

REVIEW

Non-heart beating organ donation: overview and future perspectives

Cyril Moers, Henri G. D. Leuvenink and Rutger J. Ploeg

Surgery Research Laboratory, Department of Surgery, University Medical Center Groningen, Groningen, The Netherlands

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Correspondence

Cyril Moers, MD, Surgery Research Laboratory, Department of Surgery, University Medical Center Groningen, CMC V, Y2.144, PO Box 30001, 9700 RB Groningen, The Netherlands. Tel.: +31 50 3611976/+31 6 19602619; fax: +31 50 3632796; e-mail: c.moers@chir.umcg.nl

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Summary

New indications for organ transplantation combined with a stagnating number of available donor grafts have severely lengthened the waiting list for almost all types of transplantations. This has led to a renewed interest in non-heart beating (NHB) donation, as a possible solution to bridge the gap between supply and demand. In this review, we present an overview of current NHB donation practice, outcome, existing problems and future perspectives. We focus on possible improvements in donor management, recipient care and new methods of organ preservation that may be better suited for these marginal organs. Successful institution of NHB protocols depends on adapting current transplantation practice at all levels, which is one of the greatest challenges for researchers and professionals in this interesting re-emerging field.

Introduction

The concept of non-heart beating (NHB) donation is not new. In the early days of organ transplantation, all deceased donor grafts were retrieved from donors who had suffered cardiac death [1–3]. When legal definitions for brain death became available in the 1960s [4], most centers established transplant programs based on organ retrieval from heart beating, brain dead (BD) donors, thus avoiding the warm ischemic damage that NHB donor organs by definition have sustained [5].

However, organ donation has become a victim of its own success. In the last decades, indications for transplantation have become broader, whereas donor organ availability did not increase substantially. Partially due to improved traffic safety regulations, the number of BD organ donors has dropped: in the Eurotransplant region the relative amount of donors with cerebral trauma decreased from 43% in 1990 to 35% in 2005 [6,7]. Attempts to improve the willingness of the public to donate their organs after death have been only marginally successful. All these factors contribute to an ever increas-

ing number of patients on the waiting list. Within Eurotransplant alone, on December 31, 2005, more than 15 000 patients were waiting for an organ. Less than 6000 transplants were performed in that same year and almost 1400 patients died while on the waiting list [7].

In an effort to enlarge the donor pool, living donation has made a valuable contribution to kidney transplantation programs, and living split-liver donation is a promising method for the future in liver transplantation [8,9]. However, such programs will never yield sufficient new donor organs to bridge the gap between supply and demand. Therefore, many centers are now actively re-establishing the practice of NHB donation [10]. This is a logical step, for the potential pool of these donors is many times larger than the amount of available BD donors [5,11,12]. In the late 1980s and early 1990s, a few hospitals had already re-introduced NHB protocols. The group from Maastricht, led by Kootstra and co-workers [13], was one of the pioneering centers. In 1995, at the first international workshop on NHB donors in Maastricht, consensus was reached about donor management protocols and four different categories of NHB donors

Table 1. Maastricht classification of non-heart beating donors.

Category	Description	Procurement
I	Dead on arrival	Uncontrolled
II	Unsuccessful resuscitation	Uncontrolled
III	Awaiting cardiac arrest	Controlled
IV	Cardiac arrest while brain dead	Uncontrolled

were defined (Table 1) [14]. Ever since, the practice of NHB donation has increasingly become a part of transplant programs all over the world. Within Eurotransplant, 6% of all kidney donors in the year 2005 were NHB donors. Of these donors, 91% came from the Netherlands. In the Netherlands, already 47% of all donors were NHB donors in that same year, in most cases Maastricht category III [7]. In Spain, although nationwide only 4% of the donor pool consists of NHB donors, the Hospital Clinico in Madrid has developed a well-established NHB program as well, with approximately 25% of all deceased donors being NHB (percentages adopted from website Spanish National Transplant Organization, <http://www.ont.msc.es>). In the UK, 11% of all deceased donor kidney grafts came from NHB donors in 2005 [15]. Worldwide, several centers in the USA and Japan have started extensive NHB programs [16–19]. Kidneys are by far the largest group of transplanted NHB organs. Scarce data are available on NHB liver donation [20–22], and some centers have instituted the practice of NHB lung transplantation [23,24].

To date, more than 10 years after the workshop in Maastricht, many centers have published results of their NHB programs. Focusing on the kidney, it has become evident that NHB grafts have a significantly inferior short-term function, with reported delayed graft function (DGF) rates of 28–88% compared with 13–35% for organs retrieved from BD donors. For primary nonfunction (PNF) these rates are 1–18% and 0–10%, respectively [16,17,19,25–36]. Interestingly, although, medium- and long-term graft survival (GS) as well as acute rejection (AR) rates do not differ between these two types of donors. Brook *et al.* have summarized outcome after NHB kidney donation in several centers, reporting 14–66% AR and 54–84% 5-year GS. None of the studies included in this review found any significant difference in AR and GS rates for NHB donor kidneys, when compared with renal grafts derived from BD donors [37]. Although outcome of NHB donor organs may eventually be similar to kidneys procured from BD donors, the high rate of PNF and DGF causes considerable morbidity in the recipient, augments the length of post-transplant dialysis requirement and prolongs duration of in-hospital stay. All these factors eventually account for a significantly higher cost after transplantation.

Considering the abovementioned facts, the challenge for the near-future is to improve short-term outcome after NHB transplantation. Such an improvement can be sought in several different fields.

Donor management

In this respect, adequate donor management is essential for a successful NHB program. A major cornerstone of NHB donor management is the reduction of warm ischemia (WI). Ischemia in general, whether warm or cold, causes graft injury by means of several mechanisms. Depletion of cellular energy stores leads to inhibition of membrane transport systems, which in turn causes intracellular accumulation of ions and water resulting in cell swelling. After reperfusion, injury becomes manifest through up-regulation and surface expression of adhesion molecules, which activate host leukocytes. By binding to the endothelium and releasing oxygen free radicals and inflammatory mediators, polymorphonuclear leukocytes will contribute to vascular injury. Furthermore, cytokine release by infiltrated lymphocytes and macrophages may trigger allograft immunogenicity, rendering the organ more susceptible to a host immune attack. As nucleotides are rapidly lost during a prolonged period of ischemia, the tissue will fail to regenerate after restoration of blood flow at time of reperfusion [38,39]. The major difference between warm and cold ischemia is the rate at which injury develops. Detrimental effects of ischemia are much more pronounced as long as organ cooling has not yet been initiated. Hypothermia will slow down tissue metabolism, which is decreased by approximately 50% for every 10 °C of organ cooling. As a result, the accumulation of ischemic injury will decrease [40]. Rapid institution of cooling and washout of blood components is therefore of essential importance. This can be accomplished in several ways ranging from emergency laparotomy with direct aortic cannulation to total body cooling using an extracorporeal pumping device. The Maastricht group and others have advocated the use of a double balloon triple lumen catheter for rapid onset of cooling [13]. Although especially useful for uncontrolled (cat. I and II) NHB kidney-only donors, NHB multiorgan donation is not possible with this technique, as only the kidneys are effectively cooled. Furthermore, reliable and objective data on the technical efficacy of cooling by this and all other methods is lacking. Very few groups have actually measured whether the desired temperature of 0–4 °C is ever reached in the time that elapses between the beginning of perfusion and actual organ retrieval [41]. In addition, the time span needed to reach adequate cooling via various techniques is largely unknown. Future research directed at characterizing and improving cooling dynamics during

donor management is most likely to be very relevant for older and more marginal donor organs, in which additional (luke-)WI is absolutely undesirable.

Before cooling is instituted, other actions can also be taken to minimize the amount of injury that donor organs sustain. The group of Madrid has published data on management of uncontrolled NHB cat. I kidney donors by rapid (<15 min) emergency service response and continuation of resuscitation after declaration of cardiac death [30,32,42]. Short-term graft function of these kidneys (DGF 68%; PNF 6%) is surprisingly similar to kidneys derived from most controlled NHB donors. A possible explanation for this finding will be offered in the next paragraph. Moreover, in Barcelona, promising results have been obtained by artificial normothermic recirculation (NR) after cardiac arrest (CA) of NHB cat. II and IV kidney donors, before consent is obtained and cooling is commenced [43,44]. A completely different improvement in NHB donor management may 1 day emerge from donor pre-treatment. To prevent blood clotting after CA and subsequent poor organ wash-out, anticoagulatory and thrombolytic agents such as heparin or streptokinase can be administered to a potential donor before withdrawal of treatment [45,46]. Administration of cytoprotective substances would be another possible approach. Although proven effective in animal studies, serious ethical considerations understandably preclude most forms of pre-treatment in these patients who may become a NHB donor [47]. If, however, some agents could be used that were both beneficial for the critically ill patient and for his potential donor organs, ethically justifiable pre-treatment could become part of the management of these patients and preserve organ function.

Most NHB protocols prefer the use of controlled, cat. III donors, as WI time is known and remains relatively short. Also, procurement can be planned at a convenient moment, which does not require the complex logistics needed for immediate action in uncontrolled donors. On the other hand, the potential pool of cat. I and II donors is considerably larger [11]. Apart from this consideration, patho-physiologic reasons may also favor the use of uncontrolled donors. From studies with organs derived from BD donors it is known that brain death itself triggers pro-inflammatory and pro-coagulatory processes, which together with subsequent ischemia and reperfusion injury significantly affect the outcome after transplantation [48–51]. Most NHB cat. III donors have also sustained irreversible brain injury, the majority being cerebral hemorrhage, but will not meet legal criteria for the diagnosis of brain death. As the medico-legal moment of diagnosis of brain death is the result of a prolonged process of progressive cerebral injury, detrimental effects

as observed in BD donors will also be present in this type of NHB donor.

In addition to the presence of harmful cerebral damage related factors, NHB cat. III donor organs will then suffer from WI damage, sometimes more than reported. Most protocols allow an up to 2-h time interval between ventilator switch off and CA, when only kidneys are procured. This is a period of near-normothermia with gradually worsening hypotension and bradycardia as a result of collapsing circulation. In this state of hypotension, tissue oxygenation rapidly falls to low levels due to the absence of ventilation. When this crucial time interval is added to the usually reported WI interval between CA and systemic perfusion, WI time in the controlled NHB setting may sometimes be even longer than in the average uncontrolled NHB donor. The groups from Madrid, Leicester and Maastricht have repeatedly presented results after transplantation of uncontrolled kidney grafts that did show an inferior initial function when compared with kidneys derived from BD donors (60–94% vs. 23–35% DGF rate), but did also show no difference in long-term function [25,36,42], compared to NHB cat. III donor grafts. These centers employ rather strict emergency service protocols to keep WI as short as possible and select only those donors that have a known short WI time.

Therefore, it may be assumed that organs procured from NHB cat. I and II donors could be a safe and valuable addition to the donor pool and kidney transplantation, provided strong efforts are made to keep the duration of WI limited. Due to the detrimental effects induced by cerebral damage in combination with the uncontrollable extra WI damage between withdrawal of treatment and CA, a program based on only 'controlled' NHB donation may not necessarily be best choice.

Preservation techniques

Static cold storage (CS) has been the golden standard for organ preservation in the last decades with University of Wisconsin solution as the usually preferred preservation medium [52]. This method combined ease of use with satisfactory post-transplant outcome, especially due to improvements in preservation fluid composition. Interesting and relevant fine-tuning of preservation solution compounds is still the subject of many studies [53,54], but as far as short-term NHB graft function is concerned, no major breakthrough can be expected in this field. Considering the increasing number of older and more marginal organs, the most promising improvement may come from shifting gears to another preservation modality.

Along with the re-introduction of NHB programs came a renewed interest in hypothermic machine perfusion

(HMP) preservation, an old acquaintance from the early days of kidney transplantation [55]. This practice had been nearly abandoned as a result of the success of simple CS, which offered a logistically more feasible method. However, CS may not provide sufficient protection for a graft that has already been damaged by WI. Ample evidence from retrospective studies indicates the superiority of HMP for preservation of marginal kidneys resulting in significant improvement of short-term graft function with as much as 20% reduction in DGF vs. CS [56–61]. As prospective clinical randomized controlled trial data are lacking, a first European large multicenter prospective clinical trial comparing HMP with CS for kidney preservation is now being conducted. All consecutive kidney donors in the Netherlands, Belgium and North Rhine Westphalia, Germany above the age of 15 are randomized, with one kidney being preserved by HMP and the other cold stored, serving as a control. With a minimum of 300 donors and 600 kidneys included, this study will soon provide the level of evidence needed for decisions on whether or not HMP should become common practice, and for which subgroups of donors it is a relevant improvement over simple CS.

Viability testing of NHB kidneys during HMP is practiced by many centers throughout the transplant community [62–66]. Most widely used parameters are intravascular resistance (calculated from flow and pressure readings) and α - or total glutathione *s*-transferase (GST) levels in the perfusate. GST, formerly known as ligandin, is a cytosolic enzyme. In the kidney, the α -isozyme is exclusively located in the proximal tubules and its release therefore indicates specific injury to these structures. As acute tubular necrosis is one of the major causes of DGF, α -GST has been postulated to predict short-term post-transplant outcome. Together with general factors such as macroscopic appearance, anatomy, and donor characteristics, viability parameters are often considered in decisions on transplanting or discarding a marginal organ. However, although such tests show a strong correlation with the length of WI time, their independent predictive value for post-transplant graft function appears to be low [63,67,68]. Furthermore, no group has been able to define reliable cut-off points beyond which a kidney should not be transplanted. Unless a new, better viability marker is discovered, it is therefore highly questionable whether GST and vascular resistance-based viability testing during HMP should be conducted at all.

Hypothermic machine perfusion without active oxygenation probably exerts its beneficial effect mainly by providing a superior organ wash-out with an optimal exposure of the organ to the preservation solution, thereby preventing cell swelling with subsequent irreversible injury. However, it is still based on the concept of hypothermia.

Although injury develops at a lower rate during hypothermia, its impact cannot be ignored. In the clinical transplantation setting, cold ischemic time (CIT) is considerably longer than WI time, and for every additional 6 h of CIT the likelihood of DGF increases by approximately 25% [69,70]. In NHB donation, WI and CI thus have additive detrimental effects. This has been shown by animal studies in which prolonged CI following a WI insult rendered donor kidneys less suitable for transplantation [71,72]. These studies also illustrate that HMP cannot prevent the cold ischemic deterioration of a graft that has sustained a prolonged period of WI.

To resolve this dilemma, several groups have suggested switching to (near-)normothermic machine perfusion (NMP) as preferred method for NHB kidney preservation. NMP at or near 37 °C does support metabolism at an almost-normal rate, and by adding oxygen to the perfusate, it prevents further ischemic damage to the graft. In contrast to HMP or CS, NMP can address essential physiologic needs of the organ. Several studies have shown that NMP is superior to HMP or CS preservation of severely WI-damaged NHB donor kidneys [72–77]. Moreover, NMP may offer a more reliable method for *ex vivo* pretransplant functional assessment of a kidney graft based on urine production, perfusion dynamics and biochemical injury markers in the perfusate [78]. *Ex vivo* functional assessment of NHB pulmonary grafts, as proposed by several groups, also depends on adequate NMP, combined with artificial ventilation [79,80].

The beneficial effect of NMP can only partially be attributed to the absence of ischemia. Rather than simply preventing additional ischemic injury, cellular reparative processes may also remain available during normothermia, thus actively reversing WI injury before the organ is transplanted. Induction of heme oxygenase-1 (HO-1) has been postulated to play an important role in such cellular repair mechanisms [81,82]. In the NHB donor situation, initial WI can be regarded as an event causing tissue stress. In the kidney, for example, a cascade of pro-inflammatory processes immediately takes place leading to apoptosis and eventually necrosis of renal cortical tubular cells via a number of different pathways [83–87]. Brasile *et al.* [81] showed that, in the presence of an inducer, HO-1 expression during NMP can have cytoprotective effects against reperfusion injury. Other heat shock proteins such as HSP-70, may also play a key role in the prevention of apoptosis and subsequent necrosis after an ischemic insult [88,89]. Bellemare *et al.* found that, although WI triggers HSP-70 activation, this process is inhibited by hypothermia. Thus, tubular cells are rendered more susceptible to inducers of apoptosis, some of which do remain active in the cold [84]. NMP could overcome this shortcoming of hypothermic preservation

by providing an environment in which such reparative processes keep functioning. Although NR before cooling of NHB organs, as practiced by the Barcelona group, is primarily meant to reduce WI time, the above-average results may also be partially attributed to processes described above. NR perhaps provides a window for repair of WI damage before hypothermia commences.

Clinical NMP preservation poses several logistical challenges. Actively warming and oxygenating the perfusate, while cautiously maintaining a correct pH and other important physiologic parameters, require voluminous, high-tech equipment and the continuous presence of well-trained perfusion staff [90]. Transporting organs within an international sharing system would become almost impossible. Therefore, other preservation methods will have to be found, which combine the beneficial effects of NMP with the ease of use that CS and even HMP preservation can offer.

Recipient management

Choosing the right recipient for a particular donor organ is essential. Although blood group and, for some organs, human leukocyte antigen matching is common in transplantation, age matching might be particularly relevant in the NHB donor setting. Donor age is one of the strongest determinants for short- and long-term graft function [91,92]. In a multivariate analysis conducted by Koning *et al.*, relative risk for DGF in kidneys derived from donors above the age of 50 was 7.12, compared to grafts coming from younger donors. Organs from older donors, when damaged by WI, show an even worse outcome, especially when long-term function is considered. In recipients of kidneys from older NHB donors, Snoeijs *et al.* reported a 5-year GS of 52% vs. 68% when organs came from NHB donors under the age of 50 [93]. Matching organs from older BD donors with elderly recipients has recently been proven a reliable method to optimize outcome after kidney transplantation. This concept preserved statistically longer functioning kidneys for younger people who have more years of life ahead of them [94]. This practice could perhaps be extended to the NHB situation.

Several studies also suggest that pre-emptive kidney transplantation has beneficial effects on both short- and long-term outcome, and may reduce the number of AR episodes [95–97]. More research is needed to determine whether NHB donor kidneys perform better when transplanted into patients who have no or only a short history of dialysis.

Furthermore, relevant improvement in short-term post-transplant NHB graft function may come from the adjustment of immunosuppressive regimens. Calcineurin inhibitors (CI) such as cyclosporine and tacrolimus are

known for their nephrotoxic side-effects, which negatively affect already poor short-term function of NHB donor kidneys [98]. Much research has been directed to avoid, substitute or delay the introduction of these drugs focusing on non-nephrotoxic immunosuppressive agents to allow recovery of graft function in the early period after transplantation with interleukin-2 receptor antagonists or polyclonal antithymocyte globulin [99–101]. Although this approach can be successful in terms of reduced DGF rates, it could lead to an elevated risk of AR episodes [102]. Finding an equally effective, but non-nephrotoxic substitution for CI is part of the Holy Grail for post-transplant management of donor kidney recipients in general and NHB renal graft recipients in particular.

Conclusion

Non-heart beating donation offers a promising resource to increase the supply of deceased donor organs, despite a large number of hurdles that still have to be taken. Combining reduction of WI time with rapid and effective organ cooling, followed by HMP, is probably the most likely approach as it is technically feasible with only minimal changes of current practice. A more difficult, but possibly more effective approach would be switching to normothermic preservation instead, with the potential of repairing WI damage *ex vivo* and conducting relevant viability tests. Especially, when other organs than the kidney are to be transplanted from a NHB donor, NMP might be the key to success. Further research in this direction will hopefully yield better insight in such possibilities. Ethically justifiable patient and graft-to-be pre-treatment as well as adjusted immunosuppressive regimens for the recipient will contribute to improved outcome after NHB transplantation. For the immediate future, the inclusion of both controlled and uncontrolled NHB grafts is worth considering, provided strict protocols are instituted to minimize WI time and guarantee optimal wash-out and preservation.

Now that organ shortage has forced the transplant community to revert to a growing pool of completely different donors, it is essential to adapt to this new situation at all levels. Protocols that are fine-tuned to optimally match transplantation of grafts derived from BD donors will not automatically be the best ones for the NHB setting. Therefore, successful (re-)institution of NHB donation poses, by all means, a great challenge for both researchers and clinical professionals in the field of organ donation and transplantation.

Conflict of interest

None.

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