CHAPTER 1

Introduction and Aim of Theses
Living kidney donation

Living kidney donation is the best available treatment option for a patient with end-stage kidney disease (ESRD), leading to better outcomes compared to dialysis or transplantation with a graft from a deceased donor \(^1^2\). Transplantation from a living kidney donor has become a very important modality \(^3\) and in the Netherlands more than 50% of all kidney transplantations are performed with a living donor (Doorenbosch et al, NTS Year report, 2016). Whereas the benefits of a successful kidney transplantation for the recipient are firmly established, living kidney donation could be considered in discordance with the medical ethical principle of non-maleficence, a principle that is well embedded in the Hippocratic Oath (’Primum non nocere’, Hippocratic Corpus – Of the Epidemics, Book I, section 11,5). However, from a slightly different perspective, living kidney donation may provide benefit for both a donor and recipient \(^4\) and the principle of ‘Nil prodest quod non laedere possit idem’ (Ovid, Tristium Liber Secundus, 2.265-6), meaning ‘nothing can benefit that cannot also harm’ may be more applicable \(^5\). Donor safety therefore is a major issue to be considered in any living donation program, and bears consequences on both pre-donation screening and donor follow-up \(^6^7\).

In early studies, the absolute risk of end-stage renal disease and premature mortality after living kidney donation were generally lower than the general population in most studies \(^6^8–10\), reflecting rigorous pre-donation screening and donor selection for above-average overall health status. However in recent years, the selection criteria for potential living donors have become more liberal, mainly driven by a persistent donor organ shortage \(^6\). The impact of the changes in donor acceptance policy for donor outcome is still largely unclear. The increasing mean age of accepted donors, as well as small but significant absolute increases in adverse cardiovascular and renal outcomes compared with matched non-donors that were observed in recent reports, seem to challenge the old adagio that ‘Kidney donors live longer’ \(^8^11^12\).

These developments underline the paramount importance of pre-donation risk assessment, in terms of both renal and cardiovascular risk factors as well as appropriate donor follow-up.

Changes in donor policy and potential implications

Over the past decades, the criteria for living kidney donation have gradually changed from very strict \(^13\), to more liberal \(^14^15\). Compared with two decades ago, accepted donors are now on average older, have a higher body mass index, and are and more likely to have hypertension \(^6\), all risk factors for cardiovascular and renal damage. The (usefulness of an) upper limit for donor age is subject of debate: the current standard of practice is to accept donors >18 years of age, without a fixed upper limit \(^13\). Accordingly, in recent years, the average age of accepted donors has increased in our center from 50 before 2010 to 54 after 2010 (University Medical Center Groningen; Figure 1). Older donors
are more likely to have co-morbidities such as hypertension or cardiovascular disease, and have a lower renal function than younger donors before as well as after donation\textsuperscript{11,16,17}. Shortly after donation, a compensatory increase in single kidney GFR occurs\textsuperscript{18}. This GFR increase is less prominent or even absent in older donors\textsuperscript{19}. Older donors, particularly donors over 60 years of age, have a higher risk of renal impairment (mGFR <60 ml/min) early after donation, independent of their pre-donation GFR (figure 2). On the other hand, while younger donors have a much lower 10-year ESRD risk, their life-time risk of ESRD is higher than that of older donors, due to their longer life expectancy\textsuperscript{16}. Thus, for an adequate risk assessment life expectancy has to be taken into account as well, as illustrated in Figure 1.

Other risk factors for ESRD in living donors are obesity, high blood pressure and smoking. Obesity is linked to small increase in post-operative mortality risk\textsuperscript{20} and with ESRD in living donors\textsuperscript{21}. Smoking is a known risk factor for ESRD in many populations\textsuperscript{22} and has also

![Figure 1. Donor Age per Donor Era and Life Expectancy of the general population in the Netherlands](image)

This figure shows the distribution of donor age at nephrectomy in donors before and after 2010. Donors who donated after 2010 were significantly older and the proportion of donors > 65 years old has doubled after 2010, indicating that more high-risk donors are accepted (50±11 vs. 54±11, P<0.001). For reference, the life expectancy of the general population in the Netherlands of 2016 has been added (CBS Doodsoorzaken, 2016 zorggegevens.nl). On one hand, this graph shows that we accept donors with a higher mortality risk at the time of nephrectomy. These risks may interact with the risk a donor has from nephrectomy (donation-attributable risk) and should be assessed/predicted in individual donors before donation. It also may have consequences for their follow-up. On the other hand, while the risks are higher, the estimated life expectancy of older donor is lower. This means that risk prediction tools only need to model risks for 15-20 years in a 70 year old donor, while they need to model 40-45 years in a 40 year old donor.
been described as a risk factor in living donor candidates. High blood pressure is a risk factor for kidney damage in the general population and in renal patients, but in currently accepted hypertensive donors (that is: with blood pressure well-controlled with no more than two different classes of antihypertensive drugs, and a good renal function) short-term renal function risk not elevated as compared to normotensive donors. More donors with these risk factors are accepted for living kidney donation. To be able to ensure donor safety while accepting more donors for living donation, more insight in the effects of nephrectomy on the donor is needed.

![Figure 2. Relation between donor Age and probability of having a GFR < 60 mL/min after donation](image)

In 992 donors, pre- and post-donation mGFR was measured. Donors were divided into quartiles based on their pre-donation mGFR. This figure shows the probability of having an mGFR 3 months post-donation below 60 mL/min per category of pre-donation mGFR and age.

**Glomerular filtration rate and prediction in living kidney donors**

Clearly, reliable measurement of kidney function, usually expressed as the glomerular filtration rate (GFR, unit: mL/min, often standardized for body surface area: mL/min/1.73m²), is an essential component of pre-donation evaluation. The gold-standard technique for measuring GFR (mGFR) is the calculation of the clearance of exogenous filtration markers, such as iohexol, inulin or iothalamate. These markers are completely filtrated by the glomerulus and (almost) not excreted, resorbed or metabolized by the tubular compartment, rendering them
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highly suitable for precise GFR measurement. The GFR can also be estimated by using the serum concentration of creatinine, a breakdown product of creatine phosphate that is secreted by muscles at a reasonably constant rate\textsuperscript{26}. Several formulas have been developed to estimate GFR (eGFR) from serum creatinine in combination with other parameters including sex, age and race\textsuperscript{27}. Limitations of these formulas are that the concentration of creatinine is influenced by tubular secretion of creatinine\textsuperscript{28}, body composition and muscle mass\textsuperscript{26}. Moreover, their performance is poor in populations without renal impairment, which is particularly relevant for donor screening. Renal function can also be calculated as the renal clearance of creatinine using 24-hour urine collections\textsuperscript{26}, although this method is sensitive to collection errors\textsuperscript{29}.

Given the limitations of GFR estimation, we perform measured GFR (mGFR) routinely in all living donors before and after donation in our center. While the mGFR is considered the gold standard\textsuperscript{30}, it is a laborious and expensive procedure, and therefore not universally feasible\textsuperscript{29}. In the current KDIGO guidelines for living kidney donor evaluation, estimated GFR (eGFR) is recommended as a first step for determining kidney function\textsuperscript{13}. When the eGFR is not ‘sufficient’ for a donor to be safely accepted for donation, an additional mGFR measurement is advised. However, in addition to the inherent limitations of the eGFR, it is currently unclear which eGFR values may be considered “sufficient”, in the absence of data linking pre-donation eGFR with post-donation mGFR values. As a result, donor selection varies considerably between centers, which may on the one hand put some accepted donors at risk, and on the other hand withhold potentially suitable donors from donation.

Assessment of Renal functional reserve in living kidney donation

Besides the GFR, it also is important to predict the adaptive capacity of the remaining kidney to donation, especially in donors with a ‘marginal’ GFR. To predict adaptive capacity several approaches have been developed, such as measuring of the renal functional reserve (RFR, also called reserve capacity) as the ability of a donor to increase his/her GFR in response to a vasodilating agent (e.g. dopamine). The RFR is thought to be marker of the capacity of the kidneys to adapt after nephrectomy (figure 3), resulting in a GFR higher than 50% of the pre-donation GFR. Therefore, the pre-donation RFR was thought to be a predictor of post-donation GFR\textsuperscript{31}. A study in 2006, showed that the pre-donation RFR is indeed associated with GFR 3 months after donation, but no data on the long-term predictive capacity exist\textsuperscript{32}, despite that fact that measurement of RFR was developed more than 30 years ago.

The concept of measuring RFR for predictive purposes has also been applied in clinical conditions where renal hyperfiltration is assumed to be involved in the susceptibility to renal damage, such as obesity. Conditions associated with an elevated glomerular filtration rate,
such as obesity, may “utilize” the RFR, leaving less reserve after kidney donation. This may explain the increased ESRD risk in living donors with obesity. During the first half of pregnancy, vasodilatory hormones from the placenta also cause an increased filtration of the mother. Lack of such renal vasodilation on the other hand, is a hallmark of preeclampsia. In 2015, it was found that living kidney donors have an increased preeclampsia risk. An insufficient RFR because of obesity and living kidney donation may therefore play a role in the occurrence of adverse pregnancy outcomes in young female kidney donors.

Figure 3. Renal adaptation after living kidney donation. At the time of nephrectomy, the donor single-kidney GFR is approximately 50% of its pre-donation value, corresponding to a reduction of renal mass by 50%. However, almost immediately after nephrectomy, GFR increased in an “early” hemodynamic response to 66% of its pre-donation value. This response is blunted after days-weeks, but the GFR continues to increase in a “late” phase that is hypothesized to be more structural in nature. This late renal adaptation varies on the individual donor and sometimes takes up to 15 years post-donation.

Beyond GFR: Tubular compartment

While the aforementioned measurements encompass the glomerular filtration rate, and are accordingly used as markers of glomerular function, the tubuli are essential for, among others, electrolyte and fluid homeostasis. Previously, tubular function was considered to be secondary to the GFR, however this paradigm has been abandoned when studies demonstrated that the extent of tubulo-interstitial damage was an independent and major predictor of end-stage renal disease, even stronger than glomerular function. Consequently, markers related to electrolyte homeostasis, including renal phosphate homeostasis, have been proposed as indicators of tubular function. Renal phosphate homeostasis is regulated...
primarily in proximal tubular epithelial cells by sodium phosphate co-transporters NaPi-IIa, NaPi-IIc and PiT-2\textsuperscript{38–40}. The renal tubular maximum reabsorption capacity of phosphate per unit of glomerular filtration rate (TmP-GFR) represents the maximum renal capacity to reabsorb phosphate and may reflect tubular function, thereby being a functional marker to study “tubular quality”. As such, the TmP-GFR could be a useful tool to evaluate pre-donation tubular function. Similarly, post-transplant renal phosphate handling, reflected by the development of hypophosphatemia, could reflect tubular function and thus predict renal outcomes. Whether these markers could potentially have additional value for predicting graft outcomes in recipients remains unclear.

**Beyond GFR: Towards predicting donation-attributable risk**

The current KDIGO living donor guidelines advise to estimate an individual donor’s donation-attributable risk of developing end-stage renal disease after donation\textsuperscript{13} (figure 4). However, it remains unclear how to implement this recommendation in daily clinical practice, in the absence of a universal risk calculator. The mGFR and donor age, the two

![Projected Lifetime Risk of Kidney Failure, %](image)

- Transplant program's threshold for acceptable postdonation risk
- Transplant program declines donor candidate
- 1) Transplant program accepts donor candidate
- 2) Candidate decides whether to proceed

**Figure 4. Demographic-related, aggregated and donation-attributable risk for living kidney donors**

Examples of the different parts of donor risk according to the 2017 KDIGO living kidney donor guideline. The black dotted line represents the transplant program’s threshold for acceptable risk after donation.
most important determinants of post-donation renal function\textsuperscript{16}, can predict only 68\% of the variation in mGFR at five years post-donation, underlining the need to identify additional factors that could facilitate personalized risk assessment, including both non-modifiable (age, disease history) and modifiable risk factors (overweight, blood pressure, lifestyle) These factors could include clinical parameters as well as lifestyle-related factors (e.g. diet, physical activity) and novel biomarkers, which should be related to long-term outcome parameters. This calls for detailed donor work-up, preferably supported by a large biobank of donor material (blood, urine, skin, biopsies), questionnaires and physical tests, together with structured long-term follow-up.

It is important to realize that renal function should not be the sole outcome of post-operative donor evaluation: evaluation of cardiovascular and overall risk, as well as donor well-being are highly important as well. Quality of life, after donation is importantly adversely influenced by the presence of pain, even despite a general increase in quality of life elicited by kidney donation in itself\textsuperscript{4,10,41}. Structural assessment of quality of life, including the presence of both short- and long-term post-operative pain in donors is an essential constituent of living donor follow-up.

**Aim of the thesis**

The aim of this thesis is to contribute to ongoing efforts to improve donor evaluation from a perspective of donor safety, with a primary focus on renal risk assessment. **Part 1** is focused on available methods of renal function evaluation, including commonly used eGFR formulas, before and after donation. **Part 2** addresses renal parameters other than GFR: the potential role of presumed markers of tubular function, as well as a potential approach to develop and validate novel biomarkers to predict donation-attributable risk for the individual donor.

**Outline of the thesis**

**Part one: GFR in living kidney donation**

Part one of this thesis focusses on the validity of established methods to estimate glomerular filtration rate. In **chapter two** we address whether the pre-donation eGFR can be used for prediction of post-donation mGFR and whether pre-donation eGFR cut-off values can be defined to yield a matching post-donation single kidney mGFR. In **chapter three** we aim to evaluate the predictive value eGFR for true kidney function (mGFR) after donation.

**Chapter four** focusses on the use of the RFR as an additional predictor of the mGFR. In this chapter we focus on the changes of RFR after donation in overweight vs. non-overweight young women of child-bearing age, since this may have implications for pregnancy after
living kidney donation. In chapter five, we look at the predictive capacity of the pre-donation RFR in donors in more detail, and investigate if it can predict mGFR after donation and the mGFR increase after a reduction of renal mass by approximately 50%.

Part two: Living donation beyond the GFR
Part two of this thesis focusses on renal parameters other than GFR; the use of tubular compartment function in donors and the development of novel biomarkers to predict donation-attributable risk for the individual donor. In chapter six, we study whether pre-donation tubular phosphate handling in the donor can predict renal function in the recipient at one year post-transplantation. Chapter seven further addresses the prognostic value of phosphate handling, specifically the development of hypophosphatemia, in kidney transplant recipients. Chapter eight addresses another important outcome after living kidney donation: chronic pain. We investigated the prevalence of post-donation chronic pain in living kidney donors, and the accompanying long-term analgesic use.

Overall, it is apparent that more insights are needed in the effects of nephrectomy on the living donor. A proposed approach for the structured collection of biomaterials and functional and clinical data is described in chapter nine. At the UMCG, we recently established TransplantLines: a biobank study covering numerous aspects of solid organ transplantation, including but not restricted to, kidney donation. TransplantLines may serve as a basis for hypothesis-generating studies that yield new insights in, among many other things, donation-attributable risks. Ultimately, such information likely will contribute to improved living kidney donor screening and follow-up, and thereby to qualitatively and quantitatively improved outcomes after living kidney donation.
References


PART ONE
GFR in Living Kidney Donation