 patients were reported on clinical LT using MP. [11,16-41] Some articles from the same center showed partial overlapping of reported cases: consequently, the global number of patients for each center was extrapolated after careful evaluation of the reported series, typically taking into consideration the most recent study. Until now, 14 LT centers have reported at least one case of clinical LT using a MP: New York (n=51), Zurich (n=31), Cambridge (n=14), Groningen (n=10), Toronto (n=10), Edmonton (n=9), Milan (n=6), Birmingham (n=6), Hessen (n=6), Turin (n=4), Pisa (n=3), Palermo (n=1), London (n=1) and Guangzhou (n=1). A multicenter study from the United Kingdom was also reported (n=20). All the cases except one were performed in Western countries, with 102 and 70 cases carried out in Europe and North America, respectively.

As for the quality of the reported studies, ten and five studies were only case reports or case series, respectively. Thus, a NOS value was correctly established only in twelve cases: the overall quality of these cohort studies was globally high, with a NOS overpassing the cut-off of 6 in all the cases (median value=8) (**Table 1**).

A great inhomogeneity was observed among the 27 studies included in the final analysis. For example, normothermic machine perfusion (NMP) was performed in 65 (37.6%) cases, controlled oxygenated rewarming (COR) in 6 (3.4%), and different types of hypothermic machine perfusion (HMP) in the remaining 102 (59.0%).

In detail, HMP was applied in 53 cases, hypothermic oxygenated perfusion (HOPE) in 31 cases, dual hypothermic oxygenated perfusion (DHOPE) in 14 patients, and normothermic regional perfusion (NRP) followed by HMP in 4 cases.

Variability was also observed in regards to the type of donor: 105 (60.7%) DBD and 68 (39.3%) DCD were considered eligible for donation. Among the DCD donors, only 4 uncontrolled cases (all receiving NPR+HMP) were reported (**Tables 2 and 3**). Moreover, a total of 58 (33.5%) cases were reported in whom a liver previously refused for LT by other centers was initially treated with MP and then successfully transplanted. [17,18,28-31,35-38]

Time elapsed from donor aortic perfusion to the beginning of machine perfusion varied widely among the different experiences, with a great span ranging from a minimum of 1.2 to a maximum of 14.5 hours. Such a discrepancy depended mainly on whether the used MP was portable or not and by the fact that some of the perfused grafts were previously discharged by other transplant centers, resulting in long cold storage. It must be underlined that this span ranging was particularly evident in HMP series because in most of them non-portable devices were used. Also, duration of perfusion was different in the reported series, with a range of 1.5-18.5 hours.

In case of NMP, lactates, when recorded, tended to decrease from an initial value ranging 5.5-13.9 mmol/L to a final value ranging 0.03-2.8 mmol/L. Similarly, final portal and hepatic artery flows increased consistently during perfusion, reaching top values of 1.3 L/min and 478 mL/min, respectively. In case of COR or HMP, a smaller number of functional aspects was available.

Post-LT transaminases peaks were not reported homogeneously among the different series, with only a few studies reporting both ALT and AST peak values.

Overall results were excellent, although follow-up times were generally short. The shorter hospital stay was of only 5 days. Only two (1.2%) cases of primary non-function (PNF) were documented. Acute kidney injury was observed in 17 (9.8%) patients. Seven (4.0%) cases of ischemic-type biliary lesions (ITBL) were reported. The overall incidence of graft losses was of 12 (6.9%) cases; in one case a successful re-transplantation was performed (**Tables 4 and 5**).

Considering patients with at least 6 months of follow-up (n=154), the overall number of reported graft losses was of 12/154 (7.8%) cases. PNF and ITBL cases were 2/154 (1.3%) and 7/154 (4.5%), respectively (**Tables 4 and 5**).

A separate analysis performed on high-quality papers with a NOS ≥6 (12 articles; n=167) did not provide homogeneous data either. When sub-normothermic and NMP were used, post-LT transaminases ranges were 152-4991 and 84-9200 IU/L for ALT and AST values, respectively, while, with HMP, they were 689->3000 and 966-3547 IU/L, respectively. In these well-selected series, the number of reported graft losses was of 12/167 (7.2%) cases. PNF and ITBL cases were 2/167 (1.2%) and 7/167 (4.2%), respectively (**Tables 4 and 5**).

Another separate analysis only looking at the livers previously discarded by other centers was performed. In the subgroup of 57 initially declined livers in which enough information on the follow-up details was reported, a total of 7/57 (12.3%) graft losses was observed. It is interesting to observe that this percentage was superior respect to the one observed in the cases in which the MP was performed for testing its safety (5/115, 4.3%; p=0.11). Interestingly enough, the two cases of PNF both happened in the group of initially discarded livers (2/57, 3.5%; p=0.11). Also, ITBL rates were higher in the initially declined grafts (6/57 vs. 1/115, 10.5 vs. 0.9%; p=0.006).

DISCUSSION

The decision whether to use or discard a marginal graft for LT is a recurring dilemma for transplant professionals: indeed, the paucity of available grafts translates into an increased risk of patients dying while awaiting a transplant. [44,45] Moral issues may arise when deciding whom to allocate a graft to, potentially favoring a patient while harming another on the same waiting list. [46] Eastern LT centers have partly overcome such an impasse adopting a policy focused on living donation. [47] Conversely in Western countries, where a living donation has plateaued around a marginal role, the only solution is to expand the pool of available grafts from deceased donors. In this specific setting, there is growing enthusiasm around MPs which could lead to a revolution in liver grafts preservation and viability, although some concerns still exist on the systematic use of the various machines which have been released on the market.

Aside from economic considerations, [48] it is strikingly evident there is great heterogeneity in literature around MP. Consequently, it is not possible to come to conclusions when commenting on selectors of graft quality from machine perfused livers.

The majority of the studies published so far are only small case series or Phase I trials aiming at assessing the feasibility and safety of MP compared to cold storage. [21] This helps explain the excellent results observed regarding post-LT clinical course: perhaps, some of the grafts transplanted after MP would have also been adequate for LT with standard cold storage preservation. However, it is of interest to underline that when MP strategies were used in case of initially declined livers, the results were inferior, clearly demonstrating that the expansion of the routine graft selection criteria should be done cautiously also using this technology

No clinical studies exist comparing NMP and HMP, making it not possible to suggest the superiority of one approach over the other.

NMP is conceptually very attractive, particularly in consideration of the high number of parameters that could be monitored during perfusion. [49] Actually, the Birmingham Group successfully transplanted five out of six livers declined by other centers showing maintained function during NMP. [29] A similar experience was reported in twelve cases by the Cambridge Group. [37] However, NMP requires more significant management expertise compared to an HMP approach. As an exemplification, two grafts were lost during NMP vs. no case with HMP. In detail, one graft was not homogeneously perfused due to the presence of an aberrant right hepatic artery which had not been reconstructed before the start of normothermic perfusion, [29] and the other one due to portal vein torsion during perfusion. [24], in both cases, the reason for discarding the livers was more related to technical problems than to graft quality.

High expectations exist on the future role of NMP in the specific setting of liver steatosis, with the intent to develop “defatting” protocols, but no clinical studies on humans exist until now, something that leave this ground of research completely unexplored.

Graft evaluation during HMP is far more difficult due to the slower metabolism of liver cells, with a smaller number of biomarkers and parameters which may be tested during perfusion. Moreover, short-term HMP is charged by a further reduced evaluation capability. However, yet in case of hypothermia, a variety of parameters may be detected in the perfusion fluid aimed at assessing the quality of the graft. [50] Of note, approximately 2/3 of all the experiences reported worldwide are based on a hypothermic approach.

If it is not sufficiently clear how to select organs during normo- or hypothermic perfusion, it looks easier to decide which graft to perfuse. A recent review from Dutkowski et al. nicely stratified liver grafts concerning different risk categories, suggesting the best preservation strategy for each class. For example, standard DBD and DCD liver grafts can be routinely well preserved only with cold storage. On the opposite, grafts presenting different combinations of long ischemia time, advanced donor age, type of donor and severe steatosis may benefit from treatment with MP. [51]

This condition is especially true in DCDs, in which livers are often turned down even before deciding to go on a MP, particularly in case of uncontrolled DCD donors. For example, in a study from De Carlis et al., livers were declined while the donor was maintained on NRP and the liver showed poor perfusion and severe fibrosis at biopsy. [25] Such an approach is very well established in previous experiences from Spain where uncontrolled DCD donation was pioneered. [52]

A recent score calculated on 1,153 DCD from the UK identified three risk classes of post-LT poor function and death: low risk (0-5 points), high risk (6-10 points) and futile (>10 points). [53] It should be intriguing to speculate that extended and even overextended DCD cases can be safely used after their perfusion. [51] A recent experience from the Zurich Group explores this specific field, reporting the use of HMP in five DCD cases with 20-40% macro-steatosis, and showing a 1-year patient survival of 100% respect to a historical group preserved with cold storage reporting only 42% of survival. [11]

Focusing on the extension of the preservation time, three cases from Milan and Cambridge reached a global time from donor aortic flush to end of machine perfusion of 18.3, 18.8 and 20.0 hours, respectively. [27,36] Being a record in this topic, the case described by Watson et al. was preserved for stunning 26 hours from donor cross-clamp to recipient declamping! [36] Indeed, the use of MP approximately tripled the cold ischemia times typically accepted for DBD (8 hours) and DCD donors (7 hours). [54] There is no doubt that the opportunity to keep the grafts perfused for a long time represents another fascinating aspect. In spite of that, additional studies are needed to better define the lower and upper limits of perfusion extension.

Lastly, clear end-points should be set up when using MPs, particularly in the context of clinical studies. Not only graft and patient survivals, but also the variables which are known to influence the long-term outcomes (ITBL, transaminases peaks and early allograft dysfunction, to name a few) should be looked at in those studies that aim to build evidence around the topic of MP. [55] Several randomized control trials are underway to investigate the role of MP, either hypo- and normothermic, in LT. [56-59]

CONCLUSIONS

Until now, no exhaustive study exists that address the role of perfusion machines in selecting liver grafts available for transplantation. All the published studies mainly focused on the feasibility and safety of this new technology. Further research investigating the selection process of marginal donors is undoubtedly required. Large prospective studies focused on secondary end-points such as reduction of biliary lesions and graft dysfunction rates are awaited. However, the future broader potentials of this technology are high, especially in the case of NMP, heralding the start of a new era in the setting of organ preservation for transplantation.

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FIGURES

Figure 1. PRISMA flowchart of the literature search and study selection.

TABLES

Table 1. Quality of Evidence in the selected papers for the systematic review according to the Newcastle-Ottawa Scale.

Author	Reference	Author Selection (Max 4 stars)	Comparability (Max 2 stars)	Outcome/Exposure (Max 3 stars)	Total of stars
Kron	11	++++	++	+++	9
Guarrera	16	++++	+	+++	8
Guarrera	17	Case series			0
Guarrera	18	++++	+	+++	8
Dutkowski	19	++++	+	+++	8
Dutkowski	20	++++	++	+++	9
Ravikumar	21	++++	+	+++	8
van Rijn	22	++++	+	+++	8
Selzner	23	++++	+	+++	8
Bral	24	++++	+	+++	8
De Carlis	25	Case series			0
De Carlis	26	Case report			0
De Carlis	27	Case series			0
Perera	28	Case report			0
Mergental	29	Case series			0
Angelico	30	++++	+	+++	8
Hoyen	31	++++	+	+++	8
Pezzati	32	Case report			0
Franzini	33	Case report			0
Ghinolfi	34	Case report			0
Watson	35	Case report			0
Watson	36	Case report			0
Watson	37	++++	++	+++	9
Di Francesco	38	Case report			0
Athanasopoulos	39	Case report			0
He	40	Case report			0
Patrono	41	Case series			0

Table 2. Liver transplants using hypothermic machine perfusions: donor- and recipient-related characteristics.

Author	Ref	Year	Centre	N	Protocol	Temp (°C)	Donor					Recipient				
							DBD	DCD	Age	Sex	Steatosis %	Age	Sex	Disease	HCC	MELD
Guarrera	16	2010	New York	51	HMP	4-8	20	-	39 (±3)	-	-	55 (±6)	-	HCV=8 Alc=2 PBC=1 NASH=1 Crypto=3	5	17 (±7)
	17 18	2015					31	-	58 (±18)	-	-	68 (±8)	-	HCV=12 HBV=2 PBC-PSC-AIH=5 NASH-Alc-Crypto=10	11	20 (±6)
Dutkowski	19 20	2015	Zurich	25	HOPE	10	-	25 Con	54 (36-63)	-	-	60 (57-64)	20M 5F	Alc=4 HCV=8 HBV=1 PBC=1 NASH=6 Other=5	18	13 (9-15)
van Rijn	22	2017	Groningen	10	DHOPE	10	-	10 Con	53 (47-57)	5M 5F	7	57 (54-62)	6M 4F	Alc=3 NASH=5 PSC=1 HCV=1	-	16 (15-22)
De Carlis	25 26 27	2017	Milan	6	NRP+HMP	10	-	4 Unc	47 (40-61)	-	<10 30 <5 15	55 (54-58)	-	HBV=1 Alc=1 NASH=1 HCV-HIV=1	4	10 (8-22)
					HMP	10	2	-	18-65	1F 1M	-	61-66	2F	HCV=2	2	22-22
Patrono	41	2017	Turin	4	DHOPE	10	4	-	79 (54-81)	-	30 in 1 case	53 (25-61)	-	HCV-Alc=1 HCV=1 Alc=1 ALF=1	2	21 (7-35)
Kron	11	2017	Zurich	6	HOPE	10	1	5 Con	65 (55-86)	-	30 (20-40)	55 (53-60)	-	-	-	11 (6-16)

Abbreviations: N, number; DBD, deceased brain donor; DCD, deceased cardiac donor; HCC, hepatocellular cancer; MELD, model for end-stage liver disease; HMP, hypothermic machine perfusion; HCV, hepatitis C virus; Alc, alcohol-related cirrhosis; PBC, primary biliary cholangitis; NASH, non-alcoholic steato-hepatitis; Crypto, cryptogenetic cirrhosis; HBV, hepatitis B virus; PSC, primitive sclerosing cholangitis; AIH, autoimmune hepatitis; HOPE, hypothermic oxygenated perfusion; Con, controlled; M, male; F, female; DHOPE, dual hypothermic oxygenated perfusion; NRP, normothermic regional perfusion; Unc, uncontrolled; HIV, human immunodeficiency virus; ALF, acute liver failure.

Table 3. Liver transplants using sub-normothermic or normothermic machine perfusions: donor- and recipient-related characteristics.

Author	Ref	Year	Centre	N	Protocol	Temp (°C)	Donor					Recipient				
							DBD	DCD	Age	Sex	Steatosis %	Age	Sex	Disease	HCC	MELD
Sub-normothermic experiences																
Hoyen	31	2016	Hessen	6	COR	10-20	6	-	59 (51-71)	1M 5F	2.5 (0-10)	53 (43-65)	6M	Alc=4 HBV=1 NASH=1	1	18 (11-23)
Normothermic experiences																
Ravikumar	21	2016	United Kingdom Multicentre	20	NMP	37	16	4 Con	58 (21-85)	-	-	54 (33-66)	-	HCV=6; Alc=5 PSC=3; PBC=2 A1AT=1; NASH=1 AIH=1; SBC=1	-	12 (7-27)
Selzner	23	2016	Toronto	10	NMP	37	8	2 Con	48 (17-75)	-	-	56 (45-71)	-	Alc=4 HCV=3 NASH=3	4	21 (8-40)
Bral	24	2016	Edmonton	9	NMP	37	6	3 Con	56 (14-71)	-	-	53 (28-67)	-	NASH=3; HCV=2 HCV-Alc=2; HBV=1 AIH=1	2	13 (9-32)
Angelico	28,29, 30	2016	Birmingham	6	NMP	37	2	4 Con	50 (21-61)	3M 3F	Mild (n=5)	55 (34-66)	4M 2F	HCV=2; AIH=1 NASH=1; Alc=1 PSC=1	-	12 (9-18)
Pezzati/Franzini/ Ghinolfi	32,33, 34	2017	Pisa	3	NMP	37	3	-	74 (33-83)	2M 1F	10 (n=1)	53 (52-53)	2M 1F	HCV=2 AIH=1	1	22 (16-23)
Watson	35,36	2015 2016	Cambridge	2	NMP	37	1 -	- 1 Con	39 57	- -	0 0	48 58	M M	PSC=1 Alc=1	- -	14-26
	37	2017		12		37	3	9 Con	56 (24-67)	-	<5%=1 Mild=1 Moderate=1	57 (46-65)	-	Alc=3; A1AT=3 CVID=1; AIH=1 PSC=1; HCV=1 Alc-A1AT=1 NASH=1	3	17 (10-26)
Di Francesco	38	2017	Palermo	1	NMP	37	1	-	82	F	0	56	M	HCV=1	1	22
Athanasopoulos	39	2016	London	1	NMP	37	-	1 Con	16	M	0	59	F	PSC=1	-	-
He	40	2017	Guangzhou	1	IFLT+NMP	37	1	-	25	M	85-95	51	M	HBV=1	1	-

Abbreviations: N, number; DBD, deceased brain donor; DCD, deceased cardiac donor; HCC, hepatocellular cancer; MELD, model for end-stage liver disease; COR, controlled oxygenated rewarming; M, male; F, female; Alc, alcohol-related cirrhosis; HBV, hepatitis B virus; NASH, non-alcoholic steato-hepatitis; NMP, normothermic machine perfusion; Con, controlled; HCV, hepatitis C virus; PSC, primitive sclerosing cholangitis; PBC, primary biliary cholangitis; A1AT def, alpha-1 anti-thrombin deficiency; AIH, autoimmune hepatitis; SBC, secondary biliary cirrhosis; CVID, combined variable immunodeficiency; IFLT, ischemia-free liver transplantation.

Table 4. Liver transplants using hypothermic machine perfusions: post-perfusion clinical course.

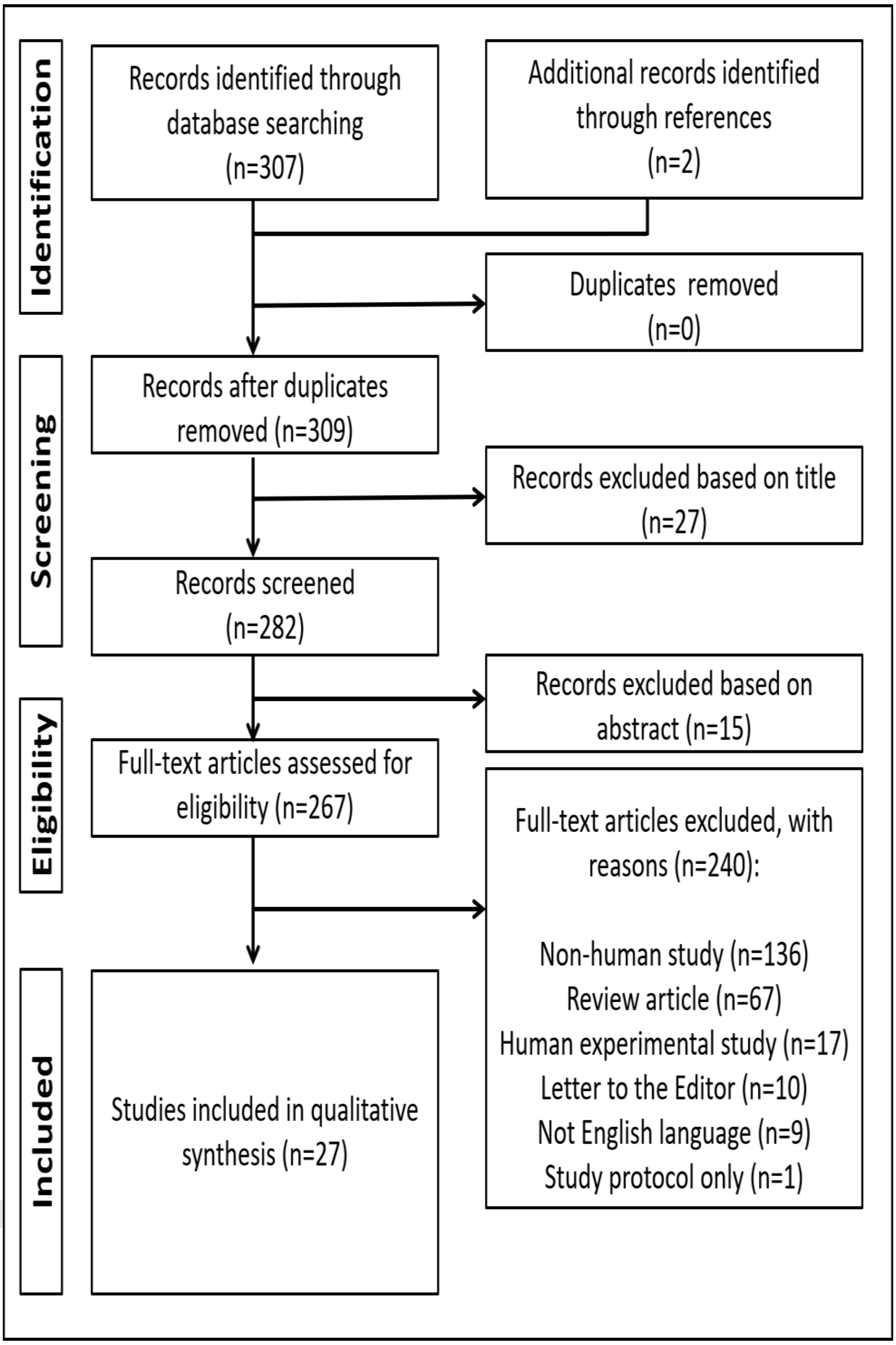
Author	Ref	N	Timing		Perfusion pressures		Lactates mmol/L		Final flow		Post-LT peak transaminases (IU/L)	Hosp stay days	FU months	Graft loss	Death	AKI	PNF	ITBL
			AF-MP	MP	HA (mmHg)	PV (mmHg)	Initial	Final	HA (mL/min)	PV (L/min)								
Guarrera	16	51	564 (±126)	258 (±54)	5.5 ±0.15	2.9 ±0.08	-	-	-	-	AST>2000=2 AST>3000=2 ALT>2000=4 ALT>3000=0	11 (±5)	12	2 (infect)	2 (infect)	1	0	0
	17,18		558 (±96)	228 (±54)	5.1 ±0.2	2.9 ±0.1	-	-	-	-	AST>2000=9 AST>3000=7	14 (±11)	12	6 (NA)	5 (NA)	3	1	3
Dutkowski	19,20	25	188 (141-264)	118 (101-149)	Not perfused	≤3 (continuous)	-	-	-	-	AST=1808 (1133-3547) ALT=1239 (689-2126) ALT>2000=6 ALT>3000=4	20 (14-23)	12	2 (NA)	NA	7	0	0
van Rijn	22	10	331 (308-376)	126 (123-135)	25 (pulsatile)	5 (continuous)	-	-	84	0.4	AST=966	22 (16-33)	12	0	0	1	0	1
De Carlis	25,26, 27	6	365 (210-735)	205 (150-360)	25 (pulsatile)	4 (continuous)	-	-	-	-	ALT=897 (120-1063)	-	3-16	0	0	0	0	0
Patrono	41	4	-	176 (150-200)	25 (pulsatile)	3-4 (continuous)	2.0 (2.0-3.5)	2.9 (1.3-4.5)	-	-	AST=685 (72-2473) ALT=348 (255-1260)	11 (8-46)	6	0	0	1	0	0
Kron	11	6	192 (150-312)	120 (108-288)	Not perfused	≤3 (continuous)	-	-	-	-	ALT=1871	3 (2-6) (ICU stay)	12	0	0	1	0	0

Abbreviations: N, number; AF, aortic flushing; MP, machine perfusion; HA, hepatic artery; PV, portal vein; LT, liver transplantation; FU, follow-up; AKI, acute kidney injury; PNF, primary non-function; ITBL, ischemic-type biliary lesion; AST, aspartate transaminase; ALT, alanine aminotransferase; NA, not available; ICU, intensive care unit.

Table 5. Liver transplants using sub-normothermic or normothermic machine perfusions: post-perfusion clinical course.

Author	Ref	N	Timing		Perfusion pressures		Lactates mmol/L		Final flow		Post-LT peak transaminases (IU/L)	Hosp stay days	FU months	Graft loss	Death	AKI	PNF	ITBL
			AF-MP	MP	HA (mmHg)	PV (mmHg)	Initial	Final	HA (mL/min)	PV (L/min)								
Sub-normothermic experiences																		
Hoyen	31	6	508 (369-870)	90	25 (pulsatile)	2-4 (continuous)	-	1.7 (0.2)	-	-	AST>2000=0 ALT>2000=0	22 (14-103)	6	0	0	0	0	0
Normothermic experiences																		
Ravikumar	21	20	-	558 (210-1110)	60-75	8	-	-	-	-	AST=417 (84-4681)	12 (6-34)	6	0	0	1	0	0
Selzner	23	10	586 (221-731)	480 (340-580)	NA	NA	-	1.5 (0.6-1.7)	300 (200-400)	1.3 (1.2-1.3)	AST=1647 (227-9200) ALT=444 (152-1460)	11 (8-17)	3	0	0	-	0	0
Bral	24	9	184 (129-208)	691 (461-769)	NA	NA	-	-	-	-	AST=1253 (383-2600)	45 (13-114)	6	1 (HCV)	1 (HCV)	1	0	0
Angelico	28,29,30	6	91 (73-117)	525 (395-605)	40-50	6-10	13.1 (5.5-13.9)	1.4 (0.7-2.8)	100-200	1.1-1.2	ALT=1242 (1188-1879)	9 (5-14)	6-30	0	0	1	0	0
Pezzati/Franzini/ Ghinolfi	32,33,34	3	226 (215-285)	255 (240-325)	From 30 to 65 in 30 minutes	From 3 to 7 in 30 minutes	-	-	-	-	AST=329 (151-2357)	140 (12-15)	1-6	0	0	0	0	0
Watson	35,36	2	350-618	132-510	From 30 to 60 in 9 minutes	From 5 to 8 in 9 minutes	7.9-9.2	0.03-0.3	-	-	960-1198	8-26	1-12	0	0	0	0	0
Watson	37	12	427 (222-877)	284 (122-530)	From 30 to 60 in 30 minutes	From 4 to 9 in 30 minutes	-	-	250	0.9	ALT=1069 (187-4991)	-	9-24	1 (PNF)	1 (PNF)	-	1	3
Di Francesco	38	1	260	210	70	9	13.5	2.8	478	1.1	-	-	NA	NA	NA	-	-	-
Athanasopoulos	39	1	129	1043	NA	NA	9.1	0.5	400	1.2	AST=395 ALT=193	11	2	0	0	0	0	0
He	40	1	-	270	NA	NA	6.8	0.3	-	-	AST=375 ALT=123	18	0.5	0	0	0	0	0

Abbreviations: N, number; AF, aortic flushing; MP, machine perfusion; HA, hepatic artery; PV, portal vein; LT, liver transplantation; FU, follow-up; AKI, acute kidney injury; PNF, primary non-function; ITBL, ischemic-type biliary lesion; AST, aspartate transaminase; ALT, alanine aminotransferase; HCV, hepatitis C virus; NA, not available.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Records excluded based on abstract (n=15)
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Full-text articles assessed for eligibility (n=267)
Full-text articles excluded, with reasons (n=240):
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Review article (n=67)
Human experimental study (n=17)
Letter to the Editor (n=10)
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