The Role of Ex Vivo Machine Perfusion in Liver Transplant Recipient Outcomes – A Systematic Review and Meta-Analysis

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STATEMENT

I, Belle Liew, confirm that all the work presented in this thesis is my own. Where information has been obtained from other sources, I confirm that this has been expressly stated in the thesis.

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ABSTRACT

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ABBREVIATIONS

ACR Acute Cellular Rejection

ALT Alanine Transaminase

AST Aspartate Transaminase

COR Controlled Oxygenated Rewarming

DBD Donation after Brain Death

DCD Donation after Circulatory Death

EAD Early Allograft Dysfunction

ECD Extended Criteria Donor

HMP Hypothermic Machine Perfusion

HOPE Hypothermic Oxygenated Machine Perfusion

IC Ischaemic Cholangiopathy

IFLT Ischaemia Free Liver Transplantation

IRI Ischaemia-Reperfusion Injury

LT Liver Transplantation

MP Machine Perfusion

NAS Non-anastomotic Stricture

NMP Normothermic Machine Perfusion

PNF Primary Nonfunction

PRS Postreperfusion Syndrome

1. INTRODUCTION

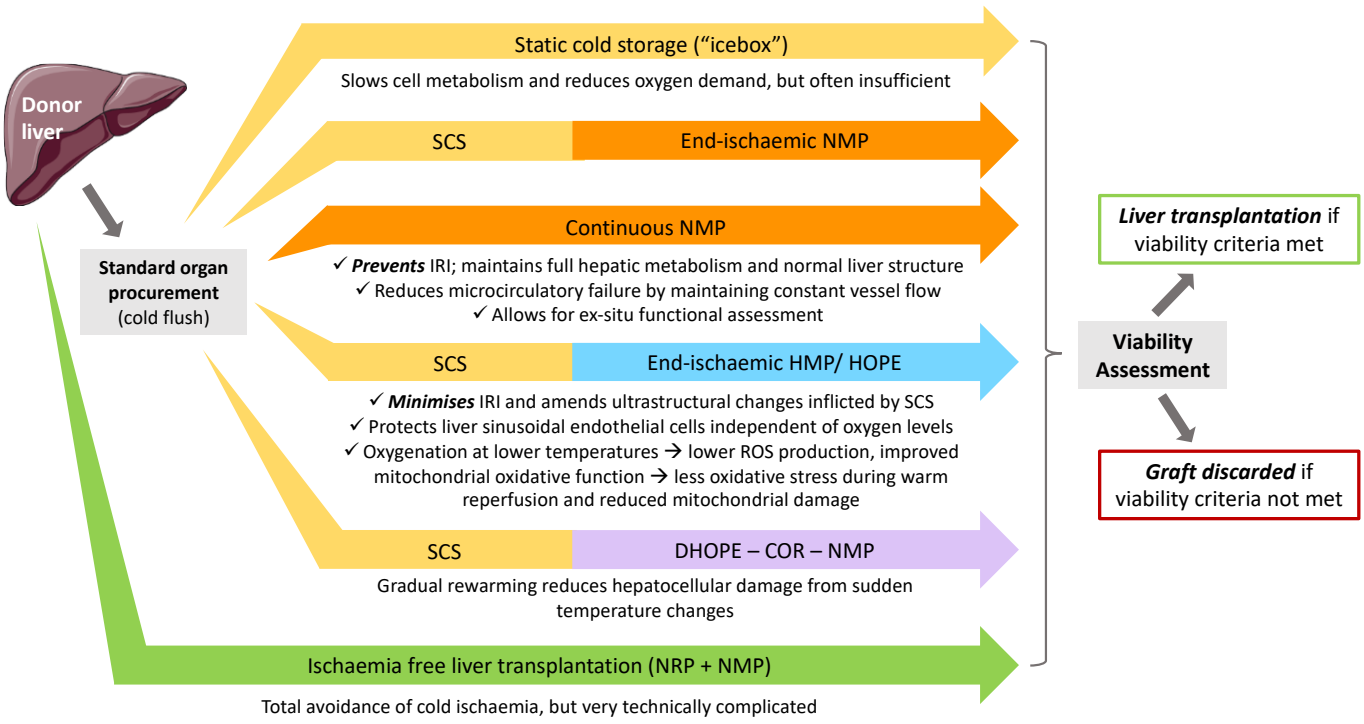
Liver disease is the leading cause of death amongst 35 to 49-year-olds, and is projected to overtake cardiovascular disease as the biggest cause of premature death in the next few years. While deaths due to other major diseases have remained constant or decreased, mortality rates due to liver failure have nearly quadrupled since 1970 (British Liver Trust). Liver transplantation is the mainstay of treatment for patients with end-stage liver disease, with a one-year survival rate of 94.2% (NHS Blood and Transplant, 2020). From 2019 to 2020, the number of patients on the active liver transplant list increased by 8%. However, access to life-saving liver transplantation is hindered by the shortage of donor organs, resulting in a waiting-list mortality of 21% in the UK.

To meet the rising demand, many centres are increasing their usage of donation after circulatory death (DCD) and extended criteria donor (ECD) livers. These grafts are associated with poorer outcomes post transplantation, with increased rates of graft loss due to primary nonfunction, early allograft dysfunction and biliary complications. This is due to the fact that they are subject to longer warm ischemic time and therefore more vulnerable to ischaemia-reperfusion injury (IRI). Static cold storage has conventionally been used to preserve donor livers but may not sufficiently sustain "marginal" high-risk grafts. Hence, there is an urgent need to improve the preservation or reconditioning strategies for these invaluable organs. Ex-vivo machine perfusion is being increasingly adopted in clinical practice with the intention of expanding the pool of transplantable livers. The two main techniques currently employed are hypothermic (oxygenated) machine perfusion (HMP or HOPE) and normothermic machine perfusion (NMP).

In hypothermic machine perfusion, the perfusate solution is circulated through the allograft at low temperatures (4-11ºC), delivering nutrients and removing metabolic waste. Active oxygenation can also be applied to the perfusate, ensuring PaO2 levels above 80kPa. In contrast, normothermic machine perfusion involves the perfusion of the donor organ with oxygenated blood or acellular oxygen carriers at physiological temperatures (approximately 37ºC). Experimental studies in animals and humans have shown that both techniques are able to alleviate or even reverse IRI-associated damage to hepatocytes and biliary epithelial cells, and the potential benefits of each are summarised in Figure 1.

Both dynamic preservation techniques share the common advantage of simulating normal blood flow through vessels. This allows maintenance of endogenous nitric oxide production, protecting liver sinusoidal endothelial cells and microcirculation, regardless of oxygenation levels. Furthermore, the delivery of nutrients and oxygen preserves hepatocellular metabolism and mitochondrial integrity, reducing the release of reactive oxygen species following reperfusion in subsequent liver implantation. By mimicking near-physiological conditions, NMP also enables ex-situ viability assessment to guide the decision on whether the donor graft is suitable for transplant.. The evolution of machine perfusion has the potential to increase the number of transplantable livers and improve outcomes for recipients of these livers.

Figure 1 Schematic illustrating the different methods of liver preservation involving ex-situ machine perfusion techniques.



Previous randomised controlled trials have compared static cold storage against either hypothermic or normothermic machine perfusion, but there is still no high level evidence comparing hypothermic against normothermic machine perfusion. Each technique has unique pros and cons, but there is controversy surrounding which strategy has superior outcomes.

The objective of this systematic review was to review existing literature on ex-vivo machine perfusion in liver transplantation, in order to inform a prospective randomised feasibility trial comparing standard organ preservation (SCS) with machine perfusion using HMP or NMP.

2. METHODS

The protocol for this systematic review was registered with PROSPERO, under registration number: CRD42021249194. This review was conducted in accordance with PRISMA guidelines.

2.1 Search Criteria

A thorough literature search was conducted in February 2021 of the online databases MEDLINE (OvidSP), EMBASE (OvidSP) and Scopus. The search algorithm "(hypothermic machine perfusion OR HMP OR hypothermic oxygenated machine perfusion OR HOPE OR normothermic machine perfusion OR NMP OR ex-vivo machine perfusion) AND (liver transplant*)" was employed, including MeSH terms for "Perfusion" and "Liver Transplantation". No limits were set for language or publication year.

Titles and abstracts were screened, and full-text articles were retrieved and further assessed for eligibility. Reference lists of the included studies were manually searched to extract any potentially relevant studies.

2.2 Inclusion and Exclusion Criteria

All experimental or observational studies reporting on the outcomes of patients receiving livers that have undergone either hypothermic machine perfusion or normothermic machine perfusion were included. Case reports or case series and cost-utility analysis were eligible for descriptive analysis. Conference abstracts and literature reviews were excluded. Non-human studies, studies focused solely on the outcomes of in-situ perfusion techniques (e.g. normothermic regional perfusion), and studies with no clinically relevant patient outcomes were excluded.

2.3 Data Management

Data was extracted from full-text studies using a pre-determined abstraction form. Variables extracted included: authors, study year, study design, ex vivo machine perfusion strategy, number of patients, donor age, allograft type, recipient age, gender, MELD score and post-transplantation outcomes.

Post-transplantation outcomes included: early allograft dysfunction (EAD), peak AST and ALT levels, primary nonfunction (PNF), acute cellular rejection (ACR), postreperfusion syndrome (PRS), biliary complications, hepatic artery thrombosis or stenosis, graft loss, retransplants, major complications, 1-year graft and patient survival, and the length of ICU and hospital stay.

EAD was defined as the presence of one or more of the Olthoff criteria (serum bilirubin ≥171μmol/L; or international normalised ratio ≥1.6; or peak ALT >2000U/L in the first seven postoperative days). PNF was defined as graft failure necessitating retransplantation or resulting in death within 10 days after transplantation. Total biliary complications encompassed

all problems with the biliary duct, such as biliary leaks, anastomotic strictures and non-anastomotic strictures, in particular ischemic cholangiopathy. PRS was defined as a decrease in mean arterial pressure of >30% from baseline lasting >1 minute during the first 5 minutes after reperfusion.

2.4 Statistical Analysis

For single group cohort studies, pooled means and pooled proportions with 95% confidence intervals (CI) will be calculated for continuous and dichotomous data respectively. For comparative studies, pooled odds ratios (ORs) will be used to assess the summary event rates. Standardised mean differences will be used to compare continuous variables. 95% CI and two-sided p-values will be calculated with $p<0.05$ considered statistically significant. Heterogeneity between the studies in effect measures will be assessed using the I^2 statistic, and I^2 values greater than 50% will be considered indicative of substantial heterogeneity. As the studies included are not expected to be functionally equivalent, a random-effects model will be used to combine the studies and pool total effect size. All statistical analysis will be performed using rBiostatistics.com.

2.5 Risk of Bias Assessment

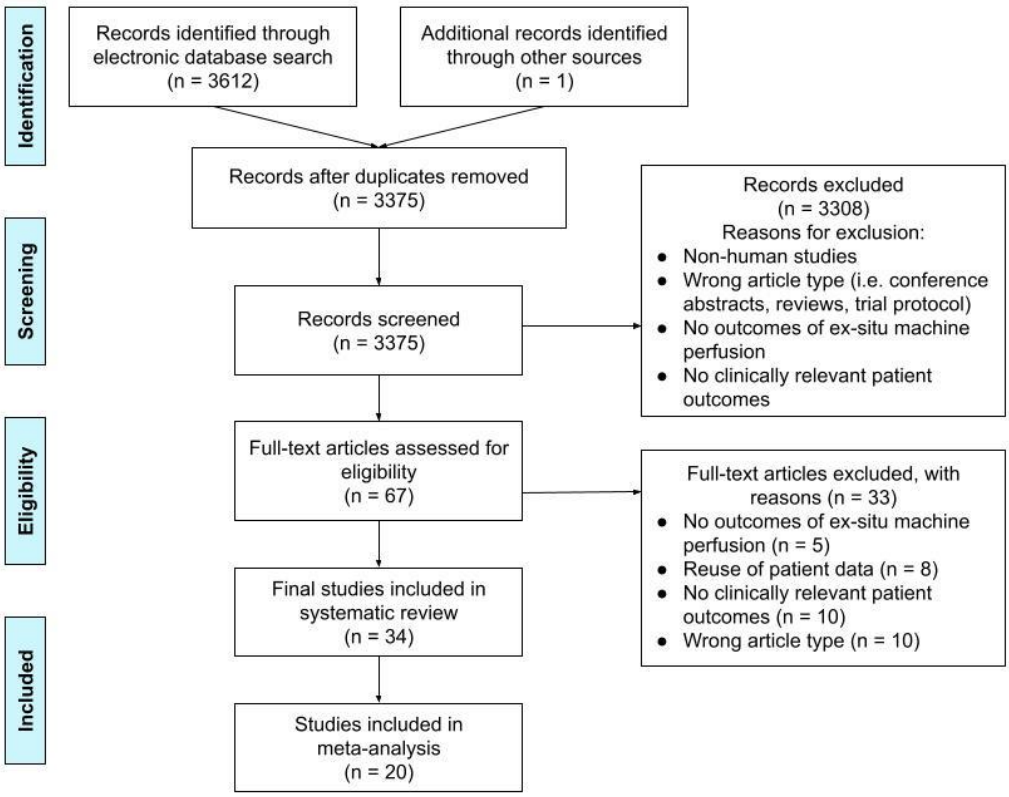
The Risk of Bias in Non-Randomised Studies – of Interventions (ROBINS-I) tool was used to assess the potential risk of bias of the comparative, non-randomised studies. The RoB2 tool was used to assess the risk of bias in randomised controlled trials.

3. RESULTS

3.1 Study Selection

The initial online database search identified 3612 papers. Following the removal of 238 duplicates, 3308 articles were excluded during title and abstract screening. Full-texts of the remaining 66 studies were further assessed for eligibility, identifying 33 studies. One additional study was identified through citation-chaining, producing the final 34 studies included in this systematic review, of which 20 were appropriate for inclusion in meta-analysis (Figure 2).

Figure 2 PRISMA Flow Diagram



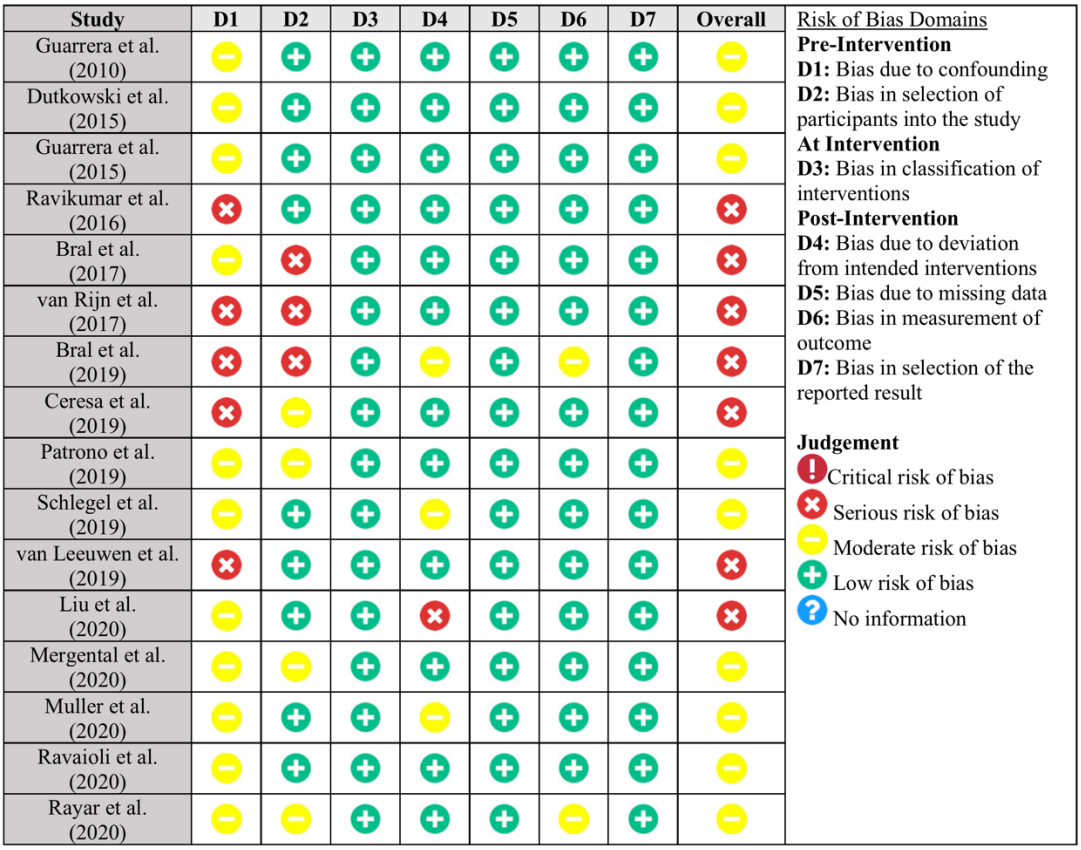
3.2 Study Characteristics and Quality

Of the 34 included articles, three were randomised controlled trials, nineteen were cohort studies (eighteen prospective and one retrospective), ten were case series or case reports, and two were cost-effectiveness studies. Table 1 summarises the study characteristics and patient demographics. The machine perfusion strategies employed are detailed in table 2.

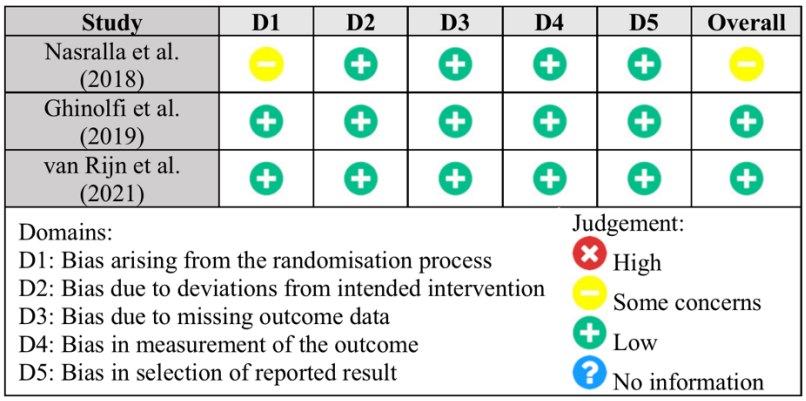
A total of 1742 liver allograft recipients were included in this review. 866 grafts were preserved with SCS (control group), 376 grafts with HMP or HOPE, and 457 with NMP. 32 grafts were procured using ischemia-free liver transplantation (IFLT), a novel procedure involving the use of both in-situ and ex-vivo NMP consecutively. Finally, eleven grafts underwent a combination of HOPE, controlled oxygen rewarming (COR) and NMP.

Using the ROBINS-I tool, nine non randomised studies were found to be at moderate risk of bias and seven studies were found to be at serious risk of bias (Figure 3A). Risk of bias assessment for the 3 randomised controlled trials using the RoB2 Tool found that two of the three studies were at low risk of bias, while there were some concerns in one study (Figure 3B).

Figure 3 Traffic light plots presenting the results of risk of bias assessment of:
(A) Non-randomised trials using the ROBINS-I tool



(B) Randomised controlled trials using the RoB2 tool



3.3 Post-Transplantation Clinical Outcomes

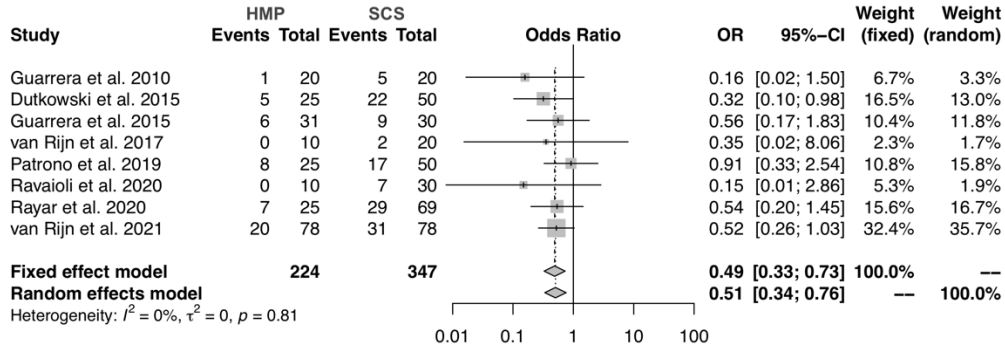
Of the included studies, nine compared HMP against SCS and six compared NMP against SCS. The main outcomes of the included studies are highlighted in Table 3; all results of the meta-analysis can be found in the Appendix.

Three studies compared the occurrence of **post-reperfusion syndrome** in HMP vs SCS, and found no significant difference in incidence (OR = 1.77, 95% CI = 0.06-54.53, p = 0.74) . There was also no significant difference in post-reperfusion syndrome in NMP vs SCS, as shown by two studies (OR = 0.81, 95% CI = 0.07-9.24, p = 0.87).

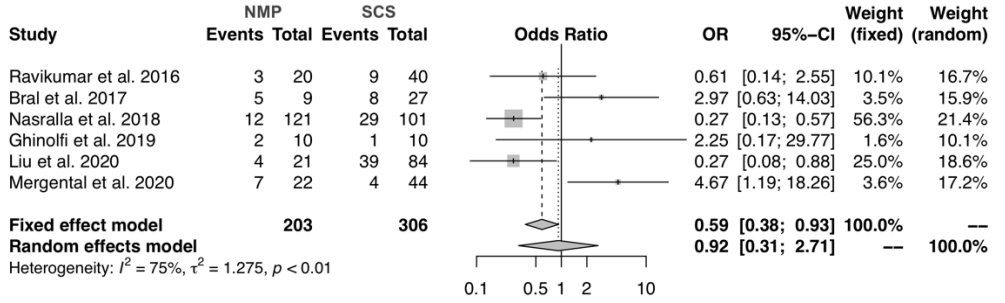
Eight studies compared the incidence of **early allograft dysfunction** in HMP versus SCS, and meta-analysis demonstrated a pooled odds ratio favouring HMP (OR = 0.51, 95% CI = 0.34-0.76, p = 0.001) (Figure 2A).

Six studies reported on the incidence of EAD in NMP, and found no significant difference between NMP and SCS (OR = 0.92, 95% CI = 0.31-2.71, p = 0.88) (Figure 2B). The pooled proportion of EAD was 30.6% (95% CI = 24.8-37.2) in the SCS group, 23.3% (95% CI = 11.9-40.7) in the HMP group and 20.6% (95% CI = 12.9-31.2) in the NMP group.

Figure 2 Forest plots demonstrating prevalence of early allograft dysfunction in:
(A) HMP vs. SCS (OR = 0.51, 95% CI = 0.34-0.76, p = 0.001)



(B) NMP vs. SCS (OR= 0.92, 95% CI = 0.31-2.71, p = 0.88)



Five studies assessed the mean difference in **peak AST and ALT levels** between HMP and SCS groups. The SCS cohort had significantly higher peak AST (mean difference = –1085.30 IU/L, 95% CI = -1977.90--192.71, p = 0.017) and significantly higher peak ALT (mean difference = –516.49 IU/L, 95% CI = p = 0.0023) compared to HMP. In contrast, meta-analysis

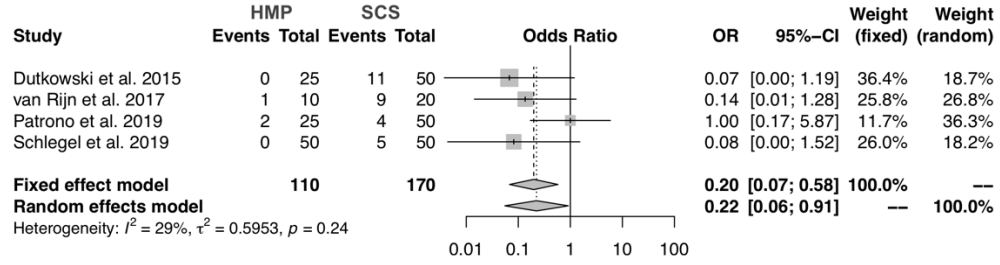
of the three studies comparing peak AST in NMP vs SCS found no significant difference between the two groups (mean difference = –716.82 IU/L, 95% CI = -1855.11-421.88, p = 0.22).

Nine studies evaluated **primary nonfunction** in HMP versus SCS cohorts and found that there was no significant difference (OR = 0.65, 95% CI = 0.22-1.95, p = 0.44). Six studies compared PNF between NMP and SCS and also found no significant difference in frequency (OR = 1.28, 95% CI = 0.13-12.57, p = 0.83). The overall rate of PNF in the SCS group was 3.3% (95% CI = 2.0-5.2%), while that in the HMP group was 3.6% (95% CI = 2.0-6.5%) and 2.4% (95% CI = 1.0-5.7%) in the NMP group.

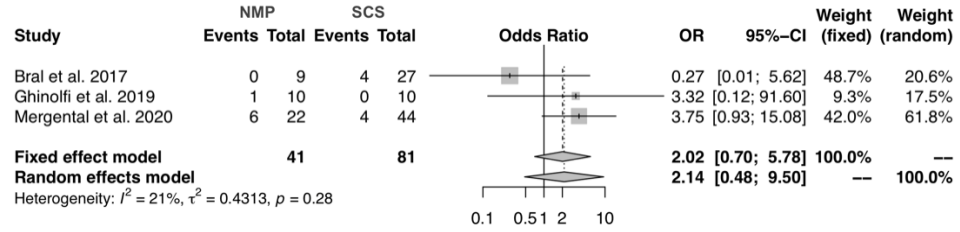
Five studies reported on the incidence of **hepatic artery thrombosis** in HMP compared to SCS, and found no significant difference between the two groups (OR = 0.50, 95% CI = 0.19-1.31, p = 0.16). Likewise, three studies reported on HAT in NMP versus SCS and also found no significance difference in incidence (OR = 0.71, 95% CI = 0.12-4.32, p = 0.71). The pooled incidence of HAT in the SCS cohort was 6.3% (95% CI = 4.1-9.6%), 3.1% (95% CI = 1.6-5.9%) in HMP and 4.1% (95% CI = 1.8-34.8%) in the NMP cohort.

Nine studies reported on the occurrence of **total biliary complications** in HMP vs SCS. There was a significantly lower incidence of biliary complications in the HMP group (OR = 0.58, 95% CI = 0.40-0.84, p = 0.0037). Four studies looked specifically at ischemic cholangiopathy rates between the two groups, and found that this occurred less frequently in the HMP group (OR = 0.22, 95% CI = 0.06-0.91, p = 0.037) (Figure 3A). Another four studies looked at the occurrence of non-anastomotic strictures, which was significantly lower in the HMP group (OR = 0.30, 95% CI = 0.14-0.63, p = 0.0017). There was no significant difference in the prevalence of anastomotic strictures (OR = 1.14, p = 0.60) and biliary leaks (OR = 0.68, p = 0.36) between HMP and SCS liver allografts. Meanwhile, the three studies comparing total biliary complication in NMP versus SCS found no significant difference between the two cohorts (OR = 2.14, 95% CI = 0.48-9.50, p = 0.32) (Figure 3B). There was also no significant difference in anastomotic strictures (OR = 0.84, 95% CI = 0.49-1.45, p = 0.53) and non-anastomotic strictures (OR = 2.14, 95% CI = 0.17-26.83, p = 0.55). The overall incidence of IC was 12.7% (95% CI = 4.8-29.7%) in the SCS group, 5.4% (95% CI = 2.0-13.6%) in the HMP group and 4.0% (95% CI = 0.7-20.7%) in the NMP group.

Figure 3 Forest plots demonstrating the prevalence of:
(A) Ischemic cholangiopathy in HMP vs. SCS (OR = 0.22, 95% CI = 0.06-0.91, p = 0.037)

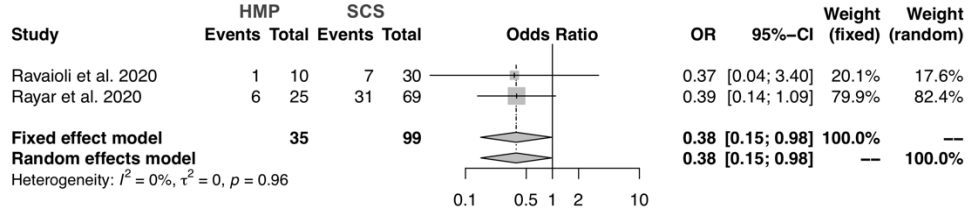


(B) Total biliary complications in NMP vs. SCS (OR = 2.14, 95% CI = 0.48-9.50, p = 0.32)

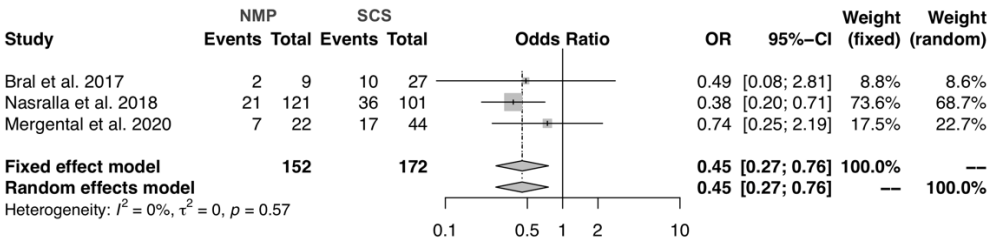


Major complications (Clavien-Dindo Grade ≥ III) were found by two studies to be significantly less common in the HMP than SCS group (OR = 0.38, 95% CI = 0.15-0.98, p= 0.045) (Figure 4A). Similarly, in three studies comparing NMP and SCS, the prevalence of major complications was significantly lower in the NMP group (OR = 0.45, 95% CI = 0.27-0.76, p = 0.0025) (Figure 4B). The pooled rate of major complication was 37.5% (95% CI = 31.6-43.7%) for SCS, 21.0% (95% CI = 10.3-38.1%) for HMP and 20.5% (95% CI = 15.4-26.9%) for NMP.

Figure 4 Forest plots demonstrating the prevalence of major complications in:
(A) HMP vs. SCS (OR = 0.38, 95% CI = 0.15-0.98, p= 0.045)



(B) NMP vs. SCS (OR = 0.45, 95% CI = 0.27-0.76, p = 0.0025)



RCT data?

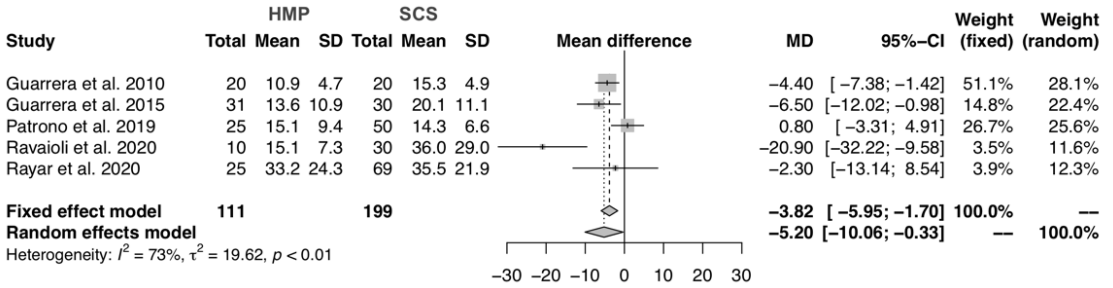
3.4 Duration of ICU and Hospital Stay

Two studies reported on the mean duration of ICU stay in HMP vs SCS, and found no significant difference between the two groups (mean difference = 1.97 days, 95% CI = -4.52-8.46, p = 0.5516). The length of ICU stay was shorter in the NMP than SCS group in three studies, but this was not found to be statistically significant (mean difference = –0.56 days, 95% CI = -16.54-8.96, p = 0.89).

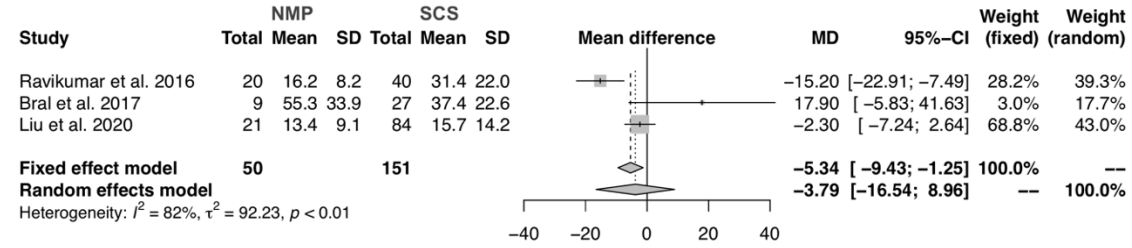
Five studies found that the mean length of hospital stay was significantly shorter in the HMP group compared to SCS (mean difference = -5.20 days, 95% CI = -10.06-0.33, p = 0.036)

(Figure 5A). Three studies also reported shorter mean hospital stay for the NMP group, but this did not reach statistical significance (mean difference = -3.79 days, 95% CI = -16.54-8.96, p = 0.56) (Figure 5B).

Figure 5 Forest plots demonstrating the standardised mean difference in hospital stay in:
(A) HMP vs. SCS (mean difference = –5.20 days, 95% CI = -10.06--0.33, p = 0.036)



(B) NMP vs. SCS (mean difference = -3.79 days, 95% CI = -16.54-8.96, p = 0.56)



RCTs data

3.5 Graft and Patient Survival

Three studies reported on the incidence of graft loss in HMP vs SCS, which was found to be significantly lower in the HMP group (OR = 0.25, 95% CI = 0.11–0.55, p = 0.0006). However, no significant difference in graft loss was demonstrated by the three studies evaluating this in NMP vs SCS (OR = 1.30, 95% CI = 0.42–4.03, p = 0.65).

Eight studies reported on 1-year graft survival in the SCS group, and the pooled rate was found to be 85.4% (95% CI = 75.6–91.7%). Six studies found that the 1-year graft survival rate was 86.5% (95% CI = 80.8–90.7%) in the HMP group, and five studies found that this was 90.7% (95% CI = 83.9–94.8%) for NMP.

1-year patient survival in the SCS cohort was evaluated by eight studies, and the overall survival rate was 90.7% (95% CI = 85.5-94.2%). Six studies assessed 1-year patient survival for HMP and found this to be 90.6% (95% CI = 85.3-94.1%). Four studies found that 1-year patient survival rate was 96.0% (95% CI = 91.7-98.1%) in recipients of NMP preserved grafts.

3.6 Cost Effectiveness

Javanbakht et al. performed a cost-utility analysis of normothermic machine perfusion using OrganOx Metra. Over a lifetime time horizon, the total costs per patient were higher for liver transplantation using NMP compared to SCS (£46,711 vs £37,370) but the total effectiveness per patient was also higher in the OrganOx Metra group (10.27 QALYs vs 9.09 QALYs). While

the total cost of OrganOx Metra for the entire cohort was higher compared to SCS (£20.1 million vs £16.1 million), this was due to the extra available transplantable grafts compared to SCS, as the perfused grafts were 50% less likely to be discarded, increasing post-transplantation costs. The estimated incremental cost-effectiveness ratio (ICER) was £7,876 per each QALY gained. The authors concluded that the use of OrganOx Metra for liver perfusion is a cost-effective strategy.

Webb et al. also reported on the cost-effectiveness of NMP using OrganOx Metra. The cumulative operative cost for one run of the OrganOx machine was $Can18,593.02 – 20,241.35. They found that the cost difference between liver transplantation using OrganOx and standard liver transplantation was statistically significant for both in-providence ($p = 0.042$) and out-of-province ($p = 0.024$) transplants. Additionally, the authors also found that due to the implementation of NMP, 84.48% (49 of 58) of OrganOx liver transplants at their centre between 2015 and 2019 switched from a potential night-time to daytime transplantation. In contrast, 64% of SCS liver transplants were completed in the daytime, suggesting that NMP has the potential to save costs from the decreased premium night-time salaries.

Two studies considered the economic benefit of hypothermic machine perfusion. Dutkowski et al. (2014) reported that the HOPE-DCD group had lower hospital costs. Rayar et al. performed cost-analysis of HOPE using the Liver Assist machine, and found that the total additional cost for HOPE was around €5,298 per patient. The average difference between cost and revenue for hospital stay from the hospital perspective was not statistically significant between HOPE and SCS groups (+€3,023±€16,537 vs +€4,059±€16,266, IC [-€5,470, -€8,652]).

3.7 Novel Perfusion Techniques

Four studies assessed the outcomes of ischemia free liver transplantation, a procedure involving the utilisation of in-vivo NMP followed by ex-situ NMP. Three case reports described relatively uneventful postoperative course, with no occurrences of EAD, PNF, PRS and biliary or vascular complications. Zhang et al. (2020) designed a prospective cohort study of 28 recipients of IFLT liver grafts, and reported only one incidence of EAD but this was suggested to be due to a large haematoma and not perfusion. Median peak ALT and AST levels were 156 U/L and 739 U/L respectively, and ALT and AST release declined quickly.

Van Leeuwen et al. (2019) conducted a prospective clinical trial of a new technique comprising sequential dual HOPE, COR and NMP. The authors reported that all eleven livers which met viability criteria were successfully transplanted, with 100% 6-months survival. Introduction of HOPE-COR-NMP also increased the number of transplantations by 20%.

3.8 Safety and Feasibility

None of the case reports or series reported any major clinical complications experienced by recipients of machine-perfused liver allografts, with 100% graft and patient survival. There were also no technical difficulties or device malfunction during machine perfusion.

Manzia et al. and Rayar et al. reported on their experiences transplanting liver grafts from advanced age donors (>80 years) after NMP and HOPE respectively. All patients had a relatively uneventful postoperative recovery, and demonstrated good clinical status and graft function at the last follow-up. Werner et al. described the first successful transplantation of a paediatric liver graft after HOPE, whereby the recipient showed completely normal liver function 1-year postoperatively with no biliary complications. Bogensperger et al. presented a case in which the donor liver was subjected to a prolonged period of NMP (10 hours 36 minutes) due to delays from SARS-CoV-2 screening and limited operating room capacity. The recipient had good initial liver function and was discharged on POD 16 after a smooth clinical course.

4. DISCUSSION

As this systematic review takes into account the recently published results of the randomised controlled trial by van Rijn and colleagues (2021), it is currently the most thorough and updated critical analysis of the outcomes of liver transplantation following machine perfusion. Our study shows that HMP is superior to SCS with regards to reducing the risk of EAD, IC, NAS, total biliary complications, major complications and graft loss. HNP is also associated with a reducedhospital stay. While NMP was shown to reduce the incidence of major complications, the present meta-analysis could not identify any other significant effects to favour the use of NMP over SCS. This may be due to the paucity of data from which to draw firm conclusions about the benefit of NMP compared to traditional methods of preservation, given the limited number of clinical trials. Our results hence indicate that machine perfusion could indeed be a better way of preserving organs.

Ischemia reperfusion injury is the primary cause of morbidity and mortality following liver transplantation. Hepatic IRI manifests clinically as higher rates of PRS, EAD, PNF and acute cellular rejection. In the long term, it could induce biliary complications including ischemia cholangiopathy. Histological evidence of biliary IRI has also been associated with the development of NAS. Such complications contribute to poor graft and patient survival. The findings of this systematic review corroborate previous experimental studies that suggest that machine perfusion could indeed be a better way of preserving organs and improve patient outcomes.

Serum transaminase levels are markers of hepatocellular damage, and higher levels of AST and ALT are correlated to EAD and graft loss following liver transplantation. Our meta-analysis demonstrates that HMP was able to significantly decrease both AST and ALT post-transplantation, which could account for the lower incidence of EAD in the HMP group compared to SCS. A reduction in peak AST and EAD was observed for NMP preserved grafts, but this was not statistically significant. These findings indicate that machine perfusion could protect against the damage caused by ischemia reperfusion injury through decreased transaminase release.

Another key endpoint of this review was the incidence of biliary complications, particularly ischemic cholangiopathy. The risk of developing non-anastomotic strictures is three times higher in DCD compared to DBD recipients, and is the primary obstacle hindering the use of extended criteria donor livers. It has been proposed that machine perfusion can maintain peribiliary vascular microcirculation and normal physiology, thus reducing biliary IRI. Pooled single-cohort data revealed that the overall rate of IC in both HMP and NMP was less than half than in the SCS cohort. Meta-analysis found that HMP was able to significantly reduce total biliary complications, IC and NAS. Higher powered clinical trials are needed to assess the impact of using NMP compared to SCS in this regard, but it is reasonable to assume that machine perfusion could protect against the development of biliary complications.

Hepatic artery thrombosis is the most serious vascular complication after liver transplantation, often resulting in graft dysfunction and loss. As the hepatic artery is the sole blood supply to the bile ducts, HAT can lead to grave ischemic damage to hepatocytes and cholangiocytes, causing subsequent biliary lesions such as bile leaks and IC (Pastacaldi, 2001). While the exact pathogenesis of HAT has yet to be elucidated, numerous surgical and nonsurgical risk factors have been proposed (Mourad, 2014). Van Rijn et al. (2017) posited that combined portal and arterial perfusion during MP could mechanically damage the hepatic artery, leading to a higher incidence of HAT post-transplantation. However, no significant difference in HAT rates was found with either HMP or NMP when compared to SCS. This supports the use of dual perfusion in machine perfusion, as it does not appear to increase arterial complications.

The one-year patient survival for adult elective deceased donor first liver transplants was 94.2% from 2015 to 2019 (NHS Blood and Transplant, 2020). The 1-year patient survival rates in our study were lower than this figure for SCS and HMP, but higher in NMP. However, this is most probably due to the short follow-up duration of most studies. Our meta-analysis did not identify any significant effects favouring the use of machine perfusion in increasing patient survival, but graft loss was significantly lower in the HMP group.

One of the hurdles facing widespread implementation of machine perfusion is its logistical complexity and financial demand. NMP in particular is more technologically challenging than HMP, and cost-utility analyses of the OrganOx Metra indicate higher costs per liver transplant compared to SCS. Nonetheless, while machine perfusion might incur greater sunk costs, it can lead to sustainable savings in the long term by reducing the expenses associated with clinical complications and lengthened ICU or hospital stay. Most importantly if MP increases the availability of usable grafts and takespatients off the waiting-list, healthcare costs of treating advanced liver disease will be avoided. .

The inclusion of case reports and series in our systematic review allowed for further investigation into rarer perioperative problems that could happen in machine-perfused livers, of which none were identified. Notably, many of these cases involving extended criteria donors (advanced age, paediatric) or initially declined grafts that had successful outcomes, highlighting the great potential of machine perfusion in salvaging grafts previously deemed unsuitable fro transplant. Multimodal perfusion strategies, such as the DHOPE-COR-NMP approach applied by Van Leeuwen's team, could combine the benefits of both techniques while compensating for the shortcomings in each. More large-scale randomised controlled trials, especially in higher-risk grafts, are needed before this can be conclusively proven.

This study has several limitations. The groups being compared were homogenous in terms of donor graft type, patient characteristics, ischemia time and preservation duration. However, different institutions utilised different machine preservation strategies, and there was a lack of standardisation in perfusate composition, perfusion route and gas delivery. There was also no stratification between continuous and end-ischemic NMP, which could have introduced analytical bias. Furthermore, studies comparing NMP vs. SCS were highly heterogenous (I^2 >

50%), thus the pooled data may have been unreliable. Given the small number of studies, an assessment of publication bias using funnel plot analysis could not be performed. Majority of the studies included were small cohort studies, which had moderate-high risk of bias due to the inevitable effects of confounding. Only three RCTs were included, thus the overall level of clinical evidence was not high.

5. CONCLUSION

With the rising demand for life-saving organ transplantation comes the pressing need for more donors. Extended criteria donors represent an underutilised source of invaluable grafts, which were previously deemed unsuitable for transplantation. Ex vivo machine perfusion, at low or physiological temperatures, has the potential to minimise ischemia reperfusion injury and even repair such grafts, lowering the risk of postoperative complications. The findings of this study support the use of machine perfusion over static cold storage, as it offers a promising solution to the current organ shortage and could increase the safety of liver transplantation.

FUTURE WORK

A multicentre randomised controlled trial with a parallel three-arm design (HOPE vs NMP vs SCS) is currently being planned. This systematic review was conducted as part of the application process to evaluate the feasibility of such a study. The results of this review and future RCT will be able to advance our understanding of machine perfusion and determine if one technique is more advantageous than the other, consequently directing the course of liver transplantation.

TABLES

Table 1 Study and Patient Characteristics

Author (Year)	Study design	Study location	Level of Evidence	Group(s)	No. of patients	No. DBD or DCD grafts	Donor age (years)	Recipient age (years)	Recipient gender	Recipient MELD score
Guarrera et al. (2010)	Prospective cohort study	USA; 1 centre	III	HMP vs. SCS	H: 20 S: 20	H: 20 DBD S: 20 DBD	H: 39.4 ± 2.5 S: 45.6 ± 2.1	H: 55.4 ± 6.2 S: 52.7 ± 8.9	NA	H: 17.2 ± 7.4 S: 16.8 ± 6.8
Dutkowski et al. (2015)	Prospective cohort study	Europe; 3 centres	III	HMP vs. SCS	H: 25 S: 100	H: 25 DCD S: 50 DCD	H: 54 (36-63) S: 48 (33-51)	H: 60 (57-64) S: 56 (49-59)	H: 20M, 5F S: 35M, 15F	H: 13 (9-15) S: 16 (10-21)
Guarrera et al. (2015)	Prospective cohort study	USA; 1 centre	III	HMP vs. SCS	H: 31 S: 30	H: 31 ECD S: 30 ECD	H: 57.5 ± 17.8 S: 57.9 ± 16.9	H: 57.5 ± 8.0 S: 58.4 ± 9.6	NA	H: 19.5 ± 5.9 S: 21.4 ± 6.3
Mergental et al. (2016)	Case series	UK; 1 centre	IV	NMP	N: 5	1 DBD, 4 DCD	49 (range 29-51)	56 (range 46-66)	4M, 1F	8 (range 7-17)
Ravikumar et al. (2016)	Prospective cohort study	UK; 2 centres	III	NMP vs. SCS	N: 20 S: 40	N: 16 DBD, 4 DCD S: 32 DBD, 8 DCD	N: 58.0 (range 21-85) S: 58.5 (range 21-82)	N: 54.4 (range 33-66) S: 55 (range 27-65)	NA	N: 12 (range 7-27) S: 14 (range 6-25)
Bral et al. (2017)	Prospective cohort study	Canada; 1 centre	III	NMP vs. SCS	N: 10 S: 30	N: 6 DBD, 4 DCD S: 22 DBD, 8 DCD	N: 56 (range 14-71) S: 52 (range 20-77)	N: 53 (range 28-67) S: 59 (range 43-69)	NA	N: 13 (range 9-32) S: 19 (range 7-34)
van Rijn et al. (2017)	Prospective cohort study	The Netherlands, 1 centre	III	HMP vs. SCS	H: 10 S: 20	H: 10 DCD S: 20 DCD	H: 53 (47-57) S: 53 (47-58)	H: 57 (54-62) S: 52 (42-60)	H: 6M, 4F S: 11M, 9F	H: 16 (15-22) S: 22 (17-27)
He et al. (2018)	Case report	China; 1 centre	IV	IFLT (in-situ + ex-situ NMP)	N: 1	ECD-DBD (steatotic liver)	25	51	Male	NA
Nasralla et al. (2018)	Randomised controlled trial	Europe; 7 centres	II	NMP vs. SCS	N: 121 S: 101	N: 87 DBD, 34 DCD S: 80 DBD, 21 DCD	N: 56 (45-67) S: 56 (47-66)	N: 55 (48-62) S: 55 (48-62)	N: 86M, 35F S: 74M, 27F	N: 13 (10-18) S: 14 (9-18)

Pavel et al. (2018)	Case report	Spain; 1 centre	IV	IFLT (NRP + NMP)	N: 1	DCD	43	43	Male	28
Watson et al. (2018)	Prospective cohort study	UK; 1 centre	III	NMP	N: 22	N: 6 DBD, 16 DCD	57 (range 24-71)	NA	NA	NA
Zhao et al. (2018)	Case report	China; 1 centre	IV	IFLT (in-situ + ex-situ NMP)	N: 2	N: 2 DBD	43, 32	45, 56	2 males	17, 15
Bral et al. (2019)	Prospective cohort study	Canada; 1 centre	III	Continuous NMP vs. Post-SCS NMP	Continuous: 17 Post-SCS: 26	Continuous: 13 DBD, 4 DCD Post-SCS: 20 DBD, 6 DCD	Continuous: 40 (range 14-71) Post-SCS: 37 (range 15-67)	Continuous: 59 (50-63) Post-SCS: 57 (40-63)	NA	Continuous: 25 (21-32) Post-SCS: 22 (17-24)
Ceresa et al. (2019)	Prospective cohort study	UK; 3 centres	III	Continuous NMP vs. Post-SCS NMP	Continuous: 104 Post-SCS: 31	Continuous: 73 DBD, 31 DCD Post-SCS: 23 DBD, 8 DCD	Continuous: 55 (range 17-83) Post-SCS: 58 (range 17-78)	Continuous: 56 (range 20-73) Post-SCS: 58 (range 25-73)	Continuous: 74M, 30F Post-SCS: 22M, 9F	Continuous: 13 (range 6-33) Post-SCS: 14 (range 7-24)
Dondossola et al. (2019)	Case series	Italy; 1 centre	IV	HMP	H: 6	4 DCD, 2 DBD	H: 52 (range 36-72)	H: 59 (range 47-66)	NA	H: 17 (range 12-35)
Ghinolfi et al. (2019)	Randomised controlled trial	Italy; 1 centre	II	NMP vs. SCS	N: 10 S: 10	N: 10 DBD S: 10 DBD	N: 81 (77.5-87.2) S: 80 (72-87.2)	N: 57 (46-61) S: 55 (43-61)	N: 9M, 1F S: 8M, 2F	N: 12.5 (9-16) S: 9.5 (8-15)
Manzia et al. (2019)	Case report	Italy; 1 centre	IV	NMP	N: 1	DBD (steatotic, nonagenarian liver)	88	53	Male	7
Patrono et al. (2019)	Prospective cohort study	Italy; 1 centre	III	HMP vs. SCS	H: 25 S: 50	H: 25 DBD S: 50 DBD	H: 74.3 ± 10.9 S: 74.9 ± 10.3	H: 56.3 ± 9.0 S: 55.9 ± 7.4	H: 15M, 10F S: 37M, 13F	H: 15.3 ± 8.6 S: 15.5 ± 8.5
Rayar et al. (2019)	Case report	France; 1 centre	IV	HMP	H: 2	DBD (octogenarian livers)	80, 83	65, 64	Males	11, 10
Schlegel et al. (2019)	Prospective cohort study	Switzerland; 2 centres	III	HMP vs. SCS	H: 50 S: 100	H: 50 DCD S: 50 DCD	H: 57 (47-67) S: 53 (33-60)	H: 58 (56-62) S: 57 (51-61)	NA	H: 11 (8-14) S: 11.8 (8.5-15.8)

van Leeuwen et al. (2019)	Prospective cohort study	The Netherlands; 1 centre	III	DHOPE-COR-NMP vs. SCS	DHOPE-COR-NMP: 11 S: 60	DHOPE-COR-NMP: 11DCD S: 24 DCD	DHOPE-COR-NMP: 63 (52-72) S: 52 (48-56)	DHOPE-COR-NMP: 61 (55-66) S: 56 (48-61)	DHOPE-COR-NMP: 7M, 4F S: 13M, 11F	DHOPE-COR-NMP: 14 (13-15) S: 18 (11-24)
Werner et al. (2019)	Case report	The Netherlands; 1 centre	IV	HMP	H: 1	DCD	13	16	Female	NR
Bogensperger et al. (2020)	Case report	Austria; 1 centre	IV	NMP	N: 1	DBD	66	29	Female	20
Javanbakht et al. (2020)	Cost-utility analysis	UK	NA	NMP vs. SCS	NA	NA	NA	NA	NA	NA
Liu et al. (2020)	Prospective cohort study	USA; 1 centre	III	NMP vs. SCS	N: 21 S: 84	N: 13 DBD, 8 DCD S: 52 DBD, 32 DCD	N: 35.0 ± 12.7 S: 34.8 ± 15.0	N: 57.0 ± 7.1 S: 57.4 ± 8.4	NA	N: 19.1 ± 7.7 S: 19.4 ± 8.7
Mergental et al. (2020)	Prospective cohort study	UK; 1 centre	III	NMP vs. SCS	N: 22 S: 44	N: 12 DBD, 10 DCD S: 24 DBD, 20 DCD	N: 56 (45-65) S: NA	N: 56 (46-65) S: NA	N: 14M, 8F S: 28M, 16F	N: 12 (9-16) S: NA
Muller et al. (2020)	Retrospective cohort study	International; 7 centres	III	HMP vs. NRP	H: 93 NRP: 132	H: 93 DCD NRP: 132 DCD	H: 61 (52-71) NRP: 50 (39-59)	H: 59 (54-63.6) NRP: 59.5 (54.5-63)	NA	H: 12 (9-16) NRP: 12 (8-16)
Ravaioli et al. (2020)	Prospective cohort study	Italy; 1 centre	III	HMP vs. SCS	H: 10 S: 30	H: 10 ECD-DBD S: 30 ECD-DBD	H: 77.5 (range 60-84) S: 75.5 (range 53-85)	H: 57.5 (range 50-68) S: 60.5 (range 48-68)	NA	H: 13 (range 7-16) S: 13.5 (range 7-20)
Rayar et al. (2020)	Prospective cohort study	France; 1 centre	III	HMP vs. SCS	H: 25 S: 69	H: 25 ECD-DBD S: 69 ECD-DBD	H: 70 (range 45-87) S: 72 (range 25-88)	H: 63 (range 43-69) S: 62 (range 36-70)	H: 20M, 5F S: 57M, 12F	H: 18.3 (range 7-37) S: 18.3 (range 5-40)
Reiling et al. (2020)	Prospective cohort study	Australia; 1 centre	III	NMP	N: 10	N: 5 DBD, 5 DCD (all ECD)	N: 33 (range 18-53)	N: 60 (54-62) (range 51-65)	NA	N: 16.5 (range 9-31)

Webb et al. (2020)	Cost-effectiveness analysis	Canada	NA	NMP vs. SCS	NA	NA	NA	NA	NA	NA
Zhang, Ju et al. (2020)	Case series	China; 1 centre	IV	NMP	N: 4	N: 1 DBD, 3 DCD (all ECD)	N: 21 (range 19-26)	N: 57 (range 31-63)	N: 3M, 1F	N: 26.5 (range 9-40)
Zhang, Tang et al. (2020)	Prospective cohort study	China; 1 centre	III	IFLT (in-situ + ex-situ NMP)	N: 28	N: 28 DBD	N: 37.2 ± 14.8	N: 50.3 ± 11.5	N: 26M, 2F	N: 15.6 ± 6.7
van Rijn et al. (2021)	Randomised controlled trial	Europe; 6 centres	II	HMP vs. SCS	H: 78 S: 78	H: 78 DCD S: 78 DCD	H: 52 (43-57) S: 49 (37-59)	H: 60 (52-65) S: 60 (52-65)	H: 55M, 23F S: 52M, 26F	H: 14 (10-19) S: 16 (10-22)

Data is presented as mean ± SD or median (interquartile range) unless otherwise indicated.
COR = controlled oxygenated rewarming; DBD = donation after brain death; DCD = donation after circulatory death; ECD = extended criteria donor; IFLT = ischaemic-free liver transplantation; HMP (H) = hypothermic machine perfusion; NMP (N) = normothermic machine perfusion; NRP = normothermic regional perfusion; SCS (S) = static cold storage

Table 1 Summary of the machine perfusion strategies utilised in each study

Study	Perfusion technique	Perfusion parameters (Device; route; perfusate; temperature; oxygen)	CIT (h)	Perfusion duration (h)	fWIT (min)	Total preservation time (h)
Guarrera et al. (2010)	Post-SCS HMP	Modified Medtronic PBS®; PV + HA perfusion; Vasosol; 4-8ºC; no active oxygenation	H: 9.4 ± 2.1 S: 8.9 ± 2.8	4.3 ± 0.9	H: 44.3 ± 6.5 S: 45.1 ± 6.7	NA
Dutkowski et al. (2015)	Post-SCS HOPE	Liver Assist; PV perfusion only; UW gluconate solution (KPS-1); 10ºC; pO_2 80-100kPa	H: 3.1 (2.4-4.4) S: 6.6 (5.8-7.5)	H: 2.0 (1.7-2.5)	H: 31 (26-36) S: 23 (20-29)	H: 5.3 (4.7-6.5) S: 6.6 (5.8-7.5)
Guarrera et al. (2015)	Post-SCS HMP	Modified Medtronic PBS®; PV + HA perfusion; Vasosol ; 4-8ºC; no active oxygenation	H: 9.3 ± 1.6 S: 8.6 ± 2.4	3.8 ± 0.9	H: 45.6 ± 7.3 S: 40.0 ± 8.3	NA
Mergental et al. (2016)	Post-SCS NMP	Liver Assist or OrganOx Metra; PV + HA perfusion; 3 units packed RBCs + 1L 5% albumin solution; 37ºC	7.0 (range 6.5-7.9)	5.8 (range 5.1-9.4)	33.5 (range 19-109)	13.3 (range 12.1-15.9)
Ravikumar et al. (2016)	Continuous NMP	OrganOx Metra; PV + HA perfusion; 3 units packed RBCs + 1 unit Gelofusine; 37ºC; PaO_2 ~12kPa	N: NA S: 8.9 (range 4.2-11.4)	N: 9.3 (range 3.5-18.5)	N: 21 (range 14-31) S: 15 (range 9-23)	N: 9.3 (range 3.5-18.5) S: 8.9 (range 4.2-11.4)
Bral et al. (2017)	Post-SCS NMP	OrganOx Metra; PV + HA perfusion; 3 units packed RBCs + 0.5L Gelofusine	N: 2.8 (range 1.6-4.9) S: 3.9 (range 1.1-14.8)	N: 11.5 (range 3.3-22.5)	NA	N: 13.1 (range 5.1-27.2) S: 3.9 (range 1.1-14.8)
van Rijn et al. (2017)	Post-SCS HOPE	Liver Assist; PV + HA perfusion; 4L Belzer UW MPS; 10ºC; 500ml/min 100% O_2 flow (PaO_2 >450mmHg)	NA	H: 2.1 (2.1-2.3)	H: 15 (13-17)* S: 16 (14-18)*	H: 8.7 (7.8-9.9) S: 8.4 (7.9-8.8)
He et al. (2018)	In-situ + ex-situ NMP (IFLT)	Liver Assist; PV + HA perfusion; 1.3L leucocyte-depleted washed RBCs + 1.3L succinylated gelatinor	NA	N: 4.5	NA	NA
Nasralla et al. (2018)	Continuous NMP	OrganOx Metra; PV + HA perfusion; 3 units packed RBCs + 0.5L Gelofusine; 37ºC; physiological PaO_2	N: 2.1 (1.8-2.4) S: NA	N: 9.1 (6.2-11.8)	N: 21 (17-25) S: 16 (10-20)	N: 11.9 (9.0-14.6) S: 7.8 (6.3-9.6)
Pavel et al. (2018)	NRP + NMP	OrganOx Metra; PV + HA perfusion; 3 units packed RBCs + 0.5L colloid	NA	NRP: 3.5 NMP: 12.7	157	NA
Watson et al. (2018)	Post-SCS NMP	Liver Assist; PV + HA perfusion; 1L leucocyte-depleted RBCs + 1L Gelofusine or Steen solution; 35-37ºC; >95% oxygen delivery or air with O_2 supplementation	6.4 (range 3.7-14.6)	NA	12 (range 5-30)	NA
Zhao et al. (2018)	In-situ + ex-situ NMP (IFLT)	Liver Assist; PV + HA perfusion; RBC-based perfusate; 37ºC; active oxygenation with 30% O_2	NA	2	NA	NA

Bral et al. (2019)	Post-SCS NMP vs. Continuous NMP	OrganOx Metra; PV + HA perfusion; 3 units packed RBCs + 0.5L Gelofusine; 37ºC; physiological PaO_2	Continuous: 3.2 (range 1-5.4) Post-SCS: 6.0 (range 3.9-8.4)	Continuous: 10.3 (range 3.3-22.4) Post-SCS: 7.8 (range 4-16.8)	Continuous: 21 (range 18-25) Post-SCS: 20 (range 14-42)	Continuous: 13.3 (range 6.1-27.3) Post-SCS: 14.3 (range 10.5-24.4)
Ceresa et al. (2019)	Post-SCS NMP vs. Continuous NMP	OrganOx Metra; PV + HA perfusion; 3 units packed RBCs + 0.5L Gelofusine; 37ºC; physiological PaO_2	Continuous: NA Post-SCS: 6.0 ± 1.3	Continuous: 12.1±4.2 Post-SCS: 8.4±4.1	Continuous: 20 (range 10-35) Post-SCS: 16 (range 12-28)	Continuous: 12.1 ± 4.2 Post-SCS: 14.2 ± 4.8
Dondossola et al. (2019)	Post-SCS HOPE	Liver Assist; PV + HA perfusion; 3/4L Belzer UW MPS; <10ºC;0.25L/min 100% O_2 flow	H: 9.5 (6.8-13.5)	H: 4.0 (range 3.0-5.3)	39.5 (range 25-37)	NA
Ghinolfi et al. (2019)	Post-SCS NMP	Liver Assist; PV + HA perfusion; blood-based perfusate; 37ºC; 4L/min 30% O_2 flow (PaO_2 200-250mmHg)	N: 4.7 (4.0-5.0) S: 6.6 (6.1-7.8)	N: 4.2 (3.3-4.7)	N: 74 (70-82) S: 69 (62-78)	NA
Manzia et al. (2019)	Continuous NMP	OrganOx Metra; PV + HA perfusion	NA	N: 8.0	NA	NA
Patrono et al. (2019)	Post-SCS HOPE	Liver Assist; PV + HA perfusion (except for 2 cases PV perfusion only); 3L Belzer UW MPS; 10ºC; PaO_2 600mmHg	H: 5.2 ± 0.9 S: 6.5 ± 1.2	H: 3.1 ± 0.8	H: 23 ± 7 S: 24 ± 5	H: 8.3 ± 1.0 S: 6.5 ± 1.2
Rayar et al. (2019)	Post- SCS HOPE	Liver Assist; PV perfusion only	H: 9.2; 5.3	H: 1.6; 1.8	NA	H: 10.7; 7.1
Schlegel et al. (2019)	Post-SCS HOPE	Liver Assist; PV perfusion only; 3L Belzer UW MPS; 10-12ºC; PaO_2 80-100kPa	H: 4.4 (3.5-5.2) S: 4.7 (4.3-5.3)	2 (1.6-2.4)	H: 31 (27-36) S: 17 (15-19)	H: 6.0 (5.0-7.0) S: 4.7 (4.3-5.3)
van Leeuwen et al. (2019)	Post-SCS DHOPE-COR-NMP	Liver Assist; PV + HA perfusion; HBOC-201 perfusion solution; 8-12ºC (1hr DHOPE), 37ºC (NMP); 1L/min 100% O_2 flow (PaO_2 >80kPa)	DHOPE-COR-NMP: 4.5 (4.0-4.9) S: 7.4 (6.3-8.2)	NA	DHOPE-COR-NMP: 16 (14-16) S: 16 (14-20)	DHOPE-COR-NMP: 14.5 (13.4-15.4) S: 7.4 (6.3-8.2)
Werner et al. (2019)	Post-SCS HOPE	Liver Assist; PV + HA perfusion; 4L Belzer UW MPS; 10ºC; 1L/min 100% O_2 flow (PaO_2 >70mmHg)	6.4	2.1	34	NA
Bogensperger et al. (2020)	Post-SCS NMP	Not reported	5.6	10.6	NA	16.2
Javanbakht et al. (2020)	NMP	OrganOx Metra	NA	NA	NA	NA
Liu et al. (2020)	Post-SCS or continuous NMP	Non-commercial, institutional perfusion device; PV + HA perfusion; 4 units FFP + 4units PRBCs + 200mL 25% albumin; 36ºC; active oxygenation	N: 3.4 (range 1.5-5.0) S: NA	N: 4.9 (range 3.4-7.9) S: NA	N: 21 ± 5 S: NA	N: 8.8 ± 1.1 S: 8.3 ± 1.5

Mergental et al. (2020)	Post-SCS NMP	OrganOx Metra; PV + HA perfusion; Belzer UW MPS; active oxygenation	N: 7.5 (5.3-10) S: NA	N: 9.8 (7.5-11.8) S: NA	N: 22.5 (19.0-35.0) S: NA	N: 17.9 (16.3-21.8) S: NA
Muller et al. (2020)	Post-SCS HOPE	Liver Assist; PV perfusion only; 3L Belzer UW MPS; 4ºC; 150-300mL/min 100% O_2 flow	H: 4 (3.1-5) NRP: 5.7 (4.7-6.6)	H: 2.2 (1.8-2.8) NRP: 3.1 (2.7-3.5)	H: 31 (26-35) NRP: 22 (19-26)	H: 6.4 (5.6-7.4) NRP: 5.7 (4.7-6.6)
Ravaioli et al. (2020)	Post-SCS HOPE	Non-commercial, institutional perfusion device; PV perfusion only; Belzer UW MPS; 4ºC; PaO_2 600-750mmHg	H: 7.1 (range 6.1-9.6) S: 7 (range 5.4-10)	2.2 (range 1-3.5)	NA	NA
Rayar et al. (2020)	Post-SCS HOPE	Liver Assist; PV perfusion only; 2L Belzer UW MPS; 11ºC; 1L/min 100% O_2 flow	H: 8.8 (range 6.3-13.7) S: 9.3 (range 3.5-12.0)	2.0 (range 1.3-4.2)	NA	NA
Reiling et al. (2020)	Post-SCS NMP	OrganOx Metra; PV + HA perfusion; 0.5L Gelofusine + 3units O- packed red cells; 37ºC	5.1 (range 3.0 – 7.1)	12.3 (8.8-14.6)	17 (14-26)	NA
Webb et al. (2020)	NMP	OrganOx Metra	NA	NA	NA	NA
Zhang, Ju et al. (2020)	Post-SCS NMP	Liver Assist; PV + HA perfusion; 6 units leucocyte-depleted red cells + 0.6L succinylated gelatin; 37ºC; PaO_2 200mmHg	8.0 (range 5.6-10.6)	5.2 (range 4.3-6.3)	8 (range 0-22)*	NA
Zhang, Tang et al. (2020)	In-situ + ex-situ NMP (IFLT)	Liver Assist; PV + HA perfusion; 1.2L leukocyte-depleted red cells + 1.2L succinylated gelatin; 36-37ºC; 300-500ml/min 100% O_2 flow (PaO_2 200mmHg)	0	3.7 (range 1.5-9.5)	NA	3.7 (range 1.5-9.5)
van Rijn et al. (2021)	Post-SCS HOPE	Liver Assist; PV + HA perfusion; 4L Belzer UW MPS; 10ºC; 500ml/ min 100% O_2 flow	H: 6.2 (5.3-6.9) S: 6.8 (5.9-8.0)	2.2 (2-2.6)	H: 11 (8-13)* S: 11 (8-15)*	H: 8.7 (7.8-9.3) S: 6.8 (5.9-8.0)

Data is presented as mean ± SD or median (interquartile range) unless otherwise indicated.

*Primary or asystolic warm ischaemic time (duration from cardiac arrest to administration of cold perfusion fluid)

CIT = cold ischaemic time; COR = controlled oxygenated rewarming; fWIT = functional (or true) warm ischaemic time; HA = hepatic artery; HMP = hypothermic machine perfusion; HOPE = hypothermic oxygenated machine perfusion; IFLT = ischaemia-free liver transplantation; NMP = normothermic machine perfusion; NRP = normothermic regional perfusion; PV = portal vein; SCS = static cold storage

Table 3 Main outcomes of studies included in meta-analysis

Study	Group	N	EAD (%)	PNF (%)	PRS (%)	ACR (%)	HAS (%)	HAT (%)	Major complications (%)	Total Biliary complications (%)	Anastomotic strictures (%)	Bile leaks (%)	IC (%)	NAS (%)	Retransplant (%)	Graft loss (%)	1-year graft survival (%)	1-year patient survival (%)
Guarrera et al. (2010)	SCS	20	5 (25.0)	0 (0)	NA	NA	1 (5.0)	NA	NA	4 (20.0)	3 (15.0)	1 (5.0)	NA	NA	NA	NA	NA	18 (90.0)
	HMP	20	1 (5.0)	0 (0)	NA	NA	0 (0)	NA	NA	2 (10.0)	1 (5.0)	1 (5.0)	NA	NA	NA	NA	NA	18 (90.0)
Dutkowski et al. (2015)	SCS	50	22 (44.0)	3 (6.0)	NA	8 (16.0)	NA	3 (6.0)	NA	23 (46.0)	NA	NA	11 (22.0)	NA	9 (18.0)	15 (30.0)	35 (70.0)	NA
	HMP	25	5 (20.0)	0 (0)	NA	3 (12.0)	NA	1 (4.0)	NA	5 (20.0)	NA	NA	0 (0)	NA	0 (0)	2 (8.0)	23 (92.0)	NA
Guarrera et al. (2015)	SCS	30	9 (30.0)	2 (6.0)	NA	NA	NA	2 (6.0)	NA	13 (43.0)	NA	3 (10.0)	NA	NA	NA	NA	24 (80.0)	24 (80.0)
	HMP	31	6 (19.4)	1 (3.2)	NA	NA	NA	1 (3.2)	NA	4 (12.9)	NA	1 (3.2)	NA	NA	NA	NA	25 (80.6)	26 (83.9)
Ravikumar et al. (2016)	SCS	40	9 (22.5)	0 (0)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1 (2.5)	NA	NA
	NMP	20	3 (15.0)	0 (0)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0 (0)	NA	NA
Bral et al. (2017)	SCS	27	8 (29.6)	0 (0)	NA	NA	NA	0 (0)	10 (37.0)	4 (14.8)	NA	NA	NA	NA	NA	NA	NA	NA
	NMP	9	5 (55.5)	0 (0)	NA	NA	NA	0 (0)	2 (20.0)	0 (0)	NA	NA	NA	NA	NA	NA	NA	NA
van Rijn et al. (2017)	SCS	20	2 (10.0)	0 (0)	NA	NA	NA	2 (10.0)	NA	15 (75.0)	3 (15.0)	NA	9 (45.0)	7 (35.0)	5 (25.0)	6 (30.0)	13 (65.0)	17 (85.0)
	HMP	10	0 (0)	0 (0)	NA	NA	NA	0 (0)	NA	6 (60.0)	2 (20.0)	NA	1 (10.0)	1 (10.0)	0 (0)	0 (0)	10 (100)	10 (100)
Nasralla et al. (2018)	SCS	101	29 (28.7)	0 (0)	32 (31.7)	13 (12.9)	3 (3.0)	4 (4.0)	36 (35.6)	NA	34 (33.7)	NA	1 (1.0)	8 (7.9)	NA	4 (4.0)	97 (96.0)	98 (97.0)
	NMP	121	12 (9.9)	1 (0.8)	15 (12.4)	12 (9.9)	5 (4.1)	2 (1.7)	21 (17.4)	NA	35 (28.9)	NA	1 (0.8)	7 (5.8)	NA	6 (5.0)	115 (95.0)	116 (95.9)
Watson et al. (2018)	NMP	22	1 (4.5)	1 (4.5)	5 (22.7)	NA	NA	NA	NA	NA	NA	NA	4 (18.0)	NA	NA	NA	NA	NA

Bral et al. (2019)	NMP*	26	5 (19.2)	0 (0)	NA	NA	5 (19.2)	NA	NA	4 (15.4)	2 (7.7)	NA	0 (0)	2 (7.7)	1 (3.8)	NA	NA	NA
Ceresa et al. (2019)	NMP*	31	4 (12.9)	NA	3 (9.7)	NA	NA	2 (6.5)	7 (22.6)	NA	NA	NA	NA	NA	2 (6.5)	2 (6.5)	26 (83.9)	NA
Ghinolfi et al. (2019)	SCS	10	1 (10.0)	0 (0)	1 (10.0)	NA	NA	0 (0)	NA	0 (0)	NA	NA	NA	NA	0 (0)	0 (0)	10 (100)	9 (90.0)
	NMP	10	2 (20.0)	0 (0)	3 (30.0)	NA	NA	1 (10.0)	NA	1 (10.0)	NA	NA	NA	NA	1 (10.0)	1 (10.0)	9 (90.0)	10 (100)
Patrono et al. (2019)	SCS	50	17 (34.0)	0 (0)	10 (20.0)	6 (12.0)	NA	NA	NA	9 (18.0)	6 (12.0)	NA	4 (8.0)	NA	NA	NA	NA	NA
	HMP	25	8 (32.0)	0 (0)	1 (4.0)	4 (16.0)	NA	NA	NA	6 (24.0)	4 (16.0)	NA	2 (8.0)	NA	NA	NA	NA	NA
Schlegel et al. (2019)	SCS	50	NA	2 (4.0)	NA	14 (28.0)	NA	6 (12.0)	NA	23 (46.0)	9 (18.0)	1 (2.0)	5 (10.0)	11 (22.0)	NA	18 (36.0)	NA	NA
	HMP	50	NA	0 (0)	NA	2 (4.0)	NA	2 (4.0)	NA	20 (40.0)	12 (24.0)	1 (2.0)	0 (0)	4 (8.0)	NA	7 (14.0)	NA	NA
Liu et al. (2020)	SCS	84	39 (46.4)	0 (0)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	NMP	21	4 (19.0)	0 (0)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Mergental et al. (2020)	SCS	44	4 (9.0)	1 (2.3)	NA	NA	NA	NA	17 (38.6)	4 (9.0)	3 (6.8)	NA	NA	1 (2.3)	NA	NA	38 (86.3)	42 (95.4)
	NMP	22	7 (31.8)	0 (0)	10 (45.0)	NA	NA	NA	7 (31.8)	6 (27.0)	2 (9.0)	NA	NA	4 (18.0)	NA	NA	19 (86.3)	22 (100)
Muller et al. (2020)	HMP	93	63 (67.7)	4 (4.3)	NA	NA	5 (5.4)	2 (2.2)	NA	32 (34.4)	24 (25.8)	6 (6.5)	NA	8 (8.6)	NA	24 (25.8)	80 (86.0)	86 (92.5)
Ravaioli et al. (2020)	SCS	30	7 (23.3)	2 (6.0)	NA	4	NA	NA	7	3	NA	NA	NA	NA	NA	NA	27 (90.0)	27 (90.0)
	HMP	10	0 (0)	0 (0)	NA	1 (10.0)	NA	NA	1 (10.0)	1 (10.0)	NA	NA	NA	NA	NA	NA	10 (100)	10 (100)
Rayar et al. (2020)	SCS	69	29 (42.0)	2 (2.9)	0 (0)	NA	NA	NA	31 (44.9)	8 (11.6)	3 (4.3)	4 (5.8)	NA	0 (0)	2 (2.9)	NA	62 (89.9)	63 (91.3)
	HMP	25	7 (28.0)	2 (8.0)	13 (52.0)	NA	NA	NA	6 (24.0)	2 (8.0)	1 (4.0)	1 (4.0)	NA	0 (0)	2 (8.0)	NA	22 (88.0)	23 (92.0)
Reiling et al. (2020)	NMP	10	5 (50.0)	NA	NA	0 (0)	NA	NA	2 (20.0)	2 (20.0)	1 (10.0)	1 (10.0)	0 (0)	0 (0)	0 (0)	0 (0)	10 (100)	10 (100)

van Rijn et al. (2021)	SCS	78	31 (39.7)	1 (1.3)	19 (24.4)	16 (20.5)	NA	2 (2.6)	NA	44 (56.4)	22 (28.2)	8 (10.3)	NA	14 (17.9)	6 (7.7)	NA	NA	NA
	HMP	78	20 (25.6)	0 (0)	9 (11.5)	9 (11.5)	NA	2 (2.6)	NA	34 (43.6)	23 (29.5)	6 (7.7)	NA	5 (6.4)	3 (3.8)	NA	NA	NA

* Only data for post-SCS NMP was used for single-group meta-analysis in studies comparing continuous with post-SCS NMP.

ACR = acute cellular rejection; EAD = early allograft dysfunction; HAS = hepatic artery stenosis; HAT = hepatic artery thrombosis; HMP = hypothermic machine perfusion; IC = ischaemic cholangiopathy; NA = not applicable; NAS = non-anastomotic strictures; NMP = normothermic machine perfusion; PNF = primary non-function; PRS = post reperfusion syndrome; SCS = static cold storage

REFERENCES

APPENDIX