Editorial Commentary

The conclusion of reducing acute rejection after liver transplantation by machine perfusion should be extrapolated with caution

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There is a theory that the unavoidable graft damage caused by ischemia-reperfusion injury (IRI) during liver transplantation (LT) can lead to severe IRI-related inflammation and trigger an early activation of the innate immune response mediated by T-cells, which potentially worsening the acute cellular rejection (ACR) cascade. As a result, machine perfusion (MP) has been placed great expectations for the potential to diminish post-LT ACR and other related immune responses by alleviating IRI through removing harmful substances and restoring cellular metabolism homeostasis (1,2). However, there has been much debate about MP's benefits on ACR as relative data is limited.

An excellent study, published in September 2023 in *Hepatology* (3) by Maspero *et al.*, might establish an new era for MP's research on ACR. We read with great interest the systematic review and meta-analysis, which shows first that MP techniques are associated with a reduction in ACR after LT compared to static cold storage (SCS). This meta-analysis comprised of six studies on hypothermic oxygenated perfusion (HOPE), one study on normothermic machine perfusion (NMP), and one study on normothermic regional perfusion (NRP), and the following conclusions were drawn: (I) MP was associated with a reduction in ACR compared to SCS [odds ratio (OR) =0.55; 95% confidence interval (CI): 0.33–0.91; P=0.02], and this benefit remained significant when considering HOPE alone (OR =0.54; 95% CI: 0.29–1.00; P=0.05); (II) further subgroup analysis confirmed significant effects of reducing ACR by MP in studies including only donation after cardiac death (DCD) grafts (OR =0.43; 95% CI: 0.20–0.91; P=0.03) and only HOPE-DCD grafts (OR =0.37; 95% CI: 0.14–1.00; P=0.05). The authors are to be commended for conducting the first meta-analysis to assess the prognosis of ACR after MP compared with SCS. Their foundings are really intriguing and exciting as this is the first time to detect that MP may be associated with a reduction in ACR after LT compared to SCS. Notably, their study presents novel evidence that HOPE has a favorable impact on the

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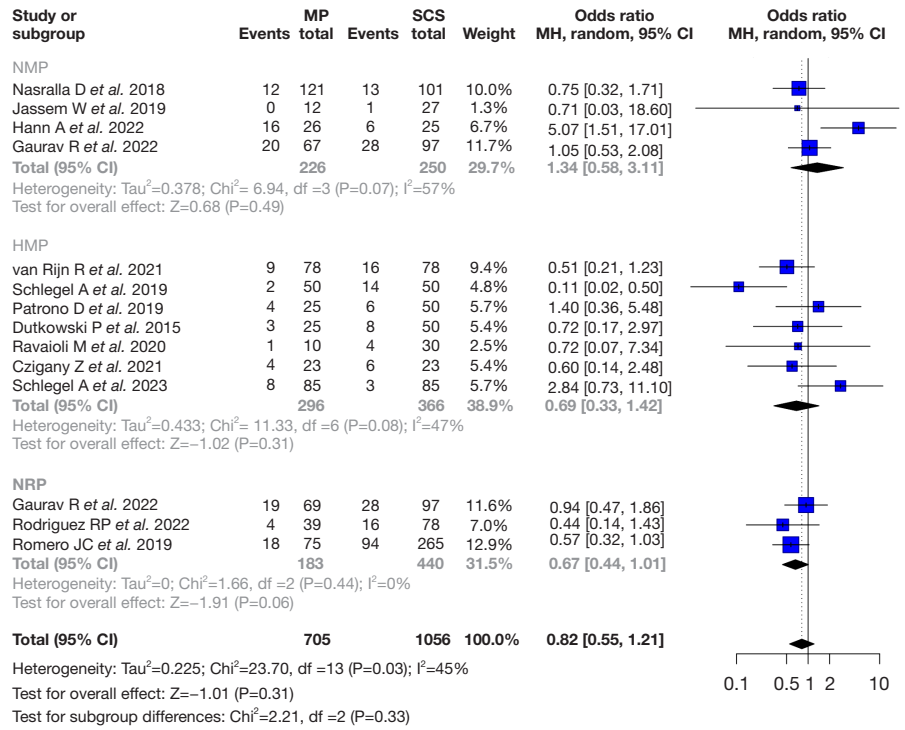

Figure 1 Forest plots of ACR in OLT after machine perfusion compared with SCS. MP, machine perfusion; SCS, static cold storage; MH, machine perfusion; CI, confidence interval; NMP, normothermic machine perfusion; HMP, hypothermic machine perfusion; NRP, normothermic regional perfusion; ACR, acute cellular rejection; OLT, orthotopic liver transplantation.

management and prognosis of ACR, which may provide medical practitioners with improved treatment alternatives and a thread to investigate the underlying mechanisms.

However, the conclusion of MP's benefits on reducing ACR should be more cautious and needs further rigorous evaluation as we found that several important studies has been omitted to be included in meta-analysis by Maspero *et al.* (3). Under the retrieval time of the article by Maspero *et al.*, 1 HOPE study by the author themselves, 3 NMP studies and 3 NPR studies were missed.

In fact, we are currently conducting a similar analysis with Maspero *et al.* and some interesting finding has been detected. To address this issue, we are now engaged further analysis and statistical examination of the included articles. The updated results of meta-analysis on ACR are presented here.

In line with Maspero *et al.*'s inclusion criteria, we updated one multi-center randomized control trial (RCT) on HOPE by Schlegel *et al.* (1), three NMP studies [including a large-scale multi-center RCT by Nasralla *et al.* (4)]. and three NRP studies [as the original data of Muñoz's (5) study and Rodriguez's (6) study are cross-linked, we chose Rodriguez's (6) data which is more updated and has a larger sample size]. Therefore, Muñoz's (5) data [NRP-DCD *vs.* SCS-donor after brainstem death (DBD)] was included in subgroup analyses to maximize uniformity of baseline values.

Ultimately, 7 HOPE studies, 4 NMP studies, and 4 NRP studies were included. The same random effects model was used to compare the effects of MP *vs.* SCS on ACR after LT. Two subgroup analyses, the first for DCD group alone, and the second for HOPE with extended criteria donors (ECD) (i.e., ECD-DCD, ECD-DBD), were performed.

The general characteristics of included studies are summarized in figures. The results obtained are present as follows:

(I) MP was not associated with a reduction in ACR compared to SCS (OR =0.82; 95% CI: 0.55–1.21; P=0.31); this effect was not significant when considering HOPE alone (OR =0.69; 95% CI: 0.33–1.42; P=0.31) (*Figure 1*); NRP tend to reduce the rate of ACR compared to SCS (OR =0.67; 95% CI: 0.44–1.01; P=0.06) (*Figure 1*);

(II) In a subgroup analysis of studies including only DCD grafts, the rate of ACR was significantly reduced by MP compared to SCS (OR =0.62;

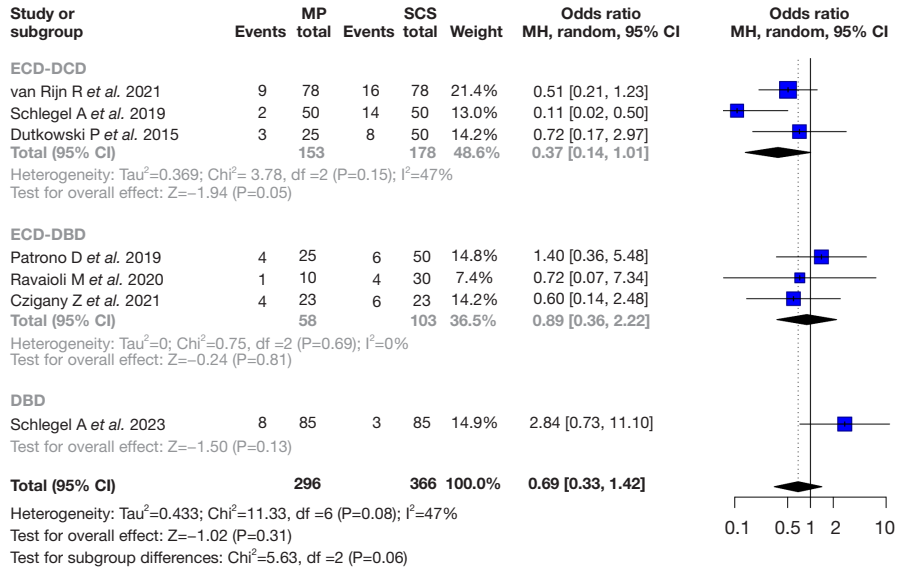

Figure 2 Forest plots of ACR in all types donor-OLT after HMP compared with SCS. HMP, hypothermic machine perfusion; SCS, static cold storage; MH, machine perfusion; CI, confidence interval; ECD, extended/expanded criteria donor; DCD, donation after circulatory death; DBD, donor after brainstem death; ACR, acute cellular rejection; OLT, orthotopic liver transplantation.

95% CI: 0.41–0.94; P=0.03) (Figure S1); when considering only studies on HOPE-DCD and NRP-DCD, ACR was significantly diminished by MP (subgroup analysis, OR =0.62; 95% CI: 0.36–0.86; P<0.01) (figure not shown); when considering only studies on HOPE-DCD, the strongest protecting effect was seen with the use of HOPE compared to SCS (OR =0.37; 95% CI: 0.14–1.01; P=0.05) (*Figure 2*), which is consistent with the results by Schlegel *et al.*;

(III) In a subgroup analysis performed on HOPE studies, HOPE reduced the incidence of ACR only in ECD-DCD (OR =0.37; 95% CI: 0.14–1.01; P=0.05), but did not maintain this benefit in ECD-DBD (OR =0.89; 95% CI: 0.36–2.22; P=0.81) (*Figure 2*).

Our results have further updated and deepen the study by Schlegel *et al.*, elucidating that HOPE and NRP, but not NMP, might be associated with a reduction in ACR compared to SCS. And meanwhile, this effect was only seen with DCD grafts. HOPE didn't show protective effects on diminishing ACR in ECD-DBD or DBD subgroup; and NMP showed no benefits on ACR in DCD either.

However, there are some limitations making these results not enough to be convincing. As mentioned by Schlegel *et al.*, most of the included studies had a fairly small sample size. There are also significant heterogeneities among study design, organ perfusion protocols, clinical characteristics, and perioperative management. More importantly, the diagnosis of ACR is ambiguous and reporting on ACR events is poorly among different included studies. Only a minority of studies report ACR while diagnostic criteria often varies between studies without standard biopsy-proven ACR. Therefore, the conclusion of reducing ACR after LT by MP should be extrapolated with caution. The underlying mechanisms of ACR are still unclear. It has been postulated that the inevitable IRI during LT may lead to early activation of T-cell mediated innate immune response and IRI-associated inflammation, which may then exacerbate the ACR-cascade (7). MP, therefore, has been expected to play an important role in diminishing ACR and other related immunological processes by alleviate IRI through washing out deleterious factors and restoring the homeostasis of cell metabolism. Theoretically, if it is true that IRI is the root of the ACR-cascade, the greater degree of IRI is alleviated by MP, the better effects could be seen in diminishing ACR by MP. Compared with DBD, DCD grafts will endure exposure to a period of warm ischemia, which exacerbates the adverse effects of IRI (8). This partly explains that the protecting effect on reducing ACR was only seen with DCD grafts as MP could remarkably protect graft from severe IRI suffered by SCS-DCD grafts. Yet

DBD grafts may confront relatively minor IRI than DCD grafts so that no significant effect on diminishing ACR could be detected between MP and SCS. It is worth noting that the impact of distinct donor types on ACR remains unclear. Further exploration of the relationship between ACR and graft type is needed. On the other hand, NMP may induce certain levels of IRI during *ex-situ* perfusion as current technology cannot warrant sufficient perfusion under long-time normothermic temperatures and therefore introduce another period of warm ischemia, especially for high risk donors. NMP might also result in an elevated graft immunogenicity. Neutrophil dominant leukocyte efflux into the perfusate during NMP could lead to the general inflammatory response and subsequent T cell priming (9), thereby increasing the risk of T cell-mediated rejection despite using NMP (10). In contrast, hypothermic machine perfusion (HMP) could enhance protective changes in mitochondrial metabolism, such as increasing the availability of adenosine triphosphate (ATP) and alleviating mitochondrial reactive oxygen species initiate IRI-cascade, thereby might ensure a protective metabolic environment for the graft and reduce the IRI-associated innate immune response (1). In other words, HMP might better alleviate IRI to diminish ACR compared to NMP. This hypothesis deserves further investigation. Based on our results, it is reasonable to speculate that HMP and NRP are important techniques with considerable potential to reduce the risk of ACR. A combination of NRP and HOPE might integrate the advantages of both perfusion strategies to further reduce IRI to achieve better effects on diminishing ACR. Recently, combined NRP&HOPE, NRP&NMP or HOPE&NMP has been investigated in preliminary studies, demonstrating great potential to enable safe transplantation of initially declined high-risk donor livers (11,12). In the future, multi-mode perfusion strategies may be able to maximize the improvement of post-operative ACR. Combined perfusion strategies will provide us with a new direction to improve ACR or other complications.

In conclusion, HOPE and NRP have great potential to diminish ACR in DCD LT compared to SCS. However, the conclusion of reducing ACR by MP should be extrapolated with caution due to limited evidence. Despite that, the interesting effects and the underlying mechanisms deserve further investigation.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Supplementary

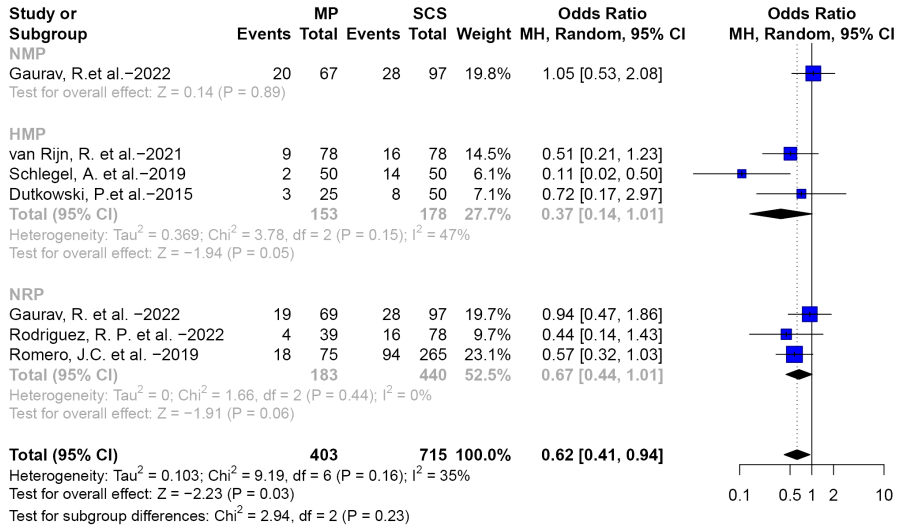

Figure S1 Forest plots of ACR in DCD-OLT after machine perfusion compared with SCS. MP, machine perfusion; SCS, static cold storage; MH, machine perfusion; CI, confidence interval; NMP, normothermic machine perfusion; HMP, hypothermic machine perfusion; NRP, normothermic regional perfusion; ACR, acute cellular rejection; DCD, donation after circulatory death; OLT, orthotopic liver transplantation.

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