

## REVIEW

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### NON HEART-BEATING DONORS IN ENGLAND

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When transplantation started all organs were retrieved from patients immediately after cardio-respiratory arrest, i.e. from non-heart-beating donors. After the recognition that death resulted from irreversible damage to the brainstem, organ retrieval rapidly switched to patients certified dead after brainstem testing. These heart-beating-donors have become the principal source of organs for transplantation for the last 30 years. The number of heart-beating-donors are declining and this is likely to continue, therefore cadaveric organs from non-heart-beating donor offers a large potential of resources for organ transplantation. The aim of this study is to examine clinical outcomes of non-heart-beating donors in the past 10 years in the UK as an way of decreasing pressure in the huge waiting list for organs transplantation.

**KEYWORDS:** Transplantation. Liver. Kidney. Donors.

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The imbalance between supply of organs for transplantation and demand for them is widening all over the world. In the United Kingdom (UK) the figures are not different, by the end of December 2004 there were over 6000 people on the active waiting list for organ transplantation. Recent data suggest that over 400 of these people will die each year before a new organ becomes available.<sup>1</sup>

When transplantation started all organs were retrieved from patients immediately after cardio-respiratory arrest, i.e. from non-heart-beating donors (NHBDs). After the recognition that death resulted from irreversible damage to the brainstem by Harvard Medical Committee<sup>2</sup> and the subsequent introduction in 1976<sup>3</sup> of direct brainstem testing to determine when death has occurred, organ retrieval rapidly switched to patients certified dead after brainstem testing. These heart-beating-donors (HBDs) have become the principal source of organs for transplantation for the last 30 years.

The number of HBDs are declining and this is likely to continue for two major reasons: fewer young people are dying as a result of severe injury or catastrophic cerebro-

vascular events<sup>4</sup>, and improvements in diagnosis and management of severe brain injuries mean that fewer fulfil the brainstem testing criteria.

Although numbers of NHBDs are slowly increasing, they still accounted for only 85 of 750 (11.3%) UK cadaveric donors in 2004-2005.<sup>5</sup>

The fundamental problem with NHBDs is warm ischemia which may lead to suboptimal transplanted organ function. Developments in organ protection will only lead to more successful outcomes from NHBD if strategies can be devised to keep warm ischaemia times as short as possible.

The aim of this study is to examine clinical outcomes of NHBDs in the past 10 years in the UK as an way of decreasing pressure in the huge waiting list for organs transplantation.

A literature review was performed based on a Medline (Pubmed from 1997 to 2006) search to identify articles on clinical NHBDs in the UK.

Information on the rates of primary non-function (PNF), delayed graft function (DGF), acute rejection, graft and patient survival were registred. Also NHBDs technique, perfusion and recipient immunosuppression were mentioned.

All centres have developed programmes based on the Maastricht protocol,<sup>6</sup> which includes the following principles:

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1. approval by local medical ethics committee,
2. diagnosis of death by doctors who are independent of the transplant team,
3. the 10-min rule (after declaration of cardiac death, the body is left untouched for a period of 10 min prior intervention),
4. rapid *in situ* cooling using a catheter inserted into the aorta, and organ retrieval using standard surgical techniques. Declaration of cardiac death implies irreversible cessation of heart function and the 10-min hands-off period ensures the process of irreversible brain death has begun.

We also recorded the criteria for exclusion of NHBDs, the Maastricht classification, the current criteria for both brain-stem death for HBDs and cardiac death for NHBDs, and finally ethics and legal issues involved in the NHBDs transplantation.

### The first International Workshop in Maastricht<sup>7</sup> defined four categories of NHBD:

#### UNCONTROLLED

**Category I** – includes victims of accident and suicide ( some centers exclude suicide victims from their programs ) who are found dead at the scene and resuscitation is deemed pointless (e.g. fatal cervical spine fracture). These are the worst group of potential donors because of the unknown primary warm ischemic time.

**Category II** – donors are mostly victims of sudden cardiac (the majority) or cerebral catastrophe who are brought to emergency departments while being resuscitated by ambulance personnel or who died in the department. Other sources include patients suffering isolated brain injury, anoxia and stroke, and victims of major trauma who died soon after hospital admission.

#### CONTROLLED

**Category III** – encompasses patients who are dying, often on an intensive care unit. These are the patients awaiting cardiac arrest where the treating clinicians have decided to withdraw treatment and not commence resuscitation for various reasons.

**Category IV** – comprises patients who suffer unexpected cardiac arrest during or after determination of brain death.

### Ethics and Legal Issues

There is an increasing worldwilde discrepancy between

**Table 1 - Non-Heart-Beating Donors - Maastricht classification<sup>7</sup>**

Description		
I	Dead on arrival	Uncontrolled
II	Unsuccessful resuscitation	Uncontrolled
III	Awaiting cardiac arrest	Controlled
IV	Cardiac arrest while brain dead (death during procurement; death during explantation)	Controlled

**Table 2 - Criteria for exclusion of non-heart-beating donors<sup>8</sup>**

1. Cardiac and circulatory arrest does not last longer than 40 minutes.
2. The patient is between 16 and 60 years old.
3. The patient does not belong to a high risk group for immunodeficiency virus (HIV), or hepatitis B or C infection. There should be no signs of intravenous drug abuse.
4. The patient has no history of primary kidney disease, uncontrolled hypertension, or complicated insulin-induced diabetes mellitus (IDDM). There are no signs of intravascular coagulation with anuria and no signs of malignancy other than a primary (non-metastatic) cerebral tumor.
5. There are no signs of sepsis or serious infection.
6. Patients who have died after assisted suicide or euthanasia are excluded from some protocols.

**Table 3 - Criteria for brainstem death apply to heart-beating donors<sup>8</sup>**

1. The underlying pathologic lesion should be understood;
2. There should be no pharmacologic, metabolic, or hormonal influence;
3. Pupillary, corneal, occulo-cephalic, vestibule-ocular, and gag reflexes should be absent;
4. No pain response to stimulation in the distribution of the fifth cranial nerve; and
5. A re-breathing test with 100% oxygen should be delivery to maintain satisfactory oxygenation, while ventilation is switched off. The rise in arterial pCO<sub>2</sub> should not stimulate respiration.

# These tests are performed by two experienced clinicians on two separate occasions.

**Table 4 - Criteria for cardiac death apply for non-heart-beating donors<sup>8</sup>**

1. Deep coma
2. Absence of pulse
3. ECG evidence of asystole

# Cardiac death in the context of potential organ donation is defined as occurring after 30 minutes of unsuccessful cardiopulmonary resuscitation under hospital conditions.

# Resuscitation must include external cardiac massage, intubation, ventilation, defibrillation (if indicate), and appropriate intravenous medication.

# Unsuccessful means that these measures did not achieve spontaneous contractile cardiac activity or peripheral circulation.

the availability of, and need for organs allografts. It has been estimated that the number of donor organs could rise by 25% through the expanded use of NHBD<sup>9</sup> making it inevitable in an era of universal shortage of donors.

All transplant procurement strategies come with an ethical dimension but this particular process raises multiple concerns, not least of which is ambiguity as to the timing and definition of death.

The principal ethical issues concerning NHBDs programs are the use of *in situ* perfusion to consent the diagnosis of death by cardiac rather than brain-stem criteria, and at what time after pronouncement of death the *in situ* cooling should be started (dead donor rule).

English law does not require consent for prolonged ventilator support or placement of the double-balloon triple-lumen (DBTL) catheter, but catheter placement is an invasive procedure and the family members may not wish for this<sup>10</sup>.

The dead donor rule<sup>11</sup> is pivotal to NHBD organ donation and states that the donor should not be killed by the act of donation (i.e. the donor must be dead by cardiac criteria at the time of retrieval). This became relevant when a decision is taken to discontinue life support measures in a patient who does not meet the criteria for brain-stem death and raises the question of when death should be pronounced after cardiac arrest<sup>12</sup>.

There is a period when the patient is dead by cardiac criteria but not by brain-stem criteria<sup>13</sup> and 10 minutes is considered sufficient for the discrepancy to be corrected. There has been considerable debate over the length of time that the patient should be left<sup>14</sup>. For ideal preservation the kidneys should be perfused as soon as the 10-minute waiting period is over.

These considerations were taken into account at the Maastricht workshop (1995). The conclusions of this discussions was that it was safer to apply the dead-donor rule only after a 10-minute period of asystole<sup>7,15</sup>. There can be little doubt that after normothermic ischaemia for this period, it would be impossible to restore myocardial function and there will be irreversible loss of all neurological function. Then, the criteria for cardiac and brain death will have been satisfied simultaneously.

### Warm Ischaemia Renal Injury

The period of warm ischaemia is usually defined as the time between cardiac arrest and the start of cardiopulmonary resuscitation.

This could more accurately be described as the absolute warm time because there may be other less obvious periods of warm ischaemia. The efficacy of external car-

diac massage is achieving renal perfusion is not definitely known and will vary according to how well resuscitation is performed. It is likely that a degree of renal warm ischaemia is common during cardiopulmonary resuscitation and this could be described as relative warm time.

Conversely cardiac massage and ventilation which are effective in oxygenating the kidneys after a period of circulatory standstill, may also be deleterious by initiating the reperfusion injury syndrome.

Warm ischaemia is known to be a major determinant of renal function after kidney transplantation. However, the amount of reversible warm ischaemic injury that the human kidney can sustain is still not known for certain. Most human NHBD kidney transplant protocols exclude kidneys which such prolonged warm times, the usual cutoff being in the of 30 to 45 minutes<sup>16,17</sup>.

Renal allografts from controlled NHBDs sometimes function immediately suggesting that the human kidney tolerates short periods of warm ischaemia quite well<sup>18</sup>. In uncontrolled NHBDs the warm time is not always accurately known. It may need to be determined by taking a history from relatives, ambulance staff, and medical personnel and in some cases this will provide only an approximate estimate.

### Surgical Technique

#### 1. Leicester Model for NHBD

##### (In situ kidney perfusion/Cooling and kidney retrieval in NHBD)

NHBDs kidneys were perfused and cooled *in situ* using a DBTL aortic catheter placed via femoral artery cut-down in the groin. The technique as described originally by Garcia-Rinaldi et al<sup>19</sup>, involves insertion of the DBTL catheter into the abdominal aorta via femoral arteriotomy. Inflation of the caudal balloon at the aorta bifurcation and inflation of the cranial balloon isolates the segment of aorta from which the renal arteries originate (vertebral level L1). A plain abdominal radiograph can be taken to show the position of the catheter, which is radio-opaque.

Wheatley et al<sup>20</sup> modification is to mix a small amount of Conray (May and Baker, Dagenham, UK) radio-contrast dye with saline injected into the balloons to allow easier identification of the balloons on radiography. Using a mixture of Conray and saline in a ratio 1:10 rather than neat Conray makes injection of the viscous liquid much easier.

The balloons were inflated with radiographic contrast medium, and the positioning checked by plain abdominal x-ray<sup>20</sup>.

The system was vented by placing a Foley catheter into

the inferior vena cava via the right femoral vein. Following *in situ* renal perfusion the NHBD was transferred to an operating theatre for bilateral donor nephrectomy.

The perfusion fluid used for NHBD was a 10 to 20 L Marshall's hyperosmolar citrate solution at 4 C infused under gravity and all kidneys were held in static ice storage before transplantation.

## 2. Newcastle upon-Tyne Model for NHBD (NHBD retrieval ; pumping perfusion system)

In situ organ perfusion was performed by cannulating the femoral artery using a DBTL cannula (TXF Medical, High Wycombe, UK). The preservative solution was cold (4-8 C) heparinized (1000 IU/L) Marshall's solution. The venous venting was through placement of a cannula into the femoral vein. No radiological confirmation of placement was done. After retrieval the kidneys were cold stored and transported to the Hospital where machine perfusion and viability assessment were carried out<sup>21</sup>.

### Pumping perfusion system

A Belco BL 760 blood pump module was used for perfusion. One pump in the system provided fluid to the renal artery and the other retrieved it through a heat exchanger. The temperature of the system was maintained between 4 and 9 C. The pump was capable of delivering a flow rate of 28-480ml/min and pressure were maintained at 45-60 mm Hg. Thus a closed system of perfusion was achieved.

A viable kidney tends to have a flow rate of more than 50 ml perfusate/minute per 100 g of kidney and a glutathione S-transferase level less than 200 IU/L per 100 g kidney<sup>22</sup>.

## 3. Cambridge Model for NHBD

Following certification of death on the ward, the donor was transported to the operating theatre. Laparotomy was performed, a large bore (22F) aortic cannula inserted and the kidneys were perfused *in situ* with at least 3L of cold University of Wiscosin (UW) solution containing 20 000 units of heparin per litre.

The supra-coeliac aorta was cross-clamped to facilitate perfusion of the kidneys and the venous system was decompressed via inferior vena cava. To provided additional cooling, 2 L of crushed frozen saline were applied around the kidneys during perfusion. Donor nephrectomy was then performed<sup>23</sup>.

## 4. London Model for NHBD

An interval of 15 minutes after cardiac arrest was always allowed before cold *in situ* perfusion or crash retrieval was commenced. All donors were cooled using Soltran Kidney Perfusion Solution (Baxter Medical, Houston, TX, USA) with an additive of 20,000 IU of heparin via femoral access using a DBTL inserted into the femoral artery. Cannulas were placed only after consent of relatives was given.

The kidneys of the controlled donors were perfused via rapid aortic cannulation after laparotomy or crash retrieved with no flush and thereafter perfused with ice-cold solution on the bench<sup>24</sup>.

Pulsatile kidney perfusion with the RM3 pump (Water Medical Systems, Rochester, MN, USA) for organ assessment and preservation of up to 12 hours was used.

## RESULTS

During ten-year period analysed, NHBDs were used mainly for kidney transplantation in four centres in the UK as summarized in (Table 5).

NHBDs in the UK centres according to the Maastricht classification are shown in (Table 6).

Comparing NHBD with HBD the results of PNF, only 3 out of 24 articles have shown significant differences. As far as DGF is concern 8 out of 24 articles have shown significant differences. Acute rejection was reported significant in only 1 out of 24 articles (Table 7).

Graft survival was equal for 1, 2,3 and 5 years when comparing NHBD with HBD except in 1 article that has shown significant differences in 3 and 5 years time, (Table 8).

Patient survival was equal in 1, 3 and 5-years when comparing NHBD with HBD as shown in (Table 9).

United Kingdom centres and variations of NHBDs technique, use of double-ballon triple-lumen, machine perfusion, type of organ perfusion solution as well as immunosuppression are demonstrated in (Table 10).

## DISCUSSION

Cadaveric organs from NHBD have been used for decades. Since the introduction of "brain-stem death" criteria in 1968, NHBDs have been largely abandoned in favor of brain-dead donors<sup>47</sup>

NHBD offers a large potential of resources for renal transplantation. The process of graft selection involves a significant number of potential grafts being discarded because they are judged to be nonviable. The reported dis-

**Table 5** - England non-heart-beating donors transplant details

Author	Year	Centre	No. patients	Organ
Nicholson et al <sup>25</sup>	1997	Leicester	30	Kidney
Butterworth et al <sup>26</sup>	1997	Leicester	37	Kidney
Nicholson et al <sup>27</sup>	2000	Leicester	77	Kidney
Balupuri et al <sup>21</sup>	2000	Newcastle upon-Tyne	15	Kidney
Balupuri et al <sup>28</sup>	2001	Newcastle upon-Tyne	28	Kidney
Metcalfe et al <sup>29</sup>	2001	Leicester	72	Kidney
Gok et al <sup>30</sup>	2002	Newcastle upon-Tyne	43	Kidney
Gerstenkorn et al <sup>31</sup>	2002	London	202	Kidney
Gok et al <sup>32</sup>	2002	Newcastle upon-Tyne	46	Kidney
Sudhindran et al <sup>23</sup>	2003	Cambridge	42	Kidney
Gok et al <sup>33</sup>	2003	Newcastle upon-Tyne	25	Kidney
Brook et al <sup>34</sup>	2003	Leicester	55	Kidney
Gerstenkorn et al <sup>35</sup>	2003	London	41	Kidney
Gok et al <sup>36</sup>	2004	Newcastle upon-Tyne	72	Kidney
Brook et al <sup>37</sup>	2004	*	285	Kidney
Gok et al <sup>38</sup>	2004	Newcastle upon-Tyne	02	Kidney
Wilson et al <sup>39</sup>	2005	Newcastle upon-Tyne and Leicester	51	Kidney
Bains et al <sup>40</sup>	2005	Leicester	37	Kidney
Navarro et al <sup>41</sup>	2006	Newcastle upon-Tyne	05	Kidney
Gok et al <sup>42</sup>	2006	Newcastle upon-Tyne	19	Kidney
Navarro et al <sup>43</sup>	2006	Newcastle upon-Tyne	81	Kidney
Sohrabi et al <sup>44</sup>	2006	Newcastle upon-Tyne	05	Kidney
Sohrabi et al <sup>45</sup>	2006	Newcastle upon-Tyne	36	Kidney
Muesan et al <sup>46</sup>	2006	London	07	Liver

\*Leicester, Cambridge, London, Newcastle upon-Tyne (combined results of renal NHBD transplantation in the UK from 1988-2001)

**Table 6** - Non-heart-beating donors in England centres according to the Maastricht classification.

Centre	NHBD* not mentioned category (n=365)	NHBD Uncontrolled Category I e II (n=575)	NHBD Controlled Category III e IV (n=398)	NHBD Total (n=1334)
Cambridge	-	0	42	42
Leicester	144	144	20	308
London	202	8	40	250
Newcastle upon-Tyne	19	178	201	398
Cambridge, Leicester, London, Newcastle upon-Tyne	-	217	68	285
Leicester, Newcastle upon-Tyne	-	24	27	51

\*NHBD-non-heart-beating donor

card rate of kidneys from NHBD is significant, with estimates ranging from 50 to 65% with uncontrolled donors<sup>48</sup>.

NHBD programs remain unpopular despite the potential to increase the donor pool by up to 30%<sup>49</sup>. A number of legal, ethical and logistic reasons as well as medical concerns are responsible for this and have even compromised existing NHBD programs<sup>50</sup>.

Legal and ethical issues such as cannulation of the femoral vessels for *in situ* cooling prior to consent by the relatives and an undefined interval of no-touch between cardiac arrest, declaration of death and organ resuscitation/preservation efforts remain an unsolved problem in the United Kingdom. If relatives were present the consent rate for organ donation after cardiac arrest was more than 70%.

In addition, the logistic requirements for a successful, financially efficient NHBD program are immense and re-

quire good planning, management and organization. Transplant surgeons and coordinators must be within close reach of potential donor locations in emergency departments, intensive care units, hospital wards, and hospices. Furthermore a sufficient number of trained staff members are required to share a rota for uninterrupted on-call cover. This might be possible only for a large transplant units or particularly dedicated centers. Organ preservation by perfusion is an additional issue that requires further expertise and is expensive<sup>21,51</sup> but it might be the only reliable technique for assessing the viability of NHBD kidneys and marginal donors organs<sup>52</sup>.

The 125 NHBD transplants performed in 2005-2006 (82 kidneys only; 36 kidney/liver; 1 kidney, thoracic & liver, 2 kidney, liver & pancreas and 4 liver only) rose 44% comparing with 87 NHBD in 2004-2005 but still represent a

**Table 7** - Comparison of the results of NHBD/HBD in England relating to primary non-function, delayed graft function and acute rejection.

Author	NHBD/HBD	PNF(%) NHBD/HBD	DGF(%) NHBD/HBD	AR(%) NHBD/HBD
Nicholson et al <sup>25</sup>	30/114	3/13(s)	25/87(s)	48/27(s)
Butterworth et al <sup>26</sup>	37/91	11/1(s)	100/28(s)	27/36(ns)
Nicholson et al <sup>27</sup>	77/224	9.1/2.7(s)	84.3/21(s)	28.6/32.6(ns)
Balupuri et al <sup>21</sup>	15/nm	6.6/-	66.6/-	nm
Balupuri et al <sup>28</sup>	28/nm	3.57/-	91.6/-	nm
Metcalfe et al <sup>29</sup>	72/192	7/4(ns)	80/19(s)	24/31(ns)
Gok et al <sup>30</sup>	43/nm	-	-	-
Gerstenkorn et al <sup>31</sup>	202/nm	21.8/-	82.3/-	13.1/-
Gok et al <sup>32</sup>	46/46	8.7/2.2	94.9/41(s)	52.2/41.3(ns)
Sudhindran et al <sup>23</sup>	42/84	0/2	50/17(s)	33.3/40.5(ns)
Gok et al <sup>33</sup>	25/nm	2/nm	75/nm	-
Brook et al <sup>34</sup>	55/69	5/3	93/17	24/23(ns)
Gerstenkorn et al <sup>35</sup>	41/0	14.6	80	-
Gok et al <sup>36</sup>	72/nm	7.85/-	75.6/-	41.4/-
Brook et al <sup>37</sup>	285/0	15	79.7	41
Gok et al <sup>38</sup>	2/0	0/0	100/0	100/0
Wilson et al <sup>39</sup>	51/nm	2/nm	7/nm	-
Bains et al <sup>40</sup>	37/75	0/0	31/8(s)	7/27(ns)
Navarro et al <sup>41</sup>	81/-	nm	nm	nm
Gok et al <sup>42</sup>	19/15	nm	57.9/45.5(s)	36.8/20(s)
Navarro et al <sup>43</sup>	5/-	nm	nm	nm
Sohrabi et al <sup>44</sup>	5/-	0/-	80/-	nm
Sohrabi et al <sup>45</sup>	36/-	6.3/-	nm	35/-
Muiesan et al <sup>46</sup>	07/0	0	0	3

s-significant; ns- not statistically significant; nm- not mentioned; nhbd-non-heart-beating donor;hbd-heart-beating donor; pnf-primary non-function;dgf-delayed graft function;ar-acute rejection.

**Table 8** - Graft survival in NHBD in England.

		1 y(%)	2 y(%)	3 y(%)	5 y(%)
Nicholson et al <sup>25</sup>	NHBD	78	75	73	-
	HBD	90	85	82	-
Butterworth et al <sup>26</sup>	NHBD	73	73	73	-
	HBD	83**	79**	77**	-
Nicholson et al <sup>27</sup>	NHBD	85**	87**	87**	79**
	HBD	85	82	80	75
Balupuri et al <sup>21</sup>	NHBD	91.7	-	-	-
Balupuri et al <sup>28</sup>	NHBD	88.1	-	-	-
Metcalfe et al <sup>29</sup>	NHBD	81	-	77	73
	HBD	86**	-	78**	65**
Gok et al <sup>30</sup>	NHBD	89.6	-	-	-
	HBD	91.4**	-	-	-
Gerstenkorn et al <sup>31</sup>	NHBD	86.9	-	75.5	65.5
Gerstenkorn et al <sup>35</sup>	NHBD	-	-	82.9	-
Brook et al <sup>34</sup>	NHBD	79	-	75	69
Gok et al <sup>32</sup>	NHBD	89.6	-	89.6	-
	HBD	91.4**	-	91.4**	-
Sudhindran et al <sup>23</sup>	NHBD	84	-	80	74
	HBD	89**	-	85**	80**
Brook et al <sup>37</sup>	NHBD	88	-	84	84
	HBD	82**	-	73*	62*
Gok et al <sup>36</sup>	NHBD	92.1	-	-	-
Sohrabi et al <sup>45</sup>	NHBD	-	-	95	-
Muiesan et al <sup>46</sup>	NHBD	84	-	-	-

\*\* not statistically significant; \* statistically significant;nhbd-non-heart-beating donor;hbd-heart-beating donor; y-year.

**Table 9** - Patient survival in NHBD in England.

		1 y(%)	3 y (%)	5 y(%)
Gok et al <sup>30</sup>	NHBD	87.9	87.9	-
	HBD	89.7	89.7	-
Gerstenkorn et al <sup>31</sup>	NHBD	93.1	85.3	76.2
Sudhindran et al <sup>23</sup>	NHBD	91	91	84
	HBD	94	92	90
Gok et al <sup>36</sup>	NHBD	92.1	-	-
Muiesan et al <sup>46</sup>	NHBD	87	-	-

nhbd-non-heart-beating donor;hbd-heart-beating donor;y-year.

small proportion of the total transplant activity on the UK<sup>53</sup>.

The average NHBD retrieval rate for 2001 was 1.3 million population (pmp) for the four centres reported here. This compares with an average national rate for HBD kidneys of 23.5 pmp (United Kingdom Transplant Data<sup>54</sup>. Although the number of NHBD transplants is small the potential is greater; the most encouraging figures come from Daemen et al<sup>(55)</sup> who report 40% of their kidneys were accounted for by NHBDs.

Varty et al<sup>49</sup> reported 38% of donors were NHBD in 1991 and later Nicholson<sup>56</sup> reported that NHBD accounted for 21% of transplant activity. Light et al<sup>57</sup> stated that the number of NHBD transplant opportunities equals that of HBD whilst other authors have suggested that there are

**Table 10** - England centres and variations of non-heart-beating donors programmes.

Centre	NHBD technique	In situ Perfusion	Immunossuppression
Cambridge	Laparotomy + aortic cannula	UW	CSA/AZA/PRED
Leicester	Femoral arteriotomy + DBTL catheter	Marshall's	CSA/AZA/PRED or TAC/MMF/PRED
London	Kidney-femoral arteriotomy +DBTL	Marshall's	CSA/AZA/PRED
	Liver- PV cannulation	UW	TAC/PRED
Newcastle upon-Tyne	Femoral arteriotomy + DBTL catheter + MP system	Marshall's	Ab induction; TAC/MMF/PRED

UW-University of Wisconsin; DBTL – double-balloon triple-lumen; SKPS-Soltran kidney perfusion solution; PV- portal vein; MP – machine perfusion; CSA-cyclosporine(7mg/Kg/d); TAC-tacrolimus (0.1mg/kg/d); AZA-azathioprine(1mg/kg/d);PRED – prednisolone(20mg/d reducing to 5 mg/d at 6 months); MMF – mycophenolate mofetil (2g b.i.d.); Ab – interleukin-2 receptor antibody.

twice as many potential NHBD as there are HBD<sup>58</sup>. It has been suggested that if all potential NHBD kidneys were retrieved waiting lists for kidney transplantation could be eliminated<sup>59</sup>.

Two methods of harvesting of NHBDs have been advocated to decrease ischemic insult: 1. procurement methods include *in situ* intravascular cooling (universal), intra-peritoneal lavage and cooling (used in the United States), hypothermic and normothermic partial cardiopulmonary bypass (used in Spain) and hypothermic extracorporeal membrane oxygenation (used in Taiwan); and 2. machine preservation methods include: pulsatile intermittent perfusion and continuous-and-constant perfusion.

Ischemic injury is almost inevitable in NHBD renal grafts and primarily results from prolonged hypotension, cardiac arrest and warm ischemia during the primary warm ischaemia time (WIT). Development of allograft dysfunction can result in DGF and PNF.

As far as the NHBD for liver transplantation is concern Muiesan et al<sup>46</sup> based their donor selection on several factors including age less than 40 years, short intensive care unit stay, normal liver function tests, short interval from withdraw of therapy to cardiac arrest, short WIT and good appearance and perfusion of the liver, as judged by an experienced transplant surgeon. When the majority of these parameters were satisfied they provided organs suitable for bench segmental reduction.

They started the procedure of reduction/splitting as soon as the recovery team had returned to their centre. Care was taken to keep the temperature of the University of Wisconsin (UW) solution at 4 C and to avoid re-warming of the graft. One split procedure was performed. The left lateral segment was transplanted with 7 h of cold ischemia and provided excellent immediate function with an International Normalized Ratio of 1.2 on the second post-operative day. The adult recipient who received the right lobe split was transplanted sequentially with a cold ischemia time of 14.3 h and experienced PNF. He died of multi-organ failure after emergency re-transplantation. Subsequent reduction of NHBD grafts was performed with transplantation only of

the left lateral segment or left lobe in children<sup>46</sup>.

The recent largest retrospective study comparing outcomes of adults recipients of NHBD and HBD hepatic allografts between 1993 and 2001 based on the United Network of Organ Sharing database confirmed their greater vulnerability to cold ischemia and the higher incidence of PNF among a group of 144 NHBD livers<sup>60</sup>.

Abt et al<sup>61</sup> have emphasized the importance of short cold ischemic time for liver grafts. When the cold ischemic time was less than 8 h there was 10.8% of graft failure within 60 days of transplantation which increased to 30.4% and 58.3% when the cold ischemia time was greater than 8 and 12 h, respectively. The combination of warm and cold ischemia in NHBDs appears to make these grafts more susceptible to biliary complications. A higher incidence of ischemic cholangiopathy has been reported<sup>61,62</sup>.

D'Alessandro et al<sup>63</sup> also recognized increased incidence of ischaemic biliary complications in NHBD liver graft recipients from donors older than 40 years of age.

To date there have been no vascular complications and no evidence of major biliary complications with particular reference to ischaemic cholangiopathy in these children after a mean follow-up of 19 months<sup>46</sup>.

Reddy et al<sup>64</sup> described various cytoprotective strategies involving administration of drugs before cardiac arrest that have been successfully used in NHBD liver transplantation.

Kidneys from different Maastricht categories recovered at different rates although they were all similar at 3 months. Kidneys from NHBDs have undergone more ischemic insult than kidneys from HBDs. This occurs because of the prolonged early (primary) warm ischemia.

For Maastricht category II donors this the period between collapse and effective resuscitation, variably effective resuscitation and the “no-touch” period before perfusion.

For category III donors after withdrawal of support there can be an agonal period of hypotension followed by the “no-touch” period and then perfusion.

Category IV donors are known to be brainstem dead and are the closest to HBDs. They often arrest in theatre, there-

fore the primary ischemic time does not include the “no-touch” period. Thus the primary warm ischemic insult is likely to be maximal for category II and minimal for IV with III in-between (II greater than III greater than IV)<sup>36</sup>.

With maximal primary warm ischemic damage comes the increasing chance of *in situ* thrombosis, greater oxygen debt, and increasing acidosis and reduction of cellular adenosine triphosphate. Viability testing excludes those organs that are so badly damaged they are not likely to function. As a consequence the prolonged warm ischemia and reperfusion syndrome is likely to be more severe for kidneys from category II donors as opposed to III or IV.

A contrary argument has been put forward that a person who is hospitalized and in an intensive therapy unit for a protracted period is more likely to have inotropic support and sepsis which may result in unmeasured hypoperfusion of kidneys. Therefore, kidneys from a sudden collapse (category II) are possibly healthier than those from a hospitalized patient<sup>8</sup>.

Gok et al<sup>36</sup> demonstrated that the PNF rate is slightly higher for category II donors (13.5%) than category III (2.2%) and category IV (0%,  $p < 0.05$ ). In addition DGF incidence is greatest for category II donors (83.8%), less for category III (67.4%) and best for category IV (0%).

The introduction of pulsatile hypothermic machine-perfusion of NHBD kidneys, along with viability testing has resulted in a viable grafts acceptable survival rates with DGF in recipients. The main advantages of machine perfusion are the provision of metabolic support via replenishment of ATP by high energy yielding phosphate bonds from the perfusate (UW solution). Apart from the mechanical flush provided by continuous perfusion, the natural ischemia-induced capillary vasoconstriction is reversed as demonstrated by the reduction of intra-renal vascular resistance. This in turn is reflected in lower incidence of delayed graft function. Machine perfusion also allows monitoring and assessment of viability in an NHBD kidney graft.

Opelz and Terasaki concluded no added advantage of machine perfusion in HBD<sup>65</sup>. Machine perfusion fell into disrepute due to its complex logistics needs<sup>66,67,68</sup>. It was also reported in some cases that machine-perfusion was damaging to the graft<sup>69</sup>.

Renewed interest in this method of preservation came about through the use of marginal organs and improved preservative solutions (UW). With the improved solutions, the incidence of DGF could be reduced using kidneys from HBDs or those with prolonged cold ischaemia<sup>70,71</sup>.

Increased demand for viable organs has led to a recent upsurge in retrieval from the NHBDs. The difficulty experienced here was the viability of such kidneys. As the primary warm ischaemia times are prolonged with such do-

nors, viability assessment and organ modulation have been done by machine perfusing the NHBD kidneys before transplantation<sup>72,73,74,75</sup>.

The measurement of intra-renal vascular resistance and alpha GST is practiced by the Maastricht group, who have increased their donor pool by 20%<sup>76</sup>. Most of the studies have demonstrated a beneficial effect of pulsatile perfusion in NHBD kidneys<sup>77,78</sup>. Machine perfusion has been shown to improve the graft function in cases of marginal kidneys as well as those with prolonged cold ischaemic times<sup>78</sup>.

In the setting of NHBD kidney transplantation, PNF represents the transplant of an organ irreparably damaged by warm ischemia. WIT correlates well with graft damage and there is a point at which organs become non-viable. There is no strict maximum WIT in NHBDs beyond which transplantation is contraindicated. Limits of 30 min<sup>79</sup>, 35 min<sup>25</sup> and 45 min<sup>80</sup> have been advocated. Functional recovery in animal models has been achieved after substantially longer periods of 120 min<sup>79</sup> and 140 min<sup>81</sup>.

Renal transplants with prolonged WIT, that is primary warm ischemia, demonstrated a relative increase in free radical generation during NHBD renal transplantation. Use of traditional tissue injury markers LDH, AST and lactate and the specific markers of tissue injury Ala-AP and FABP during kidney transplantation complemented the finding of free radical injury in NHBD renal transplants. Combined markers enabled the monitoring of different types of cell injury. This correlates with the high incidence of acute tubular necrosis and subsequent DGF in NHBD renal transplants.

Reperfusion injury is such that antioxidant strategies may be of benefit. Potential measures that could be used are nitric oxide addition<sup>82</sup> and free radical scavengers (eg allopurinol, superoxide dismutase, catalase and dimethyl sulfoxide) with antioxidant supplementation (eg glutathione, vitamin E and ginkgo biloba)<sup>83,84</sup>, cytokine-chemokine suppressors, adhesion molecule blockers<sup>85,86</sup> and neutrophil-endothelial cell blockade<sup>87,88</sup>.

The role of free radicals in the pathophysiology of ischaemia-reperfusion injury in transplantation is increasingly recognized. Free radical formation appears to occur in 2 phases, characterized as reperfusion mediated injury (early and short-lived, that is seconds to minutes) and neutrophil mediated injury (late and long-lived, that is minutes to an hour). In reperfusion mediated injury adenosine triphosphate depletion during hypoxia results in the accumulation of hypoxanthine which is converted to free radicals (superoxide and hydrogen peroxide radicals) upon reperfusion by xanthine oxidase activation<sup>89</sup>. In neutrophil mediated injury reperfusion results in the activation of neutrophil NADPH oxidase and the release of further free radi-



cals (superoxide, hydrogen peroxide and hydroxyl radicals)<sup>90</sup>.

In kidney allograft free radicals can be formed throughout the renal parenchyma in the intracellular, intravascular and injury compartments. Ischaemia-reperfusion injury consist of 2 injury mechanisms namely ischemia characterized as oncotic necrosis and reperfusion injury. In the clinical setting this damage may delay the recovery of renal function prolong or complicate postoperative recovery or precipitate immunological reactions involved in the subsequent rejection reaction<sup>42</sup>.

In practice allowable maximum WIT varies in a qualitative manner i.e. a young and previously fit donor may be allowed a longer WIT than an older donor. The PNF average rate in the current study was 6.17% which is consistent with the 8 – 15 % reported previously from NHBDs<sup>55,91</sup>. This is higher than 2-5% quoted for HBD kidneys<sup>26,92</sup>. Shiroki et al<sup>93</sup> claimed that PNF rate is highest in those NHBD kidneys with warm time of > 30 min. Tanabe et al<sup>94</sup> disagreed stating that warm time bore no relationship to PNF rate but no WIT was > 30 min in their study. Brook et al<sup>5</sup> showed no episode of PNF in controlled donors with a 20% PNF rate in uncontrolled NHBD kidneys, indicating an association of warm time with PNF.

The reported incidence of PNF after transplantation using kidney from NHB donors varies from 4 to 40%, although most studies do not make distinction between kidneys from controlled and non-controlled NHB donors when describing the incidence of PNF<sup>22,27,58,75,91,94-99</sup>. The PNF is probably a consequence of ischaemic cortical necrosis<sup>5</sup>.

The perceived risk of PNF and poor graft outcome has limited the adoption of NHB donor programmes to a relatively small number of transplant centres. Some of these have used machine perfusion preservation to try and reduce the risk of PNF, although direct evidence as to its effectiveness is lacking<sup>48</sup>.

In Newcastle upon-Tyne the introduction of machine perfusion coincided with a fall in the incidence of PNF from 54 to 7%<sup>21,22</sup>. This most probably reflects the ability to perform viability testing and in Newcastle and other centres, kidneys are discarded if during machine perfusion intra-renal resistance measurements or levels of S-transferase are abnormal<sup>21,22,74</sup>.

DGF occurs more frequently in NHBD (50-100%)<sup>92,94</sup> than in HBD (20-60%) kidneys<sup>57,91,100</sup>. This increased DGF rate in NHBD kidney is likely to be a consequence of acute tubular necrosis (ATN) secondary to warm ischaemic damage. Not all DGF is due to ATN, although ATN is the most common cause and it is not therefore surprising that NHBD kidneys have a higher incidence of ATN than those from HBD<sup>101</sup>.

Brook et al<sup>37</sup> showed in controlled donors 48% of DGF in both NHBD and HBDs whilst the uncontrolled donors had a significantly higher rate ((88%). It is important to note that warm time is not the only variable that influences DGF; duration of pre-transplant dialysis and recipient body weight also correlate with post-transplant early graft function<sup>102</sup>.

The influence of DGF on kidney allograft survival is controversial. A number of studies have demonstrated poorer survival in HBDs with DGF<sup>103,104,105</sup> compared with HBDs with immediate function, while others authors have reported no such effect, independent of acute rejection<sup>106,107</sup>.

Brook et al<sup>37</sup> demonstrate that serum creatinine is stable in recipients of kidneys from NHBDs from 3 months to 7 yr post-transplant. There is disagreement in the literature over NHBD post-transplant renal function. In some studies NHBD kidneys achieved early serum creatinine levels in the normal range<sup>26,49,58,108,109</sup> whilst others studies reported poor graft function than HBD in both the short and medium term<sup>73,91</sup>.

NHBDs kidneys in the medium term achieve a good level of renal function with a mean serum creatinine at 12 months of 174  $\mu\text{mol/L}$ <sup>76</sup> and a median of 199  $\mu\text{mol/L}$  at 18 months<sup>49</sup>. So far, there is no evidence of accelerated deterioration of NHBDs kidneys because of a reduced functioning glomerular mass due to initial ischaemic damage.

Acute rejection is thought to occur more frequently and with greater severity in kidneys with prolonged ischaemia and DGF<sup>110</sup>. This may be a result of the increased rate of detection of sub-clinical rejection because biopsies are taken more frequently in the presence of DGF<sup>111</sup>.

Brook et al<sup>34</sup>, demonstrated for the first time that the high rate of DGF associated with renal transplantation from NHBDs does not lead to poor graft survival when compared with HBDs with DGF.

In recent years there has been a re-evaluation of the use of NHBDs for renal transplantation. While some studies have shown poorer graft survival for NHBD kidneys<sup>103,105</sup>, others have demonstrated favourable graft survival compared with HBDs<sup>25,91,112,113</sup>. This is despite the detrimental effects of warm ischaemic damage in NHBDs with consequent high rates of DGF.

Brook et al<sup>37</sup> reported that despite the detrimental effects of long WIT in NHBD with consequent high rates of PNF and DGF, there have been favourable comparisons with HBD in terms of graft survival also demonstrating that NHBD kidneys display parity with HBD meeting the British Transplant Society guidelines<sup>114</sup> for HBD allograft survival at 1 and 5 yr of 80 and 60% respectively.

It would seem that long ischaemic time causes reversible graft problems at an early stage in terms of PNF and

DGF. Later NHBD grafts appear to perform at least as well as those from HBDs.

Finally, as previously published there are many ways to make transplantation demand compatible with organ supply<sup>115,116,117,118,119,120</sup>. We have always to bear in mind that the gap between demand and supply of organs for transplantation continues to grow. One solution to this problem has been to return to the practice of using grafts from NHBDs. The challenge of NHBD transplantation is to minimize the first period of warm ischaemia and the consequent reperfusion injury.

## CONCLUSION

NHBDs kidney transplants are associated with allograft dysfunction as PNF and DGF which is related to primary warm ischaemic injury. This warm ischaemia is more deleterious in uncontrolled than controlled NHBDs.

Kidney transplant from NHBDs can be performed successfully. The significant degree of warm ischemic injury suffered by NHBD kidneys leads to a high incidence of DGF but the data available so far suggest that this does not adversely influence long-term graft survival.

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