





# Early outcome of machine perfusion vs static cold storage of liver graft: A systemic review and meta-analysis of randomized controlled trials

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## Abstract

The use of marginal grafts is very challenging and is associated with post-reperfusion syndrome and early allograft dysfunction. The outcomes of machine perfusion for the preservation of marginal grafts have been compared with that of static cold storage, with studies reporting a reduced risk of ischemic cholangiopathy and graft loss. We performed this systematic review and meta-analysis of randomized controlled trials (RCTs) comparing outcomes of machine perfusion of liver grafts to static cold storage (SCS) of liver grafts during liver transplantation. Two independent researchers thoroughly searched for literature in the following databases: PubMed (Medline), Cochrane Central Register of Controlled Studies (CENTRAL), clinical trial registry, ResearchGate, Google Scholar, and Scopus (ELSEVIER) databases (last search: November 2023). The search terms used were: “dynamic perfusion,” “normothermic perfusion,” “hypothermic perfusion,” “liver transplantation,” “static cold storage,” “NMP,” “HOPE,” “extended criteria grafts,” “marginal grafts,” “RCTs,” “randomized controlled trials,” “warm ischemia,” and “cold ischemia.” Eight RCTs published between 2019 and 2023 were included in the data synthesis and meta-analysis. The primary outcome considered was the overall incidence of early allograft dysfunction (EAD) between the two methods of graft perfusion after liver transplantation. The secondary outcome considered was the rate of retransplantation. Our meta-analysis revealed that SCS is associated with more EAD when compared with machine perfusion, with a p-value of <0.00001. We also found that the rate of retransplantation is higher among patients who received a liver preserved by SCS, with a p-value of 0.02. The use of machine perfusion in the preservation of liver grafts showed a significant reduction in early allograft dysfunction and retransplantation.

**Keywords:** Early allograft dysfunction; liver transplantation; machine perfusion; static cold storage.

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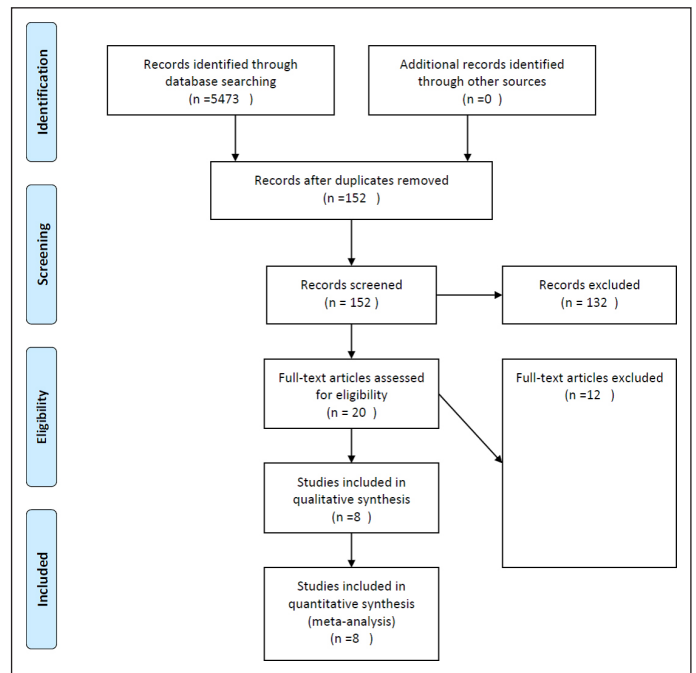
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## Introduction

Since liver transplantation became an acceptable therapeutic option for selected liver diseases, there has been a marked increase in the demand for liver grafts, resulting in a lack of adequate ideal grafts for liver transplantation. In an effort to expand the donor pool, transplant surgeons have increasingly used extended criteria grafts, domino liver grafts, and living donor grafts.<sup>[1–3]</sup> The use of extended criteria grafts is very challenging and is associated with more complications such as post-reperfusion syndrome, early allograft dysfunction (EAD), primary nonfunction (PNF), and vascular and biliary complications, among others. These complications may be associated with graft loss or even the mortality of patients.<sup>[4–7]</sup> One of the risk factors for post-transplant liver dysfunction is ischemic reperfusion injury. Liver transplantation is associated with two forms of liver ischemia, both inducing hepatocellular injury.<sup>[8–10]</sup> The first is cold ischemia, which occurs during the retrieval of the graft when the liver is cooled, perfused, and then stored in a cold preservation solution (static cold storage {SCS}). The second form of ischemia is warm ischemia, which is encountered during implantation, from the removal of the organ from ice until reperfusion, or the ischemia encountered during organ retrieval, from the time of cross-clamping (or of asystole in non-heart-beating donors) until cold perfusion is commenced.<sup>[11]</sup> The graft is metabolically inhibited during warm and cold ischemia and becomes more dysfunctional by reperfusion injury after revascularization and reoxygenation.<sup>[12,13]</sup> An ideal graft can tolerate a long period of cold ischemic time with minimal permanent sequelae. However, extended criteria grafts cannot tolerate prolonged periods of ischemia.<sup>[14]</sup> To reduce or eliminate these ischemic periods, especially in extended criteria grafts, dynamic preservation techniques using ex situ liver perfusion have been utilized.<sup>[15–17]</sup> There are two main types of ex situ liver perfusion that are clinically available. The first method is hypothermic oxygenated perfusion (HOPE), which utilizes a highly oxygenated (pO<sub>2</sub>>60 kPa) artificial solution at hypothermic temperatures (8–12°C). This method is routinely performed after the transport of the graft to the recipient center, so it is considered end-ischemic. The second method of ex situ perfusion is normothermic machine perfusion (NMP), which aims for a “near-physiological” environment. It utilizes a blood-based perfusate to perfuse the graft at 37 °C.<sup>[15–17]</sup> The outcomes of dynamic perfusion have been compared with that of static cold storage, with initial studies reporting a reduced risk of ischemic cholangiopathy and graft loss in patients who received machine perfusion when compared to static cold storage.<sup>[18]</sup> We perform this systematic review and meta-analysis of randomized controlled trials comparing the outcome of machine perfusion of liver grafts compared to SCS of liver grafts during liver transplantation.

**Methods**

This systematic review was performed in compliance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. We prospectively registered the protocol for this systematic review in the International Prospective Register of Systematic Reviews, PROSPERO (CRD42023481913). Search strategy Two independent researchers thoroughly searched for literature in the following databases: PubMed (Medline), Cochrane Central Register of Controlled Trials (CENTRAL), clinical trial registry, ResearchGate, Google Scholar, and Scopus (ELSEVIER) databases (last search: November 2023). The search terms used were: “dynamic perfusion,” “normothermic perfusion,” “hypothermic perfusion,” “liver transplantation,” “static cold storage,” “NMP,” “HOPE,” “extended criteria grafts,” “marginal grafts,” “RCTs,” “randomized controlled trials,” “warm ischemia,” and “cold ischemia.” The terms were combined using Boolean logic. Related articles and reference lists were searched to ensure the completeness of the search. Conflict was resolved by involving a third researcher. Eligibility criteria The inclusion criteria for a study to be included in the review are as follows: 1. Studies published from 1990 to date. 2. Randomized controlled trials that compared outcomes of liver transplantation in patients whose graft was preserved using SCS and those whose grafts were preserved using machine perfusion. 3. Studies with full texts. Exclusion criteria are as follows: 1. Conference presentations, editorials, and commentaries. 2. Studies in which the relevant data are absent. 3. Studies with a total sample size of fewer than 10. Quality assessment and risk of bias assessment The Jadad score, which was developed by Jadad et al.,<sup>[19]</sup> was used to assess the quality and bias of the included RCTs. The score ranges from 0-5. A score of 3 and above was considered a good quality study. Publication bias If 10 or more studies were included in the meta-analysis of a particular outcome, then publication bias was evaluated using a funnel plot. Data extraction Data extraction was performed by 2 independent researchers. The following information was extracted from each study: first author, year of manuscript publication, study design, number of patients in each group, gender of patients per group, mean age of patients in each group, type of organ preservation technique, and outcome data. In case of conflicts between the two researchers, a third researcher was involved to resolve the conflict. Outcome The primary outcome of interest is the incidence of PNF per group. The secondary outcome of interest includes the incidence of EAD per group. Other outcomes of interest include post-reperfusion syndrome, incidence of retransplantation, vascular complication, biliary complication, ICU stay, mortality, and graft survival at 1 year. Statistical



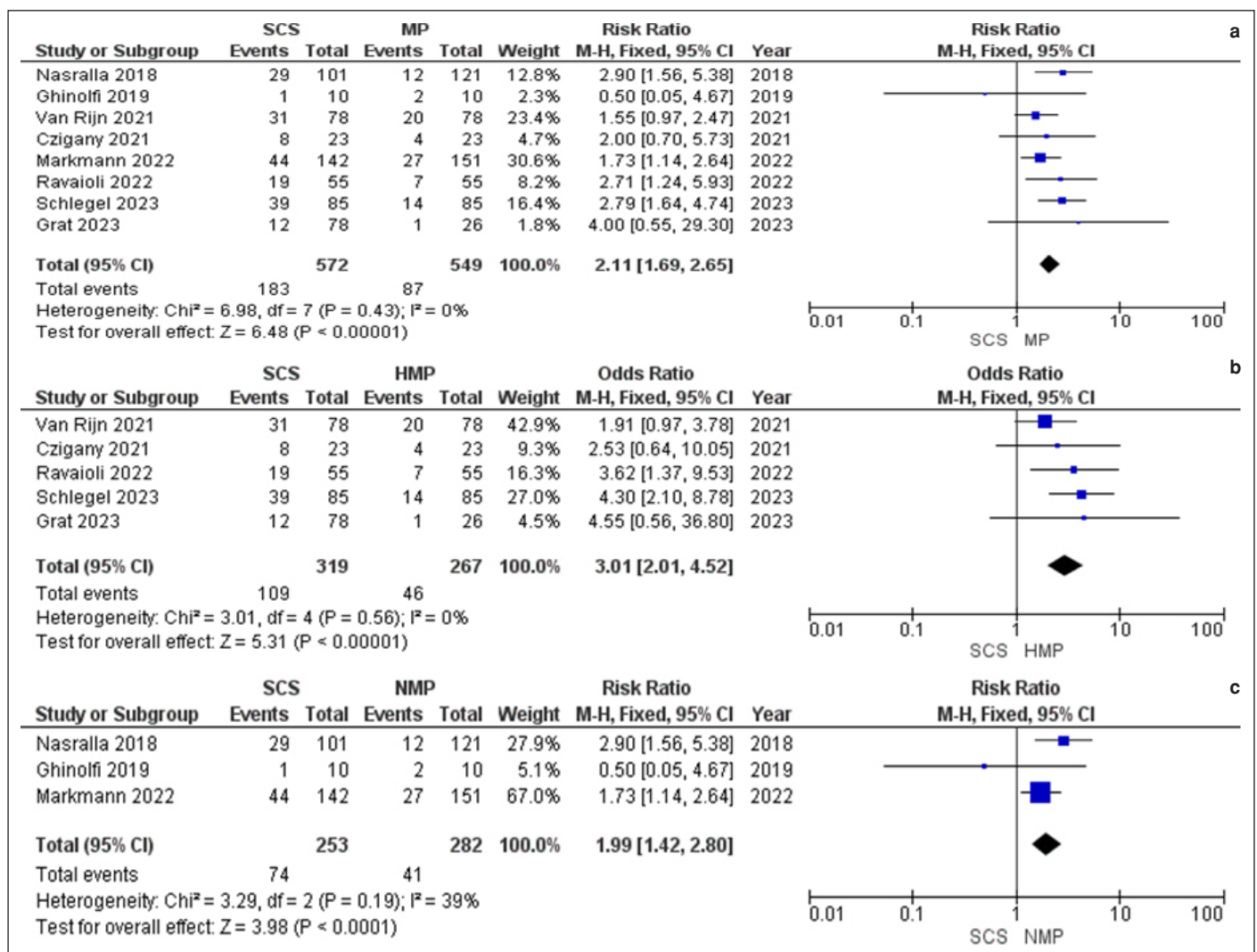
**Figure 1.** Study selection process.

analysis Statistical analyses were done using RevMan software (version 5.4.1). If the variable is dichotomous, the pooled risk ratio (RR) was calculated with a 95 percent confidence interval. However, if the variable is continuous, the weighted mean difference or standardized mean difference with a 95 percent CI was calculated from the mean and standard deviation reported from individual studies. If a study did not report the mean and standard deviation, the Wan et al.<sup>[20]</sup> method of extracting mean and standard deviation from the median and interquartile range was utilized. A fixed-effects model was used to calculate the pooled effect sizes if the data were not significantly heterogeneous. Otherwise, a random-effects model was used. Heterogeneity was assessed using the I<sup>2</sup> statistics. I<sup>2</sup>>50% was considered as statistically significant heterogeneity. Sensitivity analysis was done by sequential elimination of each of the included studies in the meta-analysis to identify the main source of heterogeneity. Publication bias was evaluated using the funnel plot and Egger’s test if 10 or more studies were included in the meta-analysis of a particular outcome, as recommended by the Cochrane handbook.

**Table 1.** Characteristics of included studies

S/N	Author	Year of publication	Sample size per group		Jadad score	Quality of the study
			SCS	MP		
1	Schlegel et al.	2023	85	85	4	Good quality
2	Van Rijn et al.	2021	78	78	4	Good quality
3	Ravaioli et al.	2022	55	55	4	Good quality
4	Ghinolfi et al.	2019	10	10	4	Good quality
5	Markman et al .	2022	142	151	3	Good quality
6	Czigany et al.	2021	23	23	4	Good quality
7	Grat et al.	2023	78	26	3	Good quality
8	Nasralla et al.	2019	101	121	3	Good quality

SCS: Static cold storage; MP: Machine perfusion. <http://www.wjnet.com/1948-5182/full/v8/i1/36.htm>



**Figure 2.** (a) Meta-analysis comparing early allograft dysfunction between MP and SCS. (b) Meta-analysis comparing early allograft dysfunction between HMP and SCS. (c) Meta-analysis comparing early allograft dysfunction between NMP and SCS.

## Results

Results were reported in accordance with the PRISMA checklist.

### Study Selection Process and Description of Selected Studies

We identified 5,473 references during the initial search. Out of these, 5,321 articles were excluded because of duplicate publications (Fig. 1). The 152 remaining references were further assessed in terms of titles and abstracts. One hundred and thirty-two references were excluded for lack of relevant data. Twenty full-text articles were retrieved, but 12 articles were excluded for lack of a control arm. Eight were included for data synthesis and meta-analysis.<sup>[21–28]</sup> The studies included were all randomized controlled trials (RCTs) published from 2019 to 2023. Five of the studies<sup>[21–23,27,28]</sup> compared SCS to HOPE, while three of the studies<sup>[24–26]</sup> compared SCS to NMP. Details of selected studies are displayed in Table 1.

### Primary Outcome

#### Early Allograft Dysfunction

The primary outcome compared was the overall incidence of EAD be-

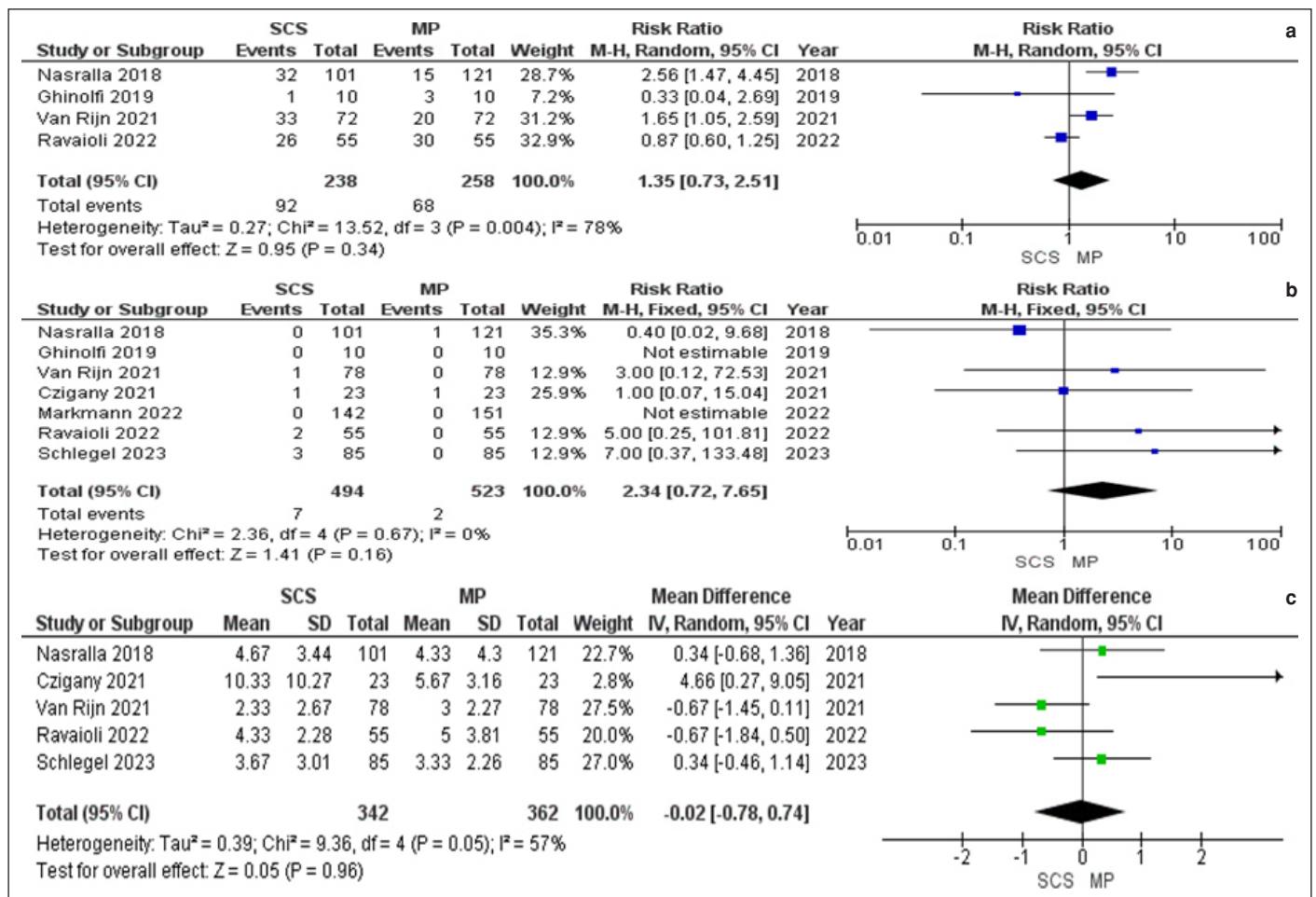
tween the two methods of graft perfusion after liver transplantation. All eight included studies<sup>[21–28]</sup> compared the incidence of EAD. In our meta-analysis, we found that patients who received a liver graft preserved with machine perfusion tend to have a lower incidence of EAD. The difference between the two groups was statistically significant with a RR of 6.48 and a  $p < 0.00001$ . There was no significant heterogeneity between the studies with  $I^2 = 0\%$  (Fig. 2a). Publication bias was assessed by visual inspection of the funnel plot and was found to be symmetrical, revealing no bias.

We also performed a subgroup analysis comparing the various methods of machine perfusion to SCS. We found that hypothermic machine perfusion is associated with less EAD when compared to SCS (Fig. 2b, RR=5.31,  $p < 0.0001$ ). A similar finding was also observed when NMP was compared to SCS (Fig. 2c, RR=3.98,  $p < 0.00001$ ).

### Secondary Outcomes

#### Post Reperfusion Syndrome

Four studies<sup>[22–25]</sup> comprising 496 patients compared post-reperfusion syndrome between the two groups of patients. Our pooled meta-analysis revealed that PRS occurred in 92 patients who received a



**Figure 3.** (a) Meta-analysis comparing post reperfusion syndrome between MP and SCS. (b) Meta-analysis comparing primary non-function between HMP and SCS. (c) Meta-analysis comparing ICU stay between NMP and SCS.

liver graft preserved with SCS as opposed to 68 patients whose liver grafts were preserved using machine perfusion. This difference was not statistically significant with an RR of 0.95 and a p-value of 0.34. There was significant heterogeneity among studies included in the meta-analysis with I<sup>2</sup>=78%, so the random effect model was used to estimate the pooled effect size. The detailed meta-analysis of post-reperfusion syndrome is displayed in Figure 3a.

**Primary Non-Function and Ischemic Cholangiopathy**

Seven of the studies<sup>[21–27]</sup> included compared primary non-function (PNF) between the two methods of liver graft preservation. The pooled sample size of the studies is 523 patients in the machine perfusion group and 494 patients in the SCS group. Pooled analysis revealed that 7 patients had PNF in the SCS group while only 2 patients had PNF in the machine perfusion group. However, this difference was not statistically significant with an RR of 1.41 and a p-value of 0.16. There was no significant heterogeneity between the included studies with an I<sup>2</sup>=0%. The detailed meta-analysis of PNF is displayed in Figure 3b.

Ischemic cholangiopathy was compared in only two studies<sup>[24,25]</sup> among included randomized controlled trials. Our pooled analysis showed that 3 out of 111 grafts preserved by SCS developed ischemic cholangiopathy as opposed to 2 out of 121 grafts preserved

with machine perfusion. The difference is not statistically significant with a RR of 0.56 and a p-value of 0.57.

**Duration of Stay in Intensive Care Unit**

Five studies<sup>[21–23,25,27]</sup> consisting of 702 patients compared the duration of stay in the intensive care unit (ICU) between machine perfusion and SCS of liver graft. Pooled analysis of these studies revealed that the duration of stay in the ICU is similar among the two groups of patients with a mean difference of 0.05 and a p-value of 0.96. There was significant heterogeneity among the studies included in the analysis with an I<sup>2</sup>=57%, so the random effect model was used in estimating the pooled effect size. The detailed meta-analysis of ICU stay is displayed in Figure 3c.

**Retransplantation**

Five studies<sup>[21–23,25,27]</sup> consisting of 702 patients compared the rate of retransplantation between machine perfusion and SCS of liver graft. Pooled analysis of these studies revealed that the rate of retransplantation is higher among patients who received a liver that was preserved by SCS. The difference was found to be statistically significant with an RR of 2.30 and a p-value of 0.02. There was no heterogeneity between the studies included in the analysis with an I<sup>2</sup>=0%. The detailed and graphical representation of this meta-analysis is displayed in Figure 4.

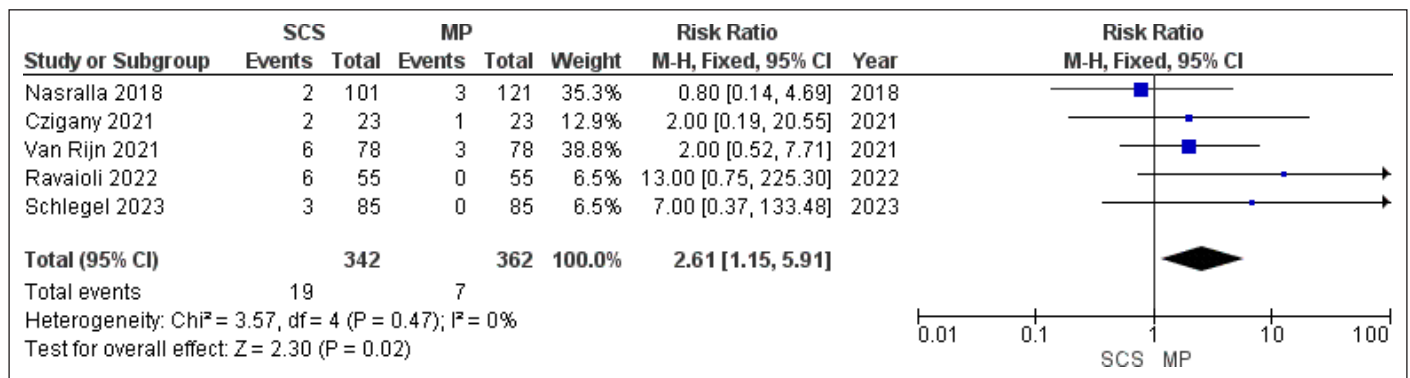


Figure 4. Meta-analysis comparing retransplantation between MP and SCS.

## Discussion

The increased demand for liver grafts has led to a scarcity of donors, resulting in the heightened use of extended criteria donors or marginal donors. The definition of a marginal or extended criteria donor varies, but the term is commonly used to describe grafts obtained from donors aged above 65 years, donors who have spent at least 7 days in the ICU, obese donors, donors with fatty livers, prolonged cold ischemia time of over 12 hours, and donors with elevated liver enzymes or hypernatremia at any time.<sup>[29,30]</sup> Reports suggest that extended criteria donors may constitute up to 50% of total liver donors in some European countries.<sup>[4,31,32]</sup> A persistent problem associated with the use of extended criteria donation is that the grafts are susceptible to ischemic reperfusion injury, resulting in an increased risk of EAD and primary non-function.<sup>[33–35]</sup> These complications predispose recipients to an increased risk of sepsis, graft loss, longer stays in the ICU, and longer hospital stays.<sup>[18]</sup>

One of the main aims of machine perfusion is to reduce ischemic reperfusion injury and, by extension, reduce the risk of EAD and its complications.<sup>[18,36]</sup> Excellent results have been reported with the use of machine perfusion in the preservation of marginal kidney grafts. Initial reports on the use of machine perfusion for graft preservation have also been encouraging, with reports of reduced risk of ischemic cholangiopathy and graft loss in patients who received machine perfusion compared to static cold storage.<sup>[18,36]</sup> In this meta-analysis, we compared early outcomes among patients whose grafts were preserved by SCS and machine perfusion.

Our meta-analysis revealed that machine preservation of liver grafts is associated with a reduced risk of EAD compared to SCS. We also performed a subgroup analysis and found that both hypothermic machine perfusion and NMP are associated with a reduced risk of EAD, similar to previous meta-analyses by Jia et al.,<sup>[18]</sup> Yang et al.,<sup>[36]</sup> and Parente et al.<sup>[37]</sup> The reduction in EAD among grafts preserved by machine perfusion may be a result of the reduction in ischemic reperfusion injury after machine preservation. In our meta-analysis, patients whose grafts were preserved with machine perfusion tended to have less post-reperfusion syndrome compared to SCS, but the difference was not significant. However, there was marked heterogeneity among the included studies. We also found a reduction in primary non-function among grafts preserved with machine perfusion, but the difference was not statistically significant compared to SCS, similar to the findings of Jia et al.<sup>[18]</sup> and Parente et al.<sup>[37]</sup>

Retransplantation after liver transplantation is a dreaded complication and can be due to a multitude of factors, including vascular complications, technical issues, immunological tissue rejection, allograft dys-

function, or non-function.<sup>[38,39]</sup> In this meta-analysis, we found that the rate of retransplantation is higher among patients who had SCS preservation of their liver graft. This may be related to the increased risk of EAD and the tendency for primary non-function to occur in this group of patients, as reported by Yang et al.<sup>[36]</sup> and Parente et al.<sup>[37]</sup>

Some limitations of this meta-analysis include the fact that some studies have small sample sizes, making them susceptible to higher risks of bias. Additionally, the fact that only studies published in English were included poses a potential for overlooking studies not published in English.

## Conclusion

The use of machine perfusion in the preservation of liver grafts showed a significant reduction in EAD and retransplantation compared to static cold storage.

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