Estimated glomerular filtration rate in older adults: validation, correlations and implications. Data from the general population study “Good Aging in Skåne”

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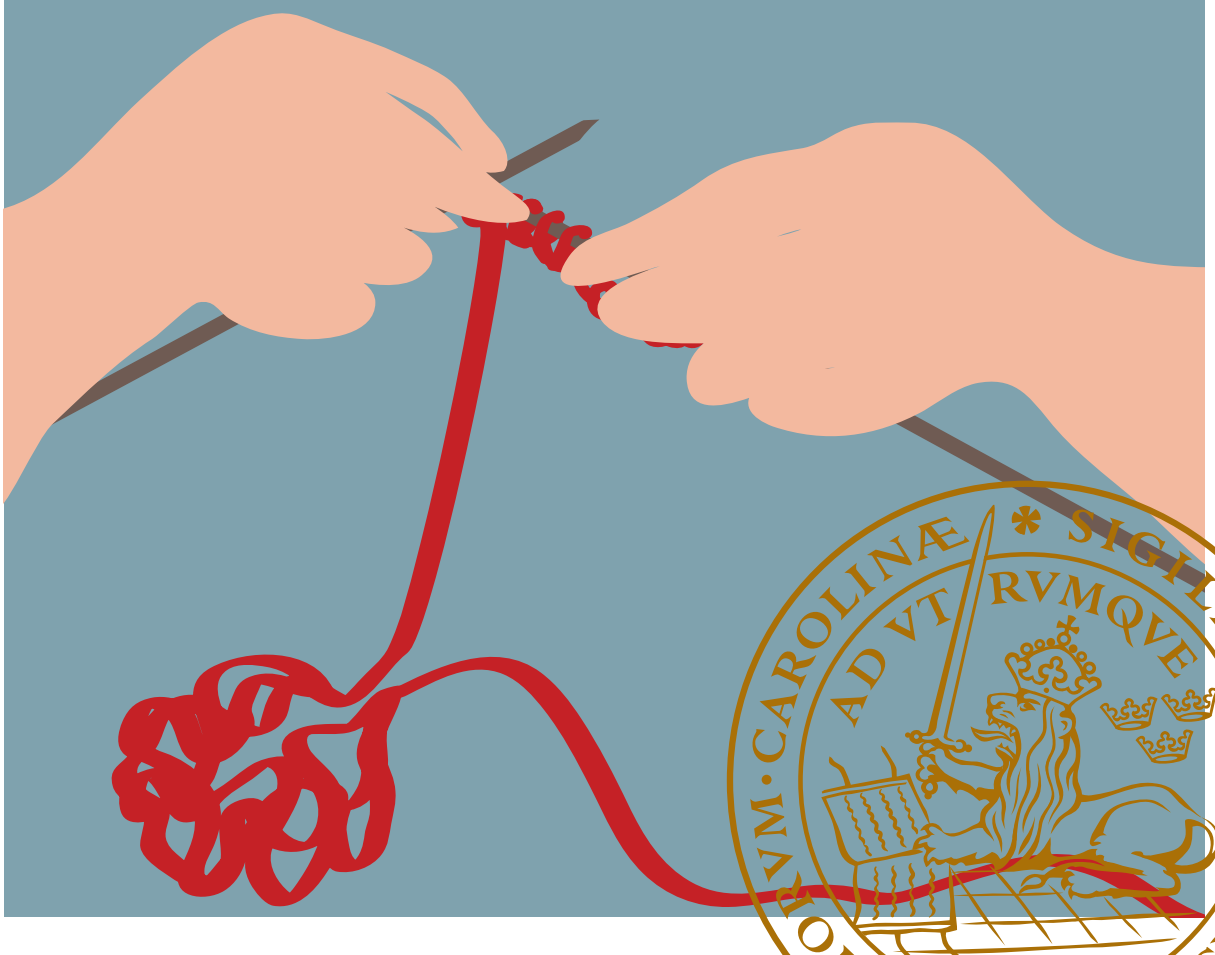

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Karin Werner

LUND
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Abstract

There are uncertanties about the role of old age in kidney function and the role of kidney function in older adults. The overall aim of this thesis is to expand the knowledge about eGFR and associated factors such as GFR markers, cardiovascular disease and mortality in older adults. Of particular interest is eGFR below 60 mL/min/1.73m2, the threshhold for the diagnosis of chronic kidney disease. The four papers are based on data from Good Aging in Skåne (GÅS) participants, a randomized sample from community-dwelling older adults in a southern region of Sweden.

Paper I (n=1252, mean age 69 years) is cross-sectional and looks at cystatin C levels and eGFR in older adults without overt vascular disease, known kidney disease, or diabetes. In a multiple linear regression analysis, male sex was associated with a more pronounced effect of age on cystatin C. In the participants 80 years and older 55% and 74%, using eGFR based on creatinine and cystatin C, respectively, had eGFR below 60 mL/min/1.73m2.

Paper II (n=126, mean age 82 years, mean eGFR 50 mL/min/1.73m2) compared eGFR based on creatinine, cystatin C, β2-microglobulin, and β-trace protein to mGFR based on single sample iohexol clearance. The accuracy (percentage of estimates ±30% of mGFR) was above 90% for all equations based on both cystatin C and creatinine. No equation was significantly more accurate than CKD-EPI based on creatinine and cystatin C.

Paper III (n=2815, mean age 73 years) is a cross-sectional study of the difference between GFR estimates based on creatinine and cystatin C. In 19% of the GÅS participants the difference exceeded 30%. A difference was associated with the known non-GFR determinants of smoking, age, body mass index (BMI), C-reactive protein (CRP), and glucocorticoid use.

Paper IV (n=2815, mean age 73 years, mean follow-up time 11 years) is a longitudinal study that looks at eGFR level and subsequent mortality, morbidity and change in eGFR. The mortality increased with lower eGFR, corresponding to the stages in chronic kidney disease. The mean decline in eGFR was 0.9mL/min/1.73m2 per year.

In summary, equations for eGFR based on both creatinine and cystatin C, but not equations with only creatinine, are sufficiently accurate for clinical use in older adults. Furthermore, it is common that eGFR based on cystatin C and eGFR based on creatinine differ substantially. Even in the absence of overt vascular disease or diabetes there is an age-related decline in eGFR in older adults. Over the age of eighty years a majority of individuals have an eGFR below the threshold for chronic kidney disease. An eGFR below this treshold is associated with a higher mortality hazard.

Key words: aging, older adults, kidney function, eGFR, GFR, cretatinine, cystatin C, longitudinal, mortality, CKD

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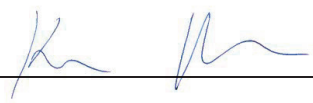
Signature  Date 2018-03-29

Estimated glomerular filtration rate in older adults: validation, correlations and implications

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Till Mathilde och Gustav

Kunskap (*Av Karin Boye, ur För trädets skull)*
Alla de försiktiga med långa håvar
träffar havets jätteskratt.
Vänner, vad söker ni på stranden?
kunskap kan aldrig fångas,
kan aldrig ägas.

Men om du rak som en droppe
faller i havet att upplösas,
färdig för all förvandling --
då skall du vakna med pärlemorhud
och gröna ögon
på ängar där havets hästar betar
och vara kunskap.

Knowledge *(By Karin Boye, translation by Jenny Nunn)*
All the mindful ones with their long nets
meet with a great laugh from the sea.
Friends, what are you seeking on the beach?
knowledge can never be caught,
can never be owned.

But if you fall like a drop
straight into the sea to be dissolved,
ready for any transformation -
then you shall wake with mother-of-pearl skin
and green eyes
on fields where the sea's horses graze
and be knowledge.

Table of contents

Original papers

This thesis is based on four papers subsequently referred to by their Roman numerals. The papers and their supplements can be found at the end of the thesis. Permission to publish the papers in this dissertation has been granted by the publishers when applicable.

I. Werner K, Elmståhl S, Christensson A, Pihlsgård M. Male sex and vascular risk factors affect cystatin C-derived renal function in older people without diabetes or overt vascular disease. Age and aging. 2014;43:411-417

II. Werner K, Pihlsgård M, Elmståhl S, Legrand H, Nyman U, Christensson A. Combining cystatin C and creatinine yields a reliable glomerular filtration rate estimation in older adults in contrast to beta-trace protein and beta2-microglobulin. Nephron. 2017;137**:** 29-37

III. Legrand H, Werner K, Christensson A, Pihlsgård M, Elmståhl S. Prevalence and determinants of differences in cystatin C and creatinine-based estimated glomerular filtration rate in community-dwelling older adults: A cross-sectional study. BMC Nephrol. 2017;18:350

IV. Werner Karin, Legrand Helen, Pihlsgård Mats, Sterner Gunnar, Christensson Anders, Elmståhl Sölve. Cystatin C and creatinine based eGFR levels and their correlation to morbidity and mortality in older adults. *Submitted manuscript*

Abbreviations

ACE	Angiotensin converting enzyme
B2M	β2-microglobulin
BTP	β-trace protein
CHF	Congestive heart failure
CKD	Chronic kidney disease
Cr	Creatinine
CT	Computed tomography
CVD	Cardiovascular disease
Cys	Cystatin C
eGFR	estimated glomerular filtration rate
$eGFR_{cr}$	eGFR based on creatinine
$eGFR_{cys}$	eGFR based on cystatin C
ESRD	End-stage renal disease
GFR	Glomerular filtration rate
GÅS	Good Aging in Skåne
IDMS	Isotope-dilution mass spectrometry
KDIGO	Kidney Disease: Improving Global Outcomes Work Group
MI	Myocardial infarction
N	Number
NSAID	Non-Steroidal anti-inflammatory drugs
P rate	Participation rate
RKFD	Rapid kidney function decline

Papers at a glance

Paper	I[1]	II[2]	III[3]	IV
Aim	To explore the variations in concentrations of cystatin C in relation to age, sex and vascular risk factors in healthy older adults.	To compare mGFR from iohexol clearance to both established and newer estimation equations for GFR in adults older than 70 years.	To describe the prevalence of differences between $eGFR_{cys}$ and $eGFR_{cr}$ in older adults and to explore which subsets of individuals may be most affected by differing estimations.	To explore the correlation of baseline eGFR with mortality, incident CVD and RKFD in older adults during a mean follow-up of 11 years.
Population	Healthy subgroup from GÅS. Mean (SD) age 69 (9) years	Sampled from GÅS baseline, V2, V4 during 2013-2015. Mean (range) age 82 years (72-98)	GÅS baseline participants with eGFR. Mean (SD) age 73 (11) years	GÅS baseline participants with eGFR. Mean (SD) age 73 (11) years
Design	Cross-sectional	Validation study	Cross-sectional	Longitudinal
Number	1252	126	2815	2815
Main statistical method	Multiple linear regression analysis	Bootstrapped CI for bias, precision and accuracy	Multiple linear regression analysis	Cox regression, logistic regression
Main result	Male sex is associated with a more pronounced age effect on cystatin C levels in healthy older adults.	All equations incorporating both cystatin C and creatinine are of adequate accuracy in older adults.	Nineteen % of the study population had a difference between $eGFR_{cys}$ and $eGFR_{cr}$ exceeding 30%.	CKD stage 3a was significantly associated with higher mortality than CKD stage 2.

Introduction

In modern society a disease is no longer automatically a state that implies suffering or even symptoms; early diagnosis can sometimes postpone or prevent overt signs of medical conditions. Aging on the other hand, can cause symptoms and suffering without obvious disease. To differentiate normal aging from aging with disease is challenging on the population level and individual level.

At the heart of the matter is that the aging process involves deterioration in functions that can mimic a disease process. Aging entails an ever increasing exposure time to all kinds of extrinsic and intrinsic damage to the organism.[4] It is regarded as an important risk factor for most disease groups including cardiovascular disease, diabetes, cancer and dementia.[5] Despite the essentially inevitable nature of aging there is debate on whether the condition itself should be called a disease.[6]

In his book "Time of our lives" Tom Kirkwood wrote:[7]

> "…grasping the correct distinction between normal aging and disease smacks of a semantic quibble, but words are powerful and the consequences of how we use them can be far-reaching".

With a majority of people living long enough to become older adults and experience aging in first person, our society and medical community must decide whether it makes sense and is beneficial to have the same disease criteria for all ages. For the diagnosis of chronic obstructive pulmonary disease, for example, the medical community employs two parallel systems, with one fixed and one age-dependent set of criteria.[8] However, when it comes to the kidney, the disease criteria for chronic kidney disease (CKD) are fixed and independent of age despite the difficulties in distinguishing the influence of aging from that of disease.

This thesis explores aspects of kidney function and kidney disease in community-dwelling older adults by focusing on the estimated glomerular filtration rate (eGFR) and its validation, its correlation with demographic and cardiovascular risk factors, as well as its long-term implications for morbidity and mortality.

Background

Kidney aging

The aging process affects kidney structure on the macroscopic and the microscopic level. Our understanding of the difference between the effects of "pure" aging and the effects of age-related disease comes largely from studies of living kidney donors where there is access to results from intraoperative kidney biopsies.[9] To be eligible for organ donation, potential donors have gone through rigorous health examinations, often with CT scans and laboratory testing. A downside to studying living donors in order to isolate aging effects is that organ donors typically are not older than 75 years.

Although our understanding of age-related changes is limited by the weaknesses of the existing research, it is now generally accepted that normal aging does affect the kidney.[10-12] The age-related microscopic changes (see Fig 1) involve the four histologic compartments of the kidney: glomeruli, tubules, interstitium and vessels, and can be summarized by the term nephrosclerosis. These compartments are intimately related and changes in one will affect the others. The biopsy from an aged kidney typically has fewer glomeruli than in younger persons, which has given rise to the idea that glomeruli can disappear, or at least shrink to microscopic invisibility, with aging.[13] In principle, ischemic lesions are thought to cause nephrosclerosis but there are alternative theories.[14] There has recently been focus on the podocytes, which are crucial cells for maintaining the function and integrity of the glomerulus. Observations of their age-related depletion and detachment have made podocytes into an alternative starting point for the chain of events in nephrosclerosis.[15]

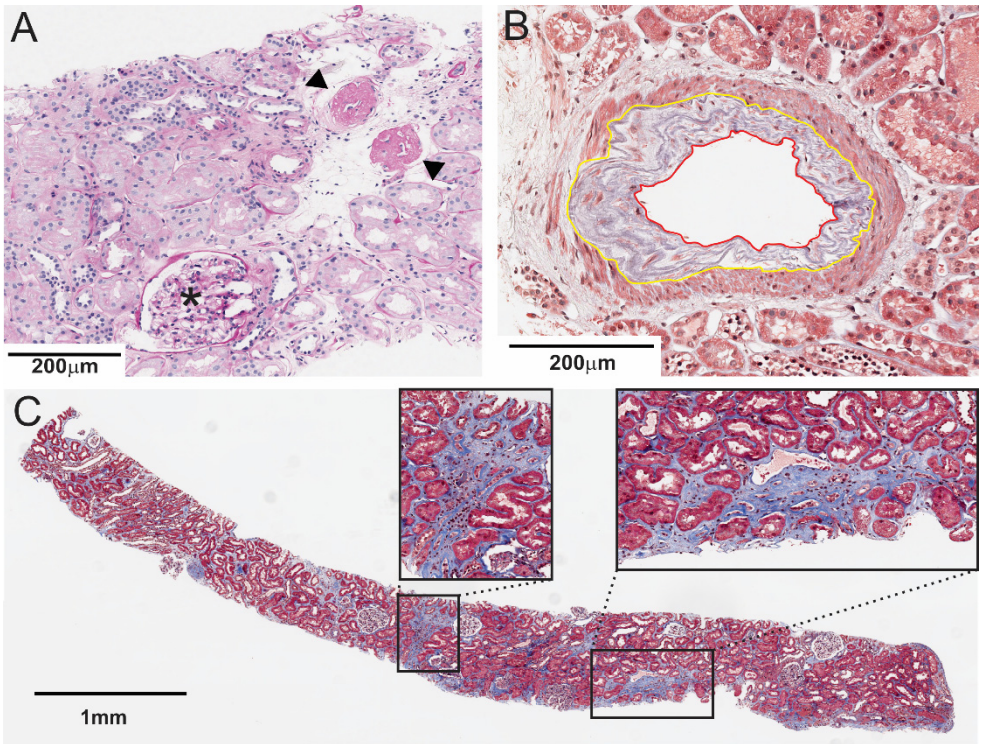


Fig 1 Microscopic changes in the aging kidney
Nephroscelrosis: A) Two globally sclerotic glomeruli (GSG) are labeled with black arrowheads. Non-sclerotic glomerulus (NSG) is labeled with a black star. GSG are surrounded by tubular atrophy. **B)** Thickened intima of a small to medium size artery (the area between red and yellow boundaries). **C)** Two foci of tubular atrophy and interstitial fibrosis are magnified. *Picture used with permission from the journal Advances in Chronic Kidney disease, first published as a supplement.*[9]

Zooming out to a macroscopic view (see Fig 2), the microscopic findings lead to smaller kidneys, which become apparent after the sixth decade.[16] Even before this, the ratio of cortex to medulla changes; the cortex size decreases and the medulla volume initially increases. This may be related to expansive fibrosis in the medulla and more aggressive glomerulosclerosis of the superficial glomeruli.[14, 17] The number and size of renal cysts increase with age[18] and there are more scars and surface roughness; vessels show signs of atherosclerosis.[14]

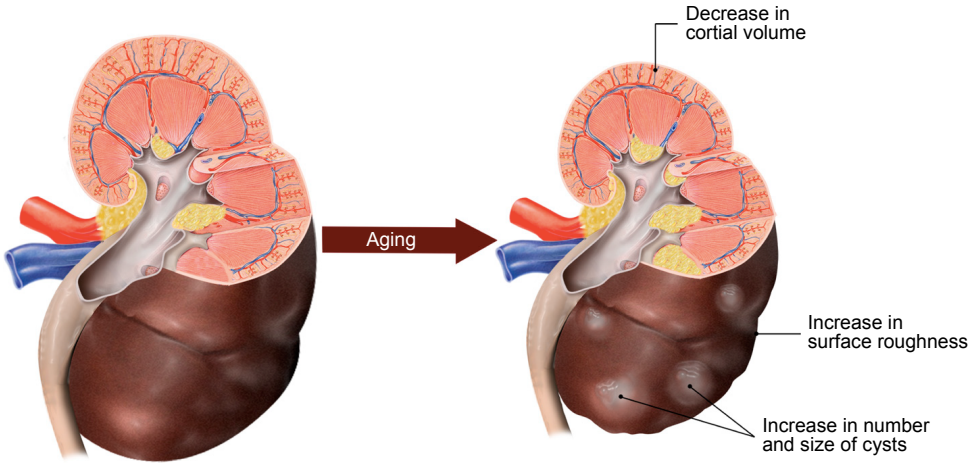


Figure 2 The effects of the aging process on the macroscopic structure of the kidney
Picture used with permission from The American Journal of Kidney Disease.[14]

These structural changes come hand in hand with functional changes.[19] The most obvious one is the age-related decline in glomerular filtration rate (GFR). The loss of functional nephrons estimated from kidney biopsies is thought to be just above 6000 nephrons per year;[13] the concordant decline in GFR is about 0.75 to 1 mL/min/1.73m^2 per year, which has been investigated through cross-sectional and longitudinal studies with different GFR estimating methods.[20-23] Though less well studied, other changes with aging seem to affect the urine concentration ability and the potassium secretion ability of the tubuli as well as some of the kidney´s endocrine functions.[9, 24-26]

Added to the effects of aging are the effects of age-related risk factors and disease such as hypertension and diabetes. These factors result in an accentuation of the described changes, but they can also bring about clearly pathological kidney damage that is not seen in normal aging, such as hyperfiltration giving rise to increased glomerular size and glomerular dysfunction resulting in abnormal albuminuria.[14]

Why GFR?

The kidney performs vital endocrine, excretory and metabolic functions in the body. These functions can in part be measured and evaluated in clinical practice. Due to the great reserve capacity of the organ, clinical and laboratory signs of disease in several of these domains may occur late in pathologic processes involving the kidney.

At the center of the kidney's excretory function is glomerular filtration. A reduction in the glomerular filtration rate can be measured or estimated over a wide range of

kidney function, from asymptomatic early stages of kidney disease to kidney failure. When other functions of the kidney deteriorate they often decline in synchronicity with a decline in GFR. These factors and the possibility of estimating the GFR with minimal invasiveness and at a low cost has put GFR and the estimation thereof at the center of diagnosis of chronic kidney disease.

mGFR

The GFR cannot be measured in human beings as it is the sum of the filtration of every glomerulus active at a moment in time. It is not constant, but changes with time of day,[27, 28] meal composition and diet,[29-31] and activity level.[32]

For simplicity we still use the term *measured* GFR (mGFR) to refer to methods that deduce a glomerular filtration rate through the clearance of an exogenous substance from plasma/urine. The principal method is essentially a continuous administration of the exogenous substance and measurement of its clearance in the urine. A variation on this concept involves administrating an exogenous substance and measuring its rate of disappearance from plasma by means of repeated blood samples. The clearance of the substance inulin is regarded as the gold standard. In clinical practice today however, the most widely used substances are iohexol[33, 34] and iothalamate[35], but EDTA[36] and DTPA[37] are other examples.[38]

Techniques have evolved to simplify the procedure even more by reducing the number of measurements from the conventional four to a single sample clearance where one injection of iohexol at baseline is followed by one plasma concentration of iohexol after an exact time interval.[39-41] The choice of time interval relies on previous estimation of the individual's GFR. The reason for an adaptable time interval is to capture an ideal moment in the theoretical slow phase of the substance elimination, which almost entirely depends on the elimination through the glomerulus, whereas the initial rapid phase also depends on body distribution. The initial iohexol concentration in plasma is calculated by the known amount of iohexol injected and the theoretical blood volume calculated by means of weight and height.

Relative GFR

The true GFR and the measured GFR are absolute values, normally with the unit mL/min. To relate the filtration rate to the body size of the subject, and thus facilitate comparison between individuals, it is often presented relative to a standardized body surface area with the unit mL/min/1.73m^2. The body surface area of an individual is calculated by an equation that has been in use for more than 100 years.[42, 43] The "standard" human could, for example, be a person with a height of 170 cm weighing

63 kg, resulting in a body surface area of 1.73m^2. It is important to recognize that when it comes to dosage of medication the relative GFR, deduced from mGFR or eGFR, can be misleading for patients with a body surface area that deviates from 1.73m^2.

eGFR equations

The equations in use for estimating GFR have three important components: First, they include the plasma or serum concentration of one or more markers of GFR. Second, they convey the exponential relationship between the marker and GFR, resulting in a numerically similar change in the concentration of the marker having greater impact at a high GFR level (low marker concentration) than at a lower GFR level (higher marker concentration). As an example for a 70-year old woman a change in creatinine concentration from 50 to 40 µmol/L changes eGFR from 87 to 98 mL/min/1.73m^2 whereas a numerically equal drop in creatinine from 250 to 240 µmol/L changes eGFR from 16 to 17 mL/min/1.73m^2. Last, the equations will commonly include variables representing non-GFR determinants specific for the marker used.

The non-GFR determinants are any physiologic or pathophysiologic factors other than GFR that affect the concentration of the GFR marker. What they are depends on the marker being used. Age and sex are often proxy markers for muscle mass. Age can also be a proxy for unknown non-GFR determinants that may be related to inflammation. The mathematical conversion of these factors into numbers has been deduced from big cohorts and often the makeup of the cohort will affect the applicability of the equation in a given population.

The equations are validated in a range of cohorts to demonstrate their external validity. The validation entails comparison of eGFR to a gold standard which can be any of the acknowledged mGFR methods. The comparison usually has three legs: accuracy, precision, and bias, to capture the truthfulness of the eGFR equation.[44] Commonly accuracy (%) is the proportion of participants that has an eGFR that lies within mGFR±30%, bias (mL/min/1.73m^2) is the median absolute difference between mGFR and eGFR, and precision (mL/min/1.73m^2) is the interquartile range of absolute differences.

The modern equations are always expressed as a relative rate with the unit mL/min/1.73m^2.

Table 1 Equations used to calculate eGFR in mL/min/1.73m^2

eGFR	Equation
creatinine (µmol/L)	
BIS1[45]	$3736\times(cr/88.4)^{-0.87}\times Age^{-0.95}\times 0.82$ [if female]
LM-REV[46]	$e^{X-0.0158xage+0.438xln(age)}$, If female and cr <150 X=2.50+0.0121×(150-cr), If female and cr ≥150 X=2.50-0.926×ln(cr/150), if male and cr<180 X=2.56+0.00968×(180-cr), If male and cr≥180 X=2.56-0.926×ln(cr/180)
CKD-EPI[47]	$141\times \min((cr/88.4)/k, 1)^{a}\times\max((cr/88.4)/k, 1)^{-1.209}\times 0.993^{Age}\times 1.018$ [if female]×1.159 [if black], k is 0.7 for females and 0.9 for males, a is -0.329 for females and -0.411 for males, min indicates the minimum of (cr/88.4)/k or 1, and max indicates the maximum of (cr/88.4)/k or 1.
MDRD[48]	$175\times(cr/88.4)^{-1.154}\times age^{-0.203}\times 0.742$ [if female]
FAS[49]	$107.3/(cr/Qcr)\times[0.988^{(Age-40)}$ when age>40 years] For age >20 years Qcr is 80 for men and 62 for women
cystatin C (mg/L)	
CKD-EPI[50]	$133\times\min(cys/0.8, 1)^{-0.499}\times\max(cys/0.8, 1)^{-1.328}\times 0.996^{Age}\times 0.932$ [if female] min indicates the minimum of cys/0.8 or 1, and max the maximum of cys/0.8 or 1.
CAPA[51]	$130\times cys^{-1.069}\times Age^{-0.117}-7$
FAS[52]	$107.3/(cys/Qcys)\times[0.988^{(Age-40)}$ when age>40 years], For age ≥70 years Q_{cys} is 0.95
cystatin C (mg/L) and creatinine (µmol/L)	
CKD-EPI[50]	$135\times\min((cr/88.4)/\kappa, 1)^{\alpha}\times\max((cr/88.4)/\kappa, 1)^{-0.601}\times\min(cys/0.8, 1)^{-0.375}\times\max(cys/0.8, 1)^{-0.711}\times 0.995^{Age}$ [×0.969 if female] [×1.08 if black], κ is 0.7 for females and 0.9 for males, α is -0.248 for females and -0.207 for males, min indicates the minimum of (cr/88.4)/κ or 1, and max indicates the maximum of (cr/88.4)/κ or 1.
BIS2[45]	$767\times cys^{-0.61}\times(cr/88.4)^{-0.40}\times age^{-0.57}\times 0.87$ [if female]
MEAN-LM-CAPA[53]	0.5×LM-REV+0.5×CAPA
FAS[52]	$107.3/(\alpha\times cr/Q_{cr}+(1-\alpha)\times cys/Q_{cys})\times[0.988^{(Age-40)}$ when age>40 years], for age ≥70 years Q_{cys} is 0.95, for age >20 years Q_{cr} is 80 for men and 62 for women, α can take a value between 0-1
eGFR other markers: β2-microglobulin (B2M) and β-trace protein (BTP) in mg/L[54]	
CKD-EPI$_{B2M}$	$133\times B2M^{-0.852}$
CKD-EPI$_{BTP}$	$55\times BTP^{-0.695}\times 0.998^{age}\times 0.899$ [if female]
CKD-EPI$_{B2M-BTP}$	$96\times BTP^{-0.278}\times B2M^{-0.588}$

Endogenous markers of GFR and their non-GFR determinants

To avoid the invasiveness and costliness of measuring GFR with exogenous markers, endogenous markers are used to estimate the GFR. An ideal marker has 1) constant production, 2) no protein binding, 3) elimination only through free glomerular filtration, and 4) should preferably be easy to measure at a low cost. No marker fulfills these requirements, meaning it is necessary to take into account non-GFR determinants as discussed in the previous section.

Creatinine is a 113 Dalton molecule that is produced almost completely in muscle from the precursor creatine at a regular rate.[55] The production rate is therefore closely related to the muscle mass which makes muscle mass the most important non-GFR determinant of creatinine. Intake of creatinine-containing foods such as red meat also contributes to the serum level. Creatinine is eliminated through free filtration in the glomeruli but especially at higher concentrations there is partial and variable elimination through the tubuli and the intestines.[56] Its concentration in plasma or serum has been used as marker of kidney disease for almost a century. It has also been used as an endogenous clearance measurement method when the plasma or serum concentration was measured in addition to the concentration in a 24-hour urine sample. This is rarely practiced today due to the method´s major sources of error.[55] Since the development of equations such as Cockroft-Gault[57] and MDRD[58, 59] 45 years ago, serum/plasma creatinine has been used to estimate GFR. Consequently, it is the most widespread marker of GFR and one of the most ordered analyses in clinical laboratories today.

There are two principle ways of analyzing the concentration of creatinine in plasma or serum in clinical use today: The Jaffe method and the enzymatic method. All calibrators of these assays should be traceable to the gold standard isotope dilution mass spectrometry (IDMS), which has been used to determine the creatinine concentration of the standardized reference material (SRM) 967.[60] Calibration addresses the problem of systematic bias which can compensate for the well-known overestimation of the Jaffe method, but not for the lack of precision in part caused by unpredictable analytic interference.[61, 62]

Another GFR marker is cystatin C which is a 13 kDalton protein that is produced in all nucleated cells in the body at a rather constant rate, freely filtered in the glomeruli, and reabsorbed and degraded in the tubuli.[63] The non-GFR determinants of cystatin C are less certain but there are indications that high doses of corticosteroids, hypo- and hyperthyroism, obesity, as well as inflammation may interfere with its production.[64-69] There is no gold standard method of analysis such as the IDMS for creatinine,[62] but a standardized reference material, ERM-DA471/IFCC, was introduced in 2010,[70] and there are ongoing studies for a future IDMS calibration.[71, 72] There are two types of analysis methods used in clinical practice today, the nephelometric and the turbidometric assays.[73] They are both based on the same principle of cystatin C antibodies attached to latex particles that create cloudiness depending on the concentration of the immune complexes. The turbidity of the sample can thus be evaluated by the diffusion of light (nephelometry) or the transmission of light (turbidimetry). None of these two methods have been proven superior to the other.

Cystatin C[74 73, 75] and, even more clearly so, creatinine[76, 77] in serum and plasma are regarded to be stable through cycles of freezing and thawing.

Newer proposed markers that have been less investigated as markers for the glomerular filtration rate are β2-microglobulin and β -trace protein.[54, 78, 79] They are also stable after a freeze-thaw cycle.[80]

Chronic kidney disease

The current definition of chronic kidney disease (CKD) stems from the 2012 guidelines of the KDIGO workgroup.[81] The group defined CKD as abnormality in the structure or function of the kidney lasting for 3 months or longer with implications for health irrespective of cause. This means that even in the absence of other signs of kidney damage or disease, a persistent reduction in the glomerular filtration rate is considered kidney disease. Chronic kidney disease is further categorized into stages (see Table 2).

Table 2 CKD stages 1-5

CKD stage	Relative mGFR or eGFR (mL/min/1.73m^2)	Description
1	>90	Normal renal function but other signs of kidney damage
2	60-89	Mild reduction in renal function with other signs of kidney damage
3a	45-59	Mildly to moderately reduced GFR
3b	30-44	Moderately to severely reduced GFR
4	15-29	Severely reduced GFR
5	<15	End-stage renal disease (ESRD)

The thresholds have been chosen on the basis of cardiovascular risk, mortality, risk of end-stage renal disease (ESRD), and associations with other impacted kidney functions.[82]

Increasing awareness of CKD and the use of eGFR equations most often based on creatinine have led to the discovery of a growing epidemic of CKD. The prevalence of CKD in in the general population in the western world is about 10%, and half of these have CKD stage 3 or worse. In older adults (70 years and older) the prevalence is more than doubled and almost all affected have CKD stage 3 or worse.[83, 84]

For older adults, particular focus has been put on the eGFR level 60 mL/min/1.73m^2, which is a level of kidney function where a GFR reduction suffices to make a diagnosis and at which the line between normal aging versus pathology can be hard to distinguish. The kidney changes that precede eGFR decline according to the CKD

conceptual model[82] may in some regards be the same[9] for normal aging and disease, but the mechanisms differ and the prognosis differs.

In this gray area of CKD, stage 3a in particular, a debate has arisen. On the one hand there are the well-accepted guidelines that are the basis of diagnosis and also solid arguments for non-age-specific criteria, most importantly the rise in absolute and relative risk for morbidity and mortality with CKD stage 3a.[12, 85] On the other hand, opponents argue that the limit for normal is set too high for older adults considering the expected age effect on kidney function, the impact of competing risks with aging, the more benign progression of CKD in older adults, and a higher risk of adverse drug reactions if all older adults with CKD underwent treatment, in addition to the potential stigma that comes with the disease label.[86-91]

To dissect the risk factors and complications that are of importance in the context of CKD in older adults is challenging since the kidney is in no way an isolated entity of the body. When the kidney is injured, this injury is likely to affect multiple organ systems, the chain of events even forming a vicious circle.[92] Epidemiological studies, especially those that are cross-sectional but even those that are longitudinal, may capture relevant correlations, but the direction of the association can be a chicken-and-egg dilemma. Risk factors for CKD such as dyslipidemia can simultaneously be complications of CKD.[93, 94] A risk factor may also be an independent cause of a CKD complication as well as causing CKD. Despite this complicated interplay, the next few items attempt to look at some of the implications and risk factors of CKD separately. Of note is that although individual variation is large, later stages of CKD, especially CKD stage 4 to 5, are often associated with clinically relevant deficiency in all functions of the kidney and cause symptoms such as anemia, acidosis and mineral bone disorder. These consequences of renal disease will not be in focus here.

Consequences of CKD

CKD in its earlier stages is often asymptomatic and the reason for labelling it a disease is mostly the increased risk of unwanted outcomes that CKD brings along. CKD in older adults differs greatly from CKD in younger adults when it comes to the disease's complications.[87] Furthermore, the etiology behind a case of CKD naturally influences the expected outcomes.

For younger people with CKD the risk of progression and even reaching ESRD is a highly relevant threat. The definition of ESRD varies and although often equated to GFR<15 mL/min/1.73m^2 it can also mean the stage where renal replacement therapy such as dialysis is warranted.[95] For older adults competing risks and perhaps the nature of their CKD makes the incidence rate for ESRD much lower than in younger

adults; the incidence rate per 1000 person-years being 10 for 75 year-olds compared to 47 for middle-aged adults at CKD stage 3a.[85, 96]

The greater vulnerability that CKD entails, which is emphasized in older adults due to aging effects, increases the susceptibility to acute kidney injury (AKI) when the kidney is exposed to a stressor related to surgery, sepsis, shock, or nephrotoxic agents.[97] Complete recovery can rarely be expected and consequently, progression of CKD usually follows.[97]

Similarly, cardiovascular disease and atherosclerosis are risk factors for, as well as important outcomes of CKD. This will be addressed more in depth in a subsequent section.

The risk of death, often resulting from the outcomes listed so far, increases for older adults with CKD compared to those without. The eGFR level at which the risk increases significantly varies across studies between 45 and 60 mL/min/1.73m^2.[85, 88, 98, 99]

Risk factors for CKD and its progression

The term risk factor has been in use at least since 1961 when used to describe factors of risk for coronary heart disease.[100] Its definition varies from it strictly being described as a *cause* fulfilling causation criteria[101] or more loosely as an *indicator* of risk for an event or disease, sometimes interchangeably used with the terms risk marker or biomarker.[102] Moreover, risk factors can be divided into non-modifiable risk factors such as age, sex and nephron endowment at birth, or modifiable risk factors such as smoking and weight. Their effect on the outcome can be direct or indirect. Of relevance in the area of CKD is that risk factors for the occurrence and progression of CKD are often but not always the same. To further complicate the picture, the interaction with age may change the scenario with risk factors' importance varying over the course of life.[103, 104] The importance of a risk factor in epidemiological studies depends in part on the strength of the association, for example the proportion of people with a certain characteristic that eventually develop the disease, and in part on the prevalence of the risk factor in the population of interest. What follows is not an exhaustive list of all risk factors and markers relevant to CKD, but a selection relevant to the present thesis.

Since chronic kidney disease can affect anyone with a condition causing damage to the kidney,[82] the causes of chronic kidney disease are diverse. All conditions that promote atherosclerosis are direct causes of chronic kidney disease with diabetes and hypertension being especially important ones in most countries.[105] The intricate relationship between CKD and CVD is discussed in the next section. There are, however, kidney-specific causes of CKD like glomerulonephritis and inherited

kidney diseases such as polycystic kidney disease. Moreover, systemic conditions such as amyloidosis, lupus or vasculitis can affect the vessels of the kidney. The kidney may also suffer permanent damage through obstructions of the urinary system downhill from the kidney, such as kidney stones, an enlarged prostate gland, or a tumor. Repeated urinary tract infections and exposure to nephrotoxic agents may eventually damage the kidney and cause CKD.

Acute kidney injury (AKI) is strongly associated with the development of chronic kidney disease,[106] although the causation is not certain.[107]

Old age is an important risk factor for CKD. It is open for debate whether aging causes chronic kidney disease or if aging is just a risk factor for other conditions that cause CKD. It is somewhat contradictory to say that normal aging is the cause of any disease but the structural and functional changes described earlier that come along with aging certainly increase the vulnerability of the kidney as one or more insults occurs.

Another non-modifiable risk factor for CKD is sex. The prevalence of CKD is higher in women.[84, 108] A lower nephron endowment in women at birth, sources of error in GFR estimation, and longer life expectancy meaning more years at risk for women may explain some of the higher prevalence in women.[108] However, female sex seems to entail some sort of protection as the rate of decline in eGFR and the progression of CKD is less steep in women compared to men.[108, 109] Proposed protective mechanisms are higher estrogen and lower androgen levels exerting a beneficial effect on the nitric oxide system, RAAS-activity, and proteases preventing matrix expansion.[11, 110-113]

An essential risk factor to consider is the starting point, i.e. the nephron endowment at birth. All human nephrons develop in utero until the last month of a normal pregnancy. After this, only change in single nephron filtration rate can affect the maximal GFR. With premature birth, unfavorable conditions during gestation, or certain genetic predispositions, the nephron number may be smaller than the average million per kidney. The number of nephrons varies 10-fold between 250 000 nephrons per kidney to 2 500 000.[114] Not only does a smaller nephron number at birth mean a smaller reserve capacity in the event of kidney disease, but it seems as if it also predisposes to hypertension.[115]

CKD and CVD: a vicious cycle

Dysfunction of the heart and kidney go together like a horse and carriage, a phenomenon referred to as the cardiorenal syndrome.[116] Chronic kidney disease increases the risk of cardiovascular disease and vice versa. The conditions have traditional cardiovascular risk factors in common: hypertension, diabetes,

dyslipidemia, obesity, and smoking. The most important pathway to the cardiorenal syndrome is thus probably atherosclerosis, but the whole story is not embedded in vascular pathology. The bidirectional interplay between the organs also work through other hemodynamic, endocrine, and metabolic mechanisms.[117-119] Exogenous substances given in good faith for heart failure may have a negative impact on the kidney. Although all cardiovascular disease is associated with chronic kidney disease, to the clinician, the relationship between heart failure and kidney failure is particularly challenging. A narrower definition of the cardiorenal syndrome involves only heart failure and late stage kidney failure.[120] The importance of proposed and acknowledged mechanisms for the initiation and aggravation of the cardiorenal syndrome depends on the stage of CKD, but the link between CKD and CVD only grows stronger with increases in the severity of either condition.

In terms of aging the role of BMI, cholesterol, and blood pressure may change over the lifespan[121] and their effects may even reverse them into markers of lower risk, as has been speculated in areas other than nephrology.[104]

The effect of aging and atherosclerosis on the kidney are to a large extent similar, but there are important differences. As nephrosclerosis progresses, the number of active nephrons is naturally reduced. If this reduction is not matched with a lower demand, the remaining nephrons will compensate and operate at a higher capacity. A rise in single nephron GFR occurs naturally in healthy individuals with physiological variations but a prolonged, even constant, activity at maximal production capacity is called hyperfiltration and will influence the nephron negatively and eventually accelerate the sclerosis.[122] This occurs in hypertension and obesity,[123] for example, but seemingly not in normal aging. The most documented cause of hyperfiltration, however, is diabetes where the hyperglycemia by itself induces greater glomerular filtration.

Diabetes is one of the most important causes of CKD and ESRD. The prevalence of CKD in diabetics is 30-40 %.[122] Diabetes is the direct cause of diabetic nephropathy but also an indirect cause of kidney disease through atherosclerosis with both pathways to CKD being captured in the concept of diabetic kidney disease.[124] Since hyperfiltration is not readily discovered through routine laboratory work, the first sign of diabetic kidney disease is often microalbuminuria.

Diabetes and hypertension are dominant causes of CKD and lead to vicious cycles where the dysfunctional kidney further contributes to worsening of hypertension[94] and poorer glycemic control.

Rapid kidney function decline (RKFD)

The concept of rapid kidney function decline (RKFD) can be used for clinical management,[81] as a risk factor or exposure variable in epidemiological research,[125, 126] and as an outcome in epidemiological research and clinical trials.[127] Perhaps the multiple purposes make a common definition difficult to agree on. The choice of eGFR equation, the population characteristics, the underlying cause of CKD and the follow-up time are also factors to take into consideration. Principally there are four types of definitions: 1) absolute decline per year, 2) relative decline per year, 3) total relative decline since baseline, and 4) a worsened CKD stage with a minimal relative decline.

Aims

The overall aim of this thesis is to expand the knowledge about eGFR and associated factors such as GFR markers, cardiovascular disease and mortality in older adults.

The specific aims are:

- To descriptively and statistically explore the levels of cystatin C in older adults without known diabetes or overt vascular disease in order to support or reject the notion of an age-related decline in kidney function. Moreover, we wish to look specifically at sex and vascular risk factors previously found to be related to kidney function. Lastly, we wish to describe the proportion of older adults that would be diagnosed as having CKD with the prevailing criteria. (Paper I)

- To compare mGFR from iohexol clearance to both established and newer estimation equations for GFR based on four markers: creatinine, cystatin C, β-trace protein, and β2-microglobulin. We seek to determine which equations are optimal for use over a range of CKD stages in adults older than 70 years. (Paper II)

- To determine the prevalence and size of differences in GFR estimation using creatinine based ($eGFR_{cr}$) or cystatin C based ($eGFR_{cys}$) equations in community-dwelling older adults. The main objective of the study is to explore correlations between previously described non-GFR determinants for cystatin C and creatinine and differences in eGFR based on these biomarkers. (Paper III)

- To explore the difference in mortality rate, incidence of acute CVD and risk of CHF and RKFD between groups of older individuals based on eGFR categories equivalent to CKD-stages 1, 2, 3a, and 3b. Furthermore, we wish to describe the trajectory of eGFR in older adults. (Paper IV)

Methods

Study population

The population study Good Aging in Skåne (GÅS) is one of four participating sites in the Swedish National Study on Aging and Care (SNAC).[128] The participants of GÅS were randomized from the National Municipality Register, the regional part of the Swedish Population Register, in five municipalities covering urban and rural areas. Men and women older than 60 years from nine age cohorts were invited by letter and those who did not respond were reminded by letter and telephone. There was a relative oversampling of the youngest and the oldest aiming for a total participation number of between 2950 and 3000 (700 for the ages 60 and 66 years, 250 for the ages 72, 78, 81, 84, and 87 years, 200 90-year-olds, and a 100 93-year-olds).[128, 129] For the purpose of this thesis with included papers all these age groups are referred to as *older adults*.

The study visits usually took place during one or two days at a study center or at home. The visit included several questionnaires on current and former life style, social circumstances, day-to-day functioning, diseases, and medications. A standardized examination was carried out. The examination included medical history and physical examination by a doctor; a cognitive test battery by a professional in behavioral sciences trained in cognitive testing; and functional tests, anthropometrics, laboratory tests, and spirometry by a registered nurse. Follow-up visits similar to the baseline visit were carried out every six years for participants younger than 78 years of age and every three years for older participants.

The recruitment of the first GÅS baseline cohort took place between 2001 and 2004 and has been followed up by return visits numbered 1, 2, 3, and 4. Two additional cohorts of 60-year-olds and 81-year-olds have been similarly recruited at later stages and are named Version 2 and Version 4 respectively.

Eligibility criteria for the GÅS study were: the participant being alive, reachable, able to communicate in Swedish, and not having moved out of the area within three months of invitation.

Paper I

A healthy subgroup with laboratory measurements at the first GÅS visit was included. The exclusion criteria were: missing laboratory measurements, diabetes, hypertension, myocardial infarction, signs of past myocardial infarction on EKG, congestive heart failure (CHF), angina pectoris, coronary bypass surgery, PTCA/PCI, cerebral infarction, TIA, aortic aneurysm, intermittent claudication, renal artery stenosis, ischemic kidney disease, glomerulonephritis, tubular disease, kidney malformations, and medical treatments that could be assumed to treat these conditions specifically. The diagnoses were gathered from medical examination, history and medical records. Medication was self-reported.

Paper II

The participants were recruited from the participants of GÅS independent of which of the three GÅS cohorts (GÅS baseline, Version 2, or Version 4) they originally belonged to. We selected a limited number of individuals to cover a wide range of GFR, ages, and both genders to achieve balanced data. We used the eGFR categories <30, 30–60, and >60 mL/min/1.73m^2 and the age categories 70–80, 80–90, and 90+years for both genders. The lower age limit was higher than in the GÅS study since we found that the knowledge gap in eGFR equation validation was primarily for ages above 70 years. The eGFR criteria corresponded to the CKD- stages.

Ideally, seven participants would be recruited for each combination of features for a total number of 126 individuals. There was, however, a lack of possible recruits in the oldest age group as well as in the category of eGFR <30 mL/min/1.73m^2. To compensate for this disparity, we oversampled participants over 87 years and those with eGFR between 30 and 45 mL/min/1.73m^2. The participants were contacted by a letter informing them of the study. They were thereafter contacted by phone to make an appointment with one of two research nurses. Participants who found it inconvenient to come to the research unit were offered a home visit. Recruitment was ongoing during the period of data collection and was stopped when 126 participants had been recruited. Data were collected between 2013 and 2015.

Paper III

All GÅS baseline participants with laboratory measurements at their first visit were included in the descriptive part. For the final analysis all participants with complete data were included.

Paper IV

All GÅS baseline participants were included in the basic descriptive part. In subsequent analysis those without laboratory measurements were excluded. For the endpoints acute CVD and CHF an additional exclusion criterion was previous CVD. For the endpoint RKFD and the descriptive part on change in eGFR only those with at least one follow-up laboratory measurement were included.

Laboratory Methods

Analyses of creatinine and cystatin C were carried out on defrosted plasma from the GÅS study biobank for samples collected up until 2013. From 2013 and onwards fresh plasma was used for analysis. Analysis was performed according to manufacturers' instructions at the clinical laboratory of Skåne University Hospital, Malmö, Sweden. The quality was ensured by internal quality control and participation in an external quality assessment program for clinical laboratory investigations provided by Equalis (www.equalis.se, Uppsala, Sweden).

The laboratory analyses of cystatin C were all carried out with particle-enhanced immunoturbidimetric assay. Creatinine was analyzed by a Jaffe method correlated to an IDMS method for the first visit. For the follow-up visits creatinine was analyzed by an enzymatic method calibrated to values assigned by IDMS and traceable to SRM 914a.

A list of reagents, instruments and plasma conditions used for each paper can be seen below and further details on the CV and calibrators can be found in the respective paper. Biobank plasma refers to plasma that has been stored in a biobank at -70˚C. For the measurement of iohexol, β2-microglobulin, and β-trace protein see Paper II.[2]

Table 3 Laboratory methods for creatinine and cystatin C relevant to the thesis.

Laboratory Method	Paper I	Paper II	Paper III	Paper IV
Cys: Biobank plasma analyzed in 2007, reagent Gentian on Beckman Synchron LX20	X		X	X (visit y 0)
Cys: Biobank plasma analyzed in 2014/2015, reagent (Gen 2) from Roche on Cobas 8000				X (visit y 3, 6, 9)
Cys: Fresh plasma analyzed in 2013/2014, reagent Roche gen 1 on Cobas 8000		X		
Cys: Fresh plasma analyzed in 2014/2015/2016/2017, reagent Roche gen 2 on Cobas 8000		X		X (visit y 12)
Cr: Biobank plasma analyzed in 2007, by Jaffe method, reagent CREm from Beckman on Beckman UniCel DxC800	X		X	X (visit y 0)
Cr: Biobank plasma in 2014, by enzymatic method, reagent Creatinine plus ver 2 from Roche on Cobas 8000				X (visit y 3, 6, 9)
Cr: Fresh plasma analyzed in 2014/2015/2016/2017, by enzymatic method, reagent Creatinine plus ver 2 from Roche on Cobas 8000		X		X (visit y 12)

X indicates that the laboratory method was used in the study, y indicates years after baseline examination.

Outcome definitions

The variables included in the four studies are described in detail in the papers. Of note is that certain variables included in more than one paper have been defined or categorized differently:

- Smoking was dichotomized as former/current or never-smoker in Paper I but in Paper III and IV there were three categories: current, former, and never-smoker.
- Hypertension was an exclusion criterion in Paper I and defined as a diagnosis of hypertension or use of antihypertensive drugs. In Paper III and IV, however, hypertension was used as a covariate and defined by a yes or no answer to the question "Is the patient currently under treatment for hypertension?"
- We have generally used the CKD-EPI equations in these papers. In Paper I, II and III we used eGFR based on creatinine and cystatin C separately since we wished to compare the two. For Paper IV we wanted eGFR to be as good

an estimate for the GFR as possible and therefore we chose to use eGFR based on both markers. In Paper I, for the multiple linear regression we chose to look at cystatin C concentration instead of eGFR in order to avoid using sex and age on both the exposure and outcome side of the equation.

Paper I

Factors expected to be associated with the plasma concentration of cystatin C due to previously described relationships with kidney functions measures were investigated.

Paper II

The accuracy (percentage of estimates ±30% of mGFR, P30), precision (interquartile range, IQR) and bias (median difference: eGFR-mGFR) of different eGFR equations based on one or more biomarkers (cystatin C, creatinine, β2-microglobulin, and β-trace protein) were investigated using single sample iohexol clearance as a reference method. In a secondary analysis the accuracy of all the eGFR equations were compared to the accuracy of CKD-EPI based on creatinine and cystatin C. CKD-EPI based on both markers was chosen as a benchmark equation since it was considered to be one of the most reliable in older adults from earlier studies.[130, 131]

Paper III

The relative difference between cystatin C based and creatinine based eGFR was calculated by ($eGFR_{cys}$–$eGFR_{cr}$)/mean eGFR. Previously described markers for non-GFR determinants of cystatin C and creatinine were included as the exposure variables. The size and direction of the relative difference between $eGFR_{cys}$ and $eGFR_{cr}$ in relation to the exposure variables was investigated.

Paper IV

The time to death or acute CVD as well as the risk of incident congestive heart failure (CHF) and RFKD for older adults with different levels of eGFR were investigated. The different levels of eGFR in the study were based on the CKD stages, rendering possible an evaluation of a possible risk increase below the current eGFR threshold for CKD. An eGFR between 60 and 90 mL/min/1.73m^2 was chosen

as a reference as this level can be considered a normal GFR level for the investigated age groups.

In contrast to the earlier papers this study relied on linking the data on the GÅS participants by means of the personal registration number to the National Patient Register, which records data on hospital stays, and the regional municipality register, which records death data. The National Patient Register automatically receives data from all hospitals in the 21 Swedish county councils monthly.[132] This data includes the patient's identity, geographical, administrative, and medical data; and most importantly the ICD diagnosis codes. The diagnoses from the register are regarded as reliable for all CVD but better for acute CVD.[133]

The county municipality register communicates with The Swedish Tax Agency that receives information from the mandatory death certificates issued by a doctor for every death.

Statistical methods

The statistical analysis in Paper III and IV and basic data management for all papers were conducted using SPSS (Version 22.0. Armonk, NY: IBM Corp.). In Paper I and II the main statistical analysis was done with statistical software SAS 9.2, Cary, NC.

No statistical comparison of the descriptive data was performed in any of the papers.[134] The exposure variables were selected on theoretical grounds and included if we had access to them and their measurement was considered to be sufficiently accurate. They were included simultaneously in the regression models of Paper I and II and cases with missing data points were excluded listwise. For Paper IV the variables included for each model were entered simultaneously (model 1 sex, age and eGFR level; model 2 all co-variates). A two-sided p-value of <0.05 was considered statistically significant.

For the continuous outcomes in Paper I and III multiple linear regression models were used. In Paper I interaction effects with age for predefined variables were included. To account for multiple testing, Bonferroni correction was conducted to achieve more reliable p-values.

A multiple logistic regression was used for the binary outcomes CHF and RKFD in Paper IV. To account for the time to each event and not only its occurrence, Cox regression with fixed covariates was also used in this paper to investigate the outcomes death and incident acute CVD. The resulting effect measure reflects the chance of the event occurring or not occurring at a certain time with all predictive variables being the same. The proportional hazard assumption means that the

proportional hazard for one subject compared to the others should not vary across time. Observations should be independent.

In contrast Paper II used no regression models. The performance of each investigated eGFR equation was compared to a pre-chosen benchmark equation. The performance measure used was accuracy, which in this paper denoted the percentage of GFR estimations by a certain equation occurring within 30% of the measured GFR. A simple approach would be to look at confidence intervals for the performance of each eGFR equation and see whether they overlap. This is intuitive but not entirely statistically correct and therefore a pairwise comparison of the accuracy of each equation to the benchmark was done. The difference in their accuracy levels was regarded as significant if its confidence interval did not include zero. To minimize multiple testing, the equations were only compared to the benchmark equation. All the confidence intervals in this paper were calculated through nonparametric bootstrapping which did not require us to make any assumptions regarding the shape of the data.

Results

Participation rate

A total of 2931 individuals agreed to participate at the baseline examination making up the GÅS baseline cohort which is the basis for Papers I, III, and IV. Using the predefined eligibility criteria, the participation rate was 60% (see Table 4 for details). An overview of the samples used in the respective papers can be seen in Fig 3.

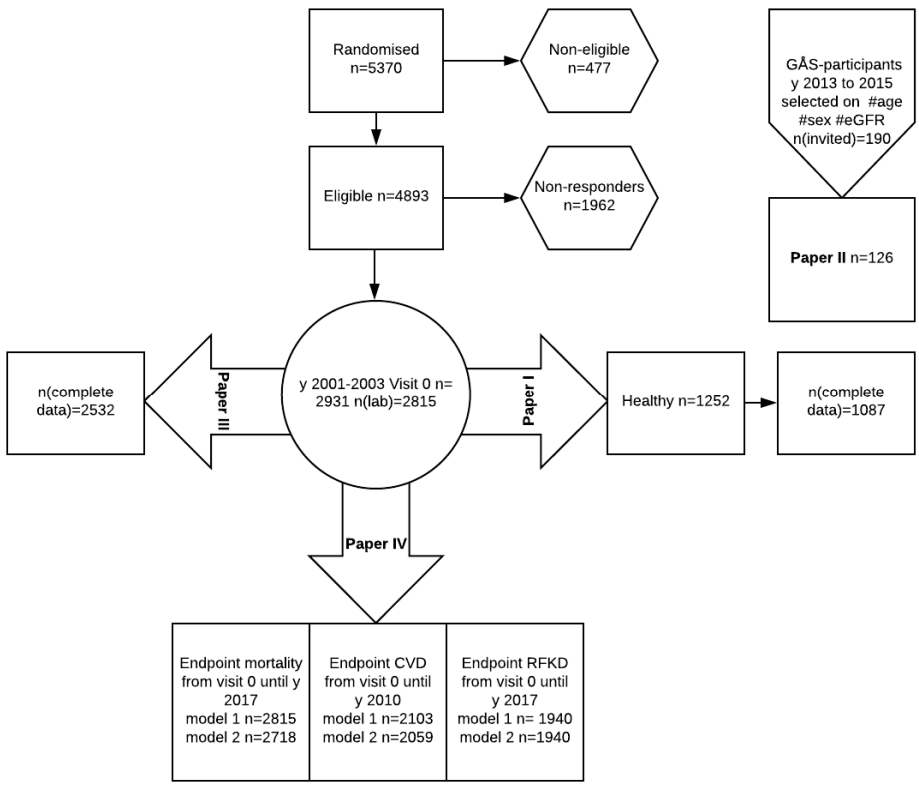


Fig 3 An overview of the number (n) of participants and the year (y) when data was collected in the four papers included in this thesis.
Visit 0 is the baseline visit for the GÅS baseline cohort and took place between 2001 and 2004.

Of all 2931 participants a majority were examined at the research center (n=2575, 88%).

Table 4 Participation rate at baseline visit (visit 0) for the GÅS baseline cohort

Age group at randomization	n randomized	n eligible	n of participants (p rate in %)	n of female participants (%)	n of participants with eGFR
All	5370	4893	2931 (60)	1636 (56)	2815
60-69	2234	2111	1383 (66)	689 (50)	1338
70-79	911	861	560 (65)	309 (55)	540
80-89	1779	1567	836 (53)	530 (63)	792
90+	446	354	152 (43)	108 (71)	145

P rate (participation rate) denotes percentage of eligible that participated at visit 0.

In Paper IV data from the follow-up visits are used as well as follow-up data on death and disease. For all participants, data on death is 100% complete. For incident acute CVD and CHF the data can be assumed to be 100% complete for all participants treated in a Swedish hospital for either of the two diagnoses. Data on change in eGFR was available for 1940 out of 2815 participants (69%) at the baseline visit.

Table 5 Participation rate at follow-up visits for the GÅS baseline cohort

Visit	n randomized	n eligible	n of participants (p rate in %)	n of participants with eGFR
Visit y 0	5370	4893	2931 (60)	2815
Visit y 3	993	924	680 (74)	630
Visit y 6	2275	2193	1832 (84)	1598
Visit y 9	603	576	393 (68)	316
Visit y 12	1562	1544	1196 (77)	992

P rate denotes percentage of eligible for each visit that participated.

In Paper II the selection of participants was based on age, sex and eGFR level and was not randomized. Each of the categories was filled when enough participants had been found through the two GÅS research centers. A total of 190 individuals were invited to participate and 126 (66%) accepted.

Figure 4 GÅS participant in his home

Paper I

There were 1252 of the GÅS participants that met the inclusion criteria and met the criteria for a healthier subgroup. For the multiple linear regression analysis an additional 165 participants were excluded due to incomplete information about variables.

In the linear regression model including vascular risk factors we found that:

- Age was associated with higher cystatin C values. Every year of older age was associated with a 0.015 mg/L higher cystatin C level ($p<0.001$). This is roughly equivalent to an eGFR loss of 1 mL/min/1.73m^2 per year for a 70-year-old Caucasian man.
- Male sex was associated with lower cystatin C values but with a more pronounced age effect on cystatin C values compared to female sex. Being a man compared to a woman, all other factors equal, was associated with an additional 0.004 mg/L increase in cystatin C level per year ($p=0.030$).
- A 1 mmol/L increase in HDL had a mean estimated decrease of 0.038 mg/L on cystatin C ($p=0.004$). Each 1 mmHg increase in diastolic blood pressure was associated with a 0.002 mg/L higher cystatin C ($p=0.038$). Smoking was associated with a 0.495 mg/L increase in cystatin value ($p=0.019$).

These results are displayed in Table 6.

Table 6 Association of sex, age, and, atherosclerotic risk factors in non-diabetic older adults without vascular disease (n=1087)

Covariate	Estimated effect on cystatin C (mg/L)	95% CI	p-value
Age (year)	0.015	0.011 to 0.020	>0.001*
Male	-0.245	-0.420 to -0.069	0.006
Current/former smoker	0.495	0.080 to 0.919	0.019
Diastolic blood pressure (mmHg)	0.002	0.000 to 0.003	0.038
HDL	-0.038	-0.063 to -0.012	0.004
Age×Male (year)	0.004	0.001 to 0.006	0.030*

The table displays a selection, based on statistical significance, of estimated effects by included covariates from a multiple linear regression model. The model includes age, sex, smoking, physical inactivity, lipid-lowering medication, BMI, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL, and interaction terms for age with sex, smoking, physical inactivity, smoking, smoking and physical inactivity as well as smoking and physical inactivity. × denotes interaction. *denotes a p-value adjusted by the Bonferroni method.

The prevalence of an eGFR equivalent to CKD was higher in older age groups both by cystatin C and creatinine based eGFR (see Fig 5).

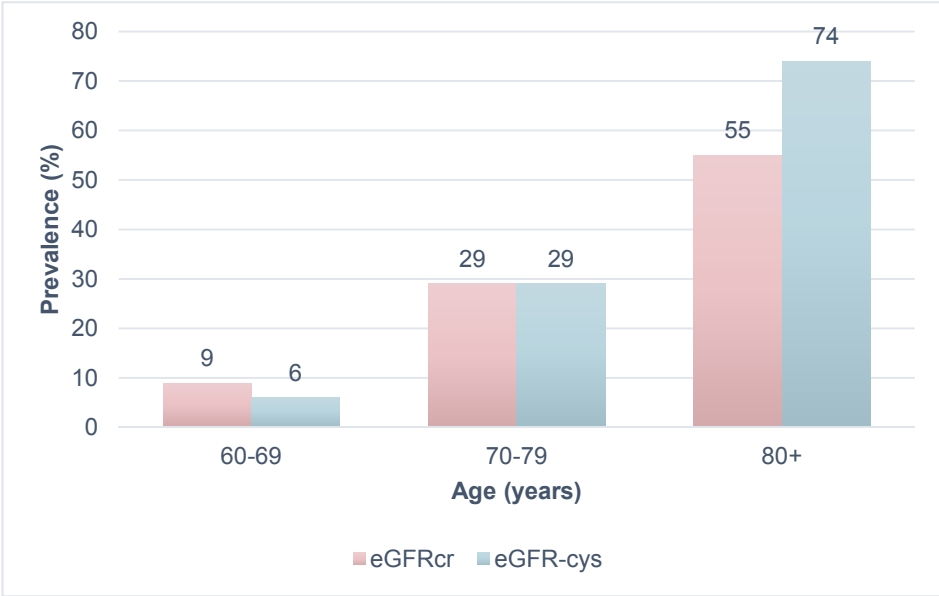


Figure 5 Prevalence of CKD stage ≥3 (eGFR<60) in non-diabetic older adults without vascular disease (n=1252)
A healthy subset of the GÅS baseline cohort with eGFR calculated by the CKD-EPI equations.[135]

Paper II

There were 126 participants with a mean age of 82 years (range 72-98 years) and 51% were men. The accuracy of the equations and the difference in accuracy compared to the benchmark are displayed in Table 7. Below is a summary of the point estimates for the items of comparison: bias, precision, and accuracy.

- Bias: All cystatin C based equations and the β-trace protein based equation underestimated mGFR while the β2-microglobulin based equations overestimated mGFR. For the creatinine based equations there was a mix of positive and negative biases.
- Precision: Equations based on cystatin C were more precise than those based on other markers.
- Accuracy: One creatinine based equation (LM-REV), two cystatin C based equations (CKD-EPI and CAPA), all equations based on both markers and none of the equations based on B2M or BTP reached an accuracy≥90%.
- Comparison to CKD-EPI$_{cr\text{-}cys}$: The benchmark equation outperformed all equations not based on cystatin C except LM-REV.

Table 7 Accuracy of equations for eGFR in the GÅS iohexol study (n=126)

Equation	P30	P30 >45	P30 <45	ΔP30
eGFR creatinine				
BIS1	88.9 [84.1 to 94.44]	97.6 [95.3 to 100.0]	70.5 [58.5 to 85.4]	***8.7 [4.0 to 13.4]***
LM-REV	93.7 [89.7 to 97.6]	97.6 [95.3 to 100.0]	85.4 [75.6 to 97.6]	4.0 [0.0 to 7.9]
CKD-EPI	84.1 [77.8 to 90.5]	90.6 [84.7 to 97.6]	70.7 [56.1 to 85.4]	***13.5 [7.1 to 19.0]***
MDRD	81.7 [75.4 to 88.1]	89.4 [83.5 to 96.5]	65.9 [51.2 to 80.5]	***15.9 [9.5 to 21.4]***
FAS	86.4 [80.8 to 92.0]	97.6 [92.9 to 100.0]	78.0 [65.9 to 90.2]	***6.3 [1.6 to 11.1]***
eGFR cystatin C				
CKD-EPI	92.1 [87.3 to 96.8]	90.6 [84.7 to 97.6]	95.1 [90.2 to 100.0]	5.6 [0.0 to 10.3]
CAPA	94.4 [90.5 to 98.4]	96.5 [92.9 to 100.0]	90.2 [82.9 to 100]	***8.7 [3.2 to 14.3]***
FAS	88.9 [83.3 to 94.4]	87.1 [80.0 to 94.2]	92.7 [85.4 to 100.0]	3.2 [-0.8 to 6.3]
eGFR cystatin C and creatinine				
CKD-EPI	97.6 [95.2 to 100.8]	100.0 [100.0 to 100.0]	92.7 [85.4 to 100.0]	1.6 [-2.4 to 5.6]
BIS2	96.0 [92.9 to 100.0]	96.5 [92.9 to 100.0]	95.1 [90.2 to 100.0]	0.8 [-2.4 to 4.0]
MEAN-LM-CAPA	96.8 [94.4 to 100.0]	100.0 [100.0 to 100.0]	90.2 [82.9 to 100.0]	0.8 [-0.8 to 1.6]
FAS	96.8 [94.4 to 100.0]	97.6 [95.3 to 100.0]	95.1 [90.2 to 100.0]	1.6 [-2.4 to 5.6]
eGFR other markers				
CKD-EPI$_{B2M}$	85.6 [80.0 to 91.2]	95.3 [91.2 to 100.0]	65.0 [50.0 to 80.0]	***12.7 [6.7 to 18.3]***
CKD-EPI$_{BTP}$	86.4 [80.8 to 92.0]	96.5 [92.9 to 100.0]	65.0 [50.0 to 80.0]	***11.9 [6.3 to 17.5]***
CKD-EPI $_{B2M-BTP}$	84.8 [79.2 to 90.4]	97.6 [95.3 to 100.0]	57.5 [42.5 to 72.5]	***13.5 [7.9 to 19.0]***

Accuracy P30: percentage of estimates ±30% of mGFR, Accuracy P30 >45: percentage of estimates ±30% of mGFR for individuals with mGFR≥ 45 mL/min/1.73m², Accuracy P30 <45: percentage of estimates ±30% of mGFR for individuals with mGFR< 45 mL/min/1.73m². ΔP30: P30(CKD-EPIcr-cys)-P30(eGFR). Bootstrapped 95% confidence interval within brackets. Italic bold highlights that CKD-EPIcr-cys has a statistically significant higher accuracy than the eGFR equation tested.

Paper III

There were 2815 participants in the descriptive part of the study and 2532 in the analytic part.

- Two thirds of participants had a difference between $eGFR_{cr}$ and $eGFR_{cys}$ exceeding 10%.
- One fifth of the participants had a difference between $eGFR_{cr}$ and $eGFR_{cys}$ exceeding 30%. Large differences between GFR estimates were especially common in the oldest age group (80+) and in those with eGFR<45 mL/min/1.73m^2.

- Smoking, age, body mass index (BMI), C-reactive protein (CRP), glucocorticoid use, and mean eGFR were significantly associated with a difference between $eGFR_{cr}$ and $eGFR_{cys}$.

Paper IV

Half of the participants died during the follow up period. The cumulative survival for groups based on eGFR level can be seen in Fig 5 with separate curves for participants younger and older than 80 years.

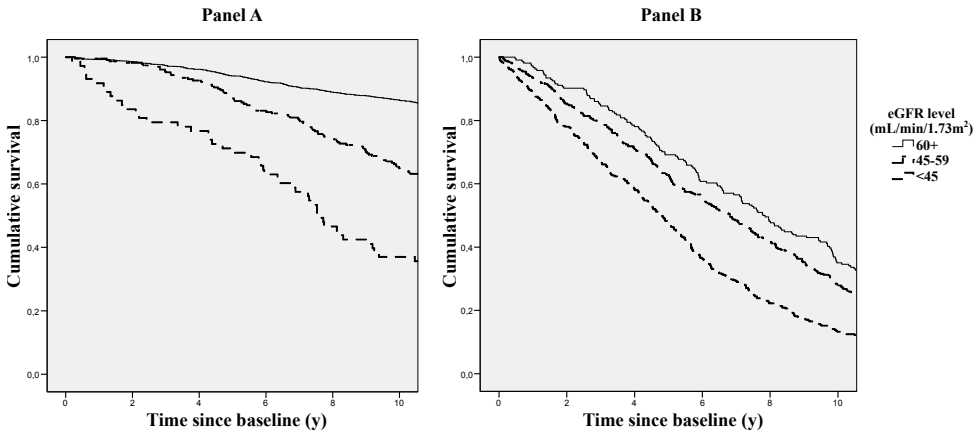


Fig 5 Cumulative survival associated with eGFR level for older adults younger than 80 and those 80 years or older in the Good Aging in Skåne baseline cohort.
Kaplan-Meier curves for participants younger than 80 years (n=1826) in **Panel A** and for participants 80 years and older (n= 989) in **Panel B**, eGFR was calculated by the CKD-EPI equation[135] based on cystatin C and creatinine with the unit mL/min/1.73m^2

There were 480 incident cases of acute CVD in total and 266 of these were in participants without prior diagnosis of MI, stroke, or CHF (n=2103). There were 404 participants diagnosed with CHF and of these 186 did not have a history of MI, stroke or CHF at the baseline examination. There were only 10 cases of ESRD diagnosed in the National Inpatient Registry from baseline until the end of 2010.

- Compared to the reference eGFR level 60-90 mL/min/1.73m^2 there was a 22% increased mortality hazard for those with eGFR between 45-60 mL/min/1.73m^2. Those with the lowest eGFR had an even more pronounced risk with a 58% increase compared to the reference.
- The level of eGFR was not significantly related to the risk of incident cardiovascular disease.

- The mean rate of change in eGFR was a decline of 0.9 mL/min/1.73m^2 per year. Relative to the baseline eGFR the average change was a yearly loss in eGFR by 1.4% over the course of the study period.

Table 8 Hazard ratios for the outcomes death and acute CVD in the GÅS baseline cohort

	Mortality model 1	Mortality model 2	CVD model 1	CVD model 2
eGFR (mL/min/1.73m^2)				
≥90	0.85 (0.63 to 1.13)	0.90 (0.67 to 1.21)	0.49 (0.26 to 0.93)	0.53 (0.28 to 1.00)
60-89	1 (ref)	1 (ref)	1 (ref)	1 (ref)
45-59	1.26 (1.09 to 1.46)	1.22 (1.05 to 1.41)	1.30 (0.93 to 1.80)	1.21 (0.87 to 1.69)
<45	1.79 (1.52 to 2.10)	1.58 (1.34 to 1.88)	1.54 (1.03 to 2.31	1.23 (0.81 to 1.87)
Age, per 1 year increase	1.11 (1.10 to 1.12)	1.12 (1.11 to 1.13)	1.06 (1.04 to 1.08)	1.07 (1.05 to 1.09)
Female	0.64 (0.57 to 0.71)	0.67 (0.59 to 0.76)	0.67 (0.52 to 0.86)	0.73 (0.56 to 0.95)

Results from Cox regression analyses. Outcomes were death (model 1 n=2815, model 2 n= 2718) and incident acute CVD (model 1 n=2103 model 2 n=2059) with model 1 (light blue) adjusted for sex and age and model 2 fully adjusted for smoking, diabetes, hypertension. History of CVD was an exclusion criteria for mortality models and adjusted for in CVD models.

Ethical considerations

All studies in this thesis had ethical approval from Lund University. Papers I, III, and IV were based on previously collected data and were included in a general ethical approval for the GÅS population study (LU 744-00) whereas Paper II required a new ethical approval (REPN 2013/1). The studies were conducted in compliance with the established ethical guidelines of the Declaration of Helsinki.[136] All participants provided informed consent.

For Paper II there was an obvious medical risk to the participants as there was an invasive procedure involved (injecting iohexol), which can result in an allergic reaction. The risk of such a serious consequence is very small but is it worth the risk when the medical benefit to the individual is negligible? We had not anticipated that there would be other benefits to the individual except finding out their exact GFR. During the data collection process several participants mentioned personal gains such as having a chat with a nurse and sometimes a doctor, making a contribution to society, and doing something other than the usual routine.

For the other papers the necessary ethical considerations may be of a more theoretical nature. Yet, behind every number in the database a person had given his or her time, energy, blood, and has agreed to have his or her personal information in the database of an institution. The data was already collected when this project started; it is unethical *not* to use the data as the costs have already been paid. The data should be used in the best possible way since a publication also hinders another researcher from using the data in a similar way.

For the GÅS population study there are large financial costs paid by society and there are other costs for the individual who participates. In fact, there may even be a cost to the person who declines participation. He or she might feel guilty for not contributing, especially since the invitation is made more personal when it is repeated by phone to those who don't actively decline. Some participants benefit from their day of participation; with attention, a hot meal, coffee and the relief of finding out their health status. However, there are also people who are confronted with a previously unknown medical condition or functional limitations, especially during the cognitive testing as testing cognition is not as common as having a medical examination. For some, the day of their visit to the research center could be they day they realized they were actually older than they felt, which can be a painful revelation.

Discussion

Methodological considerations

There are two important types of error in these research studies as in all epidemiological studies: the systematic error that results in bias of different types and the random error that results in imprecision. The effect of random error would decrease if we increased the size of the study.[137] The systematic error, or bias, has to be acknowledged and addressed during study design as well as data collection, analysis and interpretation. For any identified bias one needs to consider whether it will underestimate, exaggerate, or fully distort the result compared to the unknown truth.

Selection bias

There is potential selection bias on several levels at baseline data collection in the GÅS study:

1. The initial sampling was done by randomization from the Swedish Population Register.[138] The register has almost complete coverage but residents of Sweden who are undocumented, have no address, or are asylum seekers are not included.
2. The randomization was restricted to predefined age groups and geographic locations. There was oversampling in certain age groups.
3. In the group of randomized individuals, who were all invited by letter, there are eligible and non-eligible persons defined by the study-specific eligibility criteria.
4. The eligible can be divided into either non-participants or participants depending on their decision to participate in the study or not.
5. We have only used data from participants with complete data for the variables of interest for each analysis.

The most problematic selection bias probably occurs in the fourth step of the process. Cumulatively, the bias can be suspected to influence the study sample to

be healthier than the target population which is older adults in the general population of Skåne or Sweden. Non-participants are usually expected to be less healthy than participants and this effect might be more pronounced in studies of older adults.[139] This means that some covariates that we look at in Paper III and IV may be rarer than in the target population as well as the outcomes. If this influences the strength of associations, we expect it to be towards null. For Paper I we consciously selected a healthier subgroup of the sample and therefore the described selection bias is not of importance.

Between the study visits there is once again risk of selection bias. One is a systematic part of the study design as only the oldest participants (>78 year-olds) were invited every three years compared to the standard six years. However, there is also attrition between the visits, which is mostly due to mortality or choice to not continue. The effects of this are more difficult to predict,[140] but since the most common reason for attrition was death, it can be assumed that the participants who came to follow-ups were healthier. This is important in Paper IV where attrition complicates our interpretation of the rate of decline in kidney function and results in risk for an underestimation of the true rate of decline in eGFR.

The best way to counteract the influence of selection bias on the results is to ensure a correct randomization procedure from the population of interest, to augment the participation rate at baseline and most importantly encourage participants to stay in the study. The participation is facilitated if the location and logistics involved are convenient for the participant and if reminders are used for those who do not reply to the invitation.[141]

Since participation in studies luckily is not mandatory, a researcher has to consider the perceived costs and benefits for the participant that may affect participation. An obvious potential benefit to the individual, such as receiving a promising new treatment for a disease, might be more tempting than the participation in a population study without intervention.

In the GÅS study measures have been taken to enable as high a participation rate as possible and to counteract attrition at follow-up. For those who were recruited outside the municipality of Malmö there was a mobile research unit that changed location regularly between the smaller municipalities. For those unable to make it to a research unit, home visits were offered. Reminders were carried out through letters and phone calls. The study was made known to the public though meetings and exposure in the media. To motivate participants to continue in the study, events with lectures on GÅS research results have been organized. The team of a secretary, nurse, doctor and psychologist work to make the study visit pleasant and informative for the participant.

Information bias

Information bias concerns the measurement of exposure, outcome, and confounders where incorrect measurement will introduce a random error or a systematic bias.

For all papers in this thesis eGFR is key and also introduces different measurement errors. First of all, the laboratory measurements of the GFR markers introduce uncertainty that is likely to be nondifferential. Since the study stretches over many years there were both method changes at the laboratory and changes in sample handling, with the first analyses done from thawed plasma and the later ones from fresh plasma. Although both creatinine and cystatin C are regarded as stable in freeze-thaw cycles, there can be unforeseen effects with any sample manipulation. Since an error in measurement of a marker gives a bigger effect on eGFR at lower plasma concentrations (i.e. higher GFR), eGFR becomes more imprecise in the normal levels, where luckily exactness is of lesser importance. There is no evidence, however, that this would introduce a systematic bias; erroneous laboratory analyses would risk weakening associations but should not distort them.

Second, the eGFR equations have been developed to compensate for the influence of non-GFR determinants, which may be different in other populations than those used to develop each equation. Since the most common equations were validated in Paper II we know that, if not perfect, the CKD-EPI equations (and others) are functional for our intentions. Using eGFR in multivariable regression analyses also introduces issues that are difficult to predict since age and sex will appear as part of the equations and as covariates of their own. This was the reason why the outcome in Paper I was cystatin C concentration and not eGFR.

If a single eGFR measurement is used for the diagnosis of CKD there is a risk of misclassification bias with an overestimation of the true prevalence, since a repeated measurement might not have crossed the threshold for disease. We only had access to one eGFR measurement per visit and therefore we discuss the eGFR equivalent to each CKD stage to emphasize that we cannot diagnose CKD based on our data. Without a urine sample or other measures of kidney damage we are unable to evaluate CKD stage 1 and 2. Indeed, most categorical variables such as age categories or CKD stages risk introducing misclassification bias since information is removed in the categorizing process. This price can be worth paying for a more straightforward interpretation of the results.

Just like this thesis discusses the relevance of the criteria for chronic kidney disease, other conditions, only briefly referred to here, entail similar problems. Defining hypertension might be quite straightforward in middle-aged adults but in older adults the same numerical blood pressure might have a very different meaning. This is one of the reasons for our choice not to choose a cutoff such as 140/90 mmHg as

the definition for hypertension in Paper I, III, IV; the other reason being the risk of an inaccurate blood pressure due to white-coat hypertension.

Finally, all sources of information should be scrutinized. Self-reported data, in contrast to what we retrieved from measurements, is prone to errors especially if not well validated.[142, 143] The use of the variable physical inactivity in Paper I is an example, and it is questionable whether it mirrors the physical inactivity that we wished to investigate. Physical inactivity was non-significantly associated with lower cystatin C levels (corresponding to higher GFR), see Table 3 Paper I.

Confounding

A confounder is a factor that is associated with the exposure as well as the outcome and confounds the association between the exposure and outcome. It should not be on the causal pathway between the exposure and the outcome. Especially in Paper IV this is a theoretical problem since atherosclerosis is biologically tightly intertwined with the exposure eGFR level, the outcome mortality, and several of the identified confounders. Moreover, the relationships can be bidirectional.

The first step in handling confounding is to identify confounding factors through biological knowledge and previous studies and then measure them in a validated way if possible. When the data is already collected as in Paper I, II, and IV the measurement cannot be changed but the way of handling the data can be.

- Restriction: To control for the confounding of cardiovascular disease, hypertension, and diabetes in Paper I we excluded all individuals with these conditions.
- Stratification: In Paper IV we used stratification for age and sex for the Kaplan-Meier analysis. The ideal would be to stratify in even smaller age groups to decrease the heterogeneity within the groups, but this would result in samples that are too small.
- Multivariable regression models: These make it possible to handle several confounders, categorical or not, in the analyses. Different regression models were the basis for analysis in Papers I, III, and IV.

There is possible residual confounding caused by unknown factors. The residual confounding might also be caused by a known confounder that has not been measured or has been measured inadequately. Due to the omnipresent problem of confounding, multiple studies are needed.

Interpretation bias

Once data is collected and analyzed, the same results can be looked at through different lenses: interpretation is in the eye of the beholder. A biased interpretation can be introduced from the researcher's own previous knowledge or beliefs, financial or other affiliations, in the literature search where negative results may not have been published (known as publication bias[144]), and in the attempt to make the results understandable, coherent, and publishable. To counteract this and other biases previously discussed, systematic ways to carry out epidemiological research, such as those outlined in the STROBE guidelines, are recommended.[134] These guidelines were used for Paper III and IV.

Interpretation of results

Any of the associations we have found can either be biologically true, biased because of methodological faults, or just be arrived at by chance. If indeed the associations exist in the general older population they can either be due to direct causation, although the direction can only be guessed, or they can be indirectly associated through an unknown confounder. Population studies like the present one used in Papers I, III, and IV can rarely sufficiently demonstrate causation. They can however serve as efficient ways to generate hypotheses for further research that may reveal mechanisms behind the associations, or directly test the hypotheses through randomized controlled trials.

Validity

The internal validity depends on the quality of data and data analysis but also on the representativeness of the sample for the population of interest. Therefore, the representativeness is linked to the selection bias addressed previously. Forty percent of the eligible persons at baseline chose not to participate and all we can analyze is their age, sex and municipality. For the oldest participants more than half of those eligible at baseline declined participation, which is truly problematic for the representativeness of this subsample. The sampling of participants for the validation study in Paper II differs from the other studies in this thesis. They were sampled from the GÅS population but the sampling was not randomized. In order to validate the equation in both sexes, over a prespecified age range and across higher and lower eGFR levels we consciously oversampled in several ways, and the sample is not representative for the GÅS population nor for Swedish older adults regarding age distribution. The sample is, however, retrieved from community-dwelling older

adults and not an in-patient population, which is perhaps more relevant for the validation of eGFR equations. Furthermore, the population in all studies was retrieved from both urban and rural areas thereby increasing generalization to older adults.

The external validity depends on the internal validity and whether the results from the study population are generalizable to other populations. This can concern geographical populations but also age, sex and disease status. For example, pathophysiological relationships between the exposure and the outcome will often be common to humans all over the world; in the words of Dr. J Mark Elwood:

> "…biological relationships are generalizable although the individuals studied are not".[145]

Yet, the distribution of risk factors will be unique in every population. Furthermore, as noted previously, the effect of a risk factor might change as we age, even its direction.

Bearing this in mind and thus interpreting the results with caution, we consider our main results generalizable for community-dwelling older adults in high income countries, and in line with well-described biological mechanisms and previous epidemiological studies.

Paper I

The age effect on cystatin C in healthy older adults almost exactly replicated that found in a large cross-sectional study based on pooled data on 2846 individuals from four studies.[146] It is not possible to directly compare this result to the well-known Baltimore Longitudinal Study of Aging. They used repeated creatinine clearance to estimate GFR and found that one third of the healthy subjects had no decrease in clearance.[20] This could not be investigated in the cross-sectional data of Paper I.

The finding of a stronger age effect on the cystatin C level for men compared to women confirmed previously described biological mechanisms[11, 110-113] and resonates with the more rapid eGFR decline observed in men with CKD.[108]

Paper II

The study confirmed previous findings of the performance of eGFR equations in older adults.[130] This first study on older adults also showed that current equations with β-trace protein and β2-microglobulin are not good alternatives at this point.

The lack of a gold standard in the laboratory methods of these markers, in addition to the unknown influence of non-GFR determinants could contribute to this finding.

Paper III

The concern about the influence of non-GFR determinants on the accuracy of eGFR has been a hot topic in the clinical setting and in epidemiological studies. Paper III confirms that the worry is relevant and that several previously described non-GFR determinants are implicated in differences between GFR estimations.

Paper IV

The increased mortality hazard found in paper IV in older adults with eGFR equivalent to CKD 3a was similar to that found in a previous large meta-analysis.[85] Surprisingly, risk of CVD was non-significantly and only modestly elevated. CVD can however be suspected to be an important contributor to the observed increased mortality.

Despite the large loss to follow-up, the rate of decline confirmed the rate found in most earlier studies.[20-22] However, it is possible that the low incidence of RKFD is an underestimation.

Clinical implications

In paper II the results were somewhat relieving since they demonstrate that the accuracy of some of the eGFR equations is as good in older adults as in younger adults. The results do, however, call into question the use of creatinine-only equations, especially MDRD that is still widely in use. This could be a cause for concern regarding the interpretation of older studies that have used MDRD as the measure of kidney function. However, we cannot know for certain that this knowledge can be directly translated to the clinical setting. The clinician has more knowledge about the specific patient and can therefore consider the weaknesses and strengths of the marker(s) and other factors included in each equation. Facing a patient with an extremely low or high muscle mass for his/her age, it might be wise to use an equation without creatinine. In general, it is of interest to see whether the results by two different markers are similar, which will not be the case in at least 20% of older patients, according to the results in paper III. If the measure of eGFR do differ, this should be considered, and if the reason is not clear a GFR with an exogenous substance be considered.

We have also seen that a majority of 80-year olds without known elevated cardiovascular or renal risk have an eGFR equivalent to kidney disease. This emphasizes that we should be concerned about renal function when treating all older adults. On the other hand, such a high prevalence of low eGFR also calls into question the relevance of the current threshold for kidney disease.

The increased mortality seen in paper IV with eGFR below the limit for CKD could provide further evidence for the current disease label and enable more active secondary prevention strategies. On the other hand, in case of a lower eGFR in older adults without other signs of disease, the low eGFR could instead be viewed as a risk factor or an indicator of biological age. A high biological age is practically synonymous with the term frailty,[147] which is a condition that is increasingly frequent in epidemiological studies and is being introduced in clinical practice.[148] The difference between biological age compared to chronological age has been defined by John Grimly Evans:

> "Chronological age is measured by the simple passage of time- the number of candles on a birthday cake. Biological age tries to express how far we have travelled along the road from birth to death of old age"

The reason for the heterogeneous aging process behind the discrepancy between biological and chronological age is not solely disease. For the kidney, sex and a reduction in the original endowment of glomeruli due to a variety of genetic or epigenetic factors[149] can be part of the explanation.

On a final note, health is sometimes looked upon as the absence of disease, but is the absence of health always disease? The first principle in the constitution of the World Health Organization (WHO) from 1946 states that health is:[150]

> "…a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity"

Perhaps it is time to look closer at the gray area between health and disease where some of the signs of aging might fall. To diagnose a disease in an older adult we should perhaps use normality as the reference and not health.

Future research

The eGFR equations that have been published in the last decade have vastly improved the estimation of eGFR in general and in older adults in particular. Is there a need for further improvements in GFR estimation, and are improvements possible? Creatinine and cystatin C are not ideal markers and a new marker with no

or easily accounted for non-GFR determinants is of interest. Regarding the markers in use today, a gold standard laboratory analysis similar to the one for creatinine is lacking for cystatin C. To improve our use of these markers more research on the behavior of creatinine and cystatin C on the glomerular level is needed. Theories on the shrunken pore syndrome,[151] referring to the pores of the glomerulus and thus affecting the clearance of molecules of different sizes, could perhaps be relevant to the aging kidney.

Looking at the bigger picture, the kidney is not an isolated entity and the aging process affects the whole being and every being. Bernard Strehler described normal aging as cumulative, universal, progressive, intrinsic and deleterious several decades ago.[152] His theory has not been contradicted but there are many aspects of the aging process that are yet to be understood: Is aging uniform to the whole body and if not, what factors can explain this? How does the aging process progress: linearly, accelerating, stepwise? Are there individuals with an exceptionally slow biological aging process and what would be the explanatory protective mechanisms?

Conclusion

This thesis is based on the analysis of data collected in the general population study of older adults known as "Good Aging in Skåne" and resulted in the following conclusions:

- In older adults without overt vascular disease or diabetes, eGFR level declines with older age, supporting the notion of a direct effect of age on kidney function. Half of the adults aged 80 and above have eGFR levels corresponding to those of chronic kidney disease.
- In older adults without overt vascular disease or diabetes male sex is associated with a more pronounced age-related decline in kidney function based on cystatin C level.
- Existing equations for GFR estimations, based on both cystatin C and creatinine, are good enough to use in older adults. However, it is of concern that one-fifth of older adults can be assumed to have a difference between GFR estimated by creatinine and cystatin C exceeding 30%.
- A difference observed between creatinine and cystatin C based eGFR can in part be explained by smoking, age, BMI, CRP, and glucocorticoid use.
- There is no improvement in estimation of GFR in older adults with the newer GFR markers β2-microglobulin and β-trace protein.
- Mortality in older adults increases at eGFR levels below the threshold for chronic kidney disease, and the mortality increases further at eGFR levels corresponding to increasing severity of CKD.
- The mean rate of change in eGFR was a decline of 0.9 mL/min/1.73m^2 per year, with 9% declining at a rate exceeding 3 mL/min/1.73m^2 per year.

Sammanfattning på svenska

Bakgrund och målsättning

Åldrande är ett fenomen som alla människor kommer i kontakt med, för de flesta både som anhörig och huvudperson. Åldrandet innebär successiv förändring i struktur och funktion av kroppens organ. Hög ålder är en riskfaktor för folksjukdomar såsom hjärt-kärlsjukdom, diabetes, demens och cancer. Att skilja ut konsekvenser av rent åldrande och konsekvenser av åldersrelaterade sjukdomar är ofta svårt. Detta dilemma är högst centralt när det gäller njurfunktion och kronisk njursvikt.

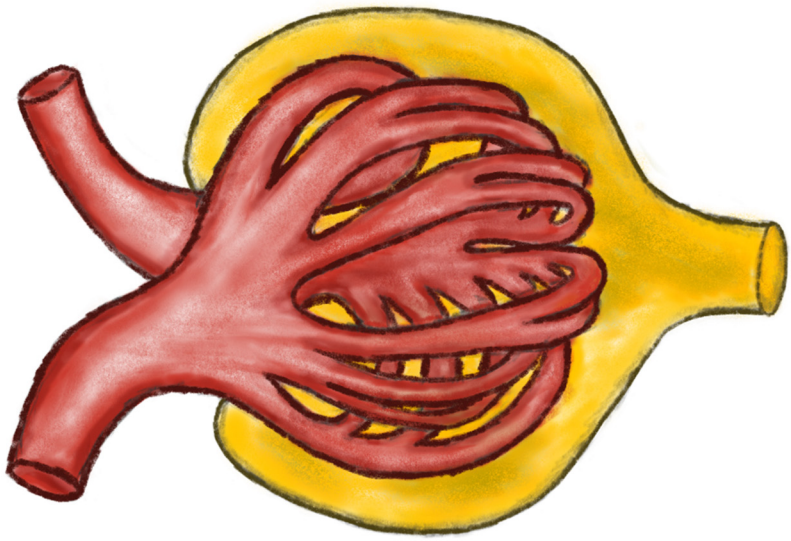
Njurarna är våra reningsverk som genom att skapa urin ger kroppen förutsättning att befinna sig i det tillstånd av balans som kallas homeostas. Njuren har ett intrikat samspel med kroppens alla organ och påverkar vätskebalans, saltbalans, pH, blodbildning, skelett, och blodtryck. Dess betydelse avspeglas i att vi är utrustade med två njurar. Reservkapaciteten är så stor att vi kan ge bort en och klara oss utmärkt ändå. Åldrande och åldrandets sjukdomar knaprar dock på reserven i det tysta.

Med boken du håller i handen beskriver jag hur vi genom fyra studier har försökt kartlägga samspelet mellan åldrande och njurfunktion. Vår utgångspunkt var följande frågor:

1. Påverkar normalt åldrande njurfunktionen?
2. Är våra mått på njurfunktion korrekta hos äldre?
3. Hur stor blir skillnaden i uppskattad njurfunktion beroende på vilken markör för njurfunktion vi mäter i blodet hos äldre?
4. Vad har njurfunktionen för konsekvenser hos äldre vad gäller död, hjärtkärl-sjukdom och fortsatt njurpåverkan?

Studierna bygger på en av njurens huvudsakliga funktioner som är filtrering av blod till primär-urin som sedan bearbetas och koncentreras till den urin vi kissar ut. Njuren besitter en miljon små silar vilka kallas glomeruli, nystan på latin. Nystanets garn är tunna blodkärl försedda med hål som vätska och små ämnen såsom salter kan passera igenom. Om njuren påverkas av åldrande eller sjukdom

kan de små silarna sluta fungera och omvandlas till ärr. Den totala silnings-kapaciteten minskar då från den ursprungliga dryga decilitern per minut.



Glomerulus
Glomerulus är benämningen på det lilla kärlnystan av kapillärer som genom små öppningar filtrerar blodet till primärurin. Primärurinen samlas i Bowmans kapsel (gul på bilden) och kommer sedan bearbetas och koncentreras till den urin som sedermera kissas ut. I varje njure finns ca en miljon glomeruli. Tecknad av Carl Loodberg.

Silningsfunktionen kan mätas genom att man sprutar in en substans i blodbanan och sedan ser med vilken hastighet den dyker upp i urinen. För att förenkla detta omständliga och kostsamma sätt att mäta har man identifierat kroppsegna ämnen som substitut. Dessa kroppsegna markörer bör bete sig på liknande sätt som tillförda markörer. De ska helst silas ut lika lätt som vattenmolekyler i glomerulus och sedan ska de inte påverkas under färden nedströms i njuren. Genom att mäta koncentrationen av sådana ämnen kan man genom en matematisk formel beräkna den glomerulära filtrationshastigheten (=silningshastigheten) så kallat eGFR (e för estimated=uppskattat).

Det är GFR som i stor utsträckning ligger till grund för de kriterier som finns för diagnosen njursjukdom i idag. Kriterierna är de samma för alla åldrar vilket betyder att man inte tar hänsyn till den förväntade påverkan normalt åldrande har på njurarna.

GFR är inte bara är ett mått relevant för njursjukdom utan också för dosering av många vanliga läkemedel och dessutom en indikator på risk vid både akut och kronisk sjukdom. Kroppsegna GFR-markörer, oftast kreatinin eller cystatin C, tillhör därför de absolut vanligaste blodproven i vården idag.

Tillvägagångsätt

För att besvara våra frågor använde vi oss av data från en befolkningsstudie som heter Gott Åldrande i Skåne där personer mellan 60 och 100 år lottas från folkbokföringsregistret för att erbjudas deltagande. Lottningen ökar chansen att studiedeltagarna är en representativ grupp som kan ge oss ledtrådar om hur äldre generellt fungerar.

1. För att ta reda på hur normalt åldrande påverkar njurfunktionen valde vi att bara ta med dem, som inte hade någon känd riskfaktor för njursjukdom såsom hjärtsvikt, hjärtinfarkt, stroke, högt blodtryck och diabetes. Vi tittade sedan på hur njurfunktionen såg ut i olika åldersgrupper och vilka faktorer som hängde ihop med njurfunktionsnivån.
2. För att undersöka hur väl ekvationerna för eGFR fungerar hos äldre valde vi att mäta GFR på ett noggrant sätt genom att tillföra röntgenkontrastmedel och se hur snabbt det försvann ut blodbanan. Sedan kunde vi se hur väl olika ekvationer baserade på kroppsegna ämnen kunde uppskatta njurfunktionen.
3. Vi jämförde resultaten av en eGFR-ekvation baserad på kreatinin med en baserad på cystatin C.
4. Slutligen tittade vi på längre sikt, i snitt mer än 10 år. Utifrån deltagarnas njurfunktion såg vi vad konsekvenserna blev i form av död och sjukdom.

Resultat

1. Njurfunktionen försämras med åldern även hos friska. Den försämras till och med i så hög utsträckning att hälften av friska 80-åringars njurfunktion är lägre än gränsen för njursjukdom tillåter. Män har en högre åldersberoende försämringshastighet.
2. Flera formler som används idag för att uppskatta njurfunktion är tillförlitliga. Bäst är de ekvationer som är baserade på både kreatinin och cystatin C. För de med sämre njurfunktion är det särskilt viktigt att använda både cystatin C och kreatinin. Den sämsta formeln (som dock är en av

världens mest använda) visade fel i upp till 35% av fallen. Motsvarande för formler med både cystatin C och kreatinin var mindre än 10%.

3. Hos en femtedel av de äldre skiljer sig GFR uppskattat från kreatinin och cystatin C mer än 30%.
4. En sämre njurfunktion hos äldre är förknippat med en ca 20% högre dödlighet när gränsen för njursjukdom har passerat. Liknande tendens kan anas vad gäller risken för hjärtkärlsjukdom vid nedsatt njurfunktion men utan tillräckliga statistiska bevis. Försämringshastigheten är i medeltal en minskning av uppskattat GFR med 1 ml per år.

Slutsats och ansats

Sammanfattningsvis så har denna avhandling bekräftat att vi på ett enkelt och tillförlitligt sätt kan bedöma njurfunktionen hos äldre med vanliga blodprov. Den visar att åldern i sig har betydelse för njurfunktionen och att en stor andel av äldre delvis till följd av detta uppfyller dagens kriterier för njursjukdom. När njurfunktionen passerar gränsen för njursjukdom så innebär det en högre risk för död oavsett ålder och andra riskfaktorer.

Förhoppningsvis kommer resultaten att uppmuntra läkare att använda sig av två olika markörer för GFR: Kreatinin och cystatin C när man vill uppskatta njurfunktionen tillförlitligt hos äldre. Den kan också bidra till sjukvårdens förståelse för förekomsten av nedsatt njurfunktionen samt storleksordningen på risken som njurfunktion förknippas med för äldre.

Med denna avhandling vill jag belysa behovet av att vi som samhälle reflekterar kring begreppet sjukdom i olika åldrar. Vi lever allt längre och gränslandet mellan sjukdom och åldrande blir alltmer betydelsefullt.

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