Protocol biopsies after kidney transplantation

Impact paragraph

Renal transplantation is the preferred treatment option for most patients with end stage renal disease (ESRD). However, graft function deteriorates with time posttransplant and eventually retransplantation or dialysis may be needed. Already in the first year after transplantation, histological signs of chronic injury, including interstitial fibrosis and tubular atrophy (IF/TA), can be observed in (protocol) renal biopsies before deterioration of renal function occurs (1). In this thesis, several histological parameters were assessed in protocol biopsies taken at transplant and during the first year after renal transplantation in a single centre cohort in order to study the prognostic value of these parameters for renal transplantation outcome.

Research and clinical implications

The decision of acceptance of a potential postmortal donor kidney is partly based on the macroscopic evaluation of the kidney by the transplant surgeon at time of organ procurement. This evaluation is mandatory in the Eurotransplant region. In our nation-wide study, macroscopic arteriosclerosis of the renal artery is associated with higher discard rate of donor kidneys. However, in kidneys that were transplanted, presence of macroscopic arteriosclerosis was not associated with a worse graft survival or worse renal function compared to kidneys without macroscopic arteriosclerosis. Furthermore, there was no correlation between the grade of macroscopic arteriosclerosis and microscopic intra-renal arteriosclerosis in the donor kidney. Macroscopic arteriosclerosis is usually scored at the aortic patch, near the ostium of the renal artery. At the ostium there is often macroscopic arteriosclerosis present, while further on the renal artery has no sign of macroscopic arteriosclerosis. Therefore, the current method of assessing macroscopic arteriosclerosis seems inadequate to assess the quality of the donor graft. Donor surgeons should be trained to score macroscopic arteriosclerosis in het renal artery and the Eurotransplant guidelines for the assessment of donor kidney quality should be adapted. After standardising scoring of macroscopic arteriosclerosis, future studies can be performed to evaluate whether renal arteriosclerosis is associated with graft function and microscopic vascular damage.

During the process of donation and transplantation, ischemia reperfusion injury is inevitable and it is associated with the occurrence of delayed graft function (DGF) and primary nonfunction (2, 3). However, there is no histological standard scoring for ischemia reperfusion injury (4, 5). We performed an explorative study in which we studied parameters for ischemia reperfusion injury in protocol renal transplant biopsies. We showed that loss of brush border is associated with delayed graft function and decreased renal function at year one after transplantation. Furthermore, presence

of neutrophils in PTCs were associated with higher IF/TA scores at one year after transplantation. There was no association of tubular cell necrosis or presence of neutrophils in glomeruli with outcome. Loss of brush border and presence of neutrophils in PTCs could be an interesting read-out for ischemia reperfusion injury in future studies. However, the parameters to score ischemia reperfusion injury are not well defined neither in our study nor in literature (5). Probably as a result of the poor definition, we and others found low interobserver agreement of these parameters (4). Future research should focus on a better definition and standardisation of the histological ischemia reperfusion injury parameters. This is needed before any conclusion about clinical applicability can be drawn. Additionally, in our study we scored the ischemia reperfusion injury parameters in reperfusion biopsies, i.e. in biopsies taken after the graft is transplanted. Hence, these parameters cannot be used for clinical decision making on suitability of the potential donor graft for transplantation. On the other hand, the parameters may be used in an assessment of baseline quality of the kidney graft.

We studied a cohort of consecutive renal transplants, on a tacrolimus-based immunosuppressive regimen, with available protocol biopsies taken at time of transplantation, and 3 and 12 months after transplantation. We assessed progression of fibrosis in relation to clinical outcome parameters. Our studies confirm that IF/TA is an important predictive parameter for graft function (eGFR) at one year posttransplant, and therefore, IF/TA is an important parameter in the assessment of renal transplant biopsies. We confirm that IF/TA development is associated with ischemia reperfusion injury and inflammatory events (6) and that higher IF/TA scores one year after transplantation are associated with lower graft survival (7-9). We showed that there is more IF/TA progression in DCD vs. DBD in the first year after transplantation, which may be related to more ischemia reperfusion injury. IF/TA progression was also dependent on the immunosuppressive regimen. In kidneys *without* any pre-existent IF/TA, there was less progression of IF/TA when sirolimus was used as additional immunosuppressant to tacrolimus while in kidneys *with* pre-existent IF/TA addition of mycophenolate mofetil to tacrolimus gave less IF/TA progression. A strategy to decrease IF/TA progression in the first year after transplantation, and with that improve graft survival, could be tailoring of immunosuppressive regimen dependent on the IF/TA score at time of transplantation.

Although our studies underline the importance of IF/TA, we have demonstrated, in line with literature, a moderate interobserver agreement for scoring IF/TA in categories according to the Banff classification (10-12). We examined whether visual renal fibrosis assessment is feasible on a continuous scale, i.e. as percentage affected area of the cortex. Interobserver agreement was numerical slightly better for assessment on

continuous scale compared to the Banff classification. Furthermore, we compared visual renal fibrosis assessment with computerised evaluation of fibrosis in percentages on Sirius red stained biopsies. Agreement of assessment on a continuous scale and Sirius red evaluation did not notably differ in our cohort. For clinical use we propose a continuous scoring system, since it does not need extra time of technical investment. However, it needs validation in future (prospective) studies. In research settings computerised evaluation of fibrosis could provide more objective fibrosis data (13).

Decreased peritubular capillary (PTC) density is associated with higher IF/TA scores and lower eGFR in cross sectional studies in chronic kidneys disease and allograft dysfunction (14-16). However, most studies focused on late stages of graft dysfunction and not much in known about PTC stability in early stages post- transplant. Our studies underscore the relation between peritubular capillary (PTC) density and IF/TA in renal transplantation settings. We showed that decrease in PTC density occurs more in DCD than in LD and DBD. Secondly we showed that decrease in PTC density occurs very early (in the first weeks) after transplantation in recipients with complications shortly after transplantation (a rejection and/or delayed graft function). Furthermore, we observed that early decrease in PTC density precedes later progression of IF/TA. Moreover, decrease in PTC density was associated with a lower eGFR at one year after transplantation and more often development of proteinuria up to ten years after transplantation. Decrease in PTC density may therefore serve as a surrogate marker for later graft function. However, assessment of PTC density is still at a too preliminary phase to implement already in clinical settings. There are several assessment methods used to score PTC density in studies and our method is a time-consuming and tedious method. Automated assessment of PTC density might be a solution for using PTC density assessments at a large scale.

Social implications

Compared to patients on the waiting list, transplanted patients have a lower mortality risk and a better quality of life (17-21). Transplanted patients have more quality adjusted life years (QALYs) over ten years than dialysis patients: 5.2-6.3 QALY's versus 4.0 QALY's (22). Furthermore, the costs per QALYs were less for transplantations compared to dialysis (22, 23). Hence, not only ESRD patients but also the society and economy benefit from kidney transplantation. There is however a shortage of donor kidneys: implementation of recommendations from our studies could, in the future, lead to more kidney transplantations with longer graft survival. As described above, we have shown that arteriosclerosis in the renal artery was not associated with worse graft function or survival. Implementing this knowledge in donor quality assessments may decrease unnecessary donor discard and, with that, enlarge the donor pool.

Furthermore, scoring ischemia reperfusion injury parameters provides a new early readout to evaluate interventions before en during donation and transplantation. This could potentially give insight, already early after interventions, which (new) interventions are successful. We have demonstrated that DCD kidneys and grafts with DGF develop more IF/TA, which is associated with worse renal function and shorter graft survival (6). Furthermore, we showed that decrease in PTC density precedes IF/TA progression. Early interventions to stabilise PTC density and interventions that reduce IF/TA progression may protect the graft from decline of function and a longer graft survival, which will increase the gained QALYs in transplantation patients and reduce costs.

Concluding remarks

Protocol biopsies taken after renal transplantation provide a useful tool to study early histological markers for short-term and possibly long-term renal function, which might be implemented in clinical care in the future. A few ischemia reperfusion injury parameters, scored in reperfusion biopsies are associated with clinical course posttransplant. Progression of IF/TA and the decrease of PTC density during the first year posttransplant may be used as indicators for long term renal function. In addition, the findings in this thesis support the development of strategies aimed at prevention of ischemia reperfusion injury and preservation of PTC density, especially in DCD donor kidneys, that may ultimately lead to their increased graft survival and/or an expansion of the donor pool.

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