Aging and Physical Activity at the Interface of Cardiovascular Risk in Renal Patients

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Aging and physical activity at the interface of cardiovascular risk in renal patients

Veroudering en fysieke activiteit op het grensvlak van cardiovasculair risico bij nierpatiënten

Proefschrift

ter verkrijging van de graad van doctor aan de

Erasmus Universiteit Rotterdam

op gezag de

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Chapter 1

General Introduction

Aging is a natural, progressive and inevitable biological process characterized by a gradual decline of cellular function as well as progressive structural changes in many organ systems. These anatomic and physiological changes delineate the process of *senescence*, a term that describes more predictable age-related alterations as opposed to those induced by diseases. Like other organ systems, the kidneys also go through the process of normal senescence, including both anatomical and physiological changes. These changes in a normal aging kidney are distinct from those in kidney diseases such as diabetic nephropathy or nephroangiosclerosis, which are relatively common in elderly. Nonetheless, it is often challenging to distinguish an inevitable organ-based senescence from a disease-mediated structural and functional changes in the elderly. Yet, it is important to emphasize that age-related diseases, when superimposed on those of normal senescence, can significantly alter the rate of functional decline, exhaust renal functional reserve and predispose these patients to cardiovascular complications or even death, particularly when a frank chronic kidney disease (CKD) is manifested. The association between CKD and cardiovascular disease (CVD) is now largely acknowledged. Cardiovascular mortality is about twice as high in patients with stage 3 CKD (estimated glomerular filtration rate (GFR) 30–59 mL/min/1.73 m^2) and three times higher at stage 4 (GFR 15–29 mL/min/1.73 m^2) compared to individuals with normal kidney function (1). In end-stage kidney disease (ESKD) dialysis patients, the mortality risk becomes 10 to 30 times higher than in the general population (2).

Most of the traditional CVD risk factors, such as older age, diabetes mellitus, systolic hypertension, left ventricular dysfunction (LVH) and low high-density lipoprotein (HDL) cholesterol, are highly prevalent in CKD. However, although the cardiovascular risk conferred by these factors may somewhat parallel the relationships described in the general population, several cross-sectional studies have suggested that the Framingham risk equation does not fully capture the extent of CVD risk in subjects with CKD (3). Discovering new risk factors or prognostic indicators is therefore of foremost importance to refine outcome prediction and drive therapeutic management in this particular disease setting.

Similar to the decline in organs' function, it is well known that an impairment in physical capacity represents a major feature of the senescence process. A reduced physical activity, poor fitness or even mobility impairment of various degrees are frequent characteristics of

elderly individuals. Although no amount of physical activity can stop the biological aging process, regular exercise can counteract some of the adverse physiological, psychological, and cognitive consequences of aging (4). Aging and physical inactivity are primary and indirect risk factors for a long list of adverse chronic conditions (5), whereas increasing physical activity from midlife to old age results in reduced rates of chronic disease and death. Due to fatigue and muscle weakness, patients with CKD also have low levels of physical activity. Such a reduced fitness capacity is noteworthy as it is associated with deconditioning and muscle wasting, declining kidney function and an increased risk of comorbidities such as cardiovascular disease. Thus a downward spiral between disease, disuse and deconditioning exists leading to a reduced quality of life, increased hospitalization rates and mortality (6).

In this thesis, I aimed at summarizing the cross-linked relationships between aging, physical activity and chronic kidney disease when looking at the exceedingly high cardiovascular risk which characterizes individuals with impaired renal function. In the first part, I focused on the myriad of epidemiological, pathophysiological and functional aspects characterizing normal and pathological renal senescence through a systematic approach to the existing literature, throwing also an eye on futuristic strategies to retard kidney aging (**Chapter 2**). Attention is paid to pulmonary hypertension (PH) as an emerging but still underestimated risk factor for mortality that worsens cardiovascular outcomes in the CKD setting. First, I summarized current evidence on the diagnostic and prognostic implications of this issue (**Chapter 3**) and then presented findings from a multicenter clinical investigation specifically aiming at testing the predictive role of PH in a large cohort of early CKD individuals with respect to hard patients' endpoints (**Chapter 4**). In the second part, I focused on physical activity and its impact on cardiovascular outcomes, particularly in aging and advanced CKD. To provide insights into the physiology of vascular pressor responses handgrip exercise across different age strata was performed in an experimental pilot trial of healthy individuals (**Chapter 5**). Thereafter, ESKD patients, a high risk population that is also acknowledged to be exceedingly sedentary (7), were analysed to explore the relationships between poor physical performance/impaired mobility and cardiovascular outcomes

(**Chapters 6 and 7**). These latter points have been addressed by analyses of the EXerCise Introduction To Enhance Performance in Dialysis (EXCITE) study.

This study is a large multicenter, randomized trial designed to evaluate if a model of intervention based on a low-grade physical program prescribed in the dialysis unit and performed at home can modify the physical activity and quality of life, reduce the risk of cardiovascular and all-causes mortality, non-fatal cardiovascular events and vascular access failure in dialysis patients (8).

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PART I
THE KIDNEY, AGING AND EMERGING RISK FACTORS

Chapter 2

The Aging Kidney Revisited: A Systematic Review

ABSTRACT

As for the whole human body, the kidney undergoes age-related changes which translate in an inexorable and progressive decline in renal function. Renal aging is a multifactorial process where gender, race and genetic background and several key-mediators such as oxidative stress, the renin-angiotensin-aldosterone (RAAS) system, impairment in kidney repair capacities and background cardiovascular disease play a significant role. Features of the aging kidney include macroscopic and microscopic changes and important functional adaptations, none of which is pathognomonic of aging. The assessment of renal function in the framework of aging is problematic and the question whether renal aging should be considered as a physiological or pathological process remains a much debated issue. Although promising dietary and pharmacological approaches have been tested to retard aging processes or renal function decline in the elderly, proper lifestyle modifications, as those applicable to the general population, currently represent the most plausible approach to maintain kidney health.

Keywords: renal aging, renal senescence, chronic kidney disease, renal function decline, aging processes.

1. INTRODUCTION

Human lifespan has substantially increased over the last century and the projected increase of elderly people over the next two future decades is impressive. Persons aged 65 years or more were 420 million in 2000 (1). By 2030, the number of these individuals is expected to be 550–973 million (1). By that date, elderly people will account for approximately 20%, 24.8% and 33% of the global population in the US, China and Europe respectively, exceeding the number of children below 15 years (2). The average age is now 76.5 years in economically developed- and 65.4 years in economically developing-countries (2).

Population based studies documented that impaired renal function is common in the elderly. In the US population, renal dysfunction has a 15% prevalence in persons older than 70 years (3). In the third National Health and Nutrition Examination Survey (NHANES III), 35% of the elderly population had stage 3 chronic kidney disease (CKD) (4). The prevalence of the most severe CKD stage (stage 5 or end-stage kidney disease; ESKD) is age-dependent (4, 5). In the United States Renal Data System (USRDS) the prevalence of the age-stratum 65-74 years is 11% and 14% for those older than 75 years (6) and similar findings have been reported also in European cohorts (7-9). In this systematic review, we describe the main anatomical and functional changes in the kidney associated with senescence and will provide updated information on the main molecular and biological pathways involved in renal aging. The criteria adopted for literature search and selection for this review are detailed in **Figure 1**.

Figure 1. Review criteria

REVIEW CRITERIA

Relevant articles were identified by searches of MEDLINE, PubMed and references from relevant articles combining the search terms "kidney" or "renal" with "aging" or "ageing" or "age" or "elderly" or "senescence". Search results were further combined with the terms "function" or "glomerular filtration rate" or "GFR" and "decline" or "impairment" or "decrease". Articles were included without language, methodology or date restriction. Only studies specifically dealing with the epidemiology, the anatomical, pathological and functional changes related with renal aging were considered.

2. EPIDEMIOLOGY OF RENAL FUNCTION DECLINE WITH AGE

Changes in renal function associated with aging have been estimated in 9 cross-sectional and in 3 cohort studies. In these studies, the annual average GFR reduction ranged from 0.4 to 2.6 mL/min (**Table 1**). The cross-sectional nature of most of these analyses and the fact that four of them were performed in living kidney donors (10-13), a highly-selected population where the absence of CKD and other co-morbidities is a pre-requisite for kidney donation, limits the generalizability of these findings and leaves open the question whether the decline in renal function is an inexorable process.

In studies based on inulin clearance performed in the fifties in a group of 70 men, including healthy volunteers but also hospitalized patients affected by hypertension, cancer, arteriosclerosis and various infective diseases, the GFR was by the 46% lower in the very old (90 years) as compared to the young people (14) and these findings were confirmed in a survey based on urea clearance (15). In the Baltimore Longitudinal Study of Aging (BLSA) (16), a longitudinal study based on serial creatinine clearance measurements in 254 men without kidney disease or hypertension, the average decline in GFR was 0.75 mL/min/year, an estimate very close to that described in a recent cross-sectional study in 1203 living kidney donors (0.63 mL/min/year) (13). In the Baltimore study, the rate of GFR loss was tripled (~1.51 mL/min) in subjects aged 40-80 years as compared to subjects aged 20-39 years (0.26 mL/min). Similar observations were reported more recently in a longitudinal study in healthy Chinese people (17). Both in the Baltimore (16) and in the Chinese (17) study the GFR remained constant overtime in 36% and 44% of subjects respectively. In the Bronx longitudinal age study (18, 19) in very elderly subjects, just a small increase in serum creatinine occurred after 3 years in long term survivors and similar observations were reported in a subgroup of 31 subjects with mildly raised serum urea at baseline, suggesting that renal function decline may not be an obligatory consequence of the aging process. In a cross-sectional analysis of the BLSA study (20) focusing on 548 healthy subjects, creatinine clearance was 140 ml/min/1.73m^2 at age 30 to fall to 97 ml/min/1.73m^2 at age 80. In the inception cohort of the Nijmegen Biomedical Study (21), including 869 apparently healthy persons aged>65 years, the annual GFR decline (as estimated by the MDRD$_{185}$ formula) was approximately 0.4 mL/min/year. In a mixed population of adults aged≥65 years including

participants with major co-morbidities (CKD included), the InCHIANTI study, creatinine clearance estimated by the Cockcroft formula showed a 2.6 mL/min/year decline over a 3-year follow up (22). Overall, these studies clearly document that on average renal function declines overtime but also show that in about one third of elderly individuals the GFR remains remarkably constant.

Table 1. Main studies on renal function decline with aging

Author	Year	Methods	Results
Davies and Shock (15)	1950	-Cross-sectional analysis of a miscellaneous population of 70 men aged 25 to 89 years including healthy subjects and hospitalized patients. -mGFR by inulin clearance.	-Linear 46% decline in mGFR from 123 (at the age of 30) to 65 (at the age of 89) mL/min/1.73 m^2 .
Smith et al. (14)	1951	-Cross-sectional analysis of general population. -Renal function measured as urea clearance.	-Decrease in urea clearance from 100% at the age of 30 years to 55% at the age of 89 years.
Rowe et al. (20)	1976	-Cross-sectional analysis of an inception cohort of 548 men (aged 20-80 years) from the BLSA. -eGFR by creatinine clearance.	-Progressive linear decline (31%) in eGFR from 140 (at age 30) to 97 (at age 80) mL/min/1.73 m^2.
Lindeman et al. (16)	1985	-Prospective study of an inception cohort of 254 men (aged 20-80 years) without kidney disease from the BLSA followed over 5 to 14 years. -eGFR by creatinine clearance.	-The mean decrease in eGFR was 0.75 ml/min/year. -Annual eGFR changes were different between the age class 20-39 (0.63 mL/min/year) and 40-80 (1.51 mL/min/year). -36% of all subjects followed had no absolute decrease in renal function. -A small group of patients showed a statistically significant increase in creatinine clearance with age.
Feinfeld et al. (18, 19)	1995	-Prospective study of 141 very elderly subjects followed over 6 years. -Renal function assessed by BUN and serum creatinine.	-Small but significant decline in BUN and creatinine at 3 years, which persisted at 6 years.
Rule et al. (11)	2004	-Retrospective analysis of 365 potential living kidney donors. -mGFR by iothalamate clearance, eGFR by MDRD and Cockroft-Gault formulas.	-Men at the age of 20 years had an estimated mean GFR of 129 mL/min that declined by 4.6 mL/min/decade. -Women at the age of 20 years had a mean GFR of 123 mL/min that declined by 7.1 mL/min/decade.
Fehrman-Ekholm et al. (12)	2004	-Cross-sectional analysis of 52 elderly "healthy" persons aged 70-110 years.	-mGFR decreases by approximately 1.05 ml/min per year in very old persons.

		-mGFR by iothalamate, eGFR by Cockroft-Gault, MDRD and Walser formulas.	
Wetzels et al. (21)	2007	-Cross-sectional study of an "healthy" inception cohort of 3732 subjects from the Nijmegen Biomedical Study, of whom 869 were elderly (age>65 years). -eGFR by MDRD.	- eGFR declined by 0.4 mL/min/year
Lauretani et al. (22)	2008	-Cross-sectional and prospective analysis (3 years follow-up) of 931 adults (aged≥65 years) from the InCHIANTI study. -eGFR by Cockroft-Gault formula.	- eGFR declined by 2.6 mL/min/year
Poggio et al. (10)	2009	-Cross-sectional analysis of 1057 prospective kidney donors. -mGFR by iothalamate clearance.	-mGFR was reduced by 1.49+/-0.61 ml/min per 1.73 m^2 per decade of testing. -Significant doubling in the rate of GFR decline in donors over age 45 as compared to younger donors.
Rule et al. (13)	2010	-Cross-sectional analysis of 1203 adult living kidney donors. --mGFR by iothalamate clearance, eGFR by MDRD and Cockroft-Gault formulas.	- reduction in mGFR by 6.3 mL/min per decade
Jiang et al. (17)	2012	-Prospective study of middle-aged and elderly 245 healthy individuals evaluated over a 5 years follow-up. -eGFR by creatinine clearance	-eGFR decreased from 98.1+/-15.6 to 90.4+/-17.3mL/min/1.73m^2. -43% of participants did not experience a decline in eGFR during follow-up.

BLSA: Baltimore Longitudinal Study of Aging; **BUN**: Blood Urea Nitrogen; **eGFR**: estimated glomerular filtration rate; **mGFR**: measured glomerular filtration rate; **MDRD**: modification of diet in renal disease (formula).

3. ISSUES WITH ASSESSMENT OF RENAL FUNCTION IN THE ELDERLY

Because sarcopenia and body weight loss reduce the daily generation of creatinine and creatinine levels are influenced by protein intake and hydration, these factors concur in making serum creatinine a suboptimal indicator of renal function in the elderly (23). The reference range for creatinine considered as normal in the healthy young is inappropriately high in the elderly and serum values in the upper normal range may underlie early renal dysfunction (24). In 20 years old individuals a creatinine value of 1 mg/dL may correspond to an estimated GFR of 120 mL/min while the same value in 80 years-old persons might reflect an eGFR of 60 mL/min (25-27). Traditional formulas for GFR estimation based on serum creatinine are notoriously unreliable in the elderly, particularly in the presence of multiple co-morbidities (28, 29). In old subjects, GFR is systematically underestimated by

the Cockcroft-Gault (CG) formula (30, 31). The Modification of Diet in Renal Disease (MDRD) MDRD equation is generally considered more accurate than the CG to estimate GFR in old people (32). However, like the CG formula, the MDRD equation has not been specifically validated in the elderly and the discordance of estimates between these two formulas is such that the MDRD GFR may be by the 60% higher than the CG-GFR in patients over 65 years (33). In a study involving 100 individuals aged 65-111 years no correlation was found between the two formulas (34). In the elderly cohort of the InCHIANTI study, creatinine clearance <60 mL/min calculated by the CG formula predicted all cause and cardiovascular mortality while the MDRD formula did not (35). In a study comparing the most recent three CKD Epidemiology Collaboration (CKD-EPI) formulas implementing creatinine (CKD-EPI Cr), cystatin-C (CKD-EPI Cys) or both (CKD-EPI Cr-Cys) in 394 elderly subjects with median age of 80 years (36), these formulas appeared less biased and more accurate than the MDRD Study equation but no equation achieved sufficient accuracy when tested against the golden standard (GFR measured by Iohexol). Other formulas such as that proposed by Keller (37) and the HUGE (hematocrit, urea and gender) formula (38) apparently improve the precision of GFR estimation in the elderly but neither of these has yet been externally validated. Serum cystatin measurement, especially when compared with reference values adjusted for age, represents a promising marker to measure renal function in the elderly (39) but cystatin-based formulas are not superior to the MDRD equation for estimating renal function in old people with GFR<60 mL/min/1.73m^2 (36). Nevertheless, formulas based on cystatin-C predict morbidity and mortality better than creatinine-based equations (40), a phenomenon likely depending on the fact that serum cystatin-C in part reflects inflammation, i.e. a strong predictor of clinical outcomes in the elderly (41). The Berlin Initiative Study (BIS)-1 (creatinine-based) and the BIS-2 (cystatin-based), have been recently developed in a cohort of 610 individuals aged 70 years or older with no or mild-to-moderately reduced kidney function (GFR <60 mL/min per 1.73 m^2) using Iohexol plasma clearance as golden standard (42). Interestingly, the BIS-2 equation yields the smallest bias followed by the creatinine-based BIS-1 and Cockcroft-Gault equations, while all the other formulas overestimate to an important extent the golden standard. These formulas are of obvious relevance but still lack external validation in other cohorts and, most importantly,

in different ethnicities. Kidney disease improving global outcomes (KDIGO) guidelines set at 60 mL/min/1.73 m² the GFR threshold below which renal function should be considered as clearly impaired (43). As this universal cutoff does not take into account age, it is much debated whether healthy elderly subjects with a GFR in the 45-60 mL/min/1.73 m² range, particularly in the absence of proteinuria and urine abnormalities, should really be considered as "diseased" or not (44). Considering these subjects as affected by CKD may allow increased cardiovascular and renal surveillance but may engender harm and increased cost because of inappropriate and over-diagnosis of CKD in low risk elderly. As for other parameters, such as blood pressure and serum glucose, perfect thresholds to distinguish between safe and risky values do not exists. However, thresholds can be useful for treatment recommendations and for the identification of subpopulation at high risk of complications. In the CKD-EPI consortium meta-analysis (45), individuals >65 years with a GFR 45-59 mL/min/1.73 m^2 had a 44% excess risk for cardiovascular death as compared to those with GFR falling in the "normal" range (>90 mL/min/1.73 m^2). In this meta-analysis there was no effect modification by age on the cardiovascular risk associated with reduced GFR. In elderly individuals aged ≥75 years with GFR 45-59 mL/min/1.73 m^2 the risk for end stage kidney disease was similar to that found in individuals aged 18–54 years with the same GFR, i.e., four times higher than that in individuals of the same age-categories and a GFR=80 mL/min/1.73 m^2 (46). No classification system is perfect and clinical judgment is important, particularly around the diagnostic thresholds. Therefore, when evaluating the GFR in the elderly, clinicians should consider co-morbid conditions, life expectancy and the time-trajectory of GFR. Renal senescence is a complex, multifactorial process characterized by anatomical and functional changes accumulating during life span. Several factors, spanning from the genetic background to exposure to chronic diseases and environmental factors generate a "multi-hit" scenario where the renal phenotype of elderly individuals shows high inter-individual variability (**Figure 2 and 3)**.

Figure 2. Main mechanisms leading to renal senescence

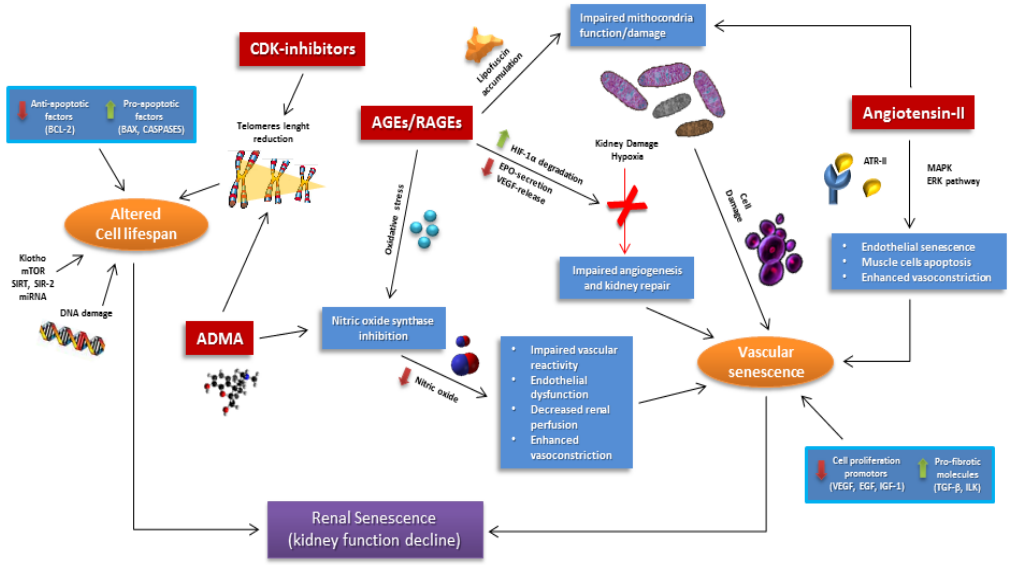
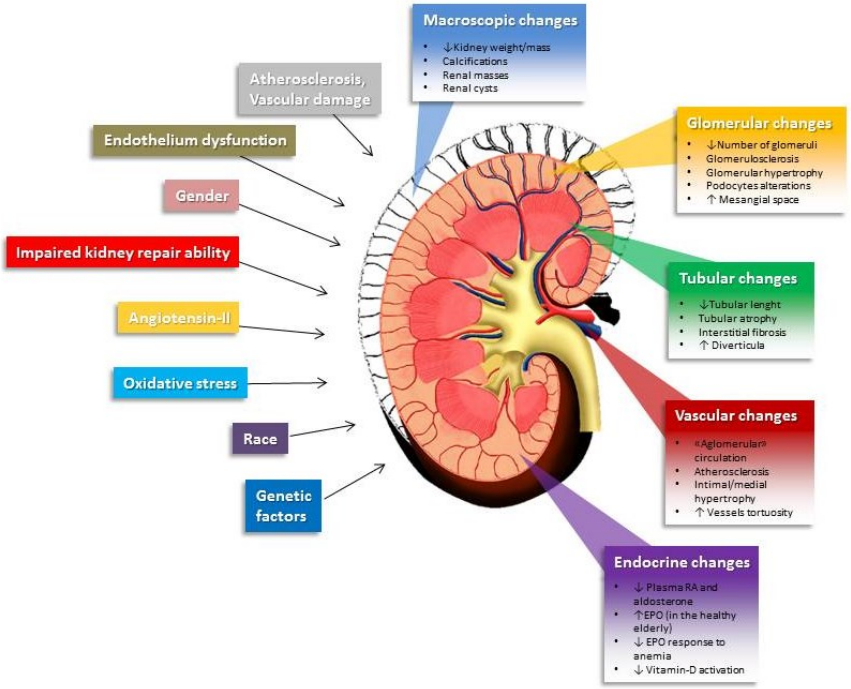


Figure 3. Macroscopic and microscopic changes in the aging kidney and risk factors



4. FACTORS ASSOCIATED WITH RENAL AGING

4.1 Gender

In experimental models, male gender enhances the age-related decline in renal function (47, 48). Accelerated GFR loss in males is androgen-dependent (48). Castration in mice limits reno-vascular aging (49) while therapy with estrogens may prevent this phenomenon (50). Studies of the effect of aging on renal function and anatomy abound but mechanistic knowledge on gender-dependent influence on this phenomenon is limited (51).

4.2 Race

Black race is an established risk factor for kidney dysfunction and for the risk of progression to end stage kidney failure (52, 53), particularly in diabetic patients (54). African descent is strongly associated with the risk of hypertensive nephrosclerosis (55).

4.3 Genetics

The genetic background plays a major role in renal senescence and genotypes exist which regulate the number of nephrons during life (56). Epigenetics - the process whereby neutral cells evolve into highly differentiated cells to constitute specialized tissues- has a prominent role in kidney aging. Regulatory genes and post-transcriptional processes, such as acetylation and methylation, are crucial for the control of the differentiation of kidney cells and for maintaining cell function during life span. In a rat model of normal aging (57), there is de novo glomerular expression of proteins which remains silenced in the young glomerulus. Accelerated renal aging including diffuse glomerulosclerosis and interstitial fibrosis occurs in differentiated podocytes after manipulation of methylation pathways (58). Fusion of foot processes and disorganization of foot structures in podocytes as well as proteinuria are hallmarks of aging kidneys in the rat and these alterations set the stage for glomerular rarefaction and functional decline. Spontaneous gene mutations in somatic and mitochondrial DNA accumulate with normal aging in kidney cells (59). Premature aging in the progeria

syndrome is characterized by focal renal scarring, glomerulosclerosis, tubular atrophy and interstitial fibrosis and associates with mutations in genes involved in DNA repair, transcription and replication (60). Functional genomics studies showed that over 500 genes are differently expressed in human kidneys across age-strata encompassing neonate's (8 weeks) and elderly's kidneys (88 years). Kidneys of elderly individuals overexpress proteins involved in the immune response, inflammation, extracellular matrix synthesis and turnover while under-express genes involved in oxidative processes, lipid and glucose metabolism and collagen degradation (61). In another study, more than 900 different age-dependent genes were identified and the expression of these genes changed in parallel in the cortex and in the medulla (62). Genes that impact upon the aging process and influence life span have been identified (63). However, only some of these seem to be involved in kidney aging. The senescence marker protein (SMP) 30-knockout mouse displays accumulation of lipofuscin and electron-dense material and lysosomial enlargement in the proximal tubules, which are alterations peculiar to kidneys of elderly individuals (64). Polymorphisms in the alpha-adducin gene predicted renal function decline in a population-based study in apparently healthy Chinese people (65). In vivo, senescent renal cells, particularly in the renal cortex, express high levels of cellular proliferation inhibitors, such as p16 and p27 (66), and the expression of these proteins goes along with the severity of age-associated glomerulosclerosis, tubular damage and interstitial fibrosis (67). The target of rapamycin (TOR) is a highly conserved gene pathway modulating the influence of nutrients on life span (68). Selective TOR-inhibition dramatically increases life span and this effect is prevented by caloric restriction (69). TOR expression increases with age in rat kidneys, particularly so in mesangial cells, and the inhibition of this pathway by rapamycin attenuates the aging-related phenotype in this model (70). The sirtuins (SIRT), a gene family homolog of the Silent information regulator 2 (Sir2) gene (71), are also implicated in renal aging. Sir2, another highly conserved longevity gene, is implicated in nutrient-dependent changes in life span (72) as well as in the prevention of DNA damage (73). SIRT-6 knock-out rats are characterized by premature aging in various organ systems including the kidney (74). SIRT-1 over-expression promotes

antifibrotic and antiapoptotic effects in renal interstitial cells and caloric restriction slows-down kidney ageing by enhancing SIRT-1-mediated mitochondrial autophagy in mice (75). Klotho is perhaps the most powerful "aging-suppressor" gene (76). The Klotho knock-out mouse exhibits aging-related diseases like atherosclerosis, osteoporosis, vascular and tissue calcifications and chronic kidney disease (77) and genetic polymorphisms in the Klotho gene have been associated with altered life span (78), accelerated vascular disease (79) and osteopenia (80). Klotho operates in concert with FGF-23 because the main receptor for this growth factor, the FGF receptor, is activated by FGF-23 only in the presence of Klotho in most tissues (81). In the kidney, Klotho is predominantly expressed in the distal convolute tubule. Furthermore, Klotho exerts a series of potentially nephroprotective actions including: 1) reduction of oxidative stress via inhibition of the insulin/IGF1 signaling and induction of the manganese superoxide dismutase (82); 2) fine-tuning of calcium-phosphorus homeostasis by down-regulation of vitamin-D synthesis and phosphaturia (83); 3) modulation of calcium channel activity in renal tubular cells (84); 4) regulation of endothelium-dependent vascular reactivity (85). Sustained oxidative and metabolic stress (85), angiotensin-II (AT-II) (86) and chronic kidney disease (87) down-regulate Klotho m-RNA expression. Transfection of the Klotho gene attenuates tubular-interstitial fibrosis and vascular wall thickening in renal vessels induced by chronic AT-II stimulation (88). Thus, Klotho hypo-regulation might at least in part explain the link between renin-angiotensin system and renal senescence (see below). Telomeres, the nucleoprotein complexes located at the end of chromosomes which serve to prevent the fusion and degradation of chromosomes, are synthetized by the enzyme telomerase. Kidney cells do not express the enzyme telomerase (89). Therefore, in these cells telomeres shorten progressively after each cell division, a process triggering cellular and organ senescence (90). In the human kidney, telomeres shortening increases with age and is more rapid in the cortex (91). Telomerase-deficient mice show reduced glomerular, tubular and interstitial cell proliferative capacity and limited ability to recover after acute kidney injuries (92). MicroRNAs (miRNAs) are fundamental modulators of cell function which regulate important biological events like cell-

differentiation and apoptosis. Global disruption of miRNAs in mice is associated with rapidly progressive chronic kidney disease (93). However, studies of renal aging based on selective manipulation of the m-RNA system are lacking and the possibility of interfering with kidney senescence at m-RNA level is still little explored.

4.4 Oxidative stress and the nitric oxide system

Free-radicals generation increases with lifespan (94). A substantial increase in kidney levels of oxidative stress markers like F2 isoprostanes, advanced glycosylation end-products (AGEs) and their receptors (RAGEs) occurs in the aging kidney (95). As to the AGE-RAGE system, it was found that aging also de-regulates kidney expression of AGE-R1, a receptor preventing AGEs-mediated injury (96). AGEs and circulating RAGEs are independently associated with decreased renal function and predict GFR decline in elderly community-dwelling women (97). In a secondary analysis of the BLSA cohort, serum levels of L-carboxymethyl-lysine (CML), one of the main AGE products, were independently associated with CKD and eGFR (98). AGEs promote degradation of the hypoxia-inducible factor (HIF)-1α, thereby limiting the response of renal cells to hypoxia. This phenomenon attenuates the secretion of EPO and the release of VEGF, a growth factor crucial for angiogenesis (99). AGEs and other oxidants reduce telomeres length and cell lifespan (see above) (100). Furthermore, AGEs are powerful inhibitors of Nitric oxide (NO)-synthase (NOS) activity in renal tubular cells (101, 102). Reduced NO bioavailability plays a major role in the structural and functional adaptations of the aging kidney. NOs inhibition by L-NAME produces a stronger vasoconstriction in old than in young renal vessels (103, 104), suggesting that endogenous NO production is of particular relevance for the control of renal circulation in aging animals. In aging rats, total body NO generation is reduced (103, 105), particularly so in the endothelium of peritubular capillaries (eNOS), suggesting that the tubular-interstitial ischemia and fibrosis typically associated with renal senescence is at least in part causally related to oxidative stress-mediated NOS inhibition (106). Female aging rats show relatively conserved levels of eNOS in renal capillaries (107) and neuronal (n)NOS in renal cortex (108) as compared to aging male rats, a phenomenon depending on the stimulatory

effects of estrogens on eNOS synthesis. Renal microvasculature is particularly sensitive to the vasodilator effect elicited by NO, a response fundamental for the control of renal blood flow and pressure-natriuresis (103, 109). The endogenous inhibitor of NOS Asymmetric dimethylarginine (ADMA) accumulates in aging rats (105) and high ADMA appears to be involved in telomeres shortening (110). In elderly patients, high ADMA is a strong predictor of death and cardiovascular events (111). Notably, this methyl-arginine may be an important effector of the age-related decrease of renal perfusion because high circulating ADMA levels go along with reduced renal perfusion in old people (112). Oxidative damage derives mainly from free radicals generated during metabolic processes at cell level. However, high dietary oxidant load with diet may contribute as well. Studies based on the ARIC cohort documented that subjects with scavenger receptors defects and high-fat diets develop atherosclerosis and severe impairment in kidney function (113). In the rat, a diet enriched of antioxidants (such as vitamin-E) reduces kidney RAGEs and F2 isoprostanes levels and increases the GFR by the 50% (95). Similarly, caloric restriction in aging rats increases kidney levels of ceruloplasmin, a powerful anti-oxidant produced by parietal epithelial cells of the Bowman's capsule in response to aging (114). Lipofuscin, a complex found in the cytosol of aging cells, is formed by free-radicals damaged proteins and fats. This complex, which is resistant to degradation, substantially impairs mithocondrial function (115). In rat models, lipofuscin accumulation in the kidney is linearly related with age and lipofuscin cell levels are 28-fold higher in very old as compared to very young rats (116).

4.5 Angiotensin-II

Angiotensin II (AT-II) regulates a variety of biological functions within the kidney including vascular tone, aldosterone release, tubular sodium reabsorption and sympathetic nerve stimulation. In addition, this peptide has important effects on cell plasticity in the kidney because it induces fibroblast differentiation into myofibroblasts, vascular hypertrophy, mitogenesis and promotes the release of various cytokines and growth factors (such as TGF-β1) (117). AT-II receptors with opposite vascular effect exist. Indeed, the angiotensin receptor-1 (ATR)-1 mediates vasoconstriction while ATR-

2 promotes NO/cGMP-mediated vasodilatation (118). In addition, ATRs stimulation by AT-II via the MAPK and ERK pathways leads to endothelial senescence and triggers endothelial and muscle cells apoptosis (119, 120). ATR-1 in the kidney are more abundant than ATR-2. During lifespan, the number of ATR-2 increases, which would favor renal vasodilation. However, due to the reduced renal flow and the attenuated NO-mediated vasodilatation discussed before, in the elderly the renal response to angiotensin-II is a sustained vasoconstriction (121). Furthermore, ATR-1 stimulation promotes mithocondrial damage and reactive oxygen species production, both of which in turn trigger age-related vascular changes (122). In the ATR-1 knockout mice, oxidative stress is markedly reduced in the kidney and in the hearth and renal tubular cells show a higher number of mithocondria as compared to wild-type controls (123). Of note, ATR-1 knockouts also outlive the wild-type controls by 26% and this increase in lifespan has been attributed to an up-regulation in the kidney of genes associated with survival (such as the sirtuin-3 or the NAMPT). Accordingly, ATR-blockade by selective inhibitors effectively improves renal function and vascular structure in aging rats (117).

4.6 Impairment in kidney repair ability

Cell proliferation is crucial for normal tissue turnover and for tissue regeneration. In the adult kidney less than 1% of renal cells maintain proliferating potential and this fraction further declines with aging (124). Such phenomenon is multifactorial in nature and it is often defined as “cellular senescence” to differentiate irreversible and specific morpho-functional changes associated with physiological cellular aging from other forms of cell cycle arrest. In aged mice kidneys, a clear age-dependent decline in the proliferative potential of proximal tubular cells occurs after ischemia/reperfusion injury (125), a phenomenon secondary to modifications of various cell-cycle regulators and to enhancement of apoptosis. The cyclin-independent kinase (CDK) inhibitor $p16^{INK4A}$, a powerful blocker of the cell-cycle, is up-regulated in epithelial and interstitial cells of aging mouse and in human kidneys as well (89, 126). Similarly p21, a CDK-inhibitor which induces proliferative arrest, apoptosis and cellular hypertrophy, increases with

age in rats (127). Both $p16^{INK4A}$ and p21 promote renal tubular senescence by enhancing telomeres shortening (91) and by upregulating the activity of senescence-related enzymes, such as β-galactosidase (89). The Caspase family includes several cysteine protease involved in apoptosis induction (128). Caspases 3,9 and the caspase-9 activator cytochrome-c are upregulated in the kidney of aging rodents (128, 129) as it is the pro-apoptotic protein Bax (125). Conversely, the expression of Bcl-2, a powerful apoptosis inhibitor, is reduced in renal tissue of aging rats (125). Overall, multiple alterations in systems controlling apoptosis explain the very high apoptosis rate in aging kidneys (106, 129). As previously alluded to, this pro-apoptotic pattern can be largely prevented by a low-calories diet adopted at young age (130). Growth factors are key players in kidney repair and an age-driven impairment in the pathways activated by these factors has been advocated to explain the inadequate regenerative capacity of the aging kidney (131). The expression of factors promoting cell recruitment and cell proliferation such as the epidermal growth factor (EGF), the insulin-like growth factor (IGF)-1 and the vascular endothelial growth factor (VEGF), decline in an age-dependent fashion (132-134) while the expression of pro-fibrotic factors like transforming growth factor (TGF)-β and integrin-linked kinase (ILK) increases (127, 135). A variety of other factors have been implicated in the impaired repair ability of senescent kidneys. The potential role of these factors in kidney aging was reviewed in detail elsewhere (136).

4.7 Cardiovascular disease and risk factors

The age-related decline in renal function is amplified in subjects with pre-existing cardiovascular disease and/or risk factors. In a cohort study of 1456 elderly individuals, the components of the metabolic syndrome and insulin resistance predicted the risks of prevalent and incident CKD (137). Hypertension, a classical age-dependent disease (138, 139), associates with typical changes in renal structure and function (140). High BP amplifies age-related vascular stiffness and atherosclerosis and vice versa (141). Endothelial dysfunction, disturbed regulation of the renin-angiotensin system and increased sympathetic tone are critical factors at the hypertension-renal ageing interface. Furthermore, due to age-related tubular-interstitial alterations, elderly

subjects are salt-sensitive, i.e. predisposed to aggravation of renal damage by hypertension-dependent and independent mechanisms when exposed to excessive salt intake (142). In this respect, it is well documented that salt-restriction programs in the elderly allows better blood pressure control and improve clinical outcomes (143).

Even though the causal nature of the link of aging with hypertension, arterial stiffness and renal dysfunction is reasonably well established, in the healthy elderly cohort of the BLSA study BP failed to predict the age-associated decline in creatinine clearance (144) while carotid intima-media thickness had no prognostic value for renal function in a community cohort in China (145). In the Italian Longitudinal Study on Ageing (ILSA) cohort in 2981 subjects aged 65-84 years with normal renal function (146), renal function loss as defined by an increase in serum creatinine >26.5 micromol/L associated with current smoking status (OR=2.3; 95% CI=1.0-5.3), fibrinogen levels>3.5 g/l (OR=2.2; 95% CI=1.6-3.3), diabetes (OR=1.8; 95% CI=1.1-2.8) and systolic hypertension (OR=1.6; 95% CI=1.0-2.6). Similarly, in the Cardiovascular Health Study (147, 148) smoking, systolic blood pressure, internal carotid artery thickness and retinal microvascular abnormalities independently predicted renal function decline overtime. Similar findings were reported in two large community-based cohort studies (149, 150) and in a recent study we briefly alluded to before (17). The severity of systemic atherosclerosis has been indicated as one of the major determinants of age-related glomerulosclerosis and decline in renal function (151).

5. STRUCTURAL AND FUNCTIONAL CHANGES IN THE AGING KIDNEY

The main anatomic and functional modifications which characterize the aging kidney are summarized in **Figure 3 and Table 2**. Kidney mass progressively increases from birth to the fourth decade of life, peaking at 250-270 g (152) and gradually regresses thereafter at a 10% reduction rate *per decade* (153-155) (**Figure 4**). In the seventh and eighth decades, kidney mass is therefore at least 20-30% less than in the fourth decade (156) and the reduction is more pronounced in the renal cortex than in the medulla (155, 157). As expected, kidney size follows the same temporal trend (153). In a series of 1957 potential kidney donors undergoing pre-donation renal imaging studies by

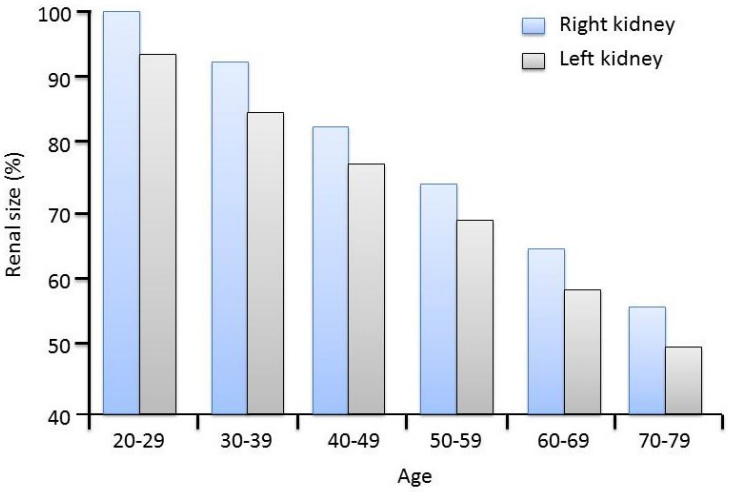
computer tomography kidney aging was accompanied by parenchymal calcifications and by a rising prevalence of simple renal cysts (158). From a microscopic point of view, the aging kidney displays glomerular, vascular and tubular-interstitial changes of various type which we will discuss in some detail in the following paragraphs.

Table 2. Main functional changes of the aging kidney (see text)

Glomerular
• ↓GFR
Tubular
• Impaired sodium balance • Impaired fluid balance • ↑ potassium retention • ↓ capacity to dilute urines • ↓ capacity to lower urine pH
Vascular
• ↓ ERPF (mostly in the cortex; conserved in the medullary) • ↓ capacity to lower urine pH • ↑ filtration fraction • ↑ post-glomerular RVRs • impaired vasodilatory responses
Endocrine
• ↓ plasma RA and aldosterone • ↑ EPO (in the healthy elderly) • ↓ EPO response to anemia • ↓ Vit-D activation

EPO: erythropoietin; **ERPF**: effective renal plasma flow; **GFR**: glomerular filtration rate; **RA**: renin activity; **RVRs**: reno-vascular resistances.

Figure 4. Renal size (%) by age among 360 healthy adults. The size of the right kidney in the group aged 20-29 was considered as reference value. Redrawn from (155).

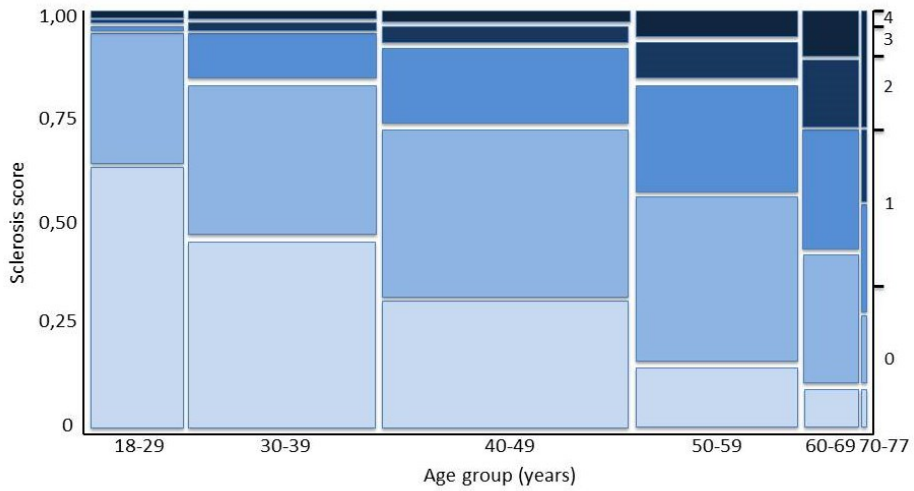


5.1 Glomerular changes

The number of glomeruli in the adult kidney ranges from 330000 to 1100000 (159). Race, gender and birth weight are the main determinants of glomerulogenesis. The number of functioning glomeruli decreases during life-time (159, 160) while the proportion of hyaline and sclerotic glomeruli increases (13, 161-163) (**Figure 5**). Glomerular obsolescence goes along with intrarenal arterial changes, particularly with intimal fibroplasia (151). In very old living kidney donors, the prevalence of glomerulosclerosis, which can be as high as 70% (13), can be predicted by the formula: (age/2)-10 (164). Sclerotic glomeruli prevail in the subcapsular cortical zone in the elderly (165) and glomerulosclerosis purely attributable to aging is a multifactorial process which should be suspected when the renal interstitium shows scarce infiltration in the absence of changes characteristically seen in hypertensive and diabetic patients. Human podocytes are unable to undergo cell division and the number of these cells decreases with age (166, 167). In aged rats, podocytes undergo hypertrophy which eventuates in apoptosis, podocytopenia and glomerulosclerosis (168, 169). Brenner

hypothesis of renal aging holds that an altered control of glomerular hemodynamics increases glomerular plasma flow and intra-capillary pressure, leading to glomerulosclerosis (170, 171). Glomeruli spared from this process are hyper-perfused and hypertrophic and these functional adaptations may serve to maintain global glomerular filtration rate (172-175). However, this process becomes “maladaptive” in the long term, because glomerular hyperperfusion goes along with glomerular hypertension (170). Low glomerular density is a powerful predictor of renal function decline in patients with glomerulonephritides (176, 177). In elderly donors glomerular density is related in an inverse fashion to the proportion of sclerotic glomeruli (178). Glomerular basement membrane thickening is another typical feature of the aging glomeruli (179) as it is mesangial expansion (180). Direct shunts between afferent and efferent arterioles bypassing the glomerulal tuft in iuxta-medullary nephrons is an additional anatomo-pathological alteration commonly seen in kidneys of elderly subjects (180, 181).

Figure 5. Sclerosis scores by age group among 1.203 living kidney donors defined as: (1) any global glomerulosclerosis, (2) any tubular atrophy, (3) interstitial fibrosis >5% and (4) any arteriosclerosis. In the figure a score of 0 is azure, a score of 4 is deep blue and intermediate scores are on a blue scale. Redrawn from (13).



5.2 Tubulo-interstitial changes

The age-dependent decline in renal size and renal mass rests more on tubulo-interstitial changes than on glomerular or vascular changes (182, 183) and the same holds true for renal function (183). As described for glomeruli, the overall number of tubules decreases with age (184). Tubular length and volume are also markedly reduced and sparse areas of scarring, tubular atrophy and tubular diverticula are common in kidneys of elderly individuals (163, 185, 186). Tubular diverticula localize mainly in the distal convolute tubule and in the collecting duct and may give rise to form simple renal cysts (187), an alteration observed in about a half of subjects ≥40 years (188). Tubular dilatation may be accompanied by accumulation of hyaline material and basement membrane thickening. When extended, this process may lead to a sort of "thyroidization" of the kidney, a common feature in end-stage kidney disease. Wrinkling and thickening of basement membrane and simplification of the tubular epithelium is frequently observed in old kidneys, while the so called "endocrine" transformation with thin basement membranes and numerous mitochondria is a relatively rare involution pattern (184). Expanded interstitial volume, infiltration of mononuclear cells and diffuse areas of fibrosis are all hallmarks of the aging kidney (56). Excessive collagen deposition and structural changes in extracellular matrix, altered regulation of the expression of metalloproteinases and TGF-β, activation of fibrosis- and hypoxia-related genes all concur to the pathogenesis of tubulo-interstitial fibrosis in aging kidneys (189-192). Alterations in tubular function go along with anatomical involvement. Enhanced proximal sodium reabsorption coupled to reduced distal fractional reabsorption allows maintenance of a normal sodium balance under steady-state conditions in the elderly (29). However, this functional resetting limits the ability to conserve sodium in response to low salt intake and makes elderly people predisposed to volume depletion and acute kidney injury (193). Inadequate activation of the renin-angiotensin system and reduced aldosterone secretion (hyporeninemic hypoaldosteronism) play a leading role into this phenomenon (194) as well as in nocturnal natriuresis, another frequent alteration in old people (195, 196). On the other hand, aged individuals display also a relative inability to excrete sodium excess in

response to salt load, a multifactorial alteration predisposing to salt retention, hypertension and cardiovascular congestion. Resistance to the natriuretic effect of atrial natriuretic peptide is a key step into this process (142). Alterations in tubular handling of electrolytes extend to potassium. Due to tubular atrophy and tubular-interstitial scarring, Na-K ATPase activity is reduced in the elderly, resulting in a high risk for hyperkalemia. Reduction in GFR, hyporeninemic-hypoaldosteronism, dehydration, metabolic acidosis, all enhance the tendency to hyperkalemia in the elderly and the administration of potassium-sparing drugs may precipitate serious clinical events in individuals harboring these risk factors (197). The capacity of diluting and concentrating urine decreases with age in humans (198, 199). Reduced expression of urea transporters in the inner medullary collecting ducts impairs the capacity of appropriately raising urine concentration in aged rats (200) which also show a down-regulation of vasopressin-2 receptors in the collecting duct and a reduced expression of the water-channels aquaporin 2 and 3 (201-203). Nocturia, which is in part a consequence of a reduced concentrating ability, is a typical feature of old age (198, 204). On the other hand, elderly people exhibit also impaired urine diluting capacity which expose them to an increased risk of hyponatremia after water load (205, 206). Even though the renal regulation of acid-base balance is globally conserved in the aging kidney (207), the capacity of generating ammonia is clearly impaired (208). Elderly subjects are more prone than young individuals to develop acidosis in response to acid load (such as after a high-protein meal or in stress conditions which activate proteolysis) mainly because of the incapacity to increase ammonia and H^+ synthesis (209-211). Impaired proton pump activity in the cortical collecting duct is a critical element in the deranged response to acid load in the elderly (208, 212). Renal-dependent metabolic acidosis has been implicated in a constellation of alterations in the elderly including hypercalciuria, decreased citrate excretion, enhanced protein catabolism, muscle wasting, bone dissolution, cardiomyopathy and progression of CKD (213).

5.3 Vascular changes

Structural changes in renal vasculature are similar to those observed in vessels in other organ systems and include intimal and medial hypertrophy, arteriolosclerosis and overt atherosclerotic lesions (214). Post-mortem angiograms and histology studies show increased irregularity and tortuosity of pre-glomerular vessels, direct shunts between afferent and efferent vessels (see above), wall thickening and narrowing of the vascular lumen of afferent arterioles (215, 216), an alteration mainly depending on vascular smooth muscle cells proliferation (154). In addition, micro-infarctions triggered by cholesterol emboli are often observed along with atherosclerosis of the aorta and renal arteries in elderly patients with diabetes and hypertension. Interlobular arteries in the elderly show fibro-intimal hyperplasia (214), a feature typically observed in patients with chronic hypertension regardless of age.

From adulthood to the age of 80 years, renal plasma flow (RPF) (15) and effective RPF (ERPF) exhibits a steady decline (29). Reduction in RPF mainly occurs in the renal cortex while medullary flow is relatively well preserved (214). Accordingly, the contribution of iuxtamedullary glomeruli to global GFR increases (29). Due to an increase in post-glomerular renovascular resistances (RVR), the GFR is relatively better preserved than ERPF both in healthy elderly people and in elderly subjects with hypertension, heart failure and other cardiovascular co-morbidities (29). Reduced ERPF has obvious causal links with structural changes in the renal vasculature, particularly at post-glomerular level (181). Furthermore, the reno-vascular response to vasodilatory agents (217) and the sensitivity of renal arterioles to endogenous and exogenous vasoactive substances (103, 218, 219) is overtly altered in the elderly.

5.4 Endocrine changes

5.4.1 Renal Autacoids

Autacoids, including prostaglandins, prostacyclins, thromboxanes and leukotrienes, are powerful endogenous vasoactive agents which also modulate platelet aggregation. The synthesis and the associated signaling transduction pathways of these complounds are altered by the aging process (220). Young and old rats fed at normal or low-salt diet,

have comparable levels of PGE2 and PGF2-α in the renal interstitial fluid (221). However, PGF2-α production is reduced and PGE2 enhanced in old rats as compared to young rats after sodium overload (221). In human studies, PGF1-α production is age-dependent while the synthesis of other prostaglandins (such as PGE2 and PGF2-α) is in large part preserved in kidneys of elderly individuals (222). A defect in prostaglandin modulation has been postulated to explain the altered adaptive capacity of the aging kidney to respond to sympathetic stimulation, such as that by mental stress (223), particularly in elderly persons with isolated systolic hypertension (224). On the other hand, the inhibition of prostaglandin synthesis produces similar functional derangements in healthy elderly and young subjects (225).

5.4.2 RAS system

Plasma renin activity (226, 227) and aldosterone (228) are about halved in elderly subjects, a phenomenon mainly due to limited synthesis and release of renin, particularly under stress conditions (229). As alluded to before, reduced activation of the renin-aldosterone system (RAS) contributes to the development of various fluid and electrolyte abnormalities and partly accounts for the higher risk of dehydration, hypernatremia and hyperkalaemia which characterize elderly persons.

5.4.3 Erythropoietin

Circulating levels of erythropoietin (EPO) are higher in the healthy elderly as compared to younger individuals (230). Increased EPO production in the elderly is interpreted as a counter regulatory mechanism aimed at preserving normal red blood cells mass in response to a higher turnover, as well as to EPO resistance. However, EPO levels are reduced in anemic elderly individuals, suggesting an impaired counter-regulatory response to low hemoglobin levels (231). In a secondary analysis of the InCHIANTI study, old age went along with reduced EPO levels, anemia and impaired renal function (232).

5.4.4 Vitamin D

Elderly people may develop vitamin D deficiency due to the impaired capacity of the aging kidney to convert 25-hydroxy vitamin-D to 1,25 dihydroxy vitamin-D (233) but extra-renal factors, i.e. 25-OH-vitamin D availability, affect at least equally vitamin-D sufficiency in the elderly. In a cohort study in 168 elderly patients with various degree of renal impairment, reduced 25-OH-vitamin D levels were independent predictors of progression to dialysis and death in the long term (234). In a secondary analysis of the BELFRAIL study, in individuals>80 years with conserved renal function higher 25-OH-vitamin D levels associated with exposure to sunshine and with an active lifestyle (235) but not with renal function. CKD may worsen vitamin D deficiency in the elderly. Indeed, in post-menopausal women, the presence of CKD predicts the risk for bone fractures while calcitriol supplements reduce the incidence of falls, a protective effect which may depend on improved muscle strength promoted by up-regulation of vitamin D receptors (236).

6.FUTURE PERSPECTIVES FOR RETARDING RENAL AGING

As briefly alluded to before, dietary interventions to retard systemic and kidney aging have been extensively studied in animal models. Isocaloric diets with low-AGE content reduce kidney and cardiovascular damage associated with age and extend lifespan in rat models (237). Furthermore, powerful anti-oxidants, such as the methylglyoxal, potentiate the protective effects of low-AGE diets (238). A diet enriched of antioxidants (such as vitamin-E), reduces kidney lipid peroxidation and accumulation of F2 isoprostanes and increases markedly the GFR (by 50%) in aging kidneys (95). Long-term administration of the NO precursor and ADMA antagonist L–arginine in aging rats ameliorates proteinuria and renal function (239). It was hypothesized that the beneficial effects of the “Mediterranean” diet on general health and lifespan might depend on the very low content in AGEs and on the high content of anti-oxidants of this diet (240). Caloric restriction retards age-related structural changes in the kidney, including glomerulosclerosis, ischemic injury, vascular wall thickening and tubular-interstitial fibrosis (241). These beneficial effects associate with reduced expression of

the matrix-metalloproteinase-7, kidney injury molecule-1 and claudin-7 (242), as well as with a reduction in renal lipid accumulation (243), ceruloplasmin production (114) and apoptosis (130). In animal models of aging, such as the 24-months F344BN old rat, caloric restriction reduces aging-related proteinuria and extracellular matrix accumulation and these effects are apparently mediated by reduced expression of vascular endothelial growth factor (VEGF), plasminogen activator inhibitor (PAI)-1 and other connective tissue growth factors (244). Caloric restriction also preserves renal SIRT-1 expression, a sensor of redox and energy state with antiapoptotic and antifibrotic effects which is considered as a main factor in the cytoprotective mechanisms which may retard kidney aging (see above). No studies documenting a beneficial effect of long-term, low-calories diets on renal function exist in humans. However, long term caloric restriction ameliorates hypertension and the metabolic profile and retards atherosclerosis (245) and the decline in diastolic function in humans (246). Observations in the Nurses' Health Study (247) would support the contention that low protein intake may limit age-related decline in renal function in humans. Indeed, in a subgroup of women with normal renal function, the estimated change in GFR attributable to excessive protein intake was 0.25 mL/min/1.73 m^2 per 10-g increase in protein intake over a 11-year period. Salt intake is another important modifiable factor which may retard renal function decline, mainly because low salt diets improve blood pressure control (248). In a small cohort of elderly hypertensive patients, the average salt excretion and the baseline eGFR were the only independent predictors of renal function decline (249). However, the observational nature of findings reporting a protective effect of low protein and sodium diets prevents causal interpretations and no recommendation for public health and clinical practice can be made on the basis of these data. Few drugs have been tested so far for retarding renal aging. In experimental studies, PPAR-γ agonists limit parenchimal sclerosis, alleviate cell senescence and improve GFR and proteinuria (250, 251). Increased renal expression of Klotho and reduced oxidative stress have been proposed as potential mechanisms to explain improvements by PPAR-γ agonists. Chronic treatment with angiotensin-converter enzyme inhibitors (ACEi) or ATR-blockers (ARBs) reduces age-related

glomerulosclerosis, mesangial expansion, tubular-interstitial fibrosis and mononuclear cells infiltration along with renal mitochondria damage (252). Furthermore, selective ATR-1 blockade prevents renal damage by increasing NO bioavailability and by reducing oxidative stress in aged spontaneous hypertensive rats (253).

7.CONCLUSIONS

Aging has been defined as "the collection of changes that render human beings progressively more likely to die" (254). This view implies the existence of an inexorable functional decline in biological systems in the whole organism. Whether aging is a disease or as the inevitable consequence of being human is a philosophical and a scientific question. Renal aging is a complex multifactorial process and ascertaining to what extent renal lesions in the elderly represent the life course exposure to chronic diseases or the local manifestation of systemic aging is tantalizing. Progress in genetics and proteomics provide promising new insights on renal aging. Proper lifestyle modifications, as those applicable to the general population, including the adoption of low-calories and low-AGEs diets with high content in anti-oxidants currently represent the most plausible approach to maintain kidney health.

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Chapter 3

Pulmonary pressure as a novel prognostic biomarker in renal patients

ABSTRACT

Renal patients are notoriously at high risk for cardiovascular complications but such risk is not fully explained by traditional and Chronic kidney disease (CKD)-related risk factors. New prognostic biomarkers are therefore needed to refine outcome prediction in this population. High pulmonary pressure (PP- also known as pulmonary hypertension) is remarkably prevalent among persons with CKD, particularly in hemodialysis patients. High PP is a powerful and independent predictor of death in the general population and in subjects with heart or lung diseases. In renal patients, there is now evidence showing that PP may hold the same prognostic utility. High PP predicts adverse cardiovascular outcomes in dialysis and pre-dialysis populations. In kidney transplant recipients, high PP is associated with worse renal outcomes. In this chapter, we will focus on high PP in the CKD population, spanning from the main techniques for assessing PP to the pathophysiology of pulmonary hypertension (PH) in renal patients. Prognostic implications of PH in CKD patients for risk stratification and therapeutic management will also be discussed.

Keywords : pulmonary pressure, pulmonary hypertension, chronic kidney disease, end-stage kidney disease, dialysis, cardiovascular risk

1. INTRODUCTION

The incidence of chronic kidney disease (CKD) and end-stage kidney disease (ESKD) is on the rise. Currently, it has been estimated that over 50 million people are affected by CKD and over 2 million persons need chronic dialysis for ESKD (1). This amount is expected to increase by 60% by 2020 (2). CKD ranks now as one of the main risk factors for cardiovascular (CV) mortality and morbidity with a substantial impact on health resources. In the US, annual costs attributable to manage CV complications of CKD patients rank from 1700 (KDOQI CKD-stage 1) to 12700 (CKD- stage 4) dollars (3). Although a large percentage of patients with CKD have traditional CV risk factors such as diabetes, hypertension and lipid abnormalities, interventions targeting these factors have failed to significantly decrease CV mortality and morbidity. Similarly, normalization of other non-traditional risk factors peculiar of CKD including anaemia, microalbuminuria, inflammation, oxidative stress and altered mineral metabolism, was not totally effective in improving event-free survival.

High pulmonary pressure (PP) has recently emerged as a novel CV risk factor in the general population. In a surveillance from 1980 to 2002 (4), the Centers for Disease Control and Prevention identified increasing rates of hospitalization associated with high PP and stable death rates ranging from 5.2 to 5.4 per 100.000 persons. Conversely, during the last decade, an increasing trend in mortality was documented with an estimated age-adjusted death rate of 4.5 to 12.3 per 100.000 (5). High PP rarely presents as an idiopathic condition, being more frequently associated with systemic, cardiac or lung disorders which may affect the pulmonary vascular circuit.

There is now accruing evidence indicating that masked, non-symptomatic high PP is exceedingly prevalent in CKD persons, particularly in ESKD patients on chronic renal replacement therapy (6). Several underlying conditions, such as volume overload, the presence of high-flow artero-venous fistulas, breath disorders and sympathetic hyper-activation have been postulated to explain such a high frequency. Nevertheless, PP is emerging as a novel, important prognostic biomarker in the renal population. In fact, the presence of high PP in CKD is generally associated with poor outcomes, spanning from increased mortality to higher rate of CV events and delayed graft function in renal

transplanted patients (6). The evaluation of PP might therefore represent an additional, helpful tool for risk assessment and risk stratification of renal patients.

2. PULMONARY CIRCULATION AND PULMONARY PRESSURE ASSESSMENT

Less known to nephrologists, who are more familiar with the systemic circulation, the pulmonary circulation is a delicate and exclusive low-resistance, low-impedance, high-capacitance and high-flow circuit. Under physiological conditions pressure levels in the pulmonary arteries are roughly one fourth to one sixth of those normally found in the systemic circulation (7). Medial thickening of the major pulmonary arteries is notably lower than that of systemic arteries, being more similar to the structure of large veins. The normal pulmonary circulation therefore consists of highly compliant pulmonary arteries and a vast capillary network with large recruitment capability which is able to accommodate large increases in blood flow without significant increases in PP, e.g. during sustained exercise or in the presence of left-to-right congenital intra-cardiac shunts. How can PP be assessed in clinical practice? The gold standard of measurement is represented by right heart catheterization (RHC), an invasive procedure which consists in reaching the right heart with a catheter inserted via a peripheral vein (8). The catheter can be moved until the right atrium or even further, reaching the right ventricle and the main pulmonary artery branches. As long as the catheter proceeds through the right heart to the pulmonary circulation this gets characteristic pressure responses, very similar to a sequence of spikes, peaking at about 20-25 mmHg. When in the distal branch of the pulmonary artery, values of the measured PP of about 14 +/- 3 mmHg are considered as normal. The assessment of the so-called pulmonary artery wedge pressure (PAWP), that is the pressure measured by wedging a Swan-Ganz catheter with an inflated balloon into a small pulmonary arterial branch, may give additional information, particularly in the presence of pathological PP values (see below). In fact, PAWP allows to assess the pulmonary vascular resistance (PVR), expressed as the ratio between the difference of mean PP and PAWP and the cardiac output. RHC is crucial for assessing PP but as it is an invasive procedure it may be associated with an increased risk of dangerous complications. Therefore, with some

exceptions, cardiac catheterization in daily practice is usually not considered as the first line approach to evaluate PP. In most cases, PP is firstly estimated by non-invasive procedures, like a simple transthoracic Doppler echocardiography. PP estimation with such technique is based on the eventual finding of tricuspidal regurgitation, a phenomenon that can be minimally present also in apparently healthy subjects. If tricuspidal regurgitation is present, the echocardiography instrument can automatically assess the Vmax, that is the maximum tricuspidal jet velocity. This parameter is important to finally estimate the PP (ePP) according to the so-called Bernoulli's equation as the product of the square of the Vmax by 4 ($4x\ Vmax^2$) (9). However, this equation often gives a too much rough estimate of ePP that can be further refined by implementing also information about the estimated right atrial pressure (RAP) in the "modified" Bernoulli's equation ($4x\ Vmax^2 + RAP$) (10). RAP is supposed to range from 10 to 30 mmHg in case of absence or presence of relevant inferior vena cava collapse.

3. DIAGNOSING HIGH PP

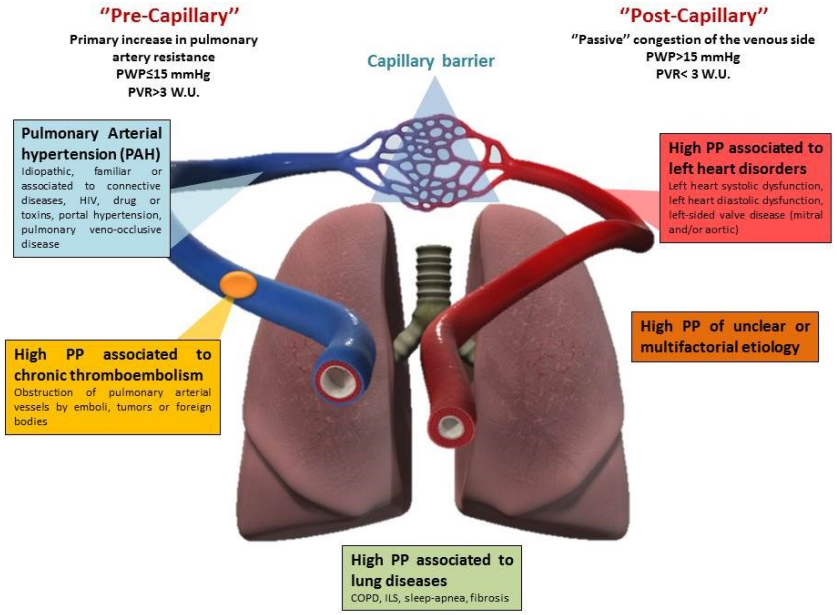
A recent joint guideline (11) made by the European Society of Cardiology (ESC) and the European Respiratory Society (ERS) has established the main criteria for the definition of "pathological" PP values, finally making clearness in a very controversial and debated topic. High, pathological PP (a condition also known as Pulmonary Hypertension-PH) is defined by a documented increase in the measured PP (mPP)≥25 mmHg at rest.

However, although not diagnostic, ePP values 35-49 mmHg (roughly corresponding to Vmax values of 2.8-3.4 m/s) can be considered suggestive of PH while values ≥50 mmHg are strongly indicative of the true presence of PH. Echocardiography estimation can then be useful as screening test for selecting patients who deserve more invasive exams for a clear-cut diagnosis of pathological PP. Even though is of foremost importance to be sure about the true diagnosis/presence of pathological PP, it is also important to identify the underlying cause of such alteration. As briefly alluded to before, mPP or ePP values alone are not sufficient to make a differential diagnosis of PH and additional parameters, such as PAWP and PVR, are required. According to the ESC-ERS guideline, which has recently been endorsed by a WHO document, different types of PH exist,

each one with its peculiar natural history and clinical approach. The WHO classification (12) of the different types of PH mostly looks at the pathogenesis and the anatomic location of the primary alteration responsible of increased PP (**Figure 1**). As mentioned, the pulmonary circulation consists of an arterial side that mostly recall the characteristics of systemic veins in terms of compliance and sectional structure and a venous side bringing the oxygenated blood back to the heart and, from there, to the systemic circulation. The capillary barrier, that is ideally in the middle between the two sides, can be useful to distinguish pathological conditions characterized by a primary increase in pulmonary artery resistances, the so-called "pre-capillary" pulmonary hypertension, from those mostly secondary to a passive venous congestion ("post-capillary"). PAWP and PVR can be helpful in discriminating pre-capillary from post-capillary forms of high PP. In pre-capillary forms, PAWP is low (<15 mmHg) while PVR are expectedly high (> 3 Woods units); conversely, in post-capillary forms PAWP values are increased (>15 mmHg) with usually low PVR (<3 Woods units) (8). Pre-capillary forms of PH include the so-called "Pulmonary arterial hypertension" (PAH) which encompasses idiopathic (IPAH), familial (FPAH) or forms associated (APAH) to congenital heart disease, connective tissue diseases, drugs and toxins, HIV infection, portal hypertension or pulmonary veno-occlusive disease. In addition, pre-capillary PH can arise as consequence of chronic thromboembolism such as in the presence of obstruction of pulmonary arterial vessels (proximal or distal) by thromboemboli, tumors, or foreign bodies. Post-capillary forms are most frequently found as associated to left heart disorders such as systolic or diastolic dysfunction or valve diseases (mitral and/or aortic). High PP can be found also in the presence of other conditions primarily affecting the lungs (e.g. chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD), sleep apnea) or as manifestation of several systemic diseases (12). These two latter cases can present either with a prevalent pre-capillary or post-capillary involvement so that a proper differential diagnosis based on PAWP and PVR is not always possible. **Figure 2** depicts a diagnostic algorithm that can be useful for approaching patients with suspected pathological PP. As said, the first approach would be to perform a non-invasive assessment of PP by echocardiography. ePP of 35-49

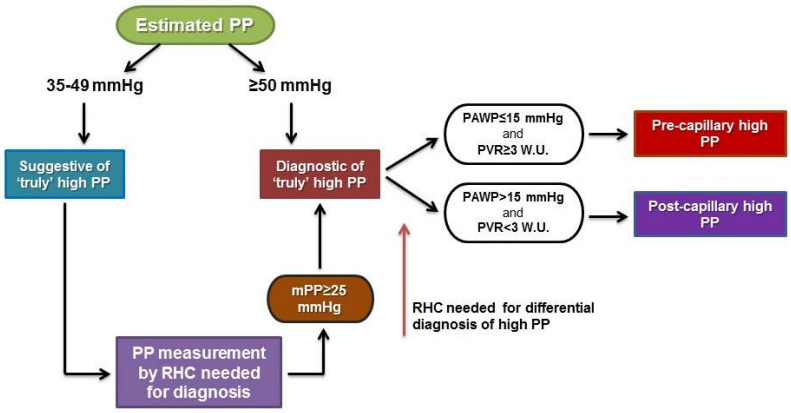
mmHg or Vmax 2.8-3.4 m/s are suggestive of PH but would require RHC for the final diagnosis. ePP>50 mmHg or Vmax> 3.4 m/s might be considered diagnostic of PH, but RHC is needed for further characterization of PH (pre- or post-capillary) on the basis of PAWP and PVR values.

Figure 1. Differential diagnosis of high pulmonary pressure in relation to the anatomic site of disease



COPD: chronic obstructive pulmonary disease; ILS: interstitial lung syndrome; PAWP: pulmonary artery wedge pressure; PP: pulmonary pressure; PVR: pulmonary vascular resistance

Figure 2. Diagnostic algorithm for approaching patients with suspected high pulmonary pressure



mPP: measured pulmonary pressure; PAWP: pulmonary artery wedge pressure; PP: pulmonary pressure; PVR: pulmonary vascular resistance; RHC: right heart catheterization

4. SIGNIFICANCE OF HIGH PP IN THE GENERAL POPULATION AND IN RENAL PATIENTS

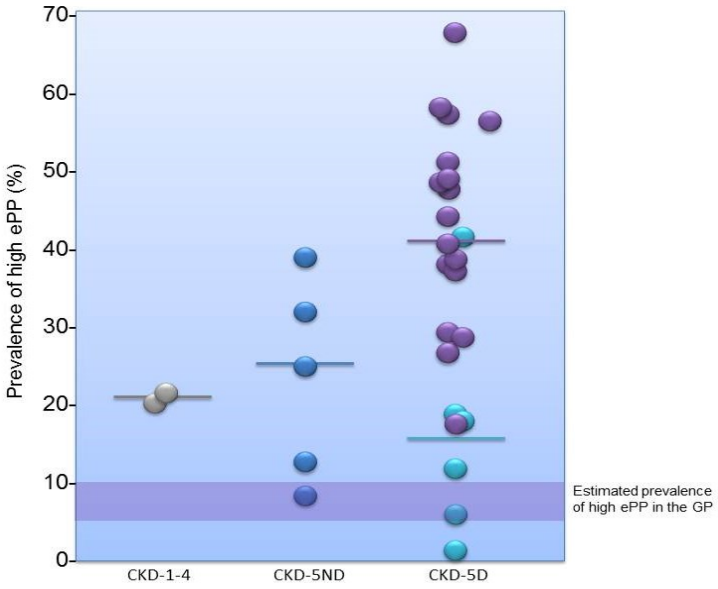
What is the epidemiological impact of elevated PP? In other words, what do studies and registries report about the penetrance and diffusion of this condition? Recently, evidence is accruing showing that high PP is much more prevalent in the general population than expected (13). This condition might remain undetected because of the absence of symptoms in the early phases and suspected only when clinical signs of right ventricular dysfunction (dyspnea, fatigue, non-productive cough, angina pectoris, syncope, peripheral edema and, rarely, hemoptysis) are manifested (8). In the Olmsted county study (14), a general population study conducted in a random sample of the same county, the prevalence of high PP defined by a Doppler-derived PP>35 mmHg was about 5% in individuals older than 45 years. Most cases of high PP detected in this population were secondary to concomitant left heart disorders and the presence of high PP was predicted by diastolic dysfunction as measured by the E/e' (early trans-mitral flow velocity [E] to early mitral annular tissue velocity [e']) ratio and by the presence of systemic hypertension and high pulse pressure. In a survey on 4579 patients undergoing echocardiographic examinations (15), the prevalence of high PP

(>40 mmHg) was 10.5%. Among the 483 cases with elevated PP 78.7% had left heart disease, 9.7% had lung diseases, 4.2% had primary pulmonary artery hypertension and 0.6% had pulmonary thromboembolism. In another study (16), the prevalence of PH in patients with chronic heart failure increased with the progression of NYHA class. Up to 60% of patients with severe LV systolic dysfunction and up to 70% of patients with isolated LV diastolic dysfunction had pathologically high PP. Pre-capillary forms of PH are less frequent with an annual incidence of about 2-3 per million and an estimated prevalence of about 15 cases per million (13). Adult females are almost three times more likely to present with PAH than males. In children, the presence of PAH is equally split along gender lines. But what happens if we refer specifically to renal patients? Do things change in this particular population? Although epidemiological data are scarce and sparse and mainly based on retrospective studies, high PP appears to be exceedingly prevalent among renal patients and not only confined to connective tissue and systemic diseases (**Figure 3**). Among pre-dialysis patients, the prevalence of PH is about 2 to 8 times higher than in the general population, ranging from 9% to 39% (6). PH prevalence is higher in the dialysis population (CKD-5D) than in CKD-ND patients. With regard to dialysis modality, the prevalence of PH is lower in patients on peritoneal dialysis (from 0% to 42%) than in hemodialysis patients (from 18.8% to 68.8%) (6). Unfortunately, there is a lack of uniformity among studies with respect to the ePP cut-offs considered as “pathologic” (ranging from 25 to ≥45 mmHg). Such a variability in the diagnostic criteria explains the wide range of PH prevalence reported in CKD patients and hampers the possibility to perform rough comparisons between studies and to provide reliable overall estimates of the frequency of high PP among renal patients.

But what kind of PH do renal patients have? Understanding the type of PH would be useful to better understand the pathophysiology of this condition and, eventually, to plan also the best treatment in such patients. Unfortunately, as stressed before, the only way to characterize the nature/origin of high PP is to perform RHC to measure PP but also to assess PAWP and PVR. In the only study measuring PP by RHC (17), PH was present in 81% of HD and 71% of pre-dialysis patients. The prevalence of (pre-capillary) high PP was 6% in CKD stage 4-5 patients and 13% in HD patients and the prevalence of

post-capillary PH was 71% and 65% respectively. These observations, although partly biased by the strict inclusion criteria of the study population (all subjects underwent RHC for characterization of an unexplained dyspnea after exclusion of other potential causes), seem to indicate that high PP is mostly post-capillary also in the renal population.

Figure 3. Reported prevalence of high estimated pulmonary pressure (ePP) in renal patients. Each dot represent a single prevalence reported in a different study cohort (see text). Grey dots indicate prevalence of high ePP in CKD stage 1-4 populations; blue dots in CKD stage-5 not on dialysis; purple dots in CKD stage-5 on chronic hemodialysis treatment; azure dots in CKD stage-5 on chronic peritoneal dialysis treatment. Correspondent lines indicate the median (calculated) value of high ePP prevalence in a given CKD class.



ePP: estimated pulmonary pressure; CKD 1-4: chronic kidney disease stage 1-4; CKD 5ND: chronic kidney disease stage 5 not on dialysis; CKD 5D: chronic kidney disease stage 5 on dialysis; GP: general population

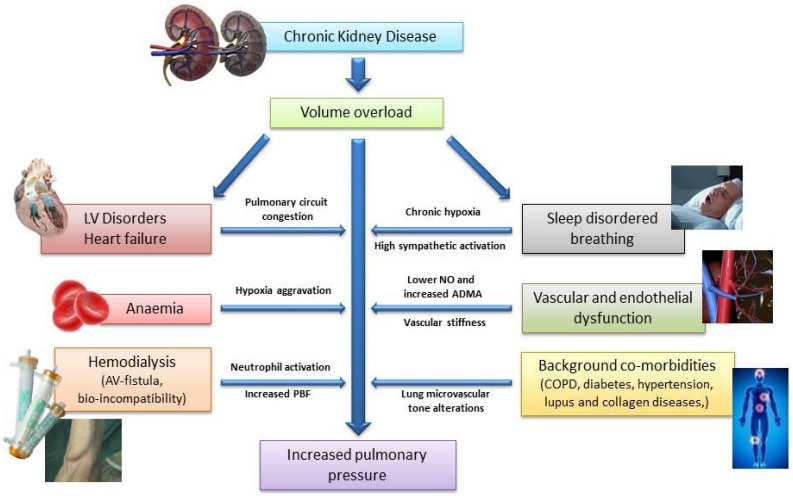
5. RISK FACTORS OF HIGH PP IN RENAL PATIENTS

As alluded to before, post-capillary high PP is likely to depend on the presence of LV disorders. Heart dysfunction is prevalent in the renal population, particularly in chronic hemodialysis patients. This condition can trigger volume overload but can be aggravated itself by fluid excess, therefore generating a vicious circle that can contribute to pulmonary congestion and high PP. Nevertheless, other risk factors/conditions may trigger and/or exacerbate PH in renal patients (**Figure 4**). Artero-venous fistula (AVF) is known to induce a decrease in systemic vascular resistances, which increases venous return and cardiac output to maintain proper blood flow to peripheral tissues. These hemodynamic effects might increase pulmonary blood flow and set the stage for high PP. Accordingly, high PP are more prevalent among HD than PD patients or CKD patients not on dialysis; furthermore, PP rises in strict parallelism with AVF creation (18) and tends to worsen overtime in the HD population (19, 20). However, evidence that renal transplantation may revert to normal PP in patients who still have a functioning AVF (21) indicates that other risk factors are involved in the genesis of high PP in CKD. Endothelial dysfunction is a major determinant of PH in the general population (22) and is exceedingly frequent in renal patients (23). Plasma levels of the powerful, endothelial-derived, vasodilator nitric oxide (NO) are more reduced in HD patients with higher PP (24). Furthermore, Asymmetric dimethyl-arginine (ADMA), an endogenous inhibitor of NO synthase which is copiously synthesized at lung level (25), has been strongly involved in experimental (26) and in primary forms (27) of PH and accumulates in subjects with renal function impairment (28). Interestingly, ADMA is increased in patients with sleep breathing disorders (29). Sleep breathing disorders, particularly sleep apnea, are highly pervasive in both pre-dialysis (30) and dialysis patients (31) and nocturnal hypoxemia by sleep apnea, is a strong trigger of high PP by enhancing sympathetic activation (32, 33).

Chronic exposure of blood to dialysis membranes causes reversible neutrophil sequestration in the lung and neutrophils activation (34) which may contribute to micro-vascular lung disease and PP increase in HD patients (35). The control of micro-vascular tone in the lung might also be affected by other diseases, such as diabetes,

connective, liver, infectious and hematologic diseases. Severe anaemia is a recognized cardiovascular risk factor in renal patients and its impact on the cardiovascular system includes direct effects to the pulmonary circulation. Indeed, anaemia could worsen PH by aggravating hypoxia (36). As a consequence of the overall dysfunction in mineral metabolism, arterial rigidity is increased in renal patients and calcium deposits have been found even in the pulmonary artery of CKD patients (37). These findings are in line with observations in the general population showing that stiffening of the pulmonary artery is significantly correlated to high PP (14).

Figure 4. Main pathophysiologic mechanisms leading to an increase in pulmonary pressure in renal patients



ADMA: asymmetric dimethyl-arginine; AV-fistula: Artero-venous fistula; COPD: chronic obstructive pulmonary disease; LV: left ventricular ; NO: nitric oxide; PBF: pulmonary blood flow.

6. POTENTIAL APPLICATIONS TO PROGNOSIS, OTHER DISEASES OR CONDITIONS

What is the prognostic impact of high PP? Do we have to fear the presence of PH in CKD patients? A large US survey (4), which collected data on PH at the community level over a 22 year-period (1980-2002), documented a stable death rate in patients with pathologically high PP, ranging from 5.2 to 5.4 per 100.000. Conversely, over the last

ten years, an increasing trend in mortality was documented with an estimated age-adjusted death rate of 4.5 to 12.3 per 100.000 (5). In addition, high PP was also associated with steadily increasing rates of hospitalizations (4). With this background in mind, one would easily argue that this trend simply reflects that one of "common" cardiovascular or pulmonary diseases; in this view, PH would be nothing more than a simple complication of traditional heart or lung disorders. However, a milestone study on PH in cardiopathic subjects has promptly rejected this hypothesis (38). The Authors studied over four hundred patients with known or presumed heart failure. After echocardiographic assessment of PP and LV ejection fraction patients were followed for up to 5.5 years. Patients presenting with a substantial increase in PP (>39 mmHg) had a significantly increased mortality in the long-term (5 years) and even in the short term (1 year) with respect to those with lower PP values. Interestingly, these observations remained unaffected if patients were stratified according to the presence of a conserved or impaired LV function (≤50% or > 50%). Multiple Cox analyses apportioned a 9% increase in mortality per each 5 mmHg increase in PP, independently of age and presence of chronic lung disease, heart failure and impaired renal function. These solid findings indicate that the prognostic impact of high PP is not influenced by traditional risk factors, including heart dysfunction. Similar observations have been extended to other populations, such as patients with primary PH or PH mostly secondary to chronic lung disease (39). What happens in renal patients? Do high PP hold the same prognostic power even in this particular, high risk population? A study in 2003 (40) tested for the first time this possibility in a cohort of chronic hemodialysis patients. Indeed, at unadjusted Kaplan-Meier survival curves, patients with ePP≥35 mmHg showed poorer survival with respect to those with lower ePP over a 5-year follow-up. These preliminary observations prompted the Authors to carry out an observational study (41) on a wider cohort of 127 ESKD patients initiating chronic renal replacement therapy. Patients' survival was assessed over a longer follow-up period (7 years) and subjects were stratified according to the absence of PH (ePP<45 mmHg) or to the presence of prevalent PH (ePP>45 mmHg already present before starting HD treatment) or incident PH (ePP>45 mmHg developed after starting HD). Overall, the presence of high PP

conferred a higher risk of death (hazard ratio 2.1 and 3.6 for incident and prevalent PH, respectively) and patients already having abnormal PP values before starting dialysis had poorer survival as compared to those developing PH after dialysis initiation. Furthermore, the prognostic power of high PP was independent of gender, age at onset of HD and presence of diabetes mellitus, left ventricular dysfunction and clinically relevant valve disease. In this study each 10 mmHg increase in ePP was associated with a fully adjusted increased risk of mortality of 50%. Similar findings were reported in another cohort of 278 patients on chronic renal replacement therapy who were followed for 24 months (42). In this series, the prevalence of ePP>35 mmHg was as high as 35%. Subjects with baseline PH had a hazard ratio of cardiovascular death of 2.36 with respect to those with "normal" ePP. This increased risk was fully independent of several potential confounders, such as gender, age at HD onset and duration of HD, pre-dialysis diastolic blood pressure, serum phosphorus, urea levels, presence of diabetes, systolic dysfunction and history of CV events. Of note, the presence of high ePP was also a powerful predictor of non-fatal cardiovascular events (hazard ratio 2.27), even after adjustment for the above-mentioned risk factors plus medication with ACEi or ARB, dialysis adequacy and C-Reactive Protein. In another study of 288 chronic HD patients (43), high ePP (>35 mmHg) was found in 38% of subjects and its presence predicted by left atrial dysfunction, urea removal ratio and use of vitamin-D receptor activators. During follow-up (Median 2.15 years) patients with higher ePP had significantly worsen cumulative hazard estimates of cardiovascular fatal events (53%, crude mortality rate 168.9/1000 patient-years vs. 22%, crude mortality rate 52.5/1000 patient-years). After adjustment for race, age, vascular access, serum albumin and history of CV disease, the presence of ePP>35 mmHg still conveyed a higher risk of death (hazard ratio 2.17) in this population. Another study (44) found abnormal ePP values (defined as Vmax>2.5 m/s) in 42/90 HD patients (47%). Subjects were followed over 12 months to assess all-cause mortality and hospitalizations. There was a statistically significant parallelism among severity of PH (defined as low: Vmax<2.5 m/s, mild: Vmax 2.6-2.9 m/s or severe: Vmax>3m/s) and mortality trend but no differences were found in all-cause hospitalizations. Recently, the prognostic impact of PP was

extended also to persons with early CKD (KDOQI stages 2-4) (45). In a series of 468 subjects with early renal impairment, the estimated prevalence of PH according to an ePP cutoff of 35 mmHg was 23% and the presence of high ePP was predicted by age and left atrial volume. Patients were followed over time (median 4 years) to assess the incidence of a combined endpoint including cardiovascular death, acute decompensated heart failure, coronary artery disease events as well as cerebrovascular and peripheral artery events. In a Cox multivariate model adjusting for age, eGFR, haemoglobin, left atrial volume, left ventricular mass and presence of diabetes mellitus and background CV disease, high ePP predicted a high risk for the combined cardiovascular end-point (Hazard Ratio 1.75). Thus, PH is remarkably prevalent also in patients with non-advanced CKD and holds its prognostic capacity with respect to adverse CV outcomes independently of classical and CKD-specific risk factors.

Accruing evidence is indicating that the prognostic utility of PP might be extended also to outcomes of kidney transplant recipients. In a retrospective, 3-year observational study (46) 55 patients undergoing renal transplantation were studied with respect to the incidence of early graft dysfunction (EGD), defined either as delayed or slow graft function. The percentage of patients developing EGD was higher within the subgroup of patients with high ePP (>35 mmHg). Furthermore, this increased risk (odds ratio 15.0) was fully independent from other traditional risk factors such as age of recipient, history of coronary artery disease, left ventricular ejection fraction, pre-transplant HD, presence of functional AVF, age of donor and cold ischaemia time. Another retrospective study (47), analyzed the clinical course of 215 transplant candidates. The Authors stratified these subjects into three different ePP categories (<35mmHg; 35-59 mmHg and >60mmHg). Despite most of them had ePP apparently falling within normal values, more than one third presented with frankly pathological values. Roughly 10% of the study cohort had even seriously high ePP (>60mmHg). The severity of ePP was directly correlated to dialysis vintage and the presence of a severe PH (ePP>50 mmHg) was a significant predictor of death after transplantation even after adjustment for age, reduced left ventricular ejection fraction, serum albumin and delayed graft function.

Table 1 summarizes the main findings from prognostic studies of high PP in renal patients.

Table 1. Main prognostic studies of high PP in renal patients

Studies looking at mortality and CV outcomes						
Study/year	**Country**	**Population**	**High ePP prevalence**	**ePP Cutoff**	**Follow-up**	**Results**
Yigla et al. 2003	Israel	58 HD 5 PD	39.7% HD 0 % PD	≥35 mmHg	>5-years	At unadjusted Kaplan-Meier survival curves, patients with ePP≥35 mmHg had poorer survival with respect to those with lower ePP
Yigla et al. 2009	Israel	127 HD	29.1%	≥45 mmHg	7-years	High PP conferred a higher risk of death (HR 2.1 and 3.6 for incident and prevalent PH, respectively) with a fully adjusted HR of mortality of 1.5 (1.2–1.9) for each 10 mmHg increase in ePP; (p=0.0007)
Li et al. 2013	China	278 HD	64.7%	≥30 mmHg	2-years	Subjects with baseline high PP had a HR of CV death of 2.36 with respect to those with normal ePP. High ePP was also an independent predictor of non-fatal CV events (HR 2.27)
Agarwal 2012	US	288 HD	38%	≥35 mmHg	2.15-years (median)	The presence of ePP>35 mmHg conveyed a higher risk of death (HR 2.17) after adjustment for race, age, vascular access, serum albumin and history of CV disease
Ramasubbu et al. 2010	US	90 HD	47%	Vmax≥2.5 m/s	>1-year	Statistically significant parallelism between severity of PH (defined as low: Vmax<2.5 m/s, mild:

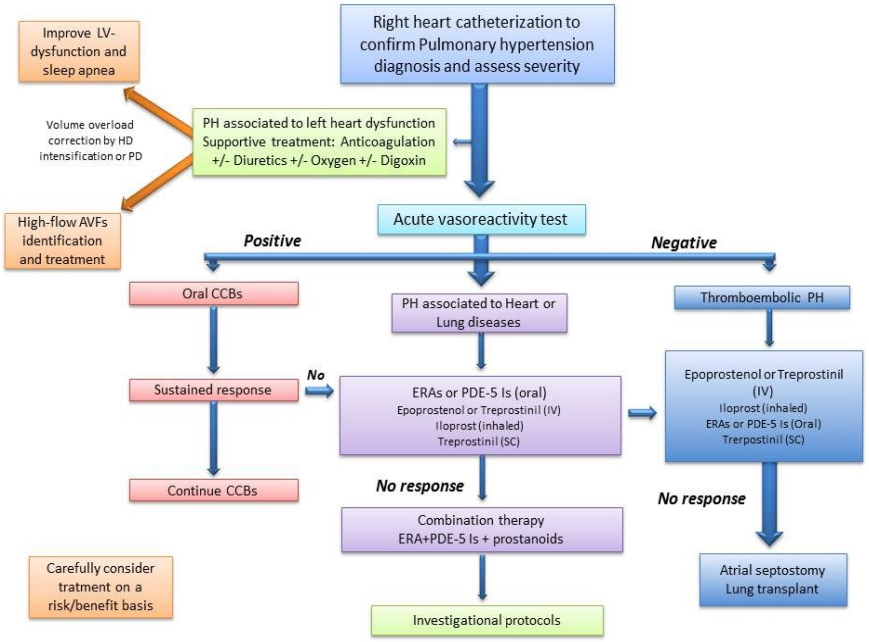
						Vmax 2.6-2.9 m/s or severe: Vmax>3m/s) and mortality trend but no differences in all-cause hospitalizations
Bolignano et al. 2015	Italy/ Germany	468 CKD	23%	≥35 mmHg	4-years (median)	In a Cox multivariate model adjusting for age, eGFR, haemoglobin, left atrial volume, LVM and presence of diabetes mellitus and background CV disease, high ePP predicted a high risk for a combined CV end-point (HR 1.75)
Studies looking at renal outcomes in kidney transplant recipients						
Study/year	**Country**	**Population**	**High ePP prevalence**	**ePP Cutoff**	**Follow-up**	**Results**
Zlotnick et al. 2010	US	55 HD	38%	≥35 mmHg	3-years	Higher percentage of patients developing EGD within the subgroup with high ePP (>35 mmHg) with a fully adjusted OR of 15.0
Issa et al. 2008	US	215 CKD/HD/PD	32%	≥35 mmHg	2.5-years	The presence of a severe PH (ePP>50 mmHg) was a significant predictor of death after transplantation after adjustment for age, reduced left ventricular ejection fraction, serum albumin and delayed graft function

CKD: chronic kidney disease; CV: cardiovascular; EGD: early graft dysfunction; eGFR: estimated glomerular filtration rate; ePP: estimated pulmonary pressure; HD: hemodialysis; HR: hazard ratio; LVM: left ventricular mass; OR: odds ratio; PD: peritoneal dialysis; PH: pulmonary hypertension; PP: pulmonary pressure; Vmax: maximum tricuspidal jet velocity

Although few doubts exist on the prognostic usefulness of PP in this population, one question still remains open –that is: is pathologically high PP also as a modifiable factor that deserves correction? Unfortunately, to date there are no specific studies of PH

treatment in CKD patients. In the absence of such evidence one can refer to the therapeutic algorithm proposed by current guidelines on PH (11) (**Figure 5**). In the general population, the first step is basically to confirm the presence of high PP and to assess the severity and possible type of PH by right heart catheterization. The second step would be to tailor the best treatment to the patient according to the (invasive) vasoreactivity test and the individual response to drug. Nevertheless, given the peculiar characteristics of renal patients, is there any additional approach that could be particularly useful in the CKD setting? Of course, the ideal solution would be to encourage kidney transplantation since accumulating (although not univocal) evidence indicates that PP may revert to normal values after receiving a renal transplant. Yet, as briefly alluded to before, a higher risk of death may persist in kidney transplant recipients with previous PH even after normalization of PP (47). Volume overload correction by ultrafiltration intensification or peritoneal dialysis might improve LV dysfunction and sleep apnea. The identification and surgical treatment of very high flow AVF would probably be helpful as well. Finally, when a pharmacological approach is needed, this should be always weighted on a risk/benefits balance, considering that most drugs for PH treatment can be dangerous in patients with impaired renal function or on dialysis.

Figure 5. Potential therapeutic approach to high pulmonary pressure in renal patients



AVFs: artero-venous fistulas; CCBs: calcium-channel blockers; ERAs: endothelin-receptor antagonists; HD: hemodialysis; IV: intravenous; LV: left-ventricular; PDE-5 Is: phosphodiesterase-5 inhibitors; PD: peritoneal dialysis; PH: pulmonary hypertension; SC: subcutaneous.

7. CONCLUSIONS

Evaluation of pulmonary pressure might represent an additional prognostic tool for risk stratification of renal patients. Pulmonary hypertension is highly prevalent in CKD patients, particularly in stage 5 patients on chronic HD. PH in CKD is a potentially reversible process because it may regress after kidney transplantation. However, in dialysis patients with established PH the excess risk for death may persist after kidney transplantation. Accruing evidence indicates that high PP predicts a high risk of death, particularly in ESKD patients, and worse outcomes in kidney transplant recipients. Large prospective studies adopting well standardized criteria of PP measurement, such as

right heart catheterization, are needed to clarify the exact risk of PH in persons with CKD.

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Chapter 4

High estimated pulmonary artery systolic pressure predicts cardiovascular outcomes in stage 2-4 chronic kidney disease

ABSTRACT

High estimated pulmonary artery systolic pressure (ePASP) is an established risk factor for mortality and CV events in the general population. High ePASP predicts mortality in dialysis patients but such a relationship has not been tested in early CKD patients. In this study we estimated the prevalence and the risk factors of high ePASP in 468 patients with CKD stage 2-4 and investigated its prognostic power for a combined endpoint including cardiovascular death, acute heart failure, coronary artery disease, cerebrovascular and peripheral artery events. High ePASP (≥35 mmHg) was present in 23% (n=108) of CKD patients. In a multivariate logistic regression model adjusted for age, diabetes, hemoglobin, left atrial volume (LAV/BSA), left ventricular mass (LVM/BSA) and history of CV disease, age (OR 1.06; 95% CI 12 1.04-1.09; p<0.001) and LAV/BSA (OR 1.05; 95% CI 1.03-1.07; p<0.001) were the sole independent predictors of high ePASP. High ePASP predicted a high risk for the combined cardiovascular end-point both in unadjusted analyses (HR 2.70; 95% CI 1.68-4.32; p<0.001) and in analyses adjusting for age, eGFR, haemoglobin, LAV/BSA, LVM/BSA and presence of diabetes and CV disease (HR 1.75; 95% CI 1.05-2.91; p=0.03). High ePASP is exceedingly prevalent in patients with stage 2-4 CKD and predicts adverse CV outcomes independently of established classical and CKD-specific risk factors. The question whether high ePASP is a modifiable risk factor in CKD patients should be addressed in appropriate randomized clinical trials.

Keywords: Pulmonary hypertension, chronic kidney disease, cardiovascular events, cardiovascular death.

1. INTRODUCTION

Patients with chronic kidney disease (CKD) are notoriously at very high risk for cardiovascular events and premature death (1). Data extracted from extensive databases in Canada document that the death risk of subjects with a GFR <60 ml/min/1.73 m² is of the same order of that in patients who had suffered a myocardial infarction and about twice higher than that in diabetic patients without CKD (2). Such a high risk derives from exposure to classical cardiovascular risk factors prior to CKD and thereafter to CKD-specific risk factors, encompassing anemia, protein wasting, inflammation and CKD-metabolic bone disorders (CKD-MBD) whose burden progressively increases as CKD evolves toward kidney failure (3). Left Ventricular Hypertrophy (LVH) and LV function disorders are highly prevalent in CKD and these alterations are considered as powerful integrators of the overall burden of classical and CKD-specific risk factors on the cardiovascular system in this population (4). However, even when considered in a context including simultaneous vascular disease, these disorders largely fail to explain in full the risk excess for cardiovascular events portended by CKD, indicating that other, hitherto overlooked, cardiovascular disorders play a role in the high risk for such events. High pulmonary artery systolic pressure estimated by echocardiography (ePASP) is an emerging, novel CV risk factor in the general population (5) (6). This condition is exceedingly prevalent in asymptomatic dialysis patients (7). Several risk factors such as volume overload, the presence of high-flow artero-venous fistulas, sleep apnea and sympathetic hyper-activation have been postulated to explain the high risk for elevated ePASP in these patients and some studies also documented that elevated ePASP predicts a high risk for all-cause and cardiovascular mortality in this population, as reviewed recently (7). ePASP evaluation in pre-dialysis CKD patients has received very scanty attention. Only one study has so far analyzed ePASP in early CKD stages (stage 1 to 3) (8) while studies looking at ePASP in advanced CKD included mainly CKD-stage 5 patients and just a small number of CKD-stage 4 patients without providing separate data or targeted analyses (9, 10). In this study we therefore aimed to estimate the prevalence and prognostic implications of ePASP in a large cohort of stage 2-4 CKD patients gathered by combining two cohorts

in a department of Nephrology in Germany (the CARE FOR HOMe study cohort) and in a Renal Unit in Italy (the MAURO study cohort).

2. METHODS

2.1 Patients and Baseline Data

Individual patient data were pooled from two inception cohorts with available echocardiography data of the MAURO study (n=80; Reggio Calabria, Italy) and from the CARE FOR HOMe study (n=388; Homburg, Germany). The patients flow diagram is depicted in **Figure 1**. Study cohorts, research protocols and main objectives of the MAURO and CARE FOR HOMe studies have extensively been described elsewhere (11, 12). Briefly, the two cohorts had very similar main inclusion criteria including: CKD-KDOQI stage 2 - 5 ND (MAURO) and CKD-KDOQI stage 2-4 (CARE FOR HOMe); age 18 - 75 years (MAURO) and age > 18 years (CARE FOR HOMe). Both cohort excluded patients with acute kidney injury; transplant patients, pregnant women and patients with active cancer or systemic diseases in the terminal phase. Patient enrollment was performed between October 2005 and November 2007 (MAURO), and between September 2008 and November 2012 (CARE FOR HOMe). The main demographic and clinical characteristics of the patients in the combined cohort are given in **Table 1.** The study protocol conformed to ethical guidelines and was approved by ethics committees of the nephrology units that participated. Written informed consent was obtained from each patient.

Figure 1. Study flow diagram

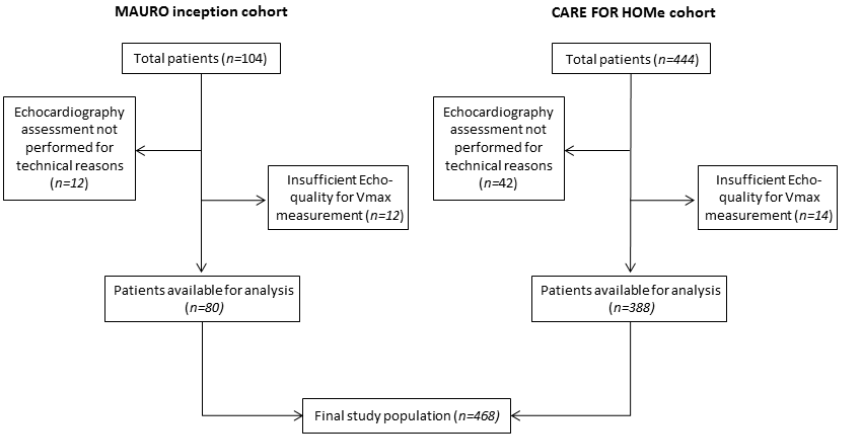


Table 1. Demographic, somatometric and clinical data of the study population

	Whole Cohort (N=468)	ePASP<35 mmHg (N=360;77%)	ePASP≥35 mmHg (N=108;23%)	p
Age (years)	64±12	62±13	71±9	**<0.001**
Male sex n. (%)	280 (60)	212 (59)	68 (63)	0.50
BMI (kg/m^2)	29±5	29.6±5.2	29.1±4.88	0.40
Systolic blood pressure (mmHg)	149±24	149±24	153±27	0.11
Diastolic Blood Pressure (mmHg)	85±13	86±13	83±13	0.16
Diabetes n. (%)	165 (35)	118 (33)	47 (43)	**0.04**
Current smokers n. (%)	59 (13)	51 (14)	8(7)	0.06
Total cholesterol (mg/dl)	190±42	191±42	184±42	0.13
Haemoglobin (g/dl)	13.4±1.6	13.5±1.6	12.9±1.6	**<0.001**
Albumin (g/dl)	4.4±0.4	4.4±0.4	4.7±0.4	0.87
Serum phosphate (mg/dL)	3.4±0.7	3.4±0.7	3.5±0.8	0.17
eGFR (mL/min/1.73m^2)	45.2±17.6	46.3±17.7	41.5±16.9	**0.01**
Albuminuria (g/g creat)	0.04 (0.007-0.21)	0.04 (0.006-0.22)	0.04 (0.009-0.21)	0.94
ePASP (mmHg)	10 (10-34)	10 (10-18)	41 (38-45)	**<0.001**
Creatinine (mg/dl)	1.66±0.67	1.65±0.68	1.72±0.63	0.33
LVEF (%)	63±10	57±21	53±23	0.17
LVM/BSA (g/m^2)	97.0±30.7	95.2±29.2	103.0±34.4	**0.03**
LAV/BSA (mL/m^2)	35.3±12.7	32.7±11.2	43.5±13.7	**<0.001**
LAD (parasternal view; mm)	37.5±12.5	37.1±11.7	38.7±15	0.31
Fractional Shortening (%)	38.0±8.2	38.4±7.9	36.8±9.1	0.12
Anti-hypertensive drugs Diuretics n (%) Beta blockers n (%)	 356 (76) 262 (56)	 260 (72) 179 (50)	 96 (89) 83 (77)	 **<0.001** **<0.001**

ARBs n (%)	223 (48)	161 (45)	62 (57)	**0.021**
ACEi n (%)	200 (43)	160 (44)	40 (37)	**0.17**
CCBs n (%)	236 (50)	184 (51)	52 (48)	**0.57**
Etiology of CKD n. (%)				
Cystic diseases	23 (5)	22 (6)	1 (1)	-
Diabetic nephropathy	43 (9)	31 (9)	12(11)	0.43
Glomerular diseases	66 (14)	54 (15)	12(11)	0.31
Nephroangiosclerosis	189 (40)	133 (37)	56(52)	**0.006**
Interstitial-/Pyelo-nephritis	21(4)	21 (6)	0 (0)	-
Other	126 (27)	99 (27)	27(25)	0.61
History of CV comorbidities n. (%)	141 (30)	98 (27)	43 (40)	**0.01**
TIA n. (%)	20 (4)	16 (4)	4 (4)	0.74
Peripheral vasculopathy n. (%)	29 (6)	21 (6)	8 (7)	0.55
Coronary Stent n. (%)	59 (13)	39 (11)	20 (18)	**0.04**
Myocardial Infarction n. (%)	52 (11)	34 (9)	18 (17)	**0.04**
Stroke n. (%)	31 (7)	21 (6)	10 (9)	0.21

Legend: ACEi, angiotensin converting enzyme-inhibitors; ARBs, angiotensin receptor blockers; BMI, body mass index; CCBs, calcium channel blockers; CV, cardiovascular; ePAP, estimated pulmonary artery pressure; LVEF, left ventricular ejection fraction; LVM/BSA, left ventricular mass indexed by body surface area; LAV/BSA, left atrial volume indexed by body surface area; LAD, left atrial diameter; TIA, transient ischaemic attack.

2.2 Patients, Laboratory and Echocardiography assessment

Blood samples were taken in fasting condition in the morning, and the second morning urine of the day was also collected. Common biochemical parameters were measured at baseline in all patients, according to standard methods in the clinical laboratories of the two cohorts. eGFR was assessed using the CKD-EPI creatinine (13) formula. Plasma samples were stored at −80 °C until batch analyses. All analyses were done blinded to clinical information. Patients' history was carefully recorded by interview and confirmed by checking patients' record, drug prescriptions included. All echocardiographic measurements were carried out according to the recommendations of the American Society of Echocardiography (14) by an observer unaware of biochemical results. Left ventricular ejection fraction (LVEF) was estimated by the Teichholz formula (15). Left ventricular mass (LVM) was calculated according to the Devereux formula (16) and indexed to body surface area (LVM/BSA). Left atrial volume was calculated from area-length method using apical 4-chamber and apical 2-chamber

(*A2C*) views, and indexed to body surface area (LAV/BSA). Left atrial diameter (LAD) was assessed by parasternal view.

2.3 Pulmonary artery pressures evaluation

Estimation of pulmonary artery systolic pressure (ePASP) was carried out by measuring maximal tricuspid regurgitation velocity (Vmax) and applying the modified Bernoulli's equation (17) to convert this value into pressure values:

$$ePASP = 4 \times (Vmax^2) + RAP$$

Right atrial pressure (RAP) was estimated to be 10 mmHg or, in case of presence of a moderate or severe tricuspid regurgitation, 20 or 30 mmHg respectively. High ePASP was defined according to an ePASP cut-off value of 35 mmHg (18). A sensitivity analysis adopting a more conservative threshold for the definition of high ePASP (>40 mmHg) was also performed.

2.4 Prospective follow-up and study end-point

The primary study outcome was a combined end-point including coronary, cerebrovascular and peripheral artery events, acute heart decompensation or cardiovascular death. All events required hospitalization and in both cohorts the same events were adjudicated after careful review of clinical files whenever needed. A detailed description on end-point definition in the MAURO and CARE FOR HOMe studies has been provided elsewhere (11, 12).

2.5 Statistical analysis

Normally distributed data were summarized as mean ± SD, non-normally distributed data as median and inter-quartile range, and binary data as percentage. Comparisons between groups were made by unpaired t-test, Mann-Whitney test or Chi Square Test, as appropriate. High ePASP was considered as categorical variable according to a cut-off of ≥ 35 mmHg. Variables associated with high ePASP were identified by logistic

regression analyses. Variables associated with the combined endpoint were identified by univariate Cox's regression and further tested by multivariate Cox regression analysis. Patients who progressed to stage 5D CKD during the follow up were maintained in all analyses, including the analysis of the combined end-point. In the multivariate analysis we included the set of variables (age, diabetes, hemoglobin, eGFR, LAV/BSA, LVM/BSA and CV comorbidities) that were associated (P<0.10) both with ePASP at logistic regression and with the combined outcome at Cox univariate analyses. A potential center effect was accounted for by data stratification. Data analysis was carried out by a commercially available statistical Package (SPSS for Windows, version 21, New York, USA).

3. RESULTS

3.1 Patients baseline characteristics

The main baseline characteristics of the study cohort are summarized in **Table 1**. Mean age of patients was 64±12 years and 60% (n=280) of them were male. One hundred sixty-five (35%) patients were diabetics and 59 (13%) were current smokers. One hundred forty-one patients (30%) had a history of cardiovascular disease, such as TIA, stroke, peripheral vascular disease or ischaemic cardiac disease. All patients had CKD stage 2-4. Mean serum creatinine was 1.66±0.67 mg/dl with a mean estimated GFR of 45±18 mL/min/1.73 m^2 (CKD-EPI). Mean systolic and diastolic BP were 150±25 and 85±13 mmHg, respectively. Median albuminuria was 0.04 (IQR 0.007-0.21). Mean LVEF was 63±10%. Mean LVM/BSA, LAV/BSA and LAD were 97.0±30.7 g/m^2, 35.3±12.7 mL/m^2 and 37.5±12.5 mm, respectively. High ePASP values (≥ 35 mmHg) were present in 23% (n=108) of patients in the study population and the prevalence of this alteration rose progressively across CKD stages (stage 2: 17%; stage 3: 24%; stage 4: 27%). In a sensitivity analysis adopting ePASP≥40 mmHg as criterion, the overall prevalence of high ePASP remained high (14%) and proportional to the degree of renal dysfunction (stage 2: 9%; stage 3: 15%; stage 4: 16%). The overall prevalence of mild to severe mitral or aortic valve disease was 6% (29/468): 7/108 (7.5%) in patents with high ePASP and 22/360 (6.1%) in those without.

3.2 Univariate and multivariate logistic regression analyses of high ePASP

On univariate logistic regression, the presence of high ePASP was significantly associated with age (OR 1.08; 95% CI 1.05-1.10; p=0.001), diabetes mellitus (OR 1.58; 95% CI 1.02-2.45; p=0.04), haemoglobin (OR 0.78; 95% CI 0.68-0.90; p<0.001), eGFR (OR 0.98; 95% CI 0.97-0.99; p=0.01), LVM/BSA (OR 1.01; 95% CI 1.01-1.02; p=0.02), LAV/BSA (OR 1.07; 95% CI 1.05-1.09; p<0.001) and history of cardiovascular disease (OR 1.77; 95% CI 1.13-2.77; p=0.01). In a multivariate regression model including all univariate associated, only age (OR 1.06; 95% CI 1.04-1.09; p<0.001) and LAV/BSA (OR 1.05; 95% CI 1.03-1.07; p<0.001) maintained a statistically significant association with high ePASP values. Data on logistic regression models are reported in **Table 2**.

Table 2. Univariate and multivariate logistic regression analysis of high ePASP (≥ 35 mmHg)

Variable	Univariate		Multivariate	
	OR (95% CI)	p	OR (95% CI)	p
Age (years)	1.08 (1.05-1.10)	0.001	1.06 (1.04 -1.09)	**<0.001**
Diabetes mellitus (yes/no)	1.58 (1.02-2.45)	0.04	1.18 (0.70-1.96)	0.53
Haemoglobin (g/dl)	0.78 (0.68-0.90)	<0.001	0.89 (0.75-1.06)	0.20
CKD-EPI (mL/min/1.73 m^2)	0.98 (0.97-0.99)	0.01	1.00 (0.98-1.02)	0.89
LVM/BSA (mL/m^2)	1.01 (1.01-1.02)	0.02	1.00 (0.99-1.01)	0.74
LAV/BSA (g/m^2)	1.07 (1.05-1.09)	<0.001	1.05 (1.03-1.07)	**<0.001**
History of CV disease (yes/no)	1.77 (1.13-2.77)	0.01	0.81 (0.46-1.41)	0.45

3.3 Cardiovascular combined endpoint during the follow-up period

During follow-up (median: 3.0 years; IQR 1.9-3.8), 76 subjects (16%) reached the composite endpoint. In particular, 11 subjects died due to CV events (6 arrhythmia, 1 ischaemic cardiomyopathy and 4 cardiac decompensation) while 65 subjects had a non-fatal CV event (25 coronary events, 2 arrhythmia, 11 cerebrovascular disease, 16 cardiac decompensation, 11 peripheral vascular disease). Data on separate outcomes in patients with or without high ePASP are shown in **Table 3**. Kaplan-Meier survival curves for the combined endpoint in patients with or without high ePASP are presented in **Figure 2**. Patients with high ePASP experienced a significantly faster evolution to the combined endpoint (crude HR 2.70; 95% CI 1.68-4.32; p<0.001 Log-rank test; χ^2 18.06). Results remained substantially unchanged when the 11 deaths were censored

(p<0.001; Log-rank test; χ^2 26.48). Interestingly, exploratory ROC curves showed that the optimal ePASP value for discriminating patients reaching the endpoint was nearly identical to the currently recommended high ePASP echocardiographic cut-off adopted in this study (36 vs 35 mmHg; **Figure 3**).

Table 3. Data on separate cardiovascular events in patients with ePASP<35 mmHg or ≥35 mmHg.

Separate CV events	n	ePASP<35	ePASP≥35
Coronary	26	14	12
Arrythmia	8	4	4
Cerebrovascular	11	8	3
Cardiac decompensation	20	14	6
Peripheral vascular disease	11	6	5
Total	**76**	**46**	**30**

Figure 2. Kaplan-Meier survival curves for the combined CV endpoint in patients with ePASP ≥ 35 mmHg or ePASP < 35 mmHg.

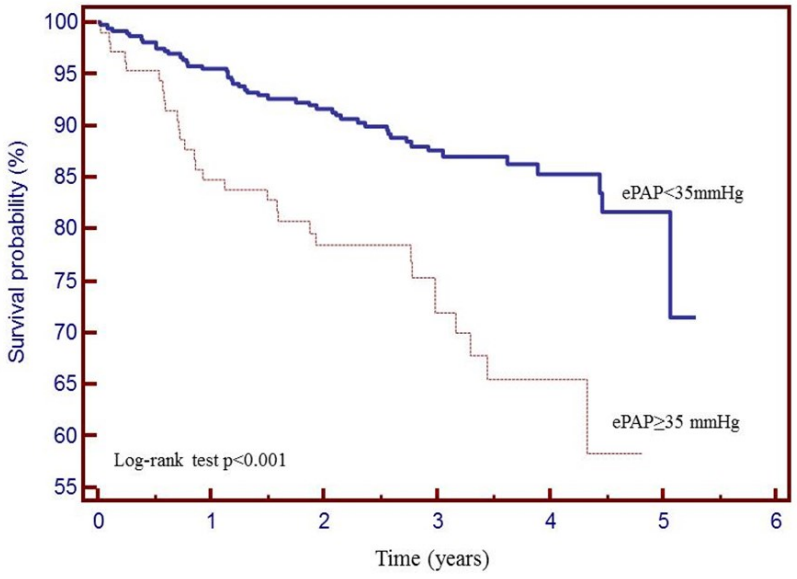
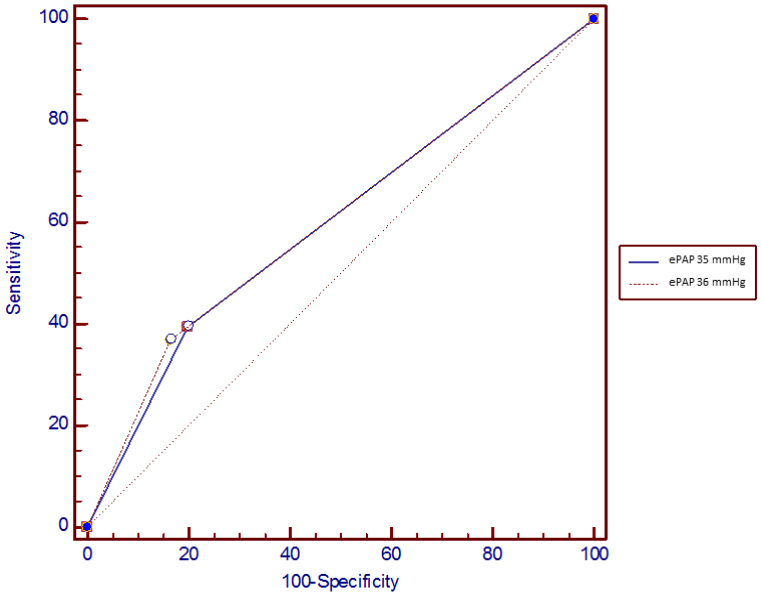


Figure 3. ROC curves comparing the optimal ROC-derived ePASP value (36 mmHg) to the currently suggested echocardiographic cut-off for high ePASP (35 mmHg) for discriminating patients reaching the endpoint.



3.4 Cox's regression models for the cardiovascular combined endpoint

At univariate Cox regression models, high ePASP, older age, presence of diabetes mellitus, lower hemoglobin, higher LAV/BSA, higher LVM/BSA, history of CV comorbidities, and lower eGFR all predicted adverse cardiovascular outcomes (**Table 4**)**.** In analyses adjusting for baseline associated to high ePASP ($p<0.10$) and including all univariate predictors of the combined end-point only high ePASP (HR 1.75; 95% CI 1.05-2.91; $p=0.03$), diabetes mellitus (HR 1.61; 95% CI 1.02-2.54; $p=0.04$), LAV/BSA (HR 1.02; 95% CI 1.01-1.04; $p=0.04$), history of CV disease (HR 3.63; 95% CI 2.18-6.05; $p<0.001$) and eGFR (HR 0.97; 95% CI 0.96-0.99; $p<0.001$) maintained an independent relationship with the same combined end-point. In these adjusted analyses LVM/BSA, haemoglobin levels and age were no longer associated with the incidence of the combined end-point (**Table 4**).

Table 4. Cox proportional hazards regression models for the combined outcome.

Variable	Units of increase	Univariate			Multivariate		
		HR	95% CI	p	HR	95% CI	p
ePASP ≥ 35 mmHg	yes/no	2.70	1.68 to 4.32	**<0.001**	**1.75**	**1.05 to 2.91**	**0.03**
Age	1 year	1.05	1.03 to 1.07	**<0.001**	1.01	0.98 to 1.03	0.50
Diabetes mellitus	yes/no	1.91	1.21 to 3.00	**0.005**	**1.61**	**1.02 to 2.54**	**0.04**
Haemoglobin	g/dl	0.77	0.67 to 0.88	**<0.001**	0.91	0.78 to 1.07	0.26
CKD-EPI	mL/min/1.73 m^2	0.96	0.95 to 0.98	**<0.001**	**0.97**	**0.96 to 0.99**	**<0.001**
LAV/BSA	mL/m^2	1.04	1.03 to 1.06	**<0.001**	**1.02**	**1.01 to 1.04**	**0.04**
LVM/BSA	g/m^2	1.01	1.01 to 1.02	**<0.001**	1.01	1.00 to 1.01	0.06
History of CV disease	yes/no	5.00	3.13 to 8.01	**<0.001**	**3.63**	**2.18 to 6.05**	**<0.001**

Forcing albuminuria and the use of ACEi, ARBs, CCBs, beta-blockers and diuretics into the model, ePASP≥35 mmHg remained significantly associated to the combined outcome (HR 2.01; 1.18-3.43, p=0.01).

4. DISCUSSION

In this study in a population of pre-dialysis CKD of various severity, from stage 2 to stage 4, high pulmonary artery systolic pressure as assessed by echocardiography was present in about ¼ of patients and predicted adverse cardiovascular outcomes independent of background cardiovascular events, LVH and other major risk factors in this population. Overall the present data extend to mild to moderate CKD stages observations made in dialysis patients. The prevalence of high ePASP by echocardiography was estimated in two large population based studies, the Olmsted county study (19) and the Armadale echocardiography study (20). In both studies the prevalence of high ePASP was unexpectedly high being about 5% in the first study and 9.1%, in the second study. The vast majority of patients with high ePASP in these large surveys had concomitant cardiac disease and resulting LV dysfunction.

In patients with advanced, stage 5 CKD before or after initiation of dialysis, the prevalence of high ePASP largely exceeds estimates in the general population, ranging from 9% to 39% in patients maintained on conservative treatment and from 18.8% to

68.8% in patients on chronic renal replacement therapy (7) suggesting that CKD severity may trigger and/or worsen the increase in ePASP. Our observations indicate that high ePASP is exceedingly prevalent also in CKD patients with less advanced renal dysfunction (K-DOQI stages 2-4), being as high as 23% by the ePASP>35 mmHg criterion and 14% by the ePASP>40 mmHg criterion. A very recent study in 101 Chinese patients with CKD stage 1-3 reported a 23.7% prevalence (8), a value very close to that observed in our cohort by adopting the ePASP>35 mmHg criterion. Multiple risk factors may be implicated in the high prevalence of elevated PASP values in CKD. In the present study, high ePASP was associated with age, low GFR, high left atrial volume, low hemoglobin and major comorbidities such as diabetes and background CV disease. As expected in pre-dialysis CKD patients, anemia was of mild to moderate degree which may help to explain the fact that this risk factor had no independent relationship with pulmonary pressure. Sleep apnea (21), increased sympathetic activity and high levels of the endogenous inhibitor of NO synthase Asymmetric Dimethyl-arginine (ADMA) are implicated in pulmonary hypertension (PH) in individuals with primary forms of pulmonary artery hypertension (22, 23). The relationship of these factors with high ePASP in CKD patients needs to be investigated in specifically designed studies in this population. Elevated pulmonary pressure was associated with high pulse pressure in the Olmsted study (19) suggesting that stiffening of the pulmonary artery might in part explain high pulmonary pressure in the general population. Although increased arterial rigidity is one of the main features of CKD (24), we did not observe an association between pulse pressure and pulmonary pressure, suggesting that the contribution of arterial stiffening to high pulmonary pressure in earlier CKD stages is of limited importance. In the general population, primary or secondary left ventricular disorders are the most common triggers of pulmonary hypertension (19) and up to 60% of patients with severe LV systolic dysfunction and up to 70% of patients with isolated LV diastolic dysfunction may have concomitant PH (25). In our study high ePASP strongly associated to higher LV mass index and larger left atrial volume, and the association with left atrial volume remained significant even after adjustment for potential confounders. These findings, which are in keeping with previous studies of PH in dialysis

patients (9, 26-28) support the hypothesis that in most cases PH in CKD is post-capillary in nature (29), i.e. that it depends on passive congestion of the venous side of the pulmonary vasculature rather than by an increase in pulmonary artery vascular resistances. Subtle or clinically manifest volume expansion, a hallmark of CKD, together with LV diastolic dysfunction, is a relevant determinant of left atrial volume (30) and therefore may contribute to raise pulmonary pressure in CKD patients. As most relevant finding of our study, high ePASP emerged as a strong predictor of a combined end-point including fatal and non-fatal CV events. Indeed, independently of other risk factors, CKD patients with ePASP≥35 mmHg had a 75% risk excess for developing the combined endpoint as compared to those with ePASP<35 mmHg. Overall, our data extend to earlier CKD stages observations made in two cohort studies among dialysis patients (28, 31). Our study has several limitations. The main limitation is the fact that we estimated PASP by echocardiography. Echocardiographic estimates of PASP are just an approximation of the true (directly measured) pulmonary artery systolic pressure. However, echocardiography is considered an acceptable approach for investigating PASP in large scale epidemiologic studies (19, 20) and the criteria for estimating PASP and for defining elevated ePASP adopted in the present study are identical to those adopted in the largest studies in the general population (19, 20). Although high ePASP above 35 mmHg may reflect the true presence of PH, guidelines recommend that the diagnosis of PH should be confirmed by a direct measurement of pulmonary pressure (18). Until now, direct pulmonary pressure measurement in CKD was performed in a single study including 31 hemodialysis patients and 31 stage 5 CKD patients before starting dialysis treatment (29). The overall prevalence of PH in this series was very high (81%), which may reflects selection bias, as patients were selected for invasive measurement of pulmonary pressure because of the presence of dyspnea unexplained by concomitant cardio-pulmonary diseases. Although the combined data-base of our two cohorts was fairly large and based on standardized echocardiographic criteria, the prevalence of high ePASP we found cannot be generalized to the CKD population at large. Further studies in other ethnical groups will be needed to allow solid estimates of such prevalence in early CKD patients. Finally, as expected in an early to moderate

CKD population, the rate of CV death (2.3%) and non-fatal CV events (13.8%) was relatively low. Overall the number of clinical events was insufficient to perform separate analysis of mortality and relevant cardiovascular outcomes. In conclusion, we observed a high prevalence of elevated PASP as assessed by echocardiography among patients with CKD stage 2-4. The presence of high ePASP independently predicts adverse CV outcomes, even after adjustment for CV risk factors, eGFR, prevalent cardiovascular disease and echocardiographic markers of left heart disease. Our findings need to be confirmed in studies adopting invasive measurements of PASP in patients with high ePASP as indicated by echocardiographic screening. Future studies should address the issues whether CKD patients with high ePASP should undergo further investigations, and whether tailored therapeutic approaches to normalize pulmonary pressure will beneficially affect their outcome.

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PART II

AGING AND PHYSICAL ACTIVITY MPACT CLINICAL OUTCOMES IN RENAL PATIENTS

Chapter 5

Short term vascular hemodynamic responses to isometric exercise in young adults and in the elderly

ABSTRACT

Background: Vascular ageing is known to induce progressive stiffening of the large elastic arteries, altering vascular hemodynamics under both rest and stress conditions. In this study, we aimed to investigate changes in vascular hemodynamics in response to isometric handgrip exercise across ages. ***Methods:*** We included 62 participants, divided into three age categories: 20-40 (n=22), 41-60 (n=20) and 61-80 years (n=20). Vascular hemodynamics were measured using the Mobil-o-Graph based on the pulsatile pressure changes in the brachial artery. One-way ANOVA test was performed to analyze the changes induced by isometric handgrip exercise. ***Results:*** After isometric handgrip exercise, aortic PWV increased by 0.10 m/s in the youngest, 0.06 m/s in the middle age and 0.02 m/s in the oldest age category. Changes in PWV strongly correlated with those in cSBP ($r=0.878$, $p<0.01$). After isometric exercise mean change of SBP was -1.978% in the youngest, 0.61.27% in the middle-aged and 8.27.24% in the oldest subjects. in the oldest subjects. Increasing handgrip strength was associated with an increase in SBP and cSBP (respectively 1,08 and 1,37 mmHg per 1 kg increase in handgrip strength, P=0.01). Finally, PWV was significantly associated with increasing handgrip strength with an increase of 0.05 m/s per 1 kg higher increased handgrip strength (P=0.01). ***Conclusion:*** This study found increased blood pressure levels after isometric challenge and a strong association between handgrip strength and change in blood pressure levels and aortic stiffness in elderly subjects.

Key words: vascular aging, isometric stress, blood pressure, pulse wave velocity

1.INTRODUCTION

During isometric exercise, the autonomic nervous system elicits a cardiovascular response by an increase in the sympathetic nerve activity. Two neural systems contribute to sympathetic activation: the 'central command' and the 'exercise pressor reflex' (1). Signals arising in the motor cortex activate both the cardiovascular control areas, to modulate sympathetic and parasympathetic activity, and skeletal muscle contraction (2). The exercise pressor reflex (or muscle reflex) is generated by contraction of skeletal muscle via mechanically (muscle mechanoreflex) and chemically (muscle metaboreflex) skeletal muscle receptors (3). During handgrip exercise the pressor reflex occurs when the muscle fatigues and/or when there is a mismatch between blood supply and metabolic demand (4). While the increase in arterial pressure during isometric exercise is a well-established response (5), the response of other vascular hemodynamics to exercise, such as arterial stiffness, is yet unclear. Age is the most important determinant for changes in the cardiovascular system. Vascular aging results in loss of elastic properties, which leads to progressive stiffness of the large elastic arteries (6). Several studies have shown that arterial stiffness is an independent predictor of chronic heart disease (7,8), stroke (8,9), all-cause mortality and cardiovascular mortality (10) in hypertensive patients and it is associated with increased risk for cardiovascular events in the general population (11,12). Measurement of pulse wave velocity (PWV) is the leading noninvasive method of assessing arterial stiffness which is calculated by dividing the pulse distance travelled by the time travelled (13). Data on the effects of isometric exercise on arterial stiffness are limited. Several studies found an increase in central large artery stiffness in response to isometric handgrip exercise in hypertensive (14) and healthy participants (14-16) suggesting that the increase in arterial stiffness could be due to the increased vasoconstriction from sympathetic system activation. However, these studies included participants within a range of age between 30-54 years. Since age is an important determinant for stiffening of the elastic arteries, it is interesting to explore the effect of aging on vascular hemodynamics in response to isometric exercise. We hypothesized that changes might be more pronounced in elderly compared to young adults due to an altered autonomic

response. Therefore, we investigated the changes in vascular hemodynamics in response to isometric handgrip exercise in persons of different ages.

2. METHODS

2.1 Subjects

We included 62 participants aged 20-80 years. The participants were students, employees and apparently healthy patients from the section of Geriatric Medicine of the Erasmus University Medical Center. Informed consent was obtained from all participants and the study was approved by the local Medical Ethics Committee of the Erasmus MC (MEC 2014-336).

2.2 Protocol

The participants entered the room and were instructed to assume a supine position. An inflatable cuff was placed on the dominant arm. After five minutes of rest, baseline data were acquired using the Mobil-o-Graph® device. Patients were asked to perform maximum hand grip trial in the standing position with their arms along their body. Maximal hand grip strength was the highest score recorded. Hand Grip strength was measured using a strain-gauged dynamometer (Takei, TKK 5401, Takei Scientific instruments Co. Ltd., Japan). Participants performed a maximum voluntary contraction using a handgrip dynamometer held in the dominant hand during 30 seconds. After 30 seconds of rest, the vascular hemodynamics were measured.

2.3 Measurements

The Mobil-o-Graph® is an oscillometric blood pressure measurement validated device able to measure vascular hemodynamics such as central and peripheral blood pressure, augmentation index (AIx) and pulse wave velocity (PWV) (17). Measurements of PWV and AIx are based on the pulsatile pressure changes in the brachial artery. The pressure cuff sensors the first systolic peak of the pulse wave which corresponds to the ejection of the blood volume into the aorta. The second systolic peak represents the reflection

of the pulse wave from the periphery. PWV can be calculated by dividing the difference in time, between the beginning of the first and second systolic peak, and the distance from the jugular notch to the symphysis. Augmentation index is the difference of the amplitudes of the first and second systolic peak. Estimates of PWV are derived using ARC Solver algorithms.

2.4 Statistical analysis

All descriptive data are reported as mean ± SE. Differences in baseline hemodynamics were investigated by ANOVA. We investigated possible changes of blood pressure levels, heart rate, cardiac output, total peripheral resistance, augmentation index and pulse wave velocity induced handgrip exercise with two-way ANOVA analyses in the three different categories of ages with models adjusted for age, sex and the baseline corresponding hemodynamic value (ie baseline systolic blood pressure was included in models investigating changes of systolic blood pressure levels). We investigated correlations between hemodynamic changes and handgrip strength with the linear regression. The level of significance was taken as $p < 0.05$. Analysis were performed using IBM SPSS Statistics for Windows, Version 21.0.

3. RESULTS

Table 1 shows the mean values for the characteristics of the participants. Mean age of the youngest, middle-aged and oldest subjects was respectively: 28,8 ± 5,5 years, 49,8 ± 5,9 years and 71,0 ± 5,6 years. The results of the hemodynamic measurements at baseline are presented in **Table 2**. We found a statistically significant difference in PWV (P for trend < 0.01) and in pulse pressure between groups (P for trend < 0.05) at baseline. No differences were found in systolic or diastolic blood pressure between groups. There was no difference in maximum handgrip strength between groups with respectively 31,2± 5 kg in the youngest, 32,5± 2.2 kg in the middle-aged and 29,7± 5.4 kg in the oldest subjects (P for trend = 0.748). Changes in vascular hemodynamics induced by isometric handgrip exercise. **Figure 1** presents the changes in hemodynamic parameters after handgrip exercise. After isometric exercise mean change of SBP

(**Figure 1A**) was -1.9% in the youngest, 0,6% in the middle-aged and 8.2% in the oldest subjects. The differences in mean change of SBP between the youngest, middle aged and oldest participants were statistically different (p= 0.03) and (p= 0.01) respectively. The mean change in DBP (**Figure 1B**) after handgrip exercise was 0,7% in the youngest, 0,3 % in the middle-aged and 6.9% in the oldest subjects. The difference in mean change of DBP between middle aged and oldest participants was significant (P < 0.02). We found no difference in mean change of PP (**Figure 1C**) and heart rate (**Figure 1D**) between the youngest, middle-aged and oldest subjects with respectively -9%, -1% and 14.%. The mean change in augmentation index **(Figure 1E**) after handgrip exercise was -50 % in the youngest, 35 % in the middle-aged and 80 % in the oldest subjects. The difference in mean change of augmentation index between young and oldest subjects was statistically significant (P < 0.046). After isometric exercise all subjects shown a non-significant decrease in CO (**Figure 1F**) and a non-significant increase in total peripheral resistance (TPR) (**Figure 1G**). No difference was found in mean change of PWV after handgrip between subjects with -0,83% in the youngest, 1.0% in the middle-aged and 2.21% in the oldest subjects. Relationship between handgrip strength and changes in vascular hemodynamics. **Table 3** shows the association between handgrip strength and vascular hemodynamics, in the youngest, middle-aged and oldest subjects. In the youngest and middle-aged subjects, we found no association between handgrip strength and change in vascular hemodynamics. We found an association between handgrip strength and the change in SBP, PP, cSBP, cPP and PWV in the oldest subjects. Increasing handgrip strength was associated with an increase in SBP and cSBP (respectively 1,08 and 1,37 mmHg per 1 kg higher in handgrip strength, P=0.01). This means that per 10 kg handgrip strength, SBP and cSBP, increase with 10,8 mmHg and 13,7 mmHg. The association between increasing handgrip strength and the change in PP and cPP was also significant (P=0.01). With an increase of 1 kg handgrip strength, PP increased with 1,19 mmHg while cPP increased with 1,46 mmHg. Finally, PWV was significantly associated with increasing handgrip strength with an increase of 0.05 m/s per 1 kg increased handgrip strength (P=0.01).

Table 1. Clinical characteristics of study participants

Variables (mean ± SD)	**Age 20-40 (n=22)**	**Age 41-60 (n=20)**	**Age 61-80 (n=20)**
Age, years	28,8 ± 5,5	49,8 ± 5,9	71,0 ± 5,6
Sex, male	50 %	50 %	60 %
Height, cm	175 ± 10	171 ± 16	173 ± 10
Weight, kg	76 ± 13	83 ± 27	80 ± 14
Body mass index, kg/m2	24,7 ± 4,1	25,9 ± 3	26,7 ± 4,3

Table 2. Hemodynamic parameters at baseline

Variables (mean ± SD)	**Age 20-40 yrs (n=22)**	**Age 41-60 yrs (n=20)**	**Age 61-80 yrs (n=20)**	**P value ***
Systolic BP, mm Hg	119 ± 7,9	123 ± 3,4	132 ± 8,4	0.59
Diastolic BP, mm Hg	80 ± 4,6	81 ± 2,0	76 ± 4,9	0.46
Pulse pressure, mm Hg	39 ± 5,3	42 ± 2,3	56 ± 5,6	0.05
MAP, mm Hg	95 ± 9	100 ± 11	105 ± 14	0.96
Heart rate, bpm	68 ± 4,6	60 ± 2,0	62 ± 4,9	0.17
Central systolic BP, mm Hg	111 ± 7,5	115 ± 3,3	124 ± 8,0	0.54
Central diastolic BP, mm Hg	80 ± 4,6	82 ± 2,0	77 ± 4,9	0.45
Central pulse pressure, mm Hg	31 ± 5,0	33 ± 2,2	47 ± 5,4	0.03
Augmentation index @75, %	12 ± 8,3	25 ± 3,6	24 ± 8,8	0.24
Cardiac output, l/min	5,2 ± 0,5	4,6 ± 0,2	5,0 ± 0,5	0.09
TPR, s*mmHg/min	1,08 ± 0,22	1,36 ± 0,23	1,35 ± 0,27	0.10
Aortic PWV, m/s	7,6 ± 0,3	7,0 ± 0,1	8,2 ± 0,3	0.00
Handgrip strength, kg	31,2 ± 5,0	32,5 ± 2,2	29,7 ± 5,4	0.75

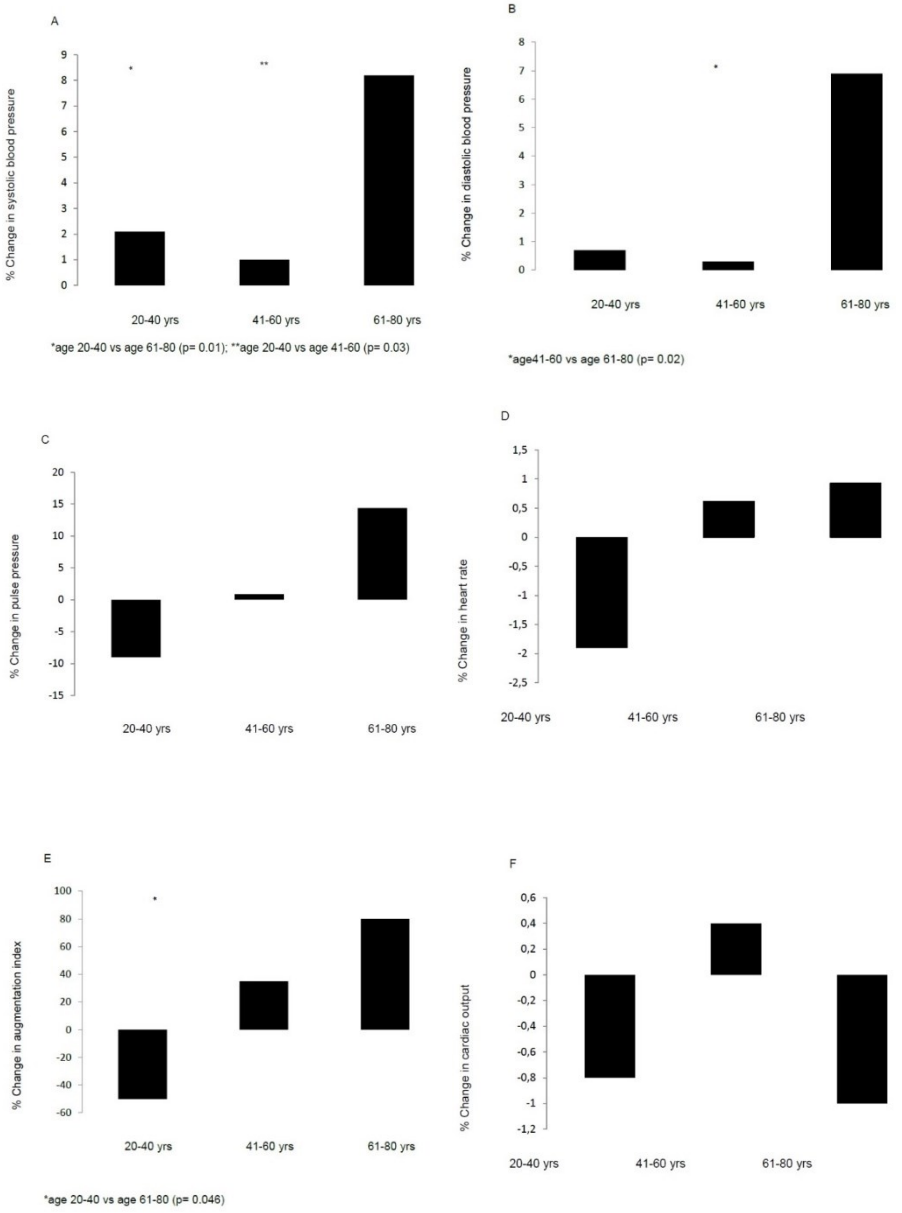
* P value for trend; BP, blood pressure; MAP, mean arterial pressure; TPR, total peripheral resistance ; PWV, pulse wave velocity

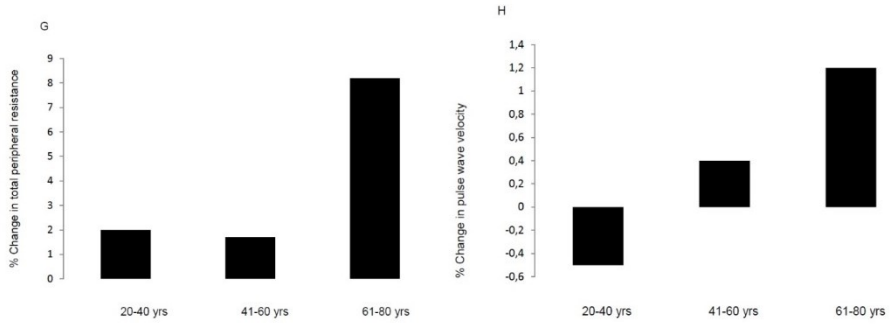
Table 3. Delta hemodynamic parameters as determinant of handgrip strength in the youngest, middle-aged and oldest subjects

	Age 20-40 years (n= 22)			Age 41-60 years (n=20)			Age 61-80 years (n=20)		
Handgrip (Kg)	**B**	**SE**	**P value**	**B**	**SE**	**P value**	**B**	**SE**	**P value**
Δ SBP, mmHg	0,20	0,12	0,12	0,15	0,16	0,34	**1,08**	**0,36**	**0,01***
Δ DBP, mmHg	-0,04	0,10	0,71	0,09	0,09	0,31	-0,10	0,23	0,66
Δ PP, mmHg	0,24	0,14	0,11	0,06	0,14	0,65	**1,19**	**0,40**	**0,01***
Δ Heart rate, bpm	-0,00	0,11	0,99	-0,02	0,06	0,70	0,12	0,34	0,74
Δ cSBP, mmHg	0,16	0,20	0,44	0,20	0,16	0,23	**1,37**	**0,44**	**0,01***
Δ cDBP, mmHg	-0,02	0,10	0,82	0,07	0,09	0,48	-0,07	0,21	0,76
Δ cPP, mmHg	0,18	0,20	0,38	0,12	0,18	0,51	**1,46**	**0,49**	**0,01***
Δ AIx @75, %	0,41	0,57	0,48	0,57	0,41	0,18	-0,32	1,19	0,79
Δ CO, L/min	-0,03	0,03	0,29	-0,00	0,02	0,89	0,02	0,06	0,72
Δ TPR, s*mmHg/min	0,01	0,01	0,24	0,00	0,01	0,86	0,00	0,02	0,91
Δ PWV, m/s	0,01	0,01	0,19	0,01	0,01	0,20	**0,05**	**0,02**	**0,01***

*, P value <0.05; B, Beta; SE, standard error; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; c, central; AIx, augmentation index; CO, cardiac output; TPR, total peripheral resistance; PWV, pulse wave velocity

Figure 1. Change in systolic blood pressure (A), diastolic blood pressure (B), pulse pressure (C), heart rate (D), augmentation index (E), cardiac output (F), total peripheral resistance (G) and pulse wave velocity (H) before and after handgrip exercise.





4. DISCUSSION

In the present study we found increased blood pressure levels after isometric challenge in elderly subjects. We also found a strong association between handgrip strength and change in blood pressure levels and aortic stiffness in the older subjects; we did not observe this association in the youngest or middle-aged subjects. Previous studies have shown an increase of blood pressure levels after isometric handgrip exercise in normotensive and hypertensive individuals (14), in healthy young subjects (16,18,19) and older subjects (20) who also observed that hemodynamic changes can considerably vary among individuals (18). Within the framework of a relatively large community-based longitudinal study, Taekema et al. (21) investigated the possible relation between blood pressure and handgrip strength in middle-aged (63 years) and old subjects (85 years). finding an association between handgrip strength and high SBP, PP only in the oldest subjects. According to Taekema we also found increased blood pressure levels after isometric challenge to be more pronounced in elderly subjects, moreover we found a strong association between handgrip strength and aortic stiffness. Several mechanisms can explain our results. First, the observed changes in blood pressure in the elderly can be explained due to failure of the aging arterial system during stress. During isometric exercise, the autonomic nervous system elicits a cardiovascular response which increases sympathetic nerve activity, arterial blood pressure and heart rate, and changes the distribution of blood flow. The arterial system in younger subjects

is capable of receiving spurts from the left ventricle of blood during isometric exercise and distribute these trough peripheral capillaries. Elastin fibers in the arterial wall minimize the fluctuations in the blood flow. Vascular ageing results in loss of elastic properties, which leads to progressive stiffness of the large elastic arteries. Stiffening leads to an increase of systolic pressure and a decrease in diastolic pressure which stresses the small arterial vessels causing damage to particularly the coronary arteries, kidney and brain (22). We found a statistically significant increase in pulse pressure and aortic stiffness with increasing age at baseline. We found significant differences in the change of SBP and DBP after exercise between the middle-aged and oldest subjects with more pronounced changes in the oldest subject. The present study has some limitations. First, we included a relatively small number of participants. Second, we performed measurements only once so we cannot exclude variation over time. Strength of this study is that we performed our measurements following standard procedure, in this way we think that exposure was similar for all participants. Moreover, we used a brachial cuff-based oscillometric device which has been shown to be reproducible (23), acceptable accurate when compared with other invasive methods (24) and useful for routine clinical practice (25). In conclusion, we found increased blood pressure levels after isometric challenge and a strong association between handgrip strength and change in blood pressure levels and aortic stiffness in elderly subjects. These findings can be possibly explained by failure of the aging arterial system during stress and increased vascular resistance with increasing age.

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Chapter 6

Physical performance and clinical outcomes in dialysis patients: a secondary analysis of the EXCITE trial

ABSTRACT

Background/Aims: Scarce physical activity predicts shorter survival in dialysis patients. However, the relationship between physical (motor) fitness and clinical outcomes has never been tested in these patients. ***Methods:*** We tested the predictive power of an established metric of motor fitness, the Six-Minute Walking Test (6MWT), for death, cardiovascular events and hospitalization in 296 dialysis patients who took part in the trial EXCITE (ClinicalTrials.gov Identifier: NCT01255969). ***Results:*** During follow up 69 patients died, 90 had fatal and non-fatal cardiovascular events, 159 were hospitalized and 182 patients had the composite outcome. In multivariate Cox models - including the study allocation arm and classical and non-classical risk factors - an increase of 20 walked metres during the 6MWT was associated to a 6% reduction of the risk for the composite end-point (P=0.001) and a similar relationship existed between the 6MWT, mortality (P<0.001) and hospitalizations (P=0.03). A similar trend was observed for cardiovascular events but this relationship did not reach statistical significance (P=0.09). ***Conclusions:*** Poor physical performance predicts a high risk of mortality, cardiovascular events and hospitalizations in dialysis patients. Future studies, including phase-2 EXCITE, will assess whether improving motor fitness may translate into better clinical outcomes in this high risk population.

Key Words: Physical performance, Six-minute walking test, Chronic kidney disease, Dialysis, Clinical Outcomes

1. INTRODUCTION

Physical activity and physical performance are notoriously poor in patients with end-stage kidney disease (ESKD) (1), a population with an extremely high risk of death and cardiovascular events (2). Even though representing strictly related phenomena, physical activity and physical performance are separated entities with different metrics. Physical activity, i.e. physical engagement in daily activities, is a well-established predictor of mortality and cardiovascular events in the general population (3) and in pathological conditions such as diabetes (4) and coronary artery disease (5) and in end stage kidney disease (ESKD) as well (6-9). To our knowledge, the relationship between actual physical performance, i.e. the objectively measured ability to perform well standardized physical efforts, and clinical outcomes in ESKD has been investigated just in a small study with a very limited number of major clinical events (just 21 deaths) (10). The Six-Minute Walking Test (6MWT) is an established test to assess physical performance in frail elderly patients (11), and this test has been applied in clinical studies in various conditions, such as heart failure (12, 13) and chronic obstructive pulmonary disease (COPD) (14). The EXCITE (EXerCise Introduction To Enhance Performance in Dialysis) study, is a large, multicentre, randomized trial whose phase – 2 (clinical outcomes and hospitalization) is still in progress. This study tests the effectiveness of an easy-to-implement program of physical training in dialysis patients. We have taken the opportunity of the EXCITE study to investigate the relationship between actual physical performance, as assessed by the Six-Minutes Walking Test, on mortality, cardiovascular events and hospitalizations in dialysis patients.

2. PATIENTS AND METHODS

The study protocol was approved by the ethical committee of our institution. All participants gave informed consent before enrolment.

2.1 Patients

The EXCITE Study is a multicentre randomized controlled trial on the effectiveness of exercise in improving physical performance and the quality of life (phase-1) and in

reducing adverse clinical outcomes (mortality, cardiovascular events and hospitalizations) (phase-2) in dialysis patients. This trial is registered in ClinicalTrials.gov (Identifier: NCT01255969). In this secondary analysis, we included 296 dialysis patients who performed the 6MWT at baseline. These patients had been on regular dialysis (HD or PD) for a median time of 44 months (inter-quartile range: 26-83). haemodialysis patients (n=247) were being treated with standard bicarbonate dialysis with non-cellulosic membrane filters of various type. PD patients (n=49) were either on 4 standard exchanges day (n=11) or on continuous cycling peritoneal dialysis (n=38). Two hundred and six patients were treated with various anti-hypertensive drugs (76 on mono-therapy with calcium channel blockers, ACE inhibitors, sartans, alpha or beta blockers, clonidine, furosemide, 65 on double therapy, 44 on triple therapy and 21 patients on quadruple and quintuple therapy with various combinations of these drugs). The main demographic, somatometric, clinical and biochemical characteristics of the study population are detailed in **Table 1**.

2.2 Laboratory measurements

Blood sampling was performed after an overnight fast. In haemodialysis patients, blood was always drawn in the morning hours (8 am – 12 am) during a mid-week day (brief dialysis interval). Serum cholesterol, albumin, calcium, phosphate, C-Reactive Protein (CRP) and haemoglobin measurements were made using standard methods in the routine clinical laboratory. Six-Minute Walking Test Physical performance was assessed at baseline with the Six-Minute Walking Test (6MWT). This test consists in a 6 minute-walk along a marked walkway on a hard, flat surface, at the maximum speed that each patient can maintain. The goal of this test is to walk as far possible in six minutes. During the walk, the patient is allowed to stop and rest whenever he/she wants, and the number of interruptions are carefully recorded by an operator. At the end of the test, the fatigue perceived by the patient is classified by the Borg Scale, a simple method that allows to rate the perceived exertion by using a scale from 0 (no exertion) to 10 (maximum exertion)

Table 1. Main demographic, somatometric and clinical characteristics in the study population and correlates of Six-Minute Walking Test (6MWT)

	Whole group (n=296)	6MWT correlation coefficient (P)
Age (years)	65±13	**-0.57 (<0.001)**
BMI (kg/m²)	25±5	-0.04 (0.63)
Male sex n. (%)	201 (68)	**0.21 (<0.001)**
Current smokers n. (%)	48 (17)	-0.04 (0.52)
Past smokers n. (%)	72 (26)	
Diabetics n. (%)	60 (21)	**-0.20 (0.001)**
On anti-hypertensive treatment n. (%)	206 (73)	0.004 (0.95)
Dialysis vintage (months)	44 (26-83)	-0.03 (0.58)
With cardiovascular comorbidities* n. (%)	226 (76)	**-0.26 (<0.001)**
Systolic Blood Pressure (mmHg)	128±20	0.07 (0.27)
Diastolic Blood Pressure (mmHg)	69±11	**0.38 (<0.001)**
Cholesterol (mg/dL)	164±38	-0.08 (0.23)
Hemoglobin (g/dL)	11.0±1.9	0.11 (0.07)
Albumin (g/dL)	3.9±0.4	**0.22 (0.001)**
hsCRP (mg/L)	0.7(0.4-2.6)	**-0.16 (0.03)**
Calcium (mg/dL)	8.7±1.4	-0.06 (0.31)
Phosphate (mg/dL)	4.9±1.5	**0.22 (<0.001)**
NYHA Class 0 n. (%)	124 (44)	
NYHA Class 1 n. (%)	95 (34)	**-0.21 (<0.001)**
NYHA Class 2 n. (%)	40 (14)	
NYHA Class 3 n. (%)	20 (7)	

* Angina, arrhythmia, myocardial infarction, coronary surgery, angioplasty, other heart surgery, claudicatio, amputations, peripheral surgery, stroke, TIA, heart failure.
Data are expressed as mean ± SD. median and inter-quartile range or as percent frequency, as appropriate.

2.3 Study end-points

In this secondary analysis of EXCITE, a composite end-point including mortality, fatal and non-fatal cardiovascular events and hospitalizations was the main study end-point. Cardiovascular events were classified as follows: stroke (ischemic or haemorrhagic) documented by computed tomography, magnetic resonance imaging and / or clinical and neurological evaluation; transient ischemic attacks (TIA); myocardial infarction confirmed by serial changes of ECG and cardiac biomarkers; ECG-documented angina episodes; heart failure, diagnosed according to criteria by the AHA guideline (15); ECG documented arrhythmia; peripheral ischemia or amputations; unexpected, sudden death highly suspected as of cardiac origin. Hospitalizations were classified in cardiovascular and non-cardiovascular using information included in the hospital

records. Cause of death was assessed by 3 independent physicians. In doubtful cases, diagnosis was attributed by consensus. During the review process, involved physician used all available medical information, including hospitalization forms and medical records. In case of death occurred at home, family members and/or general practitioners were interviewed to better understand the circumstances surrounding death.

2.4 Statistical analysis

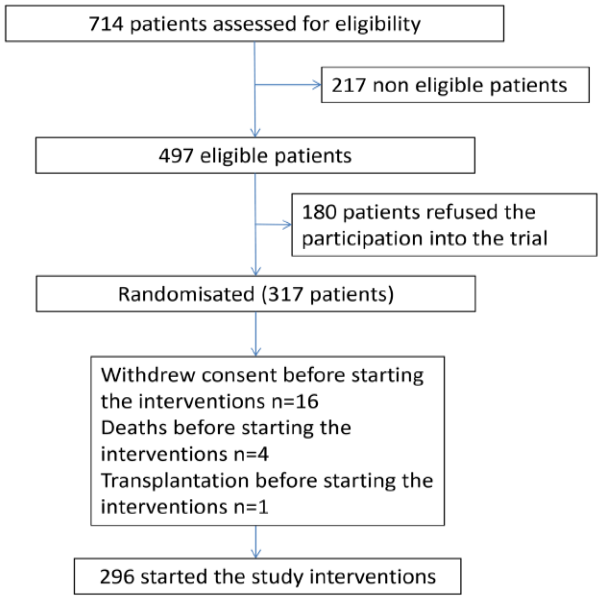
Data were expressed as mean ± standard deviation (normally distributed data), median and inter quartile range (non-normally distributed data) or as per cent frequency (categorical data). The Person correlation coefficient was used to describe correlates of 6MWT variable. The independent correlates of 6MWT were identified by correlation analysis and by multiple linear regression. Tested variables included age, gender, smoking, cardiovascular comorbidities, diabetes, antihypertensive therapy, Body Mass Index (BMI), dialysis vintage, systolic and diastolic blood pressure, cholesterol, albumin, C-reactive protein, calcium, phosphate, haemoglobin and NYHA class. All variables which correlated with 6MWT (with $P<0.05$) were jointly introduced into the same model. Survival analyses were performed by using bivariate and multivariate Cox regression models. In close parallelism with the strategy used for the identification of independent correlates of 6MWT (see above), in the multiple Cox Regression model we included all univariate correlates of the combined end point (with $P<0.05$). Statistical analysis was performed by using standard statistical packages (SPSS for Windows, Version 20, Chicago, Illinois, USA; STATA for Windows, Version 13, College Station, Texas, USA).

3. RESULTS

The flow-chart describing the recruitment basis of the study population and the subsequent selection process, from eligibility to randomisation, is reported in **Figure 1** and the baseline characteristics of patients randomised to the study intervention are described in **Table 1**. Enrolled patients had a mean age of 65 years. Sixty-eight of them

were male, 17% were current smokers, 26% were past smokers. Twenty-one per cent of patients were diabetics and 76% had cardiovascular comorbidities.

Figure 1. Flow diagram of the patients enrolled into the study (patients randomised to the physical exercise program or to standard care)



3.1 Correlation analyses

Baseline 6MWT, expressed as number of meters walked in 6 minutes, significantly correlated with age (ρ= -0.57, P<0.001), gender (ρ= 0.21, P=0.001), cardiovascular comorbidities (ρ= -0.24, P<0.001), diabetes (ρ= -0.20, P=0.001), diastolic blood pressure (ρ= 0.38, P<0.001) albumin (ρ= 0.22, P=0.001), phosphate (ρ= 0.22, P<0.001), CRP levels (ρ= -0.16, P=0.03) and NYHA class (ρ= -0.21, P<0.001). In a multiple linear regression analysis including all univariate correlates of 6MWT, only age (beta= -0.55), gender (beta= 0.16), and cardiovascular comorbidities (beta= -0.17) maintained an independent association with 6MWT (P<0.02).

3.2 Survival analyses

The median follow-up was 3.3 years (interquartile range: 2.7-3.5 years). During this period, 69 patients died, 90 had fatal or non-fatal cardiovascular events, 159 were hospitalized. Overall, 182 patients had the composite end-point death/cardiovascular events/hospitalizations. In a bivariate Cox regression model, including the allocation arm as covariate, an increase of 20 meters walked during the 6MWT significantly (P<0.001) reduced the risk of the composite end-point by 6%. Similar results were obtained in bivariate analyses of the individual end-points. In these models, an increase of 20 meters significantly reduced all-cause death by 12% (P<0.001), fatal and non-fatal cardiovascular events by 7% (P<0.001), and all-cause hospitalizations by 4% (P=0.002). The relationship between physical performance and the combined end-point was confirmed in a model adjusting for age, gender, systolic blood pressure, cholesterol, diabetes, smoking, cardiovascular

comorbidities and allocation arm (HR: 0.94, CI: 0.91-0.98, P=0.001) (**Figure 2**). By the same token, physical performance by 6MWT predicted all-cause death (HR: 0.89, CI: 0.84-0.94, P<0.001) and hospitalizations (HR: 0.96, CI: 0.92-0.99, P=0.03). A similar trend was observed for CV events, but this relationship did not reach statistical significance (HR: 0.96, CI: 0.91-1.01, P=0.10). Forcing risk factors peculiar to ESKD (haemoglobin, albumin and phosphate) into the model did not modify these relationships (**Figure 2**).

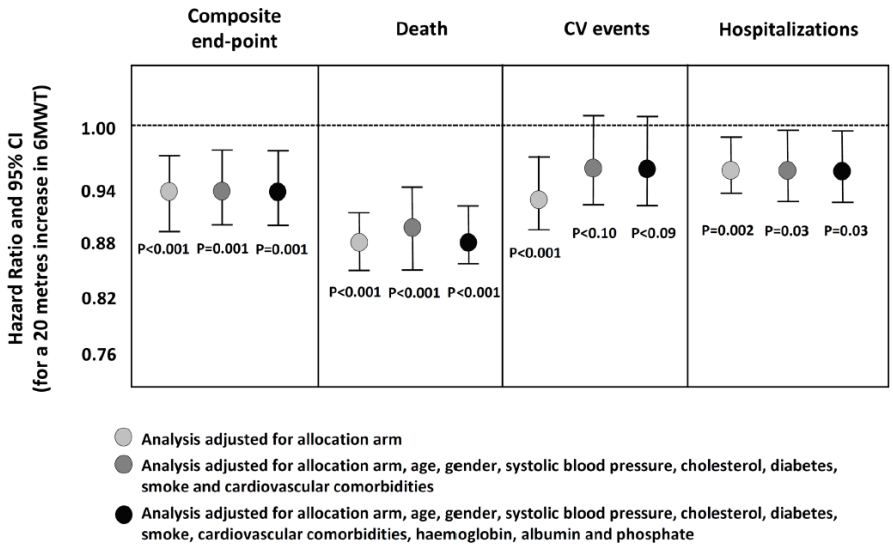
4. DISCUSSION

This study shows that 6MWT, a test commonly used to measure exercise capacity and motor fitness, predicts the risk for mortality, cardiovascular events and hospitalizations in chronic kidney disease patients on dialysis. Physical activity, either measured by questionnaires (16, 9) or by accelerometers (17) or pedometers (18), is about 50% less in dialysis patients than in age and sex matched individuals. In the Dialysis Outcomes and Practice Patterns Study (DOPPS), self-reported activity was an independent predictor of death and exercising at least once a week predicted a 27% risk reduction (19). Similar results emerged from the Wave 2 study of the United States Renal Data

System (USRDS) (7) and in a study based on accelerometry (8).While physical activity estimates engagement in daily activities, metrics of physical performance provide objective measures of motor fitness. As such, metrics of physical performance looking at walking speed like Six-Minute Walking Test (6MWT), measure cardiorespiratory endurance, muscle endurance and strength as well as balance and coordination.

The 6MWT has been applied in the whole age spectrum (19, 20) at population level and in several conditions, including chronic congestive heart failure (13, 21) and other cardiac conditions (22), COPD (14) and in hereditary diseases like cystic fibrosis (20). This test has prognostic relevance because it predicts clinical outcomes in several categories of patients including elderly patients undergoing coronary artery bypass grafting (23) and patients with chronic heart failure treated with cardiac resynchronization (24). Whether this test of motor fitness predicts mortality in dialysis patients has never been investigated. In this study, we found coherent correlations between physical performance (6MWT) and some factors which have an obvious influence on health status, such as age, gender, cardiovascular comorbidities, NYHA class, diabetes, diastolic blood pressure and other parameters (25-29). However, only age, gender and cardiovascular comorbidities maintained an independent association with the 6MWT suggesting that these factors are major determinants of motor fitness in dialysis patients. According to our working hypothesis that physical performance measured by the 6MWT holds prognostic value in dialysis patients, we found that this test is a strong predictor of mortality, cardiovascular events and hospitalizations in this population. More specifically, in adjusted analyses, we observed a reduction of 6% in the combined outcome for each increase of 20 walked meters, and a reduction of 12% and 4% for all cause death and hospitalization. Thus our data extend to the dialysis population observations made in other conditions (23, 24) and underscore the relevance of objective measures of motor fitness in assessing the overall risk profile of dialysis patients. Future studies, including phase-2 of EXCITE, will assess whether interventions aimed at improving physical fitness may translate into better clinical outcomes, including better physical performance, longer survival and reduced rate of cardiovascular events and hospitalizations in dialysis patients.

Figure 2. Hazard ratio and 95% CI associated to an increase of 20 walked metres during the six-minute walking test (6MWT) for the composite end-point (A), all-cause mortality (B), fatal and non-fatal cardiovascular (CV) events (C) and hospitalizations (D).



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Chapter 7

Fitness for Entering a Simple Exercise Program and Mortality: A Study Corollary to the Exercise Introduction to Enhance Performance in Dialysis (EXCITE) Trial

ABSTRACT

Background/Aims: In this corollary analysis of the EXCITE study, we looked at possible differences in baseline risk factors and mortality between subjects excluded from the trial because non-eligible (n=216) or because eligible but refusing to participate (n=116). ***Methods:*** Baseline characteristics and mortality data were recorded. Survival and independent predictors of mortality were assessed by Kaplan-Meier and Cox regression analyses. ***Results:*** The incidence rate of mortality was higher in non-eligible vs. eligible non-randomized patients (21.0 vs. 10.9 deaths/100 persons-year; P<0.001). The crude excess risk of death in noneligible patients (HR 1.96; 95% CI 1.36 to 2.77; P<0.001) was reduced after adjustment for risk factors which differed in the two cohorts including age, blood pressure, phosphate, CRP, smoking, diabetes, triglycerides, cardiovascular comorbidities and history of neoplasia (HR 1.60; 95% CI 1.10 to 2.35; P=0.017) and almost nullified after including in the same model also information on deambulation impairment (HR 1.16; 95% CI 0.75 to 1.80; P=0.513). ***Conclusions:*** Deambulation ability mostly explains the difference in survival rate in non-eligible and eligible non-randomized patients in the EXCITE trial. Extending data analyses and outcome reporting also to subjects not taking part in a trial may be helpful to assess the representability of the study population.

Key Words: Physical exercise, Mortality, Outcome study, Dialysis, Deambulation.

1. INTRODUCTION

Precise definition of the population investigated in clinical trials and in well-planned observational studies is fundamental to understand the potential applicability of findings in these studies in clinical practice. The CONSORT (Consolidated Standards of Reporting Trials) document recommends a specific diagram for describing the flow of participants through the main phases of clinical trials (1), from eligibility to actual enrollment and follow-up. By the same token, a similar approach has been recommended for observational studies by STROBE, another document produced under the aegis of the EQUATOR (Enhancing the QUAlity and Transparency Of health Research) initiative. The importance of accurate reporting of the selection process of subjects enrolled into a trial is of obvious relevance for the generalizability of findings in the same trial. However, outcome data in subjects screened but not enrolled in clinical trials (as not eligible or as refusing to provide consent to participate) have received very little attention and we have been unable to identify even a single study focusing on this issue. The problem appears of particular relevance in trials testing exercise programs, i.e. trials which selects individuals with an inherently lower risk profile, i.e. with a degree of fitness allowing a physical exercise program. We hereby report a study corollary to the EXerCise Introduction To Enhance performance in dialysis (EXCITE) study, i.e. a multicentric, randomized controlled clinical trial on the effectiveness of exercise for improving physical performance and the quality of life and for reducing adverse clinical outcomes (mortality, cardiovascular events and hospitalizations) in dialysis patients (NCT01255969). We specifically designed this study to investigate differences in baseline risk factors and their influence on the overall survival in non-eligible patients and in eligible patients who declined the invitation to participate into the trial.

2. PATIENTS AND METHODS

The study protocol was in conformity to the ethical guidelines of our institution and informed consent was obtained from each participant.

2.1 Patients

This is a study corollary to the EXerCise Introduction To Enhance performance in dialysis (EXCITE) study. More detailed information about this trial are available in ClinicalTrials.gov (NCT01255969). In this study we investigated the differences in baseline risk factors and their influence on the incidence rate of mortality in non-eligible patients (n=216; 90% on HD) and eligible patients who did not give the informed consent to take part into the EXCITE trial (n=116; 84% on HD). Deambulation ability was categorized as independent, assisted or total inability to deambulate (bedridden or wheel-chaired). The main demographic, clinical and biochemical characteristics of patients included in this study are given in **Table 1**.

Table 1. Main demographic, clinical and biochemical characteristics of patients

	Non eligible N=216	Eligible non randomized N=116	P
Age (years)	70±14	67±14	0.043
Male sex n. (%)	119 (56)	75 (65)	0.111
Smokers n. (%)	61 (35)	33 (49)	0.049
Diabetics n. (%)	64 (30)	24 (21)	0.063
History of neoplasia n. (%)	44 (21)	29 (25)	0.355
Myocardial Infarction n. (%)	51 (24)	18 (16)	0.077
Stroke n. (%)	29(14)	11 (10)	0.286
Transient Ischemic Attack n. (%)	44 (21)	11 (10)	0.010
Anginal episodes n. (%)	35 (16)	8 (7)	0.015
Arrhythmia n. (%)	53 (25)	13 (11)	0.003
Peripheral vascular disease n. (%)	45 (21)	4 (4)	<0.001
Heart failure n. (%)	145 (73)	96 (61)	0.02
NYHA class n. (%):			
1	54 (26)	50 (45)	<0.001
2	29 (14)	7 (6)	
3-4	70 (34)	10 (9)	
Deambulation:			
Independent	79 (37)	107 (94)	
Assisted	77 (36)	7 (6)	<0.001
Total inability (bedridden/ wheelchair)	60 (28)	0 (0)	
Systolic BP (mmHg)	127±19	133±19	0.077
Diastolic BP (mmHg)	69±11	71±10	0.239
Hypertension n. (%)	144 (67)	91 (78)	0.028
Total cholesterol (mg/dL)	164±41	171±50	0.198
Triglycerides (mg/dL)	153±73	175±106	0.05
Haemoglobin (g/dL)	11±2	11±1	0.571
Albumin (g/dL)	4.3±4.8	3.9±0.4	0.351
Calcium (mg/dL)	7.9±2.0	8.3±2.0	0.156
Phosphate (mg/dL)	4.4±1.4	5.0±1.8	0.002
CRP (mg/L)	1.3 (0.4-3.6)	0.7 (0.4-2.9)	0.020

Data are expressed as mean ± SD, median and inter-quartile range or as percent frequency, as appropriate.

2.2 Laboratory measurements

Blood sampling was performed during a midweek day (short dialysis interval). Serum cholesterol, albumin, calcium, phosphate, C-Reactive Protein (CRP) and haemoglobin measurementswere made using standard methods in the routine clinical laboratory. Follow up After the initial assessment, the median follow-up was 2.9 years in non-eligible patients (interquartile range 1.2 to 4.0 years) and 4.0 years in eligible patients unwilling to participate into the trial (interquartile range 2.3 to 4.0 years). During follow-up, death was accurately recorded. Each death was reviewed and assigned an underlying cause by a panel of 5 physicians. As a part of the review process, all available medical information about each death were collected. This information always included study and hospitalisation records. In the case of an out-of-hospital death family members were interviewed by telephone to better ascertain the circumstances surrounding death.

2.3 Statistical analysis

Data were expressed as mean ± standard deviation (normally distributed data), median and interquartile range (non-normally distributed data) or as per cent frequency (categorical data), and the comparisons among groups were made by One Way ANOVA, Kruskal-Wallis Test and Chi Square Test, as appropriate. In each study cohort, the independent predictors of mortality were identified by Kaplan-Meier survival analysis and by univariate and multivariate Cox regression models. Tested variables included age, gender, smoking, diabetes, blood pressure, total cholesterol, triglycerides, haemoglobin, albumin, calcium, phosphate, C-Reactive Protein, history of neoplasm, myocardial infarction, stroke, TIA, angina episodes, arrhythmia, peripheral vascular disease, and heart failure. We built-up multiple Cox regression models specific to each study cohort, by introducing into these models all variables which were related to all-cause mortality with $P<0.10$. To assess whether the observed difference in the death risk among the two study cohorts (i.e. non-eligible patients and eligible non-randomized patients) could be explained by differences in baseline risk factors (**Table 1**), we performed a multiple Cox regression analysis in the whole study population (n=648)

including the group (cohort) variable and a series of risk factors which were related (with P<0.10) to mortality in each study cohort (**Table 2**) and/or differed (with P<0.10) among the two cohorts (**Table 1**). By this strategy we constructed models of adequate statistical power (i.e. at least 10 deaths for each variable into the models). In the survival analysis, data were expressed as hazard ratio, 95% confidence interval and P value. The explained variation in mortality attributable to single variables was calculated by the method proposed by Hosmer and Lemeshow (2). Statistical analysis was performed by using a standard statistical package (SPSS for Windows, Version 20, Chicago, Illinois, USA

3. RESULTS

The source population of the EXCITE study included 648 patients with ESKD. Among these, 216 patients were excluded because they did not meet inclusion criteria (33%), 116 patients were eligible but did not give the informed consent (18%) and the remaining 316 patients were randomized (49%). Most non-eligible patients (64%) did not deambulate autonomously and/or needed to be assisted in everyday life. Eligible patients nonparticipating into the study were significantly younger and displayed a lower proportion of patients with angina, arrhythmia, heart failure and TIA as compared to non-eligible patients. Circulating levels of phosphate and the prevalence of smokers and hypertensive patients were higher in eligible non-randomized patients than in non-eligible patients (**Table 1**). Circulating levels of C-Reactive Protein (CRP) and the prevalence of peripheral vascular disease were higher in non-eligible patients than in eligible patients who did not give the informed consent (**Table 1**).

3.1 Predictors of mortality in eligible non-randomized patients and in ineligible patients

During the follow-up period, the number of deaths was 119 in non-eligible patients (55%) and 41 in eligible non-randomized (35%) patients. In non-eligible patients, age, triglycerides, phosphate, CRP, history of heart failure, history of peripheral vascular disease and deambulation degree (autonome, assisted or bedridden/wheelchaired)

predicted death with formal statistical significance (p<0.05) while only age, history of arrhythmia and history of neoplasia associated significantly with the same outcome in eligible patients who did not enter into the trial (**Table 2**). Non-significant associations (p>0.05, p<0.10) with death were noted for smoking (**Table 2**). In multivariate analyses mortality was predicted only by age, triglycerides, CRP, degree of deambulation and history of heart failure in non-eligible patients (**Table 3a**) and only by age in eligible non-randomized patients (**Table 3b**).

3.2 Comparison of survival in eligible non-randomized patients and in ineligible patients

The incidence rate of mortality was substantially higher in non-eligible patients (incidence rate: 21.0 deaths/100 persons-year) than in eligible non-randomized patients (incidence rate: 10.9 deaths/100 persons-year) (Log rank test: $\chi 2=13.85$, p <0.001) (**Figure 1a**). To assess whether the difference in baseline risk factors could explain the difference in the death risk of the two study cohorts, we performed two multivariate analyses including the group variable of eligible and non-eligible patients (cohorts) and a series of risk factors which were related to mortality (with p<0.10) in each study cohort (**Table 2**) and/or differed (with p<0.10) among the two cohorts (**Table 1**). To estimate the impact of the degree of deambulation impairment on mortality we tested two models, the first excluding deambulation ability and the second including this co-variate. In the first model the crude difference in the mortality risk among the two cohorts (HR 1.96; 95% CI 1.36 to 2.77; P<0.001) was reduced modestly (HR 1.60; 95% CI 1.10 to 2.35; P=0.017) (**Figure 1b** and **Table 4**). However, additional adjustement for deambulation almost nullified the excess risk of death seen in non-eligible patients (HR 1.16; 95% CI 0.75 to 1.80; P=0.513) (**Figure 1c** and **Table 4**). Of note, the explained variation in all-cause mortality was 33% for the model excluding and 38% for the model including the deambulation variable (P<0.0005). Thus, deambulation captures as much as the 13% in the explained variability in the risk of death.

Table 2. Univariate Cox regression analyses in the two study cohorts. Categories grading deambulation impairment are listed in **Table 1**

	Unit of increase	Non eligible patients*	Eligible non- randomized patients**
Age	1 year	1.04 (1.02-1.06), p<0.001	1.04 (1.01-1.06), p=0.01
Gender	0=F; 1=M	1.17 (0.81 1.68), p=0.41	0.90 (0.48 1.70), p=0.74
Smoking	0=no; 1=yes	1.41 (0.95-2.11), p=0.09	1.32 (0.60-2.94),p=0.49
Diabetes	0=no; 1=yes	1.13 (0.77-1.66), p=0.54	1.04 (0.50-2.19), p=0.91
Systolic BP	1 mmHg	0.99 (0.99-1.01),p=0.47	1.01 (0.98-1.03), p=0.56
Diastolic BP	1 mmHg	0.99 (0.97-1.00), p=0.11	0.97 (0.93-1.01), p= 0.12
Total cholesterol	1 mg/dL	1.00 (0.99-1.00), p=0.97	1.00 (1.00-1.01),p=0.35
Triglycerides	1 mg/dL	0.99 (0.99-1.00), p=0.01	1.00 (0.99-1.00)p=0.74
Haemoglobin	1 g/dL	0.98 (0.90-1.06), p=0.58	1.02 (0.82-1.27), p=0.87
Albumin	1 g/dL	0.99 (0.94-1.04), p=0.71	0.93 (0.41-2.13), p=0.86
Calcium	1 mg/dL	0.99 (0.90-1.08), p=0.79	1.02 (0.87-1.20),p=0.84
Phosphate	1 mg/dL	0.88 (0.77-1.00), p=0.05	1.00 (0.84-1.20), p=0.99
CRP	1 mg/L	1.01 (1.00-1.01),p=0.03	0.99 (0.94-1.04), p=0.57
History of myocardial infarction	0=no; 1=yes	1.25 (0.82-1.88), p=0.29	0.50 (0.18-1.41), p=0.19
History of stroke	0=no; 1=yes	1.15 (0.68-1.95),p=0.60	1.34 (0.52-3.41), p=0.54
History of TIA	0=no; 1=yes	1.23 (0.79-1.89), p=0.36	1.28 (0.50-3.26), p=0.61
History of angina	0=no; 1=yes	1.31 (0.82-2.07), p=0.26	1.00 (0.31-3.23), p=0.99
History of arrhythmia	0=no; 1=yes	1.39 (0.93-2.06), p=0.11	2.24 (1.03-4.85), p=0.04
History of heart failure	0=no; 1=yes	1.46 (1.25-1.72), p<0.001	1.05 (0.74-1.49), p=0.78
History of peripheral vascular disease	0=no; 1=yes	1.53 (1.00-2.32), p=0.05	1.51 (0.37-6.13), p=0.57
History of neoplasia	0=no; 1=yes	1.37 (0.89-2.11), p=0.15	1.98 (1.04-3.77), p=0.04
Deambulation	1 category	0.66(0.53-0.83), p<0.001	0.46(0.17-1.30), p=0.14

* HR (95% CI) and P; ** HR (95% CI) and P

Table 3. Cox regression in non-eligible patients (a) and eligible non- randomized patients (b)

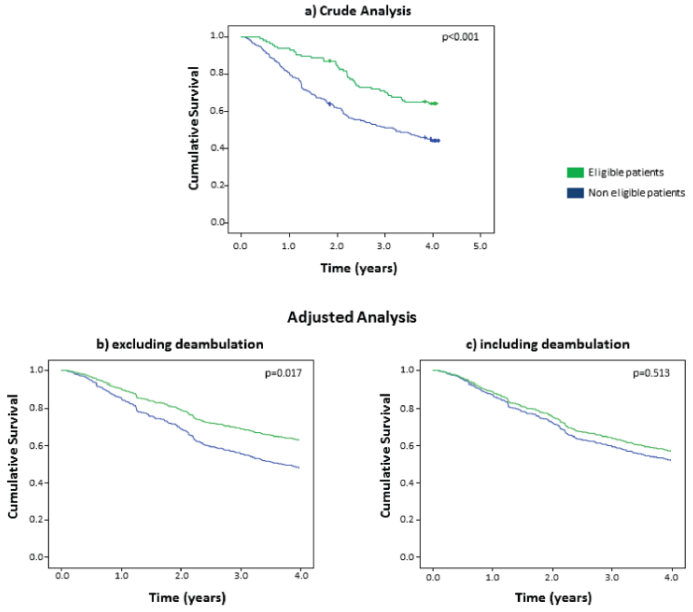
	Units of increase	HR (95% CI) and P
a) Number of deaths, n=119		
Age	1 year	1.04 (1.02-1.06), p<0.001
Smoking	0=no; 1=yes	1.13 (0.74-1.72), p=0.59
Triglycerides	1 mg/dL	1.00 (0.99-1.00), p=0.04
Phosphate	1 mg/dL	0.95 (0.82-1.10), p=0.95
CRP	1 mg/L	1.01 (1.00-1.02), p=0.05
History of peripheral vascular disease	0=no; 1=yes	1.29 (0.84-1.99), p=0.25
Deambulation	1 unit	0.72 (0.57-0.91), p=0.01
History of heart failure	0=no; 1=yes	1.81 (1.03-3.16), p=0.04
b) Number of deaths, n=41		
Age	1 year	1.03 (1.00-1.06), p=0.04
History of arrhythmia	0=no; 1=yes	1.91 (0.87-4.17), p=0.11
History of neoplasia	0=no; 1=yes	1.62 (0.84-3.15), p=0.15

Table 4. Multivariate Cox regression analysis in the whole study population

	Units of increase	Crude analysis**	Adjusted analysis	
			Excluding Deambulation**	Including Deambulation**
Eligible non-randomized patients	1	1*	1*	1*
Non eligible patients	2	1.94 (1.36–2.77), p <0.001	1.60 (1.10–2.35), p=0.017	1.16 (0.75–1.80), p=0.513
Age	1 year		1.03 (1.02-1.05), p<0.001	1.03 (1.02-1.05), p<0.001
Systolic BP	1 mmHg		1.00 (1.00-1.01), p=0.86	1.00 (1.00-1.01), p=0.77
Triglycerides	1 mg/dL		1.00 (1.00-1.00), p=0.13	1.00 (1.00-1.00), p=0.27
Phosphate	1 mg/dL		0.96 (0.85-1.08), p=0.49	0.99 (0.88-1.11), p=0.85
CRP	1 mg/L		1.01 (1.00-1.02), p=0.08	1.01 (1.00-1.01), p=0.14
Smoking	0=no; 1=yes		1.26 (0.87-1.83), p=0.21	1.27 (0.88-1.85), p=0.21
Diabetes	0=no; 1=yes		1.10 (0.75-1.60), p=0.64	0.92 (0.92-1.37), p=0.69
History of myocardial infarction	0=no; 1=yes		0.87 (0.57-1.34), p=0.53	0.87 (0.57-1.33), p=0.52
History of TIA	0=no; 1=yes		1.07 (0.71-1.62), p=0.75	0.96 (0.63-1.47), p=0.86
History of angina	0=no; 1=yes		0.97 (0.59-1.58), p=0.90	1.03 (0.63-1.67), p=0.92
History of arrhythmia	0=no; 1=yes		1.17 (0.81-1.70), p=0.40	1.20 (0.83-1.74), p=0.34
History of peripheral vascular disease	0=no; 1=yes		1.41 (0.92-2.16), p=0.12	1.38 (0.90-2.13), p=0.14
History of heart failure	0=no; 1=yes		1.46 (0.96-2.21), p=0.08	1.34 (0.88-2.04), p=0.18
Hypertension	0=no; 1=yes		0.99 (0.67-1.46), p=0.97	1.04 (0.71-1.54), p=0.84
History of neoplasm	0=no; 1=yes		1.26 (0.86-1.84), p=0.24	1.29 (0.88-1.90), p=0.19
Deambulation	1 category			0.66 (0.52-0.84), p=0.001

*Reference group; **HR (95% CI) and P

Figure 1. Unadjusted and adjusted survival analyses in the two study groups (non-eligible and eligible non randomized patients)



4. DISCUSSION

This study corollary to a multicenter randomized, clinical trial testing the effect of physical exercise in dialysis patients shows that the mortality risk is twice higher in patients who were excluded from the trial than in eligible patients who did not accept to participate. Importantly, the mortality rate in this group (10.9 deaths/100 persons-year) was lower than the average mortality rate in the ERA-EDTA registry (3) and in the Italian registry of dialysis (4). As expected, the two populations which remained external to the trial, i.e. the ineligible population and the population with sufficient fitness but unwilling to undergo the exercise program, showed several differences in baseline risk factors such as age, blood pressure, phosphate, CRP, smoking, diabetes, triglycerides, cardiovascular comorbidities and history of neoplasia. We hypothesized that these differences could explain the higher death risk of ineligible patients and tested this hypothesis by modeling death risk in an analysis where the two cohorts were nominally identified by a specific covariate. The proviso was that the inclusion of known risk factors differentiating the two cohorts should attenuate substantially or cancel out the excess death risk of unfit patients ineligible to the trial. However, in contrast to our working hypothesis, we found that adjustment for background risk factors only in limited part explained the between cohorts difference in mortality. This finding indicates that major risk factors fail to fully capture the higher probability of death in patients ineligible to clinical studies. Thus, the higher death rate in those patients must necessarily depend on unmeasured risk factors of paramount importance for human health. Most non-eligible patients (64%) did not deambulate autonomously or needed to be assisted, indicating that comorbidities limiting motor activity in everyday life mark a degree of severity that is unaccounted for by nominally defined, ungraded, major comorbidities like arrhythmia, heart failure, cerebro- and peripheral-vascular disease, ischemic heart disease and neoplasia. Indeed, when we introduced into the model deambulation impairment, the between cohorts difference in the risk of death almost disappeared (HR 1.16; 95% CI 0.75to 1.80; P=0.513). This observation is of importance because physical performance and inability to deambulate are very rarely taken into account when describing the risk profile of dialysis patients in major clinical trials and

observational studies in this population. For example, neither in the 4D (5), nor in the AURORA study (6), nor in the more recent EVOLVE trial (7), physical disability was an exclusion criterion but in none of these trials information on physical disability was given. About 1/3 (33%) of the source cohort of the EXCITE study had a relevant degree of physical impairment and it is likely that a similar proportion existed in the source populations of other trials in dialysis patients. The variability in mortality rate explained by unfitness to a simple exercise program is substantial and ignoring severe limitation in physical functioning may have a non-trivial effect for the interpretation of the risk profile of patients included in clinical studies. However, as discussed, information on ambulatory ability is almost always omitted in trials in the dialysis population. Findings in this study represent a call for systematic reporting information on physical activity and/or (motor) ability in clinical trials and observational studies in this population. Indeed –well beyond classical risk factors, background co-morbidities and risk factors peculiar to end-stage renal disease- deambulation impairment explains a relevant proportion of the variability in mortality in ESKD. Furthermore, our findings suggest that extending outcome reporting and data analyses to the whole source population of clinical trials may provide relevant information to better frame the implications of the selection process applied to restrict the trial population to patients with well-defined demographic and clinical characteristics.

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Chapter 8

Discussion

The studies included in this thesis provide new insights on the clinical impact of aging, physical activity and emerging risk factors in individuals with impaired renal function. In particular, I have found that pulmonary hypertension (PH), which is in most cases clinically unsuspected, may represent an important missing piece for improving cardiovascular risk stratification, even in those individuals with no evidence of lung or heart disease. PH is highly prevalent in CKD patients, particularly in individuals on chronic replacement therapy by hemodialysis. Apart from the presence of other co-morbidities or systemic diseases, several CKD-specific risk factors including the presence of artero-venous fistula, fluid overload, sleep breathing disorders and the exposure to dialysis membranes can be implicated at various levels in increasing pulmonary pressure. PH in CKD is a potentially reversible process because it may regress after kidney transplantation. However, the excess mortality conveyed by this condition may persist in some kidney graft recipients.

My work also accrued evidence pointing at aging as a key-determinant of major alterations in kidney biology. Although distinguishing normal renal senescence from pathological chronic kidney disease is, in most cases, far from being simple, a systematic and correct estimation of renal function in the elderly and the establishment of proper therapeutic measures in a timely manner appear of foremost importance for improving clinical outcomes in this particular population setting. In parallel to renal function, aging may also affect fitness capacity. Early changes of vascular responses to exercise prior to a clear reduced physical activity may eventually end in a severely impaired mobility. This latter trend is of importance, particularly for patients on chronic dialysis. In fact, I found that, in these individuals, a reduced exercise capacity or (even more clearly) an impaired mobility, may convey an incremental risk of worse outcomes. This suggests that appropriate and “tailored” fitness approaches in patients on dialysis may bring remarkable benefits as previously observed in other frail populations.

Influence of aging on renal biology and functionality

The dramatic population aging on a world scale may anticipate a burden of elderly individuals with end-stage kidney disease who are expected to significantly impact

health care resources. For instance, according to the US Renal Data System, during 2005–2013, the adjusted point prevalence rates per million population of reported end-stage renal disease increased from 70.5 to 82.1 (16% increase) in the age group 0–19 years, from 730.6 to 858.2 (17% increase) in the age group 20–44 years, from 2333.3 to 3150.4 (35% increase) in the age group 45–64 years, from 3627.5 to 5500.6 (51% increase) in the age group 65–74 years, and from 2762.4 to 4795.8 (73% increase) in the age group 75+ years (2). Time projections over the next 20 years seem to reinforce the concept that old and very old individuals will account for more than 50% of incident and prevalent ESKD cases worldwide. It is therefore not surprising that, in last years, the aging kidney has become a topic of great interest in geriatric medicine and clinical nephrology (1).

Through a systematic approach to the existing literature, I found collective evidence indicating that renal aging is a multifactorial process where gender, race and genetic background and several key-mediators such as oxidative stress, the renin-angiotensin-aldosterone (RAAS) system, impairment in kidney repair capacities and background cardiovascular disease, play a significant role. Features of the aging kidney include macroscopic and microscopic changes and key functional adaptations. Unfortunately, none of these can be considered pathognomonic of aging. Hence, the question to which extent renal aging should be considered as a physiological or pathological process remains mostly unanswered. Effective strategies for retarding kidney aging or for limiting its detrimental effects on pre-existing age-related kidney diseases might be essential for limiting the above-mentioned, dramatic CKD scenario. Yet, although promising dietary and pharmacological approaches have been tested to retard aging processes or renal function decline in the elderly, proper lifestyle modifications, as those applicable to the general population, currently represent the most plausible approach to maintain kidney health.

Improved understanding of renal aging might, in theory, also help optimizing the management of renal allografts obtained from older donors. For instance, it has been suggested that the early occurrence of critical telomere shortening and replicative senescence may contribute to the pathogenesis of chronic allograft nephropathy in

older allografts (3). Hence, efforts to understand the age-related changes in kidney function and mechanisms, which underlie these changes, might help identifying potential interventions for maximizing successful donations from the so-called "marginal" kidneys.

Role of pulmonary pressure as a new risk factor in renal patients

Although patients with chronic kidney disease consume an unequal share of health care resources, mortality and cardiovascular morbidity remain unacceptably high, particularly in persons with End-Stage Kidney Disease (4). Such a risk excess remains partly unexplained. The majority of interventions targeting traditional or "uremia-related" risk factors failed to significantly improve event-free survival. New prognostic indicators are therefore needed to refine outcome prediction and drive therapeutic management in this population. By approaching the existing literature in a systematic way, I found that mild-to-moderate forms of pulmonary hypertension (PH) have high prevalence among renal patients. Of note, in some hemodialysis population, such prevalence was reported to be as high as 70%. Even if clinically unsuspected, the presence of PH must deserve attention in this particular disease category. In fact, in an ample cohort of individuals with non-advanced CKD, I found that PH was a strong predictor of a combined endpoint including cardiovascular death, acute heart failure, coronary artery disease, cerebrovascular and peripheral artery events. Of note, PH remained an independent risk factor also in analyses adjusting for a series of potential confounders including age, residual renal function, haemoglobin, cardiac volumes and mass, presence of diabetes and prior CV disease. In the general population, over the last ten years, an increasing trend in mortality due to PH was documented with an estimated age-adjusted death rate of 4.5 to 12.3 per 100.000 (5). Of note, even in large cohorts of individuals with moderate to severe heart disease, the predictive power of PH remains independent from traditional cardiovascular risk factors and parameters of (left) heart dysfunction (6). Hence, in renal patients, PH may convey an incremental mortality and cardiovascular risk that reflects that evidenced at the community level and in other "high risk" populations. The question whether PH is a modifiable risk factor

in CKD patients should be addressed by targeted clinical trials. However, some issues may hinder future research in this field.

As indicated by current guidelines on PH management (7), right heart catheterization is essential for confirming the diagnosis and characterizing the disease. Yet, the vast majority of studies on PH in CKD used estimated (ePAP) rather than measured (mPAP) pulmonary artery pressure values to identify this condition. Although ePAP is considered a valid surrogate of mPAP, particularly for screening purposes, estimated values may significantly diverge from real measures and do not allow disease categorization (8). This aspect could represent a serious limit for establishing proper therapeutic approaches to treat PH in renal patients. In the general population, treatments aiming at optimizing left ventricular function and alleviating fluid overload (e.g. diuretics, beta blockers, drugs influencing the renin-angiotensin-aldosterone (RAAS) system) are fundamental for normalizing PH secondary to congestive heart failure (9,10). Conversely, the use of vasoactive agents is usually confined to patients with primary pulmonary arterial hypertension. Since left ventricular disorders are highly prevalent in the CKD population, the recommendations for the treatment of PH WHO II category (that is secondary to heart disease) in the general population (9) could reasonably apply also to this disease setting. Yet, no solid evidence on the treatment of PH in patients with CKD is available so far (10). Hence, drug therapies, whereas necessary, should be considered on the basis of the individual risk-benefit profile as most drugs may be harmful in the presence of severe renal impairment (11). In view of this “grey area” of evidence, future research including large scale, interventional studies adopting well standardized criteria of PAP measurement, such as right heart catheterization, would be of utmost importance to confirm the need for clinical attention on PH and to define benefits and harms of appropriate therapeutic approaches also in the CKD setting.

Aging, response to exercise and clinical impact of physical activity in dialysis patients

Failure of the arterial system during stress and increased vascular resistance are well-known hallmarks of the senescence process (12). Vascular ageing results in loss of

elastic properties, which triggers an increase in systolic pressure and a decrease in diastolic pressure due to progressive stiffness of the large elastic arteries (12). This may result in damage to small arterial vessels of the coronary, brain and kidney vascular districts, hence accelerating chronic cardiovascular or renal disease. No less important, poor vascular compliance to physical efforts elicits a reduced muscle tolerance to daily-life activities that accounts for most of the sedentary habits which characterizes old individuals. Similar to the elderly population, hemodialysis individuals are also characterized by a moderate-to-severely reduced physical activity (13). The increasing need for hemodialysis in our growing elderly populations will reduce the quality of live with more co-morbidities including depression (14). National Kidney Foundation Kidney Disease Outcomes Quality Initiative Guidelines formally recommend dialysis patients to be “counseled and regularly encouraged by nephrology and dialysis staff to increase their level of physical activity” (15). However, the evidence for recommending exercise training in this setting is still limited. With the EXCITE study (Exercise Introduction to Enhance Performance in Dialysis), a randomized, multicenter trial of moderate walking exercise to improve functional status in ESKD individuals, I demonstrated that reduced walking capacity predicts a high risk of mortality, cardiovascular events and hospitalizations in dialysis patients. The walking capacity encompasses cardiorespiratory and muscle endurance, muscle strength and balance and coordination, which are fundamental in daily living in patients with chronic kidney disease and in the elderly (16). Very importantly, similar observations were previously made in other frail, high-risk populations, such as elderly patients undergoing coronary artery bypass grafting (17) and patients with chronic heart failure treated with cardiac resynchronization (18). Hence, our findings extend the relevance of objective measures of motor fitness in assessing the overall risk profile to the dialysis population. Starting from these premises, future studies are required to assess whether improving motor fitness translates into better clinical outcomes in the dialysis population.

Of similar importance, in another analysis of the same trial, I also demonstrated that mobility mostly explains the difference in survival rate in non-eligible and eligible non-randomized patients. In other words, despite the two groups that remained outside the

trial (i.e. the ineligible population and the population with sufficient fitness but unwilling to undergo the exercise program) had several differences in baseline risk factors, adjustment for these background risk factors explained little mortality compared to the large effect of impaired mobility. This is important for at least two reasons. First it underlines the need for a systematic reporting information on physical activity and/or (motor) ability in clinical trials and observational studies conducted in dialysis patients. Second, and more general, it confirms that extending data analyses and outcome reporting also to subjects not taking part in a trial may be helpful to assess the representability of the study population.

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Summary

The number of elderly persons worldwide is forecasted to grow dramatically over the next decades. Hence, future medicine will inevitably face new challenges related to the several, inexorable consequences of aging. The assessment of renal function in the framework of aging is problematic and the question whether renal aging should be considered as a physiological or pathological process remains an open issue. Gender, race, genetic background and other mediators such as oxidative stress, the renin-angiotensin-aldosterone (RAAS) system, impairment of kidney repair capacities and background cardiovascular disease, are all key-players of kidney aging. Whether or not promising dietary and pharmacological approaches targeting some of these factors retard renal senescence and prevent chronic kidney disease (CKD) is currently tested.

Indeed, there is an urgent need to identify new prognostic biomarkers for refining outcome prediction in CKD individuals. Pulmonary hypertension (PH), a powerful and independent predictor of death in the general population and in subjects with heart or lung diseases, is highly prevalent also in renal patients and may hold the same prognostic utility for risk stratification and therapeutic management, particularly in persons with early CKD stages. As for the kidney and other organs, senescence is known to impair also physical capacity. In a pilot trial of healthy individuals of various ages, I found increased blood pressure levels after isometric challenge and a strong association between handgrip strength and change in blood pressure levels and aortic stiffness in elderly subjects. This altered vascular hemodynamics may reflect vascular ageing, a prelude to a decline in physical activity and mobility which characterizes the majority of old individuals. Similar to the elderly population, hemodialysis individuals have also a reduced physical activity. In an analysis of the EXCITE (Exercise Introduction to Enhance Performance in Dialysis) trial, a significantly reduced walking capacity predicted a high risk of mortality, cardiovascular events and hospitalizations in dialysis patients; this substantiates the importance of including objective measures of motor fitness in assessing the overall risk profile of dialysis and elderly study populations. In

another analysis of the same study, I focused on two groups, who remained outside the trial. Despite several differences in baseline risk factors, an impaired mobility was the sole variable clearly explaining the difference in survival rate between these two sub-populations. Future studies are required to assess whether improving fitness may translate into better clinical outcomes in this high risk population.

Samenvatting

Verwacht wordt dat het aantal ouderen wereldwijd de komende decennia dramatisch zal groeien. Daarom zal de toekomstige geneeskunde onvermijdelijk geconfronteerd worden met nieuwe uitdagingen met betrekking tot de verschillende, onverbiddelijke gevolgen van veroudering. De beoordeling van de nierfunctie in het kader van het ouder worden is problematisch en de vraag of nierveroudering moet worden beschouwd als een fysiologisch of pathologisch proces, blijft een open vraag. Geslacht, ras, genetische achtergrond en andere mediatoren zoals oxidatieve stress, het renine-angiotensine-aldosteron (RAAS) -systeem, verminderde niercapaciteit en cardiovasculaire aandoeningen zijn spelers van nierveroudering. Of op dit moment veelbelovende voedings- en farmacologische benaderingen die op sommige van deze factoren zijn gericht, de renale senescentie vertragen en chronische nieraandoeningen (CKD) voorkomen, worden momenteel getest. Er is zeker een dringende behoefte aan het identificeren van nieuwe prognostische biomarkers voor het verfijnen van de voorspelde uitkomst bij CKD-individuen. Pulmonale hypertensie (PH), een krachtige en onafhankelijke voorspeller van overlijden in de algemene bevolking en bij personen met hart- of longaandoeningen, komt ook veel voor bij nierpatiënten en kan hetzelfde prognostische nut hebben voor risicostratificatie en therapeutisch management, met name bij personen met vroege CKD-stadia. Wat de nier en andere organen betreft, is bekend dat senescentie ook de fysieke capaciteit schaadt. In een pilot-onderzoek met gezonde individuen van verschillende leeftijden, vond ik verhoogde bloeddrukniveaus na isometrische uitdaging en een sterke associatie tussen handgreepsterkte en verandering in bloeddrukniveaus en aortastijfheid bij oudere proefpersonen. Deze veranderde vasculaire hemodynamica kan vaatveroudering weerspiegelen, een opmaat naar een afname in fysieke activiteit en mobiliteit die kenmerkend is voor de meerderheid van de oude personen. Net als oudere patiënten hebben hemodialyse-individuen ook een verminderde fysieke activiteit. In een analyse van de EXCITE-test (Exercise Introduction to Enhance Performance in Dialysis), voorspelde een significant

verminderde loopcapaciteit een hoog risico op mortaliteit, cardiovasculaire gebeurtenissen en ziekenhuisopnames bij dialysepatiënten; dit onderbouwt het belang van het opnemen van objectieve metingen van motorische fitheid bij het beoordelen van het algehele risicoprofiel van dialyse en oudere studiepopulaties. Ondanks meerdere verschillen in basislijnrisicofactoren, was een verminderde mobiliteit de enige variabele die het verschil in overlevingspercentage tussen deze twee subpopulaties duidelijk verklaarde. Toekomstige studies zijn nodig om te beoordelen of het verbeteren van conditie kan leiden tot betere klinische uitkomsten in hoog risico deze populatie.

PHD PORTFOLIO

Name PhD student:	Period: **2015-2018**
Davide Bolignano	
Erasmus MC Department:	Promotors: **Prof. F.U.S. Mattace-Raso,**
Internal Medicine	**Prof. E.J.G. Sijbrands**
Research School: **COEUR**	Co-promotor: **Dr.G. Tripepi**

PhD Training:

Research skills

- Aging and cardiovascular risk factors in kidney diseases
- Exercise in dialysis patients
- Systematic reviews and meta-analyses

Invited Lectures

- Rare Disease? Challenging guideline. European Renal Best Practice CME Course at the 52nd ERA-EDTA Congress. London, May 2015
- Pulmonary hypertension in CKD patients: Relevant? Preventable? Treatable? 53rd ERA-EDTA Congress, Vienna, May 2016.
- Dialysis modality choice in diabetic patients with end-stage kidney disease. 53rd ERA-EDTA Congress, Vienna, May 2016.
- Clinical Guidance in Rare Kidney Diseases: Time to Revisiting Research Methodology? 24th Budapest Nephrology School, Budapest, August 2017
- The Conundrum of First Dialysis Modality in Diabetic Patients with ESKD. 24th Budapest Nephrology School, Budapest, August 2017
- Stealing my heart: Vascular Access and Pulmonary Hypertension. 50th ASN Kidney Week, New Orleans (US), November 2017
- Facing obesity in kidney transplantation. European Renal Best Practice CME Course at the 55th ERA-EDTA Congress, Copenhagen, Denmark, May 23 2018.

International Conferences

- 52nd ERA-EDTA Congress. London 2015
- 53rd ERA-EDTA Congress. Vienna 2016
- 54th ERA-EDTA Congress. Madrid 2017
- 24th Budapest Nephrology School. Budapest 2017
- 50th ASN Kidney Week. New Orleans (US) 2017
- 9th European Conference on Rare Diseases and Orphan Products, Vienna, 2018
- 55th ERA-EDTA Congress. Copenhagen, 2018

Other

- Editor in Chief of NDT-Educational
- Academic Editor of PLOS One
- Editorial Board member for Journal of Onco-Nephrology and Journal of Renal and Hepatic Disorders
- Reviewer for: Journal of the American Society of Nephrology, PLOS one, Nephrology Dialysis and Transplantation, Clinical Chemistry and Laboratory Medicine, Nature Reviews Nephrology, Critical Care, Kidney International, Clinical Journal of the American Society of Nephrology, American Journal of Nephrology, JAMA, Biomarkers in Medicine, International Urology and Nephrology, Journal of Nephrology, Clinical Kidney Journal, Future Medicine, World Journal of Gastroenterology, Trials, Clinical Pharmacokinetics, Obesity Research & Clinical Practice, Annals of Medical and Health Sciences Research, BMC Nephrology, Expert Review of Clinical Pharmacology, Endocrine.

LIST OF ALL PUBLICATIONS (2015-2018)

1. **Bolignano D**, Pisano A, Coppolino G, Tripepi GL, D'Arrigo G. Pulmonary hypertension predicts adverse outcomes in renal patients: a systematic review and Meta-Analysis. Ther Apher Dial. 2018 Nov 22. doi: 10.1111/1744-9987.12777
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11. Coppolino G, Cernaro V, Placida G, Leonardi G, Basile G, **Bolignano D**. Endothelial progenitor cells at the interface of chronic kidney disease: from biology to therapeutic advancement. Curr Med Chem. 2017 Sep 20.
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37. Couchoud C, **Bolignano D**, Nistor I, Jager KJ, Heaf J, Heimburger O, Van Biesen W; on Behalf of the European Renal Best Practice (ERBP) Diabetes Guideline Development Group. Dialysis modality choice in diabetic patients with End-stage Kidney Disease: a systematic review of the available evidence. Nephrol Dial Transplant, 2015 Feb;30(2):310-20

BOOK CHAPTERS

1. First Author of the chapter "Gender at the interface of renal aging: physiological and pathological perspectives" in "Principles of Gender-Specific Medicine, 3rd Edition"- Elsevier Academic Press (2016)
2. Co-Author of the chapter "Left Ventricular Hypertrophy in Chronic Kidney Disease", in the "Oxford Textbook of Clinical Nephrology2, 4th Edition- Oxford University Press (2015)
3. Co-Author of the chapter "Kidney and neoplastic disease: an overview with a particular interest to interpretation of cancer biomarkers" in "Biomarkers in Disease: Methods, Discoveries and Applications"- Springer (2015)

4. Co-Author of the chapter “Overview of Neutrophil Gelatinase-Associated Lipocalin (NGAL) as a biomarker in nephrology” in “Biomarkers in Disease: Methods, Discoveries and Applications”- Springer (2015)
5. Unique Author of the chapter “Pulmonary Hypertension in CKD - A New Problem Child” in “Cardio-Renal Clinical Challenges”-Springer (2015)

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Davide Bolignano was born in Reggio Calabria (Italy) on October 31th 1980. In 2004 he graduated in Medicine (MD) at the University of Messina and in 2009 he obtained the specialization in Nephrology, both *cum laude*. He currently holds a full position at the Institute of Clinical Physiology of the Italian National Research Council (CNR) based in Reggio Calabria, Italy, as clinical researcher. In 2012, he was honorary research fellow at the Cochrane Renal group (Sydney, Australia) to improving skills in systematic reviews, meta-analyses and guideline-making processes. In 2014, he completed the Global Clinical Scholars Research Training Program in methods and conduct of clinical research at the Harvard Medical School (Boston, US) with a special mention for the final thesis. In recognition of his achievements and contributions to the field of nephrology, in 2017, he has been awarded the Distinguished Fellow of the European Renal Association (FERA). From 2014 he is Head of the Research Unit on Systematic reviews and Meta-analysis of the Institute of Clinical Physiology of the CNR. His main scientific interests/fields of investigations are the epidemiology and pathophysiology of chronic and acute kidney diseases, renal biomarkers and cardiovascular risk. To date, he is Author/Co-Author of over 120 scientific peer-reviewed publications indexed on Pubmed and several abstracts or invited lectures at various international congresses on nephrology, cardiovascular and laboratory medicine.