Delayed graft function in renal transplantation

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CHAPTER 7

SUMMARY

Delayed graft function (DGF) in renal transplantation remains enigmatic. Progress in the research after the etiology and consequences of DGF are hampered because DGF is a poorly defined syndrome and the clinical consequences of DGF on long term graft function have been difficult to ascertain. In general renal biopsies are not taken to document the cause of DGF but to exclude latent acute rejection episodes. Large biopsy studies on DGF are not consistently available. The interest in DGF has gained renewed interest with the increased use of marginal donors, including non-heart-beating donors, donors at the extremes of age, and donors with hypertension or diabetes. This group of donors experience DGF more frequently, with reported incidences of upto 50%.
Our knowledge on DGF is mainly based on studies in experimental animals and on clinical data on acute renal failure in native kidneys (Chapter 1). This comparison has major flaws, since the risk factors and clinical setting for acute renal failure in the transplantation setting are substantially different from the risk factors for acute renal failure in native kidneys. In the transplant setting the kidney suffers from cold ischemia after the graft is harvested from the donor and cold perfusion is started. This procedure can last as long as 48 hours. Furthermore after the transplantation procedure ATN can be complicated by an increased likelihood of acute rejection episodes or drug-related nephrotoxicity.

Pathogenesis of DGF

Despite the above mentioned flaws, the underlying mechanism of DGF is considered to be related to ischemic and reperfusion damage, resulting in acute tubular necrosis.
In the pathogenesis of acute tubular necrosis, 3 stages can be recognized. The first stage is the **ischemic phase** in which ischemic and reperfusion injury (IRI) takes place and in which renal epithelial and endothelial cells are subjected to lethal insults leading to apoptosis and /or necrosis. The **maintenance phase** represents a phase of equilibrium between injury and intrinsic or up regulated defense mechanisms, events leading to cellular repair, proliferation and redifferentiation. This may lead to the **recovery phase** in which epithelial en endothelial function improve, leading to the recovery of renal function.

Lack of consistency in definition of DGF and its consequences for risk factors long term consequences

Risk factors for DGF are well known. These comprise older donor age, prolonged cold ischemia time and female gender of the donor. However there is debate on the impact of DGF on late graft outcome. Some authors reported an effect of DGF on renal allograft survival, while others only found inferior graft survival in patients who also experienced acute rejection episodes.
One possible explanation for this apparent difference in outcome may be the definition of DGF that is used. In most studies DGF is defined as the need of dialysis treatment in the first week after renal transplantation. This is a criterion that is easy to register and to obtain from large databases. However, dialysis during the first week after transplantation is also performed for other reasons than DGF, such as hyperkaliemia and / or fluid overload.

Another flaw in this definition is the inability to exclude acute rejection and calcineurin inhibitor toxicity as an additional cause of impaired graft function. To study risk factors for DGF and its clinical consequences, it is therefore important to use a definition of DGF, in which the contribution of ischemia and reperfusion injury is stressed.
In this thesis we analyze the risks and consequences of delayed graft function, using a functional definition in which acute rejection and calcineurin inhibitor toxicity was excluded and the ischemic origin of DGF was stressed:
In this thesis we use a functional definition of DGF: We diagnosed DGF retrospectively, when serum creatinine level increased, remained unchanged or decreased less than 10% per day immediately after surgery during three consecutive days for more than one week excluding acute rejection when anti rejection treatment was started within this first week. Grafts that never functioned, ultimately leading to graft nephrectomy, were also excluded.

The above mentioned introduction is a summary of **chapter 1** of the thesis
In **chapter 2** we analyzed the risk factors for the occurrence of DGF in a cohort of 734 patients transplanted between 1983 and 1997. We found that the presence of DGF was associated with classical risk factors such as older donor age, a prolonged cold ischemia time and a low mean arterial blood pressure of the recipient. The impact of DGF was restricted to the quality of renal function (creatinine clearance) within the first year after transplantation but was not associated with inferior long term graft outcome. Besides the occurrence of DGF, one year renal allograft function was associated with older donor age, female gender of the donor and the occurrence of acute rejection episodes. Moreover, graft function after this first year is mainly determined by the quality of allograft function at one year and not with a history of DGF or acute rejection.
Using these findings, we hypothesized that, since graft function is correlated with the number of functioning nephrons (functional renal mass), DGF and long term graft function are related with each other through this functional renal mass. As it is known from literature that renal mass is influenced by age and gender, this functional mass might be the connecting link that explains the relation between DGF and poor graft outcome.
In **chapter 3** we tested this hypothesis in a study that correlated the renal functional mass with long-term graft outcome. The functional renal mass was determined using the tubular function slope (TFS), a parameter in ^{99m}technetium mercaptoacetyltriglycine (^{99m}MAG-3) scintigraphy. In a group of 42 grafts, 14 experienced DGF and had a significant lower functional renal mass than 28 grafts without DGF. This difference persisted during the total follow up period of 3 years. When the creatinine clearances were analyzed in the group of 734 patients described in chapter 2, the creatinine clearances in the DGF group were significantly lower during follow up period as compared with patients with immediate function of their transplanted kidney.
Another striking finding was that, in the DGF group as well as in the PF group, the functional renal mass was severely reduced directly after transplantation. Recovery took place within 1 month after transplantation and was identical for both groups. From these findings we conclude that ischemia and reperfusion injury leads to injury that is identical for all grafts. Its effect on functional renal mass of the graft disappeared within 1 month.

Hypercalcemia is frequently seen in patients on renal replacement treatment. This is caused by the use of calcium containing phosphate binders and vitamin D analogues, in order to prevent hyperparathyroidism and related bone disease. However studies on the effect of hypercalcemia on DGF are lacking in literature. Hypercalcemia can result in renal failure in native kidneys (nephrocalcinosis), but the exact mechanism of how hypercalcemia leads to renal failure is not well known.

In experimental models 3 types of nephrocalcinosis are recognized:

1. **Macroscopic nephrocalcinosis** is characterized by calcium deposits that are recognized by ultrasound.
2. **Microscopic nephrocalcinosis** is characterized by the presence of calcium containing deposits in tubules, recognized by light microscopy.
3. **Chemical nephrocalcinosis**, is assumed when no calcium deposits are found in the presence of hypercalcemia and acute renal failure. Chemical nephrocalcinosis might be associated with high cytosolic calcium levels, that activate enzymes associated with necrosis and apoptosis.

In **chapter 4** we described, to our knowledge, for the first time the relation between hypercalcemia and the occurrence of DGF in a group of 585 recipients of a cadaveric graft. We also looked for an anatomical substrate in 71 renal biopsies that were taken within the first week after transplantation. The presence of calcium crystals and signs of tubular necrosis did not correlate with serum calcium levels, suggesting that chemical nephrocalcinosis could be a determinant of DGF.
Another striking finding was that the use of calcium channel blockers (CCBs) protected against the occurrence of DGF. In the past these CCBs were frequently studied with the idea that its vasodilatory characteristics protected against calcineurin inhibitor toxicity. The results of these studies were not uniform and therefore CCB's are not used in clinical practice to prevent calcineurine inhibitor toxicity on a regular base. We found in a group of patients in which by definition calcineurin toxicity was excluded as a cause of DGF that the use of CCBs protected against the occurrence of DGF. This is probably explained by the prevention of high cytosolic calcium levels and subsequent activation of enzymes involved in the process of necrosis and apoptosis, like calpaines and caspases, involved in the pathogenesis of ATN.
Whether DGF develops in the allograft, depends on the balance between apoptosis and necrosis inducing factors on the one hand and protective factors on the other hand. In our opinion these protective factors are important determinants the quality of the graft and consequently long term graft outcome. The superoxide dismutases (SOD) are enzymes that act as scavengers of radicals that are produced upon ischemia and reperfusion damage.
In **chapter 5** markers that are representative of the three stages in the development of ATN are studied in renal biopsies from grafts with and without DGF. Damage factors that dominate the initiation phase were visualized by morphological examination and by staining for active caspase 3, which is a major effector enzyme in the process of apoptosis. The protective response, that is important in the maintenance phase, was visualized by manganese SOD (Mn SOD). Signs of regeneration and proliferation, characteristic for the regeneration

phase are characterized by vimentin and Ki-67 staining respectively. We found that grafts without DGF had a more intensive staining for Mn SOD. The presence of tubular necrosis was, as expected, associated with active caspase-3 staining. The presence of vimentin and Ki 67 was associated with recovery of renal function but these data do not reach the level of significance. These findings support the hypothesis that the occurrence of DGF was the resultant of the balance between protective and damaging factors. Since the occurrence of DGF is associated with older donor age and female gender of the donor, the connecting link between these epidemiological and immunohistochemical factors, probably is the quality in terms of functioning nephrons of the graft. We propose that the presence of Manganese SOD in a pre-transplant biopsy can help to determine graft quality. Supplementation of Manganese SOD to grafts might be a method to diminish the occurrence of DGF and preserve renal allograft function.