The non heart-beating donor

G Kootstra, J K Kievit and E Heineman

Department of Surgery, University Hospital Maastricht, Maastricht, The Netherlands

Given the shortage of donor kidneys for transplantation, we have focused on the use of non heart-beating (NHB) donor kidneys since 1982. The major drawback for the use of NHB donor kidneys is the inherent possibility of severe ischaemic damage leading to primary non function. Thus viability assessment of ischaemically damaged kidneys is crucial, and, therefore, a machine perfusion programme was reinstituted in 1993. Machine perfusion (MP) enables viability assessment through analysis of perfusion characteristics and measurement of enzyme release into the perfusate. Of the last 100 consecutive MP NHB donor kidneys, 71 kidneys were transplanted and 29 kidneys were discarded. Nine kidneys started functioning immediately, 51 kidneys showed delayed function and 11 kidneys never functioned. When analysing in retrospect different parameters for viability assessment, only α-GST, an enzyme specific for damage of proximal tubular cells within the kidney, could discriminate between functioning and non-functioning kidneys. With this promising viability assessment, the large NHB donor potential and the good transplant results, we recommend the use of these donors.

It is evident that, so far, the problem of shortage of kidneys for transplantation has not been solved and it will not be solved in the near future. Several programmes have been launched, such as EDHEP¹ and Donor Action², to increase the number of available donors. At the national level, only the Spanish transplant organization has, with the help of grants from the government, realized a very effective network and programme, not matched by any other country or exchange organization³. In 1994 in Spain, the procurement rate was 54 kidneys per million inhabitants. In the Eurotransplant exchange organization covering over 100 million Europeans, there is a plateau for the last 5 years with a number of 27 post mortem kidneys per million⁴. Recently, the use of kidneys from living unrelated donors, especially spouses, has gained popularity⁵,⁶. However, we cannot close the gap between supply and demand with the kidneys of living (non)-related donors. So there is need for an innovative approach to relieve this short-fall. Such an innovative approach is the use of non heart-beating (NHB) donors. We
now have experience over 15 years, although only in the last 5–7 years has the number of kidneys been considerable. In 1996, NHB donation increased the number of kidneys procured in our programme by 31.

**History, definition and categories**

In the early days of transplantation after the initial successes with identical twins, the main source of kidneys were, in fact, NHB donors. Trauma patients with severe brain damage were, when the prognosis was very poor, taken to the operating theatre. The donor was disinfected with iodine and draped. When the surgeons were gowned and gloved, the ventilator was switched off and cardiac arrest was awaited. Laparotomy started at the moment of cardiac arrest and the kidneys were procured. The results of these *post mortem* kidneys were reasonable, although not really good. This was not only due to ischaemic damage but also to the lack of potent immunosuppressive drugs. Many patients suffered from delayed function of their transplanted kidney, and this so-called acute tubular necrosis (ATN) obscured the diagnosis of rejection.

Extensive studies were undertaken to determine how the function of these NHB donor kidneys could be improved and, very importantly, could be predicted\(^7\)^\(^8\). Machine preservation and testing was developed\(^9\).

With the introduction of the concept of brain death, kidneys could be procured from heart-beating (HB) donors. Kidneys from these donors performed better and NHB donorship was abandoned. The classical publication of Terasaki and Opelz\(^10\), showing that cold storage was as good as machine preservation for kidneys from HB donors, put an end to the use of machine preservation.

It is noteworthy, however, that in some countries, like Sweden, the concept of brain death has only recently been accepted and in Japan there is still no brain death legislation\(^11\). Since 1982, we have been confronted with a shortage of kidneys, and hence explored the possibility of re-introducing the concept of NHB donorship. This succeeded since 1989 on a regular basis. We re-introduced machine preservation in 1993, helped by a grant of the Dutch Government and the Health Insurance Fund. In fact, we started where in the 1970s the transplant field had stopped.

In the HB donor, the diagnosis of death is based on brain criteria and the concept is called ‘brain death’. There is extensive literature on how to diagnose brain death and it has been discussed medically, legally and from different cultural views. This now is not the case for the concept of the NHB donor. In a NHB donor, death has to be defined on cardiac
Transplantation criteria — irreversible cardiac arrest. In the UK, the NHB donor is called an ‘asystolic donor’. The question arises as to whether somebody is really dead at the very moment of cardiac arrest and whether the dead donor rule is respected. One probably could restart the heart of a person that has arrested after a ventilator switch off procedure and, therefore, it can be disputed whether someone is, in this situation, dead at the very moment of cardiac arrest. This dead donor rule states that ‘organs should be taken only after death and patients should not be killed by organ removal’. At the first International Workshop on NHB donation, it was agreed to wait another 10 min after cardiac arrest to ensure that a situation equivalent to brain death has occurred.

We have defined the potential of NHB donors into 4 categories (Table 1). The first category donors (dead on arrival) are not yet used for donation. There are too many questions regarding length of ischaemia, cause of death, etc. The second category (unsuccessful resuscitation) is new in the field of NHB donorship in so far that this category was not used in the early days of transplantation. Category three involves the ventilator switch-off type of patients, although not all category three potential donors are ventilator dependent. With this category one had experience in the early days. We have notified a fourth category. This is the category wherein donors diagnosed brain dead, or in the process of the diagnosis of brain death, have a cardiac arrest and wherein resuscitation is unsuccessful. Instead of calling off the organ donation, one has to switch to a NHB donor procedure to enable procurement of at least the kidneys.

**The NHB donor procedure**

In every NHB donor procedure there is a certain period of warm ischaemia to the kidneys and this warm ischaemia will be responsible for the delayed onset of function of the kidneys. The ischaemic damage can be reduced by bringing down the temperature, because at low temperatures there is hardly any metabolism and decay in kidneys. This cooling of the kidneys is realized by introducing a catheter through a cut-

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
<th>Location in the hospital</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dead on arrival</td>
<td>Accident and Emergency</td>
<td>Not yet accessible</td>
</tr>
<tr>
<td>2</td>
<td>Unsuccessful resuscitation</td>
<td>Accident and Emergency, Regular Ward</td>
<td>Accessible, only half of the procedures are successful</td>
</tr>
<tr>
<td>3</td>
<td>Awaiting cardiac arrest</td>
<td>Intensive Care, Regular Ward</td>
<td>Accessible, high percentage of success</td>
</tr>
<tr>
<td>4</td>
<td>Cardiac arrest during or after brain death diagnostic procedure</td>
<td>Intensive Care</td>
<td>Switch from HB to NHB saves at least the kidneys for transplantation</td>
</tr>
</tbody>
</table>
The non heart-beating donor

Fig. 1 Double balloon triple lumen cooling catheter, (AJ 6516) produced by Porgès, Le Plessis-Robinson, France. The two small outer balloons, marked ABDO and THOR reflect the status of both large balloons inside. The mark on the catheter, indicated by the arrow, is meant as point of maximum insertion.

down in the groin. The technique has been described previously\textsuperscript{16}, and is summarized below.

A longitudinal incision over the inguinal ligament is performed and a Ch16 double balloon triple lumen (DBTL) catheter (AJ 6516, Porgès, Le Plessis-Robinson, France) is inserted (Fig. 1). The catheter is advanced through the iliac artery into the aorta. The abdominal balloon is inflated and the catheter is carefully retracted until the inflated balloon hooks at the bifurcation of the aorta. Next, the thoracic balloon is inflated. Now a segment of the aorta, where the renal arteries are situated, is isolated. Through the third lumen of the catheter, the cooling solution is now introduced and the kidneys are flushed out \textit{in situ}. A cut-down in the femoral vein is the next step and a Foley catheter Ch16 is introduced up into the caval vein for decompression. The Foley catheter is connected with a large reservoir under the bed or resuscitation table. As cooling fluid we use the histidine-tryptophan-ketoglutamine (HTK) solution\textsuperscript{17}, which is supplied in 5 litre canisters. A radio-opaque solution is used to fill the balloons and an X-ray of the abdomen is performed to check whether the balloons are in proper position. When one observes that the skin in the area of the ninth to the twelfth rib is blanching and getting cold, this is additional proof that the balloons are in the proper position. The incision in the groin is provisionally closed. Now there is a stable situation and the kidneys are cooled inside the body. On many occasions in category two donors, this is the time when the family is invited to a
Fig. 2 Gambro PF-3B perfusion machine (Gambro, Lund, Sweden) Inside the sterile organ chamber, the kidney is continuously perfused with perfusate which is cooled by ice water inside the machine. Oxygen is provided. Perfusion pressure and flow can be set and temperature is recorded. Samples of perfusate can be collected from the tubing.

farewell visit with their loved one. Meanwhile, a theatre is prepared and after departure of the family the dead body with the in situ cooling apparatus is moved to the theatre for the procurement of the kidneys. Basically, this is the procedure for the category two donors. Until the consent of the family is obtained, the time is bridged by cardiac massage and artificial ventilation. This is preceded by 10 min of no-touch of the dead body after the diagnosis of cardiac death by the resuscitation team. In the category three donors, after cardiac arrest the same 10 min of no-touch is practised and then the catheter is inserted. After nephrectomy by midline incision and splitting the aorta to provide an aortic patch for each kidney, the kidneys are refilled with HTK at the back table. Then they are stored in either the cold storage solution or they are directly connected to the preservation machine, depending where the procedure...
took place. At our hospital, each kidney is connected to a separate preservation machine (Fig. 2) and tested for viability.

**Viability testing**

There is a need for an objective test that measures the ischaemic damage and predicts whether the kidney will function or not. Cold storage, the commonly used preservation method for HB donor kidneys, does not offer this opportunity, although a biopsy of the kidney can be studied. We have not found a correlation between post donor nephrectomy histological findings and post transplant function. We could not confirm in biopsies of human kidneys the work of Yin and Terasaki done in animal kidneys\(^{18}\). They tested the activity of the reduction-oxidation enzymes in the mitochondria with the reduction of tetrazolium salts to formazan.

In machine preservation, a perfusate of modified Belzer's solution is pumped through the artery into the kidney, the venous effluent is collected, cooled and reused. It is a closed circuit with approximately 500 ml of the Belzer perfusate. Machine preservation enables the study of the resistance in the kidney, the pH of the perfusate and the release of lysosomal enzymes into the perfusate. Resistance and pH have not been decisive; however, the release of damage-related enzymes is promising. \(\alpha\)-Glutathione-S-transferase is an enzyme specific for the proximal tubulus cell in the kidney\(^{19}\). In our hands the release of this enzyme correlated well with the outcome, but more data are needed to strengthen and confirm our observation\(^{20}\).

**Results**

For this review, we analysed the most recent results of our NHB donor programme. A cohort of the last 100 consecutive NHB donor kidneys analysed with machine preservation is presented. From July 1994 until January 1997 (2.5 years) we preserved 100 NHB kidneys on the machine. Of these, 58 were from our own procurement programme and 42 were sent to us for machine preservation and evaluation. 29 kidneys were considered unsuitable, based on donor history, warm ischaemia time, violence of asepsis, macroscopic appearance or a combination of these. 71 kidneys were transplanted. Of these, 9 gave immediate function, 51 showed delayed function while 11 never functioned (primary non function). In Table 2, data concerning age, warm ischaemia time, and viability parameters are shown. The reason for
Table 2  Outcome of viability assessment of machine perfused non-heart-beating donor kidneys (n = 100), tested for different parameters

<table>
<thead>
<tr>
<th>n</th>
<th>Age (years)</th>
<th>WIT (min)</th>
<th>LDH T8 (U/L)</th>
<th>IRR T8 (mmHg/ml/min)</th>
<th>α-GST T8 (microgram/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IF</td>
<td>9</td>
<td>43 ± 7</td>
<td>36 ± 9.9</td>
<td>546 ± 100</td>
<td>0.76 ± 0.08</td>
</tr>
<tr>
<td>DF</td>
<td>51</td>
<td>41 ± 2.2</td>
<td>51 ± 5.1</td>
<td>862 ± 66</td>
<td>0.87 ± 0.06</td>
</tr>
<tr>
<td>PNF</td>
<td>11</td>
<td>46 ± 4.7</td>
<td>60 ± 12.1</td>
<td>966 ± 244</td>
<td>1.07 ± 0.28</td>
</tr>
<tr>
<td>Non Tx</td>
<td>29</td>
<td>45 ± 2.3</td>
<td>78 ± 7.9</td>
<td>1653 ± 325</td>
<td>1.09 ± 0.11</td>
</tr>
</tbody>
</table>

All results are mean values ± SEM. Results for LDH and α-GST are corrected for kidney weight. IF = immediate function, DF = delayed function, PNF = primary non function, Non Tx = not transplanted, WIT = warm ischaemia time, LDH = lactate dehydrogenase, IRR = intra renal resistance and α-GST = α-glutathione S-transferase. T8 = 8 h after start of machine perfusion.

primary non function of the 11 kidneys were: acute rejection (n = 5), vascular thrombosis (n = 3) and bleeding, no perfusion on first scan and never functioning for unknown reason, in one kidney each.

With a frequency of 15%, primary non function is approximately the same as in a previous study in which we compared the outcome of 57 cold stored NHB kidneys with 114 heart beating kidneys.

In Table 3, the parameters are compared statistically, and it shows that α-GST is the only parameter which can discriminate between functioning and non-functioning grafts. Lactate dehydrogenase might be a parameter that predicts immediate function from delayed function.

Discussion

There is a considerable lack of donors and, therefore, we embarked on the concept of the NHB donor. The concept is not new and actually post mortem kidney transplantation started with the use of NHB donors, of what we call category three donors. Our inclusion of an additional group — category two is new. Through our NHB donor programme in

Table 3  Statistical analysis of parameters used for viability testing, when comparing 3 groups of machine perfused non-heart-beating kidneys (n = 71) with different outcome

<table>
<thead>
<tr>
<th>Age</th>
<th>IF vs DF</th>
<th>IF vs PNF</th>
<th>IF vs PNF</th>
<th>P = 0.025</th>
<th>IF vs DF vs PNF</th>
<th>P = 0.034</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH T8</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
<td>P = 0.017</td>
<td>n.s</td>
<td>P = 0.024</td>
</tr>
<tr>
<td>IRR T8</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>α-GST T8</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
</tr>
</tbody>
</table>

Non-parametrical Mann-Whitney U test, 2-tailed and P < 0.05 is considered significant. n.s = not statistically significant. IF = immediate function, DF = delayed function, PNF = primary non function, WIT = warm ischaemia time, LDH = lactate dehydrogenase, IRR = intra renal resistance, α-GST = α-glutathione S-transferase and T8 = 8 h after start of machine perfusion.
The non heart-beating donor

In the past 5 years, we have procured 31–40% more kidneys. These kidneys were offered to the Eurotransplant exchange organization and consequently transplanted in the selected centres. For 3 years, no kidneys have been accepted by German centres, because there is doubt within the Federal Medical Committee whether the process of confirming death in the case of a NHB donation is correct. Here, the lack of literature and expertise regarding NHB donation plays a decisive role. A special issue of *Transplantation Proceedings* devoted to NHB (Proceedings of the First International Workshop at Maastricht, 1995)\(^{15}\) is now available and it presents current opinions and positions. The 10 min ‘no-touch’ period is proposed in these proceedings to comply with the dead donor rule and to create a situation that is equivalent to brain death. These additional 10 min of warm ischaemia will be tolerated by the kidney, but the outcome of other organs of NHB donors, for instance liver and lung, are probably less favourable. Nevertheless, there are publications on successful transplantation of livers in category three NHB donors\(^{22,23}\). Transplantation of NHB donor lungs is still in the experimental phase\(^{24}\).

In our cold storage study\(^{21}\), we experienced 14% primary non function, and this was 15% in the machine preservation series published here. This percentage of primary non function in a HB series was 3.7–6.4%\(^{25}\) and in our control group of HB kidneys it was 8%\(^{21}\). However, one has to realize that the outcome of a kidney transplant not only depends on warm ischaemia, type of preservation, etc. Recipient factors, like sensitization, play an important role as well.

Nicholson\(^{26}\) has studied the workload of a NHB donor programme and the yield. Only one-third of the referrals resulted in a successful procurement procedure. In our programme, the refusal rate is, at 30%, the same for NHB and HB donors, which is remarkable because in the NHB setting there is always a time constraint.

It has been claimed that delayed onset of kidney function post transplantation obscures the diagnosis of rejection. This might be true, although nowadays, with the new immunosuppressive protocols, the percentage of kidneys that will go into rejection has decreased considerably. In most transplant centres there is experience with diagnosing rejection during ATN by comparing perfusion scans and repeated kidney biopsies. This will provide, on time, the diagnosis of rejection.

Although we expected to reduce the number of primary non function kidneys through machine preservation, this is not confirmed by the results of our recent 100 NHB donor kidneys. Opelz and Wujciak\(^{27}\) found, in a relatively small subset of transplants with prolonged warm ischaemia, no convincing evidence that machine preservation resulted in better graft outcome. Matsuno\(^{28}\) performed a prospective study in which one NHB donor kidney of the same donor was machine preservation...
preserved and the other cold stored. He found a better outcome for early graft function in favour of machine preservation. In the study presented here, we accepted kidneys with long ischaemia times and, in addition, the 10 min of no-touch was introduced in our protocol in 1995. This might explain why we were not able to improve on the result for primary non-function: we simply extended the acceptable warm ischaemia too long. Nevertheless, it is certain that the most promising, and probably only way of viability testing, is with machine preservation. Our results with the alpha-GST enzyme in the perfusate are very promising. It is so far the only parameter that discriminates functioning from non-functioning kidney grafts. This is a retrospective finding, we now are studying the predictive value of α-GST in NHB donor kidneys.

In view of the existing shortage of kidneys and the improvement of quality of life in transplanted patients, we invite transplant centres worldwide to join the study and collect experience with the use of NHB donors, which has, in our estimation, the potential to solve the shortage problem.

References

13. Arnold RM, Youngner SJ. Time is of the essence: the pressing need for comprehensive non-heart-beating cadaveric donation policies. Transplant Proc 1995; 27. 2913-7
14. Arnold RM, Youngner SJ. The dead donor rule: should we stretch it, bend it, or abandon it? Ken Inst Ethics J 1993; 3: 263
The non heart-beating donor


