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Studying the short-term complications of kidney transplantation: from bed to bench

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PART III

Chapter 8

Summary, conclusions and future perspectives

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Summary

Kidney transplantation is one of the most successful medical achievements of the 20th century and has evolved as the treatment of choice in most patients with end-stage renal disease. Although a large part of the demand for donor kidneys is currently covered by grafts retrieved from living donors to compensate the lack of suitable deceased donor organs, a persistent organ shortage remains. As deceased donation brain death (DBD) donors did not suffice, the transplant community turned towards organs donated after circulatory death (DCD) as a potentially effective means to address the severe organ shortage.[1,2] Yet, from a clinical point of view, there are several non-immunological outcomes that have resulted in a reluctant attitude towards the use of these donor organs. Much of this attitude in kidney transplantation is driven by the fear of two short-term complications: early graft loss (EGL) and delayed graft function (DGF) (defined as the situation in which the recipient is temporarily dialysis dependent after transplantation). The focus of this thesis was on the underlying mechanisms and consequences of these two early complications. To obtain better insight in the impact of EGL and DGF on clinical outcomes following deceased donor kidney transplantation, we first performed a series of epidemiological evaluations that are summarized in part I of this thesis. In the second part, we focused on DGF as the primary non-immunological complication of DCD kidney transplantation and as a clinical manifestation of ischemia-reperfusion (I/R) injury. Whilst I/R injury has been studied for over 50 years, there remains a persistent challenge in translating preclinical successes to clinical achievements. In the second part of this thesis, we have therefore explored a number of methodological challenges in an effort to bridge the translational gap between bench and bed.

Part I

Chapter 2 focuses on early graft loss (EGL) as one of the most feared complications after DBD, and in particular DCD kidney transplantation. In a nationwide evaluation that included 10,307 deceased-donor kidney transplantations performed in The Netherlands between 1990 and 2018, we show that the incidence of EGL is similar after a primary DBD and DCD transplant procedure (8.1% vs. 8.5%; p=0.54). To estimate the optimal balance between the donor pool size and impact of EGL (a reticent attitude toward high-risk organs contributes to increasing organ shortages, yet a permissive policy results in an unacceptable high incidence of EGL), we systematically evaluated the consequences of EGL. Results show that EGL associates with significant detrimental consequences for the individual patient that include profound short-term and long-term mortality rates, a reduced chance of relisting and re-transplantation, and for those re-transplanted an increased risk of recurrent EGL. These observations implicate that–while the development

of EGL and the associated poor outcomes are generally attributed to the use of suboptimal kidney grafts–recipient-related factors may also elicit the development of EGL. Further evaluation shows that, despite the profound impact of EGL for the individual patient, the 8.2% overall incidence only minimally affects the survival of the transplanted patients. This implies that a policy aimed at further minimizing the incidence of EGL will lead to avoidable deaths as a result of longer waiting-list times.

Notwithstanding the EGL equivalence after DBD and DCD kidney transplantation (**chapter 2**), the higher incidence of DGF after DCD kidney transplantation remains a major impediment to an increased utilization of these kidneys in clinical practice. Yet, emerging reports indicate that despite this different and much higher DGF incidence, long-term outcomes and survival of DCD procedures are equivalent to DBD procedures.[3-5] In **chapter 3** and **chapter 4** we have attempted to find an explanation for these contrasting short-term, but equivalent long-term survival outcomes of DBD and DCD kidney transplantation.

In light of the results obtained in **chapter 2**, namely that the incidence of EGL after DBD and DCD kidney transplantation is equal, we hypothesized in **chapter 3** that prevailing concerns regarding inferior DCD outcomes are often based on data from historical cohorts, with interference of time-related effects that may no longer apply in our current timeframe. In **chapter 3** we describe the results of a nationwide, time-dependent comparative analysis. The study confirms that conclusions with regard to inferior outcomes of DCD donor grafts are interfered by time-related effects (i.e. time-varying confounding and effect modification by time) and that outcomes of DCD procedures have improved over time. In fact, whilst the 1998-2008 era associates with compromised outcomes for DCD procedures when compared with DBD procedures (higher incidences of DGF and EGL, impaired 1-year renal function in patients without DGF and inferior 1- and 5-year graft survival rates), the 2008-2018 era shows outcome equivalence for both DBD and DCD procedures. This change has occurred despite the fact that currently more older and higher risk donors and more complex recipient risk profiles are involved. To date, only the incidence of DGF remains significantly higher after DCD kidney transplantation (DBD: 16.6% vs. DCD: 39.7%; $p<0.001$). Nevertheless, patient- and graft survival equivalence for DBD and DCD procedures can be demonstrated despite the higher incidence of DGF in DCD grafts.

The notable finding of **chapter 3** that DBD and DCD graft survival is not different, despite a persistent higher incidence of DGF in DCD grafts, suggests a different impact of DGF on DBD and DCD graft survival. Our epidemiological evaluation performed in **chapter 4**, confirms this hypothesis. In fact, whilst DGF severely impacts on 10-year graft survival in DBD grafts (HR 1.67; $p<0.001$), DGF does not impact on graft survival

in DCD grafts (HR 1.08; p=0.63). Further evaluation of explanations underlying this phenomenon, shows that the severe impact of DGF in DBD grafts is not caused by a more severe DGF phenotype. On the contrary, DCD grafts are hallmarked by a more severe DGF phenotype, as indicated by a prolonged need of posttransplant dialysis and inferior renal function. As a result we then hypothesized that the differential impact reflects a difference in graft 'resilience'–i.e. the ability of the graft to cope with negative environmental changes [6]–with DCD grafts being more resilient than DBD grafts. Immunohistochemical analysis confirms the presence of resilience enhancing factors in donor kidneys. In order to map putative molecular differences in organ resilience between DBD and DCD grafts, gene expression profiling was followed by Ingenuity Pathway Analysis in pre-reperfusion kidney biopsies. Pathways relatively enriched in DCD grafts were all part of established resilience networks. Downregulated pathways appeared to be dominated by pro-inflammatory signaling cascades. These findings suggest 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.

Although epidemiological evaluation in **part I** shows that the impact of DGF on DCD grafts may be less stringent than commonly thought, DGF remains–albeit less prevalent– an important complication after DBD kidney transplantation. In **part II** of this thesis, we have therefore performed an evaluation of I/R injury from a more molecular perspective and how certain responses may contribute to the development of DGF.

Part II

Chapter 5 provides novel insights into the pathophysiology of DGF as clinical manifestation of I/R injury were provided. This study shows that while oxidative damage is widely considered as the key driver of I/R injury,[7] clinical observations point to a postreperfusion metabolic paralysis caused by reductive stress as the actual effector mechanism of I/R injury. To be more specific, results of a more recent work (Thesis L.G. Wijermars) has identified a fully discriminatory metabolic signature in I/R injury which is hallmarked by the inability of cells to generate high energy phosphates and a comprehensive activation of oxidation routes including glycolysis, fatty acid β-oxidation, glutaminolysis, and branched chain amino acid oxidation.[8] This compensatory catabolic state triggered by high-energy phosphate depletion results in a parallel production of reducing equivalents, i.e. NADH and $FADH_2$. Whilst these equivalents are normally oxidized through the electron transport chain (respectively complex I (NADH) and II ($FADH_2$)), or by the cytosolic LDH (glycolysis), the excess reductive load and apparent I/R-related defects in the oxidative routes may result in accumulation of reducing equivalents and therefore cause reductive stress.

Exploration of processes underlying I/R injury, critically relies on preclinical–both *in vitro* and *in vivo*–models and read-outs. Unfortunately, it appears that there is a large translational gap, as most preclinical models cannot be successfully replicated under clinical conditions. In a first attempt to bridge the translational gap, we explored methodological challenges in animal (**chapter 6**) and cell culture (**chapter 7**) studies focusing on I/R injury.

Chapter 6 focuses on potential translational hurdles between preclinical research and the clinical context. A systematic literature search identified 34 preclinical studies reporting metabolic data on I/R injury. Methodologies as well as metabolic outcomes were semi-quantified and aligned with clinical data. Results show a poor alignment of the reported preclinical metabolic data with clinical observations, and also identified a series of critical methodological shortcomings in preclinical studies (e.g. experimental set-ups that do not allow discrimination between ischemic and I/R injury, inappropriate sampling techniques, and vast diversity in the reported metabolic profiles) each compromising the interpretation and translatability of the preclinical studies.

Chapter 7 elaborates on mammalian cell culture as another promising model to study the processes of I/R injury and it focuses on the Crabtree effect[9] which emerges as a major challenge in this field of research. The Crabtree effect refers to the adaptation of cultured cells to a glycolytic phenotype, away from oxidative phosphorylation. By conducting a systematic literature search we showed that circumventing the Crabtree effect (i.e. reverting the metabolism of cultured cells from glycolysis back to oxidative phosphorylation for adenosine triphosphate generation) is possible. Strategies are roughly based on the suppression of glycolysis (either direct by the use of glycolytic inhibitors, or indirect by the use of galactose medium), or alternatively on metabolic reprogramming (e.g. inhibition of HIF1α, anti-TGF-β-antibodies, H2S and irisin). The choice of strategy, however, should be dictated by the scientific question.

Conclusions

The first part of this thesis demonstrates that kidneys from DCD donors can be fully embraced, and that the perception of DCD kidney transplantation being inferior to DBD kidney transplantation – that is mainly motivated by concerns regarding high incidences of DGF and EGL – is no longer justified. One key explanation for this phenomenon is that outcomes of transplanted DCD kidneys have improved over time, with a similar incidence of EGL following DBD and DCD kidney transplantation in the current era. A further explanation is that both donor types have a different biological response to DGF: whilst DGF severely impacts on DBD graft survival, DGF has no impact on DCD graft

survival. Our molecular evaluation performed suggests that this different impact relates to donor type-specific regulation of resilience and pro-inflammatory pathways benefitting the DCD graft and its outcomes. As such, the persistent high incidence of DGF in DCD grafts should not be regarded an impediment toward the use of these donor kidneys.
The second part of this thesis identifies and explores a series of physiological (i.e. the Crabtree effect) and methodological (i.e. experimental set-ups) contrasts between preclinical I/R injury models and the clinical reality, that all may contribute to the impaired translatability of preclinical studies in clinical practice. Awareness of these challenges and pitfalls may help to improve study designs in future research, bringing us one step closer towards bridging the translational gap.

Future perspectives

As in most studies, our attempt to answer a few questions has lead to new challenges and many more questions.

One notable aspect that emerged from this thesis, is that–although the development of early and late graft loss is generally attributed to the use of suboptimal kidney grafts – the actual impact of donor characteristics on graft survival is less profound than assumed. This is not only supported by the paradoxical finding that transplant outcomes improve whilst we accept many more older and higher risk donor organs, but also by the remarkably poor association between (i) the kidney donor risk index (KDRI) and 5-year graft survival, and (ii) traditional donor/procedural risk factors and EGL (chapter 2, 3). Hence, these data suggest that we can focus less on donor characteristics. The importance of recipient factors, however, is underpinned from the observation that EGL patients have a reduced chance of relisting (for re-transplantation), and an increased risk of recurrent EGL when re-transplanted. From this perspective, it would be of interest to shift future research from a primarily *donor* focused towards a more *recipient* or *donor-in-host* focused research. This might help us to better identify recipient factors, and donor-recipient interactions that impact on transplant outcomes.

Since our results show that the incidence of EGL has been decreasing over time, we may conclude that we are heading in the right direction. An intriguing and still underexposed question, however, is whether this intuitive response is justified. A policy aimed at (further) minimizing the incidence of EGL could lead to 'cherry-picking' of organs, which would contribute to the persistent organ shortage and prolong time on the waiting list. Therefore, although *a risk-avoiding* policy may be natural among doctors facing their patients (not taking unnecessary risks for the safety of the patient), it is debatable

whether it would be more justified to strive for a *risk-accepting* policy for the 'hidden' patients on the waiting list.

Another fascinating observation in the first part of this thesis is that although DGF is often considered a relative contra-indication for the use of DCD kidneys, DGF does not impact on graft survival in DCD grafts (whereas it does negatively impact on graft survival in DBD grafts). We hypothesized that this differential impact reflects differences in graft 'resilience' (i.e. the ability of the graft to cope with negative environmental changes [6]). Indeed, molecular evaluation showed relative enrichment of resilience-associated pathways in DCD versus DBD grafts. Although these observations are only based on donor kidneys, it is of interest to test whether this observation for the kidney also applies to other organs and what molecular signals underlie the selective activation of these pathways. This can subsequently serve as a basis to investigate whether organ resilience can be modulated, which could be beneficial in terms of organ preservation and preconditioning in a broad variety of major surgical interventions that induce ischemia and reperfusion (e.g. in transplantation, cardiothoracic and vascular surgery).

In the second part of this thesis we have explored challenges and methodological shortcomings that arise in *in vitro* and *in vivo* studies focusing on I/R injury. For cell culture studies, the Crabtree effect must be considered when designing future experiments. For preclinical studies, the first step was taken to identify pitfalls and provide recommendations for future research models. Integrating this theory into research practice may be an important next step towards the ultimate goal to narrow the translational gap between bench and bed, and achieve a successful targeted intervention that will benefit our patients.

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