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Sistematic review

THE TELL-TALE HEART. MACHINE PERFUSION IN HEART TRANSPLANTATION

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Summary

Hear Transplantation (HTx) is the current best surgical treatment for patients affected by end-stage heart failure. However, the improvement of medical and interventional therapies has led to an increasingly old and high-risk population of HTx candidates. Moreover, the use of "extended" donor criteria to face the shortage of donors could increase the risk of worse outcomes after HTx. In this setting, donor organ preservation strategy could significantly affect HTx results.

The cold storage (CS) represents the standard practice worldwide for donor graft preservation. However, prolonged ischemic time is known to be an independent risk factor for mortality after HTx.

Machine perfusion (MP) systems continuously perfuse the donor heart, reducing ischemic time. Also, since MP permits to evaluate marginal donor organs, they could represent a safe and effective technique to expand the available donor pool. Despite the increasing number of donor hearts preserved with MP, whether MP could be considered superior to traditional CS still represents a matter of debate.

The aim of this paper is to summarize and critically assess the available clinical data on MP employed for HTx.

Key words: machine perfusion, heart transplantation, normothermic *ex-situ* perfusion, organ care system, organ preservation

"Yes! Yes, I killed him. Pull up the boards and you shall see! I killed him. But why does his heart not stop beating?! Why does it not stop!?"
from "The Tell-Tale Heart", Edgar Allan Poe (1809-1849).

INTRODUCTION

Heart Transplantation (HTx) represents the gold standard surgical treatment of end-stage heart failure. However, despite improvement in recipient care and management, the rate of Primary Graft Dysfunction (PGD) continues to be relatively high, still representing the leading cause for 30-day mortality after HTx [1-3]. A recent national UK study reported an overall incidence of PGD after HTx of 36%, with an incidence of moderate-to-severe PGD of 32% [4].

The interaction of donor, recipient and procedural variables may predispose to this life-threatening complication. On one hand, the improvement of medical and interventional therapies, as well as the wide use of mechanical circulatory supports (MCS), have led to an increasingly old population affected

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by multiple comorbidities that eventually receives an HTx. On the other hand, the use of "extended" donor criteria to face donors shortage has increased the risk of worse outcomes after HTx.

Considering procedural factors, donor organ preservation strategy plays a central role in development of PGD. The traditional static cold storage (CS) remains the standard practice worldwide for donor graft preservation, being user friendly and cost-effective. However, using conventional CS, prolonged ischemic time is known to be an independent risk factor for PGD and mortality after HTx [5-7]. Moreover, the negative impact of graft ischemic time is considerably hampered by other donor characteristics, as age, left ventricular hypertrophy, mild coronary artery disease, catecholamines support [7].

Machine perfusion (MP) systems permit to continuously perfuse the coronary arteries, reducing ischemic time and potentially mitigate the deleterious effects of ischemia/reperfusion injury. Despite the increasing number of donor hearts preserved with MP, whether MP could be considered superior to traditional CS still represents a matter of debate.

The aim of this paper is to summarize and critically assess all available clinical data on MP, reporting also the Udine experience with MP for donor heart preservation.

MACHINE PERFUSION

Differently from static cold storage, MP represents a dynamic method of preservation that prevents extra ischemia and potentially provides a better preservation of the donated heart, whilst providing opportunity to assess metabolic and functional status of the graft. Two types of MP have been employed in cardiac transplantation: hypothermic (HMP) and normothermic (NMP) machine perfusion.

Hypothermic MP

The rationale of hypothermic preservation consists in reducing the metabolic requirements of the heart with an optimal and homogeneous cooling (below 10°C), while providing continuous metabolic support though infusion of enriched solutions. Experimental studies performed on large animal models have suggested that compared to static cold storage, HMP could attenuate tissue injuries and provide superior myocardial function after transplantation[8], but clinical adoption of HMP has been limited due to the concerns about a reliable functional assessment: since the graft is perfused with a cold cardioplegic solution, it is maintained in a non-beating state.

Three single-center clinical studies analyzed the effect of HMP so far, with three different perfusion solutions: Wicomb et al. In 1984 used crystalloid cardioplegic solution in 4 patients [9], Hill et al in 1997 used colloid cardioplegic solution in 8 patients [10], and more recently, in 2020 Nilsson et al reported their experience employing a home-made MP with hyper-oncotic cardioplegic solution supplemented with hormones and erythrocytes, termed "non-ischemic hypothermic perfusion" (NIHP) [11]. In this series, 6 patients who received donor hearts preserved with NIHP showed better outcomes at 6-months after HTx compared to 25 recipients who received static cold-storage preserved grafts.

Based on these preliminary promising results, the Xvivo Perfusion AB (Goteborg, Sweden) has patented the NIHP and further developed it to a commercially available device, and currently a randomized clinical trial is ongoing to assess patient and graft survival comparing NIHP to SCS [12].

Normothermic MP

Normothermic MP systems perfuse the heart with oxygenated blood and enriched solutions, keeping it beating and at a near-physiological temperature (es. 34°C). Currently the Organ Care System (OCS, TransMedics Inc, Andover, MA) represents the only NMP commercially available for clinical use in HTx. *Ex-vivo* perfusion with this device is particularly attractive when evaluating "extended criteria" donor organs, since besides limiting graft ischemic time, it permits a real-time monitoring of hemodynamic parameters and of lactate concentration, the principal marker of organ metabolism, with a timely identification of potentially unsuitable grafts. Moreover, for these reasons, OCS is increasingly employed in resuscitation and assessment of organs from donation after circulatory death (DCD).

The Organ Care System (OCS)

The OCS consists of a portable platform composed of a wireless monitor/controller and a circuit in which the donor blood perfuses the beating and empty donor heart (Fig. 1A). The donor blood is enriched with a specific Priming Solution (containing mainly Mannitol, electrolytes, vitamins and antibiotics), and during *ex-vivo* perfusion two other solutions are infused into the perfusion system: the Catecholamine Solution (containing epinephrine to replenish the depleted catecholamines) and the Maintenance Solution (an adenosine-enriched solution with the purpose to modulate the coronary artery resistance).

A heater-membrane oxygenator module maintains oxygenated and normothermic (34°C) the donor blood, that is delivered into the aortic inflow cannula by a peristaltic pump after closing both venae cavae. The blood perfuses the coronary vessels and it reaches the coronary sinus and eventually the pulmonary artery, where an outflow cannula collects the blood to close the machine's circuit (Fig. 1B). The blood that does not reach the coronary sinus (because of aortic regurgitation and bleeding from cut surfaces), is

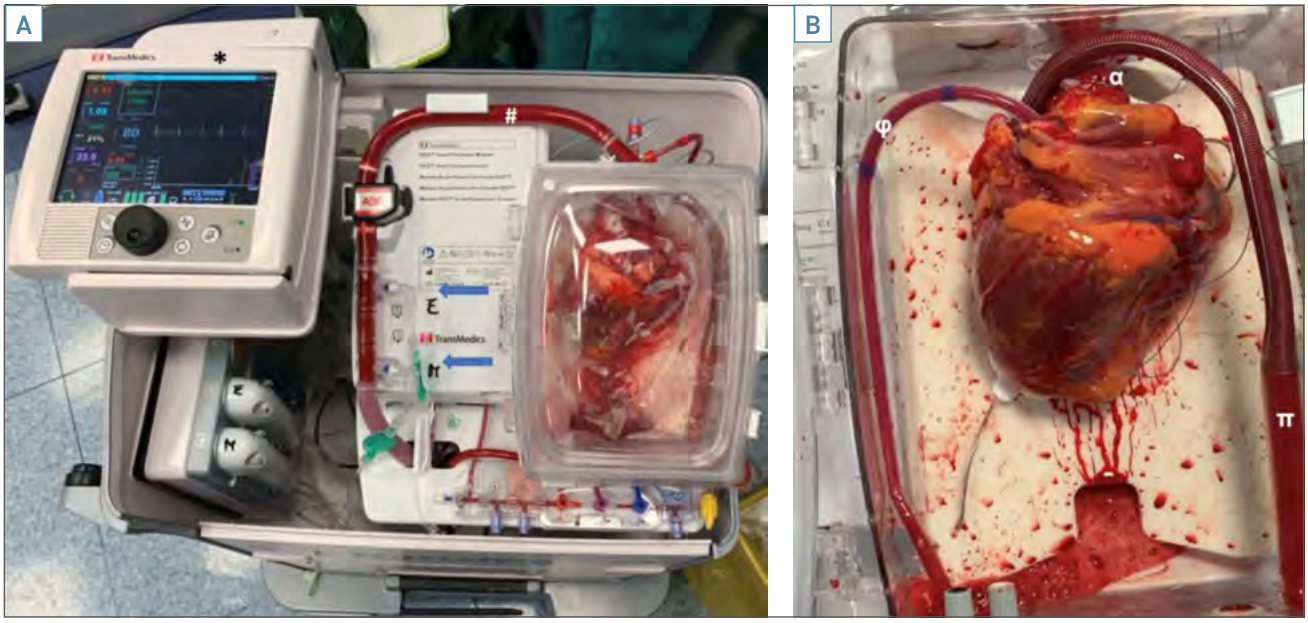

Figure 1. A) The Transmedics Organ Care System portable platform. Wireless monitor/controller (*); arterial perfusion line (#); infusion lines for epinephrine (E) and maintenance (M) solutions indicated by the arrows; 1B) the instrumented donor heart. Aortic connector (α); pulmonary artery cannula (π); left ventricular venting tube (φ).

collected and re-infused into the circuit. Flow and pressure of the blood are registered by probes. Stopcocks permit blood sampling, to control both arterial and venous lactate concentration levels. Arterial pressure and coronary flow can be manipulated by variation of the pump flow speed and/or of the Maintenance Solution infusion rate. OCS settings were adjusted to maintain the mean aortic pressure between 80 mm Hg and 100 mm Hg, and coronary blood flow between 700 mL/min and 900 mL/min. Graft function is assessed by continuous monitoring of aortic pressure, coronary flow, and the differential artero-venous lactate profile. A lactate level > 5 mmol/l is considered an index of myocardial damage and so a contraindication for using the graft, as well as an unfavorable artero-venous lactate production pattern (venous lactate concentration higher than arterial lactate level).
The OCS device could be transported either by car, plane, or helicopter.

MP employment

During the last decade, normothermic *ex-vivo* perfusion has emerged as a key factor in expanding the cardiac donor pool, as it favors a safer employment of extended-criteria donor hearts by limiting ischemic time and allowing graft assessment.

To shorten the ischemic time

The continuous coronary perfusion with oxygenated enriched blood is the main advantage of normothermic machine perfusion. Graft ischemic time is reported to be an independent risk factor for PDG after HTx and for mortality up to 15 years [5], and its negative role is amplified in extended criteria donor grafts, particularly in aged donor hearts [5-7].
The results of the PROCEED II controlled trial demonstrated the non-inferiority of the NMP compared to the traditional SCS. Patient and graft survival between both study arms were similar, although the OCS group reported a significantly shorter graft ischemic time. Interestingly, in OCS group 4 hearts were discarded because of an increasing lactate concentration and histological analysis revealed signs of infarction and contusion and severe unrecognized left ventricular hypertrophy [13]. Other single-center and retrospective studies [14-20] further reported graft ischemic time significantly shorter when grafts were preserved with NMP instead of being preserved with SCS, as reported in Table I.
Therefore, the OCS could be an effective tool to ensure graft quality when the expected ischemic exceed the traditional threshold of 4 hours, favoring long-distance organ retrieval. Two case reports presented successful HTx after preservation times as long as 10 hours [21] and 16 hours [22].
The relatively safe non-ischemic "out of body time" could also be advantageous in particular situations, such as an unexpected nodule discovered during organ retrieval that needs a histological definition [23].

To assess the organ

NMP has shown interesting and promising results when employed in extended criteria DBD donors, and donation

Table I. Studies of normothermic machine perfusion for hearts from DBD with and without CS as control group.

Author	OCS group					CS group			
	N. of patients	Donor Age	Recipient Age	Out of body time (min)	Graft Ischemic Time (min)	N. of patients	Donor age	Recipient age	Graft ischemic time (min)
Ardehali et al. [13]	67	35 (range 18-58)	56 (range 20-75)	324 ± 79	113 ± 27	63	34 (range 13-60)	57 (range 20-76)	195 ± 65
Garcia Saez et al. [14]	26	37 ± 12	43 ± 13	371 ± 102	87 ± 15	-	-	-	-
Kaliyev et al. [15]	13	43 ± 15.5	40 ± 12	330.3	83 ± 8	-	-	-	-
Koerner et al. [16]	29	36 (range 17-54)	50 (range 37-64)	297	52	130	-	50.7 (range 37-64)	-
Sponga et al. [17]	14	46 ± 11	64 (range 35-75)	452	132 ± 28	24	44 ± 13	57 (range 30-73)	225 ± 48
Sponga et al. [18]	21	47 ± 11	58 (range 24-66)	272 ± 65	145 ± 29	79	48 ± 13	60 (range 28-73)	213 ± 63
Sato et al. [19]	16	-	52 ± 15.5	362 ± 153	114 ± 51	18	-	59 ± 16	183 ± 34
Rojas et al. [20]	68	37	49 ± 13	381 ± 74	115 ± 43	51	44.5	59 ± 13	228 ± 43

after circulatory death (DCD). Donors with extended criteria were defined as those > 50 years of age, with a history of drug abuse, cardiac resuscitation, coronary artery disease (CAD), expected graft ischemia time > 4 hours, left ventricular ejection fraction (LVEF) < 50%, or interventricular septum thickness (IVS) > 14 mm.

Data regarding marginal DBD donors are derived mainly from single-center observational studies [14,17,20]. A previous report of our group was able to demonstrate that NMP, compared to CS, seems to provide more stable hemodynamic conditions, reducing complications and allowing optimal outcomes. In fact, 5-year survival of OCS preserved heart group was 100 *vs* 73% of CS control group (p = 0.04). These results are also supported by histopathological and ultrastructural evidence, suggesting better myocardial preservation in NMP grafts [17].

The EXPAND trial was designed as a single-arm multicenter study that evaluated the impact of OCS preservation in extended-criteria donor hearts. Out of 93 included donor hearts, 75 were employed for HTx (81% utilization rate). The 30-day post-HTx survival rate was 94.6% and the incidence of severe PGD in first 24 hours was 10.7%. Moderate to severe PGD was observed in 14.7% of patients [24].

In our experience, out of 67 grafts preserved with NMP, a total of 8 grafts were discarded (12%), mostly due to a progressive increase in lactate concentration, expression in most cases of severe left ventricle hypertrophy and undiagnosed coronary artery disease. In one case, NMP real-time evaluation of lactate trend permitted to discard an apparently standard organ which, at the gross pathological examination, revealed a dissection of the right coronary artery at 4 mm from its origin [25]. As regard organ assessment and expansion of donor pool instead, at our center a donor heart with a myocardial bridge was successfully transplanted in a 66-year-old recipient. A myocardial bridge in a donor graft is a relative contraindication to HTx, however, thanks to the NMP continuous evaluation of cardiac function and reduction of the potential myocardial ischemic impact of the myocardial bridge, the graft was considered suitable for HTx with consequent gratifying results [26].

Considering DCD, heart is exposed to prolonged periods of warm ischemia and to right atrial and ventricular overdistension during cardiocirculatory arrest, with possible irreversible myocardial injury. Thus, a post-asystolic functional heart assessment is of paramount importance when evaluating these hearts. In clinical practice, DCD hearts are retrieved with either direct procurement and perfusion (DPP) or normothermic regional perfusion (NRP). As shown in Figure 2, in DPP, the heart is removed

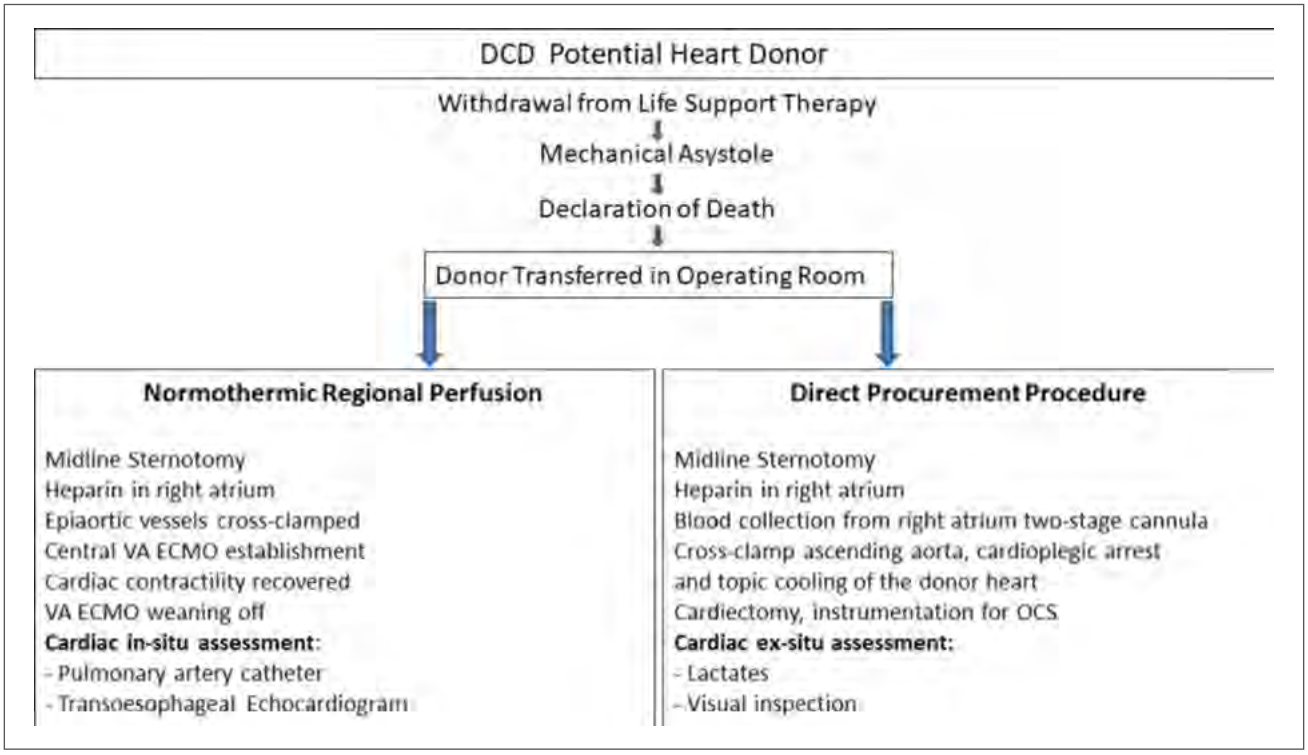

Figure 2. Diagram showing the two techniques for heart procurement in donation after circulatory death. VA ECMO: veno-arterial extracorporeal membrane oxygenation; OCS: Organ Care System.

after confirmation of death and expeditiously reperfused using the OCS [27-30]. Instead, in NRP the employment of extracorporeal membrane oxygenation (ECMO) facilitates the cardiac resuscitation. After the donor is weaned from ECMO, the heart is assessed *in-situ* and if adequate recovery is observed it is retrieved and preserved with OCS or CS [31,32].

The introduction of NMP in clinical practice has permitted to utilize DCD donor hearts with satisfactory results [27-32]. Furthermore, when compared with the current "gold standard" CS-preserved DBD hearts, the OCS-preserved DCD showed to guarantee comparable results [28,32]. The OCS DCD Heart trial compared DCD heart transplant to DBD standard criteria HTx clinical outcomes. The trial demonstrated that the use of OCS resulted in high rate of DCD heart utilization for transplantation (89%) with excellent 6-months and 1-year survival after Htx compared to DBD donor hearts (94.4% and 93.3 *vs* 88.6% and 87.3%, $p < 0.001$) [33].

The method of retrieval (DPP or NRP) was not associated with a different outcome after Htx by the series of Messer et al. [32].

To facilitate HTx in high-risk patients

Heart MP could also play a protective role in high-risk recipients, particularly in those supported by durable mechanical circulatory support or who have undergone previous complex operations [14,18,20]. HTx in these patients might be technically demanding and often require a tedious dissection and prolonged cardiopulmonary bypass times to complete the removal of intrathoracic ventricular assist devices or the isolation of the cardiac structures. The use of MP allows optimization of coordination between retrieval and implanting teams, favouring a meticulous and stress-free preparation of the recipients while the donor graft remains perfused. Reducing procedural bleeding and transfusions of blood products could potentially improve haemodynamic stability after HTx. In a previous series of patients bridged to HTx with MCS from our group, OCS perfusion conferred a protective role regarding PGD development after HTX, compared to CS (7 *vs* 42%, $p = 0.03$) [18].

Unpublished data derived from a combined series of patients from Hannover and Udine Centers confirmed the effectiveness of the OCS in 80 high-risk patients when extended-criteria donor grafts are employed. In fact, in this challenging scenario the outcomes were satisfactory, as the incidences of in-hospital mortality and severe PGD were 11 and 16%, respectively.

To recover the injured graft

Sarcomere changes, such as Z-line thickening and/or non-orthodox banding were reported in donor hearts

after immediately after the retrieval [17]. After *in-situ* reperfusion during HTx, hearts preserved with cold storage are frequently affected by myocardium injury, with damaged contractile myofilaments and organelles including mitochondria. The *ex-situ* perfusion with OCS is reported to be effective in reconditioning donor hearts, that are maintained metabolically active and able to heal ultrastructural changes [17,34].
Expanded-criteria donor hearts appear to be best treated by NMP, especially when severe hypotension or cardiac arrest occurs during the retrieval phase and could hamper the negative effect of cold storage on ultracellular cardiac function [34].

To manipulate and regenerate the organ

Normothermic *ex-situ* perfusion with the OCS has proven to restore cellular function after ischemic injury [17]. Moreover, NMP could be a useful platform for cardiac conditioning before transplantation, since it creates a 'time window' between procurement and transplantation during which the organ could potentially be manipulated. Graft immunomodulation, via infusion of viral vectors [35,36] or mesenchymal stem cells (MSC) injection [37], could modify its immunogenic capacity and reactivity. MSCs are multipotent cells isolated from the bone marrow. Their use in solid organ transplantation is emerging to promote immunologic tolerance with the result of reducing acute and chronic rejection post HTx. In HTx, donor infusion of MSCs has been shown to prolong the survival of a semi-allogeneic HTx in a mouse model through the generation of regulatory T cells [38]. In addition to MSCs, also the injection of extracellular vesicles secreted from Induced pluripotent stem Cell derived cardiomyocytes (iCM-EVs) have been documented to protect acutely injured heart from pathologic hypertrophy and lead to functional recovery. Since their content is mainly composed of miRNAs that modulate cardiac specific processes, they could represent a promising cell free alternative for cardiac recovery [39].
Dr. McCully and colleagues reported that the transplantation of viable and competent mitochondria into an ischemic zone prior to reperfusion enhances post-ischemic cellular functional recovery and viability during reperfusion. The isolated mitochondria can be delivered either by direct injection or by coronary infusion, thus NMP could again represent the ideal platform for this manipulation to improve the heart metabolic function and to reduce the ischemia-reperfusion injury [40]. Preliminary scientific reports, confirming the potential for clinical application of these techniques, underline the need for prolonged graft manipulation in order to achieve a significant effect, making NMP an irreplaceable tool [35-41].
Another ineresting application could be the use of NMP for *ex-vivo* heart surgery. The organ could potentially be removed from a patient, repaired on NMP while the patient is supported on cardiopulmonary bypass, and auto-transplanted following successful modification, such as complex tumor resection [41].

CONCLUSIONS

MP can confer the opportunity to assess and recondition the donor heart and are increasingly employed worldwide in an attempt to expand the donor pool for cardiac transplantation. However, despite interesting results, the role of MP in heart transplantation remains still debatable, principally because of higher costs and training levels than those required for CS.
At present, while the clinical effectiveness of HMP has to be investigated, NMP with the OCS seems to allow for safe utilization of DCD and extended-criteria donor organs, combining two major advantages: to limit the graft ischemic time and to assess cardiac function by means of metabolic values, visual and manual inspection, and haemodynamic parameters. The maintenance of myocardial aerobic metabolism during the preservation could lead to better donor heart preservation compared to traditional CS.
Thus, the NMP represents an effective technique that permits to expand the donor pool, allowing assessment of grafts which would have otherwise been refused, while maintaining satisfactory safety levels. In fact, NMP permits to identify unsuitable grafts and discard them before transplantation, reducing the risk of primary graft failure and its life-threatening sequelae [13,17].
MP have certainly some aspects that should be investigated in the near future to further improve the technique, such as additional metabolic support, solution components and optimal perfusion settings. Also, the identification of other functional parameters or biomarkers, apart from lactate levels, could be of paramount importance to increase the sensitivity of MP to help clinicians in deeming suitability of perfused donor grafts.
The main drawback of NMP is that it requires an experienced and well-trained professional team, to manage the interaction between the donor organ and the *ex-vivo* perfusion, and to promptly intervene in case of machine malfunction or user error. In fact, since the donor heart is preserved in a beating normothermic state, the margin of safety is limited in case of complications or non-appropriate NMP management due to the risk of catastrophic and irreversible warm ischemia of the graft.
In conclusion, the satisfactory results reported in HTx with high-risk recipients and extended-criteria donors highlight the effectiveness of NMP in complex cases, particularly in unfavourable combinations of donor, procedural and recipient characteristics. The results of ongoing multicentic clinical trials investigating on heart MP are required to confirm these expectations.

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Conflict of interest

The Authors declare no conflict of interest.

Author contributions

AL, SS, GB: writing draft; AL, SS, GB, VF: data collection; UL: supervision and final review; all Authors: critical review and final approval.

Ethical consideration

This study was based on review of published data and studies, and thus it did not need Insitutional Ethics Committee approval.
These statements are not applicable for review articles, since this study did not involved directly patients and procedures.

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