Superior Health Council

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Ex vivo Organ Preservation, Evaluation and Reconditioning:
Machine Perfusion versus Cold Storage

7 September 2011

1. INTRODUCTION

On 6 January 2011, the Superior Health Council (SHC) received a request for advice from Minister Laurette Onkelinx concerning organ donation, *ex vivo* preservation and conditioning. The SHC is required to issue a comparative advisory report on the various techniques for *ex vivo* organ preservation and monitoring, especially for kidney transplants, including kidneys from donors known as "extended criteria donors" (ECD), and to assess their efficiency and safety, both for the transplanted organ as well as for its recipient.

This question is becoming increasingly important in view of the deteriorating donor profile and the growing use that is made of ECD (= donors after brain death but with co-morbidity such as older age, hypertension, high creatinine levels, etc.) and non-heart-beating donors (NHBD of DCD = donation after cardiac death) (see appendix 5.1.).

In order to be able to provide an answer to this question, an *ad hoc* working group was set up which consisted of experts in the following fields: abdominal and thoracic transplantation, nephrology, cardiac surgery, vascular graft specialists, transplant researchers, transplant coordinators.

2. CONCLUSIONS AND RECOMMENDATIONS

1) In Belgium, the referral of patients in need of organ replacement is on the increase whereas, at the same time, waiting times are growing and more patients are dying whilst on the waiting lists.

2) There are increasingly less standard organ donors available (fewer road accidents, better medical and surgical care thanks to improved treatment of critically ill patients), making it more and more necessary to resort to extended criteria donors (ECD) and donors after cardiac death (DCD), in spite of there being a more significant risk of primary non-function (PNF), post-transplant morbidity and mortality, and related healthcare costs.

3) Compared to cold storage, which is current practice for all organs in Belgium, machine perfusion (MP) has the potential:

- to improve the evaluation of the quality of the donor organ after recovery and before transplantation;
- to reduce cold ischemic injury, improve organ quality preservation and organ function after transplantation
- to recondition damaged or even discarded organs, making them transplantable again and, in doing so, to potentially increase the number of transplantations performed;
- and, to increase organ immunotolerance in the recipient.

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4) There are sufficient animal data available in the literature that verify the effectiveness and superiority of MP for organ evaluation and preservation compared to cold storage. Human data are now available for kidneys, lungs and hearts and confirm the benefits and the safety of machine perfusion, particularly for ECD and DCD. Clinical research is ongoing for livers and pancreases.

5) Given the increasing evidence in support of the clinical and financial advantages of MP, the SHC recommends that this method be implemented in view of reducing the gap between organ demand and supply in Belgium, starting with DCD and ECD kidneys.

6) However, MP is not being used in Belgium at the moment because current reimbursement for organ procurement does not cover the cost of essential devices, disposables, and personnel.

3. FURTHER DETAILS AND ARGUMENTATION

List of abbreviations

AST	Aspartate amino transferase
CI	Cold ischemia
CIT	Cold ischemia time
DBD	Donation after brain death or HBD
DCD	Donation after cardiac death or NHBD
DGF	Delayed graft function
DNA	Deoxyribonucleic acid
EC	EuroCollins
ECD	Extended/expanded criteria donors
ECMO	Extracorporeal membrane oxygenation
EVLP	*Ex vivo* lung perfusion
HBD	Heart-beating donors or DBD
HMP	Hypothermic machine perfusion
HOPE	Hypothermic oxygenated perfusion
HTK	Histidine-tryptophane-ketoglutarate
IRI	Ischemia-reperfusion injury
ISHLT	International Society for Heart and Lung Transplantation
L-FABP	Liver type fatty acid-binding protein
LTx	Lung transplantation
MP	Machine perfusion
NHBD	Non-heart-beating donors
NMP	Normothermic machine perfusion
OCS	Organ case system
OTS	Organ transport system
PEG	Polyethylene glycol
PFC	Perfluorocarbons
PNF	Primary non-function
PGD	Primary graft dysfunction
QALY	Quality-adjusted life years
SHC	Superior Health Council
SCD	Standard criteria donor
SCS	Static or simple cold storage
SCOT®	*Solution de conservation d'organes et de tissus*
TLM	Two-layer method
TX	Transplantation
UW	University of Wisconsin

3.1. Methodology

This advisory report is based on the opinion of experts as well as a review of the literature: meta-analysis, retrospective and prospective randomised trials, and feasibility/pilot studies.

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3.2. Further details

3.2.1. Introduction

Most organs for transplantation are obtained from donors after brain death (DBD), also called "heart-beating" donors (HBD). These are donors in whom death has been diagnosed by means of brain stem tests and who are maintained on a ventilator in an intensive care unit. Organ demand clearly cannot be met by using DBD donors alone. One way of expanding the deceased/cadaveric donor pool is to use organs retrieved from DCD donors, also called NHBD. Historically, organs from DCD donors have suffered higher rates of primary non-function (PNF), delayed graft function (DGF) and poorer long-term graft survival (Moers et al., 2007) (see definition in appendix 5.1). Apart from the increased use of DCD donors, a second means of expanding the pool of deceased organ donors is by using ECDs. These are organs from donors who, in the past, would not have met the criteria for transplantation under normal circumstances because these organs would have been considered to entail too high a risk of preservation injury and of poor post-transplantation function.

For treatment in transplant medicine to be successful, it is absolutely vital that the organs be preserved in optimal conditions once they have been retrieved from the donor's body. There are two main modes of *ex vivo* preservation: first, there is traditional cooling using cold preservation solutions and topical cooling with ice (Simple or Static Cold Storage: SCS). The second are perfusion systems (machine perfusion: MP), in which the organ is continuously perfused with a special preservation fluid in order to avoid as much organ damage associated with cold ischemia time (CIT) as possible.

The MP techniques are already being commonly used in the United States to preserve kidneys (> 20 %) and are increasly being applied in Europe as well. There are a series of retrospective studies, type 1 trials (meta-analyses and randomised, controlled prospective trials) available that demonstrate that this preservation method is superior to SCS (Moers et al., 2009).

The benefits shown to be gained by using MP for kidney preservation stimulated the development of similar technologies for other organs (liver, heart, lung and pancreas). For these other organs, the clinical trials are still at the experimental stage (phases I and II). Apart from the trials on kidney preservation, no randomised trial has been published to date that compares MP preservation for these organs with SCS preservation. However, the SHC believes that it is important that the general issue of preserving organs for transplantation purposes should be approached as a whole and that current and future developments in this area should be taken into account. The SHC therefore suggests that its advisory report should not be limited to *ex vivo* preservation techniques for kidneys, but that it should also discuss those that pertain to the heart, lungs, liver and pancreas on the basis of devices that already exist as well as the data from the literature.

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3.2.2. Kidney preservation methods

3.2.2.1. Introduction

Kidney transplantation (Tx) is the treatment of choice for end-stage renal disease. Compared to dialysis, kidney transplantation improves the quality of life and the life expectancy of patients suffering from end-stage renal disease, and considerably reduces the associated costs.

Table 1: Eurotransplant[1] reported in Belgium in 2008, 2009 and 2010.

	2008	2009	2010
Kidney Tx (Belgium)	448	435	408
Kidney Tx from DCD donors (DCD kidneys from abroad included)	49 (53)	69 (71)	55 (58)
Kidney waiting list	813	866	914
Waiting list mortality	18	27	34
Total of HB (DBD) donors	223	216	218
Total of NHB (DCD) donors	42	60	45
Total number of donors (one or more organs procured)	265	276	263
Number of kidneys accepted	487	486	445
Number of kidneys accepted but not transplanted eventually	41	32	37
Number of kidney donors (two kidneys used + only one kidney used)	235 (211+24)	237 (217+20)	215 (193+22)
Number of kidneys transplanted	446	454	408

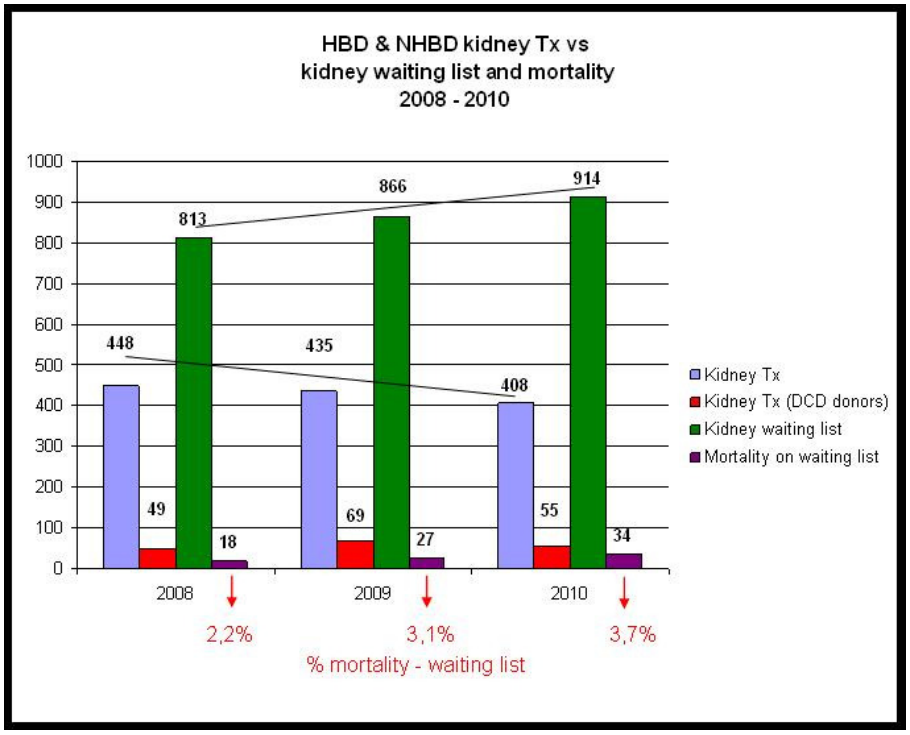

Figure 1: HBD & NHBD kidney transplantation versus kidney waiting list and mortality 2008-2010 in Belgium. Reproduced from Annual report Eurotransplant 2010.

[1] i.e. The organization responsible for the coordination and allocation of organs procured in Austria, Belgium, Croatia, Germany, Luxembourg, the Netherlands and Slovenia.

The number of "ideal" (standard criteria donors: SCD) donors has been decreasing over the years, compelling transplant surgeons to consider organs from non-ideal (higher risk) donors, viz. ECD and DCD organs.
The ECD criteria include (1) kidneys from donors over the age of 60 or (2) kidneys from donors over the age of 50 with at least two of the following features:

(1) a history of hypertension;
(2) death from a cerebral vascular accident;
(3) creatinine level over 133 µmol/l (1,5 mg/dl) (Port et al., 2002).

ECD kidneys have a higher incidence of DGF and a lower long-term survival rate, with a greater proportion of patients returning to dialysis and needing a second kidney transplant. DCD kidneys also suffer from a higher rate of DGF.

DGF refers to a kidney that does not function properly immediately after transplantation. Patients suffering from DGF require one or more sessions of dialysis until the kidney fully recovers. DGF is a frequent (20 - 30 %) complication in kidney transplantation and is an indicator for poorer post-transplant outcome. A rare and extreme form of DGF is PNF: in these cases, the transplanted kidney never recovers and the patient remains dialysis-dependent (> 1 %).

Three types of factors play a role in the appearance of DGF:

- donor factors: age, donor type (DCD and ECD kidneys have a higher rate of DGF);
- recipient factors: re-transplants, immunised patients and patients who have been on dialysis for a long time prior to transplantation have a higher rate of DGF;
- preservation period: the interval between organ procurement and transplantation (=CIT; CIT plays a major role in DGF (the longer the CIT, the higher the risk of DGF)).

All these factors are fixed and cannot be modified. However, it is possible to intervene during the preservation period, viz. both

- ***quantitatively by reducing the duration of the CIT***, and
- ***qualitatively by improving the preservation by MP.***

3.2.2.2. Kidney preservation

The oldest, standard method to preserve the kidney is SCS: the kidney is simply placed in an icebox using cold preservation solutions and topical ice cooling.

MP provides an alternative *dynamic* and more *controlled* means of preserving kidneys for transplantation. During MP, the kidney is placed in a special container and is continuously perfused with a specifically designed preservation solution at a fixed temperature (4°C) and at a given pressure. MP is more efficient in preserving the donated kidney in the interval between procurement and transplantation than SCS, which results in a significantly lower incidence of DGF, thus avoiding the need for post-transplant dialysis.

In Belgium, approximately 30 kidneys have been preserved by MP each year since 2000.

Several studies comparing MP and SCS; (Daemen et al., 1997; Kwiatkosski et al., 2007; Merion et al., 1990; Moustafellos et al., 2007; Plata-Munoz et al., 2008; Van der Vliet at al., 2001; Wight et al, 2003) have indicated that MP is superior to SCS.

Moers and colleagues – in cooperation with Eurotransplant - conducted the first large-scale prospective multicentre randomised controlled trial (involving part of Germany, the Netherlands and Belgium) (Moers et al., 2009). This study randomly assigned 672 kidneys from DCD donors and DBDs to MP or SCS. In the study, the primary end-point was DGF defined as the need for dialysis in the first week after transplantation. Secondary end-points were duration of DGF; severity of DGF defined by the rate at which the serum creatinine level decreased; PNF, rejection, and others.

Generally speaking, comparing MP kidney preservation with SCS kidney preservation (Moers et al., 2009) showed that the former resulted in

- a statistically significant drop in the DGF rate (SCS: 26,5 % - MP: 20,8 %);
- a statistically significant drop in the duration of the DGF (13 days to 10 days);

- a statistically significant increase in transplant survival (90 % to 94 %);
- a drop in the PNF rate from 4,8 % (SCS) to 2,1 % (MP) (p = 0,08).

More specifically, as regards ECD kidneys (Treckman et al., 2011), it appears that:
- the incidence of DGF is significantly less if the kidney is placed on MP compared to kidneys in SCS (SCS: 29,7 % - MP: 22,0 %);
- there is a clear statistical increase in transplant survival after 1 year (80 % to 92,3 %);
- there is a clear statistical increase in the survival of transplanted kidneys affected by DGF (41 % to 85 %).

A closer look at DCD kidneys reveals that (Jochmans et al., 2011):
- the incidence of DGF is significantly less when the kidney is placed on MP compared to kidneys in SCS (SCS: 69,5 % - MP: 53,7 %);
- there is a statistically significant drop in the duration of the DGF (13 days to 9 days);
- transplant survival after 1 year is similar;
- the length of the hospital stay is reduced (19 days to 17 days).

A British trial based on a smaller sample of DCD kidney recipients than the Eurotransplant trial did not find there to be any difference between MP and SCS as regards the incidence of DGF. In contrast to the Eurotransplant trial, the kidneys were not placed on MP immediately in this trial, but were first preserved by means of SCS; it also used other randomisation and allocation methods (Watson et al., 2010).

In sum, according to these trials, preserving kidneys by means of MP (when done immediately after procurement and continued until transplantation) is superior to SCS. This method appears to be particularly advisable for ECD kidneys (lower DGF incidence and higher transplant survival rate) and DCD kidneys (lower DGF incidence).

3.2.2.3. Cost-effectiveness / QALYs
During the first year after the operation, renal transplantation costs are in the same range as dialysis costs. However, after the first year, renal transplantation is both less costly and more effective (as a measure of QALYs: quality-adjusted life years) than dialysis (Cleemput et al., 2004). The financial impact of MP can be seen at three different levels. First, MP brings down the cost of transplantation because it reduces the rate of DGF and its associated costs (dialysis, additional investigations, biopsies, longer hospital stay, etc.). Second, MP kidney recipients display a better transplant survival whereas a higher proportion of SCS kidney recipients return to dialysis and need a retransplant. Third, it is estimated that MP will allow for more kidneys to be used to meet the increasing demand for transplantation. The Kidney Pancreas Committee of the Belgian Transplantation Society assessed that an additional 30 renal transplants could be performed each year if clinicians were able to preserve and evaluate the kidneys that are currently regarded as “too marginal” to be used. Indeed, in contrast to SCS, it is possible to determine the quality of the kidneys on MP by measuring the concentration of certain biomarkers in the perfusate (Moers et al., 2010) and by measuring the flow/resistance during perfusion (Jochmans et al., 2011). It follows that, by improving the results of and access to kidney transplantation, Belgium could achieve significant cost savings.

A specific study for Belgium has shown that this method leads to lower costs in the short term and that it is also advantageous in the long term (data not published).

3.2.2.4. Devices
See appendix 5.2.1.

3.2.2.5. Conclusion
At least one randomised clinical trial (type 1) has shown that, compared to SCS kidneys, kidneys that have been preserved by MP in the interval between procurement and transplantation work better and longer after transplantation, thus reducing the incidence of PNF and DGF, enhancing transplant function and allowing longer transplant survival. These benefits are particularly strong for kidneys obtained from ECDs (reduced DGF; better transplant survival) and from DCD donors (reduced DGF).

MP reduces the costs associated with kidney transplantation.

Improved high-risk organ preservation as well as the possibility of evaluating these organs during the preservation period will lead to increased use of ECD and DCD kidneys and will contribute towards reducing the gap between the number of available kidneys and the number of patients on the waiting list.

Given the fact that in Belgium as well as worldwide, the number of organs known as “standard” donor organs is gradually going down (fewer road accidents, better medical and surgical care), whilst the number of ECD and DCD organs is on the rise, MP provides an appreciable and by no means insignificant advantage both from a clinical and a financial point of view.

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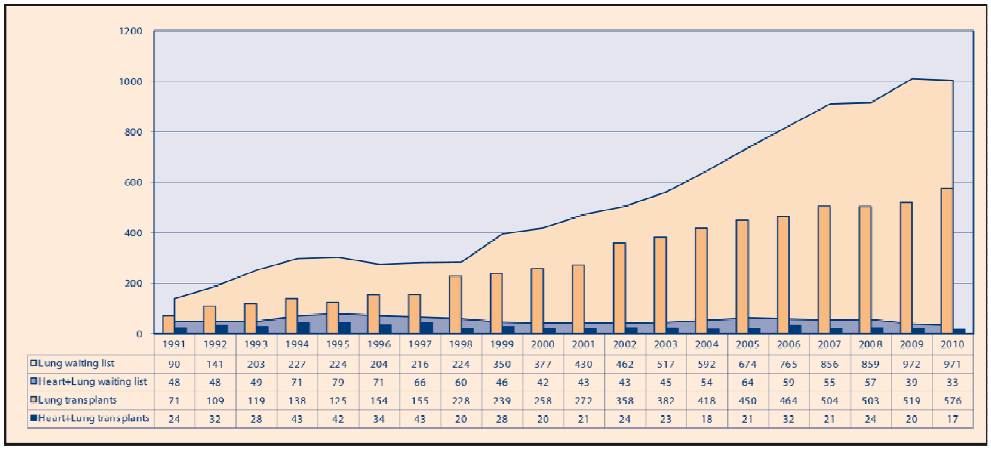
3.2.3. Lung preservation methods

3.2.3.1 Introduction

Lung transplantation (LTx) has become a standard treatment option for select patients suffering from end-stage lung disease (Christie et al., 2010). This form of therapy still faces significant challenges, including donor organ shortage, primary graft dysfunction (PGD) and bronchiolitis obliterans syndrome. According to the International Society for Heart and Lung Transplantation registry (ISHLT, 2009), PGD accounts for nearly 30 % of 30-day mortality after LTx. The early success of LTx is directly linked to the immediate resuming of organ function in the days after transplantation, with extracorporeal (cardio)pulmonary support severely compromised if needed for longer than a week. Better lung donor selection and management (Van Raemdonck et al., 2009) as well as adequate lung preservation and procurement (Shigemura et al., 2009) are key steps in the successful transition from donor to recipient that may significantly influence the quality of the transplant organ and thus the outcome in the transplant patient.

3.2.3.1.1. Eurotransplant

According to the 2010 Eurotransplant Annual Report (Eurotransplant, 2010), over 1.000 patients were waiting for a new lung on 31 December 2010. In contrast, only 593 patients actually received a lung transplant in 2010. The gap between lung donors and recipients in the Eurotransplant registry has continued to widen over the last two decades (Figure 2), resulting in 30 % of the patients remaining on the waiting list for over 24 months and 137 (about 14 % of listed patients) dying prematurely.



	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Lung waiting list	90	141	203	227	224	204	216	224	350	377	430	462	517	592	674	765	856	859	972	971
Heart+Lung waiting list	48	48	49	71	79	71	66	60	46	42	43	43	45	54	64	59	55	57	39	33
Lung transplants	71	109	119	138	125	154	155	228	239	258	272	358	382	418	450	464	504	503	519	576
Heart+Lung transplants	24	32	28	43	42	34	43	20	28	20	21	24	23	18	21	32	21	24	20	17

Figure 2: Dynamics of the Eurotransplant lung waiting list and lung transplants.
Reproduced from the 2010 Eurotransplant Annual Report.

3.2.3.1.2. Belgium

According to the 2010 Eurotransplant Annual Report (Eurotransplant, 2010), on 31 December 2010, 90 patients were registered on the lung waiting list in Belgium and 114 recipients had received a lung transplant in 2010. Nearly 72 % of the patients are transplanted within the first year and only 4,5 % of the listed patients wait for more than 2 years for a suitable donor lung. Six patients only (7 % of listed patients) died prematurely whilst on the waiting list. The situation in Belgium, which has a presumed consent law regarding organ donation, is therefore much better than that in other Eurotransplant countries with different legislations, such as the Netherlands and Germany. The current survival rate is estimated to be 90 % after one year, 80 % after three years and 70 % after five years.

Nevertheless, there are two ways in which the early outcome of LTx could still be improved in Belgium, viz.:

- by increasing the number of available organs, thereby shortening the waiting time and further reducing waiting list mortality;
- by bringing down the incidence of PGD (± 15 %) and related mortality (± 1 %). Indeed, PGD leads to lengthy stays in the intensive care unit and in hospital and to an increased risk of bronchiolitis obliterans and chronic graft dysfunction appearing at a later stage.

Further research is needed to find better preservation solutions as well as new preservation techniques in order to allow for more ECD lungs with longer CITs to be accepted and improve donor lung quality prior to LTx, respectively.

3.2.3.2 Lung Preservation

3.2.3.2.1. Cold storage

Although no large-scale randomised controlled trials in human LTx are available, existing data suggest that the extracellular-type preservation solutions (e.g. Perfadex®, Celsior®, ET Kyoto®) are superior to the traditional intracellular-type solutions (modified EuroCollins (EC) and University of Wisconsin (UW)), as they appear to be associated with a reduced incidence of ischemia-reperfusion injury (IRI) (Van Raemdonck, 2010) (Table 2). Initial antegrade cold blood pulmoplegia (through the pulmonary artery) followed by a retrograde *flush* (through the pulmonary veins) may help to improve organ quality by removing debris and microthrombi from the pulmonary vasculature, although the scientific evidence for this practice is scarce. It is also commonly accepted that lungs should be inflated with 50 % oxygen to extend the duration of tolerable cold ischemia (CI). Using the techniques mentioned above, transplant surgeons now feel comfortable with CIT reaching up to 10-12 hours.

Table 2: Studies comparing Perfadex (low-potassium dextran glucose) and modified EC in clinical LTx. Reproduced from Van Raemdonck 2010.

Reference	Study	LPDG patients	Euro-Collins patients	Survival at 30 days (%) LPDG	Euro-Collins	*P*	Initial graft function LPDG	Euro-Collins	*P*
Müller *et al.* [17]	R	32	48	94	88	0.36	159 ± 145[a]	242 ± 265[a]	0.028
Strüber *et al.* [18]	R	57	63	92	85.8	0.35	34 ± 11[b]	30 ± 10[b]	0.04
Fischer *et al.* [19]	R	46	48	93.5	89.6	0.82	370 ± 133[c]	310 ± 134[c]	0.017
Rega *et al.* [20]	R	50	50	98	90	0.04	168 ± 14[a]	249 ± 22[a]	0.001
Rabanal *et al.* [21]	R	21	25	100	88	NA	321 ± 111[c]	271 ± 103[c]	0.05
Aziz *et al.* [22]	R	32	37	90.7	89.2	0.88	266 ± 59[c]	244 ± 51[c]	0.9
Nath *et al.* [23]	R	115	116	93	95	N.S.	8%[d]	20%[d]	0.03
Oto *et al.* [24]	R	40	79	97.5	94.9	0.87	16%[d]	46%[d]	0.01
Ganesh *et al.* [25]	R	151	284	90.7	86.0	0.57	5%[e]	9%[e]	N.S.
Ferraro *et al.* [26•]	R	65	65	93.8	89.2	0.64	287 ± 137[c]	256 ± 119[c]	0.348

30-day, survival at 30 days after transplant; LPDG, low-potassium dextran glucose; NA, not available; NS, not significant; R, retrospective study.
[a] Alveolar–arterial oxygen gradient (mmHg).
[b] Lung compliance (ml/mmHg).
[c] paO_2/FiO_2 ratio at 24 h (mmHg).
[d] Primary graft dysfunction grades 2–3.
[e] Death from primary graft dysfunction.

3.2.3.2.2. Machine perfusion

History

Ex vivo perfusion of thoracic organs was historically reported on as a technique to preserve hearts and lungs during distant procurement (Hardesty & Griffith, 1987). Renewed interest has recently been shown in the use of *ex vivo* lung perfusion (EVLP) as a technique to evaluate lungs prior to transplantation. The first clinical case report of successful LTx after EVLP was published by Steen and colleagues in 2001 (Steen et al., 2001). A single left lung recovered from a Maastricht Category II NHBD after 65 min of warm ischemia and three hours of topical cooling was evaluated in an *ex vivo* circuit before it was accepted for transplantation. This unique case report demonstrated for the first time that lungs can be transplanted successfully after a period of warm ischemia, *ex vivo* perfusion and evaluation, and cold storage.

Experimental studies

The work with animals initiated in Steen's lab in Lund, Sweden (Steen et al., 2003) has prompted research groups worldwide to further investigate the technique of EVLP and its role in evaluating DCD lungs in an attempt to increase the number of lungs available for transplantation (Erasmus et al., 2006; Rega et al., 2003; Snell et al., 2006). Experimental reports on EVLP in human lungs followed (Egan et al., 2006; Neyrinck A et al., 2004; Wierup et al., 2006).

Potential applications of EVLP

It is hoped that the technique of EVLP will lead to new applications that will potentially increase the donor pool and change clinical practice for LTx in the future.

- EVLP for lung evaluation

Donor lungs are currently discarded on the basis of preset criteria such as oxygenation capacity, bronchoscopic findings, and radiographic appearance. These parameters do not always allow to obtain an accurate picture of the lung's clinical condition and therefore on-site evaluation, including macroscopic appearance, is of utmost importance. Nevertheless, the decision to discard the donor lungs for transplantation is often taken in a subjective manner, as time constraints often prevent a thorough evaluation from being carried out during organ procurement and oxygenation does not always match macroscopic findings. EVLP therefore constitutes a useful tool to re-evaluate the lungs under optimal conditions. Ventilation-perfusion matching can be optimised by means of ventilation induced dilation of all the alveoli and full microvascular perfusion. More lungs could be accepted safely if EVLP were routinely used by the most experienced member of the team to assess donor lungs in the recipient hospital.

- EVLP for lung preservation

Past attempts at machine preserving lungs for a lengthy period of time have largely failed because the integrity and normal barrier functions of the vasculature and epithelial membranes could not be maintained, which resulted in progressive deterioration of the vascular flow and the concurrent development of oedemas. The modern success of EVLP without oedema formation is partly due to the use of a buffered, extra-cellular solution with an optimal colloid osmotic pressure, such as the perfusate developed by Steen, now commercially available as Steen Solution® (Vitrolife AB, Gothenburg, Sweden). Much experimental work was recently carried out at the University of Toronto by the group led by Keshavjee. Research on pig lungs demonstrated that twelve hours of EVLP at physiological temperature using an acellular perfusate was achievable and maintained the donor lungs without inflicting significant additional injury (Cypel et al., 2008). Such extended EVLP opens perspectives to preserve and treat donor lungs for a longer period of time. Further studies in a pig LTx model nicely demonstrated that, after 12 hours of cold storage, pretransplant EVLP for 12 hours prevented further lung injury, whereas this was not the case in a control group with continued cold storage for an additional 12 hours (Cypel et al., 2009).

- EVLP for lung resuscitation and repair

Many donor lungs sustain injury before and after the onset of brain death as a result of contusion, atelectasis, aspiration, infection, or neurogenic oedema formation. Research is being conducted to investigate whether the quality of non-acceptable lungs can be adequately improved during pre-transplantation EVLP by direct pharmacological intervention on the transplant via an endotracheal or intravascular route.

Recently the Steen group in Lund reported on a series of 6 successful double transplantations of lungs that were initially discarded (Ingemansson et al., 2009). The donor lungs were reconditioned *ex vivo* in an extracorporeal membrane oxygenation (ECMO) circuit with Steen Solution® mixed with erythrocytes to form a hyperoncotic solution that can dehydrate oedematous lung tissue. Functional evaluation was performed with deoxygenated perfusate by varying the fraction of inspired oxygen. After the reconditioning, the lungs were kept immersed at 8°C in the perfusate on the ECMO circuit until the moment of LTx.

A clinical trial recently reported was performed in Toronto to assess the feasibility and safety of EVLP in ECD lungs in the hope of increasing their utilisation rates and improving transplantation outcomes (Cypel et al., 2011). So far, 20 human lungs from non-SCD (DBD or NHBD) were placed in an *ex vivo* circuit (Toronto XVivo™ system) (Figure 3) and perfused normothermically with Steen Solution® for 2 to 4 hours for physiologic re-assessment. Lungs that fulfilled the criteria of good oxygenation capacity, low compliance and airway pressures were transplanted. When compared to a control group of 136 lung recipients transplanted in the same time period with standard-donor lungs, the incidence of PGD at 72 hours after LTx was reduced by half (15 % versus 30 %, respectively; p = 0,11) with no differences in early outcomes between groups and no side effects related to the EVLP technique. A smaller experiment with human lungs has been reported on by the group at Harefield (Zych et al., 2010). Other lung transplant groups in Europe (Vienna, Hannover, Newcastle, Milan, Paris) have now embarked on the clinical transplantation of ECD lungs after EVLP (personal communication). EVLP has now provided a potential platform to repair lungs *ex vivo* prior to transplantation by allowing direct intervention on the transplant via an endotracheal or intravascular route. Possible pharmacological applications in the *ex vivo* circuit include using high osmotic perfusates or ß-adrenergic drugs to

accelerate the removal of lung oedemas, bronchodilating and vasodilating agents to improve ventilation-perfusion mismatch, antibodies blocking pro-inflammatory response, antibiotics added to the perfusate to help sterilise pneumonias, and fibrinolytics to help remove pulmonary microthrombi.

Figure 1 Schematic of the ex-vivo lung perfusion system

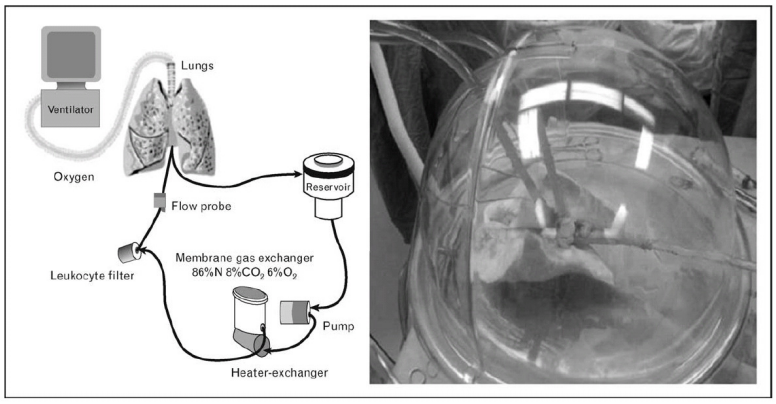


Figure 3: Diagram of the EVLP system. The lungs are placed within the XVIVO^{TM} chamber (Vitrolife AB, Gothenburg, Sweden). The perfusate leaves the lungs via the left atrial cannula and enters the reservoir. From there, the perfusate is pumped using a centrifugal pump into the oxygenator and heat exchanger where it is deoxygenated by a gas mixture (86 % N_2, 8 % CO_2 and 6 % O_2) and warmed to normothermia. The perfusate then passes through a leucocyte filter before reentering the lungs via the pulmonary arterial cannula for oxygenation. Reproduced from Cypel M et al., 2009.

- EVLP for lung reconditioning

Bronchiolitis obliterans syndrome resulting from chronic allograft rejection remains the main adverse factor affecting long-term survival after LTx. EVLP is hoped to become a technique that may help to induce tolerance to the transplanted lung in the recipient by *ex vivo* immunotherapy or gene therapy. No experimental data have been published so far using EVLP to prevent acute or chronic allograft rejection.

3.2.3.3. Devices

See appendix 5.2.2.

3.2.3.4. Conclusion

New extra-cellular preservation solutions for donor-lung cold storage have contributed to the recent reduction in the incidence of PGD over the last decade, leaving more room to extend the donor criteria and CIT.

EVLP is now on the horizon as a method to evaluate and accept marginal lungs in a more objective way, to extend the preservation period safely, and to repair lungs of inferior quality. More scientific and financial data are needed to evaluate the role of available lung preservation devices and their impact on the number and quality of donor lungs for transplantation.

3.2.4. Heart preservation methods

3.2.4.1. Introduction

The number of heart transplantations performed each year has been declining for two decades as a consequence of organ shortage. This shortage has led to longer waiting times on the transplant list (median waiting time = 76 days in 2007 vs 118 days in 2009) and a rise in waiting list mortality (Eurotransplant 2009 and 2010). For instance, in 2010, 105 new patients were placed on the waiting list for a heart transplant in Belgium, 68 received a transplant and 18 died before the operation (Eurotransplant Annual report 2010).

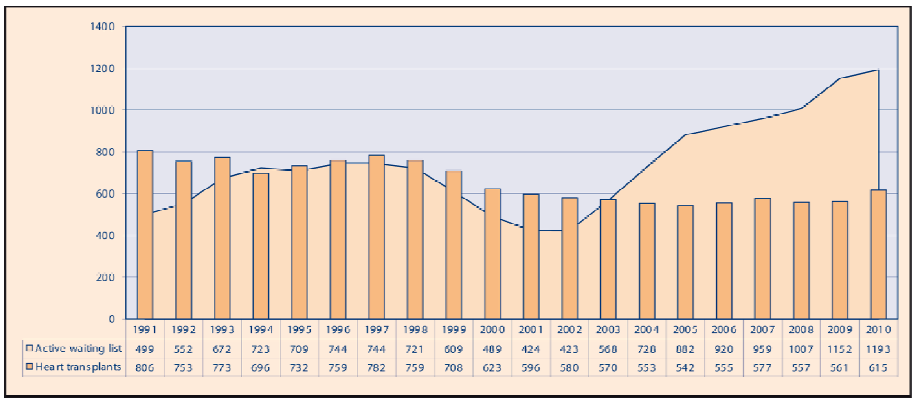


Figure 4: Dynamics of the Eurotransplant heart waiting list and heart transplants. Reproduced from Annual Report Eurotransplant 2010.

Consequently, transplant centres have developed different strategies to treat terminal heart failure patients. First, the use of mechanical circulatory support as a bridge to transplantation is becoming more and more frequent, which results in higher costs for the community (1 implantable ventricular assist device = +/- 75.000 euros). Secondly, the procurement of organs from older or marginal donors is on the rise to maintain transplant activity (Eurotransplant 2010; Stehlik et al., 2010). Since MP has been shown to be superior to SCS for the preservation of ECD kidneys (Moers et al., 2009; Moers et al., 2011), attention to this type of preservation technique has grown over the past years in the heart transplant community.

3.2.4.2. Heart preservation

Animal Studies

It appears that MP improves the maintenance of myocardial metabolism, with investigators reporting a reduction in intracellular lactate (Rosenbaum et al., 2008; Rosenbaum et al. 2007; Van Caenegem et al., 2011), a higher phosphocreatine/creatine ratio (Van Caenegem et al., 2011), an increase in glucose production (Ferrera et al., 1994) and greater fatty acid turnover in the heart (Hudin et al., 1991). These metabolic events appear to translate into improved intracellular high-energy phosphate levels (Ferrera et al., 1994; Peltz et al., 2005; Van Caenegem et al., 2011) and reduced oxidative stress in the myocardium (Fiton et al., 2004).

A decrease in myocardial damage has been observed by carrying out a quantification of ultrastructural changes in the myocyte (Ferrera et al., 1994; Ferrera et al., 1998), assays of DNA damage (Fiton et al., 2004; Fiton et al., 2005), apoptotic cell counts in reperfused heart tissue (Ozeki et al., 2007; Peltz et al., 2005; Rosenbaum et al., 2007) or by measuring cardiac enzyme levels after reperfusion (Rosenbaum et al., 2008). These benefits have been described even with prolonged preservation periods (Ferrera et al., 1994; Hassanein et al., 1998; Poston et al., 2004; Wicomg et al., 1989). Wicomb et al. (1986) report on the successful transplantation of canine hearts after 48-hour MP. Studies evaluating early post-reperfusion ventricular performances have shown improvements with MP compared to SCS, even for conventional storage periods (Rosenbaum et al., 2008; Rosenbaum et al., 2007).

Human studies

Human data are very scarce in the literature.
The PROTECT 1 study (Tenderich et al., 2007) was a safety and performance trial conducted in Europe between January 2006 and February 2007. It involved 20 patients who received a heart that had been preserved on the Organ Care System (OCS™). The mean preservation time was 298 min, but the effective CIT was 22 min. The reported survival at 30 days (Tenderich et al., 2007) and 1 year (Tenderich et al., 2008) is particularly high (100 %). The PROTECT II trial was conducted between February 2007 and December 2008. It involved 43 patients.
The PROCEED study (McCurry et al., 2008) was a safety and performance study performed in the USA between April 2007 and February 2008 with 14 patients. The 30-day survival was 11/14 patients. One patient died from PGD. The company reports that the overall 30-day survival for patients included in the 3 studies was as high as 97 %. These trials have enabled the authors to conclude that serum lactate levels and perfusion parameters (high resistance) are predictive of graft dysfunction after MP (Hamed et al., 2009).
The PROCEED II trial is currently ongoing in the USA. In this study, MP is compared to SCS for the preservation of traditional donor hearts.

Technical aspects

All the available devices use an oxygenator to supply oxygen to the perfusion solution.
The OCS™ use normothermic blood perfusion. The blood is collected from the donor at the time of procurement. This might be less than ideal, with this blood carrying inflammatory mediators (cytokines, neutrophils, activated complement, etc.) from the donor (brain death or cardiac death). The advantage is that the heart keeps on beating in the device during aerobic metabolism. The left ventricular function can be investigated. Unfortunately, this is not the case for the right ventricular function, the right ventricle not being filled. Perfusion parameters (high resistance) and final serum lactate are predictive markers of graft dysfunction after MP.

The LifeCradle® and the HeartPort® systems use hypothermic perfusion with preservation solutions. They are cheap and easy to set up. Myocardial oedema has been reported in some studies (Ferrera et al., 1994; Fiton et al., 2004). A moderate water content increase does not seem to be related to post-preservation graft dysfunction (Ozeki et al., 2007; Poston et al., 2004).

3.2.4.3. Potential benefit

Improving the preservation technique has numerous potential benefits.
Continuous perfusion may wash out metabolites that accumulate during the ischemic process.
Transplant failure is responsible for 40 % of the peri-operative deaths after heart transplantation. An ischemic time over four hours is an independent risk factor for transplant failure and may affect 1-year post-transplant survival (Stehlik et al., 2010). MP could increase the ischemic time that has no deleterious impact on the organ. MP could also increase the donor pool by extending the ischemic time and procuring hearts from a broader range of donors than is currently the case by using marginal donors and, ultimately, even NHBD.

The hypothetical economic value of MP derives from two different clinical contributions, viz. (1) improved transplant outcome and (2) increased supply of transplantable hearts. By comparing their ischemic times to data in the literature, Transmedics® assessed that the improved outcome would result in a net economic value of 51.408 euros per procedure, whereas the increased heart supply would lead to a net economic value of 273.171 euros per procedure (Transmedics® data). However, this needs to be confirmed in clinical trials.

3.2.4.4. Device

See appendix 5.2.3.

3.2.4.5. Conclusion

1. In animal studies, MP seems to improve the metabolic preservation of hearts compared to SCS. Myocardial injury resulting from ischemia appears to be less significant with MP. MP also seems to enhance functional recovery after preservation.

2. Human data are scarce. The technique is feasible and safe. Until now, no reproducible data have been published that prove that MP is clinically superior to SCS in heart transplantation. The ongoing PROCEED II trial will compare MP to SCS for the preservation of traditional donor hearts for transplantation. There are no data regarding the resuscitation of heart transplants from marginal donors.

3. No data compare hypothermic MP to normothermic beating heart perfusion preservation.

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3.2.5. Liver preservation methods

3.2.5.1. Introduction

The current standard liver preservation method is SCS. Liver transplants are first flushed-out and cooled down using UW, Histidine-Tryptophan-Ketoglutarate (HTK), Celsior, or Marshal solution and then stored in an organ preservation solution on melting ice (4° C) - the so called "ice box" - during transportation. During this period, as is the case for all other solid organs, liver transplants are thus deprived of oxygen. Tissue oxygenation is restored at the time of reperfusion in the recipient, which is a phenomenon known as IRI. SCS is still the golden standard to preserve livers, which are exposed to three types of ischemia during the transplantation process: *in-situ* CI (prior to procurement), *ex-situ* CI (during preservation) and *in-situ* ischemia (during implantation, right before reperfusion). Hypothermia slows down the metabolic rate, protecting livers - to a certain extent - against hypoxic damage to which the organs are inevitably exposed between procurement and transplantation. This is particularly true for livers obtained from "ideal" donors, which can be safely preserved by means of cold storage for 12 hours. However, SCS preservation has its limitations, especially for high-risk transplants. At 4°C, the metabolism slows down, but is still present and livers suffer progressive energy depletion, acidosis, a loss of electrolyte homeostasis (influx of Ca and Na, and an efflux of K), and cell swelling. All these changes are exacerbated immediately after reperfusion due to the production of radical oxygen species, which leads to the activation of phospholipases and protease and a series of enzymatic cascades, thus promoting inflammation. Kupffer cells and leukocytes play a central role in this process. Because ECD and DCD livers are more prone to CI and the subsequent IRI, these livers should/can be stored by SCS for only short periods of time.

With the increasing use of ECD and DCD livers comes the quest for

- improved liver preservation methods, and
- the possibility to assess the quality and viability of livers prior to transplantation.

Liver MP preservation might have the potential to fulfil these two goals.

Among the main advantages that MP is hoped to confer, there is the improved preservation of *all* (standard criteria, ECD and DCD) types of donor organs. Compared to SCS, MP provides many *anticipated* advantages including (1) continuous circulation and enhanced preservation of the microcirculation, (2) continuous nutrients and oxygen delivery to meet the organ's metabolic requirements, (3) removal of metabolic waste products and toxins, (4) opportunity to assess organ viability, (5) better clinical outcome as a result of improved immediate graft function rates, (6) prolonged preservation time without increased preservation damage, (7) administration of cytoprotective and immunomodulating substances, and (8) financial benefit due to a lower graft dysfunction incidence, shorter hospital stays, and better transplant survival rates.

3.2.5.2. Current principles of MP liver preservation

Directly applying hypothermic machine perfusion (HMP) to livers (as is the case for kidneys) does not seem to work well. This is what the Leuven group observed in a porcine model of transplantation using non-oxygenated UW for 4 hours through the portal vein and hepatic artery (Monbaliu, 2006).
There are obvious differences between livers and kidneys on a macroscopic, microcirculatory and cellular level that should be taken into account when determining the optimal settings for liver MP. There are three types of liver MP protocols, depending on the preservation temperature: normothermic (body temperature), hypothermic (4-6°C) or subnormothermic (20°C).

Table 3: Three types of liver MP.

	Hypothermic	**Normothermic**	**Subnormothermic**
Temperature	4-6°C	32-39°C	20°C
Solution	a-cellular	sanguineous	a-cellular/sanguineous
Oxygenation	diffusion or oxygen carrier	oxygen carrier	diffusion or oxygen carrier
Perfusion pressure	< physiological	physiological	not yet defined
Route of perfusion	single/dual vessel	dual vessel	single/dual vessel
Perfusion type	continuous/pulsatile	continuous/pulsatile	continuous/pulsatile

Research is currently being carried out to define and fine-tune the ideal HMP conditions for the liver. Various parameters have been tested in experimental models, viz. flows and pressures (pulsatile or not), single (hepatic artery, or portal vein) or dual perfusion, oxygenation or no oxygenation, different MP preservation solutions, and various temperatures. Several reviews on this subject are particularly worth pointing out: Dutkowski et al., 2008; Fuller & Lee, 2007; Schreinemachers et al., 2007; van der Plaats et al., 2004; Monbaliu & Brassil, 2010.
Some rodent models have been designed to investigate liver HMP. Only successful protocols in preclinical large animal models will be discussed. Besides animal studies, only one pilot trial has recently proven the feasibility of preserving standard livers by HMP and successfully transplanting them (Guarrera et al., 2009).

Animal studies

Many animal studies using rodent models suggest that liver HMP is superior to SCS (Monbaliu, 2010). Data from large animals are scarce. In 1990, Pienaar reported that 7 out of 8 dogs survived the transplantation of a liver that had been preserved by HMP for lengthy periods of time, viz. up to 72 hours (Pienaar et al., 1990). However, it has been difficult to reproduce these experiments and, what is more, it seems that canine models might not appropriately reflect the clinical situation. Pig models are a better alternative, and Dutkowski used such a model to show that a short period of oxygenated HMP improves transplant condition and post-transplant outcome (Dutkowski et al., 2009). However the most promising results for liver MP have been obtained in livers that were perfused at warmer (i.e. near to normal) temperatures in the presence of oxygen. In fact, normothermic machine perfusion (NMP) reproduces a normal metabolism *ex vivo*. NMP for the liver has been mainly developed in Oxford and Berlin. Using rigorous porcine liver transplantation models, these two groups showed beyond any doubt that transplants exposed to warm ischemia can be fully resuscitated by means of NMP (Vogel T et al., 2010).

Human studies

Data on liver MP preservation are extremely scarce, despite the advantages observed in experimental studies.
Ex vivo HMP for livers (using diluted, heparinised and oxygenated blood through the portal vein and hepatic artery) was, promptly applied by Thomas Starzl for the first 11 human liver transplantations (Starzl et al., 1968; Brettschneider et al., 1969). but was abandoned later when SCS became available.
Today only one pilot trial has proven the feasibility of preserving standard livers by HMP and successfully transplanting them (Guarrera et al., 2009). In this clinical phase I trial, a portable bypass system provided a flow-controlled perfusion (0,7 ml/g liver/min) that was split into the hepatic artery and portal vein, reaching a pressure of 3-5 and 12-18 mmHg, respectively. This trial used Vasosol (Holdings East, Glenmoore, USA), a modified UW-MP preservation solution, to which anti-oxidants, metabolic substrates and vasodilators were added. A successful outcome was achieved by transplanting 20 normal livers after 4-7 hours of oxygenated HMP. The total CIT remained below 12 hours. Low and stable perfusion pressures were reached, viz. mean pressures of 5,5 mmHg for the arterial flow and 3 mmHg for the portal flow, respectively. Although the livers were not actively oxygenated during HMP, pO_2 levels remained relatively high (mean of 137,2 mmHg) as a consequence of ambient air interchange at the organ chamber. Interestingly, the transaminase peak in the MP preservation solution (regarded as a potential marker to assess viability) after 2 hours of HMP correlated well with the transaminase serum peaks after transplantation.
Besides this hypothermic approach, a clinical trial using NMP preservation is currently planned by the Oxford team.

3.2.5.3. Potential benefit of viability assessment

There are no objective tools available to date to assist clinicians in the evaluation of human livers for transplantation. The decision to accept or discard a particular liver is still based on the judgment of the surgeon, who takes into account the medical history of the donor, the liver biochemistry, the macroscopic and – if necessary – the microscopic aspect of the liver.
Compared to SCS, HMP provides a window between procurement and transplantation during which organ quality can be directly assessed *ex vivo*. Liu from the Leuven group observed that, during porcine-liver HMP, the cumulative release of L-FABP (Liver type fatty acid-binding protein) and aspartate amino transferase (AST) can be measured and used to distinguish moderately damaged

livers from severely damaged ones (Liu et al., 2009a). Similarly, during the HMP of human livers discarded for transplantation, cumulative AST release in MP preservation solution can allow for potentially transplantable livers to be distinguished from absolutely non-transplantable ones (Liu et al., 2009b).
Functional evaluation provides another opportunity to assess viability. Ideally, such an evaluation requires normo- or near-to-normothermic conditions, including detoxification capacity, metabolic synthetic ability as well as the uptake, transportation and biliary excretion of substances. Additionally, cytosolic enzymes can be monitored to assess pre-existing or ongoing organ damage. Implementing NMP will result in more red blood cells being required, which may constitute an additional difficulty when different organ procurement/preservation teams have to retrieve/share donor blood. As a result, research into the use of artificial oxygen carriers is greatly needed.
Finally, the ultimate potential benefit will be to recondition livers during NMP.

3.2.5.4. Device
See appendix 5.2.4.

3.2.5.5. Conclusion
Animal studies have shown that *ex vivo* oxygenated HMP and NMP are clearly superior to SCS, especially for ECD and DCD livers. NMP seems particularly efficient to recondition livers that would otherwise fail. Feasibility trials will hopefully confirm the promising preclinical results of NMP.

As is the case with *ex vivo* NMP, *in vivo* artificial normothermic recirculation (which takes place in the donor prior to organ procurement) has the potential to reverse the substantial ischemic injury sustained by livers from uncontrolled DCD. *In vivo* normothermic recirculation has the potential to increase the number of liver transplantations from uncontrolled DCD donors significantly. It is currently only used in some dedicated centres in Spain (Barcelona and Madrid) and in France (Paris). One of the most important challenges for these approaches will be to reduce the incidence of ischemic-type biliary strictures responsible for a considerable amount of transplant loss.

Despite human data being very scarce, it has recently been shown that HMP is feasible for SCD livers. It is in the early stages of being extended to the preservation of ECD and DCD livers. Trials are needed to ascertain the clinical value of HMP.

3.2.6. Pancreas preservation methods

The outcome after whole organ pancreas transplantation has consistently improved over the last 20 years; islet transplant results are also improving steadily (Robertson et al., 2010). The reasons are multifactorial, including improved immunosuppression (Malaise et al., 2008), prophylaxis against infections and thrombosis (Muthusamy et al., 2010), and modifications in surgical (Squifflet et al., 2008) and islet preparation techniques (Baertschiger et al., 2008). In addition, improvements in organ preservation have undoubtedly had a significant impact on the outcome of transplantation (Kandaswamy, 2004). That is achieved by cooling the pancreas down to 4 to 8°C and maintaining a medium of reduced metabolism at low temperatures. But the pancreas has certain unique anatomic characteristics. First, when procured for whole-organ transplantation, the pancreas is taken with a segment of the bowel (duodenum). The duodenum is more prone to CI injury than other abdominal organs. Second, the pancreas is a low flow organ, in contrast to the kidney, which makes it also more prone to barotraumas from aggressive cold perfusion once the aorta is cross-clamped. It follows that the preservation tools and methods for whole-organ pancreas transplantation are not valid for islet transplant preparations. Indeed, cold storage may be adequate for preservation prior to pancreas transplantations, but insufficient when pancreases are processed for islets (in this case, several donors are required for a single transplant) or when ECDs are used. Supplementation of cold storage solutions with cytoprotective agents and MP may improve pancreas and islet transplantation outcomes in the future (Iwanaga et al., 2008).

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5. APPENDIX(ES)

5.1. Definitions

CIT *Cold ischemic time*

Time interval between the beginning of cold perfusion after organ procurement and the moment when the blood flow through the organ is re-established in the recipient.

DBD *Donor after Brain Death*

Donor who has been declared brain dead (defined as the irreversible loss of cerebral circulation with continuing heart flow) and in whom the cardio-respiratory functions are preserved until organ procurement.

DCD *Donor after Cardiac Death = Non Heart Beating Donor* = NHBD

NHBD procurement from patients in whom cardiac death has occurred or is imminent can be considered in several cases. During a consensus conference in Maastricht in 1995, 4 categories of NHBD donors were defined:

Category I	**Death prior to arrival in the hospital**
	No resuscitation Duration of cardio-respiratory arrest unknown
Category II	**Unsuccessful resuscitation**
	Resuscitation initiated outside or in the hospital, but unsuccessful. Duration of cardiac arrest known
Category III	**Programmed cardiac arrest (controlled NHBD)**
	Patients with no vital prognosis but who do not develop the criteria for brain death and in whom a therapeutic arrest is programmed
Category IV	**Cardiac arrest after brain death**
	Mainly patients who have been declared brain dead and in whom organ procurement is programmed, but whose condition quickly grows unstable and on whom an emergency procedure is carried out in order to be able to procure the abdominal organs in spite of the cardiac arrest.

At present, the NHBD procurement protocol is almost exclusively concerned with categories III and IV.

ECD *Extended/expanded Criteria Donor*

The definition of these criteria depends on the procured organ, but mainly concerns donors aged > 50-55, with a history of cardio-vascular disorders, addiction, resuscitation or lengthy stay in an intensive care unit.

DGF *Delayed Graft Function*

= “delayed” and especially insufficient function of the transplanted organ.

5.2. Organ perfusion devices

The list with the organ perfusion devices is provided for information purposes only. It is not exhaustive and only provides a snapshot of the devices available.

5.2.1. Kidneys

As regards kidneys, the portable devices are marketed by **www.organ-recovery.com**.

5.2.2. Lung

Portable machines similar to recently developed heart support systems are currently being designed for EVLP to make these potential applications a clinical reality. Three different companies have now marketed commercial devices (OCS® from Transmedics, Andover, MA, USA; Vivoline LS1® from Vivoline Medical, Lund, Sweden; Lung Assist® from Organ Assist, Groningen, the Netherlands). One other company is also working on a device that is not yet available for clinical use (LifeCradle® LU from Organ Transport Systems, Frisco, Tx, USA). Experimental and clinical data assessing the feasibility and safety of these transport devices are still awaited.

5.2.3. Heart

There are three devices that are in the clinical or pre-clinical development stages

1. The OCS™ from Transmedics®. It is the only device available for clinical application.

2. The LifeCradle® system from Organ Transport System Inc. OTS® is a start-up company derived from the Texas University Health Science Center. It performs research in the field of organ preservation. OTS® has developed a MP with specific devices for heart preservation (LifeCradle® HR) but also for the liver (LifeCradle® LI), kidneys (LifeCradle® KI) and lungs (LifeCradle® LU). They are currently in a pre-clinical phase, only in the USA.

3. The HeartPort® system from Organ Recovery System®. The HeartPorth® system is a modified LifePort® system (used for kidneys) with an oxygenator and a pulsatile flow perfusion. It is currently under development in animal studies.

5.2.4. Liver

There are currently 3 companies that are developing and/or testing commercial devices for liver MP preservation:

- Organ Assist, Groningen, the Netherlands
- Organ Recovery Systems, Chicago, USA
- Doorzandt Medical Device, Amsterdam, the Netherlands.

A fourth company, viz. Transmedics®, has developed OCS™, which is a system for normothermic lung and heart preservation and which might become available in some devices for liver preservation in the near future.

5.3. Additional information on pancreas and pancreatic islet transplantation

5.3.1. Pancreas preservation for pancreas transplantation

The two main methods used for experimental and clinical pancreas (organ) preservation are SCS and MP (Iwanaga et al., 2008). The hypothermic pulsatile MP technique originally developed by Carrel and lately popularised by F.O Belzer (Belzer et al., 1967) has been widely used for clinical kidney transplants but not for clinical pancreas preservation.

Early experiments with canine segmental grafts, reported by Florack et al. (Florak et al., 1983) demonstrated that failure rates with MP were 30 % at 24 h and 40 % at 48 h. There were no failures at 24 and 48 h with SCS. These results, along with the complexities associated with MP of the pancreas, have made cold storage the preferred and most widely used method for pancreas preservation (Iwanaga et al., 2008).

For the SCS preservation of pancreas transplants, the first solutions that were used were Collins (Collins et al., 1969; Hardie et al., 1977), Sacks (Sacks et al., 1973), and EC solution (Dreikorn et al., 1980). But UW (Walhlberg et al., 1987; Walhlberg et al., 1986) became the standard preservation solution for pancreas transplantation and has remained so for almost 20 years (Iwanaga et al., 2008). Recently, multiple reports have suggested that other preservation solutions may be effective alternatives to UW (table 4).

Table 4 Preservation solutions.

Components (mmol/l)	EC	UW	HTK	Celsior	SCOT 15
Na^+	10	28	15	100	143
K^+	113,4	125	18	15	5
Ca^{++}			0,015	0,26	1,7
PO_4	57,6	25	9		
HCO_3	10				25
Glucose	194				11
Raffinose		30			
Lactobionate		100		80	
Gluthation		3		3	
Allopurinol		1			
HES		50 g/l			
PEG 20					15 g/l
Viscosity	1,18	3,156		1,15	1,05

EC: EuroCollins; UW: University of Wisconsin; HTK: Histidine-Tryptophane-Ketoglutarate
SCOT®: *Solution de Conservation d'Organes et de Tissus*.

Using an HTK solution, comparative studies (with UW) have been performed demonstrating similarities between both solutions in the context of low-to-moderate flush volume and short CIT (≤ 10h) for HTK (Becker et al., 2007;Englesbe et al., 2006; Fridelle et al., 2010; Schneeberger et al., 2009;). By contrast, other studies found a higher incidence of postoperative complications in pancreases flushed with HTK, including graft pancreatitis, octreotide use and a lower insulin-independence rate at hospital discharge (Alonso et al., 2008; Stewart et al., 2009a; Stewart et al., 2009b).

Celsior, an extracellular, low viscosity preservation solution originally designed for heart transplantation, has also been used for experimental pancreas preservation with controversial results: Baldan et al. (2001) found it to be an effective alternative to UW, whereas Uhlmann et al (2002) reported increased IRI. For other organs, such as lungs (Thabut et al., 2001) and livers (Cavallari et al., 2003; Lama et al., 2007; Maggi et al., 2000), Celsior solution gave comparable clinical results. The first prospective, randomised study comparing UW with Celsior for clinical pancreas transplants was reported by Boggi et al. (2004), demonstrating similar safety profiles for pancreas preservation. This was also reported by Manrique et al (2006): 2-year graft survival rates, pancreas leakage rates, and clinical graft pancreatitis rates were similar using either one or the other solution. That is also true for kidney preservation (Faenza et al., 2001).

A new preservation solution, viz. SCOT® 15, which contains an extracellular ionic composition including polyethylene glycol (PEG) as a colloid, was recently used by Hauet & Eugene (2008) for experimental organ preservation. However, there is still a lack of clinical data for pancreas preservation.

5.3.2. Pancreas preservation for islet transplantation

UW has also been the solution used since the 1980's to preserve pancreases for clinical islet transplants (Zuker et al., 1989). When other preservation solutions were available, Salehi et al. (Salehi et al., 2006) reported that islet yields from human pancreases preserved in HTK or UW were equivalent. Another study by Hubert et al. (2007) demonstrated that the islet isolation yields from pancreases preserved with Celsior were 2.1-fold lower than those obtained with UW. That study suggests that colloid-free preservation solutions might be suboptimal for pancreas perfusion and cold storage prior to islet isolation and transplantation.

Conversely, Giraud et al. (2009) showed that there is a potential clinical application for SCOT® and that it could increase islet yield and reduce graft immunogenicity in pancreatic islet transplantation. By using SCOT®, these authors were eventually able to improve the pancreatic islet isolation process in a murine model using NHBD (Giraud et al. 2009).

Based on that early experience, there is a consensus among the main islet transplantation centres that islet yields and quality can be improved with better pancreas procurement techniques and by using cold-preservation techniques that are not necessarily needed for whole-pancreas transplants (Iwagana et al., 2008). The two-layer method (TLM) for pancreas preservation is an example of a technique for improving islet yield and quality by increasing pancreas oxygenation during preservation. Based on several studies (Hering et al.,2002; Fraker et al., 2002; Lakey et al., 2002) the TLM has been widely used by islet transplant centres worldwide, but the mechanisms through which it improves human islet yield and quality are not yet fully understood. Matsuda et al. (2003) have suggested that TLM cold storage protects isolated islets against apoptosis through the mitochondrial pathway. Moreover, Noguchi et al. (2010) reported on the basis of a pig model that the islet yield from pancreases preserved with the TLM and a modified so-called "classic solution" was significantly higher when compared with the TLM using UW. They hypothesised that their own solution is less likely to inhibit collagenase activity than UW (Contractor et al., 1995). Therefore, the TLM could be a promising technique for both pancreas and islet transplantation (Fujino, 2010), even though other preservation methods are used.

The basic principle of the TLM is the use of a cold solution, mainly UW, in combination with an oxygen carrier solution, viz. perfluorocarbons (PFC), which have a higher specific gravity. Therefore the PFC solution settles at the bottom and the UW lies above it. The pancreas is suspended at the interface of the two solutions (Figure 5).

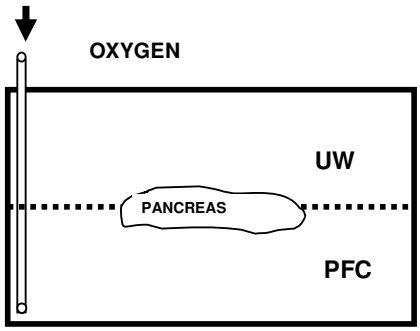

Figure 5: The TLM: UW solution is at the top and PFC solution at the bottom.

Forty-minute PFC-oxygenation prior to pancreas placement suffices to maintain adequate O_2 concentrations for up to 24 hours. The oxygenation must be carried out using a 10 to 12 mm Hg gas pressure and a 50 to 100 cc/min flow rate. Afterwards, the pancreases can be transported without the oxygenating apparatus. Moreover, it has been shown that maintaining the temperature of the medium at 8°C rather than 4°C results in superior islet function (Kandaswamy, 2004). The beneficial role of

oxygenation in improving pancreas quality and islet isolation was also demonstrated experimentally by Hackl et al (2010), who simply preoxygenated different preservation solutions (UW, HTK, Celsior…), and Scott et al (2010), who used persufflation.
It follows that the one – and two – layer methods with PFC (which are inert solutions with a high capacity for dissolving oxygen or oxygenation with other tools) have proved successful for SCS pancreas preservation. Taking into account that PFCs can be formulated as an emulsion, there is a huge renewal of interest in using the emulsion for continual MP or as simple flush solution (Hosgood & Nicholson, 2010). Indeed, Taylor et al (Taylor et al., 2010) reported that islet isolation from juvenile porcine pancreases can be successful after 24-h HMP preservation; HMP is well tolerated, leading to a moderate oedema but no loss of function of the harvested islets. Moreover, the oedema appears to aid in enzymatic digestion, producing a greater islet yield and purity compared to pancreases subjected to 24 h of SCS. In parallel, Karcz et al. (2010) have developed a model of MP for porcine pancreases which is simple, reliable, and protects the histopathologic integrity of the graft. The model can be used in further studies to improve the quality of pancreas preservation, and assess the condition of borderline pancreatic grafts and enhance their viability.

5.3.3. Conclusion

With the number of pancreas transplantations on the increase and following the advent of clinical islet transplantations, there will soon be a significant shortage of pancreases. It follows that more effective preservation methods will be required, and that the cold storage of donor pancreases will not suffice on its own. As controlled NHBD may be a potential source for pancreases, other preservation methods might be considered. Indeed, with NHBD, significant ischemic damage and post operative complications are liable to occur, especially in the event of prolonged cold storage preservation (Humar et al., 2000). Thus, the TLM has been shown to be promising in extending clinical pancreas preservation times and improving warm ischemic insult (Kandaswamy, 2004).
Therefore, there is an urgent need for access to the TLM technology: not only for pancreas preservation prior to islet transplantation, but also for pancreas transplantation. The MP technique is also a pancreas preservation method which will need to be reevaluated in the future in the light of new technologies using other perfusion fluids, temperatures and oxygenation for pancreatic transplant conditioning.

6. RECOMMENDATIONS FOR FURTHER RESEARCH

As was the case with the prospective randomised studies for kidneys mentioned above, trials will need to be conducted that compare the preservation of the liver, lungs, hart and pancreas with MP and SCS.

7. COMPOSITION OF THE WORKING GROUP

All experts joined the working group ***in a private capacity.*** The names of the members and experts of the Superior Health Council are indicated with an asterisk*.

ANGENON Elyane	Transplant coordinator	ULB
ANTOINE Martine	Cardiac surgery	ULB
GIANELLO Pierre*	Abdominal and experimental surgery	UCL
JASHARI Ramadan	Cardiac surgery, cardiovascular tissue banking	EHB
MEYNS Bart	Cardiac surgery	UZLeuven
MIKHALSKI Dimitri	Transplantation surgery	UZBrussel
MONBALIU Diethard	Abdominal transplant surgery	UZLeuven
PEETERS Patrick	Nephrology	UZGent
PIRENNE Jacques	Abdominal transplant surgery	UZLeuven
SQUIFFLET Jean-Paul	Abdominal transplant surgery	ULg
VAN RAEMDONCK Dirk	Thoracic surgery	UZLeuven
VAN CAENEGEM Olivier	Cardiology, intensive care	UCL
YSEBAERT Dirk	Abdominal transplant surgery	UZA

The sub-working group was chaired by Martine ANTOINE, the scientific secretary was Muriel BALTES.

The following experts read and approved the advisory report: to be complete

BAUDOUX Etienne*	Medicine, cell therapy	ULG
BEELE Hilde*	Medicine, dermatology	UZ Gent
BOUTSEN-ECTORS Nadine*	Medicine, anatomical pathology	KUL
DELLOYE Christian*	Medicine, orthopaedic surgery	UCL
GUNS Johan*	Medical-social sciences	UZ Brussel
HEINEN Ernst	Human histology	ULG
PIRNAY Jean-Paul*	Medical sciences	QAMH
VAN GEYT Caroline*	Medical-social sciences	UZ Gent
VANDERKELEN Alain*	Medicine, general surgery	QAMH
VERBEKEN Gilbert*	Biology, QA/QC/RA	QAMH

The Administration was represented by:

BONTEZ Walter	Coordination Blood, Cells, Tissues and Organs	FAMHP

The working group was chaired by Hilde BEELE; the scientific secretary was Muriel BALTES.

About the Superior Health Council (SHC)

The Superior Health Council is a federal body that is part of the Federal Public Service Health, Food Chain Safety and Environment. It was founded in 1849 and provides scientific advisory reports on public health issues to the Ministers of Public Health and the Environment, their administration, and a few agencies. These advisory reports are drawn up on request or on the SHC's own initiative. The SHC takes no decisions on the policies to follow, nor does it implement them. It does, however, aim at giving guidance to political decision-makers on public health matters. It does this on the basis of the most recent scientific knowledge

Apart from its 25-member internal secretariat, the Council draws upon a vast network of over 500 experts (university professors, members of scientific institutions), 200 of whom are appointed experts of the Council. These experts meet in multidisciplinary working groups in order to write the advisory reports.

As an official body, the Superior Health Council takes the view that it is of key importance to guarantee that the scientific advisory reports it issues are neutral and impartial. In order to do so, it has provided itself with a structure, rules and procedures with which these requirements can be met efficiently at each stage of the coming into being of the advisory reports. The key stages in the latter process are: 1) the preliminary analysis of the request, 2) the appointing of the experts within the working groups, 3) the implementation of the procedures for managing potential conflicts of interest (based on the declaration of interest, the analysis of possible conflicts of interest, and a referring committee) and 4) the final endorsement of the advisory reports by the Board (ultimate decision-making body). This coherent set of procedures aims at allowing the SHC to issue advisory reports based on the highest level of scientific expertise available whilst maintaining all possible impartiality.

The advisory reports drawn up by the working groups are submitted to the Board. Once they have been endorsed, they are sent to those who requested them as well as to the Minister of Public Health and are subsequently published on the SHC website (www.css-hgr.be), except as regards confidential advisory reports. Some of them are also communicated to the press and to target groups among healthcare professionals.

The SHC is also an active partner in developing the EuSANH network (European Science Advisory Network for Health), which aims at drawing up advisory reports at the European level.

In order to receive notification about the activities and publications of the SHC, you can send an e-mail to **info.hgr-css@health.belgium.be**

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