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The Neglectable Impact of Delayed Graft Function on Long-term Graft Survival in Kidneys Donated After Circulatory Death Associates With Superior Organ Resilience

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Objective: To explore putative different impacts of delayed graft function (DGF) on long-term graft survival in kidneys donated after brain death (DBD) and circulatory death (DCD).

Background: Despite a 3-fold higher incidence of DGF in DCD grafts, large studies show equivalent long-term graft survival for DBD and DCD grafts. This observation implies a differential impact of DGF on DBD and DCD graft survival. The contrasting impact is remarkable and yet unexplained.

Methods: The impact of DGF on DBD and DCD graft survival was evaluated in 6635 kidney transplants performed in The Netherlands. DGF severity and functional recovery dynamics were assessed for 599 kidney transplants performed at the Leiden Transplant Center. Immunohistochemical staining, gene expression profiling, and Ingenuity Pathway Analysis were used to identify differentially activated pathways in DBD and DCD grafts.

Results: While DGF severely impacted 10-year graft survival in DBD grafts (HR 1.67; $P < 0.001$), DGF did not impact graft survival in DCD grafts (HR 1.08; $P = 0.63$). Shorter dialysis periods and superior posttransplant eGFRs in DBD grafts show that the differential impact was not caused by a more severe DGF phenotype in DBD grafts. Immunohistochemical evaluation indicates that pathways associated with tissue resilience are present in kidney grafts. Molecular evaluation showed selective activation of resilience-associated pathways in DCD grafts.

Conclusions: This study shows an absent impact of DGF on long-term graft survival in DCD kidneys. Molecular evaluation suggests that the differential impact of DGF between DBD and DCD grafts relates to donor-type specific activation of resilience pathways in DCD grafts.

Keywords: delayed graft function, donation after brain death, donation after cardiac death, kidney transplantation, resilience

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In an era of severe donor organ shortage and growing waiting lists for renal transplantation there is an increased reliance on expanded criteria donors and organs donated after circulatory death (DCD). While DCD donor kidneys constitute a large potential donor pool, higher incidences of primary non function and particularly delayed graft function (DGF) are regarded as major impediments.

Notwithstanding the higher incidence of DGF in DCD compared to donated after brain death (DBD) grafts, large cohort studies from the United Kingdom and The Netherlands show equivalent survival for kidneys DBD and DCD grafts.[1–3] This observation suggests a differential impact of DGF on DBD and DCD graft survival.

The apparent differential impact of DGF on DBD and DCD graft survival is remarkable and yet unexplained. One possible explanation for this phenomenon is that the type of DGF in DBD grafts reflects more severe transplantation-related injury. An alternative and mutually nonexclusive explanation is that the differential impact reflects differences in graft ''resilience''—ie the ability of the graft to cope with negative environmental changes[4]—with DCD donor kidneys being more ''resilient'' than DBD grafts. Tissue resilience is an established phenomenon in cancer biology, and negatively associates with patient prognosis.[4] However, in the context of transplantation biology, resilience could be a beneficial factor potentially contributing to better transplantation outcomes.

Considering the emerging epidemiological evidence for a different impact of DGF on DBD and DCD graft survival and its clinical relevance, we have focused in this hypothesis generating study on this putative differential impact and also attempted to explore its biological basis.

METHODS

Study Population

The impact of DGF (defined as the need for dialysis in the first postoperative week(s)) on long-term graft survival was evaluated in 6635 deceased donor kidney transplants performed between January 2000 and January 2018 in the Netherlands (Netherlands Organ Transplant Registry (NOTR)). Combined organ procedures, procedures in recipients younger than 12 years and uncontrolled circulatory death donor procedures were excluded.

The impact of donor type on DGF phenotype and functional recovery dynamics was assessed for 287 DBD and 312 DCD kidney transplants performed at the Leiden University Transplant Center between 2007 and 2018. A more detailed description of the methods is given in the Supplemental Data, http://links.lww.com/SLA/B725.

The clinical nomenclature and different phases included in this paper are illustrated in Figure 1.

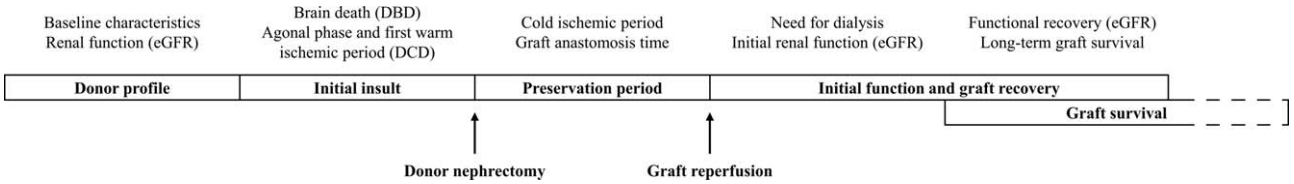

FIGURE 1. The clinical nomenclature and different phases included in this paper.

Histology and Gene Expression

Pre-reperfusion tissue biopsy samples from 80 donor kidneys were randomly selected based on donor type and the presence or absence of DGF (n = 20 per group, Supplemental Table 1, http://links.lww.com/SLA/B725). Immunohistochemical staining was performed for BCL2, IGF-1R, p53, PCNA, phospho-EGFR, phospho-MAPK14, phospho-mTOR, PPARγ. Details of the antibodies and procedures are summarized in the Supplemental Data, http://links.lww.com/SLA/B725 and Supplemental Table 2, http://links.lww.com/SLA/B725.

Gene expression profiling of 23 DBD and 16 DCD pre-reperfusion renal biopsies was followed by Ingenuity Pathway Analysis (IPA, QIAGEN, USA) to identify differentially regulated pathways (Supplemental Table 3, http://links.lww.com/SLA/B725).[5,6]

All renal biopsies used in this study were collected after static cold storage, and prior to reperfusion. Further details of the analyses are provided in the Supplemental Data, http://links.lww.com/SLA/B725.

Statistical Analysis

STATA/SE version 12.0 (StataCorp, Texas) and IBM SPSS Statistics 23.0 (Amsterdam, The Netherlands) were used for statistical analysis. Comparisons between groups were analyzed using standard statistical methods. Cox proportional hazards models, adjusted for donor/recipient age and sex, and cold ischemic period were used to evaluate differences in impact of DGF on 10-year graft survival. Univariate analysis was followed by multivariate regression analysis to identify factors associated with DGF. A detailed description of the statistical analysis is given in the Supplemental Data, http://links.lww.com/SLA/B725.

RESULTS

Epidemiological Evaluation

Putative differential impacts of DGF on DBD and DCD graft survival were evaluated in 6635 kidney transplants (43.6% DCD procedures) that were performed between 2000 and 2018 in The Netherlands (Supplemental Table 4, http://links.lww.com/SLA/B725). The registry data confirmed a higher incidence of DGF in DCD grafts (DCD: 42.2% vs. DBD: 17.8%; $P < 0.001$) but also showed differential impact of DGF on long-term graft survival per donor type. In fact, while DGF severely impacted 10-year graft survival in DBD donor kidneys [adjusted DGF-associated hazard ratio (aHR) for graft loss: 1.67 (95% CI 1.35–2.08); $P < 0.001$], no impact on survival was observed for DGF in DCD donor kidneys [aHR for graft loss: 1.08 (95% CI 0.82–1.39); $P = 0.63$]. Interaction testing confirmed the differential impact of DGF on DBD and DCD long-term graft survival (P for interaction < 0.001).

The differential impact of DGF on long-term graft survival may relate to a greater threshold to develop DGF in DBD grafts (ie, that development of DGF in DBD grafts requires a more severe insult). This hypothesis was tested by using a qualitative and quantitative evaluation of risk factors associated with DGF. An inventory of risk factors associated with occurrence of DGF (multivariate analyses) revealed clearly qualitative differences between the 2 donor types. The first warm ischemic period, a discriminant factor of DCD grafts, was positively associated with DGF in DCD grafts. Both donor types shared cold ischemic period as a risk factor for developing DGF. Donor age was a significant risk factor for DBD grafts, but an association with DGF in DCD grafts did not reach statistical significance ($P = 0.11$). The last serum creatinine value in the donor, human leukocyte antigen (HLA)-DR mismatch, and graft anastomosis time exclusively associated with DGF in DBD grafts but not in DCD grafts (Supplemental Table 5, http://links.lww.com/SLA/B725).

Quantitative analysis showed that DGF in recipients of DBD grafts was associated with a slightly unfavorable donor and procedural profile as reflected by the 2-year difference in donor age, higher donor serum creatinine concentrations, and 8% and 12% longer cold ischemic and graft anastomosis times (Table 1). However, this less favorable risk profile did not result in a more severe DGF phenotype in DBD grafts. On the contrary, recipients of DCD grafts with DGF required longer dialysis, and had profoundly inferior renal function (eGFR) in the first week following the last dialysis ($P < 0.001$) (Table 2).

The above results did not point to a more profound DGF phenotype as underlying cause of the negative impact of DGF on long-term graft survival in DBD grafts. Alternatively, the differing impact may reflect differential resilience between the 2 donor types, with DCD grafts being more resilient than DBD grafts. A concept that is supported by the superior functional (eGFR) recovery dynamics in DCD grafts (Fig. 2).

Histology and Gene Expression

To explore the presence of resilient enhancing factors, we mapped several molecular upstream regulators associated with resilience in the context of tumor biology (eg, p53, phospho-EGFR, IGF-1R, phospho-mTOR, phospho-MAPK14, PCNA, BCL2 and PPARγ).[7–13] The immunohistochemical analysis demonstrated

TABLE 1. Comparison of Risk Factors Associated With DGF in DBD and DCD Graft Recipients

	DBD DGF + n = 667	DCD DGF + n = 1219	*P* Value
Donor age (yrs)	52.1 (14.4)	50.2 (14.5)	0.006
Donor last creatinine (μmol/L)	77.0 [60.0–100.0]	68.0 [54.0–83.5]	<0.001
Mismatch HLA-DR			0.004
0	243 (36.5%)	362 (29.9%)	
1	360 (54.1%)	752 (62.0%)	
2	62 (9.3%)	98 (8.1%)	
Cold ischemic period (h)	18.4 [14.4–23.0]	17.0 [13.1–21.0]	<0.001
Graft anastomosis time (min)	35.0 [26.0–42.0]	31.0 [25.0–40.0]	<0.001

Data are presented as mean ± standard deviation (SD) or as number (%) or as median [25 and 75 IQR].

TABLE 2. DGF Phenotype in DBD and DCD Graft Recipients

	DBD DGF + n = 80	DCD DGF + n = 179	*P* Value
Duration of dialysis (d)	7.5 [5.0–12.0]	9.0 [6.0–13.8]	0.039
Number of dialysis	3.5 [3.0–5.8]	4.0 [3.0–6.0]	0.462
First autonomous eGFR	20.3 [14.4–35.7]	13.4 [9.3–22.8]	<0.001

Data are presented as median [25 and 75 IQR].

expression of the aforementioned resilience factors in pre-reperfusion kidney biopsies, indicating that aspects of the molecular mechanisms associated with tissue resilience are present in both donor types (Supplemental Figures 1, http://links.lww.com/SLA/B725 and 2, http://links.lww.com/SLA/B725).

With the aim of evaluating putative differential activation of molecular pathways associated with resilience in DBD and DCD grafts, an unbiased pathway analysis was performed on the gene expression profiles in pre-reperfusion kidney biopsies from DBD and DCD donors. There were no differences in baseline characteristics between DBD and DCD donors (Supplemental Table 3, http://links.lww.com/SLA/B725). Using DBD grafts as the comparator, 6 differentially activated ($P < 0.05$) upstream regulatory pathways, and 13 differentially inhibited regulatory pathways were identified in DCD grafts (Fig. 3). All upregulated pathways belonged to a family of factors responsible for renal development, cell fate, organogenesis, and stem cell maintenance. Pathways inhibited in DCD grafts included the p53 pathway, and a cluster of pro-inflammatory factors (IL6, TNFα, RANKL (TNFSF11), CEBPβ, TICAM1) (Fig. 3). Functionally, the strongest influence was found by pathways associated with cardio-vascular diseases (P value range 2.5×10^{-10} to 2.2×10^{-3}), in particular a gene cluster mapped by IPA as ''advanced stage peripheral artery disease'' (P value 2.5×10^{-10}). This cluster is dominated by upregulation of heat shock proteins (Supplemental Figure 3, http://links.lww.com/SLA/B725).

DISCUSSION

While a high incidence of DGF after DCD kidney transplantation is considered a major obstacle toward a more liberal use of these grafts, recent epidemiological observations suggest that this concern might be unjustified. This integrative epidemiological and molecular analysis has clearly shown a differential impact of DGF on DBD and DCD graft survival, with no impact of DGF on DCD graft survival. This finding may reflect a more favorable baseline molecular resilience signature in DCD donor kidneys.

Transplants procedures with DCD donor kidneys are associated with a twofold to threefold increased incidence of DGF.[2,3,14] DGF is an established risk factor for premature graft loss, and as such the higher incidence of DGF with DCD grafts is considered a relative contra-indication for the use of DCD grafts by some transplant centers. This notion has recently been challenged by cohort studies showing equivalent graft survival for DBD and DCD grafts despite the difference in incidence of DGF: an observation that implies a differential impact of DGF on DBD and DCD graft survival. In this context it should be noted that the conclusions regarding the negative association between DGF and long-term outcomes are mainly based on studies from an era with an almost exclusive use of DBD grafts.[15–19] Moreover, it cannot be excluded that conclusions for DCD grafts are confounded by factors that relate to both DGF and graft survival.

The differential impact of DGF on graft survival was confirmed by the outcome data for almost 6700 deceased donor kidney transplantations performed in The Netherlands, a country with a longstanding liberal tradition toward the use of DCD grafts (currently 50% of all deceased kidney transplantation procedures). While regression analysis confirmed the impact of DGF on long-term graft survival in DBD grafts, DGF did not affect graft survival in DCD grafts.

In an effort to understand the different impact of DGF on graft survival we first tested in this study whether the apparent impact on DBD grafts reflects the presence of a more severe DGF phenotype. This hypothesis was not supported by the clinical data. On the contrary, transplants with DCD grafts were hallmarked by a more

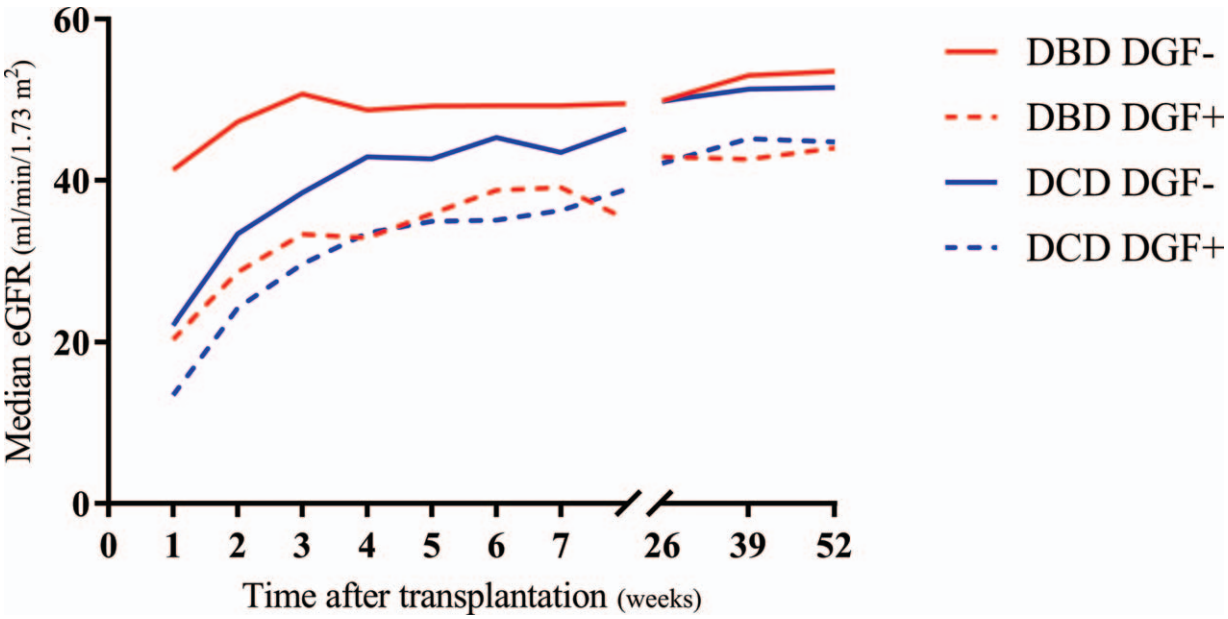

FIGURE 2. Functional renal recovery (eGFR) after kidney transplantation.

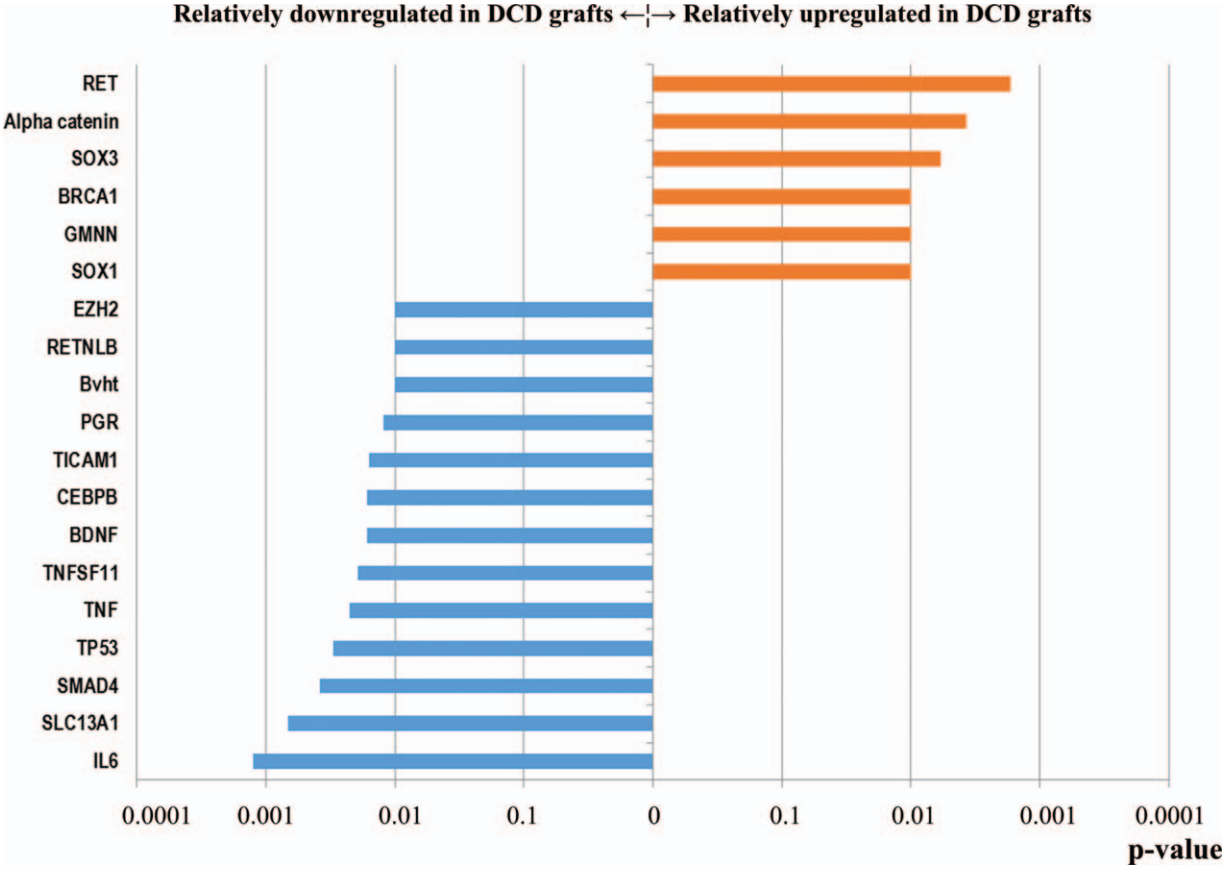

FIGURE 3. Differentially regulated upstream regulators in DBD and DCD donor kidneys based on Ingenuity Pathway Analysis (DBD is reference). BDNF (Brain-derived neurotrophic factor); BRCA1 (Breast cancer-associated gene 1); Bvht (Braveheart); CEBPB (CCAAT/Enhancer Binding Protein-β); EZH2 (Enhancer of zeste homolog 2); GMNN (Geminin); IL6 (Interleukin-6); PGR (Progesteron Receptor); RET (Rearranged during transfection); RETNLB (Resistin-like molecule β); SLC13A1 (Solute carrier family 13 member 1); SMAD4 (Sma (*Caenorhabditis elegans*) Mothers Against Decapentaplegia homologue 4); SOX1 (Sex determining region Y-box protein 1); SOX3 (Sex determining region Y-box protein 3); TICAM1 (Toll Like Receptor Adaptor Molecule 1); TNFα (Tumor Necrosis Factor α); TNFSF11 (Tumor Necrosis Factor ligand Superfamily member 11); TP53 (Tumor Protein p53).

severe graft injury as indicated by profoundly impaired posttransplant renal function (eGFR), and in case of DGF, a prolonged need for posttransplant dialysis. Irrespective of this, DCD grafts demonstrated an adequate functional recovery within weeks after transplantation, resulting in a renal function fully comparable to DBD grafts. The impact of DGF on ultimate eGFR was similar for DBD and DCD grafts. Thus, our clinical data do not support a more severe DGF phenotype as underlying cause of the negative impact of DGF in DBD grafts. In this light, we explored possible differences in graft resilience as an alternative explanation for the contrasting impact of DGF in DBD and DCD grafts.

Biologically, resilience is the ability of an organism to recover to normal functioning after perturbation.[20] In the context of ageing, resilience is the ability to cope with stress and re-establish homeostasis.[21] Tissue resilience is an established phenomenon in tumor biology, and a known negative prognostic factor.[4] In the context of organ transplantation, superior resilience would obviously be beneficial in terms of graft recovery and survival.

We applied gene expression profiling followed by pathway analysis to map putative molecular differences in organ resilience between DBD and DCD grafts. Pathways relatively enriched (n = 6) in DCD grafts were all part of established resilience networks. Five upregulated pathways in DCD grafts (RET, Alpha catenin, GMNN, SOX1, and SOX3) were associated with renal development and cell proliferation, and partly associate with the Wnt/β-catenin signaling pathways:[22–26] a pivotal pathway in kidney development, repair, and regeneration.[27–32] The sixth upregulated pathway was the BRCA1 tumor suppressor pathway. BRCA1 is a key player in cellular repair through its role in DNA repair and cell cycle checkpoint activation. This pathway was recently shown to be cardioprotective after myocardial infarction.[33] In contrast to the BRCA-1 tumor suppressor pathway, we observed down-regulation of the p53 network. While this downregulation is considered a negative aspect in tumor biology, it has been pointed out that downregulation of p53 is part of the normal, physiological regenerative response, and as such, could be part of an activated resilience network.[34]

Downregulated pathways in DCD grafts were dominated by pro-inflammatory signaling cascades (ie, IL6, TNFα, RANKL (TNFSF11), CEBPβ, TICAM1). This downregulation could be a consequence of an activated resilience network in DCD grafts. Other explanations included passive enrichment, reflecting differences in leucocyte influx (and thus genes associated with leucocytes) in DBD grafts,[35] as well as upregulation of parenchymal inflammation in response to brain death in DBD grafts.[36] It is unclear to what extent

the relative downregulation of inflammatory responses in DCD grafts contributes to the absent impact of DGF in these grafts. Although inflammation is often seen as a ''negative'' factor, experimental data suggests that brain death-associated immune activation may not accelerate ischemia reperfusion injury,[37] whereas other studies actually indicate aggravation of experimental ischemia reperfusion injury following interference with IL-6 or IL-9 signaling.[35,38]

A further observation is the downregulation of the BDNF signaling route in DCD grafts. Strong associations exist between BDNF and the kidney injury molecule (KIM-1), and BDNF has been recently proposed as a biomarker for glomerular injury.[39] As such, the relative downregulation of BDNF in DCD grafts might indicate that the glomerular injury is less in DCD than in DBD grafts.

On the functional level, the most influential transcriptomic signals were related to cardio-vascular diseases, in particular ''advanced stage peripheral artery disease.'' This cluster is mainly comprised of members of heat shock protein superfamily. Induction of heat shock proteins following ischemia has been well documented. In the context of brain ischemia this was correlated with the regions that ultimately survived the injury,[40] suggesting that this superfamily is part of a resilience response.

Since all renal biopsies in this study were from grafts that were maintained on static cold storage (hence a state of absent transcriptional activity), the clear differences in gene expression profiles probably reflect donor-specific aspects such as brain death.[41] An alternative and nonexclusive explanation is that the activation of resilience pathways in DCD grafts is caused by a process of ischemic preconditioning that may occur during the agonal phase and first warm ischemic period prior to donor nephrectomy in DCD donors. Ischemic preconditioning, which generally refers to a preceding state of ischemia that is followed by reperfusion, is an established phenomenon in experimental studies.[42–44] Yet, studies so far do not indicate a benefit of ischemic preconditioning for clinical kidney injury.[45] It might be speculated that the ischemia applied in clinical studies is insufficient to induce activation of resilience pathways, and that more profound and localized triggers which occur during the agonal phase and first warm ischemic period in DCD donors are required.

Our study has several limitations. It is in part based on registry data including the standard flaws of a registry with some data missing and a lack of predefined variables, leading to more heterogeneity in data registration. Outcomes are prone to confounding by indication with some clinicians being more critical than others when accepting or declining DCD grafts for transplantation. Also, exploration of molecular mechanisms is based on observational data. A more detailed experimental exploration and validation of the observed differences is compromised by the profound species differences with regard to acute injury, ischemia reperfusion, and resilience.[46,47]

In conclusion, results in this clinically relevant study show that DGF has no obvious impact on long-term graft survival in DCD grafts. As such, the high incidence of DGF in DCD grafts should not be regarded a relative contraindication or impediment toward the use of these donor kidneys. The molecular evaluation performed suggests that the different impact of DGF in DBD and DCD grafts relates to donor type-specific regulation of resilience and pro-inflammatory pathways benefitting the DCD graft and its outcomes.

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DISCUSSANTS

Michael Olausson (Gothenburg, Sweden):

This is an important paper, which demonstrates excellent results with DCD renal grafts. The study is based on registered data, but contains a large number of cases. The thought of an increased resilience toward DGF in DCD versus DBD is not new, but it is interesting.

I have a number of questions for the authors:

First, could you please tell us which category of DCD donors this is? Are they controlled or uncontrolled, and what about the Maastricht category?

Second, what factors, aside from resilience, can you think of in the DBD donors with DGF that might explain the lower graft survival?

Third, why do you think DCD grafts have an increased risk of DGF, considering how kidneys are retrieved in multiorgan donors?

Finally, in the downregulated pathways of DCD grafts, you note IL-6 and TNF-α (proinflammatory cascades) among others. The biopsies where taken before reperfusion. Do you see any problems with this? What happens after reperfusion in the DCD grafts?

Response From Michèle J.C. de Kok (Leiden, The Netherlands):

Thank you for kindly reading the manuscript and for your questions. In the Netherlands and the United Kingdom, DCD is almost consistently classified as a Maastricht Category III controlled circulatory death donation. We only have a few uncontrolled circulatory death donors. For the analyses, we choose to exclude this small group of uncontrolled circulatory death donors. Therefore, only kidneys from donors with Maastricht Category III and IV were used for the analyses.

Your second question is an important question. One obvious additional aspect is the effect of brain death. Brain death in DBD donors results in a severe autonomic storm after herniation of the brainstem, which is followed by a progressive and hostile systemic response affecting hemodynamic stability, metabolism and hormonal balance. This profound impact on metabolism and homeostasis causes significant inflammation, systemically and in potential donor organs. Although inflammation is often perceived as a "negative" factor, some studies, including previous work done by our group, suggest that inflammation can actually be beneficial in the acute phases of transplantation. Therefore, it is difficult to state whether inflammation during transplantation is a favourable or unhelpful event at this point in time.

Next, I think that DCD grafts have an increased risk of DGF, as DCD kidneys are exposed to the agonal phase and a first warm ischemic period after withdrawal of treatment. Our group has been studying the mechanisms of DGF in the context of clinical kidney transplantation, and the data have shown that DGF is preceded by a metabolic collapse due to mitochondrial injury. We also found that the warm ischemic period has a clear impact on mitochondrial function. Thus, I consider it very likely that the agonal phase and first warm ischemic period in DCD kidneys will definitely contribute to the development of DGF.

Also, in this study, we performed a univariate and multivariate regression analysis to identify risk factors associated with DGF. The results show that the first warm ischemic period, a discriminant factor of DCD grafts, is a risk factor for the development of DGF.

Finally, your question about whether inflammatory pathways are downregulated in DCD grafts, while being upregulated in DBD grafts, is very interesting. I think that it is more likely that there is an upregulation of inflammation in DBD grafts, reflecting the state of brain death in these donors.

In general, as I mentioned previously, we assume that inflammation is a negative factor in DBD grafts. However, some studies suggest otherwise. For instance, anti-TNF treatment failed to make a difference in human trials of sepsis. Also, with regard to IL-6, experimental studies showed that the neutralization of IL-6 resulted in an aggravation of renal I/R injury. As this was an observational study, answers can only be derived from experimental studies.

With regards to the biopsies taken before reperfusion, I don't think this is a problem. The molecular analyses of these back-table biopsies provide us with interesting, donor-specific information.

What happens after reperfusion is still unknown. Currently, we are evaluating the genomic responses in DBD and DCD grafts, and will relate these to the outcomes.

Antonio D. Pinna (Abu Dhabi, United Arab Emirates):

Thank you for this very interesting presentation. I have 2 questions for you. First, in order to understand why DGF in the DCD

donors is not as bad as the DBD ones, did you take into account the possibility that the management of DCD donors in the Netherlands is replicating something similar to remote ischemic preconditioning on your organs? Second, did you do a core precooling on the DCD donors before the organ procurement, as it had been suggested in a paper in the *New Journal of Medicine*?

Response From Michèle J.C. de Kok (Leiden, The Netherlands):

Thank you for your interesting questions. With regards to the first question, we consider that there might be a form of ischemic preconditioning in DCD grafts, which may occur during the agonal phase and first warm ischemic period. Although clinical trials to date have not confirmed a benefit of ischemic preconditioning in clinical kidney injury, it could be hypothesized that the degree of ischemia applied in these studies is insufficient to induce resilience, while it is not comparable to the ischemic injury that occurs during the agonal phase and first warm ischemic period in DCD donors.

As per your second question, we do not perform a core precooling of DCD donors in the Netherlands.

Stefan Schneeberger (Innsbruck, Austria):

Congratulations on an elegant trial. You established the lack of a correlation between delayed graft function and the eventual clinical outcome between DBD and DCD, and then you established a gene expression profile, where you compared DBD with DCD. So, did you manage to correlate that gene expression profile with delayed graft function in the two different groups? This essential link is missing from your presentation.

Response From Michèle J.C. de Kok (Leiden, The Netherlands):

Thank you for your kind remarks. In this study, the molecular analyses of the back-table biopsies now provide us with very interesting, donor-specific information. We have not differentiated between DBD and DCD grafts with or without DGF; however, I agree that this would be quite interesting to evaluate.