Non-heart-beating kidney donors : clinical implications

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NON-HEART-BEATING KIDNEY DONORS

- CLINICAL IMPLICATIONS -

J.H.C. Daemen

NON-HEART-BEATING KIDNEY DONORS

- CLINICAL IMPLICATIONS -

PROEFSCHRIFT

ter verkrijging van de graad van doctor
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PROCUREMENT AND TRANSPLANTATION OF KIDNEYS
FROM NON-HEART-BEATING DONORS

- an overview -

from: Procurement and Transplantation of Kidneys from Non-Heart-Beating Donors
JHC Daemen, APA Oomen, EHJ Belgers, G Kootstra
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Introduction

Progress in transplantation medicine, mainly as a result of improving immunosuppressive treatment and HLA-matching, has made kidney transplantation more and more acceptable as a routine treatment for patients with end-stage renal failure. Today, results in kidney transplantation are impressive; over the years the 1-year graft survival for cadaveric donors reached 80% and the 1-year patient survival 95% [1].

A successful kidney transplantation results in improved quality of life for the patient and reduces the costs of treatment of end-stage renal failure. However, transplant numbers cannot keep up with the demand and waiting lists are growing as organ procurement rates are not increasing. Within Eurotransplant (ET), the exchange organisation of the countries of Austria, Belgium, Germany, Luxembourg and The Netherlands, kidney recovery rates decreased 10% in 1994 when compared to 1993. In 1996, still this rate is 7% below the level of 1993. As a result, the gap between organs available and patients awaiting kidney transplantation widened; and by the end of 1996, 11,000 patients were on the renal transplant waiting list, while only 3,300 transplants could be performed that year [2]. Despite the fact that the number of potential brain dead, i.e. heart-beating (HB), and realistic donors is estimated to be as high as 43-55 per million population (p.m.p.) annually [3], the overall retrieval rate for 1996 within the ET area only was 14.5 p.m.p. This resulted in 27.3 kidneys p.m.p. available for transplantation. Obviously, more efforts are needed in effectuating potential HB donors through educating both lay public and health care providers. The persisting organ shortage, however, increases the need for alternative sources of donor organs.

Although transplantation with organs from living donors gives superior results when compared to cadaveric organ transplantation [4], the actual number of donors is limited and is unlikely ever to meet the demand. With non-heart-beating (NHB) donors, expansion of cadaveric organ donor programs is possible. In contrast to the HB donor that is declared dead after irreversible cessation of all brain stem functions, the NHB donor is declared dead after irreversible and final cardiac arrest. As a consequence, NHB donors have no circulation and immediately an ischemic state occurs, whereas potential HB donors remain ventilated and have a beating heart after the diagnosis of brain death. Therefore, in HB donation, organs can be recovered electively while they are sufficiently oxygenated and are not damaged by warm ischemia. The NHB donor is a still relatively underdeveloped source that can be very valuable in decreasing the discrepancy between demand and supply of transplantable kidneys [5,6].

Although the impression may exist that the concept of NHB donation is new, the concept is merely revitalised. In fact, before the notion of brainstem death was generally accepted, organ procurement was

only possible from donors after final circulatory arrest [7], i.e., from NHB donors. These donors then were all patients with severe brain trauma from which ventilatory support was withdrawn in the operating room (OR). After apnoea and subsequent cardiac arrest, organs could be procured. Together with the introduction of criteria for brainstem death and the use of HB donors, the use of NHB donors declined, probably because it was thought that they provide an inferior quality of organs and that the use of ischemically damaged organs should be prevented. Additionally, in the early 1970s the number of available HB donors was sufficient to meet the demand at that time. Because of the reduced organ recovery rates, nowadays there is renewed interest in NHB donors. Several emergency protocols for salvaging organs after final circulatory collapse and for keeping the option of organ donation, have been developed [8,9]. Since especially kidneys seem to tolerate ischemia well, almost exclusively kidneys may be procured following such protocols [10,11]. At the University Hospital Maastricht, a NHB donor program based on such an emergency procedure was implemented in 1980 and kidneys from NHB donors have been recovered and transplanted since [12].

Non-Heart-Beating Donors

NHB donors have in common that they sustain irreversible cardiac arrest before organs are procured. Subsequently, the kidneys suffer an ischemic insult of unknown severity. This warm ischemia time (WIT), defined as the period from circulatory standstill until the start of organ cooling, is important for the viability and posttransplant function of the grafts. Essential in the NHB donor is to limit the WIT and establish cooling of organs as soon as possible after asystole in order to slow down metabolism and prevent organs from further decay. Because final cardiac arrest may occur under very different conditions, four categories of NHB donors have been defined; category 1 *Dead on arrival*, category 2 *Unsuccessful resuscitation*, category 3 *Awaiting cardiac arrest*, and category 4 *Cardiac arrest while brain dead*, respectively [13]. These categories are described in more detail in chapter 2.

Cooling Techniques

In NHB donation warm ischemic damage is the most detrimental factor to the organ and a major determinant of function after transplantation. Therefore, rapid cooling and flushout of blood from the organs as soon as possible after cessation of circulation, declaration of death, and obtaining consent, is essential for successful organ recovery in NHB donors. By decreasing the core temperature of the kidney to 15 °C, metabolism is decreased with 90%, reflected by decreased oxygen consumption of tubular cells [14]. This is mainly because at this temperature the sodium-potassium pump, responsible for 85% of

tubular oxygen consumption, ceases to function [15]. For realising this cooling, several techniques may be employed.

The most simple way is to start intra-aortal cooling after laparotomy similar to HB donor procurement. Controlled NHB donor procedures (*category 3*) may take place in the OR and after withdrawal of ventilatory support and subsequent cardiac arrest, immediate laparotomy is performed. Rapid recovery techniques of all abdominal organs are developed; initial cooling of organs through femoral catheters and subsequent back table cooling will preserve organs for transplantation [16,17]. In renal donors, in a controlled setting, kidneys can at first be procured without cooling and, after 15-18 min of WIT flushed at the back table immediately after procurement [18]. Under noncontrolled conditions (*categories 1,2,4*) rapid intra aortal cooling requires an emergency laparotomy which might cause logistic difficulties and problems in obtaining consent for donation, if needed [19].

Devices, like the double-balloon-triple-lumen (DBTL) catheter [8], have been developed for rapid in-situ intravascular cooling. These catheters are placed in the common femoral artery, advanced into the aorta, and (after filling the balloons proximal and distal of the renal arteries) the kidneys can be flushed (fig. 1). Occasionally, introduction is impossible because of severe femoral atherosclerosis or size of the donor arteries. Experimental data revealed that a minimal perfusion pressure of 70 mmHg is needed for rapid and successful in-situ cooling of the kidneys resulting in better survival and earlier function [8,20].

Sometimes intravascular cooling has been combined with intraperitoneal cooling; although experimental data showed quicker cooling of the kidneys, no advantages of additional peritoneal cooling have been reported in clinical transplantation [21,22]. Results on initial peritoneal cooling alone, before emergency laparotomy and intravascular renal cooling, were reported by Orloff et al. [23], although in a near-to-controlled setting.

Total body cooling as method of organ preservation after cardiac arrest has been reported by several authors [24,25] using a femoro-femoral bypass and extracorporeal perfusion devices [26]. These procedures, however, are rather complicated and technical problems have to be solved when used in uncontrolled NHB donor procedures.

Simplicity of procedures is important in the acceptance of donation procedures; health care workers will probably be more reluctant towards difficult and technically demanding procedures that need the availability of specially trained personnel and equipment.

Figure 1. Schematic drawing of the DBTL catheter in a NHB donor.

A = DBTL catheter with inflated balloons positioned in the aorta

B = kidneys flushed through the renal artery

C = catheter inserted through right femoral artery

D = infusion system with cold HTK perfusion solution

A way to bridge the time period between cardiac arrest and procurement of the kidneys, is by means of a mechanical cardiopulmonary resuscitation device. Successful organ preservation was reported by employment of this method for periods up to 4 h [27].

Instead of continuing to perfuse the organs with warm oxygenated blood, others have attempted to preserve organs by means of warm preservation solution. Tojimbara et al. [28] recently reported successful experimental preservation of ischemically damaged livers through initial flush-out with warm University of Wisconsin (UW) cold storage (CS) solution. Also 6 h of ex vivo canine kidney preservation at temperatures of 25-32°C seemed feasible and resulted in successful autotransplantation [29]. This warm preservation method is based on a perfusate developed from a modified tissue culture medium and perfluorochemical emulsion as an oxygen carrier and it maintained renal metabolic function well. It also might be developed into an in-situ preservation technique in noncontrolled NHB donation procedures to bridge the period between cardiac arrest and organ procurement. As a preservation method it was also proposed to enable functional evaluation of the kidneys prior to transplantation [30].

Donor Criteria

In our program, NHB donors are considered acceptable when the duration of initial cardiac arrest, i.e. time from cardiac arrest until start of adequate resuscitation, does not exceed 30 min. Additionally, we accept a 2-hour resuscitation period (table 1). So, the total WIT, i.e. time from initial cardiac arrest until cooling of organs through an in-situ preservation (ISP) procedure can be established, may be 150 min maximum. Although no experimental data on the impact of resuscitation on perfusion of the kidneys are available, we assume that ongoing cardiac massage and ventilation are to be preferred over no support at all. Efficiency of cardiac massage and ventilation are checked by arterial blood gases.

At the University Hospital Maastricht, we consider 65 years the upper age limit for NHB donors. Other exclusion criteria are signs of sepsis or intravenous drug abuse, or a history of untreatable hypertension, kidney disease, and malignancies other than primary brain tumours. It is obvious that in some NHB donors, especially *category 1* and *category 2 NHB donors*, essential information about the patient's medical history may not be available until after the nephrectomy. Other programs may apply other criteria for age and ischemia times.

In the Maastricht recovery area with approximately 750,000 inhabitants, kidneys are recovered from NHB donors according to these criteria. In the period 1980-1992, kidney retrieval from 67 NHB donors

resulted in 79 transplants (59%); in the same period 346 kidneys were transplanted from 183 HB donors (95%). Thus NHB donors accounted for 18% of the total number of kidneys transplanted; in 1994, NHB donation even accounted for 40% of the total number of kidneys available for transplantation in our area [31]. Of course, relatively more kidneys from NHB donors are discarded for reasons of damage, bad in-situ or back table flush-out. Some kidneys were not transplanted because no suitable recipient was available (AB blood type, HCV positive donor). Others reported a similar increase in kidney procurement rates after implementing a NHB donor program [32].

Table 1. NHB donor criteria

- * Time between cardiac arrest and start cardiopulmonary resuscitation < 30 min
 - * Effective cardiopulmonary resuscitation < 2 h
 - * Age $\leq$ 65 years
 - * No signs of systemic infection or sepsis
 - * No signs of intravenous drug abuse
 - * No patient history of:
 - untreatable hypertension
 - kidney disease
 - malignancies other than primary brain tumours
-

NHB Donor Procedure

In the Maastricht program 75% of the potential NHB donors are reported from the ER, i.e. in an uncontrolled setting (*category 2*). After the team of physicians taking care of the patient and performing cardiopulmonary resuscitation has declared this patient dead on cardiac criteria, potential NHB donors are reported to the transplant co-ordinator. After accepting the donor as medically suitable, the transplant team is notified and the relatives are approached for obtaining consent. Meanwhile, cardiac massage and artificial ventilation are resumed after a waiting period of 10 min. This period is introduced to respect the dead donor rule [33] and to make it clear to anyone involved that there is a change from saving a patient's life to salvaging organs for donation. To optimise renal perfusion, heparin (20,000 IU) and phentolamine (0.125 mg/kg) are administered intravenously and flushed with saline. At consent, an arteriotomy is performed through a longitudinal incision over the inguinal ligament and a Ch16 DBTL

catheter (AJ 6516, Porgès, Le Plessis-Robinson, France)(fig. 2) is inserted. Through the common femoral artery the DBTL catheter is positioned in the aorta and ISP of the kidneys is started. As flush-out solution we use large volumes, approximately 10-15 litres are needed, of cold histidine-tryptophan-ketoglutarate (HTK, Bretschneider solution)[34]. The advantage of HTK is thought to be the strong buffer capacities at temperatures $> 10^{\circ}\text{C}$ through the histidine-HCl buffer [35], and it is available in 5-liter bags. The initial flush-out is done from a height of 100 cm or by means of a roller pump to obtain the minimally required perfusion pressure. By filling the balloons with a radiopaque solution, the position of the DBTL catheter can be checked by abdominal X-ray (fig. 3) and adjusted if necessary.

Figure 2. DBTL cooling catheter with inflated balloons.

Distance between the balloon is 23 cm. with multiple double-sided perforations (P) for easy and rapid perfusion. Rupture of balloons may be recognised through the small control balloons (B).

Figure 3. Abdominal radiograph of a DBTL catheter in situ.

(A) The catheter is inserted through the right femoral artery and is placed correctly. Note the elongated and typical contour of the abdominal balloon stuck at the aortic bifurcation; the thoracic balloon cannot be seen.

(B) The catheter is inserted through the right femoral artery; the abdominal balloon is in an incorrect position in the right iliac artery while the thoracic balloon shows an atypical position. The donor was supposed to have died of myocardial infarction but appeared to have an aneurysm of the distal aorta as well. This explains the atypical position of the DBTL catheter.

Venous drainage is secured by a simple Ch22 urinary catheter inserted into the femoral vein. An important part of our procedure is that before nephrectomy, it is arranged for relatives and/or a priest to visit the deceased. The nephrectomy is performed preferably as soon as possible after starting the in situ cooling; however, procedures have been successful with in situ cooling for up to four hours [8,36]. Near-to-controlled NHB donor procedures (*category 3*) are performed at the Intensive Care Unit (ICU)

following the same procedure using DBTL catheter and ISP. This in contrast to the controlled NHB donation procedures in an OR after withdrawal of ventilatory support and subsequent cardiac arrest; in some protocols laparotomy and cooling is started just 2-4 min after cardiac arrest [18,37].

Preservation

Simple CS is widely accepted as the method of choice for the preservation of renal grafts since it is cheap and easy. The advantages of preservation by continuous cold perfusion are most pronounced for prolonged preservation times [38] and kidneys from marginal donors with ischemic damage [39]. Recent experimental work at our center showed that preservation of ischemically damaged kidneys by machine perfusion (MP) was superior to preservation by CS with HTK solution [40]. MP resulted in better survival rates and better preservation of the microcirculatory integrity [41]. Kozaki et al. [42] and Matsuno et al. [43] confirmed these results in a clinical transplant program using NHB donors from which one kidney was preserved by MP and the contralateral by CS. All machine perfused NHB donor kidneys had earlier life-sustaining function than the contralateral renal grafts preserved by CS. D'Alessandro et al. [18] and Orloff et al. [23] achieved impressive results transplanting NHB donor kidneys after preservation by MP. Another advantage of MP is that it allows access to the kidneys for intervention and the possibility of evaluating the condition of the grafts [44].

Recently a new preservation method for NHB donor kidneys has been proposed, using MP with a feedback mechanism resulting in adequate perfusion pressure at much lower flow rates [45]. This is thought to reduce the additional vascular preservation damage by continuous perfusion. Clinical application of this MP method needs to be evaluated.

Another method to preserve ischemically damaged kidneys is the use of retrograde persufflation of oxygen through the renal vein. In animal experiments it was shown that the resynthesis of high energy phosphates was improved; survival rates, however, could not be increased [46-48]. Although experimental data were promising, so far no evident clinical improvement of transplant results has been reported [49]. Successful experimental preservation of ischemically damaged extrarenal organs has been reported; liver and pancreas metabolism could successfully be resuscitated using respectively, normothermic perfusion and two-layer (UW/perfluorochemical) hypothermic storage methods [50,51].

In 1993 we re-introduced MP as the preservation method of choice for our NHB donor kidneys, with the intention to reduce the delayed function (DF) rates. After the ISP procedure and subsequent

nephrectomy, the kidneys are prepared on the back table and the renal artery is cannulated for MP. Routinely cortical wedge biopsies are taken for histological examination. Then the kidney is placed in a specially designed organ chamber (fig. 4) fitting into a Gambro-PF-3B perfusion machine (fig. 5).

Figure 4. Organ chamber with a human donor kidney cannulated and connected to the perfusion system.

The renal artery (A) is connected to the perfusion system by a bull-dog clamp. The effluent from the renal vein (V) flows freely into a container at the bottom of the organ chamber and from there re-enters the perfusion circuit.

As a perfusate we use Belzer's perfusate, the UW solution developed for perfusion preservation. It is based on the UW CS solution (Viaspan), basically with the impermeant gluconate replacing the lactobionate [38]. In UW solution for MP the serum albumin as a colloid has been replaced by the totally synthetic hydroxy-ethyl-starch (HES)[52]. With albumin based perfusates human blood products are needed (human albumin in either plasma, protein plasma fraction, silica gel fraction or human albumin added to saline or Ringer's lactate) with all the specific risks of these blood products. Additionally, albumin has a limited use as intravascular oncotic pressure diminishes in the course of preservation [53], it is known to cause endothelial damage [54], and last but not least, plasma is known to predispose for rejection [55]. The synthetic Belzer's perfusate eliminated all these side effects of plasma derived

products and proved to reduce rates of acute tubular necrosis (ATN)[56].

Figure 5. Organ chamber placed in a Gambro-PF-3B organ perfusion machine.

After connecting the kidney to the perfusion system, perfusion flow is set to a systolic pressure of 60 mmHg and kept constant. The pressure is subsequently allowed to vary as an indicator of intrarenal vascular resistance (IRR). When IRR fails to decrease, accompanied by high lactate dehydrogenase release in the perfusate and low pH, the kidneys are not used for transplantation [44]. When donor history reveals no contraindications, histology shows no signs of renal cortical necrosis or severe pre-existent renal damage, and the kidneys do well during continuous perfusion, we consider the kidney suitable for transplantation. The kidneys are finally transported to the transplant center selected by Eurotransplant and remain in the perfusion machine until implantation.

Transplant Results

Although nowadays some programs are procuring extrarenal organs from NHB donors (mainly *category 3 NHB donors*), most centers have only experience with kidneys from NHB donors. Reports on transplant outcome show in general high rates of DF, most likely due to ATN (table 2). Early reports showed no difference in short term transplant outcome between controlled NHB and HB donor kidneys with DF rates of 40% and 31% [58,59]. More recently when transplanting NHB donor kidneys, DF rates were found to vary from 60 to 90% [43,60-65], although from the University of Rochester a DF rate of not higher than 22% was reported [23]. The long-lasting program at the University of Wisconsin reported similar results [18].

Table 2. Transplantation results with kidneys from NHB donors

Center ¹	NHB donor category ²	DF rate ³	1-yr graft/patient survival ⁴
Barcelona (E) [62]	3,4	67	90 / 92
Leicester (UK) [64]	2	90	93 / ?
Zurich (SUI) [63]	2,3	29	84 / 90
Madison (USA)[18]	3	19	83 / 94
Pittsburgh (USA) [15]	2,3	69	85 / 95
Maastricht (NL) [59]	2,3,4	65	73 / 95

1 = See reference list.

2 = Category of NHB donors; description see text and reference 13.

3 = Delayed function; reported DF rate (%).

4 = Reported 1-year graft (%) and patient survival (%).

However, all these procedures were in a near-to-controlled setting (*category 3 NHB donors*) with WIT of 20-35 min and very restrictive criteria for potential donors. Remarkably, both programs prefer MP as method of preservation for their NHB donor kidneys. Long-term graft and patient survival were reported to be equal for NHB and HB donors from almost all programs [19,24,25,32,61,66,67]. Only one program reported evidently lower 1-year graft survival. No cardiac massage, artificial ventilation, or in-situ cooling was applied in this program and all donors were NHB donors who suffered about 45 min of WIT after an agonal period in a nearby hospice [68]. Remarkable results were reported by Naqvi et al. [69], with two kidneys from NHB donors that additionally suffered extreme long cold ischemia times.

Transplantation results of all kidneys from NHB donors procured in the period from 1980 to 1992 at the University Hospital Maastricht and transplanted within the ET area, were recently analysed [60]. Transplant outcome of NHB donors (n=57) was compared to a matched HB control group (n=114). Most NHB donors were from *category 2* and occasionally *category 3 or 4* and none of the NHB donor procedures took place under controlled circumstances in the OR. Apart from the cause of death, donor data in these series were comparable. Short-term results showed almost twice as much DF (i.e., need for posttransplant dialysis) in the NHB group. Kidney function was equal for both groups within 3 months posttransplant; graft and patient survival were not different for up to 10 years.

Transplant results of extrarenal organs, including liver, pancreas and lung, are excellent and equal those obtained in HB donation as long as the procedure is performed under controlled conditions with minimal warm ischemia (*category 3*) [14,15]. Liver transplants in noncontrolled situations resulted in primary nonfunction (PNF) in half of the cases [62].

It is obvious that the circumstances under which donors sustain irreversible cardiac arrest, as well as the duration of the warm ischemic period (i.e. the respective NHB donor category), have a major impact on transplant outcome. The excellent results from Zurich [64], for instance, are achieved with kidneys from NHB donors, who are mainly trauma victims that die in the OR and whereof the kidneys are used as primary transplants only. The reports from Madison [14,18], Rochester [23], and Pittsburgh [15] are based on NHB donors who have died after withdrawal of life-sustaining treatment and therefore resulting in extreme short warm ischemic times. The results from Leicester, on the contrary, are only with grafts from donors after resuscitation in a uncontrolled setting [32].

In general though, transplant results with renal grafts from NHB donors are good and comparable to results with HB donor grafts, although with significantly increased DF rates. Particularly in *category 2*

NHB donors with long resuscitation efforts and subsequent prolonged WIT, organs may be profoundly damaged by warm ischemia and give PNF upon transplantation. Obviously, PNF and viability of the kidneys are of major concern in uncontrolled NHB donation. Parameters that indicate the ischemic damage sustained and that may predict functional outcome of the NHB donor kidneys upon grafting, will help to prevent the transplantation of organs that are not viable. Although several potentially useful parameters have been proposed, none of them are routinely used for assessment of organ viability in clinical practice [71-75]. This subject is addressed in chapters 6 and 7.

Ethical Issues

As stated before, transplant medicine started with results obtained through the use of NHB donors sustaining cardiac arrest after withdrawal of mechanical ventilation. Without this pioneering, transplant results could not have made the same progress as they did later on. As discussed by Youngner et al. [75], the reluctance towards NHB donation may be based upon the fact that there is controversy whether two basic requirements for organ donation are met [76]. Firstly, there is *the dead-donor rule: organs should be taken only after death and patients should not be killed by organ removal* [33]. In case of *category 3 NHB donation* procurement procedures are started after cardiac arrest has occurred. Although in some protocols organ cooling is already prepared before withdrawal of support, laparotomy with subsequent organ removal is started after 2-4 min of asystole [16,17]. It is disputable whether after this short period without circulation all brain functions have ceased. In favour of these procedures is the fact that it is the wish of the deceased or the relatives to withdraw life-sustaining treatment, because of incurable disease, and to donate organs. Within our NHB donor program at the University Hospital Maastricht in case of *category 3 NHB donation*, ventilation is withdrawn at the ICU and cardiac arrest is often witnessed by the next of kin. A waiting period of 10 min of asystole is respected to ascertain that there is a neurological state comparable to brain death before starting the ISP procedure. It is very unlikely that from these donors extrarenal organs can be procured and successfully transplanted since with these organs immediate posttransplant function is necessary. In *category 2 NHB donation*, patients are declared dead after unsuccessful cardiopulmonary resuscitation and may subsequently become NHB organ donors. Although the patient already is declared dead by the attending physician, according to our protocol, again the 10 min waiting period is respected, before the transplantation team takes over and restarts cardiac massage and artificial ventilation. No doubt there is a neurological state comparable to brain death. Furthermore, a separation of procedures directed towards saving patients or preserving organs is secured. Criteria for declaring patients dead under such circumstances in order to have the option of organ donation have recently been defined by the Swiss Academy of Medical Sciences [77].

They state that cardiac arrest is irreversible, whenever despite full mechanical and chemical resuscitation efforts for 30 min, there is no restoration of spontaneous circulation. In case of deep coma together with apnoea and pulselessness, the patient may be declared dead and organ procurement may follow. In countries with the legal system of presumed-consent or with systems that allow introduction of cooling catheters before consent, organs can be preserved immediately until consent for organ donation is obtained. In our program after the 10 min waiting period, cardiac massage and mechanical ventilation are resumed until organ donation has been discussed with the relatives; sometimes initially only consent for introduction of the DBTL catheter for ISP is obtained while maintaining the option of donation [78].

The second basis of organ donation is that *care of living patients cannot be compromised in favor of potential recipients*. With regard to this rule, a strict separation is needed of physicians taking care of patients and physicians directly involved in transplantation. Therefore, death must be diagnosed by someone not involved in the transplantation process. This separation is not always possible as patients will be identified as potential *category 3 NHB donors* by the attending physician who, as in HB donation, will often discuss organ donation with the relatives before death of the donor. Evaluation by external reviewers whether all steps are according to the protocol for NHB donation on a case-to-case basis may minimise the chance of a conflict of interests [80]. In case of *category 2 NHB donation*, the decision to stop resuscitation and proceed towards organ donation may be reviewed by such a committee in retrospect.

We feel that reluctance about NHB donation results from unacquaintedness with the procedure and therefore education and openness towards public and medical health care professionals are mandatory. Additionally, NHB donor programs should only be implemented based on written protocols approved by the local ethics committee. Recently, at the First International Workshop on NHB Donors, statements and recommendations on this subject have been postulated [80].

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CATEGORIES OF NON-HEART-BEATING DONORS

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Introduction

In the past decade we have accumulated considerable experience with organ donation, mainly kidney, from non-heart-beating (NHB) donors [1]. Encouraged by the results and the many discussions with colleagues at workshops and meetings, we have learned that several categories of NHB donors can be distinguished. All these categories have their own peculiarities regarding preservation, viability of organs and ethical aspects. Therefore, we consider it of importance for further development of this large potential source of organs for transplantation, that the discussion is structured and guided by the classification we have drafted. We distinguish four categories of NHB donors (table 1).

Table 1. Categories of NHB donors

Category 1	Dead on arrival	Uncontrolled
Category 2	Unsuccessful resuscitation	Uncontrolled
Category 3	Awaiting cardiac arrest	Controlled or Uncontrolled
Category 4	Cardiac arrest while brain dead	Uncontrolled

Categories of NHB donors

The first category, category 1, has a large potential and is called *"Dead on arrival"*. Individuals in this category are declared dead outside the hospital and brought to the Emergency Room (ER) for donation purposes only. An attempt to resuscitate these patients has not been made because it would be obviously senseless to do so. The potential donors may have died of severe brain trauma or cervical fractures. Probably only kidneys will be recovered and viability is a major concern; data on the time of cardiac arrest must be precise and reliable. Another problem to overcome in this *category 1 NHB donation* is the need to re-route the dead body to the ER instead of to the morgue. Obtaining consent from relatives and legal authorities might cause delay and make donation virtually impossible. By immediate intra-aortic cooling upon arrival at the ER, organs could be obtained for donation. We have no knowledge of any

program obtaining organs from *category 1 NHB donors* at this moment.

The next category is *category 2: Unsuccessful resuscitation*. This concerns patients brought into the ER while resuscitated by the ambulance crew. The resuscitation is taken over by the hospital team. When this team decides to discontinue resuscitation because continued intervention proves to be unsuccessful, the transplant team is notified. They have to go over the available data and to decide whether a NHB donor procedure should be started. Important information is: the age of the succumbed, cause of death, kidney function, other diseases and last but not least, the time period between cardiac arrest and the moment of discontinuation of treatment. When the decision is made to start the NHB donor procedure, in countries with presumed consent legislation ("opting-out") the cooling catheter can be introduced immediately. In countries without or with "opting-in" legislation, the time until consent for organ donation is obtained, has to be bridged. There are different protocols. Either cardiac massage and artificial ventilation are performed or this is not done. In Maastricht we bridge this period with cardiac massage and ventilation, although we would prefer to start the cooling instantly [2]. Legislation is on its way wherein "preparatory handling" is allowed in order to preserve the organs for transplantation before the person's or the family's wish is known.

Category 3 is called: *Awaiting cardiac arrest*. This category includes patients that are dying in the Intensive Care Unit and where the patient or the family have agreed on organ donation. When treatment is withheld, cardiac arrest is waited for. Thereafter the cooling catheter is introduced and the deceased is transferred to the operating room for removal of the kidneys and, if possible, other organs. In the early days of transplantation when the principle of brainstem death was not yet established, this was the procedure of choice. It is referred to as "ventilator switch-off" procedure. We would like to propagate the use of the description "withdrawal of support", because there might be a situation in which the patient is not ventilator dependent. In the Pittsburgh protocol the waiting time is 2 minutes before cooling is started. We prefer to wait 10 minutes after cardiac arrest, to be sure that the patient's brain is irreversibly damaged and an equivalent situation to brainstem death has been reached. These 10 minutes however, might preclude donation of other organs than kidneys. An example of a patient in this category is someone with a severe brain trauma who does not meet the criteria of brainstem death. Nevertheless, the team that treats the patient has decided that it is useless to continue treatment. Another example is a patient with an end-stage neurological disease, for instance brain tumour with coma but without brainstem death. When the cardiac death of the patient is awaited for in the operating room, and when after cardiac arrest the abdomen is opened for central cooling through the aorta, this procedure is referred to as "controlled" NHB donation. "Controlled" means that under these circumstances the ischemia time

is under control and can be kept to a minimum, and liver, pancreas and even lungs might be procured for transplantation [3].

The last category is *Category 4: "Cardiac arrest while brain dead"*. This category includes patients who suffer an unexpected cardiac arrest either in the process of being diagnosed as brainstem dead or after the determination of brainstem death, but before they are taken to the operating room and organs could be retrieved. It is likely that at first it is tried to restore circulation, but when this is unsuccessful a NHB donor procedure may be started. In most cases consent to organ donation already has been granted at time of cardiac arrest and cooling can be employed instantly at the bed site.

Discussion

We distinguish four categories of NHB donors and we have experienced that exchange of ideas and structuring of a discussion on NHB donation can be guided through this classification system. So far we have not had access to *category 1* subjects. It is difficult to realise the procedure, on the one hand because of the absence of relatives at time death - and it might take hours before they can be located - and on the other hand because of uncertainty of ischemia time. Nevertheless, the potential of donors in this category is large. Access to these donors requires a presumed consent legislation or a legislation that allows for starting of organ cooling to prevent kidneys from decay while the relatives are approached for obtaining consent for organ donation.

At the University Hospital Maastricht we have been successful in *category 2 NHB donors* and refusal rates are not higher than in heart-beating situations. In several cases the family consented to introduction of the cooling catheter but asked for more time to reach a decision on actual organ donation. The catheter for cooling is introduced and the kidneys are cooled in the intact body. Arrangements are made for family members to take leave of their deceased beloved one. In case of consent for organ donation the body is then brought to the operating room where the kidneys are removed.

The main ethical issue raised by *category 3 NHB donation* is that one decides to obtain organs before criteria for (brain) death are fulfilled. As stated above, we consider a period of 10 minutes of no intervention important, because in doing so, there will be a state that mimics the brainstem death state. Ten minutes of asystole and no blood circulation at normothermia results in irreversible damage of the brain. It will be practically impossible to diagnose brain death, and it is subject of discussion whether the waiting period with no circulation should be 10 or 8 or even 15 minutes.

Category 4 is a group of donors that obviously can serve as kidney donors when the heart function cannot be restored. We would like to stress that a set of instruments for the introduction of the cooling catheter should be available and ready at the bedside of every potential or declared donor in order to enable at least procurement of the kidneys in case of irreversible cardiac arrest. We have encountered a special subgroup within this category, namely patients in whom brainstem death has been diagnosed, but whose family cannot accept organ removal with a still beating heart. In a few such cases we have successfully obtained at the least kidneys by means of the NHB donor procedure after withdrawal of respiratory support and subsequent cardiac arrest.

In order to structure further discussion on NHB donation we propose the use of the four categories reviewed. In our experience they fulfil the goal of a better understanding of the NHB donor concept and a more clear discussion.

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THE POTENTIAL POOL OF
NON-HEART-BEATING KIDNEY DONORS

Introduction

Solid-organ transplantation has become acceptable as the treatment of choice for end stage organ failure. Ever since the routine implementation of renal transplantation, however, the number of patients awaiting transplantation outnumbered the number of kidneys available, and the total number of renal transplants performed is strictly limited by the availability of donor organs. Although within the Eurotransplant (ET) organisation more cadaveric donors came available over the years, the number stabilised in the early 1990s and failed to increase thereafter. Additionally, due to the more liberal criteria for entering the transplant waiting lists and re-entry of patients having lost their primary graft, the waiting list has grown steeply and the discrepancy between demand and supply has increased. As a result, in the ET countries Austria, Belgium, Luxembourg, Germany and The Netherlands almost 11,000 patients were awaiting a renal transplant by the end of 1996, while little over 3,000 kidney transplants were performed that year [1].

Fighting this organ shortage is one of the challenges the transplant community has to face and various possibilities to alleviate this problem have been put forward. One of the most promising ways to increase the actual number of kidneys for transplantation, is expanding the donor pool with non-heart-beating (NHB) donors. Increased numbers of valuable organs have been reported by procuring kidneys from patients who died after irreversible cardiac arrest [2] and interest in NHB organ donation is growing. Although the pool of potential brain dead organ donors has been studied extensively in various ways [3-6], no reports exist evaluating the pool of patients dying of cardiac arrest that might be suitable NHB kidney donors. We therefore estimated by retrospective death chart review the number of potential NHB kidney donors that died at the University Hospital Maastricht in one year. In this way we gain insight into the proportion of this NHB donor pool, and the significance towards alleviating organ shortage through routine procurement of kidneys from NHB donors.

Material and Methods

The University Hospital Maastricht is located in the south of The Netherlands and is a hospital with 663 beds, including 16 Intensive Care Unit (ICU) and 22 Coronary Care Unit (CCU) beds. The hospital is serving a population of 209,000 with 20,440 admissions a year; it also serves as an academic referral center for other hospitals which account for 30% of the total number of admissions. Additionally, it houses a renal and pancreas transplant program, and has a long tradition in the procurement of kidneys from NHB donors within a NHB donor program that was installed in 1980. In general, kidneys from NHB donors are procured after an in-situ preservation (ISP) procedure, using a cooling technique by

femoral catheter. Our protocol for donor treatment and procurement has been described in extenso elsewhere [7].

The study criteria were according to the NHB donor criteria used within our protocol and involved all in-hospital deaths 3-65 y of age in the year 1994, including patients that died either just before or after admission to the Emergency Room (ER). Using the computerised registration of ICD-9-CM codes, codes of the International Classification of Diseases, patients known with malignancies other than primary brain tumours, and patients at time of death known to suffer from kidney disease, sepsis, severe uncontrollable hypertension, or suspect of intravenous drug abuse, were excluded. Of the remaining patients, death charts were reviewed and abstracted (e.g. sex, age, cause of death, hospital unit, medical condition and kidney function at time of death); deaths with incomplete charts were excluded from the study. Patients with clinical signs of brain death, whether or not confirmed by isoelectric electroencephalogram (EEG), were considered potential brain dead heart-beating (HB) donors.

The charts were reviewed by transplantation experts to determine the deceased's medical suitability to be a NHB kidney donor. Since obviously not all patients are equally suitable as kidney donors, medical suitability was scored (1 to 3) according to renal function (last known serum creatinine) and the identification of risk factors that are thought to influence the outcome of NHB donor kidneys (age over 50, hypertension, diabetes mellitus) or to thwart the in-situ cooling (arterial vascular disease)(table 1). Patients medically unsuitable, regardless of the risk factors mentioned, were given suitability score 0 and were excluded. Although NHB donors have in common that organs are procured after cardiac arrest and subsequent period of warm ischemia, the total group is very diverse and categories of NHB donors have been identified and described by us [8]. These categories will be important in the transplant outcome of NHB donor kidneys. *Category 3 (Awaiting cardiac arrest)* and *category 4 NHB donors (Cardiac arrest while brain dead)* mimic HB donors with minimal ischemic damage to the organs, while organs from *category 2 NHB donors (Unsuccessful resuscitation)* have suffered considerable warm ischemia. Additionally, in *category 3 and 4 NHB donation* organ donation can be discussed before final cardiac arrest and ease preservation procedures. Logistics are important in the success rate of ISP procedures, and performing emergency procedures like ISP is easier at ICU and ER than at general wards. Therefore, also the logistic availability of potential donors (category, hospital unit) was scored (1 to 3). Again 0 scorers were expelled from further analysis (table 1). Finally, it was assessed whether suitable patients had become actual donors (i.e., ISP procedure had been started), permission for donation was refused by the relatives, or the deceased had not been identified as a potential NHB kidney donor.

Table 1. Scoring criteria for medical suitability and logistic availability of potential NHB donors.

Score	Medical suitability
0	unsuitable
1	creatinine > 100 µmol/L + 2 or more risk factors ^a
2	creatinine ≤ 100 µmol/L + 2 or more risk factors or: creatinine > 100 µmol/L + 0-1 risk factors
3	creatinine ≤ 100 µmol/L + 0-1 risk factors
Score	Logistic availability
0	dead on arrival (<i>NHB donor category 1</i>) ^b / not available
1	unsuccessful resuscitation (<i>NHB donor category 2</i>) / general wards
2	unsuccessful resuscitation (<i>NHB donor category 2</i>) / ICU, CCU, ER, OR ^c
3	awaiting cardiac arrest (<i>NHB donor category 3</i>) or cardiac arrest while brain dead (<i>NHB donor category 4</i>) / ICU, CCU

^a risk factors are: age > 50, patient history of hypertension, diabetes, or arterial vascular disease.

^b categories of NHB donors; see ref. 8.

^c ICU=intensive care unit, CCU=coronary care unit, ER=emergency room, OR=operating room.

By combining the medical suitability and logistic availability scores in a total score, the potential of NHB kidney donors and the likelihood that the donation would be effectuated successfully, were quantified. Total scores of 2-3, 4, or 5-6 points, were considered to reflect a low (group A), moderate (group B), or high potential (group C) to become a successfully effectuated NHB kidney donor, respectively. Using this potential for success, estimations for the minimum and maximum numbers of NHB donors available, were calculated. When appropriate, data are given as mean $\pm$ SEM. Statistical analysis employed non-parametric Mann Whitney-U test and chi-square tests for differences between groups.

Results

From a total of 603 in-hospital deaths at the University Hospital Maastricht in 1994, there were 175 deaths among patients 3-65 years of age; another 25 deaths in this age group were reported from the ER. Computer analysis of these deaths revealed 82 patients diagnosed at time of death with ICD-9-CM codes that precluded kidney donation. No data were available from 9 deaths; death charts of 109 patients were studied. Subsequent expert review of the charts showed that another 45 patients were in a medical condition at time of death that made kidney donation impossible (e.g. long agonal phase, suspect of carcinoma, unsuitable medical history); 8 patients seemed to have become clinically brain dead, which had been confirmed by EEG when organ donation was considered (7 cases). The potential of HB donors therefore was 8; the remaining 56 were considered to be potential NHB donors (fig. 1).

The scores for medical suitability, logistic availability as well as the combined total scores are depicted in table 2; the combined scores in table 3. The latter showed that 27 deceased patients had a high potential of becoming a successful NHB kidney donor (group C, score 5-6), while 22 patients scored 4 (group B, moderate potential) and 7 scored 2-3 (group A, low potential). Estimations of the potential number of NHB donors available therefore ranged from 27 - 56, i.e., 4.5 - 9.2 NHB donors/100 in-hospital deaths, depending on whether only potential donors from group C were included, or all potential NHB donors (groups A, B, and C).

Potential NHB donors were predominantly male (70%), aged 49.1 ± 1.8 y, and serum creatinine 100.1 ± 6.8 μ mol/L. Most of them died of acute heart failure (55%), cerebral trauma (13%), or cerebral bleeding/anoxia (14%). They died at ER (36%), ICU (20%), CCU (13%), operating room (OR)(13%), and general wards (20%). Attending physicians at time of death were cardiologists (45%), neurologists (18%), cardiopulmonary surgeons (16%) or general surgeons (7%). Forty-nine (88%) of all potential NHB donors were identified as *category 2 NHB donors*, that died after an unsuccessful resuscitation

Figure 1. Selection process for potential NHB donors

Table 2. Scoring results of potential NHB donors (n=56)

score ^a	medical suitability (n)	logistic availability (n)
1	2	11
2	24	37
3	30	8

^a See criteria outlined in table 1.

attempt. The rest was identified as *category 3 NHB donors*; none was identified as a *category 4 NHB donor*. When comparing potential donors in group C with the remaining potential donors (groups A-B), there were no differences except for serum creatinine (76.8 ± 7.9 vs. 109.5 ± 8.4 $\mu\text{mol/L}$, respectively; $p=0.01$) and for hospital unit where the patient died (24/27 at ER, ICU, or CCU vs. 11/29, respectively; $p=0.001$).

Table 3. Potential of successful NHB kidney donation

Potential	Total score ^a	Group	n
Low potential	2-3	A	7
Moderate potential	4	B	22
High potential	5-6	C	27

^a Total scores are the combined scores for medical suitability and logistic availability of the potential donors.

Of the 56 potential NHB donors at our institution, 20 were identified as such; in one case identification of the donor was not possible and consent from the next of kin could not be obtained in time to start organ preservation. Consent to donation was refused in 6/19 cases (32%), equal to our refusal rate in HB donation. Thirteen (68%) became actual donors; in 1 case legal consent was withdrawn by the District Attorney after a successful ISP procedure. Not identified and therefore missed potential donors only differed from identified potential NHB donors in their serum creatinine levels (91.9 ± 6.6 vs. 126.3 ± 17.2 $\mu\text{mol/L}$; $p=0.04$), and they were more often attended by physicians other than cardiologists ($p<0.001$). Apart from losing donors due to refused permission for organ donation, actual donors were lost because of technical failures. Through projection of the figures for refusal and technical failure onto the 27-56 estimated potential NHB donors, the number of kidneys that would actually come available is calculated to be 24.0 - 49.6 per year (table 4).

Table 4. Projected number of NHB donor kidneys available

Estimation	High	Moderate	Low	HB ^a
Potential donors	56	49	27	8
Refusal rate (32%)	17.9	15.7	8.6	2.6
Actual donors	38.1	33.3	18.4	5.4
Technical failures (35%)	13.3	11.7	6.4	--
Kidneys available	49.6	43.2	24.0	10.8

^a HB=heart-beating brain dead donors

Discussion

The success of renal transplantation is limited by the number of organs available. The growing demand for kidneys is accompanied by an insufficient supply of organs. In fact, in the ET area in 1994 donor rates decreased by 10%: 13.9 donors were effectuated per million population (p.m.p.) versus 15.4 in 1993, resulting in 26.4 kidneys p.m.p. available for transplantation [1]. Numerous studies have been conducted to estimate the potential donor supply, directed towards the identification of potentially brain dead patients. Although study designs differed (prospective-retrospective, review of in-hospital death charts at various hospital units, or identification of potential donors from large mortality data bases), invariably it was concluded that the potential number of cadaveric organ donors is far larger than the numbers effectuated. The calculated numbers of potentially brain dead donors show considerable variation, roughly from 28 to over 100 HB donors p.m.p., due to the criteria used to in- and exclude patients as suitable donors and the way of extrapolating study results to larger populations [3-6,9-12].

There are several options to fight the huge disparity between the numbers of donor kidneys demanded and available. Although the best transplant results are achieved with organs from living donors [13], the

supply will probably never suffice since their availability is limited by personal and cultural conditions. Within ET currently 7% of renal transplants are performed with organs from living related donors [1]. Organ shortage will also be alleviated by a more efficient use of the grafts available through improving transplant results. Ongoing research efforts will improve immunosuppressive regimens that might reduce the loss of transplanted grafts due to acute and chronic rejection. Additionally, overall graft survival may be increased through more strict selection of recipients with regard to age, physical condition, retransplantation, and sensitisation grade. Xenotransplantation, although maybe feasible in the future, is not an option at the moment [14].

The most direct option with the largest potential is increasing the number of cadaveric organ donors. Much can be achieved in identifying potential organ donors, improving consent rates, and increasing transplant activities by implementation of organisations for procurement, education, and transplant co-ordination [15,16]. Feest et al. [17] reported doubled donation rates after introduction of a protocol to initiate ventilatory and hemodynamical support for the sole purpose of organ donation in patients with lethal cerebrovascular incidents. Over the years donor criteria have become more liberal and so called marginal donors are increasingly used to expand the donor pool [18]. All these efforts, however, are primarily directed to increase the number of HB donors, although probably less than 6% of patients dying in hospitals will be diagnosed brain dead [10,19]. Far larger is the estimated proportion of potentially suitable donors reported to die of cardiac arrest before ventilation is started (57%), or while on respiratory support but before brainstem death can be confirmed (12-21%)[20,21]. All these patients must be considered potential NHB donors.

Organs from NHB donors can be procured and transplanted successfully, as reported by several authors [22-25]. In fact, before the notion of brain death was generally accepted all transplants were performed with organs from NHB donors; these donors sustained cardiac arrest after withdrawal of ventilatory support in the OR. Even today, in countries where brain death is not accepted, this is the only way of procuring organs. Later, emergency procedures were developed for organ salvage from donors with unexpected and irreversible cardiac arrest [26-28] and implementation of such protocols resulted in at least 20% more kidneys transplanted [2,29].

The proportion of potential NHB donors, however, is much more impressive. As revealed by this study, in addition to the potential eight HB donors, 27-56 NHB donors were found resulting in a projected annual 2 to 4.5 fold increase of kidneys available for transplantation (table 4). Nathan [30] estimated a potential of 123 (range 104-183) NHB donors p.m.p. from a computer algorithm of in-hospital deaths

in the United States. This number was twice as high as the projected pool of estimated HB donors. Introduction of routine kidney procurement from NHB donors in, for example, only the University Hospitals in The Netherlands, would already have major consequences for the Dutch renal waiting list. Assuming that all eight University Hospitals are comparable with regard to patient population and causes of death, their reported 4860 in-hospital deaths in 1994 may have included a number of 217.6 to 451.3 potential NHB kidney donors. The extrapolated total number of kidneys available from these eight centers, with presumably equal refusal and failure rates as found in our center, thus could be as high as 143.6 to 297.8 annually. For The Netherlands this would be equivalent to a rise of 9.4-19.4 kidneys p.m.p. These additional organs for transplantation would at least stop the waiting list from growing.

Through employing a scoring system in our study, we tried to correct for factors that may hamper preservation procedures and reduce the chance on a successful donation procedure. Logistically it is much more demanding to perform ISP procedures at general wards than at specialised ICU or CCUs; introduction of this kind of procedures needs education, training and dedication towards donation. However, 38 (68%) of the identified potential NHB donors in our estimate were located at ER, ICU, and CCU; hospital units traditionally well equipped with highly trained personnel that often is familiar with emergency and donation procedures. Additionally, 21 (70%) of the 30 potential NHB donors with the highest medical suitability scores were found at these units as well. Focusing on NHB donation at ER, ICU, and CCU wards might therefore be justifiable as this option is the most promising with regard to success and would still improve donation rates remarkably.

For the calculated numbers of kidneys eventually available, a technical failure rate was taken into account. In the period 1993-1995 malpositioning and impossible insertion of the cooling catheter, together with rupture of balloons, accounted for a loss of 35% of donors, *category 2 NHB donors* mostly. This figure is consistent with the failure rate of procedures reported by Dunlop et al. [31]. However, through using a redesigned cooling catheter (Porgès AJ 6516) and checking catheter position by X-ray, this failure rate could be reduced. Apparently, techniques can still be improved further which will increase the actual number of organs available per potential NHB donor. Not surprisingly, *category 2 NHB donors*, i.e., patients dying of unexpected or sudden cardiac arrest, account for 88% of the identified potential in this estimate. Essential in limiting the ischemic damage to the organs and keep the option of organ donation in this category, is to start in-situ cooling as soon as possible after cessation of circulation. In countries with opting-in legislation, an important burden in this so called uncontrolled NHB donation is the fact that also consent for preservation is mandatory before organ cooling may be initiated. Presumed-consent legal systems, or legislation that allow preservation of organs without

consent if relatives cannot be approached immediately, offer the possibility to minimise the duration of ischemia and thus optimise the success of NHB organ donation.

This study has shown that the pool of potential NHB kidney donors is considerable; 2 to 4.5 fold increased numbers of kidneys may come available from NHB donors when compared to the estimated number of HB donor kidneys. With at least a doubling of the number of renal grafts, the acute shortage of kidneys for transplantation will be fought successfully and waiting lists will no longer grow. We recommend the introduction of kidney procurement from NHB donors at ER, ICU, and CCU, as donation activities at these hospital units will be the most effective and successful. As most NHB donors are uncontrolled *category 2 NHB donors*, a legal system based on presumed-consent that allows preservation of organs without consent, enables optimal availability of the pool of potential NHB kidney donors.

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SHORT-TERM OUTCOME OF KIDNEY TRANSPLANTS
FROM NON-HEART-BEATING DONORS
AFTER PRESERVATION BY MACHINE PERFUSION

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Introduction

In the surge for ways to reduce the organ shortage non-heart-beating (NHB) donors are recognised as a valuable source of organs for transplantation. After irreversible cardiac arrest and the subsequent diagnosis of death, organs can be procured and used for transplantation, especially kidneys are known to tolerate ischemia well [1]. The University Hospital Maastricht has had a NHB donor program for over a decade and procures and transplants kidneys from NHB donors [2]. Analysis of the transplantation outcome with these renal grafts showed a high incidence of delayed function (DF) due to acute tubular necrosis (ATN) when compared to grafts from heart-beating (HB) donors [3]. From experimental data it is known that preservation of ischemically damaged kidneys by machine perfusion (MP) is superior to simple cold storage (CS)[4,5]. In order to reduce the DF rate of kidney grafts from NHB donors we recently implemented MP as the preferred method of preservation. In this study the results of 22 renal grafts from NHB donors preserved by MP are evaluated and compared with a historical control group of grafts from NHB donors preserved by CS.

Material and Methods

Patients dying after irreversible circulatory arrest in the Emergency Room or Intensive Care Unit, were considered potential NHB donors when meeting the following criteria: circulatory arrest no longer than 30 min excluding the time of effective resuscitation and a total resuscitation time not longer than 2 h. The upper age limit was considered to be 65 y and the potential donors were not known to suffer from kidney disease, uncontrolled hypertension or metastasising malignancies. Patients with signs of sepsis or intravenous drug abuse were excluded. All donors came either available after an unsuccessful attempt of cardio-pulmonary resuscitation (*category 2 NHB donors*) or sustained final cardiac arrest after withdrawal of ventilatory support (*category 3 NHB donors*)[6]. After consent, an in-situ perfusion procedure using a double-balloon-triple-lumen cooling catheter (Porgès AJ 6516) according to a technique described before, was performed [7]. Afterwards the kidneys were procured in the operating room. The organs were preserved by either CS, using Euro-Collins (EC), histidine-tryptophan ketoglutarate (HTK) or University of Wisconsin (UW) CS solutions, or by MP in a Gambro-PF3B perfusion machine using a UW derived machine preservation solution as a perfusate [8]. The in-situ flush out was performed using the respective CS solution, or HTK in all MP cases. During MP flow characteristics and enzyme loss were recorded to evaluate the viability of the kidney. Because of these parameters, among others, kidneys were discarded and not used [9]. Kidneys transplanted within the Eurotransplant area were included and data were collected by approaching the transplant centers.

Short-term posttransplant function was classified as: (a) immediate function (IF), i.e. immediate life-sustaining renal function without posttransplant dialysis; (b) DF, i.e. renal function that ultimately was life-sustaining but required one or more dialysis sessions; (c) primary nonfunction (PNF), i.e. renal function failed and the patient never was without dialysis. Renal function was recorded as serum creatinine at 1 and 3 months after transplantation. DF was quantified by urine output during the first 24 h posttransplant, the duration of DF (number of days) and number of dialysis sessions required. The warm ischemia time (WIT), i.e., time elapsed between cardiac arrest and in-situ organ cooling, tended to be longer in the MP group. Therefore, the results of transplanting kidneys with prolonged warm ischemia, WIT of 45 min or more, were analysed separately.

The results are given as mean $\pm$ SEM. Statistical analysis employed the chi-squared test with Yates' correction or Fisher's exact test, Mann-Whitney U-test and Kruskal-Wallis one-way ANOVA test for differences between the groups, as appropriate. A p-value of less than 0.05 was considered significant.

Results

Between July 1993 and December 1994, 22 kidneys were recovered from 14 NHB donors, preserved by MP and subsequently transplanted. In the control group 57 kidneys from NHB donors were transplanted after preservation by CS in the period 1980-1992. Causes of death showed more brain trauma in the CS group while more patients died after myocardial infarction in the MP group ($p=0.001$). Data concerning donor and recipient factors are summarised in table 1. Donor age was 40.7 ± 2.3 y in the CS group and 45.8 ± 3.7 y in the MP group ($p=0.21$); last serum creatinine level of the donors was 101.6 ± 4.9 and 111.6 ± 9.4 $\mu\text{mol/L}$, respectively ($p=0.35$). Kidneys in the CS group sustained a WIT of 33.1 ± 2.9 min versus 52.0 ± 7.9 min in the MP group ($p=0.10$). Forty-two (74%) were cold stored in EC, 14 (24%) in HTK, and 1 (2%) in UW; in the MP group one pair of kidneys was initially flushed with EC and all others with HTK.

Mean cold ischemia time (CIT) was 31.5 ± 1.1 h for cold-stored grafts and 30.2 ± 1.2 h for machine perfused grafts ($p=0.50$). Recipient data in either group were comparable for age, sex, number of the graft, and peak level of panel-reactive-antibodies. In the MP group the mean HLA-DR mismatch was 0.6 ± 0.1 vs 0.3 ± 0.1 in the CS Group ($p=0.03$). Mean anastomosis time was shorter in the CS group (36.2 ± 2.0 min vs 40.7 ± 3.4 min, $p=0.05$). Short-term posttransplant outcome is summarised in table 2. Results were comparable for both groups, reflected by similar immediate posttransplant urine output and equal duration of DF, number of dialysis sessions, and kidney function at 1 and 3 months. In the separate

Table 1. Donor and recipient data ^a

	CS	MP	p-value
Period	1980-1992	1993-1994	
Cases	57	22	
<u>Donor data</u>			
Age (y)	40.7 ± 2.3	45.8 ± 3.7	0.21
Creatinine (μmol/L)	101.6 ± 4.9	111.6 ± 9.4	0.35
WIT (min)	33.1 ± 2.9	52.0 ± 7.9	0.10
<u>Recipient data</u>			
Age (y)	44.9 ± 1.7	49.0 ± 3.1	0.19
CIT (h)	31.5 ± 1.1	30.2 ± 1.2	0.50
Anastomosis time (min)	36.2 ± 2.0	40.7 ± 3.4	0.05
Graft number	1.2 ± 0.1	1.1 ± 0.1	0.80
HLA-DR mismatch	0.3 ± 0.1	0.6 ± 0.1	0.03
CyA immunosuppression	35 (38%)	18 (82%)	0.05

^a Results are given as mean±SEM or numbers (%).

Table 2. Short-term outcome up to 3 months posttransplantation ^a

	CS	MP	p-value
Cases	57	22	
Immediate function	15 (26%)	8 (36%)	0.67
Delayed function	34 (60%)	11 (50%)	
Primary nonfunction	8 (14%)	3 (14%)	
Urine output (ml/1st 24 h)	836.9 ± 154.1	1465.2 ± 540.2	0.66
Duration of DF (days)	17.2 ± 1.7	18.9 ± 2.8	0.63
Number of dialyses	6.0 ± 0.8	5.5 ± 1.2	0.84
Creatinine 1 mo (μmol/L)	338.5 ± 46.1	324.5 ± 69.4	0.58
Creatinine 3 mo (μmol/L)	194.2 ± 13.8	264.8 ± 44.8	0.39

^a Results are given as mean±SEM or numbers (%).

analysis (concerning prolonged ischemia time defined as WIT of 45 min or more), 17 kidneys (30%) in the CS group sustained prolonged warm ischemia (WIT 59.7 ± 4.3 min) vs 10 kidneys (45%) in the MP group (WIT 89.0 ± 6.2 min, $p=0.002$). Results are depicted in table 3. All donor data were comparable; for recipient data again the anastomosis time was different (CS group 29.9 ± 2.3 min vs MP group 47.5 ± 4.1 min, $p=0.001$). In table 4 the posttransplant outcome of kidney transplants with prolonged warm ischemia is summarised. It is shown that this outcome was better in the MP group when compared to the CS group ($p=0.02$), reflected by lower serum creatinine at 1 month ($p=0.05$); serum creatinine at 3 months showed statistically no significant difference ($p=0.50$). Length of DF and number of dialyses required were the same.

Discussion

Although kidneys from NHB donors have been used ever since the first transplantation, ways have to be found to ameliorate ischemic damage through optimal methods of preservation and minimisation of reperfusion injury, in order to develop this valuable source of transplantable organs. Studies on the transplantation results with kidneys from NHB donors show DF rates as high as 60-75%, almost twice as high as for grafts from HB donors [3,10-15]. This delayed onset of renal function is unfavourable; DF is masking the signs of early acute rejection, will complicate the post-operative treatment and reinstitution of dialysis will have a negative impact on the patient's psyche. Consequently hospital stay will be prolonged and costs increased [16,17]. The effect of DF on graft survival is controversial; some believe DF is associated with reduced graft survival, while others suggest that DF only in combination with acute rejection is responsible for worse transplant outcome [18-23]. In spite of doubled DF rates, long-term graft and patient outcome did not differ between recipients of NHB and HB kidneys [3].

To reduce DF rates we introduced MP in 1993 as the method of preservation for NHB donor kidneys using the UW preservation solution for MP as a perfusate. Nowadays, CS is accepted as the method of choice for the preservation of renal grafts; the advantages of preservation by continuous cold perfusion are most pronounced for prolonged preservation times and for kidneys from marginal donors with ischemic damage [4,24]. Recent experimental work at our laboratories showed that preservation of ischemically damaged kidneys by MP was superior to preservation by CS. MP counteracts the intra-renal vascular resistance induced by ischemia, providing better reperfusion and delivering oxygen and nutrients to and removing waste products from the organ during preservation. This resulted in better survival of the animals and better preservation of the microcirculatory integrity [5,25]. The superiority of MP has been confirmed in clinical transplantation on several occasions and recently

Table 3. Donor and recipient data for kidneys with WIT of 45 min or more ^a

	CS	MP	p-value
Period	1980-1992	1993-1994	
Cases	17	10	
<u>Donor data</u>			
Age (y)	42.4 ± 3.9	52.6 ± 5.2	0.08
Creatinine (μmol/L)	114.6 ± 8.6	121.4 ± 5.4	0.44
WIT (min)	59.7 ± 4.3	89.0 ± 6.2	0.002
<u>Recipient data</u>			
Age	44.8 ± 3.8	51.7 ± 4.2	0.27
CIT (h)	30.8 ± 2.2	29.6 ± 1.9	0.56
Anastomosis time (min)	29.9 ± 2.3	47.5 ± 4.1	0.001
Graft number	1.3 ± 0.2	1.2 ± 0.1	1.0
HLA-DR mismatch	0.3 ± 0.1	0.5 ± 0.2	0.22
CyA immunosuppression	13 (76%)	8 (80%)	0.83

^a Results are given as mean±SEM or numbers (%).**Table 4.** Short-term outcome of kidneys with WIT of 45 min or more ^a

	CS	MP	p-value
Cases	17	10	
Immediate function	4 (23%)	5 (50%)	0.02
Delayed function	13 (76%)	3 (30%)	
Primary nonfunction	0 (0%)	2 (20%)	
Urine output (ml/1st 24 h)	959.5 ± 323.5	1734.8 ± 998.0	0.70
Duration of DF (days)	17.2 ± 2.4	12.7 ± 1.8	0.42
Number of dialyses	4.8 ± 0.9	5.3 ± 1.3	0.93
Creatinine 1 mo (μmol/L)	408.6 ± 76.9	197.3 ± 26.3	0.05
Creatinine 3 mo (μmol/L)	197.4 ± 23.0	163.3 ± 14.9	0.50

^a Results are given as mean±SEM or numbers (%).

impressive results were reported with NHB donors after preservation by MP [26-29]. Kidneys were from *category 3 NHB donors* only and sustained considerably less ischemia than our series and showed merely 19-22% DF rate [30,31]. In the present analysis, the DF rate in the MP group was 50% compared to 60% in the CS group.

Apart from donor type (cadaver vs living donor and NHB vs HB donor) and the preservation mode, several other factors have been identified as risk factors for ATN and DF. Retransplantation, warm ischemia time, type of preservation solution, preservation time and number of HLA-DR mismatches have all been supposed to worsen early posttransplant function [3,16,17,23,32,33]. In this analysis several of these factors were studied and some appeared to be statistically significant different between the study group and historical controls. For the machine perfused kidneys the anastomosis time was longer as well as the WIT; the evident worse match on HLA-DR antigens in the MP group further deteriorates the early posttransplant function of these grafts. The results of the comparison between CS and MP groups therefore may be biased to the detriment of the MP group concerning transplant outcome. However, although considered to be more profoundly damaged than the NHB donor kidneys in the CS group, the grafted kidneys preserved by MP showed equal short-term outcome when compared to grafts preserved by CS. In a previous analysis we showed that the elevation of serum creatinine at 1 month posttransplant when compared to HB donors, was transient and neutralised at 3 months, not affecting long-term graft outcome [3].

In the last few years increased efforts have been made to identify potential NHB donors and it was noticed that in our program more *category 2 NHB donors* were accepted after long resuscitation efforts. This fact is reflected by the increased number of donors dying after myocardial infarction and unsuccessful resuscitation; momentarily about half in the MP group (period 1993-1994) against one-third in the CS group (period 1980-1992). The more recently effectuated donors may therefore have suffered essentially more ischemic damage while all kidneys procured were preserved by MP. Kidneys in the historical control group on the contrary, may be less damaged while all were preserved by CS. The separate analysis of NHB donor grafts with WIT longer than 45 min, indeed showed that almost half of the kidneys in the MP group suffered prolonged WIT and that even within this subgroup WIT evidently was increased (89.0 ± 6.2 vs 59.7 ± 4.3 min for MP vs CS group, respectively; $p=0.002$). In the prolonged ischemia group, the other donor and recipient data were more comparable in CS and MP groups thus minimising their influence. Although in the subgroup kidneys preserved by MP still sustained more warm ischemic damage than their CS counterparts, the short-term results were significantly improved by MP. This improvement was reflected by a better serum creatinine level at one

month. The number of days before kidney function was life sustaining as well as the number of dialyses needed, did not differ among the groups, implying that MP did not effect the severity of DF once it had occurred. Using cyclosporin (CyA) in the immediate posttransplant period is known to prolong the duration of DF [17]. The length of DF is important since prolonged DF was observed to be detrimental to graft survival in NHB donor kidney transplants (34). In the MP group, more patients received CyA (82 vs 63%, $p=0.05$) which may oppose the positive action of perfusion preservation. The use of ATG as induction immunosuppression and delayed introduction of CyA until acceptable kidney function is established, could decrease the duration of DF [33].

Conclusion

NHB donor kidneys and their recipients need special attention with regard to preservation and postoperative treatment. High DF rates are of major concern and must be reduced to improve graft prognosis. The CS group in this analysis suffered less severe ischemic attacks than the MP group. Nevertheless, short-term outcome was at least equal in both groups; for kidneys that sustained prolonged ischemia, however, MP was superior to CS despite more unfavourable donor conditions. To verify these results and study the impact of MP on long-term outcome, a prospective study with matched controls is currently in process. We conclude that continuous cold perfusion is superior to simple cold storage as the preservation mode for NHB donor kidneys, especially after prolonged warm ischemic periods.

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EFFECT OF MACHINE PERFUSION PRESERVATION ON DELAYED
GRAFT FUNCTION IN NON-HEART-BEATING DONOR KIDNEYS
- EARLY RESULTS -

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Introduction

Organs for transplantation procured from non-heart-beating (NHB) donors sustain a period of warm ischemia once the blood stops circulating. This warm ischemic period leads to rapid loss of high energy metabolites, altered calcium metabolism, and vascular blockage, which consequently damage the organs. Basic cell functions are disturbed and reactive oxygen species are generated when the blood flow is subsequently restored [37]. As a result, upon reperfusion organ function after transplantation may be impaired or even lost. Transplanted NHB donor kidneys show high rates of delayed onset of function [16,35,38]. The impact of delayed function (DF) on the outcome of transplantation is negative and considered undesirable for both clinical and economical reasons.

Preservation by machine perfusion (MP) is thought to be advantageous for the preservation of ischemically damaged kidneys [23] and for improving the immediate posttransplant graft function [39]. Experiments have confirmed this; in fact, MP preservation of kidneys damaged by warm ischemia has been shown to be superior to preservation by simple cold storage (CS). In a recent study, MP resulted in better survival rates and improved preservation of the microcirculatory integrity [6,7].

In an attempt to improve the early posttransplant function and reduce the DF rate, we selected MP as the preferred method for kidney preservation in our NHB donor program. In this study, we evaluate the early posttransplant function of grafted NHB donor kidneys preserved by MP and compare the short-term results with those of matched heart-beating (HB) donor kidneys preserved by CS.

Patients and Methods

NHB donor kidneys were procured from patients who had suffered irreversible cardiac arrest. Patients were considered potential donors if circulatory arrest lasted less than 30 min (excluding the time of resuscitation), and cardiopulmonary resuscitation did not exceed 2 h. The donors were not older than 65 years of age and had no history of uncontrollable hypertension, kidney disease, or malignancies other than nonmetastasising primary brain tumours. The donors had no signs of systemic infection or intravenous drug abuse. The kidneys were preserved in-situ through a technique applying a femoral cooling catheter and large volumes of cold preservation solution, as soon as possible after death and consent for organ donation was obtained [20]. All kidneys were preserved by MP in Gambro PF-3B organ perfusion machines (Gambro, Lund, Sweden). University of Wisconsin (UW) solution for MP was used as the perfusate [5]. Flow was set to an initial perfusion pressure of 60 mmHg and kept constant thereafter; the kidneys were perfused continuously until transplantation. Organs not procured locally,

were initially preserved by CS and sent to our institution. Kidneys were offered by Eurotransplant and transplanted to patients at several transplant centers according to the uniform Eurotransplant allocation policy.

As controls for each recipient of a NHB donor kidney, we selected two recipients of HB donor kidneys from Eurotransplant files. This control group was stratified for transplant center to prevent bias due to differences in posttransplant management. To prevent bias due to the effect of different preservation solutions, all HB donor kidneys were preserved by UW in simple CS. The controls were matched for the number of transplant (first, second, third), current level of panel reactive antibodies (PRA; 5% or less, 6%-85%, or > 85%), number of HLA-DR mismatches (0,1,2), donor age (< 10 years difference), date of transplantation (< 5 years difference), and recipient age (15-50, over 50 years of age; table 1). Transplant centers were approached for co-operation and data were collected by questionnaire.

Table 1. Matching criteria

- Same transplant center
- CS preservation by UW ^a
- Number of transplant (1st, 2nd, 3rd)
- Current level PRA ($\leq 5\%$, 5-85%, $\geq 86\%$)
- HLA-DR mismatches (0, 1, 2)
- Donor age (+/- 10 years)
- Date of transplantation (+/- 5 years)
- Recipient age (5-50, >50 years)

^a CS=cold storage, UW=University of Wisconsin preservation solution, PRA=panel of reactive antibodies, HLA=human lymphocyte antigens

Relevant donor and recipient characteristics were analysed to ascertain the efficiency of matching. Early posttransplant function was classified as (1) immediate function (IF), i.e., life-sustaining renal function without any posttransplant dialysis; (2) delayed function (DF), i.e., ultimately life-sustaining renal

function but at least one posttransplant dialysis treatment necessary; or (3) primary nonfunction (PNF), i.e., renal function failed with continuing dialysis. Renal function was estimated at 1, 3, and 6 months posttransplant by measuring serum creatinine levels. Grafts with DF were analysed further. The number of postoperative days until the patient was without dialysis and number of dialysis sessions within this period were registered. Rejection episodes in the first 3 months were defined as the number of rejection treatments implemented, whether proven by biopsy or not.

Finally, the outcome of renal grafts from NHB donors who died after an unsuccessful resuscitation attempt (*category 2 NHB donors*) was compared to that of renal grafts from NHB donors who sustained irreversible cardiac arrest after intentional withdrawal of life-support treatment (*category 3 NHB donors*). Categorisation is described in detail elsewhere [24].

The results are given as percentages or as mean $\pm$ SD. The differences between the groups were calculated with the Pearson chi-square test and Yates' correction for discrete variables. The Mann-Whitney U-test for unrelated continuous variables, where appropriate. P values less than 0.05 were considered statistically significant.

Results

Between August 1993 and July 1995, 39 NHB donor kidneys were preserved by MP at the University Hospital Maastricht and subsequently transplanted within the Eurotransplant organisation. Two grafts that were successfully transplanted outside this area were not available for matching purposes and excluded from the study [29]. Thus, 37 kidneys with a follow-up period of at least 6 months (mean 13 ± 7), were included in the NHB group. Consequently, 74 HB controls were matched and selected for comparison. The donor data are given in table 2. The main causes of death among the NHB donors were myocardial infarction (35%), cerebral bleeding (30%), and cerebral trauma (19%). HB donor death was caused mainly by cerebral bleeding (58%) and cerebral trauma (34%; $p < 0.001$). Seventeen kidneys from *category 2* and 17 from *category 3 NHB donors* were transplanted. Three (8%) kidneys were procured from NHB donors who had sustained irreversible cardiac arrest after brain death had been diagnosed (*category 4 NHB donors*). NHB donors died either in the emergency room (41%) or Intensive Care Unit (ICU; 59%), so that none of the in-situ preservation procedures took place in the operating room under controlled circumstances [14].

Table 2. Donor characteristics ^a

	NHB donors	HB donors	p-value
Age (years)	45±16	41±15	0.23
Gender (M:F)	16:21	51:23	0.009
Serum creatinine (μmol/L)	111±58	87±31	0.009
Diuresis (mL/last 24 h) ^b	3319±2493	5337±3291	0.003
WIT (min)	49±34	0±0	<0.001

^a Values in mean±SD or numbers.

^b Whenever available in NHB donors.

Recipient characteristics are given in table 3. There were no differences between the NHB and HB donor groups for blood type, original renal disease, type of dialysis (haemodialysis in 70% vs 64%, $p=0.83$), or duration of pretransplant dialysis (36 ± 39 vs 45 ± 43 months, $p=0.32$). Immunosuppressive protocols were similar for both groups. Cyclosporin was used for 32 (86%) of the NHB donor kidneys, and for 68 (92%) of the HB donor kidneys ($p=0.93$). It was introduced preoperatively or immediately postoperatively in 23 (72%) and 47 (68%) cases, respectively. Recipients of NHB donor grafts received cyclosporin plus steroids 14 times (38%) and triple immunosuppression (cyclosporin, azathioprine, and steroids) 17 times (46%). These regimens were prescribed in 46 (62%) and 18 (24%) recipients in the HB donor group, respectively ($p=0.08$). Induction protocols using OKT3, ATG/ALG, or other agents for rejection prophylaxis were used for 8 (22%) NHB donor kidneys and for 15 (20%) HB donor kidneys ($p=0.56$).

Early posttransplant function of NHB donor kidneys was significantly different from that of HB donor kidneys (table 4). Renal function was impaired but recovered within 6 months (table 5). As a result of the high PNF rate, the survival of NHB donor grafts was reduced. Additionally, two grafts failed within the first month due to untreatable acute rejection. Thus, graft survival at 6 months was 72% for NHB donor grafts and 90% for HB donor grafts. No patients died within 6 months posttransplant in either group.

Table 3. Recipient characteristics ^a

	NHB donors	HB donors	p-value
Age (years)	48±14	47±12	0.36
Gender (M:F)	24:13	47:27	0.89
Retransplant	4 (11%)	15 (20%)	0.37
Current PRA >5%	6 (17%)	12 (16%)	0.87
HLA-DR mismatches	0.5±0.5	0.4±0.5	0.52
CIT (h)	30±6	25±9	0.001

^a Values in mean±SD or numbers (%).

A comparison of transplants with kidneys from *category 2* (n=17) and *category 3* (n=17) *NHB donors*, revealed no differences in early posttransplant outcome or renal function, despite increased age (52±14 vs 41±13 years, respectively; p=0.01), increased serum creatinine (133±52 vs 93±62 µmol/L, respectively; p=0.003), and longer warm ischemia time (WIT; 79±26 vs 21±11 min, respectively; p<0.001) for *category 2 NHB donors*. Five grafts (29%) from the *category 2 NHB donors* and one from the *category 3 NHB donors* had PNF. The difference, however, was not statistically significant (p=0.12).

Discussion

The success of transplantation has made it the treatment of choice for patients with end-stage renal disease. Yet, the increasing demand for kidneys has created a gap between the organs available and the organs needed. In renal transplantation, *NHB donors* are valuable in reducing this organ shortage and the waiting lists, and organ procurement from these donors is gaining more attention. However, *NHB donors* sustain cardiac arrest, and warm ischemia subsequently damages the kidneys. This, in turn, cause acute tubular necrosis (ATN) and impaired clinical function after grafting. Thus, there is often no immediate life-supporting function and dialysis must be continued until the transplanted kidney has recovered and regained its function.

There is much controversy about the effect of DF on graft survival [4,8,9,36]. It is thought to increase the incidence of acute rejection [22] and to reduce graft survival. Without rejection, graft survival may not be influenced by DF [34]. This suggests that, while ischemic and immunologic factors may

Table 4. Early posttransplant function ^a

	NHB donors	HB donors	p-value
Immediate function	12 (32%)	44 (59%)	0.02
Delayed function	18 (49%)	25 (34%)	
Primary nonfunction	7 (19%)	5 (7%)	
Diuresis posttransplant (mL/1st 24 h)	1511±2341	1911±2392	0.09
Duration DF (days)	18±10	14±11	0.82
Dialyses posttransplant (number)	6±4	5±3	0.74
Hospital stay (days)	33±17	33±32	0.28
Rejection episodes (number/1st 3 mo)	0.6±0.9	0.7±1.0	0.88

^a Values in numbers (%) or mean±SD.

Table 5. Serum creatinine of functioning kidneys ^a

Months posttransplant	NHB donors	HB donors	p-value
1	292±184 (26) ^b	185±114 (61)	0.01
3	217±126 (27)	152±52 (62)	0.02
6	196±117 (24)	152±69 (63)	0.22
lowest	155±64	124±42	0.04

^a Serum creatinine in $\mu\text{mol/L}$, values in mean±SD.

^b Number of evaluated grafts.

both result in ATN and DF of the transplanted kidney, DF with an immunologic component will reduce graft survival [18]. Although it may have multiple causes, DF is a serious posttransplant complication for a number of reasons. Posttransplant management is more difficult, symptoms of early rejection are disguised, and costs are increased due to longer hospital stay and continued dialysis. Furthermore, the prolonged dependence on dialysis and initial failure of the transplant are frustrating to both recipient and physician. Therefore, DF is a major concern in kidney transplantation, especially with NHB donor kidneys.

It has been reported that preservation by MP reduces DF rates and increases the prompt function of renal grafts [1,17,25,28]. Moreover, MP with the synthetic UW solution for MP (Belzer's perfusate) has been found to be superior to MP with plasma based perfusate [2]. Nevertheless, the effect of MP on graft survival remains controversial [25,30]. Because MP preservation is labour-intensive, logistically demanding, and costly, and because CS solutions are becoming increasingly safe and effective, most transplant centers have abolished the use of preservation machines. Nowadays organ preservation by simple CS is the standard. Kidney preservation by MP may still be preferable for prolonged preservation times [27] and for preservation of ischemically damaged kidneys. However, the observed prolonged cold ischemia time (CIT) in the NHB donor group, and thus in the MP preserved group, should be avoided, as it is known to lead to DF [10]. It is also reported to have a detrimental effect on graft survival in combination with DF, HLA-DR mismatch, and acute rejection [11]. In the case of organs already damaged by warm ischemia, as in NHB donor kidneys, prolonged hypothermic ischemia has an additional negative effect on transplant outcome, even if kidneys are preserved by MP. Therefore, preservation time should be kept to a minimum.

MP for preserving NHB donor kidneys has been reported to be more advantageous than CS in a study in which each kidney was compared with its own contralateral as a control [26]. The procedures, however, were fully controlled and the WIT was extremely short. The excellent transplant results with NHB donor kidneys reported by D'Allessandro et al. [14], and Orloff et al. [31] are at least partially attributable to preservation by MP.

Since no group of NHB donors was available as a control group for this study, the impact of MP on transplantation results with NHB donor kidneys had to be compared to HB donor controls. The groups were adequately matched and were comparable. Increased donor serum creatinine and decreased diuresis are typical for NHB donors and reflect the hemodynamically unstable agonal phase. MP could not correct for the differences in early posttransplant function between NHB and matched HB donor grafts.

Although DF still occurred significantly more often in the NHB MP group, our results with MP in this study suggest a reduction of DF in comparison to the previously reported DF rates of 60%-90% with NHB grafts preserved by CS [15,16,38]. Others report considerable lower DF rates with CS; however, donors were different with respect to age, cause of death, CIT and WIT [33]. Excellent results with DF rates of merely 19%-22% have been obtained with *category 3 NHB donors* and preservation by MP [14,31]. In those series, however, many viable grafts have been lost due to the extreme selection.

Whenever DF occurred, the severity did not differ among the groups. This is reflected in the equal duration of postoperative dialysis and the number of dialyses needed. After initial impairment due to more DF in the NHB donor group, renal function recovered, and within 6 months was equal for both groups. Similarly, the lowest serum creatinine reported in the NHB donor group ($155 \pm 64 \mu\text{mol/L}$), as an indication of potential renal function, was higher than the lowest level for the HB donor group, but acceptable. The higher DF rate did not result in more acute rejection episodes in the NHB donor group, confirming the suggestion that DF based on ischemia does not predispose the patient to acute rejection. Although there was a slight tendency to treat recipients in the NHB donor group with triple immunosuppressive therapy more often, induction therapy was equally employed. To prevent cyclosporin nephrotoxicity in the early posttransplant period, especially in recipients of NHB donor kidneys with a high risk of DF, induction therapy with ATG, ALG, or OKT3 and sequential delayed introduction of cyclosporin may be beneficial [19].

The PNF rate of 19% for NHB donor kidneys in our study is unacceptable. Analysis of the contralateral kidneys of the grafts with PNF, revealed one pair failing and three kidneys with not transplanted contralaterals. Excessive ischemia might have played a role in the failure of these five kidneys. The other two PNF grafts had functioning contralaterals. This high PNF rate also had a major impact on graft survival in the NHB donor group, which was reduced considerably. When considering functioning grafts only, the 6-month graft survival was similar (92% and 97%) for both groups. The increased failure rate of NHB donor kidneys underlines the importance of parameters that may give an indication of the warm ischemic damage sustained and the expected posttransplant function. Especially in *category 2 NHB donors* warm ischemic damage is unknown and may vary considerably. An adequate viability test might indicate whether posttransplant tubular necrosis in a graft is reversible; it might thus be of help in discarding nonviable kidneys [13].

In the present study, the beneficial effect of MP on warm ischemic damage in NHB donor kidneys was demonstrated by the similar results obtained with grafts from *category 2* and *category 3 NHB donors*.

Despite the older age of, and increased WIT for, *category 2 NHB donors*, transplanted kidneys functioned as well as the obviously less damaged kidneys from *category 3 NHB donors*. Apparently, MP is especially beneficial after prolonged warm ischemia, confirming earlier suggestions [12,32]. In addition to the direct effect on the organs, MP provides access to the organ for graft evaluation and intervention during preservation. Although proposed "rescue agents" did not affect the transplant outcome of canine kidneys damaged by prolonged hypothermia [32], the effect of such agents on posttransplant function of NHB donor kidneys is worth evaluating. Furthermore, in ischemically damaged kidneys, reperfusion injury might be reduced by preventing free oxygen radical formation during preservation [3,21].

From this study, we conclude that early posttransplant function of NHB donor kidneys preserved by MP is impaired versus that of matched HB donor controls preserved by CS. Still, the results suggest a beneficial effect of MP on NHB donor kidneys as the DF rates are lower than those reported in NHB donor kidneys preserved by CS. Moreover, the transplant outcome is similar for *category 2* and *category 3 NHB donors*. Apparently, MP does not repair NHB donor kidneys but merely minimises additional damage. However, our study group was rather small, and our findings need to be confirmed in the long-term. Since NHB donor kidneys are already ischemically damaged, further insults that threaten graft function, i.e., prolonged CIT and cyclosporin nephrotoxicity, should be avoided to obtain optimal transplant results. MP plays a key role in improving the preservation of ischemically damaged organs and in developing viability tests that will prevent transplantation of nonviable kidneys. Although MP is useful for preservation of NHB donor kidneys, in itself it is not sufficient to obtain optimal transplant results.

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VIABILITY TESTING DURING ORGAN PRESERVATION

- an introduction -

from: Kidney Transplantation from Non-Heart-Beating Donors
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Viability

In order to expand the non-heart-beating (NHB) donor pool safely, it is very important to be able to distinguish whether a graft is going to have primary nonfunction (PNF) upon transplantation or not. Therefore, it is crucial to be able to predict whether a kidney is viable or not because, especially in the NHB donor, kidneys might have sustained substantial warm ischemia of unknown severity and duration. Ever since kidney transplantation was implemented, efforts have been made to predict the viability of the organ before transplantation. Reports in the literature on this subject have decreased in the last decade, probably because of the almost exclusive use of heart-beating (HB) donors and the increasing safety of the preservation methods. An interesting and complicating factor as well, is the fact that even if one would be able to quantify the ischemic injury after procurement, or just before implantation, there still is an unknown injury at reperfusion to come. Therefore, posttransplant kidney function is the outcome of several cumulative ischemic attacks to the kidney.

Nucleotides

Cell anoxia causes inhibition of the oxidative phosphorylation, thus a fall in adenine nucleotide content. Although adenine tri-phosphate (ATP) content of the renal cortex and total adenine nucleotide (TAN) content could be correlated to survival of the kidneys [1], this correlation does not exist after pre-existing normothermic ischemia [2]. TAN content decreased with reduced viability but was, along with warm ischemia, influenced by the duration of cold preservation [3]. Also, when restoring TAN levels during preservation of ischemically damaged kidneys, survival was not improved [4], proving that nucleotide levels alone cannot predict organ viability. Hypoxanthine as a degradation product (DP) of adenine nucleotide catabolism estimated in the wash-out perfusate of ischemic kidneys, was brought forward as a useful parameter for assessment of ischemic damage in an experimental setting [5]. Although Maessen et al. [6] found that the ratio of DP to TAN, assessed immediately after procurement of HB donor kidneys, was strongly correlated to the sustained period of warm ischemia, i.e. warm ischemic time (WIT), it was stressed that this assessment of WIT should be regarded as a parameter of safety margins for viability rather than as a straight predictor of organ viability. An in-situ flushout, as is done in the clinical NHB donor procedure, will probably wash out the DP thus making the DP/TAN ratio useless in estimating this safety margin.

Intracellular ^{31}P phosphorus metabolites can be assessed during cold storage (CS) by magnetic resonance spectroscopy (31P-MRS), a non-invasive and non-destructive method. The subsequent degradation of ATP to adenine di-phosphate and eventually adenine mono-phosphate (AMP) releases phosphorus atoms

at each step. The phosphorus mono-esters (PME) and inorganic phosphorus (Pi) concentrations in renal tissue measured by ^{31}P -MRS, correspond to the concentrations of AMP and free phosphorus [7]. Bretani et al. [8] found a strong correlation between the PME/Pi ratio and warm and cold ischemic damage in rat kidneys, objectified by electron microscopic evaluation. These results were confirmed in canine and rat transplant models showing that the PME/Pi ratio is a sensitive indicator of a compromised energy conversion system due to hypoxia and ischemia [9,10]. In human kidneys preserved by CS, lower PME/Pi ratios were associated with prolonged posttransplant dysfunction of the grafts due to acute tubular necrosis. Pretransplant sensitivity of 75% and specificity of 87% were reported in predicting delayed function (DF) by using a PME/Pi ratio=0.5 as cut-off point [11]. However none of these kidneys sustained essential warm ischemia.

Enzymes

The lack of oxygen eventually alters cell membrane integrity, which causes lysosomal enzymes to leak into the interstitial space. Reports on using these lysosomal enzymes in estimating ischemic damage are many. Lactate dehydrogenase (LDH) is most often mentioned and directly related to ischemia and postischemic function [12]. More recently, the specific LDH4 and LDH5 isoenzymes were said to present a more sensitive parameter for hypoxic tissue damage [13]. In nonischemic kidneys, LDH (as was aspartate aminotransferase, AST) was found also to indicate preservation damage [14]. Being both lysosomal enzymes of the proximal tubular cell, N-acetyl- β -D-glucosaminidase failed to predict kidney function in canine renal transplantation [15], while ligandin was able to predict kidney function prior to transplantation in humans [16]. Most of these tests are done by estimating enzyme activity in the perfusate while the kidneys were continuously being perfused in a perfusion machine. Determination of hyaluronic acid levels in the washout effluent of CS kidneys performed just prior to implantation of the kidney failed to predict early graft function, unlike it did in liver transplants [17].

Another way to estimate the viability of kidney grafts is measuring the overall impairment of metabolic capacity of the renal tubular cells. One way of determining this capacity is by means of the principle that tetrazolium salts are rapidly reduced to formazan by intact mitochondrial reduction-oxidation enzymes, thus causing a detectable colour change. Smith et al. [18] and Yin et al. [19] described methods and found a strong correlation between ischemia times at different temperatures and the ability of renal cortex to reduce tetrazolium salts. Although proposed, trials with human material were never reported.

Electrolytes

Through the sodium-potassium pump (NaK-pump) renal tubular cells can maintain the sodium (Na^+) and potassium (K^+) gradients across their cell membrane. Since the NaK-pump is ATP dependent and during anoxia ATP levels rapidly decline, ischemia is hampering the function of the NaK-pump. The cell is unable to keep up its intracellular composition thus loses its integrity, swells, and finally the cell membrane ruptures. As a result K^+ is leaking into the interstitial space. By measuring the K/Na-ratio in human kidney tissue biopsies Sells et al. [20] were able to predict posttransplant renal function well in a retrospective analysis. By measuring the K/Na-ratio in the perfusate of dog kidneys preserved for 6 days, an additional parameter for parenchymal viability was found [21].

Recently the use of a biochemical multisensor element for estimating extracellular ion-shifts was reported [22]. By monitoring electrolyte activities at the organ surface, the authors were able to correlate posttransplant function (i.e., nonfunction vs. delayed function) to pretransplant K^+ activities in human cadaveric kidney transplants [23]. Again, these kidneys did not sustain warm ischemia.

Functional

As a reaction to the ischemic insult the vascular resistance within the kidney is increased. The precise mechanism is still unclear but it might be the result of nervous stimulation, the local release of vasoconstrictive agents, the loss in ability of the renal endothelium to release endothelium derived relaxing factor (EDRF, identified as nitric oxide) [24,25], and/or vascular blockage due to swelling of endothelial and perivascular cells and congestion of erythrocytes [26,27]. By recording flow and perfusion pressure during preservation in a perfusion machine, this increased intrarenal vascular resistance (IRR) can be estimated and function as a parameter for perfusion and ischemic damage [28]. In both experimental and clinical studies, increasing IRR patterns during machine perfusion (MP), were related to impaired posttransplant kidney function. Perfusion characteristics have been purported as a method to safely expand the donor pool with donors regarded as marginal in both HB and NHB donor programs [29-31]. High IRR and low perfusion flow are regarded as parameters to discard kidneys although different cut-off points were reported. As mentioned before, in the Maastricht NHB donor program we also discard NHB donor kidneys based on their perfusion characteristics [32]. Although identifying non-functioning kidneys seems possible, distinguishing between delayed and immediate functioning grafts upon transplantation, might be difficult by these parameters.

Another method for testing the viability of the organs during MP could be by perfusion techniques. A

washout technique with radioactive Xenon-133, reveals the redistribution of renal flow through the different compartments of the kidney; reduction of cortical flow is correlated to ischemic injury to the kidney [33]. Perfusing the kidney with technetium labelled hexamethyl propylene amine oxime (Tc-HMPAO) and scintigraphic imaging will visualise perfusion defects. Thus these parameters might be useful in predicting renal damage and posttransplant viability [34]. No clinical use has been reported yet.

A possible method was recently suggested by using tandem scanning confocal microscopy (TSCM). This form of intravital microscopy enabled Andrews et al. [35] to identify in a rat model ischemic damage *in-vivo*. Observing rat kidneys preserved by CS using this technique provided artifact-free images of the villous brush-border and tubular lumen. In this way it was possible to follow up the condition of the preserved kidney in a time course up to 72 h of CS preservation [36]. However, the hypothesis that by this method one would be able to differentiate nonviable from viable kidneys in human transplantation certainly needs further investigation.

Although several methods have been proposed to assess the ischemic damage of kidneys for transplantation, few have been studied in warm ischemia as in NHB organ donation. So far, none have found broad clinical use in predicting renal transplant outcome. The most reliable factor predicting viability is the duration the ischemic period the organ suffered, including both normothermic and hypothermic ischemia. However, even with known total ischemia time predicting transplant outcome is hazardous as posttransplant function is the result of many factors. In NHB donation, as all organs are ischemically damaged, a useful viability test, i.e., a parameter to assess ischemic damage is of utmost importance. Anyway, the ultimate viability test still is transplanting the organ and assessing its function.

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GLUTATHIONE S-TRANSFERASE AS PREDICTOR OF
FUNCTIONAL OUTCOME IN TRANSPLANTATION OF
MACHINE PRESERVED NON-HEART-BEATING DONOR KIDNEYS

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Introduction

Due to the growing demand for organs for transplantation together with a stabilising supply of brain-dead, i.e. heart-beating donors, there is a surge for alternative sources of grafts. One of these alternatives is the use of organs from non-heart-beating (NHB) donors, which might reduce the shortage of kidneys for transplantation considerably. Organs are procured from NHB donors after final and irreversible cardiac arrest; therefore, organs sustain warm ischemic damage. The warm ischemic time (WIT), defined as the period between cardiac arrest and start of the cooling of the organs, is often of unknown duration. Because WIT is one of the most important factors determining transplant outcome, organs might eventually become nonviable with increasing WIT. Therefore, estimation of the ischemic damage is important; for the safe use of kidneys from NHB donors it is essential to define reliable parameters to assess the ischemic damage and possibly predict transplant outcome.

Apart from the potential benefits on transplant results [1,2], preservation by machine perfusion (MP), i.e., perfusing the kidney continuously with cold solution, enables surgeons to judge the condition of the graft by registering flow and pressure characteristics and analysing the washout of enzymes into the perfusate. An increase in intrarenal vascular resistance (IRR) during preservation is related to impaired posttransplant function [3]; release of the cytosolic enzyme lactate dehydrogenase (LDH) into the perfusion solution correlates with ischemia time and subsequent function [4]. The appearance of α -glutathione S-transferase (α GST) in the perfusate of continuously perfused kidneys was found to predict the development of posttransplant acute tubular necrosis (ATN)[5]. However, none of these parameters was ever used to measure warm ischemic damage in relation to graft viability and transplant outcome of NHB donor kidneys. The aim of this study was to assess warm ischemic damage in NHB donor kidneys, and to investigate the relationship between perfusion characteristics and release of enzymes and the functional outcome of the grafts.

Materials and Methods

All kidneys were from NHB donors and were preserved by MP at the University Hospital Maastricht. The organs were procured after final cardiac arrest in an unsuccessful cardiopulmonary resuscitation procedure (*NHB donor category 2*) or from NHB donors that sustained cardiac arrest after withdrawal of ventilatory support (*NHB donor category 3*). Several kidneys were procured from brain-dead donors experiencing irreversible cardiac arrest before organs could be recovered (*NHB donor category 4*)[6]. After consent was obtained, an in-situ preservation procedure using a double-balloon-triple-lumen femoral cooling catheter (AJ 6516, Porgès, Le Plessis-Robinson, France) was performed, as described

in detail earlier [7]. Donor nephrectomy was performed in the operating room; subsequently, the kidneys were prepared for MP on the back table and placed in specially designed organ chambers fitting into Gambro PF-3B perfusion machines (Gambro, Lund, Sweden). Before the kidneys were connected to the machine, a wedge biopsy from the cortex was taken for histology. As a perfusate for the MP, 500mL of Belzer's perfusate, the University of Wisconsin solution for MP, was used [8]. Some NHB donor kidneys were shipped for MP to our center from other donor hospitals after a procurement procedure similar to ours; during transportation, these organs were stored on ice. One machine was used for one kidney. After the kidney was connected to the perfusion system, perfusion flow was set to a systolic pressure of 60 mmHg. The perfusion flow was kept constant, but the pressure was allowed to vary. Perfusion pressures (systolic, diastolic, and mean) were recorded at the beginning of the MP and after 1, 4, and 8 hr. IRR was calculated by dividing the mean pressure by the registered flow (in mmHg/mL/min per 100 g of kidney weight); a decrease reflected a reduced IRR. Samples for LDH and α GST analysis were taken from the perfusate at time points 0, 1, 4, and 8 hr of preservation and immediately stored at -70°C until used. α GST was quantitatively determined by using the NEPHKIT-ALPHA immunoassay designed for urine analysis (Biotrin International, Dublin, Ireland). Perfusate samples were assayed following the standard assay after being diluted 1:500. LDH was determined by standard colorimetric assay (Boehringer Mannheim, Almere, The Netherlands). Results were adjusted for kidney weight.

Kidneys were discarded and not used for transplantation whenever donor data, macroscopic appearance of the organs, perfusion characteristics and/or histological examination indicated excessive ischemic damage and raised doubt about viability [9]. Kidneys judged unsuitable for transplantation were preserved for at least 8 hr, and data were collected as described before (not-transplanted [NOT-TX] group). Kidneys considered suitable for transplantation were allocated to recipients by the Eurotransplant organisation and remained in the perfusion machine until grafting.

Data about posttransplant function were collected by questionnaire and the transplanted grafts were divided into two groups. The functioning (F) group included kidneys that gave life-sustaining renal function, whether temporarily requiring posttransplant dialysis or not. The non-functioning (NF) group included those grafts that failed to give renal function, no matter what the cause, and dialysis had to be continued. The F group was further subdivided into delayed function (DF) and immediate function (IF) subgroups according to whether posttransplant dialysis was needed.

Results are given as mean $\pm$ SD; statistical significance for differences were calculated by non-parametric Mann-Whitney U-test and chi-square test for differences between groups, where appropriate.

P values less than 0.05 were considered significant.

Results

Between July 1993 and November 1995, 67 kidneys procured from NHB donors were preserved by MP and yielded data for analysis (table 1). Twenty-one kidneys were not transplanted (NOT-TX group), 37 grafts entered the F group, and 9 entered the NF group. Basic donor data were equal for the F and NF groups (serum creatinine: 122 ± 72 and 130 ± 62 $\mu\text{mol/L}$, $p=0.28$; donor age: 41.6 ± 15.7 and 48.7 ± 16.5 years, $p=0.37$, respectively). In the NOT-TX group, serum creatinine was 139 ± 57 $\mu\text{mol/L}$ ($p=0.03$ and $p=0.30$ for F and NF groups, respectively), and donor age was 46.2 ± 15.0 years ($p=0.55$ and $p=0.39$ for F and NF groups, respectively). WIT was longer in the NF group (70.3 ± 38.2 min vs. 41.6 ± 29.9 min in the F group, $p=0.04$), whereas cold ischemic time was not different (31.1 ± 10.2 and 33.0 ± 5.1 hr in the F and NF groups, respectively; $p=0.13$). In the NOT-TX group, WIT (84.0 ± 57.1 min) was longer than in the F group ($p=0.001$) but equal to WIT in the NF group ($p=0.77$).

Table 1. Donor data for F, NF, and NOT-TX groups^a

	F	NF	NOT-TX
n	37	9	21
donor age (years)	41.6 ± 15.7	48.7 ± 16.5	46.2 ± 15.0
creatinine ($\mu\text{mol/L}$)	122 ± 72	130 ± 62	139 ± 57 ^b
WIT (min)	41.6 ± 29.9	70.3 ± 38.2 ^b	84.0 ± 57.1 ^b
cold ischemic time (hr)	31.1 ± 10.2	33.0 ± 5.1	--

^a Values are expressed as mean $\pm$ SD.

^b Significantly different from the F group.

Intrarenal Vascular Resistance. Except at 1 hr, there was no significant correlation between WIT, as determinant of ischemic damage, and IRR (p values >0.05); linear correlation was found at 1 hr ($r=0.272$; $p=0.04$). The IRR of NF grafts increased significantly when compared with F grafts at 4 and 8 hr of preservation time ($p=0.02$ and $p=0.04$, respectively; fig. 1A). The NOT-TX group was significantly

different from the F group and equal to the NF group for all time points.

Lactate Dehydrogenase. No correlation was found between WIT and LDH activity in the perfusate. When comparing the F and NF groups, no differences were found (fig. 1B). The activity in the NOT-TX group was equal to that in the NF group, but increased when compared with the F group. There was no activity at 0 hr.

α -Glutathione S-Transferase. α GST release at 4 hr and 8 hr correlated well with the WIT (fig. 2) (linear correlation: $r=0.353$, $p=0.009$, and $r=0.346$, $p=0.011$, respectively). No correlations were found between α GST and IRR for all time points ($p>0.05$). Release of α GST correlated well with the release of LDH into the perfusate, but just at 4 hr and 8 hr ($r=0.0267$, $p=0.04$, and $r=0.364$, $p=0.007$, respectively). Compared with the F group, α GST levels were significantly elevated in the NF group at all time points (p -values 0.02, 0.005, and 0.004 at 1 hr, 4 hr, and 8 hr, respectively; fig. 1C). Organs in the NOT-TX group had equally high α GST values in comparison to the NF group; values were higher in comparison to the F group, except at 1 hr ($p=0.19$).

No enzyme was detectable at the start of MP. The poorer the posttransplant graft function, the higher the level of α GST found; when the posttransplant function was examined per quartile of α GST level at 4 hr, the proportion of never-functioning grafts increased significantly (table 2; $p=0.009$).

Table 2. Posttransplant function per α GST level after 4 hr of MP

Quartiles	α GST at 4 hr ^a	n	F : NF (%) ^b
1	702 $\pm$ 184	12	12 : 0 (100)
2	1280 $\pm$ 256	12	10 : 2 (83)
3	2108 $\pm$ 288	11	9 : 2 (82)
4	3870 $\pm$ 920	11	6 : 5 (55)

^a Values are expressed as mean $\pm$ SD in μ g/100 g kidney weight.

^b The values represent the number (%) of kidneys in the F group; posttransplant function worsened with increasing α GST level ($p=0.009$).

Figure 1. IRR (A), LDH (B), and GST (C) during MP.

* Significantly different from the F group.

Within the F group, the DF (n=26) and IF (n=11) subgroups were not different for donor data and ischemia times. Results for the analysed parameters are depicted in table 3. Remarkably, neither IRR nor α GST differed for IF and DF, whereas LDH could discriminate very well between them (p-values 0.0003, 0.0003 and 0.0001 at 1 hr, 4 hr, and 8 hr, respectively).

Discussion

In the early days of transplantation, efforts were made to predict transplant outcome during preservation. Probably due to the almost exclusive use of brain-dead donors and the increasing effectiveness and safety of the preservation methods for renal grafts, interest in this subject has decreased. The increasing demand for organs, however, has renewed interest in the use of organs from NHB donors. Because all these organs are damaged by warm ischemia, parameters that may quantify this damage are currently regaining importance. Several viability parameters based on the determination of nucleotides, enzyme and electrolyte release, and functional and morphological status of the kidneys have been proposed. However, currently none of these parameters that may estimate warm ischemic damage and predict transplant outcome is routinely used in clinical practice [10-14]. Several parameters potentially useful for NHB donor kidneys were evaluated in this study.

The IRR reflects the vasoconstrictive response of the renal vascular bed to the ischemic insult. The precise mechanism of this increasing resistance is still unclear but might be the result of the local release of vasoconstrictive agents and the reduced ability of the damaged vascular endothelium to release endothelium derived relaxing factor (identified as nitric oxide)[15-17]. The congestion of erythrocytes in capillaries additionally increases the IRR and is an important factor in postischemic renal dysfunction [18]. As a result, the calculated IRR can be seen as a parameter revealing warm ischemic damage [19]. In some clinical programs, IRR and perfusion flow are used to safely expand the donor pool with kidneys from marginal donors; kidneys with high IRR are discarded, although different cut-off points are reported [20,21]. However, the IRR is dependent on perfusion flow, and thus organs must be connected to the perfusion system perfectly. Unobserved vascular leakage during perfusion and multiple arteries may make flow and pressure registrations less reliable. Therefore, despite the observed differences between the F and NF groups, we are reluctant to use IRR as a sole parameter to judge kidney viability. However, IRR may be valuable in confirming expected functional outcome predicted by other parameters.

Figure 2. Correlation between WIT and α GST after 4 hr of MP

Linear correlation: $r=0.353$; $p=0.009$.

Table 3. Values for IRR, LDH, and α GST during MP for IF and DF subgroups ^a.

T	IRR (mmHg/mL/min per 100 g kidney weight)		LDH (μ mol per 100 g kidney weight)		α GST (μ g per 100 g kidney weight)	
	IF	DF	IF	DF	IF	DF
0	0.88 $\pm$ 0.31	0.90 $\pm$ 0.54	--	--	--	--
1	0.82 $\pm$ 0.35	0.87 $\pm$ 0.45	412 $\pm$ 184	1002 $\pm$ 750 ^b	940 $\pm$ 612	1542 $\pm$ 1308
4	0.75 $\pm$ 0.36	0.76 $\pm$ 0.35	602 $\pm$ 264	1354 $\pm$ 854 ^b	1320 $\pm$ 720	1834 $\pm$ 1284
8	0.68 $\pm$ 0.24	0.74 $\pm$ 0.32	748 $\pm$ 286	1758 $\pm$ 1040 ^b	1598 $\pm$ 704	2730 $\pm$ 2120

^a Values are expressed as mean $\pm$ SD.

^b Significantly different from the IF subgroup ($p<0.001$).

Relatively independent from the perfusion flow is the absolute enzyme release into the perfusate. It was already observed by Belzer in the 1960s that, with ischemically damaged kidneys, the peak release of LDH appeared later in the course of MP when compared to undamaged kidneys, probably due to the initially impaired perfusion flow [22]. Washout of ubiquitous enzymes during single organ perfusion reflects the number of cells damaged in the organ. In the ischemic state, plasma membrane integrity is altered, and its permeability is lost through different pathophysiological pathways [23,24]. Although the subsequent release of enzymes from cells suggests severe membrane damage, it not necessarily indicates the death of these cells [25]. In this context, the cellular enzyme LDH is often reported and found to correlate with the duration of renal ischemia and postischemic function [4,22,26]. In nonischemic kidneys, it has been shown to be an adequate indicator of preservation injury [27]. Although during single-organ perfusion all LDH in the perfusate originates from the kidney, it remains unclear which renal cells are damaged specifically, since LDH is present in almost all living cells. Also, LDH is released into the perfusate by damaged erythrocytes that after the initial cold flush are trapped in the renal microvasculature and being washed out during MP. This lack of specificity may explain why in our study LDH could not distinguish between damaged grafts with and without function (F versus NF).

Glutathione S-transferase is a cytosolic enzyme present in liver, kidneys, small intestine, testes, ovaries and adrenal glands [28]. More important, within the kidney the α -isoenzyme, α GST, is confined exclusively to the proximal tubule (PT) cells [29]. Glutathione S-transferase conjugates a great variety of molecules that are considered to be detoxifying. The conjugate is further degraded by enzymes of the gamma-glutamyl cycle to become mercapturic acid, which is excreted in the urine [30]. Glutathione S-transferase, formerly known as ligandin, assessed during preservation of kidneys without ischemic damage, has been reported to predict ATN and transplant outcome well [5,31]. The determination of glutathione S-transferase isoenzymes in the urine appeared to be very useful in differentiating various renal tubular lesions [32,33]. Warm ischemia predominantly damages the PT cells [15]; therefore, the appearance of α GST in the perfusate may reflect the number of PT cells damaged. Renal grafts not functioning after transplantation because of too severe ischemic damage are considered to have irreversible tubular necrosis. The level of α GST as a specific and quantitative marker for PT damage is anticipated to correlate well with WIT and thus to predict functional outcome adequately, which is exactly what we found. The fact that α GST is not able to differentiate between IF and DF grafts (table 3) may be due to the exclusive use of NHB donor kidneys in this study. These grafts all sustain warm ischemic damage; even in the IF and DF subgroups, WIT was 44.7 ± 10.9 and 40.3 ± 27.6 min, respectively. This is enough to cause PT cell damage and the release of α GST, which does not always result in clinically evident ATN. On the other hand, that LDH is able to identify IF grafts within the F

group indicates that in general only the minimally damaged grafts can give immediate function.

The strong correlation between WIT and release of α GST and the ability of this enzyme to differentiate between functioning and non-functioning NHB donor kidneys are important because α GST can be used to define margins for the safe use of NHB donor kidneys. Classifying the graft function according to the α GST level (table 2) showed that high α GST release was associated with significantly poorer graft function. Using at random for α GST at 4 hr an upper level of 3000 $\mu\text{g}/100\text{ g}$ of kidney as the limit for the viability of organs, would have resulted in the transplantation of 37 kidneys of which 4 would not have functioned. The predictive value of this cut-off point for transplanting a viable kidney was 89%; the predictive value for discarding a nonviable kidney was 56%. Exclusion of transplanted kidneys from the NF group that failed due to other factors than ischemia, such as acute rejection or renal vein thrombosis, would improve the predictive value of a positive test. Indeed, in our study three of the four kidneys that showed no function, and had an α GST level at 4 hr of less than the proposed 3000 μg , failed for reasons other than excessive ischemic damage, proven by functioning contralateral kidneys from the same donor. Since levels for enzyme release in the NOT-TX group are close to data of the NF group, it may be assumed that these discarded kidneys would not have functioned when transplanted. Transplanting these probably nonviable grafts would have improved the predictive value of a negative test remarkably.

This study shows that MP offers possibilities for evaluating renal grafts from NHB donors. As could be expected, NF kidneys suffered from longer WIT than F kidneys. α GST, and to a lesser extent, IRR were able to distinguish viable (F group) from nonviable grafts (NF group) early in the course of preservation. Only α GST showed a strong correlation with WIT and is an adequate predictor of functional outcome. The LDH release into the perfusate could identify relative undamaged grafts but is not useful in predicting nonfunction. It should be realised, however, that transplant outcome is multifactorial and that warm ischemic damage, although very important, is just one factor. We have confirmed that MP is the preservation method of choice for NHB donor kidneys, and we are convinced that the parameters discussed, especially the measurement of α GST, will help to select nonviable kidneys from the valuable kidneys for transplantation.

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SUMMARY AND CONCLUSIONS /
SAMENVATTING EN CONCLUSIES

Summary and Conclusions

Transplantation is the preferred treatment of end stage renal failure. A successful renal transplantation improves the quality of life and at a lower cost than dialysis. However, ever since the first transplant there has been an increasing shortage of donor organs. In 1996, 11,000 patients awaiting a kidney transplant were registered at the Eurotransplant Foundation. That year about 3,300 renal transplants were performed, from which 250 from living donors. Although momentarily the potential of brain dead, heart-beating (HB), donors is not employed to its full extent, sufficient numbers of donor organs will probably never be realised by HB donation alone. Due to the shortage of donor kidneys, more attention should be paid to non-heart-beating (NHB) donation, i.e. donation by donors that died of cardiac arrest, as a possible source of donor organs. Although NHB donation exists since the beginning of transplantation, nowadays it is just employed at a minor scale.

NHB kidney donors sustain irreversible cardiac arrest and due to the circulatory collapse the organs are damaged by ischemia. In **CHAPTER ONE** the concept of NHB kidney donation is outlined. According to the donation and preservation protocols at the University Hospital Maastricht, several of the proposed cooling and preservation techniques are described and results are compared. It is obvious that not all NHB donors are comparable and in **CHAPTER TWO** four categories of NHB donors are proposed (category 1 *Dead on arrival*, category 2 *Unsuccessful resuscitation*, category 3 *Awaiting cardiac arrest*, and category 4 *Cardiac arrest while brain dead*).

In **CHAPTER THREE** the results of an important study are described. Depending on medical suitability of the NHB donor and the possibility to execute a donation procedure, annually 2 to 4.5 times more kidneys may come available from NHB donors than from HB donors. The largest proportion (68%) of these potential NHB donors is located at emergency room, intensive care and coronary care units. The potential donors at these departments are also the medically most suitable. Focusing on these hospital units for implementing routine procurement of kidneys from NHB donors is probably most effective.

Due to the circulatory arrest NHB donor organs are ischemically damaged and transplant results may be effected negatively. Earlier studies showed that NHB donor kidneys suffered more delayed function (DF) upon transplantation but that long term results were comparable to those with HB donor kidneys. From experimental work it was understood that machine perfusion (MP) preservation was superior to preservation by simple cold storage (CS). Based on these data a clinical study in which NHB donor

kidneys preserved by MP are compared to NHB donor kidneys preserved by CS, has been conducted as described in **CHAPTER FOUR**. Upon transplantation MP preserved organs functioned better than CS preserved organs, especially after prolonged warm ischemia (over 45 minutes). Just preserving NHB donor kidneys by MP is not enough to achieve a short-term transplant outcome similar to that of HB donor kidneys preserved by CS, as is described in **CHAPTER FIVE**. The DF rate is higher with NHB donor kidneys (49% vs 19%) and apparently more than MP alone is necessary to eliminate the negative effects of warm ischemia.

The ischemic insult that a NHB donor kidney suffers may be so extensive that the organ is not viable any more. Upon transplantation such a kidney will not recover but will show primary non-function (PNF). This PNF has to be prevented in order to safely expand the potential NHB donor pool. Parameters that determine ischemic damage and thus may predict transplant outcome are very important. In **CHAPTER SIX** a review is given of viability parameters as proposed in the literature.

MP enables viability assessment and in **CHAPTER SEVEN** several parameters potentially useful as viability predictors are studied during the preservation of NHB donor kidneys. The level of α glutathione S-transferase (α GST) in the perfusate appears to be a good parameter to differentiate viable from non-viable kidneys. α GST is an enzyme that in the kidney is confined to the proximal tubular cells and thus reflects proximal tubular damage. There is a strong correlation with the period of warm ischemia sustained and the functional transplant outcome can be predicted adequately. By defining a maximum level for α GST (3,000 μ g / 100 g kidney tissue after 4 hours preservation) and excluding kidneys with higher values from transplantation, transplanting NHB donor kidneys with a high risk of PNF can be prevented.

In this thesis it has been shown that the pool of potential NHB donors is at least twice as large as the pool of potential HB donors. Introduction of NHB donation on a large scale will eliminate the shortage of donor kidneys. In the future it might be possible to accept other organs than kidneys from NHB donors for transplantation (livers and lungs). Incidentally this is done in 'ideal' NHB donors from category 3. Performing this on a widespread basis might reduce or even eliminate the shortage of these organs as well.

Important at introduction of protocols is, that health care workers are familiar with NHB donation, as they have to recognise potential NHB donors and start the donation procedure. The procedure in NHB donation is relatively simple and introducing of a cooling catheter into a femoral artery, as described in

our protocol, can be done almost everywhere.

For the relatives donation by a NHB donor is as acceptable as donation by a HB donor. In our experience refusal rates are equal in NHB and HB donation. We have also experienced that potential NHB donors are not recognised as such. Therefore education of public as well as health care workers is essential.

In The Netherlands legislation recently has changed and now it allows to perform preliminary action for keeping organs suitable for transplantation. Thus, even before consent can be obtained, with this legislation introduction of a catheter and starting organ cooling is allowed for in NHB donation. Potential NHB donors will be more accessible and the donation procedure will improve. Additionally, as part of the new law a nation-wide registration system will be implemented that will give people the opportunity to be registered as organ donors. Today a large proportion of potential donations may not be realised because the deceased's wish is not known and the relatives cannot consent to donation in time. Hopefully this national registration will provide a solution to this problem and enable successful implementation of NHB donation protocols. Probably only an 'opting-out' legislation that makes everybody an organ donor unless registered otherwise, will allow for maximum profit by the NHB donor potential.

From our studies MP appears to be the superior preservation method. MP preservation gives better functional results of transplanted NHB donor kidneys than CS preservation. Furthermore through viability testing MP can prevent the transplantation of non-viable kidneys. Through assessing α GST values during MP, the ischemic damage of the kidney and thus its viability can be established. In this way the fear of transplanting a non-viable kidney can be diminished. This will improve the acceptance of NHB donation and lead to an increasing number of donation procedures. Despite MP NHB donor kidneys more often suffer from DF than HB donor kidneys. DF is a predictor of poor transplant outcome, although this is not the case in our NHB donor kidneys. In spite of the fact that DF seems not to cause rejection and graft loss in NHB donor kidneys, it is considered a negative aspect of transplanting NHB donor kidneys.

By developing preservation solutions especially for ischemically damaged kidneys the ischemic organ damage and thus DF may become treatable. From experimental studies in animals it seems possible to alleviate reperfusion damage and reduce DF rates by treating the recipient (platelet-activating-factor antagonist, adenosine inhibition). Of course additional damage to the already ischemically damaged kidneys must be prevented and the organ should be transplanted under the best conditions possible. Therefore, recipients should be selected carefully, preservation time should be kept to a minimum, and

cyclosporin nephrotoxicity may be prevented by induction immunotherapy.

MP is an important aspect in NHB kidney donation and it is desirable to designate preservation centers. Here NHB donor kidneys, and especially those with extended warm ischemic damage, can be preserved by MP and their viability can be assessed. In such preservation centers continuous research will be done focusing on preservation methods for ischemically damaged kidneys and other ways to alleviate DF.

Samenvatting en Conclusies

De voorkeursbehandeling van terminale nierinsufficiëntie is transplantatie. Een succesvolle niertransplantatie verbetert de kwaliteit van leven van de patiënt en is kostenbesparend ten opzichte van dialyse. Echter sinds de eerste niertransplantaties is er steeds een tekort aan donororganen geweest dat steeds verder toeneemt. In 1996 waren bij Eurotransplant ongeveer 11.000 patiënten geregistreerd in afwachting van een niertransplantatie. In dat jaar werden minder dan 3300 niertransplantaties verricht, waarvan 250 met nieren van levende donoren. Hoewel momenteel het potentieel aan hersendode, heart-beating (HB), donoren zeker niet volledig voor donatie wordt benut, is het onwaarschijnlijk dat van HB donoren alléén ooit voldoende transplanteerbare nieren zullen worden verkregen. In het kader van dit orgaantekort krijgt de non-heart-beating (NHB) nierdonatie, dus donatie door donoren die overleden zijn aan een hartstilstand, steeds meer aandacht als alternatieve bron van donororganen. Alhoewel NHB donatie reeds sinds het begin van de orgaantransplantatie bestaat, wordt het momenteel slechts op kleine schaal toegepast.

Hartdode nierdonoren overlijden ten gevolge van een hartstilstand waardoor er sprake is van een circulatiestilstand en er ischaemische orgaanschade ontstaat. In **HOOFDSTUK EEN** wordt het principe van NHB nierdonatie beschreven. Aan de hand van het donatie- en preservatie protocol van het Academisch Ziekenhuis Maastricht worden de verschillende koel- en preservatie technieken beschreven en worden resultaten vergeleken. Duidelijk wordt dat NHB donoren niet zonder meer onderling vergelijkbaar zijn. In **HOOFDSTUK TWEE** worden vier NHB donorcategorieën onderscheiden en beschreven (categorie 1 *Dead on arrival*, categorie 2 *Unsuccessful resuscitation*, categorie 3 *Awaiting cardiac arrest* en categorie 4 *Cardiac arrest while brain dead*).

Een belangrijke studie is gedaan in **HOOFDSTUK DRIE**. Afhankelijk van de medische geschiktheid van de NHB donor en de praktische uitvoerbaarheid van een donatie procedure, blijken er jaarlijks 2 tot 4,5 maal zoveel nieren voor transplantatie beschikbaar te kunnen komen van NHB donoren dan thans van HB donoren. Het overgrote deel van de potentiële NHB nierdonoren (68%) kan gevonden worden op de Eerste Hulp afdeling, Intensive Care Unit en Coronary Care Unit. De potentiële donoren op deze afdelingen zijn tevens medisch het meest geschikt. Om het potentieel aan NHB nierdonoren zo efficiënt mogelijk te benutten, zullen NHB donatie protocollen dan ook met name op deze afdelingen ingevoerd moeten worden.

Door de circulatiestilstand worden bij NHB donoren de organen per definitie ischaemisch beschadigd. Dit kan de transplantatieresultaten negatief beïnvloeden. Eerdere studies hebben laten zien dat bij transplantatie van NHB donornieren weliswaar meer 'delayed function' (DF) optreedt, echter dat de lange termijn resultaten vergelijkbaar zijn met die van HB donornieren. Uit dierexperimenteel onderzoek is gebleken dat preservatie van ischaemisch beschadigde nieren middels machine perfusie (MP) een beter functioneel resultaat geeft dan preservatie middels 'cold storage' (CS). Op basis van deze gegevens is in **HOOFDSTUK VIER** een klinische studie beschreven waarbij NHB donornieren gepreserveerd middels MP zijn vergeleken met NHB donornieren gepreserveerd in CS. Bij transplantatie wordt bij MP een gunstig effect op het functionele resultaat waargenomen dat met name optreedt bij lange warme ischaemie duur (langer dan 45 minuten). Uit het in **HOOFDSTUK VIJF** beschreven onderzoek blijkt het niet mogelijk te zijn met NHB donornieren de korte termijn resultaten van HB donornieren gepreserveerd middels CS, te evenaren door alleen MP toe te passen. Er wordt nog steeds een hoger DF percentage gevonden bij NHB donornieren (49% vs 19%) en kennelijk is er méér nodig dan alléén MP om de negatieve effecten van ischaemie te elimineren.

De ischaemische schade aan een NHB donornier kan dusdanig zijn dat deze niet meer vitaal is. Een dergelijke nier zal na transplantatie niet herstellen en 'primary non-function' (PNF) geven. Voor het verder uitbreiden en veilig toepassen van NHB nierdonatie moet PNF zoveel mogelijk voorkómen worden. Parameters die ischaemische schade meten en zo het transplantatieresultaat kunnen voorspellen, zijn van groot belang. In **HOOFDSTUK ZES** wordt een overzicht gegeven van in de literatuur geopperde parameters. MP biedt de mogelijkheid metingen te verrichten en in **HOOFDSTUK ZEVEN** worden tijdens preservatie van NHB donornieren verscheidene parameters bestudeerd. Het in het perfusaat bepalen van het α glutathion S-transferase (α GST), een enzym dat in de nier slechts voorkomt in de proximale tubulus, blijkt een goede parameter te zijn om avitale van vitale nieren te onderscheiden. Er is een sterke correlatie met de warme ischaemie duur en het functionele resultaat kan adequaat voorspeld worden. Door een grenswaarde voor α GST te definiëren ($3000\mu\text{g} / 100\text{g}$ nierweefsel na 4 uur preservatie) en nieren met hogere waarden niet te transplanteren, kan voorkómen worden dat NHB donornieren die met redelijke zekerheid PNF geven, getransplanteerd worden.

In dit proefschrift is aangetoond dat het potentieel aan NHB donoren minstens tweemaal zo groot is als het potentieel aan HB donoren. Het op grote schaal invoeren van NHB donatie protocollen zal het tekort aan donornieren kunnen wegwerken. Mogelijk dat in de toekomst ook andere organen van NHB donoren geaccepteerd kunnen worden voor transplantatie (bijvoorbeeld lever en longen). Dit gebeurt reeds incidenteel bij 'ideale' NHB donoren uit categorie 3. Het wijdverspreid uitvoeren van dergelijke NHB

donaties zou ook voor deze organen kunnen betekenen dat het tekort vermindert en mogelijk zelfs verdwijnt.

Belangrijk voor het op grote schaal invoeren van NHB donatie is de bekendheid van de procedure bij medisch personeel. Zij moeten immers potentiële donoren herkennen en bereid zijn een donatieprocedure in gang te zetten. De NHB donatieprocedure is relatief eenvoudig en het inbrengen van een koelcatheter in de liesarterie, zoals beschreven in ons donatie protocol, kan bijna overal uitgevoerd worden.

Voor nabestaanden lijkt donatie door een harddode donor op z'n minst even acceptabel als donatie door een hersendode donor. In onze ervaring is het weigeringspercentage bij NHB donatie gelijk aan dat bij HB donatie; potentiële NHB donoren worden echter meestal niet als zodanig herkend. Voorlichting van publiek en medisch personeel is daarom essentieel.

In Nederland staat de recent aangepaste wetgeving ten aanzien van orgaandonatie toe dat er voorbereidende handelingen verricht worden met het doel orgaantransplantatie mogelijk te maken. Het is dus bij NHB donatie toegestaan, vóórdat de expliciete wens van de overledene c.q. de nabestaanden bekend is, een koelcatheter in te brengen en met het koelen van organen te beginnen. Potentiële donoren zijn hierdoor makkelijker voor donatie beschikbaar en er kan sneller een donatieprocedure gestart worden. Daarnaast zal het nieuwe centrale registratie systeem mensen de gelegenheid bieden zich te laten registreren als donor. Momenteel wordt waarschijnlijk een flink deel van de potentiële donaties niet gerealiseerd omdat de wens van de overledene niet bekend is en nabestaanden niet op tijd hun toestemming kunnen geven. Hopelijk biedt het registratie systeem een oplossing voor dit probleem en maakt het een succesvolle invoering van NHB donatie makkelijker. Waarschijnlijk alleen een 'opting-out' wetgeving, waarbinnen iedereen donor is tenzij bij leven anderszins geregistreerd, zal een maximaal benutten van het NHB donor potentieel mogelijk maken.

Uit onze studies blijkt dat MP als preservatiemethode voor NHB donornieren de eerste keus is. Met MP is het functionele resultaat van NHB donornieren beter dan met CS. Tevens kan gedurende MP de nier getest worden en dus transplantatie van avitale nieren worden voorkomen. Door tijdens MP α GST te bepalen, kan de schade aan de nier en daarmee de viabiliteit bepaald worden. De vrees een niet functionerende nier te transplanteren kan zo worden weggenomen. Dit zal de acceptatie van NHB donatie bevorderen en leiden tot meer donatieprocedures. Ondanks MP blijft DF vaker voorkomen bij NHB dan bij HB donornieren. Hoewel DF wordt gezien als een voorspeller van een slecht transplantatie resultaat op de lange termijn, is dit niet het geval bij onze NHB donornieren. Hoewel bij NHB donornieren DF

dus niet vaker rejectie geeft noch vaker leidt tot verlies van het transplantaat, blijft het een negatief aspect van het transplanteren van NHB donornieren.

Door preservatievloeistoffen speciaal voor ischaemisch beschadigde nieren te ontwikkelen zou de ischaemische schade en dus DF behandelbaar kunnen worden. Uit dierexperimenteel onderzoek lijkt het mogelijk reperfusie schade te onderdrukken door de ontvanger te behandelen (platelet-activating-factor antagonist, adenosine remming) en zo DF te verminderen. Uiteraard dient additionele schade bij reeds ischaemisch beschadigde nieren tot een minimum beperkt te blijven en moet de nier onder de meest gunstige voorwaarden getransplanteerd worden. Zo moeten ontvangers zorgvuldig geselecteerd worden, moet de preservatietijd zo kort mogelijk zijn en zou cyclosporine nefrotoxiciteit voorkomen kunnen worden met inductie immunotherapie.

MP speelt een belangrijke rol bij NHB nierdonatie en het lijkt wenselijk preservatiecentra op te zetten zodat NHB donornieren, en dan met name die nieren met vermoedelijk uitgebreide ischaemische schade, machinaal gepreserveerd en op hun transplanteerbaarheid getest kunnen worden. In deze centra zal ook voortgaand onderzoek gedaan kunnen worden naar preservatiemethoden voor ischaemisch beschadigde nieren en andere manieren om DF te verminderen.

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ABBREVIATIONS

ALG	anti leucocyte globulines	ICU	intensive care unit
AMP	adenine mono-phosphate	IF	immediate function
ATG	anti T-lymphocyte globulines	IRR	intrarenal vascular resistance
ATN	acute tubular necrosis	ISP	in-situ preservation
ATP	adenine tri-phosphate	LDH	lactate dehydrogenase
CCU	coronary care unit	MP	machine perfusion
CIT	cold ischemia time	NF	non functioning
CS	cold storage	NHB	non-heart-beating
CyA	cyclosporine A	NOT-TX	not transplanted
DBTL	double-balloon-triple-lumen	OR	operating room
DF	delayed function	Pi	inorganic phosphate
DP	degradation products	PME	phosphate mono-ester
EC	Euro-Collins	p.m.p.	per million population
EEG	electro encephalogram	31P-MRS	phosphorus ³¹ -magnetic resonance spectroscopy
ER	emergency room	PNF	primary non-function
ET	Eurotransplant	PRA	panel of reactive antibodies
F	functioning	PT	proximal tubule
α GST	α glutathione S-transferase	SD	standard deviation
HB	heart-beating	SEM	standard error of the mean
HLA	human lymphocyte antigens	TAN	total adenine nucleotides
HTK	histidine tryptophane ketoglutarate	UW	University of Wisconsin
ICD	international classification of diseases	WIT	warm ischemia time

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De afdeling Algemene Heelkunde van het Academisch Ziekenhuis Maastricht heeft een zekere traditie opgebouwd voor wat betreft non-heart-beating nierdonatie en preservatie. Binnen deze traditie past ook het tot stand komen van dit proefschrift, waarbij vele mensen direct en indirect betrokken zijn geweest. Deze betrokkenheid heeft zich met name afgespeeld op en rond het preservatielab in het azM, doch ook op vele plaatsen ver daarbuiten. Op deze plaats wil ik graag allen die op hun eigen wijze een bijdrage hebben geleverd aan de genese van dit boekje, hiervoor hartelijk bedanken.

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Jan-Willem Henri Catharina Daemen werd geboren op 18 maart 1966 te Geleen, Nederland. Hier behaalde hij in 1984 aan de Scholengemeenschap St. Michiel zijn gymnasium-B diploma. Hij studeerde vervolgens geneeskunde aan de Rijksuniversiteit Limburg te Maastricht; het arts diploma volgde in maart 1991. Na zijn militaire dienstplicht vervuld te hebben als dienstplichtig bataljonsarts in het Noordduitse Seedorf, werd hij in augustus 1992 arts-assistent in het Scheperziekenhuis te Emmen. In januari 1994 keerde hij terug naar Maastricht om als arts-onderzoeker binnen de afdeling Algemene Heelkunde van het Academisch Ziekenhuis Maastricht, onder leiding van Prof.dr. G. Kootstra vorm te geven aan een Ontwikkelingsgeneeskunde Project naar de machinale preservatie van non-heart-beating donornieren. In 1996 werd het diepe zuiden wederom verruild voor het hoge noorden om te beginnen met de opleiding tot algemeen chirurg in de regio Groningen (opleider Prof.dr. R. van Schilfgaarde). Sinds juni van dat jaar is hij werkzaam als AGIO in het Sophia Ziekenhuis te Zwolle (opleider Dr. W. van Rooyen).