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REALITIES AND PERSPECTIVES

Complex situations: the example of renal transplantation

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Notes are linked to the references page.

Transplantation is an especially complex situation because it involves a donor, a recipient, and an organ. The donor organ may already be damaged because of the donor's age, comorbidities (especially cardiovascular), and stress related to brain death and to resuscitation. Ischaemia–reperfusion injury, which varies in severity according to the type of donor and the conditions of graft preservation, can occur at every step in the transplant process, from massive cerebral ischaemia leading to brain death to revascularisation of the ischaemic organ. Finally, recipient factors can also contribute to graft dysfunction.

FACTORS INFLUENCING GRAFT SURVIVAL

Graft survival according to donor factors

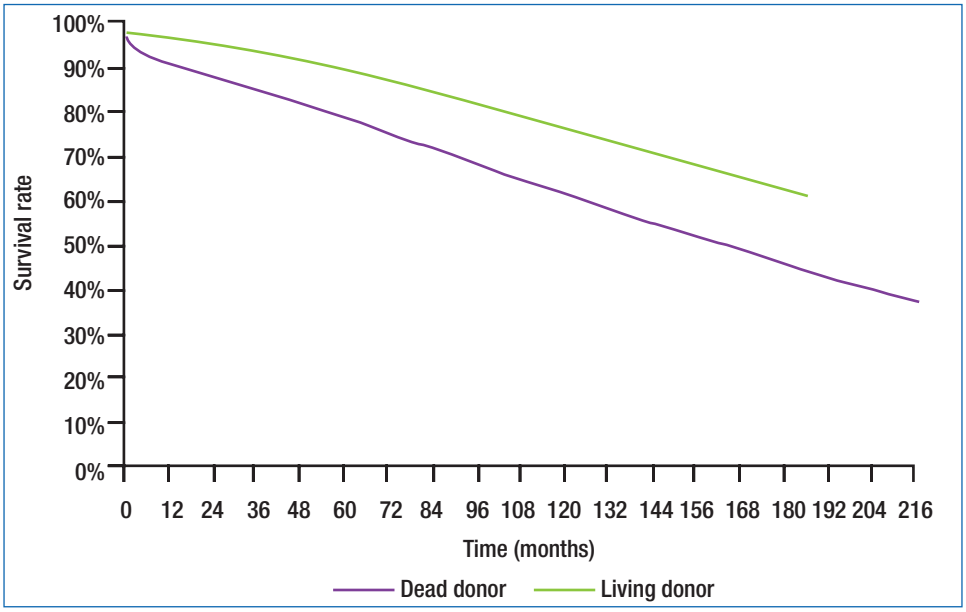
Donor organs are harvested from living donors, brain-dead donors or cardiac death donors.

- Living donors are relatively rare (comprising 16% of kidney donors in France in 2014)[1], but these donor organs are the most satisfactory owing to the good overall health of the donor, the absence of problems with graft preservation, and transplantation procedures that are well codified. However, recent data question the long-term outcome of grafts from live donors.
- Brain-dead donors are more numerous (82% in 2014)[1]. 'Standard criteria' donors, i.e. younger than 55 years and without associated comorbidities, can be distinguished from 'expanded criteria' donors who are over 55 years and/or with cardiovascular comorbidities. The latter, recruited to alleviate the shortage of donor organs, accounted for 2.2% of all organ donations in 1996 and account for 30% now. Such progression is far from benign because age-related organ damage and donor comorbidities related to the expanded criteria could lower organ quality.
- Cardiac death donors are generally people under the age of 50 years who died from a cardiac cause, without a cardiac history. These donors are classified according to the so-called Maastricht classification: categories I and II include death from unexpected cardiac arrest (uncontrolled cardiac arrest) and category III comprises donors who died from circulatory arrest following limitation or withdrawal of life-sustaining therapy (controlled cardiac arrest).

Survival rates of organ grafts from living donors are markedly higher than from deceased donors (see Figure 1A) and survival rates of organ grafts from standard criteria brain-dead donors are far higher than from expanded criteria donors[1]. Rather unexpectedly, survival rates of grafts from cardiac death donors are quite similar to those of standard criteria brain-dead donors (see Figure 1B).[1]

Figure 1. Graft survival according to type of donor[1]

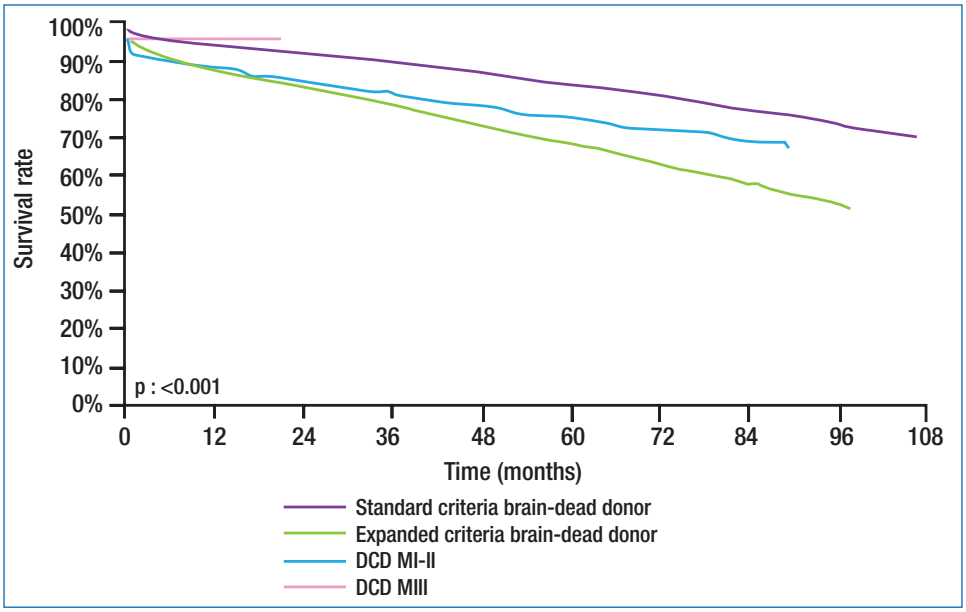
A.



Donor type	N	Survival at 1 month	Survival at 1 year	Survival at 5 years	Survival at 10 years	Survival at 15 years	Median survival (months)
Dead donor	50924	95.8% [95.6% - 95.9%]	90.9% [90.7% - 91.2%]	78.6% [78.2% - 79.0%]	61.3% [60.8% - 61.8%]	45.5% [44.9% - 46.2%]	163.2 [160.8 - 165.7]
Number of subjects at risk*		48386	44662	29675	14615	5888	
Living donor		97.9% [97.5% - 98.3%]	96.6% [96.0% - 97.1%]	89.6% [88.5% -90.6%]	76.5% [74.6% - 90.6%]	62.8% [59.8% - 65.6%]	NO
Number of subjects at risk*		4394	4051	2189	2189	269	

[ ] : Confidence interval
NO : non observable
* : Number of patients remaining to be observed at each time point and for whom no event has occurred
Data extracted from CRISTAL,02/03/2017

B.



Donor type	N	Survival at 1 month	Survival at 1 year	Survival at 5 years	Survival at 10 years	Survival at 15 years	Median survival (months)
Standard criteria brain-dead donor	13241	96,9% [96.6% - 97.2%]	94,3% [93.9% - 94.7%]	84,3% [83.6% - 85.0%]	NO	NO	NO
Number of subjects at risk*		12642	11634	5551	0	0	0
Expanded criteria brain-dead donor	10443	94.6% [94.2% - 95.1%]	87.5% [86.9% - 88.2%]	68.3% [67.2% - 69.4%]	NO	NO	NO
Number of subjects at risk*		9725	8454	3093	0	0	0
DCD MI-II	584	91.9% [89.3% - 93.8%]	88.0% [85.1% - 90.4%]	75.2% [71.0% - 78.9%]	NO	NO	NO
Number of subjects at risk*		530	486	195	0	0	0
DCD MIII	27	95.8% [73.9% - 99.4%]	95.8% [73.9% - 99.4%]	NO	NO	NO	NO
Number of subjects at risk*		21	9	0	0	0	

[ ] : Confidence interval
NO : non observable
* : Number of patients remaining to be observed at each time point and for whom no event has occurred
DCD MI-II : Death from unexplained cardiac arrest (Maastricht criteria I and II)
DCD MIII : Death from cardiac arrest after limitation or withdrawal of life-sustaining therapy (Maastricht criteria III)
Data extracted from CRISTAL, 02/03/2017

Ischaemia–reperfusion injury

All transplanted organs are exposed to a risk of ischaemia–reperfusion injury, which can result in a reduction of long-term graft survival.

Impact of cerebral ischaemia resulting in brain death

Massive cerebral ischaemia resulting in brain death causes a major inflammatory reaction in the kidney graft, leading to microvascular lesions and tubular damage. Microvascular lesions interfere with perfusion and increase cell adhesion and microvascular permeability, which, by sustaining an inflammatory state, results in persistent ischaemia. Depending on the duration of ischaemia, tubular lesions, especially of the proximal tubule, can become necrotic and impair glomerular filtration.

We will focus more specifically on the consequences of oxygen supply on mitochondrial metabolism. The lack of oxygen induces a deceleration of oxidative phosphorylation and adenosine triphosphate (ATP) production which prevents ATP-dependent transporters from functioning, thus disrupting cellular ion homeostasis and promoting acidosis, cell distention and cytoskeleton disorganisation. In parallel, the increase in intracellular calcium levels leads to activation of calcium-dependent enzymes, such as phospholipases involved in proinflammatory processes, or the calcium-dependent protease, calpain, which disrupts the cytoskeletal structure.

Depending on the duration of the ischaemic period, these processes lead to reversible mechanisms such as endothelial activation or irreversible outcomes such as cell death by necrosis or apoptosis.

Impact of hypothermia during graft preservation

To date, the most widely used method to preserve donor organs after procurement is static preservation by simple cold storage at 4°C. The principle is based on two laws of thermodynamics: the first is that metabolic activity is decreased by 50% with each 10°C drop in temperature (i.e. metabolic activity is reduced to 15% at 4°C), and the second is that low temperature slows the rate and yield of enzymatic reactions.

While reduced metabolic demand is indeed the expected effect of hypothermia, it also has deleterious effects by causing:
- disruption of the secondary, tertiary and quaternary structure of proteins, altering their functions;
- disorganisation of lipid chain alignments, opening of passive ion channels, structural disordering of membrane proteins, and loss of ion channel tightness.

This results in ATP depletion, reduced reaction rates and yields, ion disequilibrium (fall in membrane potential), calcium influx (induction of cell death-signaling pathways, impact on membranes), acidosis and cell distention. All of these effects raise the question of the relevance of the choice, totally empirical, of a temperature of 4°C for organ preservation.

Hypoxia and hypoxia-inducible factor in the early phase

Hypoxia inducible factor 1α (HIF-1α) is a sensor of oxygen deprivation. In oxygenated environments, it is hydroxylated by prolyl-hydroxylase. As it is no longer hydroxylated in a setting of hypoxic stress, it stimulates erythropoiesis (by erythropoietin release), angiogenesis (by vascular endothelial growth factor, VEGF) and anaerobic glycolysis. HIF-1α is therefore a key modulator of short-term adaptation to hypoxic conditions[2].

With respect to long-term outcomes, we compared two kidney graft protocols in a porcine autotransplantation model: standard 24-hour cold storage at 4°C and 24-hour cold storage preceded by 1 hour of warm ischaemia at 37°C (achieved by clamping the renal pedicle)[3].

During the first week of reperfusion, grafts exposed to warm ischaemia plus cold storage grafts showed a higher degree of injury. Tubular dedifferentiation was associated with delayed HIF-1α expression and loss of its role in transcription. In highly injured kidneys, deregulation of the HIF-1α pathway was also observed in the chronic phase, with reduced production of VEGF A and upregulation of VEGF receptor 1 and thrombospondin 1. The kidneys showed altered renal histology and decreased function. HIF-1α was therefore present in the underperfused tissues because of the endothelial injury, but it did not activate the system of defence against this hypoxia.

Protein families modified by cold hypoxia

We identified the main protein families modified by cold ischaemia in a human endothelial cell model after 24-hour cold storage at 4°C. We found early changes in the expression of cytoskeletal proteins and proteins involved in transcription, transregulation, intracellular transport and energy metabolism, corresponding to an adaptive response in the first few hours, that was no longer apparent after 12 hours.

A similar study on the transcriptome in our porcine renal graft model with 30-minute warm ischaemia followed by 24-hour cold storage identified 43 genes that were upregulated or downregulated in ischaemic versus normal kidney (log2, $P<0.05$). After enrichment of the storage solution, 82 genes involved in inflammation, cell adhesion, metabolism, etc. were found to be up or downregulated.

Endoplasmic reticulum stress

The endoplasmic reticulum (ER) degrades misfolded proteins. ER stress is caused by an accumulation of misfolded or unfolded proteins in the ER lumen and elicits an adaptive response known as the unfolded protein response (UPR). If this accumulation of misfolded or unfolded proteins persists, prolonged UPR signaling directs the cell towards programmed cell death. Experimental data suggest that ER stress is involved in the development of tissue injury in solid organ transplantation.

Three stress-sensing proteins are involved in the UPR signaling pathway: PERK (protein kinase RNA-like endoplasmic reticulum kinase), IRE1 (inositol-requiring enzyme 1) and ATF6 (activating transcription factor 6). Accumulation of misfolded proteins causes activation of IRE1 and transcription of the transcription factor Xbp1 (X-box binding protein 1), PERK, ATF4 (activating transcription factor 4) and ATF6, its transport into the Golgi apparatus.

We have used pharmacological agents or RNA silencing (siRNA) to modulate these pathways in a human endothelial cell model. We found that inhibition of ATF4 (expressed in early ischaemia), ATF6 (expressed after about 24 hours of ischaemia) and Xbp1 has a protective effect and enhances cell survival during reperfusion.

Consequences of reperfusion

During organ transplantation, reperfusion following the re-establishment of blood supply to the ischaemic organ results in sudden re-oxygenation, which triggers an oxidative stress response with production of reactive oxygen species towards all the major classes of biological molecules. This process is responsible for cell injury leading to cell death.

In addition, endothelial activation induced by reperfusion causes a pro-aggregating state, which impedes reperfusion in the ischaemic territory and causes hypoxia.

Finally, endothelial activation increases during early reperfusion and leads to the recruitment of inflammatory cells, mainly monocytes and neutrophils. This innate immunity is dependent on damage-associated molecular patterns, alarmins that can activate Toll-like receptors and stimulate the inflammatory process. Adaptive immunity is also activated secondarily by the recruitment of T-lymphocytes.

MECHANISMS OF CHRONIC INJURY

The purely immunological vision of graft rejection is now being called into question because even though immunological factors play a role (poor matching, human leukocyte antigen (HLA) pre-immunisation, delayed graft function, inappropriate immunosuppression, poor compliance), graft survival appears to be related mainly to non-immunological factors: donor age, poor quality graft, brain death, preservation damage, ischaemic injury, recipient comorbidities (hypertension, dyslipidemia) and immunosuppressant toxicity. Grafts from marginal donors already contain lesions, so the progression to chronic injury can be rapid, especially when the initial reserve of nephrons is compromised.

PERSONALISATION OF ORGAN TRANSPLANT MANAGEMENT

The main problem in deciding which graft preservation method to use concerns the evaluation of graft quality. Currently, creatinine remains our main biomarker but lacks specificity and sensitivity. Histology is essentially

a qualitative approach subject to wide inter-operator variability. When all is said and done, the clinician has no quantitative cut-off values to aid in decision-making.

Evaluation and prediction scores of long-term graft outcomes

Nevertheless, clinical scores have been introduced, although there is still no consensus, to estimate the risk of graft failure:

- The KDRI (kidney donor risk index) takes into account donor age, weight, height, ethnic background, creatinine, comorbidities (hypertension, diabetes), cause of death, and serology.
- The UK-KDRI (United Kingdom kidney donor risk index) takes into account donor age, history of hypertension, weight, length of hospital stay, use of adrenaline.

Histology scores, which are also not consensus-based, have also been developed:

- The kidney pathology score takes into account glomerulosclerosis, interstitial fibrosis, tubular atrophy and vascular lesions by distinguishing intimal fibrous thickening and arteriolar hyalinosis.
- The Maryland aggregate pathology index takes into account periglomerular fibrosis, glomerulosclerosis and arterial wall-to-lumen ratio.

Composite scores combining biological and histopathological criteria appear relevant but need to be validated in multicentre studies:

- The Necker composite score takes into account donor creatinine (≥1.7 mg/dL), hypertension and glomerulosclerosis (≥10%). The presence of two of these criteria is associated with a five-fold higher risk that the estimated glomerular filtration rate (eGFR) will be less than 25 mL/min. The presence of three of these criteria is associated with a 27-fold higher risk.
- The composite score developed by De Vusser's group takes into account donor age, glomerulosclerosis (≥10%) and interstitial fibrosis/tubular atrophy. Estimated 5-year graft survival is reduced by 20% for a score over 60.

CONCLUSION

The evaluation of donor organs has to be redesigned to enable effective precision medicine. It should incorporate the most comprehensive data possible on donor and recipient to establish the risk profile of the donor kidney and allow it to be directed safely towards transplantation, prior repair, or non-use.

Different techniques should help in this regard. Magnetic resonance imaging (MRI) can evaluate graft perfusion and provide the clinician with a set of markers of graft status in less than 2 hours. Multimodal assessment by MRI, nuclear magnetic resonance (NMR), spectroscopy, and other imaging modalities. allows an anatomical and functional evaluation of the graft. Combined with genomics, histology and relevant biomarkers, it could enable the development of much more effective algorithms than we currently have. Ideally, the goal would be to create perfusion laboratories, evaluation units that examine the donor organs and relay them to transplant centres or discard them in the case of poor quality.

There were more than 57,000 patients with a transplanted organ in 2015 and their number is bound to grow. Unfortunately, there is a glaring shortage of organs and compromises have to be made as to the choice of donors. Applying precision medicine to graft evaluation has the potential to significantly improve outcomes transplantation.

REFERENCES (Underlined references are linked to PubMed abstracts)

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