GFR meets mTOR: value of different methods to measure and estimate GFR & (side) effects of mTOR inhibition in renal transplantation
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Summary
PART I

Exact assessment of renal function, expressed as glomerular filtration rate (GFR), remains an important subject of research even 75 years after the discovery of creatinine as an endogenous marker to estimate renal function. Since plasma creatinine concentration is influenced by other parameters than kidney function alone, for example age, gender, race and diet, multiple formulas have been developed to overcome these factors. The most known and commonly used formulas are the Cockcroft-Gault - and MDRD formula. Furthermore, other endogenous markers have been identified, like cystatin C and beta-trace protein, possibly representing a better reflection of GFR than plasma creatinine concentration.

In chapter 1, the performance of these various endogenous markers in patients at risk or with overt renal failure, is discussed in comparison to established gold standard methods for the assessment of GFR, like $^{51}$Cr-EDTA and $^{125}$I-iothalamate/$^{131}$I-hippuran. The advantages and disadvantages of formulas based on these endogenous markers are described. Also a newly developed, still experimental method using the MRI contrast agent gadolinium-DTPA, is introduced.

Adult patients with Fabry disease, a lysosomal storage disease due to alpha-galactosidase deficiency, underwent yearly GFR measurement with iothalamate/hippuran to monitor the effect of enzyme replacement therapy. In chapter 2, these data were used to compare the value of formulas based on either plasma creatinine, cystatin C, beta-trace protein or a combination, with the gold standard GFR measurement. Bias, but especially precision and accuracy deviated considerably from gold standard. Furthermore, the creatinine-based formulas overestimated GFR in male Fabry patients, possibly reflecting decreased muscle mass in these patients compared to the normal population. Cystatin C and beta-trace protein alone did not prove to be better markers to estimate GFR. Although, we concluded that GFR estimated by the tested formulas could not replace gold standard GFR when more precise knowledge of renal function is required, the creatinine and cystatin C combined Stevens formula performed best, closely followed by the CKD-EPI and MDRD.

Chapter 3 discusses a new exogenous nonradioactive marker as a gold standard method to assess GFR, gadolinium-DTPA (Gd-DTPA, Magnevist®). We measured renal function in renal transplant recipients, candidate kidney donors, HIV infected patients and patients with Fabry disease. Unfortunately, GFR measurement using Gd-DTPA had an unacceptable bias, precision and accuracy when compared to an established radioactive gold standard method with $^{51}$Cr-EDTA and did not outperform estimated GFR with creatinine and/or cystatin C. Therefore we do not consider this method suitable to be used in the clinic.

Cystatin C is explored in chapter 4 as a marker of residual renal function in critically ill patients treated with continuous venovenous hemofiltration (CVVH). Since creatinine is removed from the blood during haemofiltration, it can not be used to determine residual renal function. However, the low molecular weight protein
Cystatin C, with a molecular weight of 13.3 kD, appeared to be a good candidate for this task. We measured pre- and post filter concentrations of cystatin C and found that the removed quantity averaged 2.13 mg/h, corresponding with less than 30% of its production. We therefore conclude that cystatin C could be representative of residual renal function.

Precise determination of GFR remains extremely difficult and the obtained kidney function can differ depending on the used method. Furthermore, additional physiologic factors like diet, fluid intake or circadian rhythm, can affect GFR not necessarily reflecting an improvement or pathologic decline of GFR. In general, exact measurement of GFR should be reserved for specific situations for example the measurement of GFR in candidate kidney donors or in research settings. Most often, it is sufficient to know whether kidney function is above or below 60 ml/min, for instance if medication has to be adapted. Creatinine-based formulas like the MDRD and recently developed CKD-EPI appear to be precise enough when GFR is between 10 and 60 ml/min. However, one should be careful in interpreting estimated GFR > 60 ml/min, since creatinine is an unreliable marker in this range. The course of estimated GFR in time is the most crucial to monitor, and action should be undertaken when significant decline is noticed.

Cystatin C could have a place in situations when creatinine is notoriously unreliable, for example in patients with extremely low muscle mass which is the case in anorexia nervosa and muscle diseases. Moreover, in the rare case of urine leakage in the peritoneal cavity and hereafter reabsorption of urinary creatinine, plasma creatinine is falsely elevated and cystatin C could be of value since it is broken down in the proximal tubule and does therefore not appear in the urine. There seems no additional value of beta-trace protein.
PART II

Inhibitors of the mammalian target of rapamycin (mTOR) were introduced in renal transplantation because of their supposed lack of nephrotoxicity, possible anticancer effects and their beneficial effects on the vessel wall. Recently a multicenter randomized controlled trial, the MECANO trial, was performed studying the effects of withdrawal of cyclosporine from an immunosuppressive regimen containing an IL-2 antagonist (basiliximab), cyclosporine, prednisolone and mycophenolate sodium early after renal transplantation. After 6 months, renal transplant recipients were (in the absence of rejection, proven by renal biopsy) randomized to one of three immunosuppressive regimens: prednisolone/ cyclosporine, prednisolone/mycophenolate sodium and prednisolone/everolimus. The prednisolone/mycophenolate arm was prematurely halted due to an increased incidence of acute rejection. From January 2005 until September 2009, the Academic Medical Center, University Medical Center Groningen and the Leiden University Medical Center participated. The studies mentioned below represent substudies of this trial.

Chapter 5 gives an overview of the mTOR pathway and the general effects of mTOR inhibition. Since the mTOR pathway is ubiquitously present in the body it is not surprising that many side effects accompany its use.

One of the most severe complications caused by mTOR inhibitors is pneumonitis, a possible life threatening condition. Chapter 6 describes a case-control study performed in renal transplant recipients treated with the mTOR inhibitor everolimus, reporting the incidence, radiologic features and risk factors of everolimus-induced pneumonitis (EIP). EIP appeared to occur relatively often with an incidence of 13%, presenting as organizing pneumonia and/or non-specific interstitial pneumonitis, the latter carrying the risk of becoming a chronic condition. Unfortunately no risk factors could be identified, especially no correlation with everolimus dose. This lead to the recommendation to withdraw everolimus completely instead of lowering the dose when EIP is suspected.

Another, much debated side effect is the often observed de novo occurrence of - or increase in proteinuria after start of an mTOR inhibitor. This issue is addressed in chapter 7, comparing proteinuria in renal transplant recipients treated with prednisolone/everolimus to those treated with prednisolone/cyclosporine and relating this to renal biopsy data analyzed with conventional light microscopy as well as electron microscopy. We found a slight increase in non-nephrotic range proteinuria in the everolimus treated patients, not accompanied by a decrease in renal function. Moreover, we found no abnormalities upon light- and electron microscopy, especially no signs of podocyte damage. The increase in proteinuria in the everolimus treated patients compared to the cyclosporine treated patients might be explained by the antiproteinuric properties of the latter.

Although chronic kidney disease and the use of immunosuppressive drugs are known to increase the risk of venous-thrombo-embolic events, the observed number of unexpected thrombo-embolic events in patients treated with the mTOR inhibitors
sirolimus or everolimus was remarkable. This was the reason to conduct the pilot study reported in **chapter 8**. Here we compared various parameters of coagulation in everolimus treated patients (mTOR group) to those treated with cyclosporine and/or mycophelate (non mTOR group). We found that in the mTOR group as well as in the non mTOR group, von Willebrand factor prothrombin fragment 1+2 were elevated, pointing to increased coagulation potential and activation of coagulation following renal transplantation. The use of mTOR inhibitors even further increased vWF levels as compared to a non-mTOR based immunosuppressive regimen. Furthermore, we observed higher F1+2, TAFI and PAI-1 concentrations in the patients treated with an mTOR inhibitor. This indicates that treatment with an mTOR inhibitor leads to increased endothelial activation, thrombin formation and impaired fibrinolysis compared to treatment with a non-mTOR inhibitor. At completion of this pilot study, the overall results of the MECANO study were analyzed and demonstrated an increased incidence of thromboembolic complications in the everolimus - compared to the cyclosporine and mycophenolate treated patients. This suggests caution in prescribing mTOR inhibitors to patients with a history of venous or arterial thrombosis.

The drop-out rate due to side effects of mTOR inhibitors in patients treated with these drugs in study context is high, sometimes mounting 50%. Side effects complicating its use vary from mild diarrhea to life threatening pneumonitis. Considering the high incidence of adverse effects, first line treatment with mTOR inhibitors in renal transplantation is not a matter of course. Nevertheless, in selected patient groups mTOR inhibitors can have a clear place. Calcineurin-inhibitor induced nephrotoxicity is a common cause of graft failure; switch to a calcineurin-inhibitor free regimen with an mTOR inhibitor can lead to stabilization or slowing down of decline in GFR. Furthermore, an increasing amount of data suggest that mTOR inhibitors have beneficial effects on the incidence of non-melanoma skin cancer and are therefore preferred as immunosuppressive drug in these patients. Moreover, it is probably effective in lymphoma and already registered for the treatment of renal cell carcinoma, both more prevalent in renal transplant recipients. If prescribed, adequate monitoring of adverse events is important. Based on the studies mentioned in Part II, next to standard measurements of kidney and liver function, differential count and cholesterol, I would recommend to monitor also pulmonary function by function tests before start and 3 monthly hereafter in the first two years. If the vital capacity or CO diffusion declines, a HRCT should be performed to exclude EIP. If proteinuria occurs or increases during treatment with an mTOR inhibitor, renal biopsy should be performed to be able to make an exact diagnosis. If mTOR inhibitors are prescribed to patients with a history of venous or arterial thrombosis, anticoagulation should be considered pending further studies.