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RESEARCH ARTICLE

Machine Preservation and *Ex Situ* Assessment of the Donor Heart: A New Paradigm in Heart Transplantation

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ABSTRACT

Heart transplantation is the gold standard treatment for advanced heart failure in selected patients. Organ shortage is however a major concern, leading transplant teams to expand the criteria for donor selection. Organ retrieval is routinely performed nowadays in donors older than 55 years, with impaired left ventricular function and/or hypertrophy, or after prolonged cardiac arrest such as after circulatory-determined death. All these conditions are associated with an increased high of primary graft failure, the main cause of early death after heart transplantation. Machine preservation technology has been recently applied to allow for extended preservation of the donor heart. Compared to conventional static cold storage, this approach can also provide viability assessment of the donor heart before transplant, especially in case of *ex situ* normothermic blood perfusion. After a brief review of currently available preservation machines, we sought to describe different approaches for assessment of the donor heart using *ex situ* organ perfusion, from metabolic monitoring to cardiac imaging and hemodynamic investigations.

Introduction

Machine preservation technology has been recently developed to allow for safer preservation of the donor heart, mainly in the setting of transplants at high risk for primary graft failure. After an overview of current challenges in heart transplantation (HT), we will describe currently available devices for *ex situ* heart preservation, underscoring advantages and limitations for each commercially available machine. We will then report approaches to assess the donor heart during *ex situ* perfusion, a major issue to improve the early outcomes in HT.

Current challenges in heart transplantation

1. *Organ shortage and donors with extended criteria*

Heart failure is the main cause of mortality in Western countries, accounting for more than one third of premature deaths. Heart transplantation (HT) is the gold standard treatment for advanced heart failure with 60 to 65% of survival at 10 years after transplant.[1,2] Current organ shortage is however a major limitation leading transplant teams to dramatically expand the criteria for donor selection. Over the past two decades a growing number of donor hearts with extended criteria have been allocated including those older than 55 years, with impaired left ventricular function and/or hypertrophy, prolonged cardiac arrest, and more recently those donated after controlled circulatory death (DCD).[3,4] All these conditions are associated with an increased risk of primary graft failure and early death after transplant.[5]

2. *Cardiac metabolism and ischemia-related injuries*

Another major issue in HT is the limited tolerance to organ ischemia. Conventional static cold storage allows for 4 hours of ischemic period from aortic cross clamping in the donor to reperfusion of the heart in the recipient. Beyond this critical time window, the risk of primary graft failure is markedly increased (especially in case of donor with extended criteria), along with a higher rate of early death during the first postoperative month after transplant.[3,5] Indeed, the heart requires the most important energy supply in the human body with a 6-kg daily consumption of adenosine triphosphate. Among other energy substrates, cardiomyocytes can metabolize glucose, ketone bodies produced from fatty acids, and lactate.[6] Cellular anoxia following myocardial ischemia rapidly inhibits mitochondrial oxidative phosphorylation, leading to major shortage in adenosine triphosphate.[7] Glycolytic shift related to anaerobic metabolism then allows for optimal use of energy supply but generates tissue acidosis. Residual adenosine triphosphate is therefore used to preserve mitochondrial membrane potential. From the first minutes of myocardial ischemia, regulation of calcium and potassium homeostasis is dramatically impaired leading to intracellular sodium overload.[8,9] The longer the ischemia period, the more extensive and potentially irreversible the lesions. Massive release of reactive oxygen species by the mitochondria results in major cell membrane injury and interstitial edema.[8] These lesions are known to be associated with impaired myocardial function, especially loss of contractility. In the setting of HT, primary graft failure may therefore occur in case of ischemia-related injuries, most of time requiring transient mechanical circulatory support until recovery.

3. *Donation after circulatory-determined death*

Donation after brain death (DBD) is the most common situation for organ procurement in HT. Historically, the first human HT performed by Christiaan Barnard at Cape Town in December 1967 preceded the worldwide accepted definition of brain death. This cardiac allograft was procured after circulatory-determined death in a young women with severe head trauma associated with irreversible brain injury.[10] The principle of donation after circulatory death (DCD) was therefore demonstrated, but has been progressively replaced in the 1970's by DBD after definition and acceptance of this approach by the medical community.[11] In the mid-2010's, two groups (Papworth Royal Hospital, Cambridge, UK and St Vincent Hospital, Sydney, Australia) initiated DCD heart transplantation programs.[12-14] The main concern of this approach is to expose the heart to prolonged warm ischemia following withdrawal of life supportive therapies. The risk of primary graft failure after DCD HT (from 17 to 30%) is correlated with the duration of functional warm ischemia before retrieval of the donor heart.[14,15] These groups observed that postoperative outcomes were more favorable when this critical period was lower than 20 minutes, whereas the risk of primary graft failure was markedly increased beyond a 30-minute period of functional warm ischemia.[14,15] To prevent the risk of early postoperative death, transplant teams assess the viability of the heart before transplantation. This approach is at the opposite of conventional static cold storage since no evaluation of the graft is possible from retrieval to transplant. *Ex situ* isolated perfusion is the most commonly applied method to investigate the quality of DCD hearts. Indeed, potential myocardial injuries that may contraindicate HT can be detected in this way. In their preliminary experience, UK and

Australian groups showed 2 and 5-year survival rates higher than 90% and 82% respectively.[14,15] These favorable outcomes, comparable with contemporary results of DBD transplantations, are a major hope for patients waiting for HT, and underscore the need for *ex situ* heart perfusion technology.

Machine preservation technology in heart transplantation

1. From static to controlled cold storage

Two different approaches can be applied during preservation of the donor heart to prevent ischemia-reperfusion injuries: 1) reduction of the metabolic demand; 2) continuous supply of energy substrates. Cold storage at 4°C achieves the first objective by preserving the heart in static condition with very low requirement for energy substrates. This most commonly used method in HT requires limited human and financial resources.[16] Along with potential unstable temperature associated with ice storage, another major concern is the limited duration for organ preservation, up to 6 hours, but with an increased risk of primary graft failure beyond 4 hours. Indeed every hour of ischemia beyond 4 hours increases the risk of primary graft failure by 43%, and one-year mortality by 25%.[5,17] Moreover impaired myocardial cooling and direct contact with ice can result in irreversible injuries for the heart, especially when tissue temperature drops below 2°C.[18] An innovative technology maintaining the temperature between 4 and 8 °C has been recently reported, allowing for safe preservation of porcine hearts up to 12 hours.[19] This approach ensures stable temperature and pressure controlled environment since the heart is immersed in a cold preservation solution for traveling. A multicenter international clinical trial including 800 patients is currently on-going to investigate the safety and efficacy of this approach in HT.(https://clinicaltrials.gov/ct2/show/nct04141605) Preliminary results show a trend for a lower rate of primary graft failure compared to conventional static cold storage (16.1% vs. 20.2%) for a mean preservation duration of 210.3 ± 52.2 min.[20] A limitation of this device is however the lack of *ex situ* evaluation of the donor heart before transplant, unlike machine perfusion technology.

2. Hypothermic perfusion

To achieve longer preservation, machine perfusion technology has been applied in the field of organ transplantation. This concept of dynamic preservation, widely applied in kidney transplantation since the late 2000's, aims at preventing ischemia-reperfusion injury through continuous perfusion of preservation solution during organ traveling. Along with the benefit of maintaining cold homogeneous temperature, this approach also provides metabolites, oxygen as well as protective drugs contained in commercially available preservation solution. In the setting of *ex situ* dynamic hypothermic preservation, perfusion set includes a circuit, a roller pump, an oxygenator and a heater-cooler unit without direct contact between ice and myocardium. In the perfusion module, the heart is completely submerged in cold preservation solution. Antegrade coronary perfusion is achieved thanks to an intra-aortic cannulation. Coronary flow is adjusted (from 35 to 200 mL/min) for temperature (from 4 to 8°C) and pressure-controlled (20 mmHg) organ protection, allowing for extended preservation of the donor heart up to 24 hours in preclinical studies.[21-23]

The *ex situ* hypothermic perfusion machine from XVivo Perfusion AB (Göteborg, Sweden) is the only commercially available device with clinical results in HT to date.[24] A multicenter randomized clinical trial is currently on-going in Europe to investigate the safety and efficacy of this technology compared to conventional static cold storage (https://clinicaltrials.gov/ct2/show/NCT03991923). In the XVivo device the heart is immerged in a reservoir filled with a buffered extracellular solution including albumin and dextran for optimal osmotic pressure (Steen solution, XVivo Perfusion AB, Sweden), combined with 450 to 500 mL of leucocyte-filtered red blood. Temperature of the perfusate is maintained at 8°C and continuous oxygenation is provided thanks to a miniaturized automated heart-lung machine.

Among the determinants of myocardial oxygen demand, contractility of the heart is the most important, while basal metabolism accounts for only 10 to 20% of total oxygen consumption.[25] Continuous oxygen supply has therefore to be maintained even if the heart is arrested during isolated hypothermic perfusion. Indeed, cellular metabolism is dramatically reduced at 8°C, but never completely nil. Compared to normothermia, energy metabolism is halved for every 10°C reduction in organ temperature according to Van't Hoff's rule.[26] Coronary flow is correlated with myocardial oxygen consumption since the main mechanism to improve myocardial oxygen delivery is by increasing coronary flow. On the other hand excessive oxygen supply during hypothermia may result in myocardial injury related to generation of free radicals.[27] The optimal oxygen supply during hypothermic organ perfusion remains a matter of debate. It is acknowledged that ATP levels in the

myocardium can only be restored when oxygen is supplied, even at low temperatures. In the setting of donation after circulatory-determined death, donor hearts are exposed to prolonged warm ischemia and therefore have very low cellular rate of ATP. Potential impact of oxygenated *ex situ* hypothermic perfusion on oxidative stress and post-transplant outcomes remains to be investigated for DCD hearts in clinical practice.

3. Normothermic perfusion

Ex situ isolated perfusion of a frog heart was first reported in 1866 by Ludwig and Cyon, then Langendorff reported in 1885 a cardiac perfusion set up for mammalians characterized by retrograde cannulation of the aorta to ensure continuous perfusion of the coronary vessels.[28] This preparation enabled major advances in experimental cardiology over the 20th century, and was recently applied in the field of HT in the mid 2000's. Preliminary experiences using Langendorff normothermic blood perfusion of porcine hearts underscored the major interest of this technology in HT.[29] The Organ Care System (OCS, TransMedics, Andover, USA) is the only commercially available machine for isolated blood perfusion of the heart in a beating state. This transportable device has enabled more than 1300 HT across the world since its first clinical application in 2008, including more than 350 DCD cases.[30,31] This approach does not tolerate any significant aortic regurgitation on the donor heart, at the risk of coronary malperfusion and thus impaired myocardial protection and assessment. The OCS technology has been demonstrated to provide comparable outcomes compared to static cold storage for "non-marginal" donor hearts.[32] One of the main advantage is to extend the preservation of donor hearts until transplantation. The longest out-of-body time reported to date for a human cardiac graft to be transplanted was 17 hours, including 16 hours on the OCS system.[33] This is a major advance in HT to reduce the risk of primary graft failure when the ischemic time is expected to be longer than 4 hours (organ retrieval associated with long transportation delay; recipient with past history of multiple open heart surgeries or with intracorporeal mechanical ventricular assist device). A second objective to be achieved with OCS is to evaluate the transplantability of marginal grafts, including DCD hearts.[13] These grafts need to be assessed before transplant to avoid irreversible primary graft failure related to prolonged warm ischemia after withdrawal of life-sustaining therapies in the donor. Emphasis has recently raised about the optimal method to investigate the quality of the donor heart during *ex situ* perfusion. The eligibility for transplant remains a though decision to be made by transplant clinicians who therefore need to be confident with machine perfusion technology along with the means to assess the viability of the donor heart in this setting.

Assessment of the donor heart during isolated *ex situ* heart perfusion

Different approaches can be applied to assess the donor heart during *ex situ* preservation, mainly depending on the physiology of the perfusion machine (resting vs. beating state).

1. Metabolic assessment

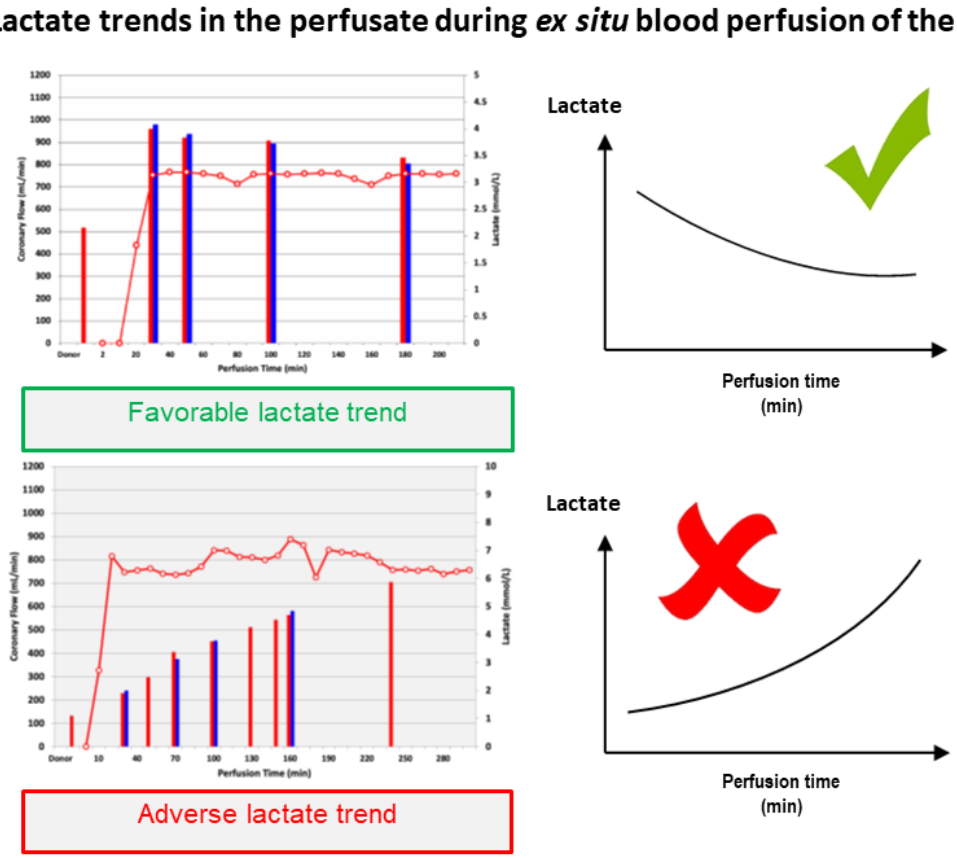
Normothermic blood perfusion is associated with the higher metabolic demand compared to other machine preservation technologies, since this is the only condition in which the heart is preserved in a beating state. However in the OCS commercially available set up, left heart cavities are unloaded (Langendorff mode), so that myocardial oxygen demand related to wall tension and muscle shortening is low.[34] Heart rate, contractility and basal metabolism are known to be the main determinants of myocardial energy consumption in this "resting mode" blood perfusion setting. Management as well as viability assessment of the beating heart on the OCS is based on lactate metabolism. Arterial and venous blood is regularly sampled from the perfusion circuit for lactate dosage. Myocardial extraction of lactate is expected. It has been reported to be associated with myocardial viability, and thus with suitability of the heart for transplant.[29,35] At initiation of *ex situ* normothermic blood perfusion, arterial lactates usually increases and efficient myocardial extraction is observed after 30 to 60 minutes otherwise mean aortic pressure and coronary flow have to be adjusted (**Figure 1**).

Even if there is no consensus considering the cut-off value for arterial lactate to decline a donor heart for HT preserved on OCS, most of transplant teams aim to achieve arterial lactate <5 mmol/L.[12] However the final value of arterial lactate depends on initial concentration of lactate in the perfusate. Indeed, blood used to prime the OCS circuit is usually collected from the organ donor. In case of a DCD heart, withdrawal of life sustaining therapies is followed by an agonal phase characterized by impaired tissue perfusion and prolonged hypoxia (warm ischemic period) until cessation of blood flow. A high rate of lactate is therefore generated (usually from 5 to 10 mmol/L) in the donor blood, and thus remarkable in OCS circuit at initiation of isolated heart perfusion. Since there is no clearance of lactate by any component of the circuit, every change in lactate concentration observed during *ex*

situ heart perfusion is directly related to myocardial metabolism. The most important is to obtain positive arteriovenous lactate values, consistent with favorable myocardial uptake, whatever the absolute value of arterial and venous lactates. In normothermic and normoxic conditions the heart is actually able to metabolize lactate. A too early decrease of arterial lactate during the first hour ex situ perfusion should however be avoided since it may be related to excessive coronary flow that may result in myocardial edema and thus progressive increase in coronary vascular resistance.

There is no consensus considering the optimal duration of isolated heart perfusion before allocation of the heart for transplantation, especially for DCD hearts. Moreover the level of lactates at the end of *ex situ* perfusion run has been poorly correlated with post-transplant outcomes to date. Further preclinical studies are needed to validate the relationship between adverse lactate trend and the risk of primary graft failure. It remains a major concern for clinicians to decide for non-eligibility of a donor heart for transplantation, just based on lactate trend.

Figure 1. Representations of favorable and adverse trends for serum lactate during isolate *ex situ* heart perfusion. Arterial lactate: red colomn; venous lactate: blue colomn; coronary flow: red line.

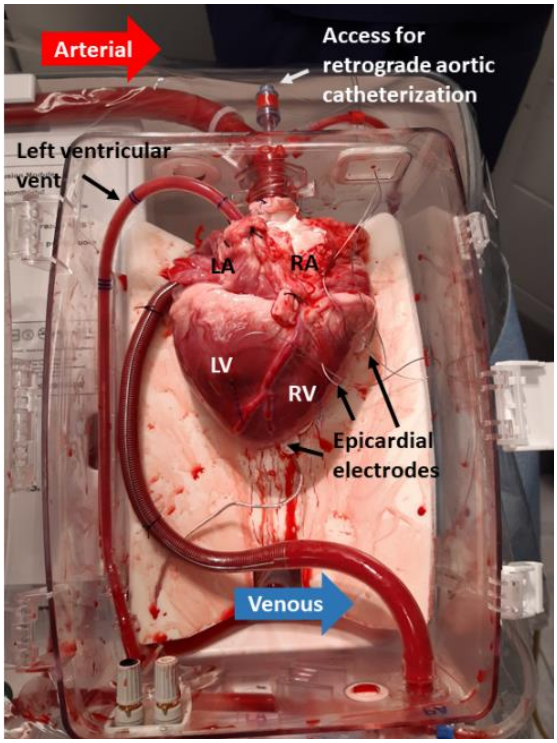


2. *Coronary imaging*

A growing number of donors with cardiovascular risk factors are proposed for procurement nowadays. Unfortunately coronary investigations are frequently unavailable at the donor site. A French retrospective study from the national transplant database showed that coronary angiography increases by 9% the acceptance of the donor heart.[36] Some groups have reported their preliminary experience of coronary angiography on isolated beating heart, mainly using OCS technology.[37,38] This approach is of major interest since donors at high-risk for coronary artery disease are routinely declined for procurement. *Ex situ* coronary imaging may therefore increase the chance for organ allocation and transplantation with reduced risk of primary graft failure. However there is no consensus considering the appropriate protocol for coronary angiography on the isolated beating heart. Indeed, the heart is hanged by the aortic cannula to ensure continuous coronary perfusion, with anterior wall of the left ventricle

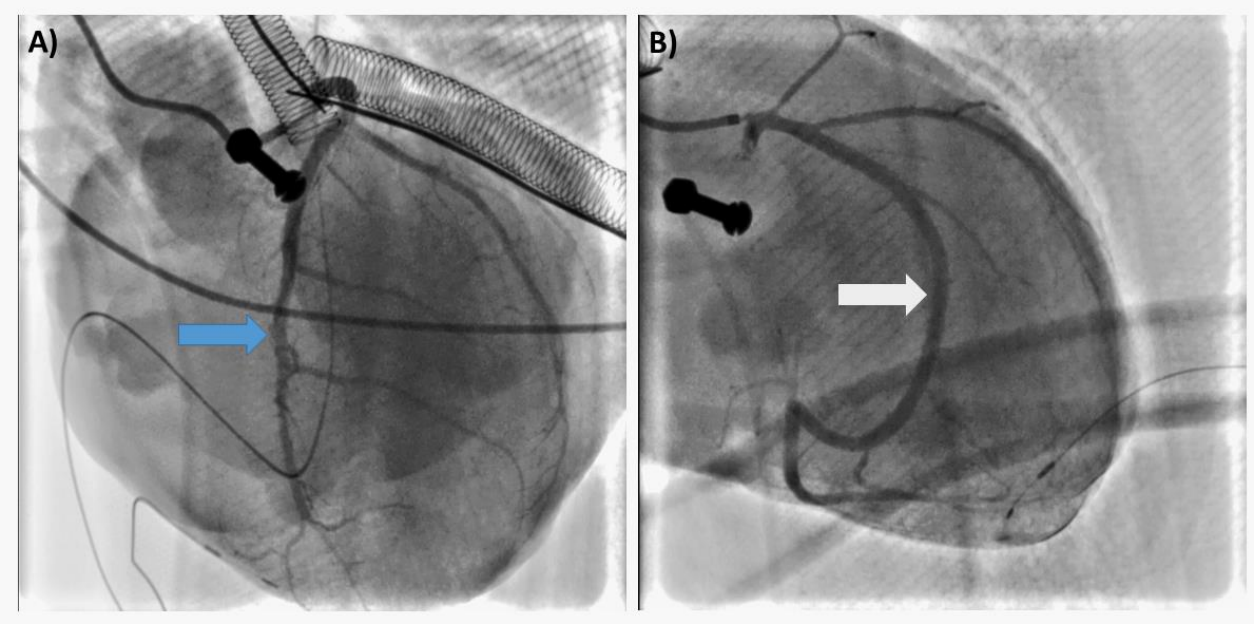
facing the bottom of the perfusion module (**Figure 2**). Conventional views usually applied for "inside the body" coronary angiography are therefore not suitable for the isolated perfused heart. In our experience, the OCS machine has to be elevated on a box to ensure safe rotations of the C-arm around the heart. A 5-French sheath has to be inserted into the secured vascular access on the arterial line in front of the aorta (**Figure 2**). A diagnostic catheter is then introduced in the aortic root using a 0.0035-inch guide wire. Sequential catheterization of the coronary ostia is performed using a 5-French left Amplatz catheter, considering the inverse position of the coronary vessels compared to *in situ* coronary angiography procedure. Rotation by 90° of the C-arm for horizontal beam orientation is necessary to record coronary angiogram (**Figure 3**).[30]

Figure 2. Instrumentation of the heart on the Organ Care System perfusion module (TransMedics, Andover, USA). Arterial access for coronary artery catheterization is remarkable at the end of the arterial line, in front of the aortic cannula. LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle.



Along with anatomical challenge to obtain coronary angiogram, this approach brings up several concerns. Radiopaque materials inside the OCS module may stop X-rays and markedly lead to misdiagnosis of coronary artery disease. Second, injection of contrast medium in a close circuit raises the issue of hemodilution. The hematocrit of the perfusate should remain above 20% as recommended by the manufacturer. Moreover, clearance of iodinated contrast agents on the OCS circuit is questionable. We hypothesize that prolonged exposition to iodinated contrast medium with low osmolality may result in deleterious myocardial edema and primary graft failure. Last, biological effects of ionizing radiation on an *ex situ* isolated perfused organ are unknown. Radiation is known to early induce endothelial cell dysfunction, characterized by increased permeability, apoptosis, and microthrombus formation.[39,40] Further studies are needed to investigate whether a dose-dependent effect along with patient's age and comorbidities (diabetes, high blood pressure) may influence the radiosensibility of the heart during *ex situ* perfusion. Other alternative approaches have been recently investigated in preclinical models for noninvasive assessment of myocardial microcirculation during *ex situ* heart perfusion including real time myocardial contrast echocardiography or 3-dimensional ultrafast echography.[41,42]

Figure 3. Left (A) and right (B) coronary angiogram performed during isolated *ex situ* perfusion of the heart. Left anterior descending artery (blue arrow) and middle right coronary artery (white arrow) are remarkable.



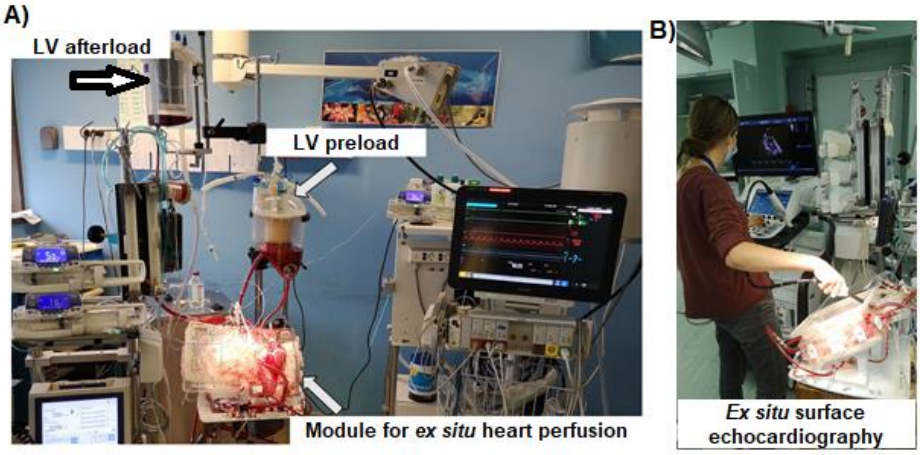
3. *Hemodynamic and functional assessment*

Cardiac function is usually investigated in the donor before organ retrieval. Recent emphasis has been observed about *ex situ* rehabilitation of donor hearts with extended criteria. Transplantability based on circulating biomarkers may be a concern, especially for DCD hearts with adverse lactate profile during *ex situ* perfusion. On the other hand, the rate of primary graft failure ranged from 15 to 30% for DCD hearts associated with favorable lactate trend before transplant.[14,15] Transplant physicians would therefore expect to get functional assessment of these marginal grafts before transplantation.

Commercially available machines for organ preservation do not provide indices for contractile performance, even during normothermic blood perfusion. In this approach, the organ is preserved in a beating state, but without loading left heart cavities (Langendorff mode). Surface echocardiography is theoretically feasible on OCS, but it doesn't provide any relevant analysis since the left ventricle is not loaded. Working mode perfusion is the only way to achieve both hemodynamic and ultrasound investigations on the isolated heart. Historically the first original OCS module was designed for working mode.[29] This approach was considered too complicated for clinical practice and Langendorff perfusion was favored.

In preclinical models, working mode conditions can be implemented for research purposes. After 30 to 60-min of stable Langendorff blood perfusion consistent with myocardial extraction of lactates, left heart cavities can be progressively filled. An additional canula should be introduced in the right atrium to achieve physiological left ventricular preload around 10 mmHg (**Figure 4**). The main challenge of this procedure is to avoid coronary air embolism. A left ventricular vent should therefore be left in place during all investigations. Similarly the aortic line has to be connected to a compliance chamber including an additional oxygenator and a cardiotomy reservoir to reproduce a left ventricular afterload of approximatively 70 mmHg (**Figure 4**). Some groups add a second centrifugal pump to ensure continuous preload for the left heart.[43,44] In working mode setting, hemodynamic and ultrasound investigations of the heart can be applied. Surface echocardiography can be performed using preferentially a transesophageal probe applied on the left atrium roof through the OCS sterile drape previously located around the heart (**Figure 4**). Transesophageal probe is manually rotated until 4-chamber view is obtained, and then adjusted to obtain 3 (100-120°) and 2-chamber (40-60°) views to allow for measurement of left ventricular ejection fraction and global longitudinal strain.[43-45]

Figure 4. (A) Custom circuit for *ex situ* isolated heart perfusion in working mode condition. Additional reservoirs allow for adjustment of left ventricular preload and afterload. (B) Surface echocardiography using transesophageal probe during *ex situ* heart perfusion. LV, left ventricle.



Pressure-volume loops is the gold standard method for assessment of cardiac hemodynamics. Initially validated in preclinical animal models, this approach has been recently applied in the field of *ex situ* heart perfusion.[44,46] After puncture of the left atrial roof, a pigtail conductance catheter can be located in the left ventricle. The position of the catheter is usually adjusted to obtain optimal volume signals. Minimum dP/dt, maximum dP/dt, left ventricular stroke work, left ventricular pre-recruitable stroke work and tau (time constant of isovolumic relaxation) can then be recorded after volume and pressure calibration steps. End-systolic elastance as well as end-diastolic pressure-volume relationship for the left ventricle can be assessed during partial transient occlusion of left atrial preload line over 5 consecutive cardiac cycles. Myocardial contractility and relaxation can thereby be discriminated independently from loading conditions. This invasive approach would however need to be correlated with ultrasound measurements or circulating biomarkers to avoid additional instrumental procedures.

Perspectives

The landscape of organ preservation for transplantation is evolving with the emergence of machine perfusion technology. This innovative approach is a major hope to safely expand the pool of donor hearts, and thereby to increase the chance for HT. Metabolic biomarkers are currently applied to ensure the transplantability of the donor heart, with limited correlations with cardiac performance to date.[47] Advances in functional assessment of the graft before transplant are expected, especially in the setting of DCD heart transplantation. Organizations dedicated for organ retrieval and preservation have to be defined, along with recognition of new professional skills. Multidisciplinary platforms for prolonged *ex situ* perfusion could soon allow for organ rehabilitation and therapeutic intervention to improve the post-transplant outcomes.[48]

Conclusion

Machine perfusion of donor hearts has recently gained major interest to improve postoperative outcomes in high-risk transplantations. Commercially available platforms for *ex situ* heart perfusion allow for longer preservation compared to static cold storage. Monitoring of the donor heart during dynamic preservation before transplant is currently based on metabolic profiling in clinical practice. Preliminary results of hemodynamic and ultrasound assessment for the isolated perfused heart are promising and require further investigations.

Conflicts of Interest Statement

The authors have no conflict of interest to declare.

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