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BIOLOGICAL AGEING AND KIDNEY TRANSPLANTATION

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BIOLOGICAL AGEING AND KIDNEY TRANSPLANTATION
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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Dedication

To my children Nellie, Adam and Gustav,
To family and friends

POPULAR SCIENCE SUMMARY OF THE THESIS

Kidney disease is a “silent” disease, affecting patients with clinical symptoms in a late stage of the disease. The kidneys have several different tasks; regulation of fluid and electrolyte-balance, correction of acid-base balance and production of erythropoietin which helps maintain adequate blood levels. The kidneys are also important for the calcium-phosphate and bone metabolism. Patients affected by chronic kidney disease in an advanced stage typically present with low hemoglobin, metabolic acidosis, Vitamin-D deficiency, low calcium-levels in the blood, excess water in the body, and fatigue due to accumulation of uremic toxins. The kidneys have an impressive reserve capacity, allowing healthy individuals with a normal kidney function to donate one of the kidneys, without any of the above symptoms. When the kidneys are affected by a disease however, such as diabetes, inflammatory kidney disease or hypertension leading to a decline in kidney function, symptoms and secondary effects become evident. Chronic kidney disease is associated with premature cardiovascular disease with an increased risk closely linked to every stage of kidney disease. Heart infarction, heart failure and sudden death are common in patients with kidney failure. Osteoporosis, fatigue, cognitive decline, muscle wasting, and depression are other common clinical manifestation of kidney failure. These manifestations are strongly associated with increasing age in the general population. Kidney failure is characterized by a faster acceleration of biological age. We do not know how different kidney failure treatments affect the rate of biological age decline (i.e., biological ageing) or which method should be used to measure biological age in this patient population.

The first study showed that the extent of comorbidity predicts patient survival after kidney transplantation, and it could serve as a pseudo-marker for biological ageing in the clinical setting. We found that the extent of comorbidity predicts patient survival with even higher sensitivity than the chronological age in elderly kidney transplant recipients. By using a risk score, Charlson Comorbidity index, we could identify patients with 4,6 times higher risk of death, ten years after transplantation. Thus, if the risk score is used preoperatively, the clinical pre-operative evaluation can be improved. By introducing an objective measurement, the risk of inadequate and subjective evaluation before kidney transplantation may be diminished.

In the second study, we examined vascular ageing and the risk of cardiovascular events such as heart infarction, stroke, peripheral vascular disease, and death depending on extent of calcification in patients´ arteries. The coronary arteries were examined by computed

tomography and one of the abdominal arteries (arteria epigastrica) was examined by a pathologist who graded the extent of calcification in the arteries. We found that moderate to severe calcification in arteria media predicted future cardiovascular events independent of other risk factors. Patients who were not eligible or did not receive a kidney transplant had a high burden of calcification in the coronary arteries and patient survival was very poor after ten years. As patients with minor or no calcification in arteria epigastrica at time of surgery had a very low risk for cardiovascular events and death it is possible to identify low risk patients by this method.

In the third study, we investigated different methods of measuring biological age by using epigenetic clocks, phenoage and skin autofluorescence. We found that patients with kidney failure have an accelerated ageing compared to a population-based control group. We also found that whereas kidney transplantation (and almost restored kidney function) mitigated accelerated ageing, dialysis treatment did not. Our findings are consistent with the notion that kidney failure accelerates the biological ageing process.

In the fourth study, we found that a circulatory DNA footprint of the gut bacteria genome indicates that patients with chronic kidney disease have an impaired intestinal integrity. Bacteria or fragments of bacteria may translocate into the blood which likely contributes to the low-grade inflammation common in this patient group. Kidney transplantation did not normalize the circulatory DNA footprint of the gut bacteria composition.

Taken together, patients with advanced chronic kidney disease are characterized by signs of early vascular and biological ageing. Scoring of the extent of vascular calcification identify high- and low-risk patients after kidney transplantation. While kidney transplantation may mitigate age acceleration we found no signs of normalization of the circulatory DNA footprint one year after transplantation. Novel treatment strategies that target the gut microbiome and the vascular calcification process should be tested in patients undergoing kidney transplantation.

ABSTRACT

The aim of this thesis was to investigate the impact of biological ageing in patients with chronic kidney disease, the effect of kidney transplantation on biological ageing and long-term outcome in patients with kidney failure. We used the national Swedish renal registry (SNR), ScandiaTransplant´s database YASWA, patient records and different methods to analyze biological ageing and circulatory microbiota to address this aim.

In study I we included a national cohort of elderly kidney transplant recipients to investigate which preoperative risk factors are associated with 1, 5 and 10-year patient and graft survival. Kidney transplantation in the elderly is becoming more common worldwide, but a global standardized preoperative evaluation does not yet exist. All patients >60 years of age (n=747) which were transplanted in Sweden between 1st of Jan 2000 and 31st of Dec 2012 were included, retrospectively. We found that 5-year patient survival was not inferior in patients ≥70 years compared to patients 65-69 years and furthermore that Charlson comorbidity index (CCI) ≥ 7 was associated with a 4.6 times higher risk of death after 10 years compared to CCI<4 (chronological age excluded). The mortality risk was lower in patients with living donors and in female recipients.

In study II we studied vascular ageing, measured as coronary artery calcification and medial calcification in arteria epigastrica as predictor of all-cause mortality and cardiovascular events (CVE) in 342 patients with kidney failure. Median follow up time was 6.4 years. Medial calcification was as good predictor as coronary artery calcification, for CVE and mortality. By dividing patients in groups of low or high grade of medial calcification we found significant differences in risk of CVE (5 % vs 28%) and death (1.6% vs 14.9%), 6.4 years after KT.

In study III biological age was measured with skin autofluorescence, epigenetic clocks and Phenoage in three groups: patients with kidney failure receiving a LDKT, patients remaining in dialysis, and a population-based control group. We found signs of accelerated biological ageing in patients with kidney failure compared to the control group. This acceleration continued in patients remaining on dialysis but was mitigated in the patients receiving a KT corroborating the hypothesis of accelerated ageing in patients with kidney failure.

In study IV we investigated the circulatory microbiome in patients with CKD stage 3-4, incident dialysis patients and in LDKT recipients. Gut dysbiosis seems to be more pronounced in incident dialysis patients than in patients with CKD stage 3-4 and LDKT recipients. Kidney transplantation did not restore circulatory microbiome, and the core microbiome remained essentially the same, one year after KT.

LIST OF SCIENTIFIC PAPERS

I. **Erlandsson H**, Qureshi AR, Scholz T, Lundgren T, Bruchfeld A, Stenvinkel P, Wennberg L, Lindnér P. Observational study of risk factors associated with clinical outcome among elderly kidney transplant recipients in Sweden - a decade of follow-up. *Transpl Int*. 2021 Nov;34(11):2363-2370.

II. **Erlandsson H**, Qureshi AR, Ripsweden J, Haugen Löfman I, Söderberg M, Wennberg L, Lundgren T, Bruchfeld A, Brismar TB, Stenvinkel P. Scoring of medial arterial calcification predicts cardiovascular events and mortality after kidney transplantation. *J Intern Med*. 2022 Feb 2.

III. Ognian Neytchev[#] and **Helen Erlandsson[#]**, Anna Witasp, Louise Nordfors, Abdul Rashid Qureshi, Ken Iseri, Hokuto Morohoshi, Colin Selman, Thomas Ebert, Karolina Kublickiene, Peter Stenvinkel, Paul G. Shiels. Accelerated uremic ageing is mitigated after kidney transplantation, but not dialysis. Submitted manuscript. [#]=shared first authorship

IV. Hannah Craven[#] and **Helen Erlandsson[#]**, Dagmara McGuinness, David McGuinness, Abdul Rashid Qureshi, Denise Mafra, Umer Z. Iljaz, Peter Bergman, Peter Barany, Paul G Shiels, Peter Stenvinkel. A circulatory footprint of the core microbiome does not normalize after kidney transplantation. In manuscript. [#]=shared first authorship

Scientific papers not included in the thesis:

Pippias M, Jager KJ, Caskey F, Casula A, **Erlandsson H**, Finne P, Heaf J, Heinze G, Hoitsma A, Kramar R, Lempinen M, Magaz A, Midtvedt K, Mumford LL, Pascual J, Prütz KG, Sørensen SS, Traynor JP, Massy ZA, Ravanan R, Stel VS. Kidney transplant outcomes from older deceased donors: a paired kidney analysis by the European Renal Association-European Dialysis and Transplant Association Registry. *Transpl Int*. 2018 Jul;31(7):708-719.

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LIST OF ABBREVIATIONS

AVC	Aortic valve calcification
AGEs	Advanced glycation end-products
BPAR	Biopsy proven acute rejection
CAC	Coronary artery calcium
CCI	Charlson comorbidity index
CKD	Chronic kidney disease
CKD G5D	Chronic kidney disease group 5 dialysis
CKD-MBD	Chronic kidney disease -mineral bone disorder
CVD	Cardiovascular disease
CVE	Cardiovascular events
DDKT	Deceased donor kidney transplantation
DKD	Diabetic kidney disease
DNAm	Deoxyribonucleic acid methylation
ECD	Extended criteria donor
ESKD	End stage kidney disease
eGFR	Estimated glomerular filtration rate
FGF-23	Fibroblast growth factor 23
FMOs	Hepatic monooxygenases
KRT	Kidney replacement therapy
KT	Kidney transplantation
LDKT	Living donor kidney transplantation
NRF2	Nuclear factor erythroid-2 factor 2
PEW	Protein energy wasting
PTDM	Post transplantation diabetes mellitus
SAF	Skin autofluorescence
SASP	Senescence associated secretory pathway

SCFA	Short chain fatty acids
SNR	Swedish renal registry
TMA	Trimethylamine
TMAO	Trimethylamine-N oxide
UNOS	United Network for Organ Sharing

1 INTRODUCTION

Chronic kidney disease is an age-associated disease and has a number of clinical manifestations associated with ageing in the general population [1]. Patients with chronic kidney disease have a decreased life expectancy, starting at eGFR <60ml/min/1,7 m^2, compared to the general population [2]. This premature survival reduction is caused primarily by cardiovascular disease (CVD). At all stages of the disease, emphasis should be put on stabilization of kidney function and preventing progression as well as treatment of cardiovascular risk factors. The risk for cardiovascular disease and mortality is increased proportionally with decreasing kidney function [1,3]. There are several existing or promising treatments for different causes of kidney failure, such as SGLT2-inhibitors in diabetic kidney disease (DKD) and CKD, GLP1-analoges in DKD, tolvaptan in adult polycystic kidney disease (ADPKD), budesonide in IgA-nephropathy, and complement-inhibitors in aHUS, IgA-nephropathy and C3 glomerulopathy.

When all therapeutic options are exhausted and kidney failure is evident, focus should shift to offering patients the optimal kidney replacement therapy (KRT) to preserve physical function and longevity. A minority of patients referred to nephrology-clinics are offered conservative treatment due to an advanced age, extensive comorbidity or personal preference. The goal, in the majority of cases, is to prolong survival and always improve quality of life in patients with kidney failure. Kidney transplantation (KT) provides superior patient survival compared to dialysis [2] . Unadjusted annual mortality in the KT group was 3.1 % and in dialysis-patients 19.9 %, in Sweden in 2020. Differences in the treatment groups exist; mean age in the transplanted group was 56 years vs 66 years in the dialysis group in Sweden 2020 (fig 1). As in most research fields, it is utterly important to adjust for confounders when comparing survival between treatment groups, and ideally, patients accepted for the waitlist but remaining in dialysis should be compared with matching patients receiving a kidney transplantation.

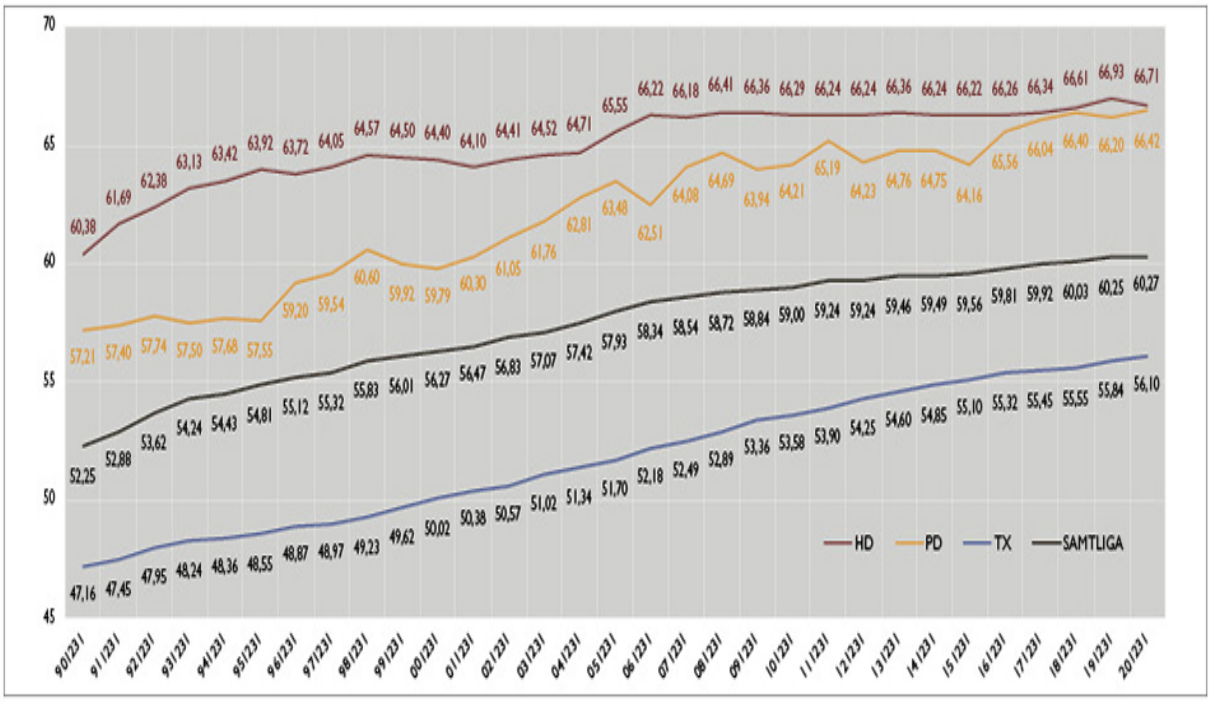


Fig 1. Mean age by year during the period 1990 to 2021, stratified by treatment modality. Reprinted with permission from SNR.

From an international perspective, waitlist times are relatively short in Sweden (median 10.7 months if PRA<80%). This creates difficulties to include a sufficient number of comparable waitlisted (not transplanted) patients, given that survival analysis ideally should be performed after a long time-period.

Kidney transplantation has become more common during the last decades globally, which in part reflects the increase of kidney failure in the world. In Sweden, patients ≥70 years of age were not considered eligible for kidney transplantation before the year of 2000. Since then, an increasing number of KT in the elderly have been performed in the country (fig. 2). This increase in elderly KT recipients emphasizes the need for greater knowledge and better understanding of which factors influence outcome in elderly kidney transplant recipients. Considering the rise in mean age of patients receiving KRT overall in Sweden (fig1), the same rise is expected in the kidney transplant-group.

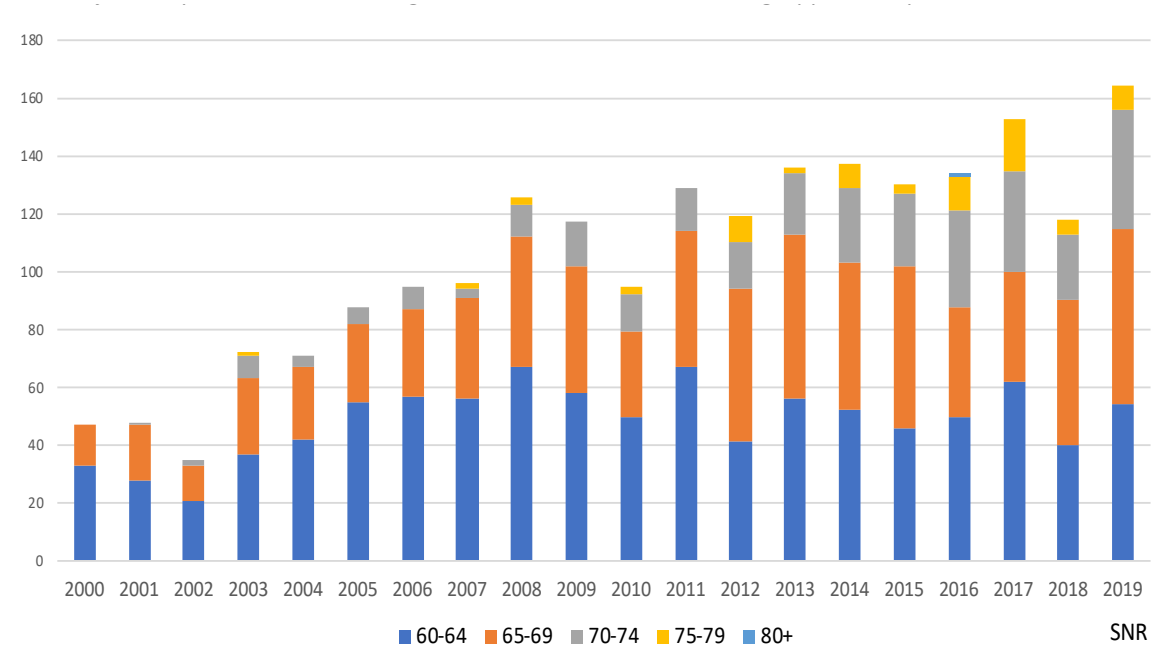


Fig 2 . Kidney transplant recipients above 60 years of age by year. 2000-2019, stratified by age-group. Data generated from the Swedish Renal Registry (SNR), printed with permission from SNR.

Some may be concerned that an increase in transplantation to the elderly will result in longer waitlist time for other adult patients, or that younger patients may not receive a kidney transplant due to the allocation to elderly individuals.

When analyzing data from SNR the last decades, evidence of this cannot be found. Waitlist times in all patients ≥18 years who are not highly immunized (PRA<80%), did not increase (fig 3).

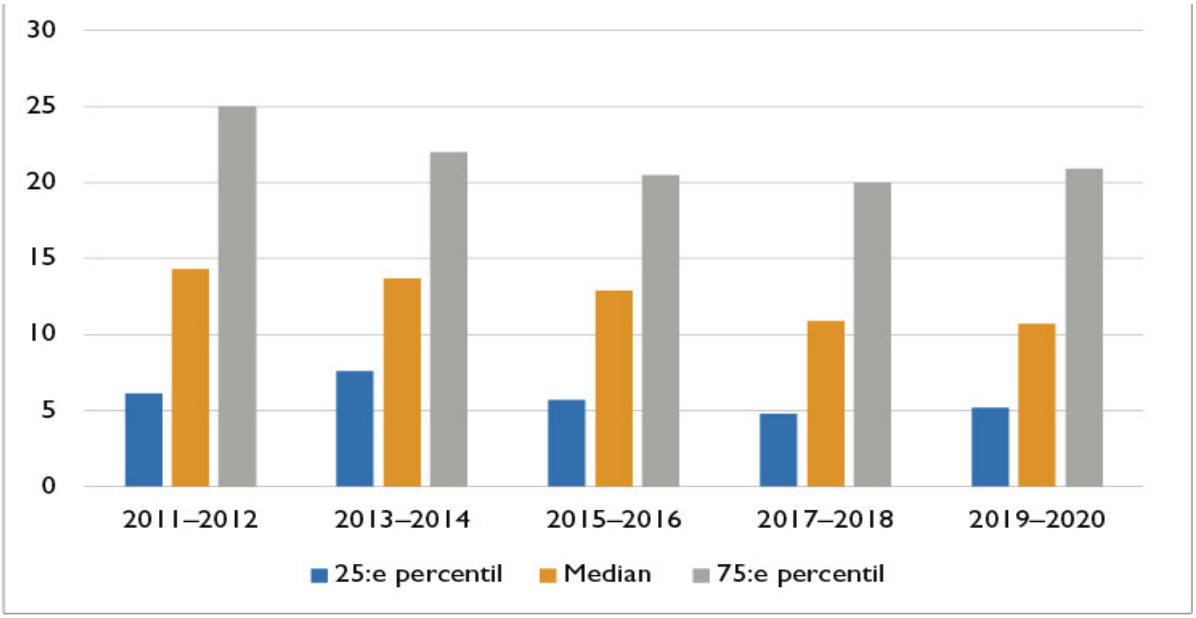


Fig 3. National waitlist times (months) until transplantation, 2011-2020. Reprinted with permission from SNR.

During the year of 2005, when only six patients ≥70 years received a KT in Sweden, the waiting time was similar to 2019, a year when 49 patients ≥70 years of age received a KT (table 1).

Year	25th percentile	median	75th percentile	KT in 60-64 years	KT in 65-69 years	KT in ≥ 70 years	KT total in ≥60 years
2005	4.1	9.7	18.7	55	27	6	88
2019	5.2	10.7	20.9	54	61	49	164

Table 1. Waitlist time in all patients >18 years in Sweden in median, 25th and 75th percentile, during the years 2005 and 2019. The annual numbers of patients receiving a kidney transplant ≥ 60 years of age almost doubled during this time period (n=88 vs n=164). Patients highly immunized (PRA>80 %) are excluded in data of waitlist time [4]. Data extracted from SNR.

Despite an increasing number of transplanted patients ≥ 60 years of age in Sweden, waitlist times remained essentially unchanged, due to a higher overall transplantation rate. During the early years of 2000, approximately 300 KT´s were performed annually in Sweden, which increased to approximately 450 transplantations annually during 2017-2019, before the Covid-pandemic (fig 4).

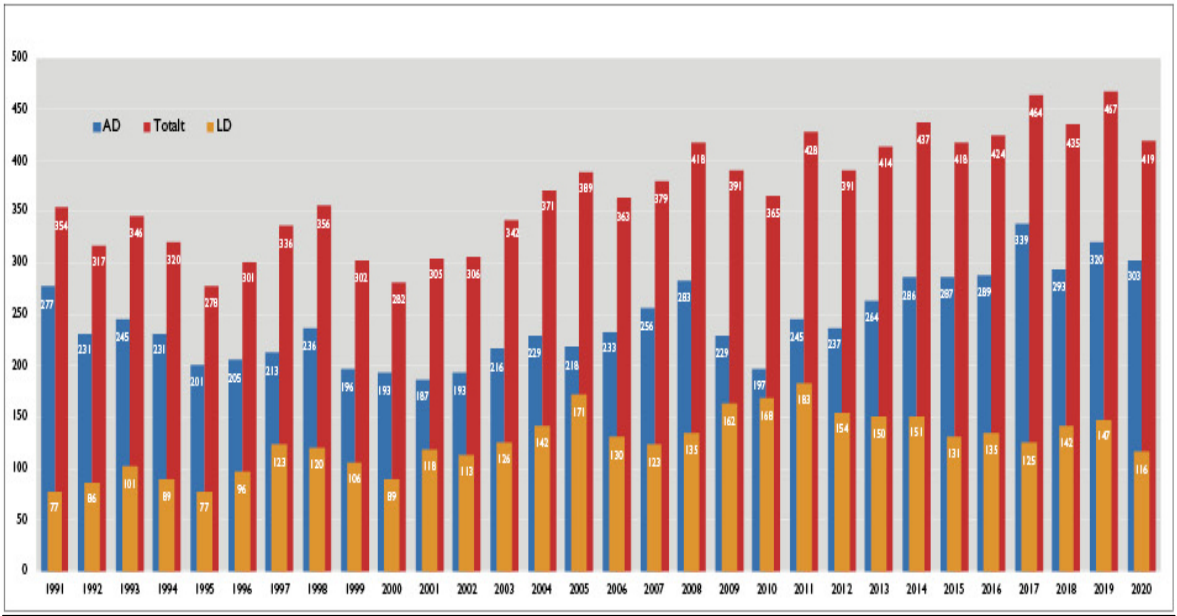


Fig 4. Number of transplants (kidneys) each year in Sweden, 1991-2020. In total (red bars), AD (deceased donor, blue bars), LD (living donor, yellow bars). Reprinted with permission from SNR.

Since the year 2000 the number of patients with a functioning graft has increased by 90% and patients in dialysis by 42 % in Sweden [4]. Overall, patients with KT´s now constitute 60 % of patients with KRT, in Sweden.

It is further encouraging that despite a significant higher number of prevalent KT patients in 2020 compared to in 2000 (6224 vs 3350 patients) we do not see a corresponding rise in death censored graft loss, presented as yellow bars in fig 5. In 2020, only 107 of 6224 patients lost their graft function which constitutes 1.7% of all kidney allografts according to data from SNR[4].

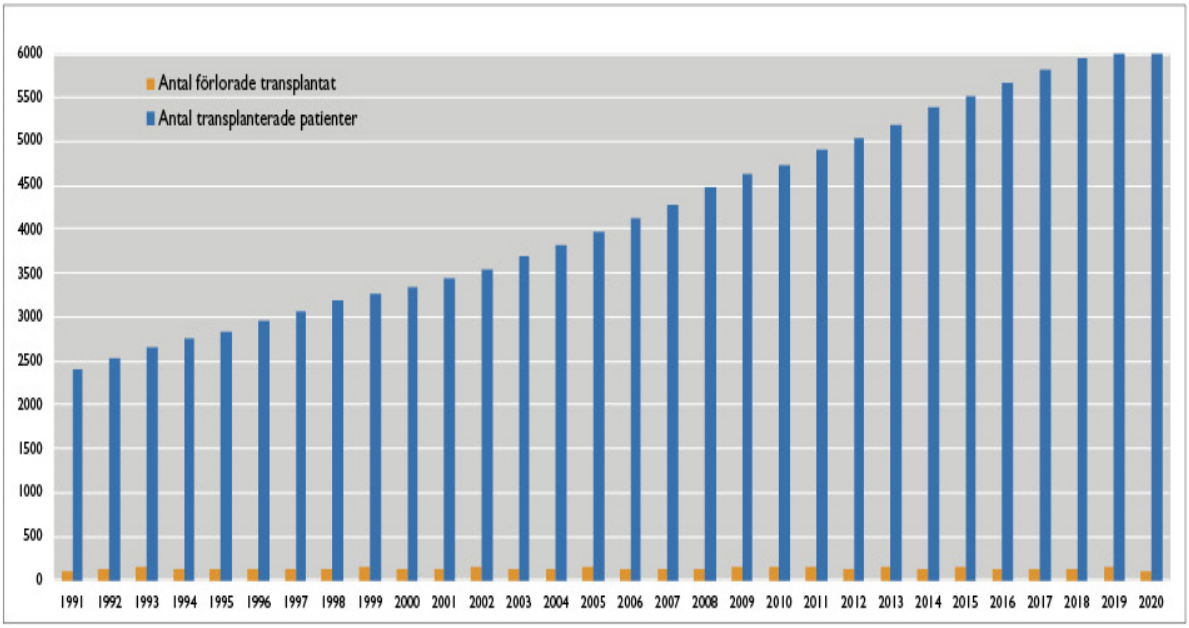


Fig 5. Number of patients in Sweden with a functioning kidney graft at the end of each year (blue bars) and the number of grafts lost annually (yellow bars), 1991-2020. Reprinted with permission from SNR.

The annual mortality rate remained stable (around 3 %) the last 30 years in the transplanted group, although mean age has risen from 47 to 56 years in the group during the same time-period (fig 6), and a doubling of annually transplanted patients >60 years the last 15 years has occurred.

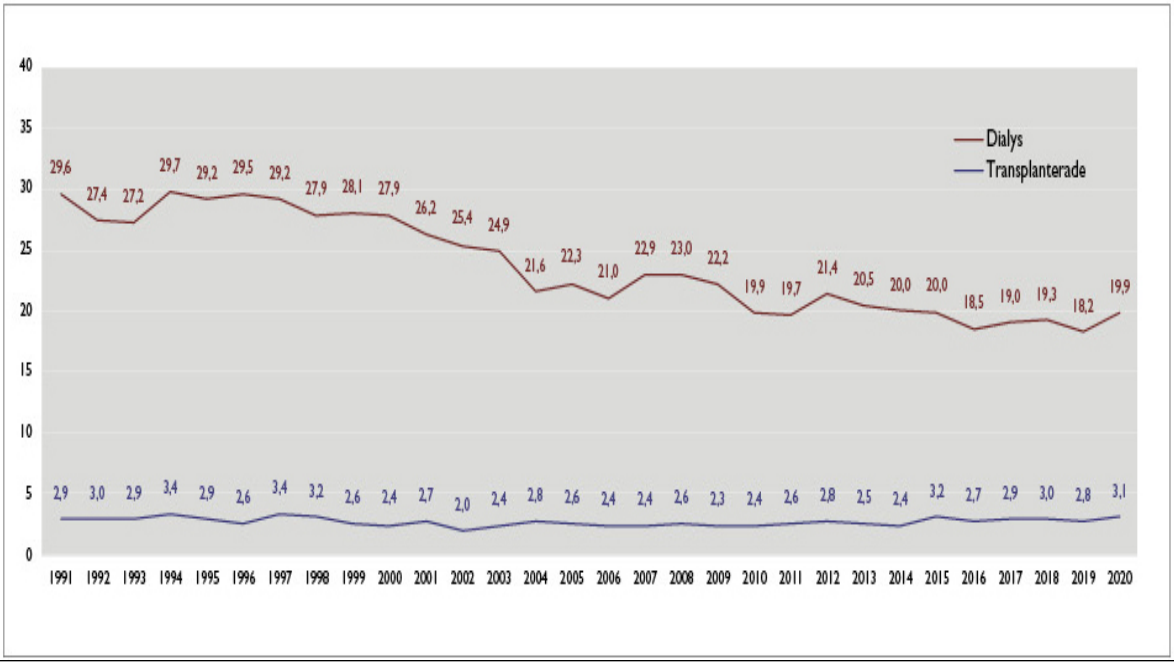


Fig 6. Annual mortality rate (%) in dialysis (red graph) and after kidney transplantation (blue graph) during the last 30 years. Reprinted with permission from SNR.

To conclude, the absolute number of death censored graft losses remains the same according to data from SNR, even though the number of KT patients living with a functioning graft has risen with 90 % the last 20 years. Annual mortality rates are essentially the same, despite an increase in mean age from 47 to 56 during the last 30 years. The introduction of transplantation to patients ≥70 years after the year 2000, which constituted 10.5 % of the total percentage of transplanted patients in 2019, has not affected mortality, death censored graft survival or waitlist times in Sweden so far. These are truly great improvements and encourages us to investigate human ageing more to see if we can make further progress in patient survival and quality of life in the ESKD-population.

Background

Kidney transplantation and biological age

Chronic kidney disease (CKD) leads to an increased risk of several diseases associated with ageing and share common features with the aging process. Osteoporosis, CVD, cognitive dysfunction, frailty, and depression are common in patients with ESKD, as in elderly patients in general. Accelerated vascular disease and muscle wasting are manifestations of premature ageing in CKD [5]. The biological process of ageing seems to be accelerated in CKD. One contributing cause is diminished filtration of uremic toxins in patients with kidney failure which speeds up accelerated ageing through different processes. In addition, patients with CKD often suffer from a complex multimorbidity, which might act as a catalysator for biological ageing. Numerous studies have shown that patients with CKD have a higher mortality risk than the general population [2,6]. The internal uremic milieu characterized by uremic toxins, inflammation, oxidative stress, hyperphosphatemia, disturbed circadian rhythm and sympathetic-vagal imbalance are potential pathophysiologic factors which may contribute to senescence and the progress of ageing in CKD patients [5]. The uremic phenotype encompasses all the established hallmarks of ageing; i.e. genomic instability, epigenetic alterations, cellular senescence, loss of proteostasis, mitochondrial dysfunction, stem cell exhaustion, telomere attrition, deregulated nutrient sensing and altered intracellular communication [8] . Ageing has been described as a loss of physiological integrity leading to increased risk of death or impaired function and affects the function of all organs. It is a systemic process and impairs response to physiological stress [8], which probably partly explains why elderly are more vulnerable to surgery, acute complications such as infections and side-effects of medication, compared to younger patients.

Ageing leads to an accumulation of senescent cells in tissues. Senescent cells impose a growth arrest or cell cycle arrest, followed by a stop of replication of damaged or old cells. Senescent cells do not divide or go into apoptosis and seems to irreversibly go into replicative arrest. Premature senescence can be an adequate cell response to proteomic, genomic and epigenomic damage because it enables prevention of mutation accrual and development of cancer [9]. Cellular senescence leads to reactive oxygen species (ROS) generation, metabolic shifts, increased protein-synthesis, and activation of senescence associated secretory phenotype (SASP). The activation of SASP leads to cellular secretion of factors leading to stem cell dysfunction and proteases which are damaging extra-cellular matrix molecules [13]. The activation of SASP also leads to the secretion of pro-inflammatory mediators, cytokines, and chemokines which attracts immune cells.

The attraction of immune cells is of interest in the kidney transplant setting since allografts from elderly donors have a higher rejection rate than kidneys from young donors, independent of recipient age-group, according to data from the US including 108 000 KT recipients [10]. A possible mechanism might be an accumulation of senescent cells in aged allografts, causing activation of SASP which attract immune cells to the graft. Accumulation of T-cells are seen in allografts with rejection. Aged allografts are also more vulnerable to ischemia-reperfusion injury caused by the donation and transplantation procedures. Donor age affects graft function at 1 year after transplantation [11]. In addition, allografts from older donors usually have more interstitial fibrosis and tubular atrophy than allografts from younger donors which further supports the principle of "age-matching" of kidney allografts [12]. Due to immune senescence, elderly recipients still have a lower rejection rate than younger recipients even though they often receive allografts from older donors [10].

With ageing, senescent cells accumulate in tissues and have been shown to be associated with several chronic burdens of lifestyle diseases [7,13]. Identification and quantification of senescent cells is challenging, which limits the understanding of their association to ageing and disease [14]. Biological age is a term often used by professionals in health care but is not well defined in the clinical setting. The term is sometimes used when a patient is the subject of a high-risk intervention, such as middle or high-risk surgery or intensive care to estimate patient prognosis in advance. If the sum of comorbidities and/or frailty can be used as a surrogate marker for biological age, risk evaluation can be more systematic by using a correct model compared to an evaluation based on the clinician´s personal experience.

During the Covid-19 pandemic, the susceptibility to severe disease and death was clearly increased in elderly, males, diabetics, ex-smokers, CKD, organ transplanted, obese, and in patients with CVD and hypertension [15,16]. Immunosuppressive agents and organ transplantation were prominent risk factors, not only for severe disease and death, but patients also had a higher risk of lack of humoral response after vaccination against Covid-19. It has been argued that patients with an underlying low-grade inflammatory state due to chronic disease have an increased risk of entering the dangerous uncontrolled inflammatory response (cytokine-storm) because of the SARS-Cov-2 virus. This may in part explain the increased risk of severe disease and death in patients with CKD and other comorbidities [17]. The Covid-19 risk of severe disease and death was clearly associated with CKD, and the risk increased with every stage of CKD [15]. It is also known that elderly >70 years suffer from immune senescence affecting all types of immune cells, which make them

vulnerable to infections [18,19]. Patients receiving immunosuppressive treatment due to solid organ transplantation typically present a weaker immune response when vaccinated leading to weaker or absent protection after vaccinations [20,21]. With every dose of repeated vaccination against Covid-19, there has still been an increase in antibody response in KT recipients in a sizeable group of patients [22,23].

Even though KT recipients, and elderly, are at higher risk for infections and death due to infections compared to the general population the risk is still lower than for patients remaining in dialysis, according to a large-scale study from the US [19,24]. This is important to keep in mind when evaluating elderly patients for KT, and when we advise elderly patients when choosing between dialysis and KT.

Measurement of biological ageing

Different individuals with the same chronological age display a range of biological ages. An individual who is 60 years old and physically active, fit, of normal weight, non-smoking, practising healthy dietary habits and free from diseases, has a remarkably different appearance in the clinical setting compared to a patient with complex multimorbidity, being physically inactive, obese, and smoking even though they share the same chronological age. For the trained clinical eye this difference is clear and evident, but we usually do not measure this difference in a standardised or systematic way. We know that patients with at higher biological age are less resilient and more prone to complications after surgery and acute illness. We lack reliable clinical diagnostic tools or methods to measure this susceptibility as well as suitable intervention strategies. It is a goal in geroscience to find reliable biomarkers of ageing which can predict onset of disease, decline of function and death on an inter-individual level [25].

Patients with a high biological age need swift diagnosis, intensive follow-up during acute illness, and prompt treatment when an impairment in the clinical condition is evident, due to their lack of margins. Early referral to intermediate or intensive care units might be of great importance in this population, to prevent a viscous circle of critical illness, for example septic shock leading to multi-organ failure and death. This would offer a possible way to improve outcome in patients with a high biological age. If a clinical tool for measurement of biological age was available, it could be used to identify vulnerable patients. In research a relevant biomarker of biological age could enable measurement of effects of interventions on biological age, at an earlier timepoint than when traditional hard endpoints are expected, such as cardiovascular events or mortality.

Epigenetic clocks

In 2013, researchers at UCLA in the US and University of Sichuan in China, developed algorithms to measure biological age by analysing DNA-methylation (DNAm) patterns. It has become evident that methyl groups bind to our DNA at specific regions, following a specific pattern of hypo and hypermethylation, as time goes by [26,27]. The accumulation of methyl-groups in our DNA regulates gene-activity. By developing algorithms, it became possible to calculate a person's epigenetic age from samples from blood or tissue [25, 28-30]. Steven Horvath who developed the DNAm clock (Horvath's clock) proposed that "DNA methylation age measures the cumulative effect of an epigenetic maintenance system" [28]. Since then, several epigenetic clocks have evolved, with the goal to develop a method which measures biological ageing and predicts morbidity and mortality with a higher precision than chronological age [31].

In 2018, Levine el al [25] developed a second generation of DNAm clock, which included ten clinically relevant parameters in the process. Well established research cohorts, such as NHANES III and IV, the Framingham Heart Study (FHS), Women's Health Initiative (WHI), the Normative Aging Study (NAS) and the Jackson Heart Study (JHS) were used in the development of the second-generation epigenetic clock, which showed a greater predictive value of mortality than the former Hannum and Horvath clocks [25]. In a first step, data from NHANES III and IV were used to develop a multisystem estimate of phenotypic age, so called "Phenoage". Validation of the clinical markers´ association with all-cause mortality, cause specific mortality, physical function and co-existing disease count was performed. The ten parameters with the strongest associations where then used to train a composite DNAm clock which incorporated the phenotypic parameters with the epigenetic clock, called DNAm Phenoage [25].

Phenoage

The parameters which showed the greatest association´s in the multisystem estimate were chronological age, serum-glucose, creatinine, C-reactive protein, albumin, alkalic phosphatase, red cell distribution width (RDW), red cell mean volume (RCV), leukocyte count, and lymphocyte percent. In the study by Levine el al [25], individuals that were 50 years old and the fastest agers had a predicted lifespan of 81 years, average agers 83.5 years, and slow agers 86 years, in a mortality prediction by DNAm Pheno Age. Importantly, DNAm Phenoage was associated with several pro-inflammatory pathways as increasing interferon (IFN) signaling, NF-Kappa B and in addition impaired DNA damage recognition and repair. IFN signaling pathways have been shown to be mediators in cellular

senescence and markers of DNA-damage [32] . This finding strengthens the concept of low-grade inflammation being a driver of biological ageing [33-35].

Levine el al [25] concluded however that Phenoage based on the ten clinical parameters alone, was more sensitive in prediction of mortality than combined in the DNAm Phenoage clock. They concluded that clinical parameters still serve as the best tool in clinical situations, but epigenetic clocks, and further development of them is valuable in research [25]. DNAm clocks could also serve as an important tool when measuring the effect of various clinical interventions on biological age in children, extremely healthy individuals and young adults who do not present with elevated CRP, creatinine, serum-glucose etc. [25,36]. In a cross-sectional analysis, accelerated DNAm Pheno ageing was associated with smoking, systolic blood pressure, triglyceride, BMI, waist-to-hip ratio, insulin and CRP. Conversely DNAm Pheno ageing was negatively associated with educational level, a proxy of intake of fruit and vegetables, HDL-cholesterol, exercise and income [25] .

Kidney transplantation and vascular ageing

Atherosclerosis is independently associated with increased chronological age [37] and the progression of atherosclerosis in the general population is clearly age-associated. Vascular ageing is caused by progressive atherosclerosis, endothelial dysfunction, and arterial stiffness [37]. Premature atherosclerotic plaque formation is a sign of biological ageing, since atherosclerotic plaques share common features with senescent cells, epigenetic changes, DNA damage and cells in growth arrest especially in vascular smooth muscle cells [38] . The activation of SASP leads to pro-inflammatory cytokine production and various other plaque destabilizing factors [39] .

The risk of cardiovascular events increases with declining kidney function [40]. Progression of atherosclerotic disease is more rapid in dialysis patients than in the general population and compared to KT recipients [41-48]. The cause is likely multifactorial and hemodynamic fluctuations caused by dialysis, fluid overload, uremic toxins, oxidative stress, down-regulation of inhibitors of calcification, chronic low-grade inflammation, gut dysbiosis and mineral bone disease disorder (CKD-MBD) may all contribute to the rapid progress of atherosclerosis and arteriosclerosis. CKD-MBD, high FGF23, hyperphosphatemia and Vitamin D deficiency is associated with CVD and mortality [46, 49-52]. Hyperphosphatemia is a common finding when kidney function declines due to decreased urinary excretion of phosphate and hyperparathyroidism. Phosphate forms crystals with calcium, so called hydroxyapatite-crystals which contributes to progression of atherosclerosis. Calciprotein particles (phosphate, calcium, and certain proteins) affect

CKD-MBD, inflammation and cardiovascular disease [53]. Conventional, so-called Framingham, risk factors contribute as well.

Chronic kidney disease is associated with progression of coronary artery calcification [41] . The risk of cardiovascular events and mortality initially increases during the first three months after KT but thereafter decreases compared to staying on the waitlist [42,54]. After KT, kidney function usually improves significantly, hypertension and circulatory effects of dialysis normalize. Despite these physiological improvements, multiple studies have shown that patients undergoing KT increase their coronary artery calcification and aorta calcification after KT but at a slower rate than patients on dialysis [41,43,44,55-58] . It seems like Patients with pre-existing coronary artery calcification seem prone to continuous progress of calcification whilst patients with no signs of calcification at baseline can continue years without any calcification [41]. The reason(s) for this are unknown. The intima vascular calcification associated with advanced atherosclerosis and calcification involving the muscular layer in medium and large-size vessels are considered to be caused by age, diabetes and CKD-MBD [59]. Baseline coronary artery calcium-score (CAC), previous cardiovascular events (CVE), dialysis vintage, low 25 (OH) D3 levels and high or low turnover bone disease are associated with progression of CAC and aorta calcification after KT [56]. There are modifiable risk factors as hyperphosphatemia, hypertension, hyperglycemia, dyslipidemia, smoking, albuminuria, and physical inactivity. In CKD patients and in KT recipient vascular calcification strongly predicts CV events and all-cause mortality, even stronger than conventional risk factors [41].

Some novel risk factors or biomarkers associated with vascular calcification (VC) have recently been proposed. As an example, Vitamin K deficiency is associated with VC in CKD and is believed to be an independent risk factor [60]. Moreover, emerging data suggests that Trimethylamine N-oxide (TMAO), a marker of gut dysbiosis which increases with decreased renal function, predicts all-cause mortality in CKD G 3-5 patients [61]. A diet of red meat, egg, dairy and fish, contains carnitine, lecithin and choline, which gut bacteria use for production of the compound TMA, which is metabolized by hepatic monooxygenases (FMOs) to TMAO. Recent studies show that TMAO is associated with heart disease, progression of CKD, inflammation, colon cancer, diabetes, and atherosclerosis [61,62]. A meta-analysis revealed an association with TMAO and major adverse cardiovascular events and death (MACE), independent of traditional risk factors [63].

Patients with CKD have an altered gut microbiota, compared to healthy patients [64]. The uremic gut microbiota is typically less diverse and more pathogenic than gut microbiota in healthy patients. Published data have confirmed the association between

gut microbiota and TMAO´s association with cardiovascular events [64-84]. A high value at baseline or an increase in TMAO over 10 years is associated with an increased risk of cardiovascular events (CVE) [67]. It is however still not clear whether there is a causal link between TMAO and CVE or if it is only an association. In a Danish cohort of patients with diabetes type 1 (n=1159) with a median follow up time of 15 years, TMAO was associated with CVE independent of traditional risk factors [84]. However, after adjustment for eGFR, the association was not evident anymore and the authors claim this might be explained by TMAO being a mediator of CVD through renal impairment or simply a marker of renal impairment.

In ESKD there is limited data on the association of TMAO with clinical outcome. In 2017 Shafi et al [75] found that a 2-fold increase in TMAO at baseline in ESKD was associated with CVE in hemodialysis patients, but the effect differed with race. Contradictory, in 2019 Stubbs et al [76] did not find any association between TMAO and CVE or all-cause mortality in hemodialysis patients with moderate-severe hyperparathyroidism. The relatively large cohort (n=1243) was enrolled from 22 countries and had a wide range of TMAO-levels. If TMAO is a mediator between gut dysbiosis, atherosclerosis progression of CKD and CVD this may offer novel possibilities for treatments targeting the microbiota [64-84], but data is so far conflicting [75,76] .

Gut dysbiosis and ageing

During the last decade, an increasing interest in the gut microbiota and its effect on health, disease and ageing has emerged. The gut microbiome colonizes the intestinal tract right after birth and is established in humans at the age of three. During childhood the microbiota changes in its abundance and composition [85]. There are several different factors contributing to this change, as differences in geographical area, ethnicity, lifestyle, diet and between individuals. Extrinsic factors as diet and physical activity have an impact in life-style related diseases such as diabetes, frailty, cancer, CVD and dementia, possibly partly mediated through the gut microbiota. The microbiota consists of viruses, bacteria, eukaryotes and archaea and the main four bacterial phyla are *Bacteroides, Firmicutes, Actinobacteria* and *Proteobacteria* [85]. These four phyla constitute 98% of the microorganisms in humans. In healthy individual, the microbiota is dominated by *Bacteroides* and *Firmicutes* [64]. The composition of microbiota over time is stable in individuals but can be altered due to changes in diet [86]. The main functions of the microbiota are;

- protection from pathogens
- absorption of food which generates vitamins and nutrients
- preservation of the intestinal integrity which prevents gut leakiness
- metabolizes fibers and are the sole producers of short chain fatty acids (SCFA) which are important for the gut integrity
- essential for development and maturation of the immune system in the host
- SCFA provides energy to the microbiota and inhibits the colonization of bacteria that are opportunistic and produces mucus
- regulates host immunity

Gut dysbiosis is associated with human ageing [85]. Pro-inflammatory commensals enrich the gut, a process which competes with more beneficial gut microbiota, in elderly. Since this change has been observed in mice that are genetically homogenous and live under similar conditions, while being fed the same nutrients the change seem to be "intrinsic to the ageing process" [85].

Patients with CKD have an altered gut microbiota, compared to healthy patients [64,87]. A meta-analysis showed decreased abundance of *Prevotella, Roseburia* and *Prevotellaceae* and greater abundances of *Streptococcaceae* and *Enterobacteriaceae,* in CKD compared to controls [87]. The uremic gut microbiota is typically less diverse and more pathogenic than in gut microbiota of healthy patients. Gut dysbiosis in CKD is caused by uremic toxins, increased urea and ammonia due to CKD, which leads to pathobiont overgrowth, decreased barrier integrity and increased inflammation [88,89,94]. Bacterial fragments, including endotoxins translocate over the impaired intestinal barrier, causing low-grade inflammation and alterations of the immune system [89-91]. Uremic toxins are produced in the gut, and a vicious cycle, called "the gut-kidney axis" further enhances disease progression [64,92]. It has also emerged convincing evidence, that gut dysbiosis compromise host immunity [93] by affecting the innate and adaptive immune system. The transformation of a healthy gut microbiota to a dysbiotic state is typically seen in patients >70 years of age, which coincides with the age when immunosenescence occur [85]. Especially frail humans display signs of dysbiosis, manifested as a reduction in beneficial microbes and in diversity of populations [85].

Kidney transplantation in elderly

-incidence and survival

Patients with ESKD are considered to have a higher biological age than non-CKD patients [5] which is driven by low-grade inflammation, called "inflammaging", oxidative stress and mitochondrial dysfunction [33-35]. According to data from the Swedish Renal Registry the annual mortality-rate in dialysis patients is 19% vs. 3% after KT. However, the comparison between dialysis patients and KT recipients is affected by selection bias, since patients not eligible for KT due to extensive comorbidities, usually receives dialysis-treatment instead of KT.

Nonetheless, in studies comparing survival in KT recipients compared to patients remaining on the waitlist, it has been shown that survival is superior after KT in all age-groups [95]. This survival benefit is evident in elderly KT recipients as well, but in some studies an increased risk for mortality during the first 1-2 years after KT has been observed [96,97]. A majority of studies with sufficient population size and follow-up demonstrate, however, a survival advantage compared to waitlisted elderly patients remaining on dialysis [98-101]. Donor type, living vs deceased, seems to have a significant effect on this survival-advantage [96, 102,103].

Still, there is probably an upper age limit when KT no longer results in a survival advantage, or any other beneficial effect compared to staying on dialysis. Elderly patients are more vulnerable to surgical trauma, immunosuppression, infections, CVE, and postoperative complications [96,103-107]. Studies have shown that health related quality of life is improved after successful KT in all age-groups [172], but the extended lifespan counted in years is shorter in the elderly, partly because of natural causes related to old age. There is still a beneficial effect on survival compared to dialysis even in patients ≥70 years [97,108,109] .

Kidney transplantation has increased in elderly worldwide during the last decades [110,111]. This reflects the increase in incidence and prevalence of ESKD. It also reflects the increased acceptance of older recipients for KT and the increased utilization of kidneys from older donors [108,109,112]. In Europe, KT in patients ≥75 years of age increased from 0.8% in 2005 to 3.2% in 2009 [112]. The allocation of kidney allografts to the elderly during this period however varied in different countries in Europe. The annual percentage of dialysis patients ≥75 years who received a KT was close to 0% in Slovenia, Denmark and Sweden and 4% in Norway during the years 2005-2014 [112]. The frequency of kidney allocation to elderly patients has subsequently increased in Sweden in recent years as in many other countries [4]. In 2000 only 16 % of KT in Sweden were ≥60 years compared to 35 % by the year 2019. No patients ≥70 years received a KT in Sweden in 2000, compared

to 10 % of the total number of transplanted in 2019 [4]. An improved preoperative evaluation as well as a better understanding of prognostic markers for clinical outcomes after KT is important in this growing and fragile elderly ESKD-population.

Some studies have reported that elderly do not have the same access to the waitlist as younger patients [112] despite the absence of significant comorbid conditions. In a French study by Legeai et al [108] , inclusion of all patients ≥70 years starting kidney replacement therapy (KRT) between 2002 and 2013 (n=41 716 patients), was conducted. The access to the waitlist in patients >70 years was 3%. Only 2% of all patients in KRT ≥70 years were transplanted during follow-up. In the dialysis group, 20% did not have co-morbid conditions i.e., CVD or diabetes, but still did not get access to the waitlist. Diabetes, CVD and dialysis-vintage >2 years was associated with an increased risk for mortality after KT in this study [108]. On behalf of European Renal Association-European Dialysis Transplant Association, Segall et al [113] authored a literature review and a position statement. It recommended that patients should not be excluded from the waitlist based on chronological age alone. Instead, factors like frailty, psychosocial issues and comorbidity scores should be included in the preoperative evaluation [113].

The impact of donor age, frailty, and comorbidities

Retrospective registry studies have shown a survival benefit in elderly KT-recipients compared to staying on the waitlist despite the increased use of extended criteria donors (ECD), according to United Network for Organ Sharing, (UNOS) criteria [98-100,114-117]. Gill et al [96] demonstrated that postoperative mortality in patients ≥65 years, compared to staying on the waitlist, varied greatly depending on type of donor in a large study (n=25 468). In patients transplanted with ECD kidneys, the postoperative mortality increased during the first 521 days compared to patients remaining on the waiting list. Patients receiving standard criteria donor (SCD) kidneys had an increased risk during the first 368 days, and after living donation for 130 days postoperatively [96]. Thus, outcome in older recipients is affected by donor type. The ECD definition has, however, been discussed since the age limit of 60 years by current contemporary standards is a relatively low age in donors compared to the mean age of deceased kidney donors in several Western countries [118-120]. Elderly patients usually have less access to living donors, hence most recipients >60 years receive ECD-kidneys in Sweden, by UNOS definition [121].

In a Polish national registry study from 2016 five-year patient survival in elderly KT recipients was inferior if donor age was ≥65 years, even after adjustment for recipient age

[122]. Ideally, adjustment for recipient comorbidity should be done when comparing the effect of donor age on patient survival, since there might be a bias in the allocation process.

Pippias el al [123] compared outcome in recipients in different age groups after transplanting kidneys from older deceased donors (55-70 years) in a European register-study. Since deceased kidney donors normally donate to two different recipients, outcome can be compared between recipients in different age groups who have received kidneys from the same donor. In this study patients were transplanted between 2000-2007 (n=1410). Kidneys from elderly donors transplanted to elderly recipients (≥70 years) hade an acceptable graft function, only six months shorter after ten years compared to the graft function in the younger recipients. The result from several studies implies that kidneys from elderly deceased donors is a good resource which should be used to enable KT in as many as possible, especially to elderly patients with a shorter life-expectancy (so called age-matching).

In some countries, kidneys from donors ≥80 years are discarded. The favorable outcome among elderly patients receiving octogenarian kidneys compared to staying on the waitlist was confirmed in a study of 2585 patients ≥60 years receiving a kidney transplant in Catalonia, Spain[124]. In the study 128 patients received a kidney from deceased donors ≥80 years. In patients (all ≥60 years) receiving kidneys from deceased donors ≥80 years compared to deceased donors 60-79 years, death censored graft survival after one and five years was poorer (86% and 64 % versus 93 % and 83 %). Adjusted risk for graft loss was 1.55 times higher (CI 95%,1.00-2.38, P=0.048). Importantly, patient survival among the recipients with deceased donors >80 years was not inferior compared to recipients receiving kidneys from deceased donors 60-79 years (7% and 21 % vs 7 % and 23%, respectively, p=0.383). Mean age in recipients who received octogenerian kidneys were 71.8 ± 4.2 years, they had a greater burden of comorbidities, and the majority were males.
What is even more important is that the study showed that patient survival compared to patients remaining on dialysis on the waitlist was significantly lower in groups with different donor age. In patients receiving grafts from donors 60-79 years (n=1084), adjusted hazard risk for mortality was 0.50 (CI 95%, 0.44-0.58, P<0.001) compared to patients remaining on dialysis and in recipients with donors ≥80 years the adjusted hazard risk for mortality was 0.54 (CI 95%, 0.38-0.77, P=0.001) after 12 months. Moreover, a lower adjusted hazard risk was observed in females and in patients transplanted during 2001-2014 compared to 1990-2000 [124].

Kidneys from older donors are associated with a lower graft survival, but they still offer a survival advantage in elderly KT recipients compared to dialysis, confirmed by several studies [115,125] . Since patient survival and quality of life improves in the elderly

(≥70 years) as well as in younger patients, it is reasonable to propose KT to elderly patients with ESKD, unless there are obvious contraindications. It is a difficult task, however, to evaluate and decide who is eligible for KT and who is too frail to tolerate surgery and immunosuppressive therapy in elderly patients. Frailty affects the risk of postoperative complications after KT, independent of other risk factors [126,127].

Frailty by Fried [128] is based on following criteria. Each criterion gives one point:

Criteria	**Explanation**
Weakness	Grip strength below established cut off (lowest 20 % by sex and body mass index)
Unintentional weight loss	Self-reported weight loss (dry weight) > 4.5 kg during the last year
Self-reported exhaustion	
Slowed walking speed	Walking time/15 feet, slowest 20% (by sex and height), below an established cut off
Low activity	Kcal/week below an established cut off

Frailty is defined by:
Non-frail: 0 p
Intermediate frail: 1-2 p
Frail: 3-5 p

Frailty has been associated with a higher risk of cognitive dysfunction, hospitalizations, mortality and lower quality-of-life in dialysis patients [129-132]. However, frail patients did improve their health-related quality of life, even more than non-frail peers, after KT in a study including 443 KT recipients by McAdams-DeMarco et al [133]. They also showed, in a multicentre study of 1975 patients that frailty did not add predictive value to a registry-based prediction model for mortality [134]. Interestingly, IL-6 and CRP as well as an inflammatory index substantially improved risk prediction of mortality [134]. Frail patients were more likely to have elevated inflammation markers. The authors concluded that this helps to "clarify the accelerated ageing in ESRD and highlight easy-to-measure markers of increased waitlist mortality risk".

In addition to frailty, physical function should be evaluated preoperatively as well. It has been shown that physical function is predictive of patient survival after KT [135-138]. At a certain biological or chronological age, the risk is probably outweighing the beneficial effects of KT. If a patient´s excepted lifespan is <2 years, no matter what reason, this has traditionally been a reason to refrain from KT. If an advanced chronological or biological

age makes it unlikely that the patient survives more than two years, it is best to decline KT. It has been suggested that if we push the age and comorbidity-limit in elderly KT recipients further, we may eventually lose the advantage of KT. The scarcity of donors and organs makes it necessary to continuously reevaluate and question kidney allocation principles. On the other hand, the increasing mean lifespan and improved health in many populations in the world [139] might entail the need to expand the upper age limit for KT. Consequently, knowledge of which preoperative clinical factors that influence KT outcome in the elderly is of vital importance. This would improve the possibility for accurately deciding which patients will likely benefit from KT and which probably would do better by remaining on dialysis.

It is likely that the estimated biological age of KT recipients could predict outcome after KT in a more precise manner than chronological age. The term "biological age" refers to a combination of the chronological age of an individual and epigenetic changes due to allostatic overload. Allostatic overload, or the wear and tear of the body, is caused by different factors for example lifestyle burden, inflammation and oxidative stress due to chronic diseases, such as uremia, cardiac failure, diabetes mellitus and others [36,140,141].

Risk score models, which are proven to be accurate enough, have the potential to enhance equality in health care by eliminating possible discrimination based on personal experience or preference of the clinician. Already existing risk scores may be good enough to motivate a more widespread use in clinical settings, after thorough evaluation in the patient cohort of interest. Preoperative prognostic risk scores have been developed which may increase the precision of risk factor evaluation in KT to elderly. Specifically, the Charlson Comorbidity Index (CCI) and the Framingham risk score (FRS) have been evaluated in earlier studies [127, 142-147]. The FRS measures the risk level of developing coronary artery disease over 10 years but it may underestimate the true cardiovascular risk in KT patients [147] .The risk factors included in FRS are age, systolic blood pressure, sex, diabetes, smoking, total cholesterol and high density lipoprotein cholesterol (HDL). The CCI is a well-established risk index for prediction of 10-year mortality in patients with comorbidities [192-194] and has been used in various clinical settings. It was developed by Dr Mary Charlson, professor of Medicine at Weill Cornell Medical College, in 1987.

Table 1. Charlson Comorbidity index (age extracted)

Medical condition	Yes	No
Myocardial infarction	1 p	0
Cardiac heart failure	1 p	0
Peripheral vascular disease	1 p	0
Cerebrovascular accident or TIA	1 p	0
Dementia	1 p	0
COPD	1 p	0
Connective tissue disease	1 p	0
Peptic ulcer diseas	1 p	0
Liver disease	Mild:1 p Moderate-severe: 3 p	0
Diabetes mellitus	Diet-controlled: 0 p Mild:1 p End organ damage: 2 p	0
Hemiplegia	2 p	0
Chronic kidney disease	Moderate-severe: 2 p	0
Solid tumor	Localized: 2 p Metastatic: 6 p	0
Leukemia	2 p	0
Lymphoma	2 p	0
AIDS	6 p	0

Nation-specific factors might affect the outcome of KT, especially in vulnerable patients. For example, varying access to organs from living or deceased donors, cold ischemia time and donor-kidneys with variable quality affect patient and graft-survival. Allocation principles and the transplant centers tolerance for accepting transplantations despite donor specific antibodies (DSA), may also have an effect on outcome. It is preferable to evaluate the outcome of KT in different clinical settings, in the country of interest, and not solely rely on data from other countries without having the above factors in consideration.

2 RESEARCH AIMS

The overall aim of this thesis was to investigate the impact of biological ageing in patients with kidney failure receiving a KT. We also studied the effect of KT on accelerated biological ageing and compared changes with patients remaining on dialysis.

The specific aims were to:

1) Evaluate chronological age and biological age as risk factors for patient and graft survival after KT in patients ≥60 years in Sweden (study 1).

2) Investigate if the degree of vascular ageing is associated with choice of KRT and study the impact of vascular ageing on CVD and mortality in patients with kidney failure (study 2).

3) Investigate if patients with kidney failure suffer from accelerated biological ageing compared to a population-based control group and compare the impact of KRT (KT vs dialysis) on biological ageing (study 3).

4) Measure and compare circulating microbiome in patients in different stages of CKD and investigate the impact of KT on the microbiome (study 4).

3 MATERIALS AND METHODS

3.1 OBSERVATIONAL STUDY OF RISK FACTORS ASSOCIATED WITH OUTCOME AMONG ELDERLY KIDNEY TRANSPLANT RECIPIENTS IN SWEDEN - A DECADE OF FOLLOW-UP

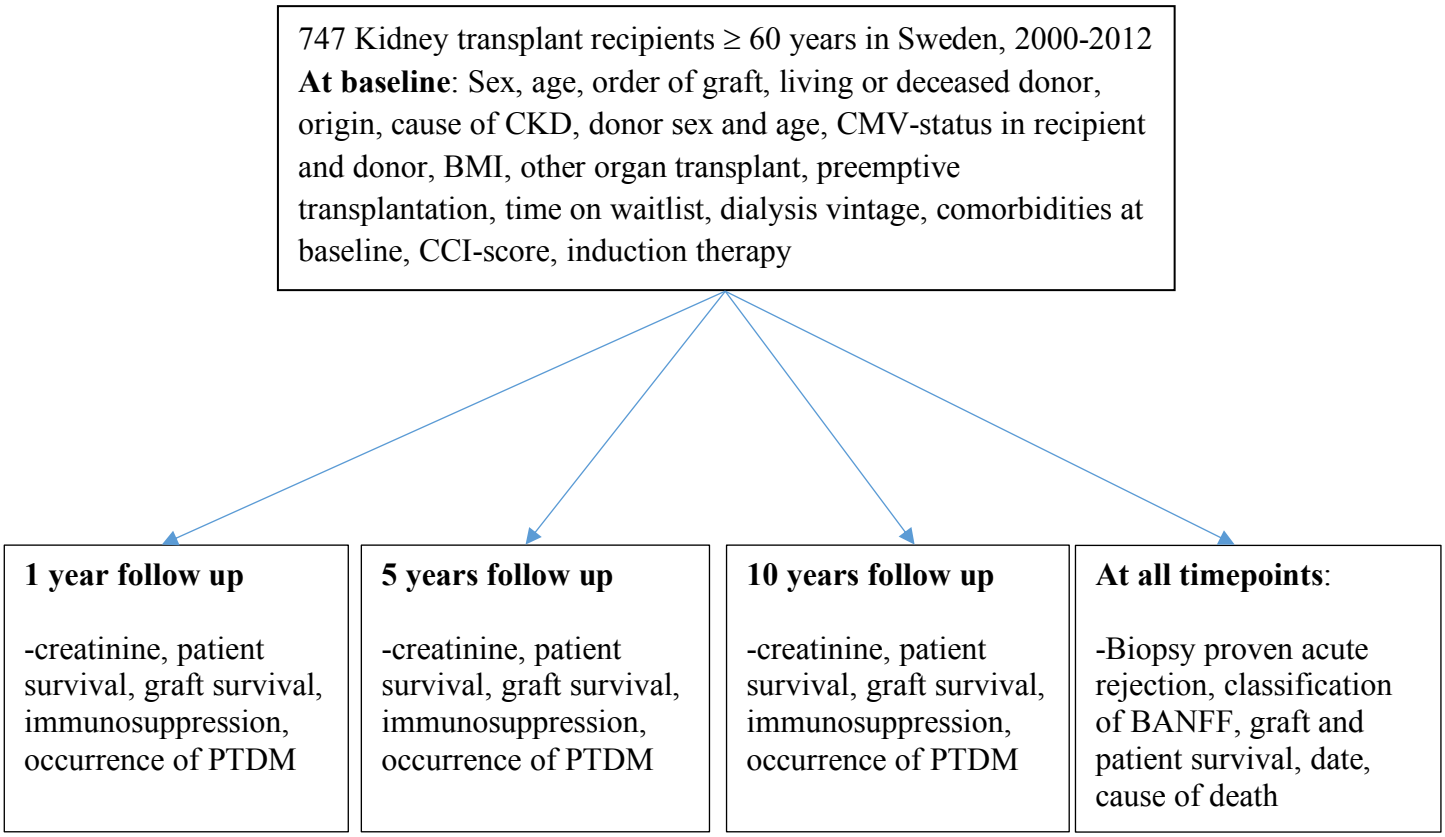
Study design: In this retrospective observational study (study 1) we analyzed the impact of chronological age and risk factors for patient and graft survival in all elderly kidney transplant recipients (n=749) ≥60 years of age, transplanted between the years of 2000 to 2012, in Sweden (Fig 1). All transplant centers (Stockholm, Gothenburg, Uppsala and Malmö) in Sweden participated. Data was collected from patient files, SNR and Scandiatransplant´s database and coded in an electronic CRF by transplant physicians or research nurses at the respective centers.

To enable clinically relevant comparison between age-groups we stratified patients in three groups: 60-64 years, 65-69 years and ≥70 years. Primary outcomes were patient and graft survival in age-stratified groups. Secondary outcome was risk factors for 10-year patient and graft survival in this elderly kidney transplant recipient group. Points of interests were age, sex, BMI, cause of CKD, living or deceased donor, donor age, donor sex, CMV-status in recipient and donor, months on waitlist prior to transplantation, months on dialysis prior to transplantation, comorbidities measured with CCI-score, immuno-suppression, induction, cause of death, occurrence of biopsy proven acute rejection (BPAR) and post transplantation diabetes mellitus (PTDM) during follow up. Scandiatransplant´s database YASWA was used to collect data concerning donor age, sex, CMV-status, living or deceased donor and months on waitlist prior transplantation. The Swedish renal registry (SNR) was used to collect data concerning months on dialysis prior to transplantation (or preemptive transplantation) and cause of CKD. Patient files or local registries at individual transplant centers were utilized to collect data on immunosuppressive treatment, induction, occurrence of BPAR, patient and graft survival, creatinine, debut of PTDM and pre-transplant Charlson comorbidity index (CCI).

Charlson comorbidity index was based on all 19 criteria; myocardial infarction (1p), congestive heart failure (1p), peripheral vascular disease (1p), cerebrovascular accident or TIA (1p), dementia (1p), hemiplegia (2p), COPD (1p), connective tissue disease (1p), mild liver disease (1p), moderate-severe liver disease (3p), diabetes mellitus none or diet-controlled (0p), diabetes mellitus uncomplicated (1p), diabetes mellitus with end-organ damage (2p), solid tumor none (0p) localized (2p) metastatic (6p), leukemia (2p), lymphoma (2p) and AIDS (6p). All patients had severe

CKD and obtained thereby 2 points. However, age was not included in the calculation of CCI, since we analyzed patients in age-stratified groups.

Figure 1. Study design



Post transplantation diabetes mellitus was diagnosed according to American Diabetes Association (ADA) criteria. Kidney biopsies were performed on clinical indication only and there were no control biopsies. The diagnosis of acute rejection was based on biopsy proven acute rejections classified according to Banff-criteria. To enable analysis of the impact of moderate-severe acute rejections and mild rejections separately we divided BPAR in two groups; borderline + Banff IA and Banff IB-Banff III when statistical analyses were performed.

Statistical analysis: Statistical significance was set at a p-value of <0.05**.** Kaplan-Meier curves were used in univariate models and patients were censored after 10 years of follow-up, time for graft failure or death. Cox multivariate regression analysis was used to control for and analyze confounding factors for patient and graft survival. Nonparametric Wilcoxon test was used for skewed continuous variables, t-test for normally distributed variables and Fisher´s test (chi-square test) was used for nominal variables when comparisons between

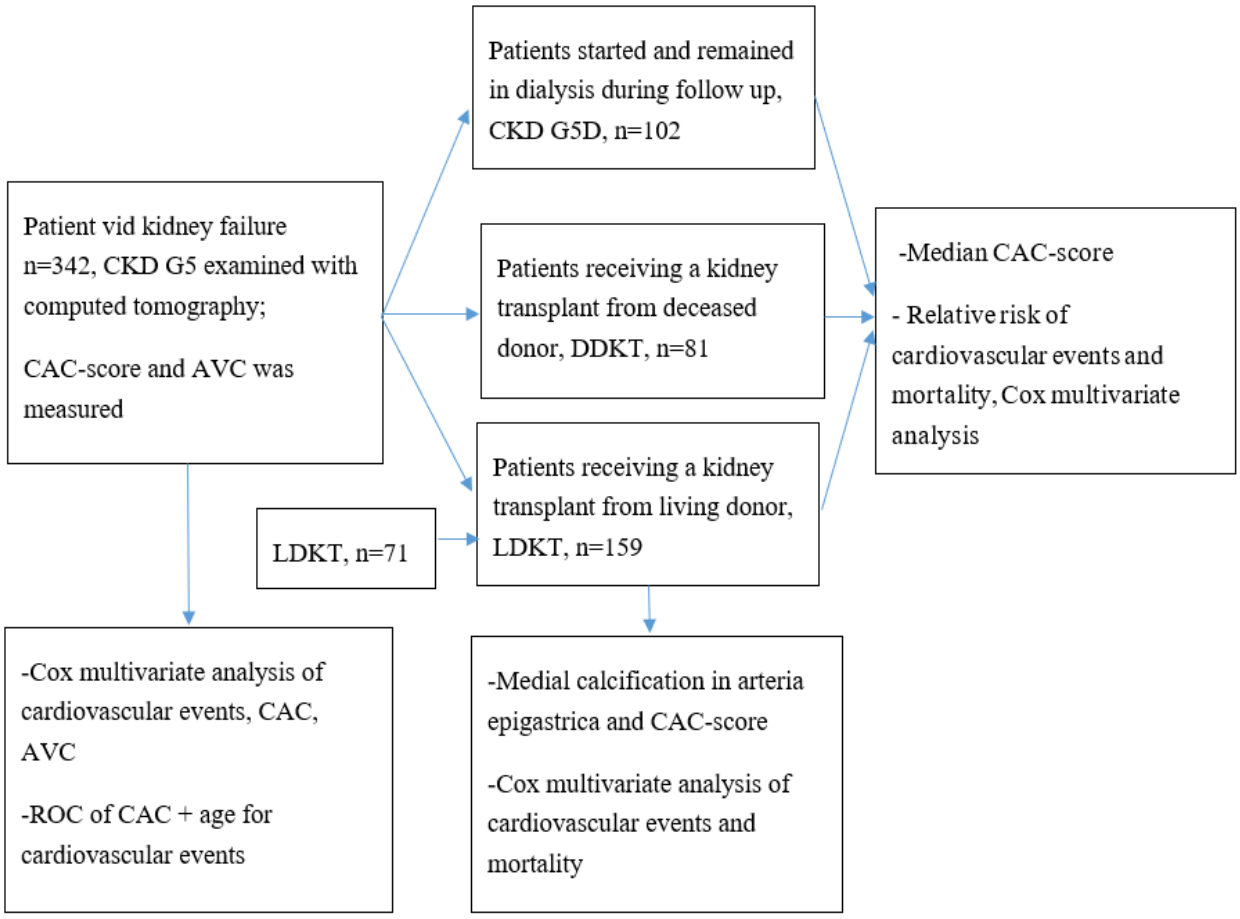
two groups were assessed. Patients lost to follow-up were censored (n=14). Software SAS version 9.4 and STATA 16.1 were utilized to perform statistical analyses.

Ethical considerations: Ethical approval was received from the Regional Ethics Committee of Gothenburg. All personal data was coded when collected in the CRF and no individual can be identified from the analyses or results. The study was conducted in accordance with the Helsinki declaration as revised in 2013. All activities in and associated with the study was consistent with the Principles of the Declaration of Istanbul as outlined in the "Declaration of Istanbul on Organ Trafficking and Transplant Tourism". At each center one transplant physician and research nurses were involved in the process of gathering all data. Individual data was handled with strict confidentiality.

3.2 SCORING OF MEDIAL ARTERIAL CALCIFICATION PREDICTS CARDIOVASCULAR EVENTS AND MORTALITY AFTER KIDNEY TRANSPLANTATION

Study design: In this prospective cohort study (paper 2), which included 342 patients who started kidney failure replacement therapy. All patients came from Stockholm. Coronary artery calcification (CAC) score and aorta valve calcification score were measured by computed tomography examinations. We included 102 patients who remained on dialysis (CKD G5D), 81 patients received a KT from a deceased donor (DDKT) and 159 patients received a KT from a living donor (LDKT). No patients were excluded. The study protocol is schematically presented below (fig 1).

Figure 1. Study design



The aim of the study was to investigate the impact of medial arterial calcification in patients with kidney failure and compare it with CAC and AVC in LDKT patients. Coronary artery calcification consists of a mix of intimal and medial calcification. The extent of medial arterial calcification was graded in biopsies from arteria epigastria. Primary outcomes were

CVEs and all-cause mortality. We also investigated the ROC of CAC in a larger cohort (CKD G5, n=102; DDKT, n=81 and LDKT, n=159) to establish a clinically relevant cut-off value to predict cardiovascular events in patients with kidney failure treated with different modalities of KRT. We performed subgroup analyses of the relative risk of CVEs and death, adjusting for Framingham risk score and type of KRT.

Patients who remained on dialysis (CKD G5D, n=102) were either not evaluated for KT, or not accepted to the waitlist, or accepted but not transplanted before their death. Removal and biopsies of arteria epigastrica were performed during LDKT (fig.2).

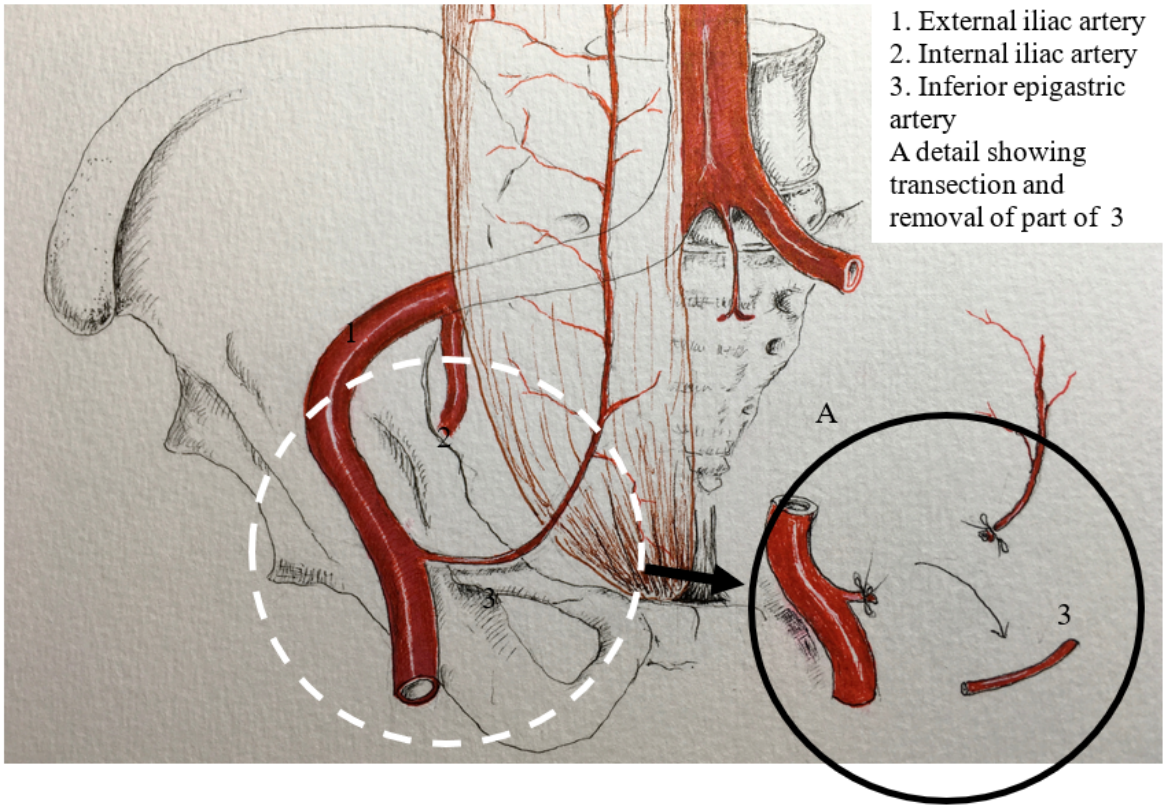


Figure 2. Removal of arteria epigastrica as a part of the routine procedure during KT. Biopsies were taken and analysed. Illustration by courtesy of Dr John Sandberg

The extent of media calcification was graded by an experienced pathologist as none, mild, moderate, or extensive (Fig. 3). To obtain useful results, we created two groups of patients 1) no media calcification + mild media calcification and 2) moderate + severe media calcification. Staining of the arterial wall was performed with the von Kossa staining before microscopic evaluation. CAC-score and AVC were determined by computed tomography of the heart, in Agatston score (AU).

The CAC-score was divided in groups; 0-100, 101-200, 201-400 and >400 AU. A cardiovascular event was defined as either: acute myocardial infarction (AMI, NSTEMI),

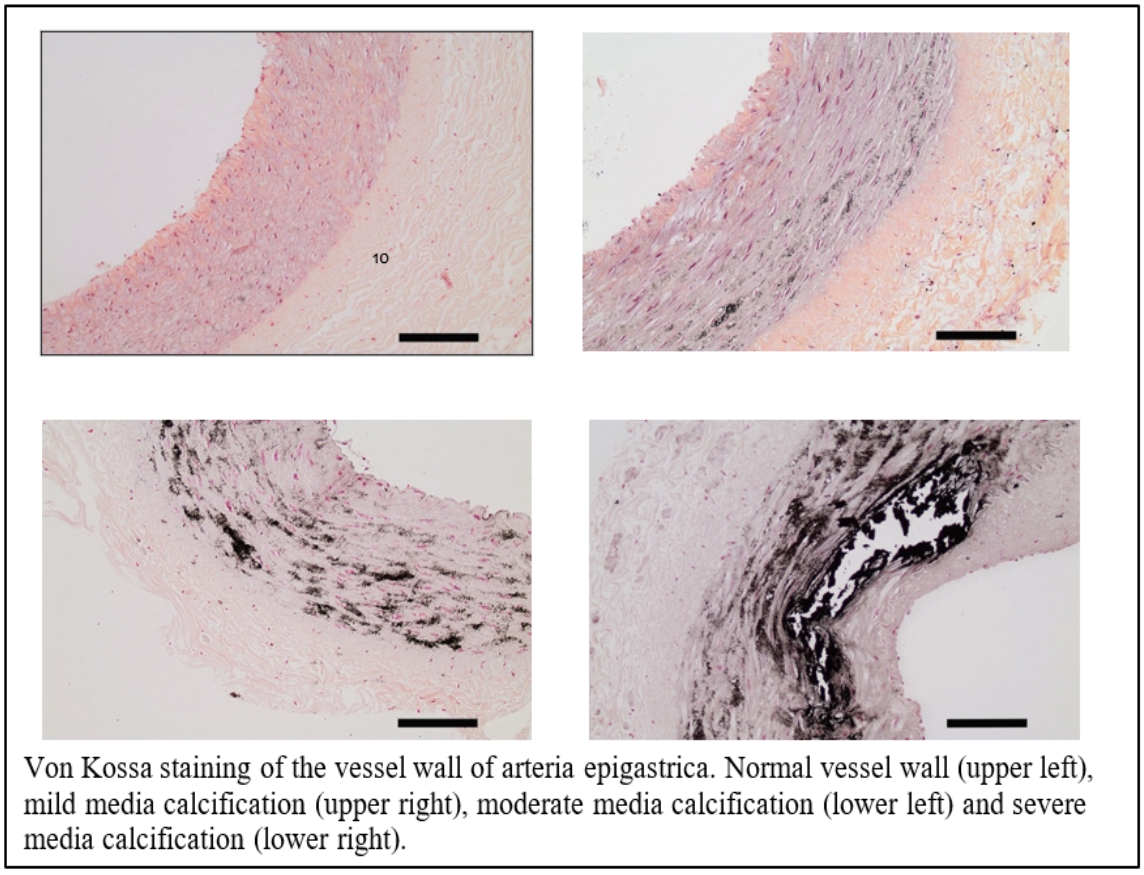
onset of ischemic heart disease requiring PCI, transitory ischemic attack (TIA), stroke, aorta valve stenosis requiring surgery or peripheral vascular ischemia during follow-up. Three patients lost to follow-up (moved abroad) were censored.

Statistical analysis: Statistical analyses were done using SAS version 9.4 and STATA version 17.0. Wilcoxon test (non-parametric) for comparisons between two groups for skewed continuous variables and Fisher´s exact test and Chi-square test for nominal variables were performed. Student´s t-test for normally distributed variables used. Kaplan-Meier survival curves for unadjusted survival analysis and Cox multivariate proportional hazard models for relative risks for CVE and all-cause mortality for one standard deviation (1-SD) increase of Framingham risk score (FRS) and hsCRP were analyzed. We also adjusted for sex, age, diabetes mellitus, hsCRP, treatment modality (CKD G5D, DDKT, LDKT) and protein energy wasting (PEW), one at a time and adding confounders stepwise in different models. The stepwise procedure was performed to find and exclude possible confounders to optimize precision. We also investigated baseline differences in CAC-score to further compare the subgroups (CKD G5D, DDKT and LDKT).

ROC-curve analysis for CAC-score and age as predictor of CVE and all-cause mortality was performed. ROC is an abbreviation for receiver operating characteristics and can be utilized to investigate a threshold's ability to discriminate. The ROC-curve shows the trade-off between sensitivity and specificity. A perfect test would have 100 % sensitivity and 100 % specificity, which means that the test identifies or predicts all cases (100% sensitivity) without any false negative cases (100% specificity). If that would be the case, the area under the curve (AUC) would be equal to 1. The closer to the left upper corner, the better performance and the closer to 1. Usually, an AUC between 0.8-0.9 would be considered an excellent predictor or test, an AUC between 0.7-0.8 acceptable, and an AUC of 0.5 suggest no discrimination. When the threshold is decreased, we get more positive values thus it increases the sensitivity and decreases the specificity. Similarly, when the threshold is increased, we get more negative values leading to higher specificity and lower sensitivity. We decided to try to determine which threshold of CAC-score is the best discriminator, or best predictor for CVE and all-cause mortality, in patients with kidney failure.

Ethical considerations: Written consent was obtained from all patients who participated in the study. The Swedish Ethical Review Authority approved the study and all research activities related to the study were in accordance with the Helsinki Declaration and the

Principles of the Declaration of Istanbul as outlined in the "Declaration of Istanbul on Organ Trafficking and Transplant Tourism." Biopsies from arteria epigastria were taken from a part of the vessel which is removed to obtain access to the urinary bladder during kidney transplantation according to routine. The procedure does not add any risk or harm to the patient during surgery.

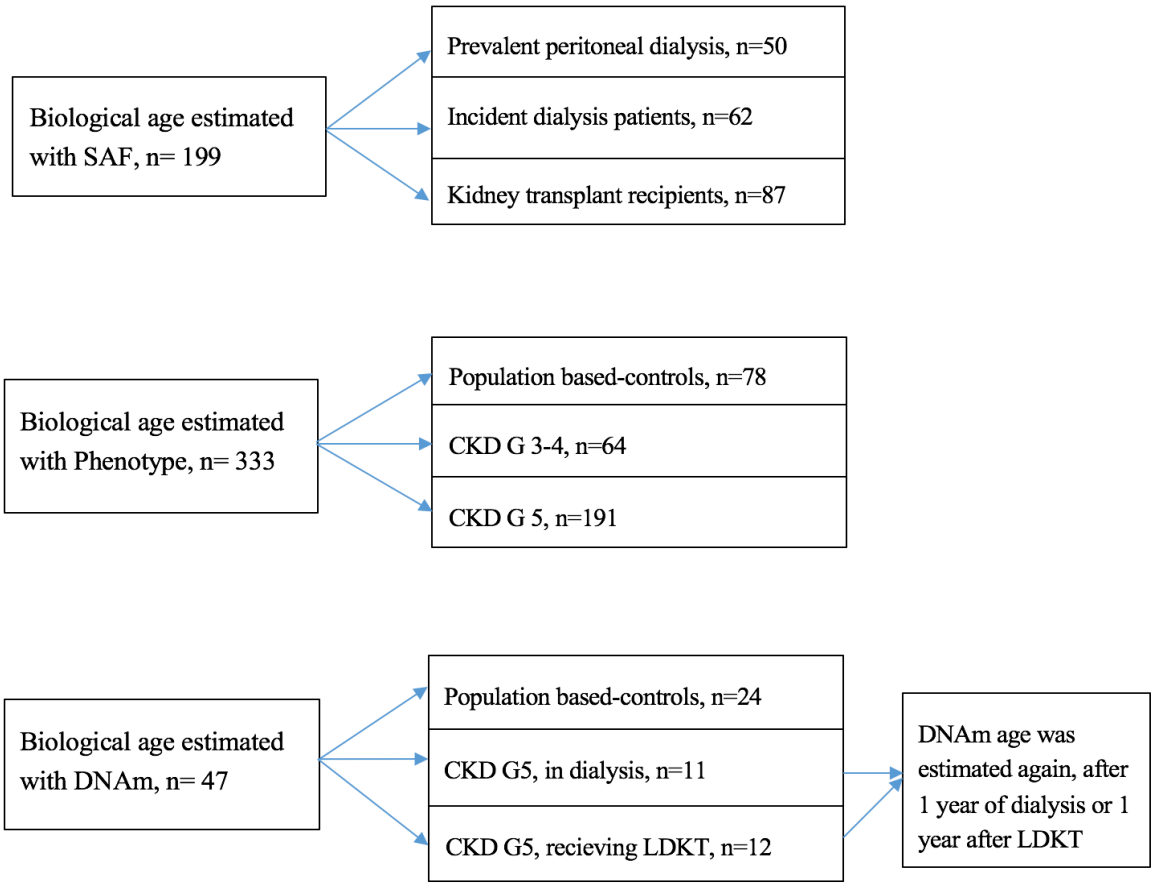


Von Kossa staining of the vessel wall of arteria epigastrica. Normal vessel wall (upper left), mild media calcification (upper right), moderate media calcification (lower left) and severe media calcification (lower right).

Figure 3. Photo by Prof Annika Wernersson, Department of Pathology, Karolinska University Hospital, Stockholm.

3.3 ACCELERATED UREMIC AGEING IS MITIGATED AFTER KIDNEY TRANSPLANTATION, BUT NOT DIALYSIS

Study design: In this prospective cohort study we investigated three different methods to measure biological ageing in patients with CKD 3-5, KT recipients and a population-based control group. We compared the biological age in patients with CKD 5 who either started dialysis or received a KT, at baseline and one year after start of dialysis/transplantation using epigenetic clocks (DNA methylation). Two other methods were used to estimate biological ageing: Skin Autofluorescence (SAF) and phenotypic age (Phenoage).



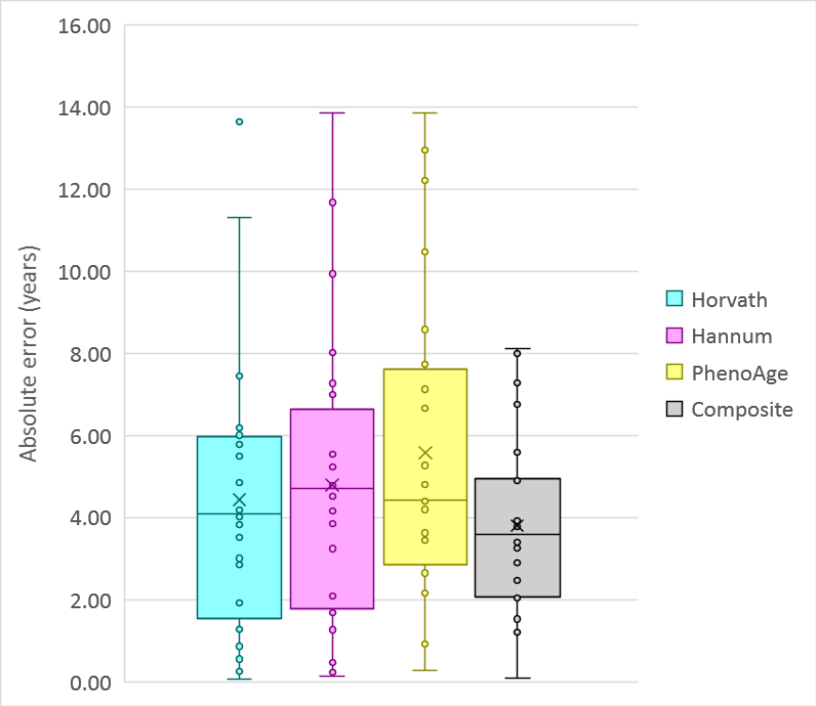
Skin autofluorescence (SAF) was measured using the Autofluorescence AGE reader™ (DiagnOptics Technologies BV, Groningen, The Netherlands). The method measures advanced glycation end products (AGEs) and is noninvasive. SAF has been shown to be associated with mortality in patients with CKD. SAF has furthermore been suggested as a potential marker of "cumulative metabolic stress" as AGEs increase during oxidative stress and hyperglycemia [148]. AGEs are at least partly excreted renally, and levels increase in CKD [149]. A spectrometer generated excitation light and the emitted fluorescence reflected from the skin was measured guarded from surrounding light. The excitation light had the wavelength between 300 and 420 nm. To calculate SAF the ratio between the average

intensity of emitted light is divided by the average intensity of excited light. This is multiplied by one hundred, expressed in arbitrary units (AU). The formula SAF age= (SAF – 0.83) / 0.024 is used to calculate an estimation of biological age [148,150,151].

Phenotypic age is an estimate for biological age based on ten parameters; chronological age, creatinine, serum-glucose, albumin, CRP (log), mean red blood cell count (RBC) volume, lymphocyte percent, alkaline phosphatase, white blood cell count and RBC distribution width (RDW) as described earlier [25] and is described extensively in the background.

DNA methylation was measured from blood samples in forty-seven subjects, taken at baseline in the in all patients, and one year after kidney transplantation in the CKD 5 and LDKT group. By using QIAamp DNA blood maxi kit, DNA was isolated and then treated with bisulfite with EZ-96 DNA Methylation kit as recommended by manufacturer´s protocols. The Illumina Infinium HumanMethylation450K BeadChip was used to measure whole-genome DNA methylation. The Illumina IDAT files using R v.4.0.0 was used to calculate the percent methylation at each locus (beta values) as done previous [152]. Beta values were used to calculate epigenetic age by the Horvath [28,29], Hannum [30] and Phenoage [25] clock. In addition, we constructed a composite epigenetic clock, which combined the results from all the clocks in to one. Absolute errors (discrepancy from chronological age) were calculated.

Figure 1. Box plot of age acceleration (difference between chronological age and DNA methylation age) in the control group, in the different epigenetic clocks. The composite clock has a median absolute error of 3.6 years and a maximum error of 8.1 years (i.e. lower than in the individual clocks).



Statistical analysis:

Differences between groups were analyzed with Kruskal-Wallis test. Chi-squared test was used for comparisons between groups for nominal variables. Statistical significance was considered when p >0.05 and p-values were not adjusted for multiple comparison. Results were expressed as median and IQR. Spearman´s rho test was used to analyze correlations between SAF age, phenotypic age and chronological age. For statistical analysis Stata version 17.0 (Stata Corporation, College Station, TX, USA) and SAS statistical software (Version 9.4; SAS Institute, Inc., Cary, NC, USA) was used and for analysis of DNAm age Microsoft Excel 365, R v.4.0.0 and Minitab 20.3. One-way ANOVA with Tukey's test were used for p-values and adjusted p-values for differences between the transplant recipients, dialysis and control patients. Shapiro-Wilks's test confirmed normality in distribution of the age acceleration. When comparing values for the same patient across two timepoints a paired t-test was used.

Ethical considerations: The Swedish Ethical Review Authority approved the study. All patients included in the study gave their consent to participate. All activities in the study were in accordance with the Principles of the Declaration of Istanbul and the Helsinki Declaration.

3.4 A CIRCULATORY FOOTPRINT OF THE CORE MICROBIOME DOES NOT NORMALIZE AFTER KIDNEY TRANSPLANTATION

Study design: In this prospective observational study we measured the circulatory microbiome in patients with CKD 3-4 (n=10), incident dialysis patients (n=10) and in LDKT patients (n=50) at baseline, and 1 year after KT. The aim was to compare the circulatory footprint of gut microbiota and degree of dysbiosis in various stages of CKD and examine the impact of a KT on gut dysbiosis/the circulatory microbiome. We used amplicon sequency variety (ASV) to investigate the microbiome of bacteria or fragments of bacteria in the circulation, as a reflection of impaired intestinal permeability in CKD patients.

Patients were recruited from ongoing studies at Karolinska University Hospital Sweden. In the longitudinal part of the study of LDKT, patients received standard immunosuppressive regime; tacrolimus, mycophenolate, and prednisolone according to the protocol of Department of Transplantation, Karolinska University Hospital, during the year of follow up. Blood samples were collected after one overnight fast and levels of hemoglobin, calcium, phosphate, iPTH, hsCRP, creatinine, betaine, choline, TMAO, total-cholesterol and triglyceride were analyzed.

By using a DNA purification kit (Maxwell^{R} 16 Blood DNA Purification kit, Promega) DNA was extracted from leukocytes collected from peripheral blood from the patient cohorts. Quantification of DNA was performed by using the high Sensitive DNA Qubit system (TermoFischer, Paisley, UK). Primers were added, specific for the V3-V4 regions of bacterial 16S and then amplified using Kapa Biosystem; Wilmington, MA, USA. The amplification is performed to make the microbiome detectable. The indexed libraries were purified and quality controlled. Negative control samples were run to control for reagent contamination. Amplicon sequence variants were identified a total of 1348 ASVs. When generating the phylogenic tree, the SILVA132 reference database was used to classify the assigned taxonomy for the ASVs.

Statistical analyses were performed in R, using the combined ASVs with their taxonomy and abundance tables. Pielou´s evenness, Shannon entropy, and rarefied richness were used to measure alpha diversity, which is a term for the abundance and variety of organisms in a community. The beta diversity is a measure of sample dissimilarities and quantifies differences in the overall taxonomic composition between two samples. The Bray-Curtis distance measure and PERMANOVA analysis was performed to investigate beta diversity. Core microbiota was determined by using the R´s microbiome package [153,154]. To

investigate how much environmental pressure affected the communities, a process called determinism, the Nearest Taxon Index (NTI) was analysed [155]. A NTI <2 indicate that the natural competition drives the community assembly whereas an NTI >2 indicate deterministic processes influencing the communities. The picante package was used to determine NTI [156]. Another method for analysis of the assembly process establishing community structure is Quantitative process estimates (QPE). This method breaks down the assembly mechanism in selection, dispersal, or drift. Dispersal limitation, homogenizing dispersal and ecological drift are stochastic processes in nature, whereas homogenizing selection and variable selection are characterized as being deterministic processes [157,158].

Ethical considerations The Swedish Ethical Review Authority approved the study. All patients gave their consent to participate, by written and oral informed consent. All activities in the study were in accordance with the Principles of the Declaration of Istanbul and the Helsinki Declaration.

4 RESULTS

4.1 OBSERVATIONAL STUDY OF RISK FACTORS ASSOCIATED WITH CLINICAL OUTCOME AMONG ELDERLY KIDNEY TRANSPLANT RECIPIENTS IN SWEDEN - A DECADE UP FOLLOW UP

Main results:

We included 747 patients ≥60 years of age, receiving a KT in Sweden during the period 2000 to 2012. In addition to the primary and secondary outcomes discussed in the method-section, we investigated if elderly KT recipients (≥70 years) had inferior patient and graft survival at 1, 5 and 10 years after KT compared to the younger patients (60-64 and 65-69 years). We investigated other potential risk factors that could affect outcome. Median age in the study was 64 years (range 60-78 years), 67 % were male, 91 % received their first KT and 12 % had prevalent diabetes nephropathy. Median CCI-score (age excluded) was 3 (interquartile range (IQR) 2-5) in all three age-groups. Median follow-up was 7.9 years (75^{th} quartile 10 years, 25^{th} quartile 6.1 years).

The main findings were:

1) Patient survival at 10 years was 65%, 56% and 44% in the age-groups 60-64 years, 65-69 years and ≥70 years, respectively (Fig.1).
2) Kidney transplant recipients ≥70 years did not have a significantly higher patient mortality after 1 and 5 years compared to patients 65-69 years (Fig. 1). This was confirmed in a Cox regression hazard analysis, adjusted for the same variates as in fig 2.
3) There were no differences in CCI between age-groups (Table 1).
4) CCI ≥7 vs < 4 (age excluded) identified patients with 4.6 times higher risk of all-cause mortality after 10 years (HR 4.57 CI 95% 2.42-8.62, p=0.0001) (Fig. 2).
5) Males had a higher adjusted hazard risk for mortality after 10 years compared to women (HR 1.39; CI 95% 1.04-1.86, p=0.024).
6) LDKT patients had a lower adjusted hazard risk for 10-year patient mortality (HR 0.64 CI 95% 0.42-0.99, p=0.049) despite having the same median CCI-score as DDKT patients (Figure 2).
7) Death-censored graft survival was 78% at 10 years. There were no significant differences in death-censored graft survival between age-groups.
8) Median time on dialysis prior to transplantation was 27 months (IQR 16-40) whereas median time on the waiting list was 8 months (IQR 1-21).

9) Main causes of death were CVD (35.6%), infections (19.4%) and malignancy (18.2%). There were no differences in causes of death between age-groups, sexes or regions.
10) Biopsy proven acute rejection did not affect patient or graft survival. Furthermore, neither did early or late rejection, nor mild (Borderline-BANFF IA) or moderate to severe rejections (BANFF II-IIIB) affect outcome. BMI, CMV-mismatch, basiliximab-induction and PTDM did not influence patient or graft survival.
11) Donor age (deceased and living) median age was 62 (IQR 55-67) years which was in line with recipient median age 64 (IQR 62-67) years (Figure 4).

Missing data was uncommon, expect for the variables BMI and PTDM (Table 2). In case of missing data patients were excluded from the analysis and we did not use multiple imputation. BMI and PTDM did not affect patient or graft survival.

Figure 1. Patient survival in age-stratified groups

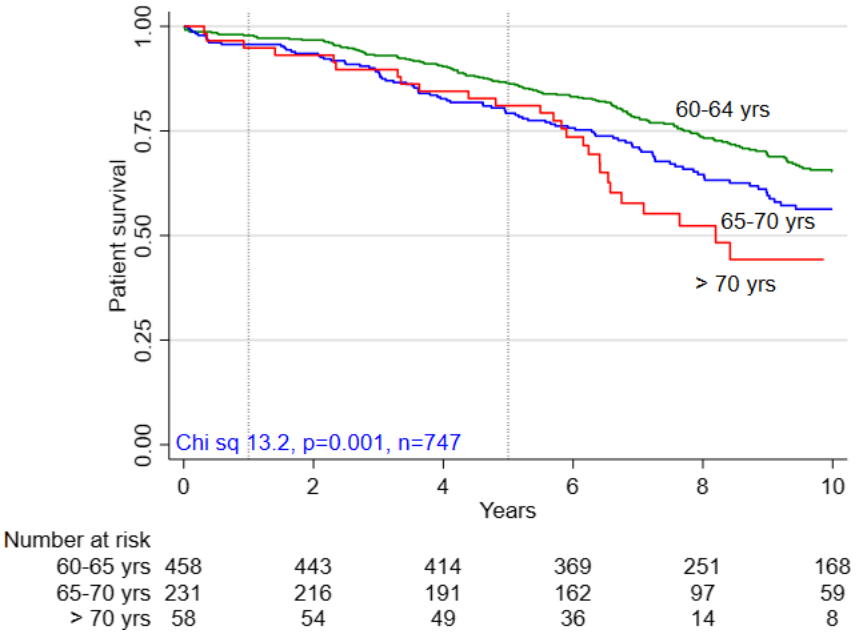


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Table 1. Charlson Comorbidity index, in age-stratified groups

	All n=559	60-64 years n=350	65-69 years n=165	>70 years n=44	p-value
Charlson Comorbidity Index	3 (2-5)	3 (2-5)	3 (2-5)	3 (2-5)	0.64

Figure 2. (a) Multivariate cox regression analysis performed for patient survival at 10 years, n = 673. **(b)** Multivariate cox regression analysis performed for patient survival at 10 years with Charlson comorbidity index, n = 500.

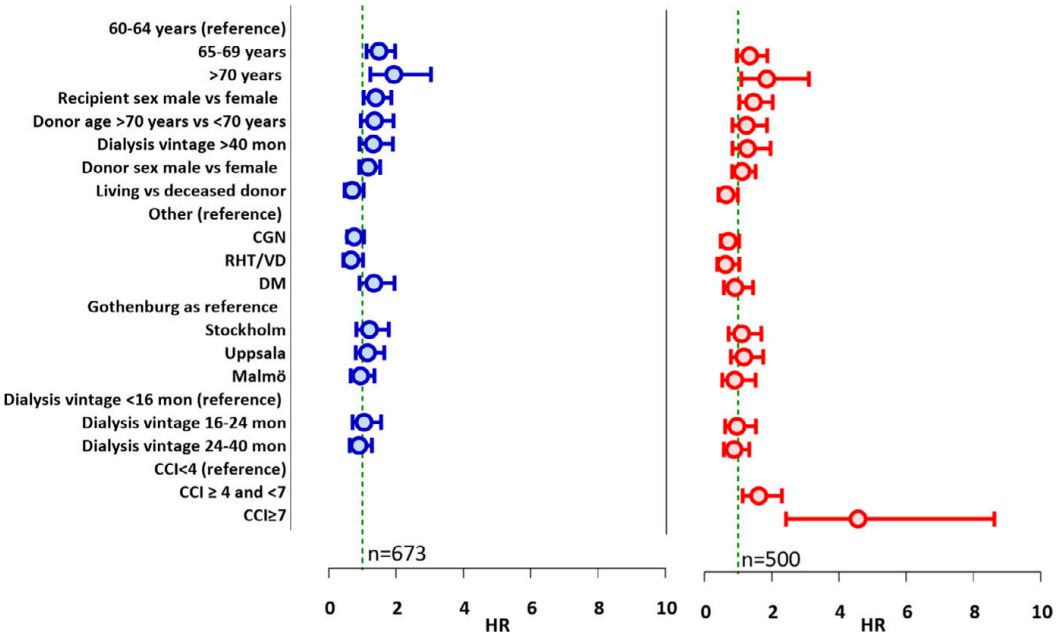

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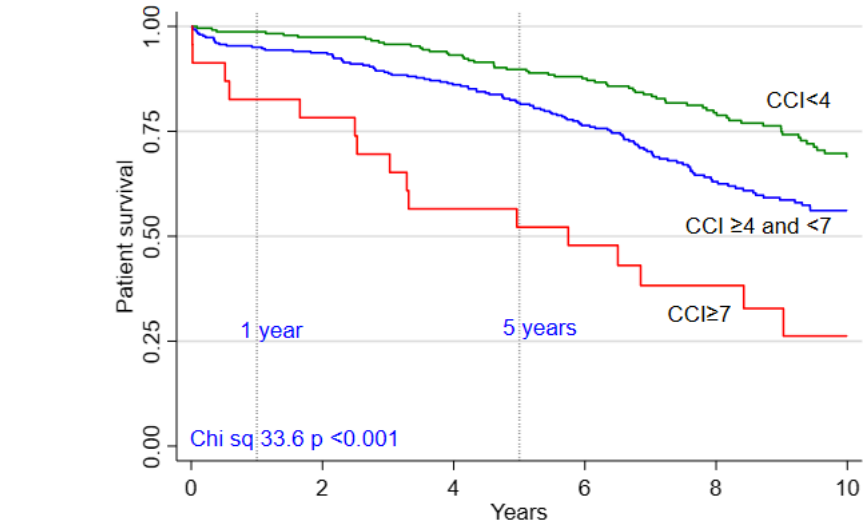
Figure 3. Patient survival stratified by Charlson comorbidity index, n = 559.



Number at risk

CCI<4	234	228	218	193	132	86
CCI ≥4 and <7	302	283	260	216	120	81
CCI≥7	23	18	13	11	7	3

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Figure 4. Donor age (blue) and recipient age (red)

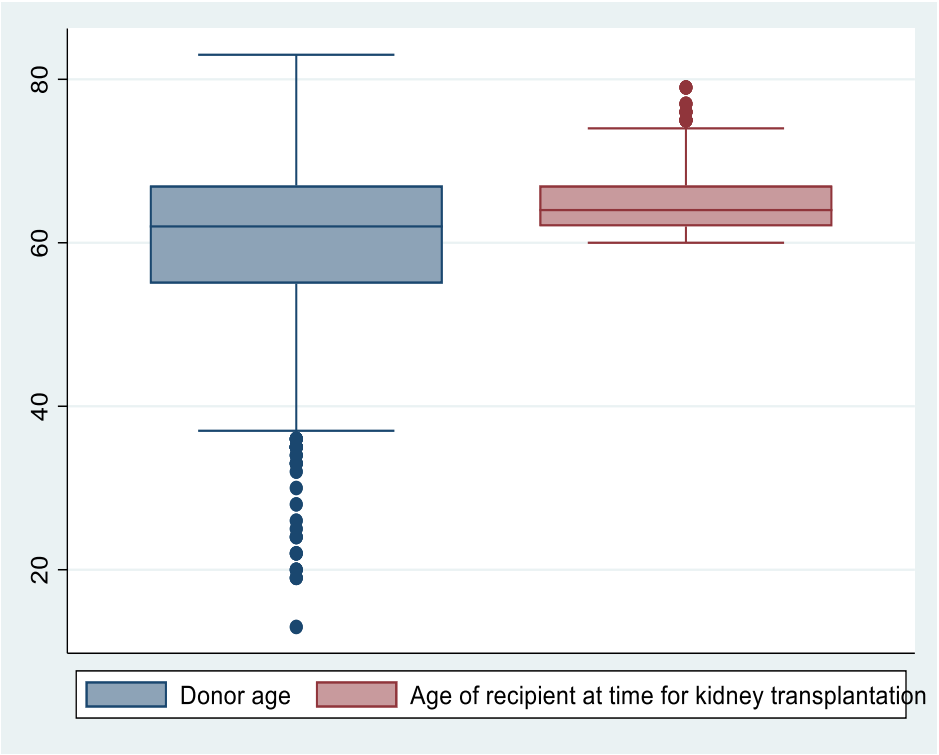

Table 2. Missing data

Variable	Missing data
Patient survival	0
Death censored graft survival	0
Cause of death n=253	0
Age	0
Sex	0
Region	0
Cause of CKD	0
Pre-emptive transplantation	1
Patients in PD/HD	71
Living/deceased donor	1
Donor age	1
Donor sex	4
Dialysis vintage	70
Waitlist time	15
First/second/third transplant	5
PTDM	175
BMI	213

4.2 SCORING OF MEDIAL ARTERIAL CALCIFICATION PREDICTS CARDIOVASCULAR EVENTS AND MORTALITY AFTER KIDNEY TRANSPLANTATION

Main results:

We included 342 patients starting KRT in this prospective cohort study. The aim was to investigate the impact of medial calcification with a mix of intimal and medial calcification represented by coronary artery calcification on CVE and mortality. Median age was 53 years, 66% were males and 17% had diabetes. There were differences in baseline characteristics between the groups; median age was 67, 53 and 47, prevalence of diabetes mellitus was 32%, 19% and 8% and the prevalence of CVD was 35%, 16% and 13%, in CKD G5D, DDKT and LDKT, respectively. There were differences in eGFR, BMI and handgrip strength at baseline between the groups. The study revealed major baseline differences in coronary artery calcification between the groups (figure 1).
The main findings in our study were:

1) Patients with none or low grade of medial calcification had a low risk for CVE (5.6% vs 28.4%) and death (1.6% vs 14.9%), compared to patients with moderate-severe medial calcification during 6.4 years of follow-up.
2) Coronary artery calcification was associated with; age, diabetes mellitus, CVD, higher Framingham risk score (FRS), higher BMI, higher IL-6, lower hand grip strength, lower albumin and aorta valve calcification (all variables with $p<0.001$). Sex ($p=0.046$) and lower diastolic blood pressure ($p=0.002$) were also associated with CAC.
3) Patients with CAC-score >400 AU had six times higher risk for CV events (HR 6.0 95% CI, 2.3-15.4, $p <0.001$) and 7.4 times higher risk for all-cause mortality (HR 7.4, 95% CI, 2.1-25.8, $p=0.002$) compared to patients with no signs of CAC (0 AU) after 6.4 years of follow-up independent of FRS.
4) Medial calcification in arteria epigastrica was associated with age, sex, prevalent diabetes and CVD, higher FRS, higher BMI, CAC-score, aorta valve calcification, CV events and mortality (all variables with p-value <0.001). Medial calcification in arteria epigastrica was also associated with systolic blood pressure ($p=0.006$) and higher hsCRP ($p=0.024$).
5) In patients undergoing LDKT, moderate to extensive medial calcification in arteria epigastrica conferred a 3.1 higher risk of cardiovascular events (HR 3.1, 95% CI 1.12-9.02, $p<0.05$) compared to patients with no or mild medial

calcification independent of age, sex and diabetes (figure 2). CAC-score was not an independent predictor of CV events in this model.

6) ROC-curve analysis showed that a CAC-score of 381 AU was the optimal cut-off value in predicting CVE (*R*-value =0.80) and a CAC-score of 371 AU was the optimal cut-off value in predicting mortality (*R*-value =0.84). Figs. 3 and 4.
7) Median CAC-score was only 3 (IQR 0-152) AU in LDKT. Still as many as 37% had moderate-severe medial calcification in arteria epigastrica.

Fig 1 Comparison of baseline median and IQR CAC-score in patients receiving LDKT, DDKT, and patients remaining in dialysis (CKD G5D).

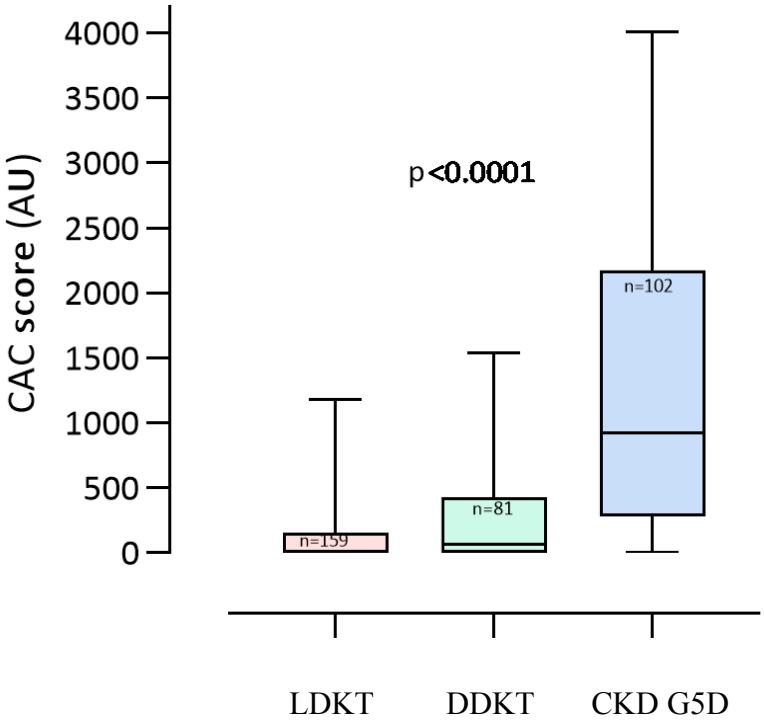

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Fig. 2 Multivariate Cox regression analysis of medial calcification (HR 3.10, 95% CI 1.12–9.02, p<0.05) and CAC (HR1.83, 95% CI 0.46–7.28) association with CVE in LDKT.

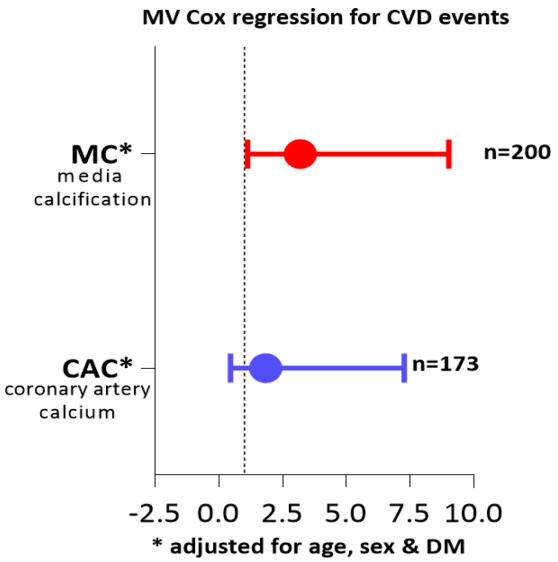

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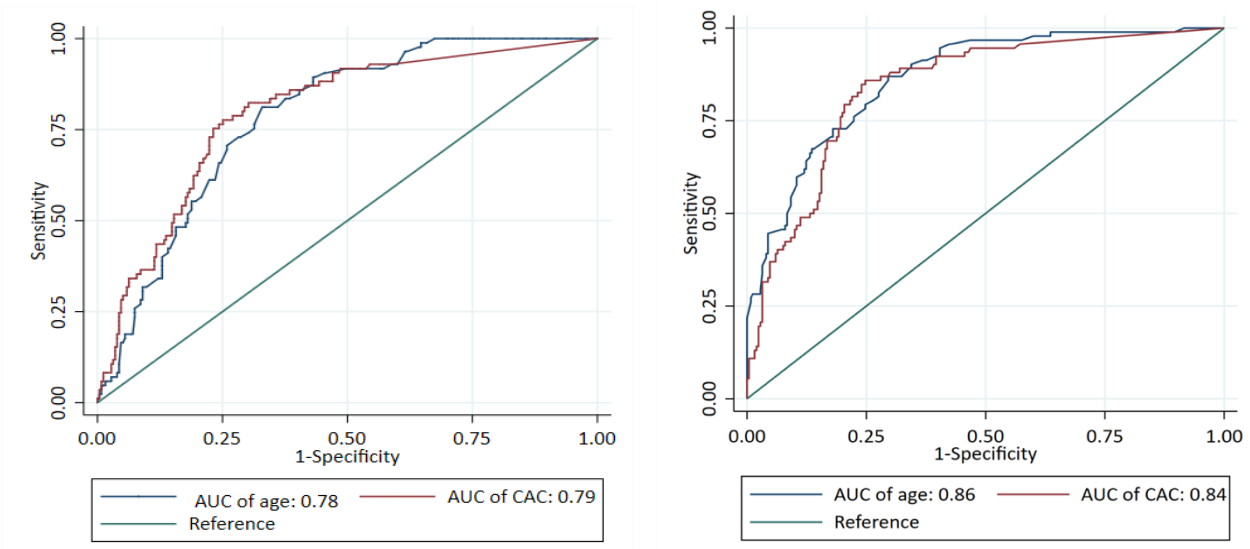
Fig. 3a (left) ROC: Area under curve (AUC) for CV-events of age and CAC-score. Cut-off level of CAC score was 381 AU (n=342). **3b** (right) ROC: Area under curve (AUC) for all-cause mortality of age and CAC-score. Cut-off level of CAC score was 371 AU (n=342)



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4.3 ACCELERATED UREMIC AGEING IS MITIGATED AFTER KIDNEY TRANSPLANTATION, BUT NOT AFTER DIALYSIS

Main results:

The main results are summarized as follows:

1) Skin autofluorescence (SAF) as an estimate of biological age was correlated with chronological age in patients with CKD G5 (rho 0.51, 0.44 and 0.25 in the LDKT, CKD 5 and PD-group, respectively, figure 1). In the LDKT and CKD 5 groups the correlations were significant ($p<0.001$ and $p=0.003$), but not in PD patients ($p=0.07$).
2) We analyzed another estimate of biological age by calculating Phenoage (PA) as described in methods in 333 subjects with different CKD stages. In the population-based control subjects the correlation with chronological age was significant and strong (rho=0.96, $p<0.001$). In the CKD G3-4 group the correlation with chronological age was weaker but still strong (rho=0.84, $p<0.001$). However, the correlation between PA age and chronological age in CKD G5 was significant but markedly lower (rho=0.27, $p<0.001$). The calculated PA overestimated the magnitude of biological age in this group of patients, and the estimate was unrealistic.
3) As expected, several common clinical parameters, higher hsCRP, IL-6, lower HGS, hemoglobin and albumin and higher prevalence of diabetes and CVD were associated with higher SAF, PA age and chronological age.
4) When analyzing DNA-methylation age, we found that incident dialysis patients and LDKT patients had an accelerated DNAm age vs chronological age compared to the control-group (fig 3). After one year of dialysis and LDKT, this acceleration was mitigated in the LDKT group, but not in the dialysis group, according to the Composite clock (fig 3). We observed a statistically significant reduction in PhenoAge acceleration (-4.4 years, $p=0.016$) and in Composite age acceleration (-2.5 years, $p=0.009$) one year after LDKT.

Fig 1. Estimated SAF age compared to chronological age in patients with CKD 5 (PD-patients, incident dialysis patients and LDKT, n=199).

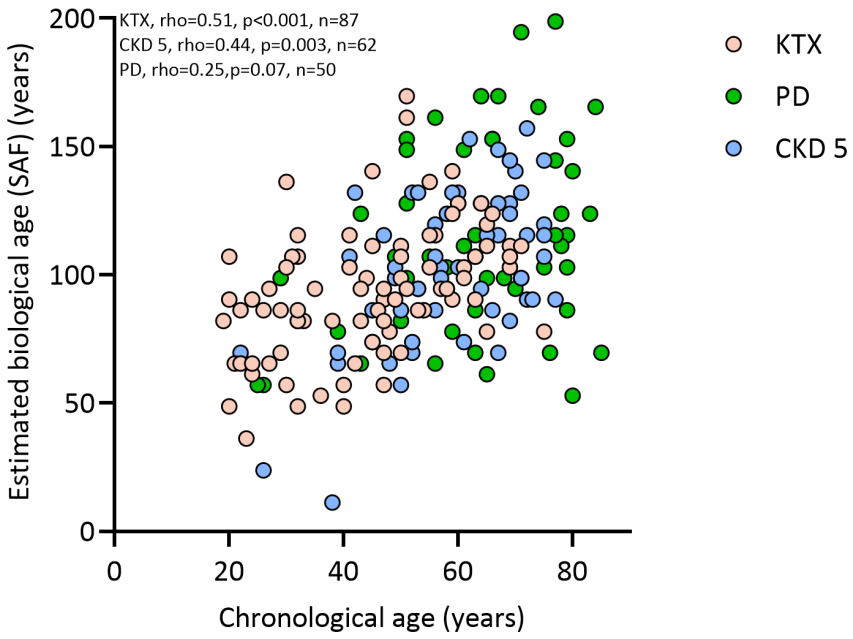


Fig 2. Estimated Phenoage (PA) in different stages of CKD (n=333). Population-based controls (HS), CKD 3-4 and CKD 5**.**

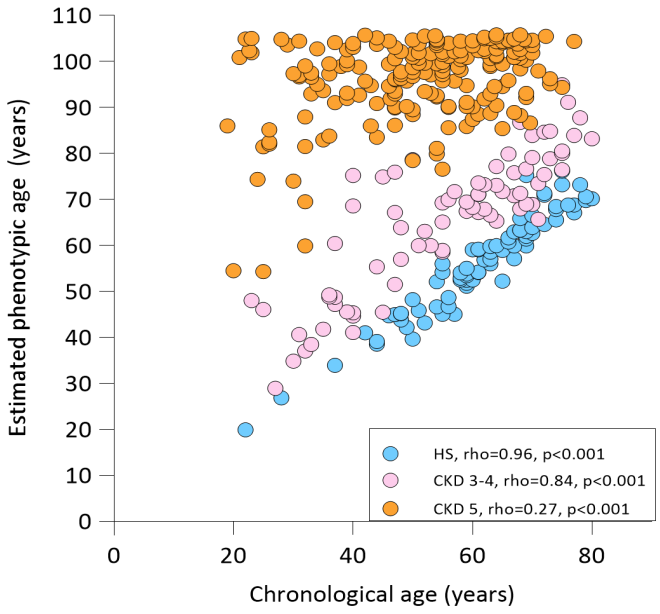
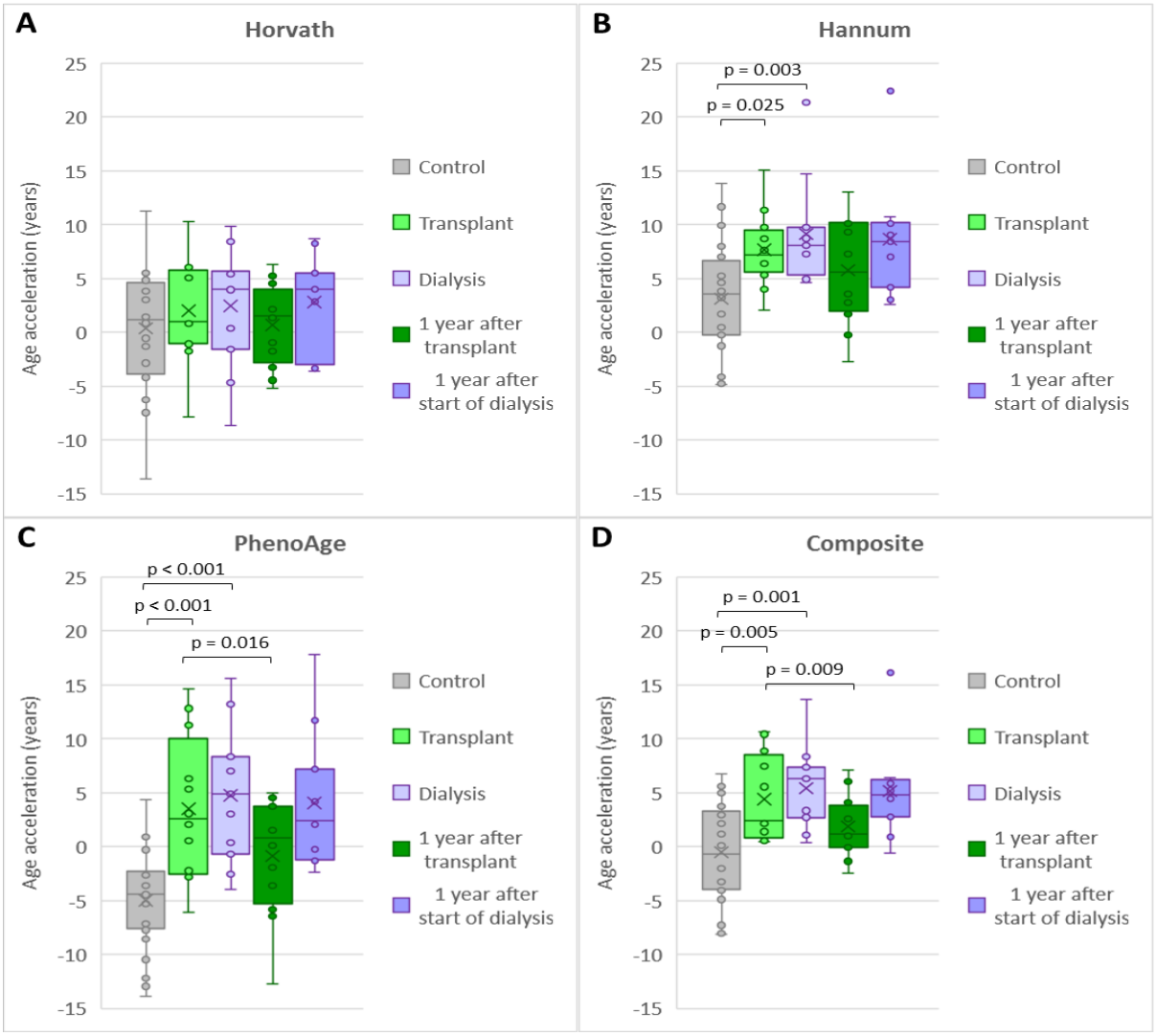


Fig 3. Box plot of age acceleration by treatment group and timepoint according to the Horvath (A), Hannum (B), PhenoAge (C) and Composite (D) clock. All clocks show a reduction in age acceleration after year one compared to baseline in the transplant group, but this difference reaches statistical significance only in the PhenoAge and Composite clock. There is no significant reduction of age acceleration after one year on dialysis.

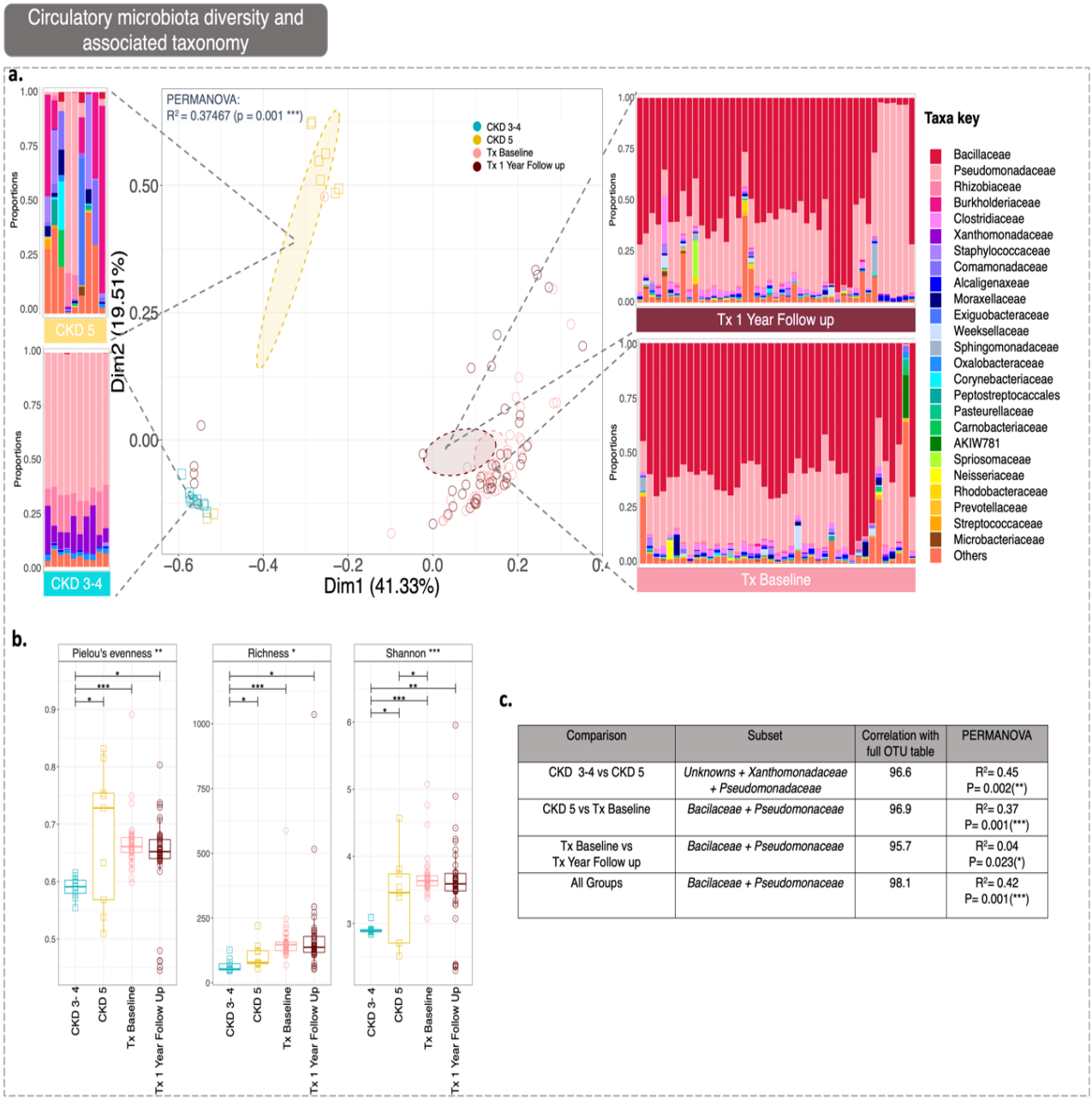


4.4 A CIRCULATORY FOOTPRINT OF THE CORE MICROBIOME DOES NOT NORMALIZE AFTER KIDNEY TRANSPLANTATION

When investigating the clinical phenotype, we found that CKD 5 had a diabetes prevalence of 50% compared to 10% in CKD 3-4 and LDKT, respectively. The prevalence of clinical CVD did not differ between the groups. Blood samples collected at baseline showed higher levels of creatinine, phosphate and iPTH in CKD 5 and LDKT compared to CKD 3-4.

Main results:

1) Beta diversity analysis showed that LDKT clustered away from CKD 5 and CKD 3-4 (1a) - a sign of having a distinct composition ($p<0.001$)
2) Samples from CKD 5 patients were significantly more diverse and even than the CKD 3-4 group (Figure 1b) when alpha diversity was determined. Peilou's evenness, Species richness and Shannon entropy was used to determine alpha diversity.
3) Patients in the CKD 3-4 and CKD 5 groups had similar core compositions (Figure 2a). Both groups had a high abundance of Pseudomonas and Stahpylococcus, which are TMA-producers.
4) LDKT´s taxa were dominated of *Pseudomonaceae* and *Bacilaceae,* at baseline and after one year post transplant. CKD 3-4´s taxa was dominated by *Pseudomonacea.* CKD 5 differed since most patients had more distinct taxa profiles.
5) In all alpha diversity measures, LDKT was significantly more diverse compared to CKD 3-4 (Figure 1b)
6) No distinct changes were observered in the core microbiotas in LDKT baseline vs one year post-transplant (Figure 2a)
7) There were only two genera which differed in LDKT baseline vs LDKT follow up. In LDKT baseline, Viellonella was increased, and in LDKT follow up, Saccarimonidales was increased (Figure 2c)
8) TMAO and betaine had a significant impact on the variation of the microbiome in CKD



Comparison	Subset	Correlation with full OTU table	PERMANOVA
CKD 3-4 vs CKD 5	*Unknowns + Xanthomonadaceae + Pseudomonadaceae*	96.6	R^2= 0.45 P= 0.002(**)
CKD 5 vs Tx Baseline	*Bacilaceae + Pseudomonaceae*	96.9	R^2= 0.37 P= 0.001(***)
Tx Baseline vs Tx Year Follow up	*Bacilaceae + Pseudomonaceae*	95.7	R^2= 0.04 P= 0.023(*)
All Groups	*Bacilaceae + Pseudomonaceae*	98.1	R^2= 0.42 P= 0.001(***)

Figure 1| Microbiota Diversity and Taxonomy (**a**) Beta diversity using Bray-Curtis distance measure and PERMANOVA results for CKD groups (3-4 and 5) and LDKT groups (Baseline and 1 year follow up), along with taxa plots representing the Top-25 most abundant families observed in all samples in the corresponding groups with the taxa key shown. (**b**) Alpha diversity (Peilou's evenness, Richness and Shannon entropy) for each group whereby lines connect two categories if the differences are significant (ANOVA) with * ($P < 0.05$), ** ($P < 0.01$), or *** ($p < 0.001$). Subset analysis (**c**) shows the combination of subsets of families which contribute significantly to changes in the microbiome.

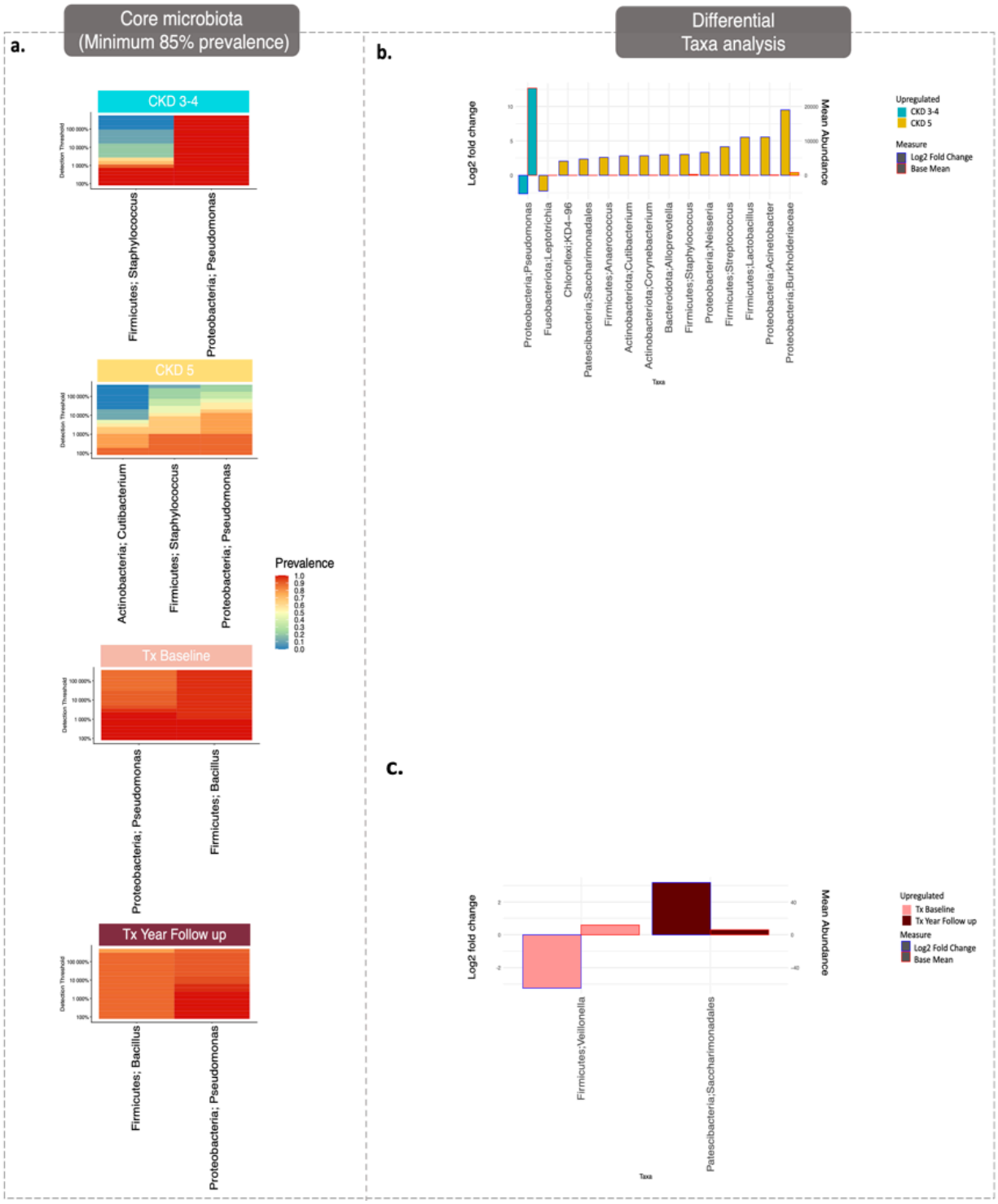


Figure 2 | Core Microbiota and Differential Taxa analysis. (a) Core microbiome analysis showing absolute abundance of genera that are present in at least 85% of samples in each group, (**b**) Genera found to be discriminately expressed based on differential taxa analyses showing which genera are up/down regulated between CKD 3-4 vs. CKD 5, (**c**) LDKT Baseline vs Follow up, where they had at least a log2 fold change (Adj p value ≤ 0.05). Bars outlined in blue shows the Log2 fold change value, and bars shown in red show the mean abundance of each genus.

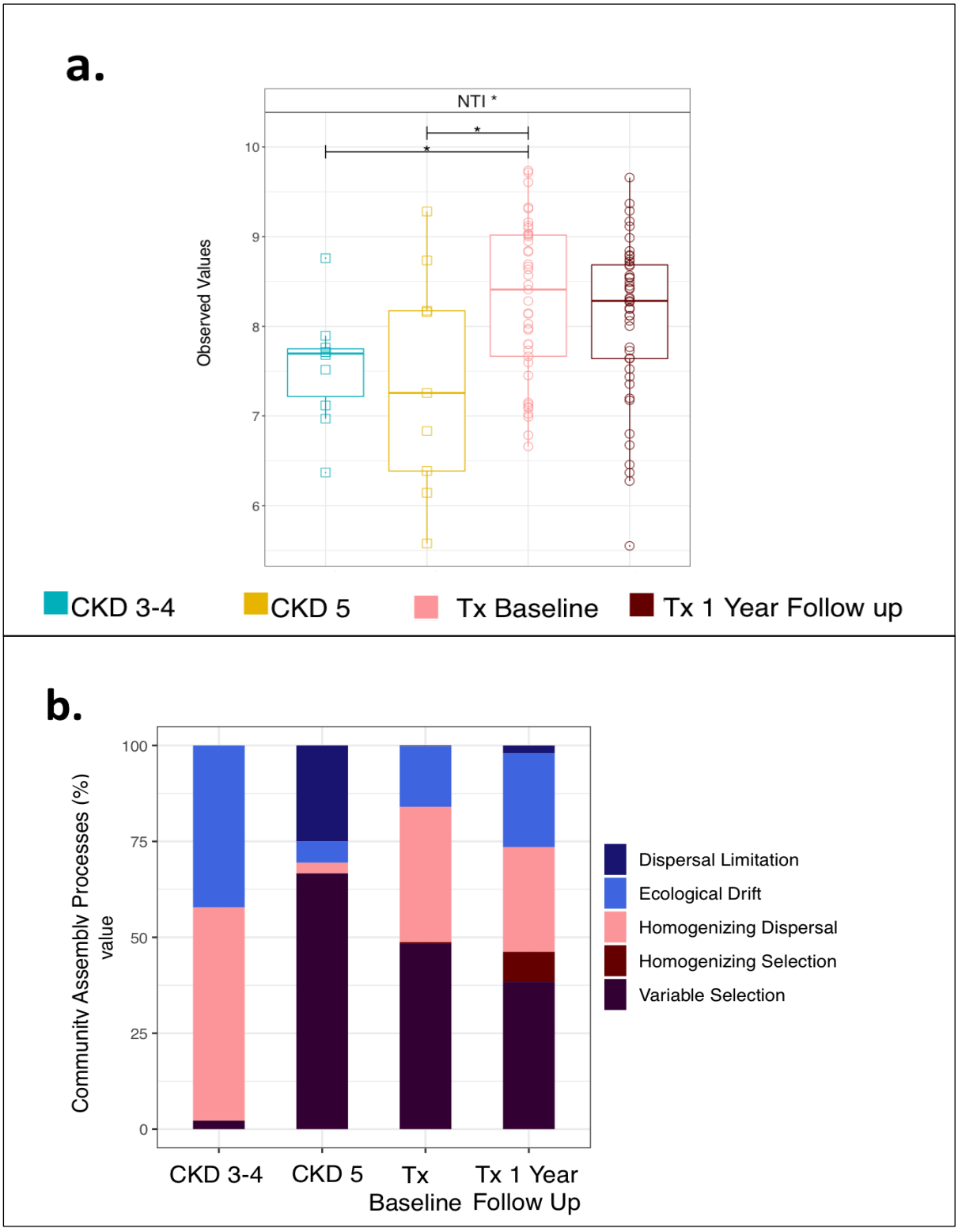


Figure 3. A combined null-model approach to identify and quantify ecological community assembly processes in CKD groups using **(a)** environmental filtering calculated as nearest taxa index (NTI) where values >+2 indicate extreme clustering in the phylogenetic tree driven by environmental pressures (determinism), where lines connecting two categories show significant differences (ANOVA) with * ($p < 0.05$), ** ($p < 0.01$), or *** ($p < 0.001$). **(b)** quantitative process estimates (QPE) approach which determines the proportion of

assembly mechanisms acting on a category within the framework of selection, dispersal and drift represented by a stacked bar chart.

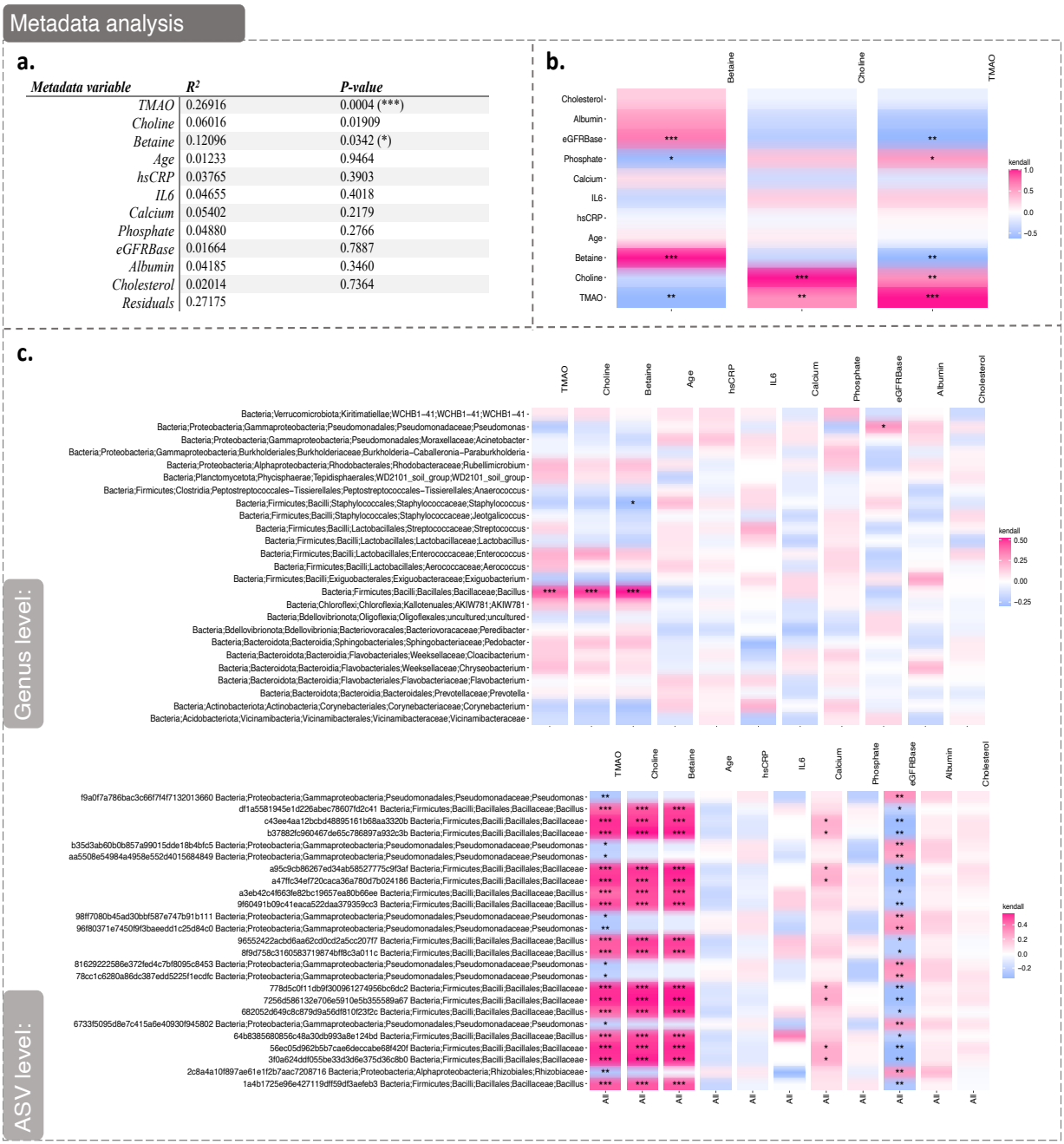


Figure 4 | Metadata analysis (a) PERMANOVA analysis of metadata whereby R^2 represents how much percentage variability in the microbiome (Bray-curtis) is caused by the metadata variable. **(b)** Kendall rank correlation analysis of metadata against elements of one-carbon metabolism, TMAO, Choline, Betaine. **(c)** Kendall Rank correlation analysis of the metadata against the 25 most abundant genera and amplicon sequence variants. Note that metadata was only available for the baseline group of the LDKT cohort, and so encompasses the CKD 5, CKD 3-4 and LDKT baseline groups only. Bonferroni correction was used to adjust for multiple comparisons. P values <0.001 is denoted with (***), <0.01 with (**), <0.05 with (*) <0.1 with (.)

5 DISCUSSION

The aims of this thesis were to explore potential ways to estimate biological ageing in a KI population and to investigate predictive markers for vascular ageing, i.e., CVE and death and compare the effect of dialysis treatment vs KT on biological aging. To do so, we included populations with CKD G5 who remained on dialysis in paper 2, 3 and 4.

5.1 OBSERVATIONAL STUDY OF RISK FACTORS ASSOCIATED WITH CLINICAL OUTCOME AMONG ELDERLY KIDNEY TRANSPLANT RECIPIENTS IN SWEDEN - A DECADE OF FOLLOW UP

5.1.1 Interpretation

In paper 1 we found that comorbidity was an even more sensitive predictor than chronological age for patient survival after KT. Since patients ≥70 years had the same 5-year patient survival as patients 65-70 years, we suggest that elderly should be offered KT in the absence of a severe comorbidity burden. Death-censored graft survival after 10 years was 78% without significant differences between age-groups, even though it is likely that the oldest patients received the oldest kidneys, which is further encouraging. It is reassuring that KT seems to be a safe procedure and long-term treatment also in patients ≥70 years. Median time on dialysis prior to KT was 27 months (IQR 16-40) whereas median time on the waitlist was eight months (IQR 1-21). The discrepancy between median time on dialysis (27 months) and median time on the waiting-list (eight months) indicates that patients probably had a substantial time on dialysis before they were referred for KT. Late initiation/examination or administrative difficulties delaying evaluation for KT and acceptance for the waitlist is an area that has the potential to improve according to our results. By increased awareness and implementing early initiation of preoperative evaluation before patients start in dialysis, we may diminish dialysis vintage before KT and thereby potentially improve patient survival.

5.1.2 Strength and limitations

The major strength of the study is the inclusion of all patients ≥60 years transplanted during a period of 12 years, the long period of follow-up (median follow-up time 7.9 years) and the participation of all transplant centers in Sweden. We also adjusted for possible confounding factors to reduce the risk of bias, both recipient and donor related. There were furthermore no exclusion criteria. Only 14 patients were lost to follow up which strengthens

the generalizability. One limitation was the lack of a comparable control-group which stayed on dialysis during follow up. HLA-status was also not available.

There are some important issues related to research in KT recipients [159]. First, a comparable control-group is often difficult to find. Even though it is possible to design a perfect randomized controlled trial in patients with CKD 5 some of the patients will get transplanted which constitutes a competing risk. As soon as a patient receives a kidney allograft, the patient switch risk-groups. Furthermore, to prove differences in mortality or CVE, it is necessary to have an extended period of follow-up, 5-10 years, and during this time, a vast majority accepted to the waitlist at baseline is already transplanted. In Sweden we are fortunate to have relatively short waitlist times compared to many other countries. There is, however, data from the US where patients accepted to the waitlist have had to wait a very long time, enabling a comparison of outcome between patients getting a transplant and accepted patients remaining on dialysis. It is possible that patients who experience a long time on the waitlist without getting a transplant represent a selective group which might affect outcome. Patients who are highly immunized with a high percentage of panel reactive antibodies (PRA) and a low matching probability are more likely to face extended waiting times. They might also have a longer history of CKD. This could introduce bias when comparing patients who receive a KT with those who do not. Patients who are highly immunized also have a higher risk of acute rejections and graft failure. Hence the degree of immunization may well serve as a confounding factor.

5.1.3 Clinical relevance

Patients ≥70 years did not have inferior 5-year patient survival compared to patients 65-69 years and KT should therefore not be denied to patients ≥70 years due to their chronological age. Based on our results, CCI is a valuable tool for risk-prediction prior KT in this national study of all patients ≥60 years. Women and recipients with living donors had a lower risk of 10-year mortality compared to males and recipients with deceased donors, after adjustment of CCI and other confounders. In clinical situations when there are doubts whether a patient should be recommended KT or not due to high age, CCI <4, female sex and living donor seem to be favorable factors for 10-year patient survival in patients ≥60 years. These factors can therefore be considered when evaluation for KT is performed in patients ≥60 years. Denial of KT based on recipient sex or type of donor should not be recommended.

5.2 SCORING OF MEDIAL ARTERIAL CALCIFICATION PREDICTS CARDIOVASCULAR EVENTS AND MORTALITY AFTER KIDNEY TRANSPLANTATION

5.2.1 Interpretation

Since atherosclerosis and arteriosclerosis is closely related to chronological age, we decided to include measurements of vascular ageing in the thesis. In paper two we found that moderate-severe medial arterial calcification was a sensitive predictor for CVE and mortality in a LDKT population who had low median CAC-scores. Notably patients with no or mild medial arterial calcification exhibited a very low risk of CVE (5.6%) and mortality (1.6 %) after 6.4 years.

According to KDIGO´s guidelines [160,161] and European Society of Cardiology (ESC) [162], all KT recipients should be treated with statins, as they reduce LDL-cholesterol. Some argue that the evidence for recommending LDL-values <1.8 mmol/L in all kidney transplant recipients is weak (level 2C) and that this recommendation should be questioned. It has also been proposed that medial calcification in patients with CKD is not promoted primarily by traditional risk-factors for CVE but may be correlated to uremia and disturbances in calcium-phosphate balance instead. In this study, we did however find strong associations with traditional risk-factors as age, sex, prevalent diabetes and CVD, higher Framingham risk score, higher BMI, CAC-score and aorta valve calcification (all p-value <0.001). Higher systolic blood pressure (p=0.006), higher hsCRP (p=0.024) and signs of PEW (p=0.008) was also associated with higher degree of medial calcification. In contrast, there was no association between phosphate, calcium or iPTH and the degree of medial calcification. Calcium and phosphate were however measured only once and is known to vary with medication and dialysis. Thus, although we cannot exclude that an association exists it was not possible to capture in this study. The associations with traditional risk factors with medial calcification as well as with CAC-score indicate that these factors are important for the development of calcification, also in CKD. The SHARP-study [163] showed a 17% primary reduction of CVE in CKD patients treated with statin and ezetimibe. According to KDIGO [161,162] and ESC [162] guidelines, it is reasonable to draw the conclusion that KT recipients do benefit from statin treatment as well, which is further supported by a post-hoc analysis of the ALERT trial [164].

5.2.2 Strengths and limitations

The strength of the study was the lack of exclusion criteria, an extended period of follow up, and a meticulous collection of baseline characteristics enabling careful phenotyping. In 342 ESKD patients CT-scan was performed, and CAC-score measured, and in 200 LDKT patients, biopsies from arteria epigastrica were collected and scored. We did not find any significant differences between included and excluded LDKT patients (study 2, table 3) which indicates that the external validity (generalizability) is sufficient. We also know from SNR data that there are no differences between patient and graft-survival between different transplant-centers in Sweden [4] which was confirmed in paper 1[121]. A limitation is the great difference in baseline calcification measured as CAC-score between the KT and dialysis patients. It is possible, that even though we adjusted for confounders in the Cox multivariate analysis (paper 2, Suppl table 2 and 3) unaccounted existing bias may remain. Another limitation is that arterial biopsies from arteria epigastrica were collected from patients selected for LDKT only and not from DDKT due to logistical reasons. Thus, the patients we studied may have been healthier than the average KT patient.

5.2.3 Clinical relevance

Markers, which may serve as possible discriminators between patients with a high or low risk of CVE and mortality after KT, are valuable to enable optimal treatment in high-risk individuals. Grading of medial calcification in none-mild or moderate-severe made it possible to discriminate patients in groups of low vs high risk of CVE (5.6% vs 28.4%, $p<0.001$) and low vs high risk of mortality (1.6% vs 14.9%, $p=0.001$) in LDKT, a group which is considered having the best outcome after KT. Making this distinction allows for improvement in precision-based medicine. We found that a CAC-score of 371 AU had the best R-value for CVE in ESKD, and this could be used as a clinical cut-off value. CAC-score >400 AU was associated with HR 5.9 (CI 95%, 2.3-15.3, $p<0.001$) for CVE, adjusted for confounding factors (paper 2, Suppl table 2) and HR 7.4 (CI 95%, 2.1-25.8, $p=0.002$) for mortality adjusted for confounding factors (paper 2, Suppl table 3). CAC-score ≈400 may thus serve as a reasonably good discriminator in the clinical situation. Notably, although p-values were low, the variance was quite large due to a limited number of patients in the study.

5.3 ACCELERATED UREMIC AGEING IS MITIGATED AFTER KIDNEY TRANSPLANTATION, BUT NOT AFTER DIALYSIS

5.3.1 Interpretation

In this study we estimated biological age using skin autofluorescence, calculated Phenoage and three epigenetic clocks based on DNA methylation (DNAm). We found that both skin autofluorescence and calculated Phenoage (PA) overestimated the magnitude of biological ageing in CKD 5. In contrast, when analyzing DNAm age (epigenetic age) we found that patients with CKD G5 presented a biologically plausible age acceleration compared to healthy subjects. When we repeated the analysis of DNAm age one year after KT or initiation of dialysis, we found indications that whereas KT mitigated age acceleration this was not evident after dialysis treatment. Our results support the proposal of CKD 5 as a clinical model of accelerated ageing [165,166]. Low-grade inflammation, oxidative stress, gut dysbiosis, immunosenescence and telomere attrition are possible factors driving accelerated biological ageing in CKD [167,168].

5.3.2 Strengths and limitations

One of the strenghts of this study is the use of several different methods when determining estimates for biological age. Since all of the methods estimated higher biological age than chronological age, our results support the concept of accelerated biolgical ageing in CKD [31]. The fact that SAF and PA was associated with clinical markers associated with chronological age indicate that both methods are of relevance, even though they overestimated biological age in advanded CKD. SAF and PA were associated with hsCRP, IL-6, higher prevalence of diabetes and CVD, low albumin, hemoglobin and handgrip strenght, which are factors associated with chronological age as well. The results showing a mitigated age acceleration one year after KT agree with observations that KT improves quality of life, physical function and life expectancy. Even though there were no differences in baseline CCI between the LDKT and the dialysis group ($p=0.39$) we cannot exclude unaccounted residual bias. Limitations also include a relatively small number of patients in the DNAm cohort, the lack of RDW values in the PA cohort. Moreover, it remains to be determined if changes in DNAm may be due to changes in the underlying disease process or if they are are causal for the ageing process. DNAm have also shown variability due to technical replicates [169].

5.3.3 Clinical relevance

If clinically useful and affordable surrogate markers of biological age can be identified, they can be used to investigate the impact and benefit of both pharmacological and life-style (such as nutritional) interventions, long before hard clinical outcomes are evident such as CVE or mortality. Our findings that hsCRP, IL-6, lower HGS, albumin, hemoglobin and higher prevalence of diabetes and CVD are associated to SAF, PA and chronological age suggests that these simple markers provide prognostic valuable information.

5.4 A CIRCULATORY FOOTPRINT OF THE CORE MICROBIOME DOES NOT NORMALIZE AFTER KIDNEY TRANSPLANTATION

5.4.1 Interpretation

Gut dysbiosis, measured as circulatory microbiome in CKD showed an increasing dysbiosis from CKD 3-4 to CKD 5. The core microbiota essentially did not change one year after KT in LDKT recipients. The resolution of the uremic milieu did not have a major impact on the composition of the circulatory microbiome. There were no signs of increased gut dysbiosis due to immunosuppressive drugs or antibiotics, one year after KT. Essentially, the circulatory microbiome remained the same, which may reflect slow changes of the microbiota in individuals, and a long-standing effect of the uremic milieu, which might have caused a sustained “wear and tear” effect affecting gut microbiota long term.

5.4.2 Strengths and limitations

The major strength was the application of a highly sensitive novel technique ASV, which in contrast to the earlier used method Operational taxonomic units (OTU), produces results which can be compared between studies. Furthermore, circulatory microbiota may reflect the loss of intestinal integrity better than analyses of fecal samples. Bacteria passing the gut barrier and entering the bloodstream probably have a greater impact on the inflammatory burden than microbiota isolated from the gastrointestinal tract. Thus, the novel opportunity to analyze the circulatory microbiome offers new insights into the loss and gain of salutogenic and pathogenic bacteria in CKD. The limitations of the study include the small number of patients and unpaired data in the analyses of CKD 3-4 vs CKD 5 patients. Differences in age and prevalence of diabetes between CKD 5 (incident dialysis patients) and patients undergoing LDKT makes direct microbiotic comparisons between these two groups difficult. The study would also have benefited from fecal microbiome analyses and analyses of biomarker(s) of loss of intestinal integrity and endotoxemia, such as

lipopolysaccharides (LPS) and CD14. The absence of recovery of dysbiosis after KT despite a major improvement of kidney function may also be explained by the relatively brief period of follow-up period. Finally, as red meat rich in L-carnitine is transformed in the gut into TMA and in addition is produced via the intermediate γ-butyrobetaine (γ-BB) metabolite, detailed information on dietary habits would also have benefited the study.

5.4.2 Clinical relevance

Chronic kidney disease is associated with low-grade systemic inflammation and immunodeficiency. Infections and CVD are the leading causes of death in CKD. Uremia causes dysbiosis by promoting overgrowth of pathogens in the gut, diminishing intestinal integrity, and causing bacteria to translocate over the intestinal wall [94]. Moreover, the gut microbiota might have an immunoregulatory role in CKD/ESKD [94]. A continuous activation of the innate immune system induces immunoregulatory mediators that inhibit adaptive and innate immunity [88, 94,170]. This dysregulated immune response and proinflammatory cytokines activated by inflammation due to translocation of bacteria or bacteria fragments, contributes to an elevated risk for CVD and may promote progression of CKD [88].

If bioactive peptides, such as prebiotics, probiotics, and synbiotics are proven to be safe and effective in improving survival of beneficial microbiota and attenuating gut dysbiosis, they could be an interesting treatment option in the CKD-population, but further studies are warranted [170,171].

6 CONCLUSIONS

The main conclusions of the thesis are:

1) Biological ageing in patients ≥60 years (estimated by the Charlson comorbidity index) identified patients with a 4.6 times higher risk of death after KT compared to patients with a lower burden of comorbidities. Charlson comorbidity index has the potential to standardize and improve preoperative evaluation of KT recipients ≥60 years of age. As patient survival after five years was not inferior in patients ≥70 years compared to 65-69 years our observation encourages KT in patients ≥70 years.

2) Scoring of the extent of medial calcification in arteria epigastrica discriminated patients with a low risk of CVE (5.6%) from patients with a high risk of cardiovascular events (28.9%) during 6.4 years of follow up after LDKT. ROC analysis identified a CAC-score of 371 AU as the best predictive value for CVE in CKD 5.

3) Patients with kidney failure present epigenetic signs of an accelerated biological ageing. The age-acceleration may be attenuated (but not normalized) after KT but not in patients remaining on dialysis.

4) A circulatory footprint of the gut microbiota worsens with progression from CKD 3-4 to CKD 5, but does not normalize one year after KT.

Further conclusions:

Chronic kidney disease displayed signs of accelerated biological aging according to all three methods used to estimate biological ageing. The more advanced stage of CKD, the higher estimated biological age. Clinical parameters, such as Framingham risk score, prevalent DM and CVD, hsCRP, IL-6, lower HGS and albumin were strongly associated with higher SAF age and PA, reinforcing their role in risk prediction in the clinical setting. The sum of comorbidities could serve as a pseudo-marker for biological ageing according to our first study. Charlson Comorbidity index (age excluded) had a stronger statistical association to death than chronological age in patients ≥60 years receiving KT.

The main cause of death after KT is CVD. Patients with a low burden of medial calcification had a low risk of CVE and all-cause mortality after KT. Patients who were not eligible for KT and remained on dialysis had a high burden of calcification and a poor outcome with a mortality approaching 100% after 10 years on dialysis. Vascular calcification is strongly associated with CVE and death. With ROC analyses we found that the magnitude of coronary artery calcification has a similar R-value as chronological age.

To improve survival in the CKD-population emphasis should be put on optimal treatment of traditional and nontraditional risk factors for CVD as well as early investigation prior to KT and early referral to transplantation to limit time on dialysis. Kidney transplantation offers the best long-term patient survival [172] and our data suggest that KT mitigates acceleration of biological ageing compared to staying on dialysis. Kidney transplantation offers a patient survival during the first five years after KT that is identical in patients ≥70 years and in patients aged 64-69 years. Based on our results from a complete national cohort with an extensive follow-up time, KT should be recommended to patients ≥70 years of age, in the absence of contraindications.

When evaluating patients for KT, focus should be on evaluation of the burden of comorbidities, chronological age, and vascular calcification. If there still is doubt whether KT should be advised or not, physical function and/or frailty may serve as additional tools. One should keep in mind however, that improvement in quality of life seems to be even greater in frail patients compared to non-frail patients after KT [133].

7 PERSPECTIVES FOR FUTURE RESEARCH

This thesis reports that CAC and medial calcification is associated with an increased risk for CVE events and mortality in CKD. The European Society of Cardiology (ESC) recommends LDL-cholesterol <1.4 mmol/L in all patients with diabetes, eGFR<30 ml/min or a history of cardiovascular events whether they have received a KT or not and <1.8 mmol/L in KT recipients without risk factors. The reasons for these recommendations are that CKD as such is a strong risk-factor for CVE especially when proteinuria is present [3]. The patients who remained in dialysis had a median CAC-score of 847 AU at baseline and a very poor prognosis and mortality close to 100% after 10 years of follow-up. The deleterious effects of vascular calcification in CKD-patients deserves attention. ESC and KDIGO´s guidelines should be followed and risk factors such as LDL-cholesterol should be treated as recommended. The SHARP study, a double-blind randomized controlled trial, showed a 17% reduction in MACEs in patients with various degrees of CKD [163]. A post-hoc analysis of the multicenter, double-blind, randomized ALERT-study demonstrated a reduced relative risk (RR 0.65, 95% CI 0.48-0.88, p 0.005), for myocardial infarction or cardiac death in KT patients receiving Fluvastatin 40-80 mg daily compared to placebo. Fluvastatin reduced LDL-cholesterol with 1 mmol/l compared to placebo and authors concluded that their findings support early initiation of statins after KT [164]. Additional therapies that can mitigate and potentially inhibit vascular calcification in CKD should be investigated.

Traditional and non-traditional risk factors, such as low-grade persistent inflammation, CKD-MBD and hyperphosphatemia should be treated optimally [173]. Future randomized controlled trials of KT recipients receiving statin (+ ezetimibe if the therapeutic goal is not reached), are needed to investigate the benefits of intensive long-term cholesterol-lowering therapy. Novel therapies, SNF472 which inhibits the formation and growth of hydroxyapatite-crystals, given as intravenous injections have been proven to attenuate the progression of VC in hemodialysis patients with pre-existing cardiovascular calcification, according to a randomized phase 2b trial [174,175]. A phase 3 study of SNF742´s effect on calciphylaxis is ongoing. A study of SNF472´s effect on peripheral arterial disease in dialysis will also be performed. Subcutaneous injections of the molecule INS3001 with similar properties as SNF472, is under investigation. The possibility of a substance, given as a daily subcutaneous injection, which attenuates progression of VC is important since it could be given to patients in various stages of CKD and to patients with peritoneal dialysis, in addition to patients on

hemodialysis. Preliminary data from the randomized VitaVask trial showed that Vitamin K1 supplementation is a highly effective treatment for correcting vitamin K deficiency and reducing progression of VC in hemodialysis patients (Ms Submitted 2022) and may be another treatment option.

Kidney transplantation should be encouraged in KRT since the progress of calcification is slower. Furthermore, transplant recipients have a lower rate of CVE compared to dialysis patients. In addition, patients are also recommended to follow a healthy lifestyle which prevents inflammation; stop smoking [176,177], avoid obesity[178,179], maintaining an adequate blood-glucose control, practicing healthy dietary habits and regular physical activity [180]. Until more knowledge based on randomized clinical trials exists, following KDIGO and ESC guidelines is recommended. The finding that biopsies of arteria epigastrica during KT surgery and pathological grading of the extent of medial calcification could serve as a risk marker for CVE after KT is novel and offers a cheap and sensitive risk prediction tool.

This thesis also demonstrates that patients with CKD display an age acceleration with a higher biological than chronological age. Moreover, whereas KT appears to mitigate the age acceleration after one year (but not normalize), no such effect was observed in patients remaining on dialysis. Given the limitations of a small number of patients our results need confirmation in larger cohorts. If reliable and readily available biomarkers of biological ageing could be identified this would benefit future intervention trials testing effects of both dietary, lifestyle and pharmacological interventions in CKD.

Given the strong link between the gut microbiome and immune system, especially in the elderly [88,170,181] a normalization of gut microbiota could be a novel strategy to improve outcome in KT patients. These patients, which are on immunosuppressants and suffer from immunosenescence are at increased risk of cancer, infections, and CVD - the three main causes of death following KT [19,24]. As gut-microbiota-targeted diets recently were shown to modulate the immune status in humans [182], studies need to test if normalization of gut dysbiosis by dietary interventions (such as fiber-rich food and fermented food), prebiotics, probiotics and synbiotics or feces transplantation, lead to dissolution of low-grade inflammation, and thereby have a potential to improve the long-term outcome by decreasing the risk of infections, cancer and CVD.

Unexpectedly, the circulatory footprint of gut dysbiosis did not improve one year after KT indicating that the core gut microbiome remained essentially the same.

Gut dysbiosis is recognized to be associated to a burden of lifestyle diseases, such as diabetes, obesity, CKD and cancer and may be a driver of premature ageing [85]. To validate diet-interventions, randomized controlled trials making proper adjustments for sex, age, and the exposome are warranted. Designing nutrition studies are always challenging, since many factors must be considered and may contribute to the diverse results of diet-interventions studies so far [183]. Adherence to the treatment arm is one of the challenges. Low-protein diets, plant-based diets, fermented foods, fish-rich diet, prebiotics, probiotics and synbiotics should be studied further, to investigate their potential in improving gut dysbiosis and decreasing intestinal permeability [184,185]. Fecal transplantation, which sometimes is given as treatment against therapy-resistant reoccurring *clostridium difficile*-infections in elderly immunocompromised patients, might be a treatment option for gut dysbiosis [186-188]. In one study of immunocompromised patients with reoccurring *Clostridie difficile*-infections, fecal transplantation had a high overall cure rate (89%), however randomized controlled trial is warranted since possible complications might occur [187,189]. By transferring healthy gut bacteria, it may be possible to induce sustained bacterial overgrowth from a healthy individual [190]. Randomized controlled trials which investigate different diets and drugs separately may be needed to expand the understanding of the importance of gut microbiota and the effect on outcome in the long term. A longitudinal study, comparing the composition and community assembly mechanisms, which controls diversity of the gut microbiota at different timepoints in patients advancing in CKD-stages would enhance the understanding of how gut dysbiosis affects the uremic phenotype and outcome. Novel techniques in ecological and genetic studies makes it possible to pinpoint effects of different interventions down to the genome and microbiome level. DNAm clocks and genome/microbiome sequencing have a potential to expand the understanding of different dietary and pharmacological interventions, long before hard endpoints, such as CVE, malignancy or mortality occur.

8 SOCIETAL IMPACTS

With better treatments an increasing expected lifespan in the general population has been observed. It is reasonable to expect lifespan to increase also in patients with chronic burden of lifestyle diseases. Medical advances in various medical fields, such as oncology, cardiology, hematology, infectious diseases and other specialties will benefit patients with CKD as well. When improved treatments, personalized medicine and drugs with less side-effects are offered in older ages, the traditional contraindications for KT have become questionable. If age-limits for different treatments expand in other fields and active treatments against conditions are offered in higher ages such as myeloma, aorta valve stenosis etc., it is reasonable to offer KT also in the elderly CKD-population. Individual circumstances and conditions should of course always be considered, especially patients´ own will. It is however important to recognize that patients´ choices often are strongly influenced by their doctor´s opinion. Thus, it is important that updated information on the excellent outcome of patient and graft survival also in older KT patients are communicated to the nephrology and transplant communities. We showed that Charlson comorbidity index and CAC-score serve as good discriminators preoperatively when predicting patient survival after KT and the risk for CVE after KT. As KT already constitutes 60% of KRT in Sweden an improvement of long-term patient survival in this group has the potential to improve the overall survival in the CKD-population.

This thesis also demonstrates that the extent of medial calcification in arteria epigastrica serve as a valuable prognostic tool for prediction of CVE and low vs high risk of mortality during long-term follow-up. If these results are confirmed also in DDKT, we suggest that biopsies and scoring of the epigastric artery calcification are introduced in the standard KT protocol. The results of this thesis offer improved tools to approach precision-based medicine in a vulnerable patient group. Finally, this thesis suggest that we should pay less attention to the patients chronological age and instead continue to search for reliable and easy-to-use markers of biological age.

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