The prevalence and identification of chronic kidney disease in Switzerland

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"Felix, qui potuit rerum cognoscere causas"

Publius Vergilius Maro, *Georgica* (70 - 19 BC)

DEDICATION

To my parents, my brothers, and my closest friends for their support and encouragement.

PREFACE

Many patients with chronic kidney disease are identified only after reaching severe stages. Is it not possible to early identify these patients in order to prevent or slow disease progression? How many persons in Switzerland may have a chronic kidney disease? Is it a real problem?
The aim of this work was to assess the prevalence of chronic kidney disease in primary care in Switzerland and to investigate neutrophil gelatinase-associated lipocalin (NGAL) as a possible biomarker of renal disease. The characteristics of chronic kidney disease and the biomarkers used for its diagnosis are first introduced. The core data is then presented in form of the following two publications:

- **The prevalence of chronic kidney disease in a primary care setting: a Swiss cross-sectional study.**

  Tomonaga Y, Risch L, Szucs TD, Ambühl PM.
  PLoS One. 2013 Jul 3.

- **Insights on urinary NGAL obtained in a primary care setting.**

  Tomonaga Y, Szucs TD, Ambühl PM, Nock S, Risch M, Risch L.
  Clin Chim Acta. 2012 Apr 11. 733-739.

The overall discussion addresses the contribution of the two reported studies. Moreover the relationships between NGAL and acute kidney injury as well as between acute kidney injury and chronic kidney disease are briefly reviewed.
The work of this thesis was performed at the Epidemiology, Biostatistics and Prevention Institute (EBPI, formerly Institute of Social and Preventive Medicine until September 2014) at the University of Zurich, Switzerland, in collaboration with the Renal Division of the Waid City Hospital, Zurich, Switzerland; and the Laboratory center Dr. Risch, Liebefeld, Switzerland. The work was partially founded by an unrestricted educational grant from Abbott AG, Baar, Switzerland.

SUMMARY

BACKGROUND: Chronic kidney disease (CKD) is characterized by a gradual loss of kidney function. Patients in early CKD stages are often asymptomatic or show non-specific symptoms. Therefore many cases of CKD are identified only in more advanced and symptomatic stages. The most common risk factors of CKD are hypertension, diabetes, and older age. CKD is usually defined and diagnosed by using the glomerular filtration rate (GFR) and the albuminuria (or the albumin-to-creatinine ratio, ACR): an estimated GFR (eGFR) <60 mL/min/1.73m^2 and/or an ACR >30 mg/g creatinine indicate that a subjects may have a CKD. Other markers of renal diseases include for example the *neutrophil gelatinase-associated lipocalin* (NGAL) and the cystatin C. NGAL in particular has first been investigated in patients with acute kidney injury (AKI), a renal problem that for many years has been considered as completely separated from CKD.

The epidemiology of CKD has been investigated in several countries and settings. The prevalence ranged from 2.6% for CKD stages 3-5 in year 2007 in Finnland to 42% for CKD all stages in year 2009-2010 in the UK. Despite such large range, the majority of the studies reported prevalence rates around 10-15%. In Switzerland, the CoLaus study and the Swiss Survey on Salt have recently investigated the CKD epidemiology. In the CoLaus study, a Swiss population-based, cross-sectional study conducted in Lausanne in 2003-2006 the prevalence of all stages CKD was 10.0%. The Swiss Survey on Salt, a prospective, nationwide survey conducted in 2010-2011 reported prevalence of about 7.7% of the included population for CKD stage 3 or higher.

The primary goal of this Ph.D. thesis was to estimate the prevalence of CKD in a primary care setting in Switzerland. Secondly, this project aimed to investigate NGAL as possible biomarker of renal disease. Moreover, the relationship between NGAL and AKI as well as between AKI and CKD has been shortly reviewed.

METHODS: A cross-sectional, multicentre, non-interventional study was conducted in seven of the 26 Swiss cantons, including all five Swiss cantons with university affiliated medical faculties (i.e. Basel, Bern, Geneva, Vaud, and Zurich), the largest canton in central Switzerland (Lucerne), and the Italian speaking canton of Ticino. Physicians invited to participate in the study were randomly selected from the total pool of general practitioners (GPs) in each canton. The study coordination centre defined the days of patient inclusion by the GPs meeting inclusion criteria (i.e. age ≥18 years and the ability to provide written inform consent). Emergency patients and patients for which the participation in the study may have caused relevant delays in patient management were excluded for ethical reasons. Otherwise, the patients were consecutively included into the study.

Socio-demographic variables, clinical status and co-morbidities were reported on a questionnaire. Urine and blood samples were sent to a central laboratory for analysis. uNGAL was assessed using

the Abbott ARCHITECT immunology module and commercially available control materials. The uNGAL values were analyzed as absolute and relative values, normalized to urinary creatinine. The eGFR was calculated with the CKD-EPI equation. All patients were stratified into CKD stages using the classification recently proposed by KDIGO. Extrapolation of CKD prevalence in primary care to national level was based first on the number of patients older than 15 years of age who had visited a GP at least once in 2007, as reported by the Swiss Federal Statistical Office. The calculations were adjusted for age and gender distributions.

The relationship between NGAL and AKI as well as between AKI and CKD has been discussed on the basis of the recently published literature.

RESULTS: The main results of this Ph.D. thesis have been reported in two distinct publications:

The Prevalence of Chronic Kidney Disease in a Primary Care Setting: a Swiss Cross-Sectional Study: Among the 1,000 individuals recruited, 57% were female, and the mean age was 57±17 years. The results of the laboratory analysis showed that mean values of many parameters were significantly different between males and females (e.g. serum creatinine, albumin in the urine, uNGAL, cystatin C, total cholesterol). However, the majority of the laboratory parameters were within normal range for both genders. Overall, 41% of the patients had normal eGFR and ACR, whereas 36% of the subjects had slightly reduced excretory renal function with physiological albuminuria based on normal ACR. Almost one fourth of the subjects (23%) had either a substantially reduced eGFR or high levels of ACR. About 10% of the patients had a substantially reduced eGFR of <60 ml/min/1.73m^2, and 17% showed relevant proteinuria (ACR >30 mg/g creatinine). At primary care level, the prevalence of CKD has been estimated to be at 19%. Until 54 years of age, between 8% and 14% of the patients visiting a general practitioner may have a reduced renal function. Thereafter the percentage of patients who may suffers from CKD strongly increase with age: 19% for patients aged 55-64 years, 29% for patients aged 56-74 years, and 53% for patients older than 75 years. Extrapolation to national level suggested that about 11% of all subjects older than 15 years in Switzerland may suffer from CKD.

Insights on urinary NGAL obtained in a primary care setting: The same population sampling mentioned above showed a median absolute uNGAL of 21 ng/L. Elevated uNGAL (>100 ng/L) together with normal kidney test results (eGFR and ACR) were found in 6.5% of all patients. Females had a significantly higher uNGAL than did males. Among a multitude of different clinical and laboratory variables, only age, gender, liver function parameters, WBC and CRP were significantly associated with uNGAL levels in a multivariate analysis. When examining the proposed KDIGO classification of CKD, the uNGAL levels at the given eGFR stages changed with increasing ACR stages and vice versa.

CONCLUSIONS: The results of this work suggest that the prevalence of impaired renal function and/or CKD in Switzerland is considerably high. Based on the data provided by the Swiss Federal Statistical Office, it may be reasonable to assume that the prevalence of renal problems may grow up in the next decades, according to the increasing prevalence of its major risk factors (i.e. diabetes, hypertension, and older age). Hence, early diagnosis, treatment of the underlying cause, and implementation of secondary preventive measures are fundamental for CKD patients in order to relieve symptoms, slow or prevent progression of the condition, and reduce the risk of developing related problems.

Concerning NGAL, the study showed that age, gender, markers of inflammation and liver function, exert influences on uNGAL concentrations. A substantial proportion of patients exhibited normal kidney testing together with elevated uNGAL, potentially identifying patients with increased renal stress and at increased risk for the development of AKI. Several studies performed in the last few years reported similar results. However, despite the fact that there is general agreement that NGAL is significantly correlated with serum creatinine and eGFR, there is not yet enough evidence concerning its predictive potential for progressive CKD. In particular it is not yet clear if adding an NGAL test to the classical prognostic factors for CKD (e.g. eGFR, albuminuria, age, gender, BMI, hypertension and diabetes) will substantially improve the prediction of outcome events in CKD patients.

In the last decades, CKD and AKI have been mostly considered as two different diseases. However, recent studies suggested that CKD and AKI may rather be two closely interconnected conditions. Indeed, each condition can be considered as risk factors for the other, and both chronic and acute diseases are risk factors for cardiovascular diseases. Elevated NGAL values have been associated with both diseases and seem to support the interconnection of the two diseases. Several studies reported that NGAL may be useful alongside serum creatinine, urine output, and other biomarker for the diagnosis and prognosis of patients with CKD or AKI. Unfortunately the validity of these results is limited to the specific settings and samplings in which NGAL and the possible outcomes have been measured. It is therefore premature to implement NGAL testing in the routine clinical use.

Prevention in form of healthy lifestyle and consequently reduced risk for CKD, and early recognition of CKD are important in order to slow or prevent progression to severe and symptomatic stages. In this optic, an increase in the CKD awareness among clinicians and patients is fundamental. Concerning the use of NGAL, more studies (ideally prospective randomized trials) are needed to increase its external validity as diagnostic/prognostic marker for CKD and AKI. Moreover, a standardisation of the measurements and clear guidelines concerning NGAL cut-off values and interpretation of the test results should be provided.

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1. INTRODUCTION

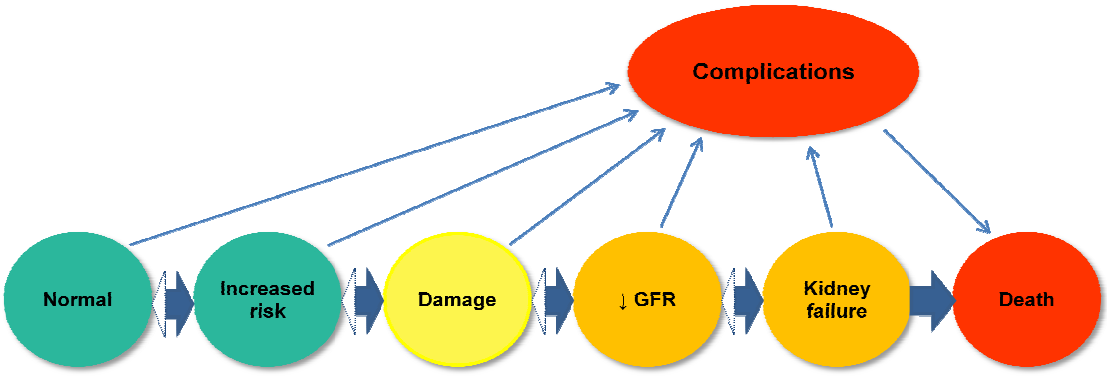
1.1. CHRONIC KIDNEY DISEASE DESCRIPTION AND SYMPTOMS

Chronic kidney disease (CKD) is a condition characterized by a progressive loss of the renal function. The gradual and usually persistent deterioration of the kidney's filtration function may last months or years (Levey et al., 2012).

The symptoms are often non-specific, and many people may not have any severe symptoms until their renal disease is in an advanced stage. The most common non-specific symptoms include feeling more tired and having less energy, having trouble concentrating, having a poor appetite, having trouble sleeping and/or muscle cramping at night, having swollen feet and ankles, having puffiness around the eyes, having dry/itchy skin, and needing to urinate more often, especially at night. As the kidney function decreases, other symptoms may appear: hypertension, an accumulation of urea that may lead to uremia, hyperkalemia and/or hyperphosphatemia (i.e. an accumulation of potassium and/or phosphates in the blood), a decreased production of erythropoietin, fluid volume overload (e.g. edema), vitamin D deficiency and hypocalcemia, metabolic acidosis, and iron deficiency anemia (Jafar et al., 2003; Levey et al., 2005; Levey et al., 2012; Locatelli et al., 2002).

The following conceptual model proposed in 2002 by the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (KDOQI) illustrates in general the continuum development, progression, and complication of CKD (Levey et al., 2010a).

Figure 1. 1. Conceptual model of chronic kidney disease development and progression.



The "Normal" and "Increased risk" circles represent potential antecedents; the "Damage", "↓GFR" and "Kidney failure" circles represent stages of CKD; the "Complication" and "Death" circles represent potential consequences; thick arrows represent risk factors associated with the development, progression, and remission of CKD. In the complications are included all complications of CKD as well as the interventions for its treatment and prevention (e.g. complication of low GFR and cardiovascular disease). CKD, chronik kidney disease; GFR, glomerular filtration rate (modified from Levey et al., 2010a).

1.2. CHRONIC KIDNEY DISEASE CAUSES AND RISK FACTORS

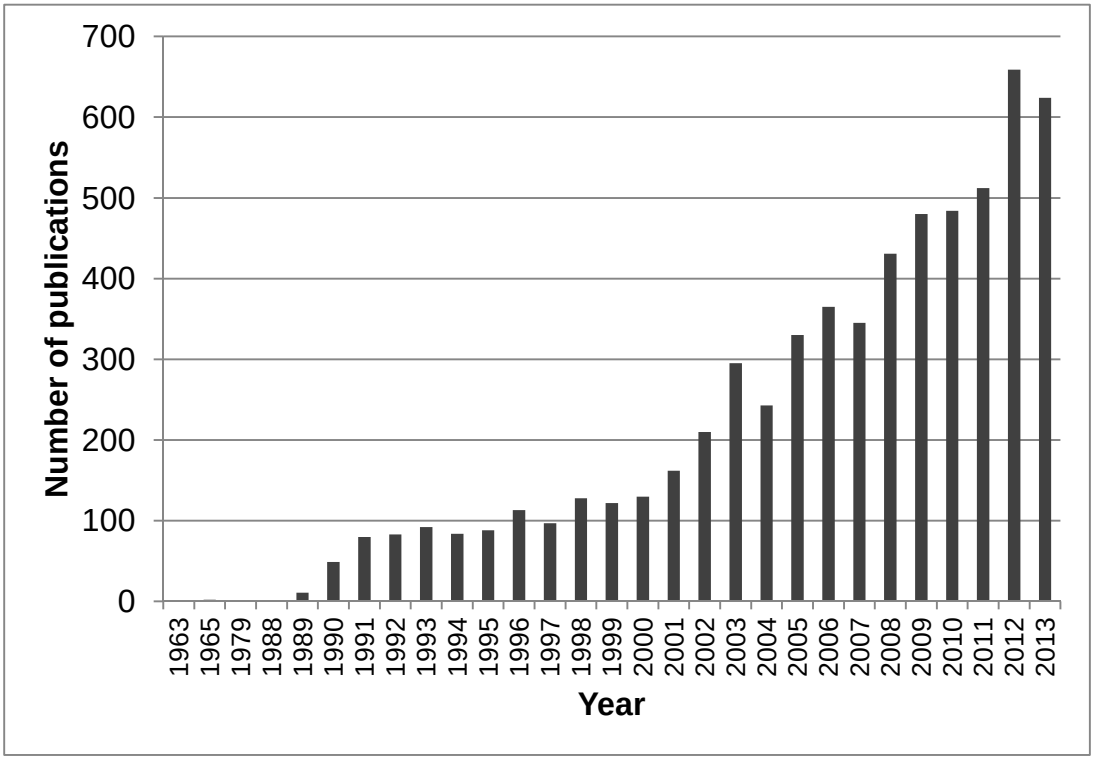
The most common causes of kidney disease are hypertension and diabetes (type 1 or 2). Other diseases and conditions that less commonly induce CKD include glomerulonephritis (i.e. the inflammation of the glomeruli, the kidney's filtering units), pyelonephritis (i.e. recurrent kidney infections), polycystic kidney disease (i.e. gradual growth of masses of cysts in both kidneys), interstitial nephritis (i.e. the inflammation of the kidney's tubules and surrounding structures), atherosclerosis, prolonged obstruction of the urinary tract (due for example to enlarged prostate, kidney stones, and some cancers), vesicoureteral reflux (i.e. abnormal backward movement of urine from the bladder into ureters or kidneys), failure of normal kidney development during pregnancy, systemic lupus erythematosus (in which the immune system attacks the kidney as if it were a foreign tissue), and the long-term consumption of some medicaments (e.g. non-steroidal anti-inflammatory drugs like aspirin and ibuprofen).

The factors increasing the risk of CKD can be divided in clinical and sociodemographic factors. The clinical factors include diabetes, hypertension, several heart diseases (e.g. heart failure, stroke), autoimmune diseases, systemic infections, urinary tract infections, urinary stones, lower urinary tract obstruction, neoplasia, family history of CKD, recovery from acute kidney failure, reduction in kidney mass, exposure to certain drugs, and low birth weight. Sociodemographic risk factors can be older age (Coresh et al., 2007; Stevens et al., 2010), ethnic group (e.g. African American, Native American, Hispanic, Asian), exposure to certain chemical and environmental conditions, low income/education (Fraser et al., 2013), and smoking (McClellan et al., 2012; KDIGO, 2013).

1.3. CHRONIC KIDNEY DISEASE EPIDEMIOLOGY

In this chapter a short overview of the available national and international literature will be provided. A short literature search in Pubmed including the search terms CKD and prevalence/incidence/epidemiology shows a constantly increasing number of CKD-related publications in the last decade. Since the beginning of this century, the publications concerning CKD increased almost linearly (from 130 publications in year 2000 to more than 600 publications in year 2013), suggesting an increasing research interest in nephrology (Figure 1.2.). Only in the last five years, more than 2'500 articles concerning and/or mentioning CKD have been published.

Figure 1. 2. Number of publications concerning chronic kidney disease epidemiology.



An overview of the papers published in the last five years (ca. 2'500 articles) suggests that the information concerning CKD prevalence in Switzerland and in general in Europe is still limited. In many publications the terms CKD and prevalence, incidence, and/or epidemiology are just used in the introduction/background chapters. Moreover, the majority of the studies investigating epidemiological aspects of CKD are related to other specific diseases and/or population groups (e.g. CKD prevalence in patients with diabetes mellitus and hypertension, CKD incidence in HIV-infected patients or cancer disease, CKD incidence after surgery or renal transplantation, etc.). Out of 2'500 articles published in the last five years, less than 80 articles investigated the epidemiology of CKD in general (at population level or in large samples). About 10 articles discuss the epidemiology of CKD as a worldwide problem. The remaining papers can be divided in four regional groups:

- United States
- Asian Countries (in particular China and Japan)
- European countries (in particular Italy and United Kingdom)
- Other countries (e.g. Arab, African, and Central American countries)

According to the Global Burden of Disease (GBD) study, CKD was ranked 27th in the list of causes of total number of deaths in 1990, but rose to 18th in 2010 (Lozano et al., 2012).

In the United States, the prevalence of CKD has been investigated with a cross-sectional analysis of the National Health and Nutrition Examination Surveys (NHANES 1988-1994 and NHANES 1999-2004), in a nationally representative sample of non-institutionalized adults aged 20 years or older. The prevalence of CKD stages 1 to 4 increased from 10.0% (95% confidence interval [CI], 9.2%-10.9%) in 1988-1994 to 13.1% (95% CI, 12.0%-14.1%) in 1999-2004 (Coresh et al., 2007). Another US study conducted by the Centers for Disease Control and Prevention (CDC) from 2007 to 2012 suggested that 15.0% (95% CI, 14.1%-15.9%) of the US population have CKD, with following stage specific rates (CDC, 2014):

- CKD Stage 1: 3.46% (95% CI, 3.06-3.88)
- CKD Stage 2: 3.55% (95% CI, 3.16-3.93)
- CKD Stage 3: 7.56% (95% CI, 6.87-8.32)
- CKD Stage 4: 0.45% (95% CI, 0.36-0.56)

CKD Stage 5 patients have been excluded because estimates of this stage are likely to be unreliable due to the likelihood that patients receiving dialysis would have a low response rate.

The estimations of the CKD prevalence in China in the last few years range from 10% to 16% (Zhang et al., 2012: 10.8%; Gu et al., 2013: 12.5%; Jiang et al., 2010: 15.2%), whereas the last calculations in Japan suggest a prevalence ranging from 12.9% in 2005 (Imai et al., 2009) to about 19% in 2002 (22.1% in males and 15.3% in females) (Nagata et al., 2010).

In a UK study of Gifford et al., the prevalence of CKD in year 2009-2010 has been estimated around 42%: 20.4% for CKD stage 1, 17.0% for CKD stage 2, 3.3% for CKD stage 3, 0.3% for CKD stage 4, and 0.04% for CKD stage 5 (Gifford et al., 2011). In another analysis including 743'935 adults in England aged 18 years and over, a national prevalence of CKD stage 3-5 of 4.3% has been estimated in 2009 (Kearns et al., 2013). In an Irish study including 2'602 patients aged 50 years or more recruited in general practice, 16.7% had CKD (Glynn et al., 2009).

In the CAHRES (CArdiovascular risk in Renal Patients of the Italian Health Examination Survey) study published in 2011, the prevalence of CKD has been evaluated in a sample of 9'020 Italian subjects aged 30-79 years (De Nicola et al., 2011). The authors reported national prevalence rates of 8.1% in males and 7.8% in females (with 3.5% and 2.4% respectively for CKD stage 3-5). In the INCIPE study conducted in North-eastern Italy and including 6'200 patients aged 40 years or more, the estimated prevalence of CKD stage 1-4 was 13.2% in 2008 (Gambaro et al, 2010). The prevalence rates for the single CKD stages from 1 to 4 were 1.7% (95% CI, 1.2-2.1), 4.3% (95% CI, 3.6-5.0), 6.4% (95% CI,

5.6-7.2), and 0.3% (95% CI, 0.1-0.4). In a Spanish study including a randomly selected sample of general population (N= 2'746) aged 20 years or older, the overall prevalence of CKD stages 3-5 was 6.8% (95% CI, 5.4-8.2) in 2001 (Otero et al., 2010). A Finnish cross-sectional population survey including patients aged 25-74 years reported a prevalence of CKD stages 3-5 of 2.6% in year 2007 (Juutilainen et al., 2012).

For the epidemiology of CKD in Switzerland, a recently published paper (CoLaus study) and a poster abstract (Swiss Survey on Salt) have been identified.

In the Swiss population-based, cross-sectional CoLaus study conducted in Lausanne in 2003-2006 and including 2'810 men and 3'111 women aged 35-75 years, the prevalence of all stages CKD was 10.0% (95% CI, 9.2-10.8%) (Ponte et al., 2013). Following stage specific CKD prevalence have been calculated:

- CKD Stage 1: 2.34%
- CKD Stage 2: 3.20%
- CKD Stage 3: 4.31%
- CKD Stage 4: 0.10%
- CKD Stage 5: 0.05%

In the Swiss Survey on Salt, a cross-sectional population-based study including a random sample of 1'377 individuals aged 15-95 years recruited in 2010-2011 from all linguistic regions of Switzerland, the total prevalence of CKD stage 3-5 was 7.7% (7.5% for CKD stage 3, 0.2% for stage 4, and 0% for stage 5) (Forni et al., 2011).

1.4. CHRONIC KIDNEY DISEASE DIAGNOSIS

CKD is normally defined and diagnosed using the criteria proposed in the guidelines of the *Kidney Disease: Improving Global Outcomes* (KDIGO) (KDIGO, 2013):

1- Glomerular filtration rate (GFR) <60 mL/min/1.73m^2 for ≥3 months, with or without kidney damage

and/or

2- Kidney damage for ≥3 months, as defined by structural or functional abnormalities of the kidney, with or without decreased GFR, manifested by *either*:

   a) pathological abnormalities; or

   b) markers of kidney damage, including abnormalities in the composition of the blood or urine, or abnormalities in imaging tests.

The best overall index of kidney function in health and disease is the GFR, whereas albuminuria is the most analysed marker of kidney damage (indicates an increased glomerular permeability).

1.4.1. Glomerular filtration rate

The GFR describes the flow rate of filtered fluid through the kidneys. The GFR test involves a blood test which measures the creatinine, a breakdown product of creatinine phosphate in the muscles. Creatinine is usually produced at a fairly constant rate by the body and is normally cleared from the blood by the kidneys. If the kidneys are damaged and the glomeruli are not filtering as much as normal, the level of creatinine in the blood increases.

In the last few years several different techniques have been developed to calculate or estimate the GFR. One of the most recent techniques to calculate the estimated GFR (eGFR) is the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula, published in 2009 (Levey et al., 2009; Levey et al., 2010b). The CKD-EPI equation, expressed as a single equation, is:

$$eGFR = 141 \times \min(Scr/\kappa,1)^{\alpha} \times \max(Scr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018\ [\text{if female}] \times 1.159\ [\text{if black}]$$

Where Scr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is –0.329 for females and –0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.

GFR (or eGFR) are commonly used to classify the renal filtration rate as indicated in following table:

Table 1. 1. Glomerular filtration rate categories in chronic kidney disease.

Category	GFR ($mL/min/1.73m^2$)	Terms
G1	≥90	Normal or high
G2	60-89	Mildly decreased
G3a	45-59	Mildly to moderately decreased
G3b	30-44	Moderately to severely decreased
G4	15-29	Severely decreased
G5	<15	Kidney failure or end-stage renal disease (ESRD)

Abbreviations: CKD, chronic kidney disease; GFR, glomerular filtration rate.

It is important to emphasize that in the absence of evidence of kidney damage, neither GFR category G1 nor G2 fulfil the criteria for CKD (KDIGO, 2013).

1.4.2. Albuminuria

Albuminuria is a type of proteinuria, a pathological condition in which a protein (i.e. albumin) is present in the urine. Since the kidneys normally do not filter large molecules into the urine, albuminuria can be used as indicator of renal damage.

Albuminuria categories are assessed using either the albumin excretion rate (AER) over 24 hours or the albumin-to-creatinine ratio (ACR). AER values below 30 mg/24h or ACR values below 30 mg/g are considered normal or mildly increased (KDIGO, 2013). Higher values are in contrast considered pathologic and may require additional controls and/or a treatment.

Table 1. 2. Albuminuria categories in chronic kidney disease.

Category	AER (mg/24h)	ACR (mg/g)	Terms
A1	<30	<30	Normal or mildly increased
A2	30-300	30-300	Moderately increased
A3	>300	>300	Severely increased

Abbreviations: ACR, albumin-to-creatinine ratio; AER, albumin excretion rate; CKD, chronic kidney disease

1.4.3. Glomerular filtration rate and albuminuria

Based on signs of kidney damage (e.g. abluminuria) and GFR, CKD is divided in 5 categories, as indicated in table 1.3..

Table 1. 3. Classification of chronic kidney disease based on kidney damage and glomerular filtration rate.

Stage	Description	GFR or estimated GFR ($mL/min/1.73m^2$)
1	Kidney damage with normal or elevated GFR	≥90
2	Kidney damage with mildly reduced GFR	60-89
3	Moderately reduced GFR	30-59
4	Severely reduced GFR	15-29
5	Kidney failure or end stage renal disease (ESRD)	<15 (or dialysis)

Abbreviations: CKD, chronic kidney disease; GFR, glomerular filtration rate.

In the *Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease*, published in January 2013 by the Kidney Disease: Improving Global Outcomes (KDIGO), the authors evaluated the risk of concurrent complications and future outcomes in relation to GFR and albuminuria category (KDIGO, 2013). The risk associations of GFR and albuminuria categories appeared to be largely independent from each other. For this reason, neither the category of GFR nor the category of albuminuria alone can fully capture the prognosis for a CKD patient. A staging system encompassing the ordered categories of GFR and albuminuria has been proposed (Figure 1.3.)

Figure 1. 3. Prognosis of chronic kidney disease by glomerular filtration rate and albuminuria category.

				Persistent albuminuria categories Description and range		
				A1	**A2**	**A3**
				Normal to mildly increased	Mederately increased	Severely increased
				<30 mg/g	30-300 mg/g	>300 mg/g
GFR categories (ml/min/1.73m^2) Description and range	**G1**	Normal or high	≥90			
	G2	Mildly decreased	60-89			
	G3a	Mildly to moderately decreased	45-59			
	G3b	Moderately to severely decreased	30-44			
	G4	Severely decreased	15-29			
	G5	Kidney failure	<15			

Green, low risk (and no CKD if no other markers of Kidney disease); yellow, moderately increased risk; orange, high risk; red, very high risk. Abbreviations: CKD, chronic kidney disease; GFR, glomerular filtration rate. (modified from KDIGO, 2013)

As illustrated in Figure 1.3., the risk of complications or future outcomes (e.g. AKI, kidney failure, cardiovascular disease or death) increases with increasing GFR and/or increasing albuminuria, emphasizing the multidimensional aspect and the complexity of CKD.

1.4.4. Neutrophil gelatinase-associated lipocalin

Neutrophil gelatinase-associated lipocalin (NGAL), also known as Lipocalin-2, is a protein belonging to the lipocalin superfamily. This protein was originally described as being covalently bound to matrix metalloproteinase 9 (MMP-9 or neutrophil gelatinase) purified from human neutrophils (Kjeldsen et al., 1993). In subsequent research, NGAL has been found to be expressed not only in the bone marrow, in which neutrophils originate, but also in tissues prone to microbial exposure and infection, as for example lungs, trachea, salivary glands, prostate, uterus, stomach, and the colon (Cowland et al., 1997; Schmidt-Ott et al., 2007; Flo et al., 2004). The expression of NGAL has also been shown to increase dramatically in renal tubular cells following tubular injury of ischemic, toxic, septic, or immunologic origin (Devarajan, 2010). The level of this biomarker increases two hours after kidney injury and is thus indicative of an AKI development (Mishra et al., 2005). For this reason it has been described as a "troponin-like marker" of the kidneys.

Beside the predictive properties of NGAL concerning AKI, a study published in 2009 suggested that NGAL may also predict the progression of CKD, independently of other confounders (e.g. eGFR and age) (Bolignano et al., 2009).

1.4.5. Other markers of kidney diseases

In the past years, several markers have been evaluated for the diagnosis/prognosis of CKD. Some of them are briefly described below:

- Cystatin C: is a cysteine protease inhibitor that is released at a constant rate by all nucleated cells into the plasma, is freely filtered by the glomerulus, and is completely reabsorbed in the tubules. Several studies reported that cystatin C appears to predict renal function (i.e. GFR) as well as creatinine in CKD, and even better than creatinine in AKI (Dharnidharka et al., 2002; Herget-Rosenthal et al., 2004; Herget-Rosenthal et al., 2005, Peralta et al., 2011). Moreover it seems that cystatin C predicts the risk of cardiovascular morbidity and mortality in patients with AKI (Shlipak et al., 2005) as well as patients with CKD (Vigil et al., 2014; Helmersson-Karlqvist et al., 2014).

- Kidney injury molecule 1 (KIM-1): this protein is upregulated in post-ischemic injury in the proximal tubule. Urinary KIM-1 has been first proposed as biomarker for the diagnosis of ischemic acute tubular necrosis (Han et al., 2002). A study published in 2007 analyzed the KIM-1 expression in biopsies of various diseases and reported that KIM-1 is elevated also in patient with diabetic nephropathy, focal glomerulonephritis, hypertension, IgA nephropathy and other renal diseases (van Timmeren et al., 2007a). KIM-1 may also be an independent predictor for graft loss in post-renal transplantation (van Timmeren et al., 2007b). Moreover, a recent study suggested

that higher urinary KIM-1 may predispose to higher risk of cardiovascular mortality independently of established cardiovascular risk factors, eGFR, and albuminuria (Carlsson et al., 2014)

- Interleukin-18 (IL-18): the pro-inflammatory cytokine IL-18, formed in the proximal tubules and detected in the urine, has been considered as potential diagnostic marker for AKI in intensive care unit and after cardiac surgery (Parikh et al., 2005; Parikh et al., 2006).

1.5. CHRONIC KIDNEY DISEASE TREATMENT

Actually there is no direct cure for CKD. Therefore, the early diagnosis, the treatment of the underlying cause, and/or the institution of secondary preventive measures are fundamental for CKD patients in order to relieve symptoms, to slow or prevent progression of the condition, and to reduce the risk of developing related problems.

The treatment usually depends on the stage of CKD. In the early stages (1-3), the treatment mainly consist in changing the lifestyle and, in some case, taking medication to control the blood pressure and lower the blood cholesterol levels (KDIGO, 2013). Lifestyle changes include stopping smoking, having a healthy and balanced diet (low in fat and cholesterol), restricting salt or potassium intake, moderating the alcohol consumption, getting regular physical exercise, and losing weight in case of overweight/obesity (Heiwe et al., 2011; Ricardo et al., 2013; Robinson-Cohen et al., 2014). For the control of blood pressure, the most used drugs are angiotensin converting enzyme (ACE) inhibitors or angiotensin II receptor antagonists (ARBs) (KDOQI, 2012; Hsu et al., 2013; Turner et al., 2012).

In more advanced stages (4-5), additional medications can be prescribed to control or prevent the symptoms of CKD. For example, erythropoiesis-stimulating agents (ESAs) can be prescribed to CKD patients with anemia, and calcium supplements are used to treat hypocalcemia (KDIGO, 2013 Lankhorst et al., 2010).

Finally, in case of kidney failure, renal replacement therapy (RRT) consisting in dialysis or kidney transplantation is required (Abecassis et al., 2008).

1.6. CHRONIC KIDNEY DISEASE AND ACUTE KIDNEY INJURY

Acute kidney injury (AKI), also called acute renal failure (ARF), is a rapid deterioration of the renal function resulting in the inability to maintain fluid, electrolyte, and acid-base balance (Basile et al., 2012; Bellomo et al., 2012). Potential complications of AKI include volume overload (leading for example to severe pulmonary oedema), hyperkalemia, hyponatremia, metabolic acidosis, and uremic syndrome (Basile et al., 2012). Compared to CKD, AKI shows a significantly faster deterioration of the kidney filtration function and is mainly symptomatic.

Some of the main risk factors associated with AKI are age ≥65 years, CKD, diabetes, heart failure, liver disease, and surgery (NICE clinical guideline 169, 2013).

AKI is primarily detected and/or monitored by serial serum creatinine and blood urea nitrogen (BUN) measurements, which rise acutely (Edelstein, 2008). Also urine output and eGFR fall can be used for detection and monitoring. Unfortunately, urine and creatinine tests are not particularly specific/sensitive for AKI (McCullough et al., 2013). Serum creatinine depends on several non-renal factors independent of kidney function (e.g. age, gender, race, muscle mass, nutrition). Moreover, several medications can alter the tubular secretion of creatinine, causing a GFR independent change in serum creatinine (Star, 1998; Waikar et al., 2011; Wu et al., 2008). BUN is also depends on non-renal factors (e.g. protein intake, catabolic state, volume status) and is therefore only a suboptimal marker for the diagnosis of AKI (Waikar et al., 2006). For this reason, in the past few years, alternative markers like NGAL, cystatin C, KIM-1, and IL-18 have been investigated as potential markers of renal function (Alge et al., 2014; Vanmassenhove et al., 2012).

For many years, CKD and AKI have been considered two separate diseases. However, the fact that both diseases share several risk factors and may lead to similar outcomes suggests that the two diseases may be linked. Whether the link is causal, unidirectional or bidirectional is not yet clear.

1.7. AIMS OF THE STUDY

Two studies, based on the data of a multicentric cross-sectional trial performed in Switzerland, form the basis for this Ph.D. thesis. Beside the CKD prevalence and the NGAL concentrations in primary care, described in the next two chapters, the relationship between NGAL and AKI as well as between AKI and CKD has been shortly reviewed in the discussion.

1.7.1. The Prevalence of Chronic Kidney Disease in a Primary Care Setting: a Swiss Cross-Sectional Study.

The aim of this study was to estimate the prevalence of CKD in a primary care setting in Switzerland. The prevalence of CKD has been assessed in a large sample of primary care patients. Thereafter, the prevalence at national level has been calculated adjusting, for age and gender distribution, the prevalence in the study sample. The results may provide important information for future national preventive programs, optimizing the resource allocation process. The estimations at national level may improve public awareness for CKD and CKD related diseases.

1.7.2. Insights on urinary NGAL obtained in a primary care setting.

This project aimed to investigate NGAL as possible biomarker of renal disease. The concentration of NGAL has been characterized in a large sample of primary care patients in order to investigate possible association with several clinical and laboratory parameters (related in particular to renal diseases). The screening of patients for increased uNGAL concentrations prior to procedures predisposed to the development of AKI may allow for the identification of patients at high risk for the subsequent development of AKI, who may require special care.

1.8. REFERENCES

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2. THE PREVALENCE OF CHRONIC KIDNEY DISEASE IN A PRIMARY CARE SETTING: A SWISS CROSS-SECTIONAL STUDY

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2.1. ABSTRACT

Chronic kidney disease (CKD) often remains clinically silent and therefore undiagnosed until a progressed stage is reached. Our aim was to estimate the prevalence of CKD in a primary care setting in Switzerland. A multicenter, cross-sectional study with randomly selected general practitioners was performed. Adults visiting their general physician's cabinet during defined periods were asked to participate. Baseline information was reported on a questionnaire, urine and blood samples were analyzed in a central laboratory. Renal status was assessed using the Kidney Disease: Improving Global Outcomes (KDIGO) classification. Extrapolation of results to national level was adjusted for age and gender. One thousand individuals (57% females) with a mean age of 57±17 years were included. Overall, 41% of the patients had normal estimated glomerular filtration rate (eGFR) and albumin creatinine ratio (ACR), whereas 36% of the subjects had slightly reduced excretory renal function with physiological albuminuria based on normal ACR. Almost one fourth of the subjects (23%) had either a substantially reduced eGFR or high levels of ACR. About 10% of the patients had a substantially reduced eGFR of <60 ml/min/1.73m^2, and 17% showed relevant proteinuria (ACR >30 mg/g creatinine). Extrapolation to national level suggests that about 18% of primary care patients may suffer from CKD. CKD prevalence in a primary care population is therefore high, and preventive interventions may be advisable, in particular as CKD prevalence is likely to rise over the next decades.

2.2. INTRODUCTION

Chronic kidney disease (CKD), defined as renal damage with persistent and usually progressive deterioration of ultrafiltration, is a worldwide public health problem (Eknoyan et al., 2004). Several studies have shown that patients with CKD have increased risk of cardiovascular events and increased risk of death (Culleton et al., 1999; Go et al., 2004; Thorp et al., 2006; McCullough et al., 2008a; Roderick et al., 2009; Ryan et al., 2009; Al-Aly et al., 2010; Wang et al., 2011; Chen et al., 2012; Hallan et al., 2012). Moreover, the ageing of the population in western countries and the generally increasing rates of obesity, hypertension, and diabetes worldwide suggest that the incidence and prevalence of CKD will rise over the next decades (Ting et al., 2009; Stevens et al., 2010).

The classification of CKD is mainly based on measured or estimated glomerular filtration rate (GFR). In CKD stages 1 and 2, kidney function is normal (GFR >90 ml/min/1.73m^2) or slightly reduced (GFR 60-89 ml/min/1.73m^2), respectively, with evidence of renal damage (e.g. proteinuria). In CKD stages 3 and 4, functional impairment is moderate (GFR 30-59 ml/min/1.73m^2) or severe (GFR 15-29 ml/min/1.73m^2), respectively. Finally, CKD stage 5 is defined by kidney failure (GFR <15 ml/min/1.73m^2) or dialysis, and is also termed end-stage renal disease (ESRD) (Levey et al., 2005).

Recently, the Kidney Disease: Improving Global Outcomes (KDIGO) foundation performed a meta-analysis to investigate the relationship of estimated GFR (eGFR) and albuminuria with mortality and kidney outcomes: the results confirmed the current definition for CKD, i.e. GFR <60 ml/min/1.73m^2 or urinary albumin to creatinine ratio (ACR) >30 mg/g (Levey et al., 2010a).

In the last decades, the majority of studies on CKD focused on its most advanced stages (stages 4-5). ESRD patients usually present with many complications, high mortality, strongly reduced quality of life, and high health care expenditures (Mucsi et al., 2008; Powe et al., 2009). At this stage renal replacement therapy (RRT), consisting in hemodialysis, peritoneal dialysis, hemofiltration, and kidney transplantation, becomes necessary (Locatelli et al., 2006).

Unfortunately these interventions are expensive and not always available. Hemodialysis costs amount to 530 Swiss Francs per session (SVK), whereas renal transplantation costs are 58,300 Swiss Francs in the first year (FOPH, 2013). Moreover, there is an increasing gap between the number of donors and the number of patients waiting for a kidney: whereas the number of kidney transplantations between 2002 and 2011 slowly increased from 204 to 282 (i.e. +38%), the number of patients on the waiting list increased from 744 to 1,185 (i.e. +59%). In 2009 the mean waiting time for a donor organ was around 700 days (Swiss Transplant, 2011).

Except for RRT, there is no other treatment for CKD patients with ESRD. Even at early stages, actual treatment options mainly aim to prevent or slow disease progression by controlling risk factors such as hypertension, diabetes, and obesity (White et al., 2005). In short, prevention plays a key role in CKD management.

One of the biggest issues in CKD prevention is actually disease awareness. In the Kidney Early Evaluation Program (KEEP), a community-based screening program, only 10.0% of the 26,213 participants were aware of suffering from CKD. The proportion in awareness was particularly low for early CKD, with 5.1%, 6.3%, and 10.0% for stages 1 to 3, respectively. In contrast, almost 40% of the patients with CKD stage 4, and 60% of those with CKD stage 5 were aware of having renal disease (Kurella Tamura et al., 2011). Thus, despite the fact that effective preventive measures exist, many CKD patients remain undiagnosed and untreated. In this regard, family physicians play a fundamental role by timely diagnosis of diabetes mellitus and hypertension in their patients, the latter being the major contributors to CKD. However, screening for signs of renal damage is required, too. Early diagnosis and treatment of CKD and CKD related complications (e.g. anaemia, dyslipidemia, metabolic bone disease, metabolic acidosis, etc.) might prevent or slow the development of further sequelae and delay the requirement for RRT (Levin et al., 2008; Murphree et al., 2010). In a large retrospective study including about 12,000 patients with stage 3 or 4 CKD in primary care, the authors reported that CKD management, especially without the involvement of a nephrologist, was not optimal: 72% of patients with diagnosed CKD lacked annual urine protein testing, 26% were not receiving appropriate angiotensin blockade, and 20% were taking potentially harmful drugs (Allen et

al., 2010). Moreover, whereas annual screening for anaemia was common (80%), annual testing for metabolic bone disease was less frequent (calcium 56%, vitamin D 26%, parathyroid hormone 13%).

One of the first steps to improve CKD management is knowledge about CKD prevalence. Consequently, in the last few years, research has focused on the epidemiology of CKD (Whaley-Connell et al., 2008; Zhang et al., 2008). Supposing that the simplest way to identify CKD is through a family doctor, the aim of this study was to estimate the prevalence of CKD in a primary care setting in Switzerland. The results of this study may provide important information for future national preventive programs, optimizing the resource allocation process. The estimations at national level may improve public awareness for CKD and CKD related diseases.

2.3. MATERIALS AND METHODS

2.3.1. Study design and patient population

A cross-sectional, multicentre, non-interventional study was conducted in seven of the 26 Swiss cantons, including all five Swiss cantons with university affiliated medical faculties (i.e. Basel, Bern, Geneva, Vaud, and Zurich), the largest canton in central Switzerland (Lucerne), and the Italian speaking canton of Ticino. The selected cantons were home to nearly 60% of the entire Swiss population in 2010 and represent all three major language regions in Switzerland (German: Basel, Bern, Lucerne, and Zurich; French: Geneva and Vaud; Italian: Ticino) (WHO, 2012). Physicians invited to participate in the study were randomly selected from the total pool of general practitioners (GPs) in each canton. Random selection was performed by a computer program generating random numbers. Physicians from 33 offices agreed to participate. The study coordination centre defined the days of patient inclusion by the GPs meeting inclusion criteria (i.e. age ≥18 years and the ability to provide written inform consent). Emergency patients and patients for which the participation in the study might have caused relevant delays in patient management were excluded for ethical reasons. Otherwise, all patients were consecutively included into the study. The study was conducted according to the Declaration of Helsinki (as revised in 2008) and with the International Conference on Harmonization-Good Clinical Practice (ICH-GCP) standards. The study was approved by all seven cantonal ethics committees: Ethikkommission beider Basel (EKBB), Kantonale Ethikkommission Bern (KEK), Commission d'éthique pour la recherche clinique dans le Canton de Genève, Ethikkommission des Kantons Luzern, Comitato etico cantonale del Canton Ticino, Commission cantonale (VD) d'éthique de la recherche sur l'être humain, and Kantonale Ethikkommission (KEK) Zürich.

2.3.2. Measures

Socio-demographic variables, clinical status and co-morbidities were reported on a questionnaire. Urine and blood samples were sent to a central laboratory for analysis. A spot urine was collected in a Greiner Vacuette tube without preservatives (Greiner Bio One, Krems, Austria), whereas venous blood was collected in Sarstedt Monovette EDTA tubes and in serum tubes containing separation gel (Sarstedt, Sevelen, Switzerland). After serum sample centrifugation, the samples were mailed to the central laboratory (*Labormedizinisches Zentrum Dr. Risch*) using overnight delivery service by the Swiss Postal Service. Laboratory analysis was performed on the day the samples were received. Laboratory parameters were determined on an Abbott ARCHITECT ci4100 analyzer platform (Abbott, Baar, Switzerland), a Sysmex XT-5000 hematology analyzer (Sysmex Digitana, Horgen, Switzerland), and a Bio-Rad D-10 HPLC system for the determination of glycated haemoglobin (HbA1c; Biorad, Pratteln, Switzerland). The following parameters were measured to assess kidney function and damage: serum and urinary creatinine using the Jaffé method, cystatin C in the serum and urinary albumin (all from Abbott, Baar, Switzerland). In our hands, the intra-assay coefficient of variation (CV; n=20) for creatinine was 1.5% at 60 µmol/L, 1.0% at 168 µmol/ L, and 0.7% at 624 µmol/L. The respective CV's were 1.8% at 0.7 mg/L and 2.0% at 3.5 mg/L for cystatin C, and 1.6% at 32.5 mg/L, 1.5% at 119.5 mg/L for urinary albumin.

2.3.3. Statistical analysis

Data were analyzed with IBM SPSS® Statistics 19.0 and Microsoft Office Excel 2007. Chi-square tests and t-tests were used for categorical and continuous variables, respectively. A two-tailed p value <0.05 was considered statistically significant.

The eGFR was calculated with the CKD-EPI equation (Levey et al., 2009; Levey et al., 2010b). All patients were stratified into CKD stages using the classification recently proposed by KDIGO (Levey et al., 2011).

Extrapolation of CKD prevalence in primary care to national level was based first on the 3,769,686 Swiss patients older than 15 years of age who had visited a GP at least once in 2007 (i.e. 62.7% of the Swiss population >15 years), as reported by the Swiss Federal Statistical Office (FSO, 2013). Secondly, using the percentages calculated in our study sample and adjusting the results for age and gender, the prevalence of CKD patients in primary care in Switzerland was estimated.

Generalized linear models were fitted to control for factors that may be related to reduced eGFR or elevated ACR. In the first model, age, gender, and clinical characteristics of the study population were entered. In the second model, the laboratory parameters were analyzed. All variables showing significant results were used in a third model. The variables confirming significance in the third

generalized linear model were finally combined in a simple linear regression analysis. The coefficient of determination (R square) and Pearson correlation coefficients were calculated. Significant variables were tested for multicollinearity by calculating the variance inflation factors (VIFs).

2.4. RESULTS

2.4.1. Socio-demographic and clinical characteristics of the study population

Among the 1,000 individuals recruited, 57% were female, and the mean age was 57±17 years. The main socio-demographic and clinical characteristics of the patients according to gender are shown in Table 2.1.. Gender comparisons revealed that males had a significantly higher BMI, higher systolic and diastolic blood pressures, a higher mean arterial pressure, and a lower heart rate. Concerning co-morbidities, males reported a significantly higher prevalence of hypertension, diabetes, and myocardial infarction. Only depression was significantly more frequent among women. No relevant differences were found regarding family history for cardiovascular disease, diabetes or CKD, which were positive in about 30%, 20% and 5-6% of the patients, respectively.

Table 2. 1. Socio-demographic and clinical characteristics of the study population according to gender.

Clinical status	**Females** Mean±SD or %	**Males** Mean±SD or %	**P**
N	567	433	-
Age (years)	56±18	57±16	0.155
BMI (kg/m^2)	27±6	28±4	0.010
Systolic blood pressure (mmHg)	133±20	138±18	<0.001
Diastolic blood pressure (mmHg)	79±11	83±13	<0.001
Mean arterial pressure (mmHg)	97±13	101±13	<0.001
Pulse pressure (mmHg)	54±18	55±17	0.334
Heart rate (bpm)	74±11	71±12	0.001
Smoker	18.9	16.4	0.311
Hypertension	29.1	38.6	0.001
Depression	15.7	7.9	<0.001
Diabetes	12.0	17.3	0.012
Myocardial infarction	2.7	6.6	0.003
Heart failure	3.5	5.8	0.062
Family history of			
Diabetes	21.9	18.8	0.133
Cardiovascular disease	31.4	29.9	0.221
Chronic kidney disease	6.6	4.5	0.109

BMI, body mass index; bpm, beats per minute; mmHg, millimetre of mercury; N, number of subjects; SD, standard deviation.

2.4.2. Laboratory parameters

The results of the laboratory analysis showed that mean values of many parameters were significantly different between males and females (i.e. serum creatinine, albumin in the urine, urinary neutrophil gelatinase-associated lipocalin (NGAL), cystatin C, total cholesterol, high-density lipoprotein (HDL), high sensitive troponin, folic acid, ferritin, chloride, inorganic phosphate, HbA1c, alanine and aspartate transaminase, gamma-glutamyl transpeptidase, bilirubin total, and albumin). However, the majority of the laboratory parameters were within normal range for both genders (Table 2.2.). For both genders, elevated values were found for total cholesterol, fasting low-density lipoprotein (LDL), parathyroid hormone (PTH), and glycated hemoglobin (HbA1c). Females showed slightly elevated values of C-reactive protein (CRP) and inorganic phosphate, whereas males had slightly elevated alanine transaminase values.

Table 2. 2. Laboratory parameters in the recruited patient population.

Laboratory parameter	**Females** Mean [95% CI]	**Males** Mean [95% CI]	**P**
Kidney			
Serum creatinine (µmol/l)	71.4 [69.8-73.0]	87.7 [85.1-90.3]	<0.001
Albumin in urine (mg/l)	19.5 [16.0-22.9]	53.9 [40.3-67.5]	<0.001
Urinary NGAL (ng/l)	64.9 [46.8-83.0]	29.1 [23.9-34.4]	0.001
Cystatin C (mg/l)	0.81 [0.79-0.83]	0.86 [0.83-0.89]	0.004
Lipids			
Total cholesterol (mmol/l)	5.64 [5.53-5.74]	5.39 [5.28-5.49]	0.001
HDL (mmol/l)	1.66 [1.62-1.69]	1.35 [1.32-1.38]	<0.001
Fasting LDL (mmol) *	3.36 [3.13-3.60]	3.29 [3.09-3.49]	0.636
Fasting triglycerides (mmol/l) *	1.45 [1.32-1.58]	1.70 [1.46-1.95]	0.066
Inflammation			
High sensitive CRP (mg/l)	5.24 [4.47-6.00]	4.33 [3.42-5.24]	0.134
Heart disease			
BNP (pg/ml)	53.75 [47.7-59.8]	61.91 [46.5-77.3]	0.289
High sensitive troponin (ng/l)	1.94 [1.45-2.43]	3.74 [2.74-4.75]	0.001
Nutritional parameters			
Vitamin B12 (pg/ml)	292.6 [272.2-313.0]	289.8 [265.2-314.3]	0.858
Folic acid (mg/ml)	19.5 [18.6-20.4]	16.8 [15.9-17.6]	<0.001
Ferritin (ng/ml)	92.9 [81.5-104.4]	194.3 [177.9-210.7]	<0.001
Electrolytes			
Sodium (mmol/l)	141.8 [141.5-142.1]	141.7 [141.3-142.0]	0.580
Potassium (mmol/l)	4.82 [4.72-4.92]	4.75 [4.65-4.85]	0.330
Chloride (mmol/l)	103.3 [103.0-103.7]	102.6 [102.2-103.0]	0.004
Calcium phosphate metabolism			
Parathormone (pmol/l)	7.40 [7.01-7.79]	7.03 [6.54-7.52]	0.237
Calcium (mmol/l)	2.40 [2.39-2.41]	2.41 [2.39-2.42]	0.461
Inorganic phosphate (mmol/l)	1.70 [1.61-1.79]	1.51 [1.41-1.62]	0.009
Diabetes mellitus			
HbA1c (%)	5.93 [5.85-6.02]	6.09 [5.98-6.19]	0.023

Liver function panel			
Alanine transaminase (U/l)	26.2 [24.7-27.7]	39.3 [36.7-41.8]	<0.001
Aspartate transaminase (U/l)	26.7 [25.6-27.8]	31.7 [30.4-33.0]	<0.001
Gamma-glutamyl transpeptidase (U/l)	30.3 [26.4-34.1]	53.9 [47.0-60.7]	<0.001
Alkaline phosphatase (U/l)	72.9 [71.0-74.9]	72.3 [69.9-74.8]	0.701
Bilirubin total (µmol/l)	9.0 [8.6-9.4]	12.1 [11.5-12.7]	<0.001
Albumin (g/l)	44.1 [43.9-44.3]	45.0 [44.3-44.7]	<0.001

BNP, brain natriuretic peptide; CI, confidence interval; CRP, C-reactive protein; HbA1c, glycated haemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NGAL, neutrophil gelatinase-associated lipocalin. * N = 107 for females and 100 for males.

2.4.3. CKD prevalence in the study population

Overall, 41.1% of the patients had normal eGFR and ACR, whereas 35.9% of the subjects had slightly reduced excretory renal function (eGFR: 60-90 ml/min/1.73m^2) with physiological albuminuria based on normal ACR (<30 mg/g). About one tenth of the patients had a substantially reduced eGFR of <60 ml/min/1.73m^2, and 17.1% showed relevant proteinuria (ACR ≥30 mg/g). Almost one fourth of the analyzed subjects had CKD, i.e. they had either a substantial reduction in renal function or high levels of proteinuria (Table 2.3.).

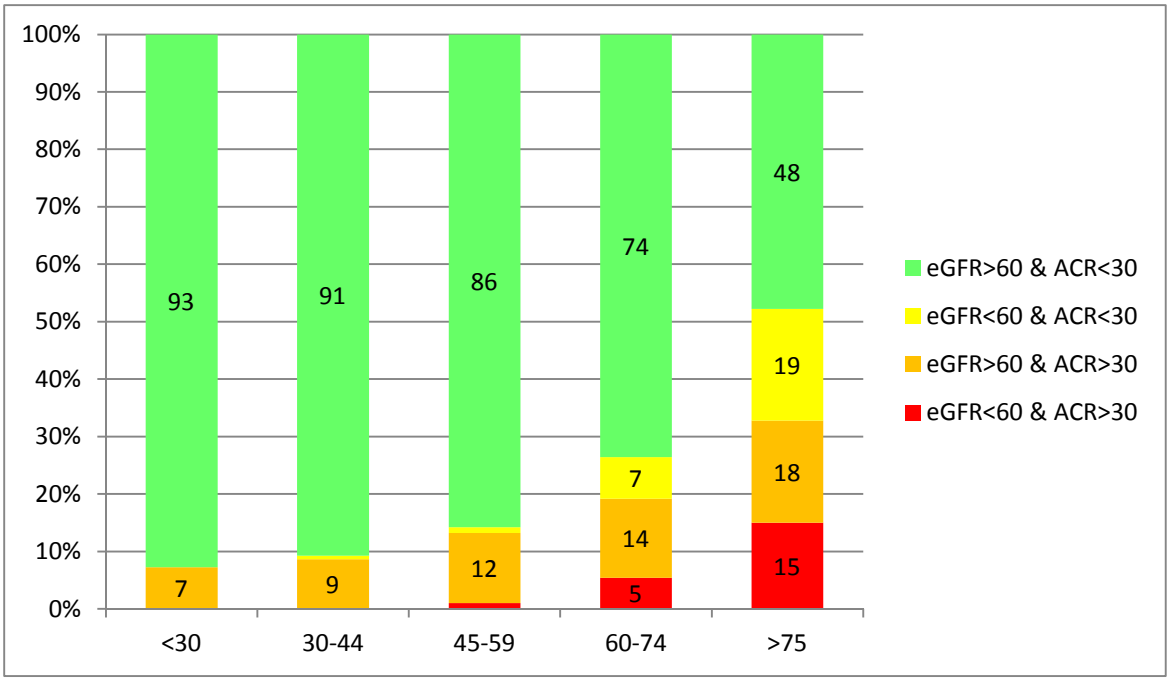
Table 2. 3. Chronic kidney disease stages, as proposed in the KDIGO classification.

	Albuminuria stage			
EGFR Stage (ml/min/1.73m^2)	**A1 (<10 mg/g)** N (%)	**A1 (10-29 mg/g)** N (%)	**A2-3 (≥30 mg/g)** N (%)	**All** N (%)
G1 (>105)	129 (12.9)	60 (6.0)	27 (2.7)	216 (21.6)
G1 (90-105)	156 (15.6)	66 (6.6)	33 (3.3)	255 (25.5)
G2 (75-89)	144 (14.4)	99 (9.9)	33 (3.3)	276 (27.6)
G2 (60-74)	59 (5.9)	57 (5.7)	33 (3.3)	149 (14.9)
G3-5 (<60)	25 (2.5)	34 (3.4)	45 (4.5)	104 (10.4)
All	513 (51.3)	316 (31.6)	171 (17.1)	1000 (100)

EGFR, estimated glomerular filtration rate (calculated using the CKD-EPI formula); KDIGO, Kidney Disease, Improving Global Outcomes.

CKD prevalence in our study population was clearly associated with increasing age: below 60 years of age CKD prevalence showed a slow increase (from 7% to 14%). Thereafter it increased faster, reaching 26% for patients aged 60-74 years and 52% for patients over 75 years of age (p<0.001; Figure 2.1.). If compared to patients without renal disease, CKD patients showed a more balanced gender distribution (51.3% vs. 58.3% females, p=0.060), but were significantly older (67±16 vs. 53±16 years, p<0.001), and had significantly higher BMI (28±5 vs. 27±5, p=0.001). Moreover they showed a significantly higher prevalence of diabetes (28.3% vs. 10.1%, p<0.001), hypertension (53.9% vs. 27.0%, p<0.001), myocardial infarction (8.7% vs. 3.0%, p<0.001), and heart failure (10.9% vs. 2.6%, p<0.001). No significant differences were found concerning family history of diabetes, cardiovascular diseases, and CKD.

Figure 2. 1. Percentage of patients with reduced eGFR and/or elevated ACR for different age groups.



ACR, albumin-creatinine ratio (given as mg/mmol); eGFR, estimated glomerular filtration rate (given as ml/min/1.73m^2).

2.4.4. Regression analyses

In order to control for factors that may be related to eGFR reduction or to an ACR increase regression analysis was performed. Concerning eGFR, in the first generalized linear model, age ($p<0.001$), gender ($p<0.001$), and heart failure ($p<0.001$) were significantly and independently correlated with eGFR. In the second model, statistically significant correlations with eGFR were found for cystatin C ($p<0.001$), total cholesterol ($p<0.001$), HDL ($p<0.001$), high sensitive C-reactive protein (CRP, $p=0.033$), brain natriuretic peptide (BNP, $p=0.007$), folic acid ($p=0.001$), sodium ($p<0.001$), inorganic phosphate ($p<0.001$), HbA1c ($p<0.001$), alanine transaminase ($p=0.001$), and albumin ($p=0.024$). In the third model combining all significant factors, gender, age, cystatin C, HDL, BNP, sodium, inorganic phosphate, HbA1c, and albumin remained significantly correlated with eGFR. The combination of the variables from the third model in a simple linear regression model confirmed a strong relationship with the eGFR ($R = 0.839$, adjusted R square = 0.701). Particularly strong correlations were found for age (Pearson correlation coefficient $\rho = -0.648$) and cystatin c ($\rho = -0.667$). No multicollinearity problems were found (Table 2.4.).

For ACR, the first model showed significant correlations with gender ($p=0.019$), age ($p=0.036$), heart rate ($p=0.021$), diabetes ($p=0.001$), and heart failure ($p=0.016$). In the second model, significant results were found for urinary NGAL ($p=0.001$), cystatin C ($p=0.001$), BNP ($p<0.001$), and HbA1c ($p<0.001$). After combining all significant factors in a third model, gender, heart rate, diabetes, heart failure, urinary NGAL, cystatin C, BNP, and HbA1c remained significantly and independently correlated with ACR. The combination of these variables in a linear regression analysis showed a weak relationship ($R = 0.439$, adjusted R square = 0.186). The highest Pearson correlation coefficients were found for BNP ($\rho = 0.322$), cystatin c ($\rho = 0.247$), and HbA1c ($\rho = 0.215$). Again, no multicollinearity problems were found (Table 2.4.).

Table 2. 4. Spearman correlation coefficients (ρ) between eGFR/ACR and the significantly correlated variables.

eGFR

Variable	ρ	P	VIF
Age	-0.648	<0.001	1.517
Gender	0.089	<0.001	1.231
Cystatin C	-0.667	<0.001	1.460
HDL	-0.043	0.012	1.253
BNP	-0.298	0.015	1.151
Sodium	-0.156	<0.001	1.111
Inorganic Phosphate	-0.256	<0.001	1.076
HbA1c	-0.229	0.017	1.145
Albumin	0.128	0.003	1.121

R = 0.839, adjusted R square = 0.701.

ACR

Variable	ρ	P	VIF
Gender	0.102	0.001	1.040
Heart rate	0.098	<0.001	1.023
Diabetes	0.169	0.049	1.545
Heart failure	0.121	0.028	1.270
Urinary NGAL	0.154	<0.001	1.027
Cystatin C	0.247	<0.001	1.213
BNP	0.322	<0.001	1.282
HbA1c	0.215	0.001	1.588

R = 0.439, adjusted R square = 0.186.

BNP, brain natriuretic peptide; HbA1c, glycated haemoglobin A1c; HDL, high-density lipoprotein; NGAL, neutrophil gelatinase-associated lipocalin; VIF, variance inflation factor.

2.4.5. Extrapolation to national level

In the extrapolation of CKD prevalence to the national level we made the assumption, that about 60% of the Swiss population visit a primary care physician at least once yearly (Table 2.5.). Of those, almost 19% (i.e. ca 700,000 patients, 11.4% of the subjects older than 15 years) may suffer from CKD, having a substantially reduced eGFR (<60 ml/min/1.73m^2) and/or relevant proteinuria (ACR ≥30 mg/g).

Table 2. 5. Chronic kidney disease prevalence in primary care.

Age group	N in CH	With at least 1 GP visit	CKD (eGFR <60 or ACR ≥30)
15-24	944 947	530 348	39 758
25-34	948 865	483 491	54 666
35-44	1 217 255	638 988	46 409
45-54	1 064 447	610 875	84 456
55-64	895 114	601 024	113 482
65-74	610 651	475 489	138 218
75+	505 433	429 471	226 665
Total	6 186 712	3 769 686	703 655
%	100.0%	60.9%	11.4%

ACR, albumin-creatinine ratio (given as mg/mmol); CH, Switzerland; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate (given as ml/min/1.73m^2); GP, general practitioners.

2.5. DISCUSSION

This study shows that CKD prevalence and/or renal function impairment in the general Swiss population is considerably high. In our sample only about 40% of the patients had a normal renal function with an eGFR ≥90 ml/min/1.73m^2 and an ACR <30 mg/g. About one third of the subjects showed slightly reduced filtration rate (eGFR 60-89 ml/min/1.73m^2) with physiological ACR, whereas 23% of the patients fulfilled the criteria of CKD (i.e. eGFR <60 ml/min/1.73m^2 and/or ACR >30 mg/g), as defined by KDIGO (Levey et al., 2011).

Our study sample, which was derived from a primary care population, is nevertheless comparable to that of the Swiss Survey on Salt, and, to some extent, to the general Swiss population. In the Swiss Survey on Salt, a prospective, nationwide survey conducted in 2010-2011 with a random sample of 1,377 subjects, the mean age was 47.3 years, with 51.2% females, 17.3% current smokers, a mean BMI of 25.1 kg/m^2, and a 25.6% prevalence of hypertension (32.3% and 19.1% for male and females

respectively) (Forni et al., 2011). At national level, the Swiss Federal Statistical Office reports the mean age of the adult patients visiting at least once a primary care physician in 2007 being 48.7 years (48.9% males) (FSO, 2013). Moreover, the following prevalence were estimated within the Swiss population in 2007: 27.9% smokers (32.3% males, 23.6% females), 15.0% hypertension (15.9%, 14.1%), 8.0% depression (6.2%, 9.8%), 3.0% diabetes (3.5%, 2.5%), and 2.1% myocardial infarction (3.1%, 1.2%) (FSO, 2013).

By extrapolation to national level, and after adjustment for age and gender, the percentage of patients in primary care that may have CKD is high, with almost 19% of the primary care population having substantially reduced renal function and/or relevant proteinuria. These results, again, are comparable to those found in the Swiss Survey on Salt, with a reported prevalence of about 7.7% of the included population for CKD stage 3 or higher. In our study, 10.4% of the patients had an eGFR <60 ml/min/1.73m^2. The difference of almost 3% may be explained by diverse recruitment strategies: whereas in our study the subjects were recruited in a primary care setting, in the Swiss Survey on Salt, the participants were recruited using a list of randomly selected households from the major Swiss telecommunication company's home phone directory. For each household, one person was randomly selected and invited to participate in the study. It is reasonable to suppose that this sample was not only younger if compared to our study population, but also healthier and therefore less likely to suffer from CKD and other diseases. In the US National Health and Nutrition Examination Survey (NHANES) 1999-2006, a representative cross-sectional national survey, 9,536 participants were interviewed at home and/or received standardized medical examination in mobile study centers (Whaley-Connell et al., 2009). The prevalence of CKD was 18.3% (9.1% for CKD stage 3-5). Again, these rates are comparable to our results.

The generalized linear regression models showed that both eGFR and ACR are strictly correlated with gender, cystatin C, BNP, and HbA1c. It is interesting to note that whereas eGFR was correlated with age and several blood/urine parameters (e.g. sodium, HDL, inorganic phosphate), ACR was age independent and directly correlated to diabetes and heart failure. These results emphasize the importance of ACR as screening and prognostic factor for young patients and for patients with diabetes and/or heart failure. For example, in the Kidney Early Evaluation Program (KEEP) Annual Data Report 2007, it has been shown that ACR is the predominant positive screening test for younger age groups: in KEEP, about 80% and 60% of the CKD patients aged 18-30 and 31-45 years, respectively, showed elevated ACR with normal eGFR. Even higher percentages were found in the NHANES cohort from 1999-2004 (McCullough et al., 2008b). In a case control study including non-diabetic and non-hypertensive patients it has been found that elevated ACR was significantly higher in patients with systolic heart failure, if compared to matched controls (Figueiredo et al., 2008).

Some limitations of the study have to be considered. Firstly, screened subjects were volunteers and therefore not necessarily representative for the overall primary care population in Switzerland. In this study, emergency patients were excluded for ethical reasons. Moreover, it is possible that patients

visiting the GP with a relatively serious/painful disease tend to refuse to participate. This may have resulted in an underestimation of the true CKD prevalence. A second limitation concerns the use of a creatinine based estimation for renal filtration function. In the last years, many formulas have been developed to calculate eGFR: the Modification of Diet in Renal Disease (MDRD) formula, the Mayo Quadratic formula, and the CKD-EPI formula (Levey et al., 1999; Rule et al., 2004; Rigalleau et al., 2007; Risch et al., 2007; Levey et al., 2009). Actually, the CKD-EPI equation seems to be the more precise formula for primary care patients (Levey et al., 2010b; Stevens et al., 2011; Matsushita et al., 2012a; Matsushita et al., 2012b). However, it is not yet recognized as the gold standard. The third limitation regards the cross-sectional nature of the study, in which only one single measurement per patient has been performed. The absence of a repeated eGFR assessment may potentially have resulted in misclassification of some patients (e.g. of individuals with acute changes in kidney function). In a study conducted by Bottomley et al., the potential overestimation of CKD prevalence after a single eGFR measurement was investigated in 512 factory workers (60.9% males, mean age 43 years) (Bottomley et al., 2010). The repeat analyses conducted 3 months after baseline evaluation revealed no significant change in the mean eGFR. However, 21% of the retested individuals had a change in their category of CKD stage and initial proteinuria was reproducible in only 48% of the cases. In a larger community based study including more than 20,000 patients over 45 years of age, Weiner et al. found that in 76.2% of the patients with initial eGFR <60 ml/min/1.73m^2 and in 83.6% of those with eGFR ≥ 60 ml/min/1.73m^2 a stable level of renal filtration function was found at follow up (Weiner et al., 2009). In this report, the study groups had a mean age of 73.4 and 59.9 years, respectively (55% females in both groups) and the follow-up was performed about 3 years after the baseline visit. In these studies, the MDRD estimating equation was used, and the participant's characteristics were clearly different from our trial. However, the results emphasize the potential of misclassification related to a cross-sectional design. Therefore, it would have been preferable to conduct a longitudinal study with multiple measurements over time to confirm and to adjust the estimated prevalence of CKD and ACR. Moreover, our study excluded paediatric patients, what confines our conclusions to adults. Beside the intrinsic limitations of a cross-sectional design, it is important to remember that the decline of eGFR with ageing is a sign of physiological senescence. With increasing age and consequent decline in muscle mass there is a consecutive reduction in creatinine generation. In the Nijmegen Biomedical Study including about 6,000 apparently healthy persons aged 18-90 years, the eGFR declined approximately 0.4 ml/min/1.73m^2 per year (Wetzels et al., 2007). Moreover, an eGFR of 60 ml/min/1.73m^2 was within the 25th and 50th percentile for men and women >65 years. In another study including more than 10,000 individuals 66 years of age or older, eGFR reductions of 0.8-1.4 and 2.1-2.7 ml/min/1.73m^2 per year were reported for non-diabetic and diabetic subjects, respectively (Hemmelgarn et al., 2006). These data emphasize that a low eGFR in elderly subjects does not necessarily imply that they have kidney disease. Unfortunately, the current NKF-CKD classification does not take into account these aspects. In general, for people with eGFR <60 ml/min/1.73m^2 or ACR >30 mg/g (i.e. a suspected CKD) further tests should be performed to determine the type and duration of kidney disease. If the duration is >3 months, CKD is formally

established. For elderly patients with slight to moderate reduction of renal function it is particularly important to monitor for rapid progression, defined as a sustained decline in eGFR of more than 5 ml/min/1.73m^2 per year (KDIGO, 2013). Moreover, some studies have shown that decreased eGFR is independently associated with increased risk of cardiovascular diseases or death (O'Hare et al., 2006). Therefore, a rapid decline in renal function may necessitate an adaptation in treatment strategy. Finally, it is important to note that the majority of previously diagnosed CKD patients, especially those with severe CKD stages requiring dialysis, are usually seen by nephrologists. Therefore, this study should be considered as representative only for adult patients in a primary care setting.

2.6. CONCLUSION

In summary, CKD prevalence in a primary care population in Switzerland is high. The growing proportion of elderly people among the Swiss population and the increasing prevalence of many risk factors will result in an increase of CKD prevalence over the next decades. Implementation of prevention and screening programs will be crucial in the managing strategies of many healthcare systems, especially in western countries. In addition, overcoming the lack of CKD awareness must become part of future strategies. Unlike the United States, where educational efforts have been made to increase CKD awareness in the general population (e.g. the formation of the National Kidney Disease Education Program by the National Institutes of Health) (NKDEP, 2013), in Switzerland and in many other central European countries, CKD is still an underestimated disease (WHO, 2012). This study, providing new information on CKD prevalence, may represent a first important step towards challenging this issue. Future steps will be to evaluate the actual burden of CKD, to investigate the prevalence of CKD in an inpatient setting, to model possible trends, and to provide suggestions to avoid uncontrolled growth of the CKD population.

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3. INSIGHTS ON URINARY NGAL OBTAINED IN A PRIMARY CARE SETTING

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3.1. ABSTRACT

Background: A majority of patients developing acute kidney injury (AKI) receive medical care from their primary care physicians prior to the occurrence of conditions that predispose them to this complication.

Methods: To characterize the uNGAL concentrations in primary care patients and to assess these concentrations with regard to different reference ranges, we conducted a multicenter, cross-sectional study with random selection of general practitioners (GP) from all GP offices in seven Swiss cantons. 1,000 adults (566 females; mean age 57±17 years) were included.

Results: The median absolute uNGAL was 21ng/L. Elevated uNGAL (>100ng/L) together with normal kidney test results (eGFR and albuminuria) were found in 6.5% of all patients. Females had a significantly higher uNGAL than did males. Among a multitude of different clinical and laboratory variables, only age, gender, liver function parameters, WBC and CRP were significantly associated with uNGAL levels in a multivariate analysis. When examining the proposed KDIGO classification of chronic kidney disease, the uNGAL levels at the given eGFR stages changed with increasing albuminuria stages and vice versa.

Conclusions: Age, gender, markers of inflammation and liver function, exert influences on uNGAL concentrations. A substantial proportion of patients exhibited normal kidney testing together with elevated uNGAL, potentially identifying patients with increased renal stress and at increased risk for the development of AKI.

3.2. BACKGROUND

Neutrophil gelatinase-associated lipocalin (NGAL) was originally described as being covalently bound to matrix metalloproteinase 9 (MMP 9 or neutrophil gelatinase) purified from human neutrophils (Kjeldsen et al., 1993). NGAL has been found to be expressed not only in the bone marrow, in which neutrophils originate, but also in tissues prone to microbial exposure (e.g., the lung, trachea, salivary gland, prostate, uterus, stomach, and colon) (Cowland et al., 1997). The expression of NGAL has also been shown to increase 1,000-fold in renal tubular cells following tubular injury of ischemic, toxic, septic or immunologic origin (Devarajan, 2010). The level of this marker increases 2 hours after kidney injury and is thus indicative of acute kidney injury (AKI) in its early development (Mishra et al., 2005).

Given these characteristics, NGAL has also been described as a “troponin-like marker” of the kidney and has been considered the most promising AKI biomarker (Devarajan, 2010). AKI is a condition that is found in approximately 7% of hospitalized patients and is associated with a variety of undesirable

outcomes that may lead to the requirement of renal replacement therapy (Nash et al., 2002; Ricci et al., 2008). Preventive measures that slow or prevent the development of AKI and factors that protect renal function have been described (Joannidis et al., 2010). Ultimately, NGAL allows for the early recognition and treatment of patients who are developing AKI.

NGAL can be measured in urine or blood (Devarajan, 2010). When urinary NGAL (uNGAL) is assayed, normalized concentrations of excreted NGAL for urinary creatinine have been proposed to account for variations in urine output (Grenier et al., 2010). Despite the fact that females display lower creatinine excretion than do males, there are no gender-specific cut-offs available for the uNGAL/urinary creatinine ratio (Perrone et al., 1992).

Before patients are placed in situations that put them at risk for developing AKI, e.g., cardiac surgery, the application of contrast media, or intensive care, they often receive medical care by their primary care physicians (Khalil et al., 2008; Weber et al., 2011). The screening of patients for increased uNGAL concentrations prior to procedures predisposed to the development of AKI may allow for the identification of patients at high risk for the subsequent development of AKI, who may require special care. However, uNGAL concentrations may be nonspecifically increased before and after such a procedure. To the best of our knowledge, little is known about uNGAL concentrations in primary care patients. We therefore conducted a multicenter study in a primary care setting. The primary objective of the present investigation was to characterize uNGAL concentrations in this cohort. As a secondary objective, we investigated the associations of uNGAL with several clinical and laboratory parameters.

3.3. MATERIALS AND METHODS

3.3.1. Study population

This cross-sectional study was performed in seven of the 26 Swiss cantons and comprised all five Swiss cantons with university-affiliated medical faculties (i.e., Basel, Bern, Geneva, Vaud, and Zurich), as well as the largest canton in Central Switzerland (Lucerne) and the Italian-speaking canton of Ticino. These seven cantons represent all three major language regions in Switzerland (i.e., German, French, and Italian) and are home to nearly 60% of the entire Swiss population. Physicians were randomly selected from the total pool of general practitioners in each canton and invited to participate using simple random selection supported by a computer program generating random numbers. Overall, physicians from 33 offices accepted the offer to participate. The study center then defined the days when the practitioners were asked to include all their patients meeting the inclusion criteria, viz., age $\geq$ 18 years and the ability to provide written informed consent. Patients presenting as medical emergencies were excluded from participation, as were patients who were unable to provide informed consent. Furthermore, patients were excluded if their participation in the study might have

caused important delays in patient management. The study was approved by all seven cantonal ethical boards (Ethikkommission beider Basel (EKBB), Kantonale Ethikkommission Bern (KEK), Commission d'éthique pour la recherche clinique dans le Canton de Genève, Ethikkommission des Kantons Luzern, Comitato etico cantonale del Canton Ticino, Commission cantonale (VD) d'éthique de la recherche sur l'être humain, and Kantonale Ethikkommission (KEK) Zürich) and is in accordance with the Declaration of Helsinki, as revised in 2004.

3.3.2. Data collection

The socio-demographic status, clinical data and anthropometric measurements of the patients were reported by the recruited physicians via a questionnaire. Study participants provided venous blood, collected in Sarstedt Monovette EDTA- and separation gel-containing serum tubes (Sarstedt, Sevelen, Switzerland). A spot urine was collected in a Greiner Vacuette tube without preservatives (Greiner Bio One, Krems, Austria). After the serum samples were centrifuged, the samples were mailed to the central laboratory using next-day delivery service by the Swiss Postal Service. Laboratory analysis was performed on the day after the samples were received.

3.3.3. Laboratory methods

Laboratory parameters were determined on an Abbott ARCHITECT ci4100 analyzer platform (Abbott, Baar, Switzerland), a Sysmex XT-5000 hematology analyzer (Sysmex Digitana, Horgen, Switzerland) and a Bio-Rad D-10 HPLC system for the determination of HbA1c (Biorad, Pratteln, Switzerland). uNGAL was assessed using the Abbott ARCHITECT immunology module and commercially available control materials, and the results showed an intra-assay coefficient of variation (CV; n=20) of 2.3% at 21 ng/L, 4.5% at 194 ng/L and 1.7% at 1,221 ng/L. Additionally, the following parameters were measured to assess kidney function and damage: serum and urinary creatinine using the Jaffe method, cystatin C in the serum and urinary albumin (all from Abbott, Baar, Switzerland). In our hands, the intra-assay CV's (n=20) for creatinine was 1.5% at 60 µmol/L, 1.0% at 168 µmol/L, and 0.7% at 624 µmol/L. The respective CV's were 1.8% at 0.7 mg/L and 2.0% at 3.5 mg/L for cystatin C, and 1.6% at 32.5 mg/L, 1.5% at 119.5 mg/L for urinary albumin. The estimated GFR (eGFR) was calculated with the CKD-EPI equation (Risch et al., 2007; Levey et al., 2009). Glucose metabolism was assessed using HbA1c, whereas lipid status was determined by measuring total cholesterol, HDL cholesterol, LDL cholesterol assessed with the Friedewald formula, and triglycerides. Additional parameters included the following: brain natriuretic peptide (BNP) and high-sensitivity troponin I for heart disease; high-sensitivity C-reactive protein (CRP) for inflammation; a liver function panel consisting of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-

glutamyltransferase (γ-GT), alkaline phosphatase (AP), total bilirubin, and serum albumin; vitamin B12 and folate; ferritin; serum electrolytes (sodium, potassium, and chloride); and markers for calcium-phosphate metabolism (calcium, phosphate, and parathormone (PTH)). Hematological parameters included a complete blood count including leukocyte differential, reticulocyte count and hemoglobin concentration in the reticulocytes. Commercially available materials were used for quality control purposes.

3.3.4. Statistical analysis

uNGAL values were analyzed as absolute and relative values, normalized to urinary creatinine. To account for the dilution of urine, the data are presented as normalized values unless otherwise stated. Spearman rank correlations between uNGAL and various demographic, clinical and laboratory variables were calculated. Proportions were compared using the Chi-squared test. Continuous variables were compared with the Mann-Whitney U test for those between two groups and with the Kruskal-Wallis test for those among three or more groups. The Bonferroni correction was applied in cases of multiple testing in the post hoc analysis. To assess for the progressive development of uNGAL concentrations with progressive disease stages such as the proposed KDIGO classification for the stages of chronic kidney disease (CKD), trend analysis was performed by means of a one-way ANOVA employing polynomial linear contrasts and relative weighted p-values (Levey et al., 2011). Overall trends as well as trends for each eGFR and albuminuria stage were calculated after logarithmic transformation of the uNGAL values was performed to normalize the concentrations. Logistic regression modeling was used to assess the effects of age and gender on the relationships among the different anthropometric measurements, laboratory parameters and uNGAL concentrations. P-values <0.05 were considered significant. All calculations were performed with SPSS version 19.0 (SPSS Switzerland, Zurich) and Excel 2007 (Microsoft, Seattle, Washington, US).

3.4. RESULTS

A total of 1,000 individuals with a mean age of 57±17 years and a female proportion of 57% were included in the study. The detailed characteristics of the study population are given in table 3.1. and reflect the expected spectrum of a non-acutely ill cohort of primary care patients. Gender comparisons revealed that males had a significantly higher BMI (28±4 vs. 27±6 kg/m^2 for females, P=0.011), higher systolic and diastolic blood pressures (138/83 vs. 133/79 mmHg, P<0.001), a higher mean arterial pressure (101 vs. 97 mmHg, P<0.001), and a lower pulse (71 vs. 74 bpm, P=0.001). Concerning co-morbidities, males had a significantly higher reported prevalence of hypertension (38.6% vs. 29.2%, P=0.001), diabetes (17.3% vs. 12.0%, P=0.012) and myocardial infarction (6.6% vs. 2.7%, P=0.003).

Only depression was significantly higher among women (15.9% vs. 8.1%, P<0.001). Table 3.2. summarizes the results of the investigated laboratory parameters.

Table 3. 1. Detailed characteristics of the investigated cohort. Where appropriate, the mean ± standard deviation (SD) is given.

Characteristic	Mean±SD or %
Female	57
Age (years)	57±17
BMI (kg/m^2)	27±5
Systolic blood pressure (mmHg)	135±19
Diastolic blood pressure (mmHg)	81±12
Mean arterial pressure (mmHg)	99±13
Pulse pressure (mmHg)	54±17
Heart rate (bpm)	73±11
Smoker	17.9
Hypertension	33.2
Depression	12.6
Diabetes	12.9
Myocardial infarction	4.3
Heart failure	4.6

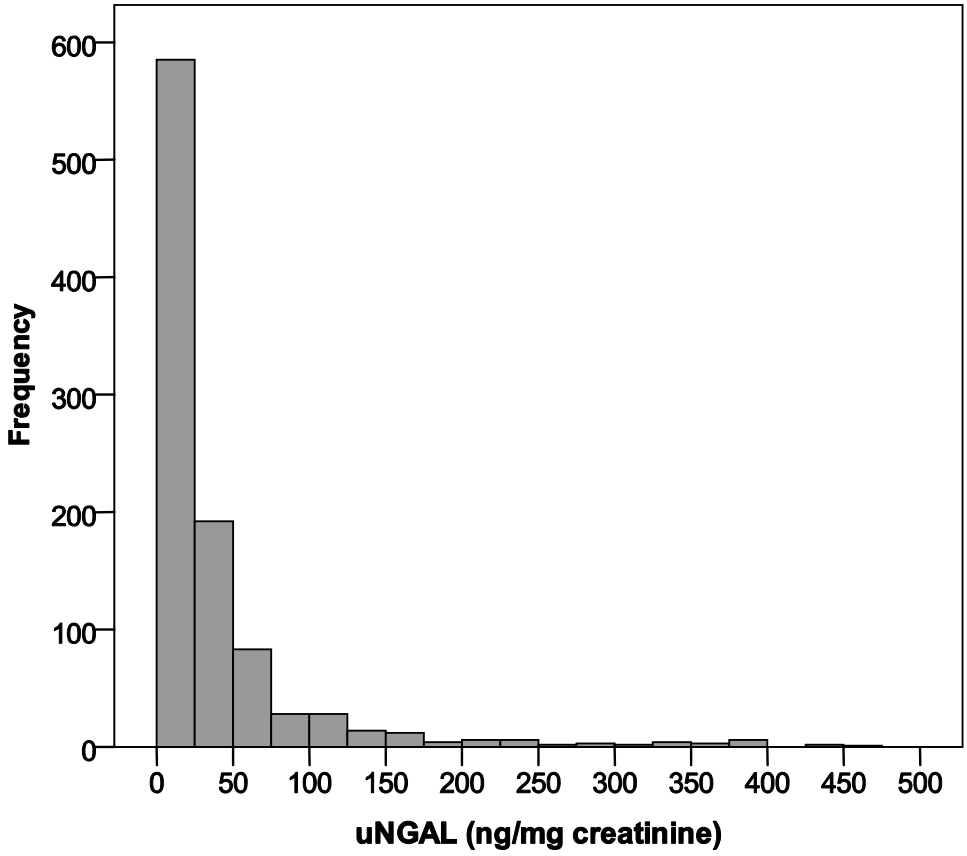
Table 3. 2. Summary of the investigated laboratory parameters grouped by pathophysiological system.

Parameter	Mean (95% CI)	Parameter	Mean (95% CI)
Kidney		***Diabetes mellitus***	
Urinary NGAL (uNGAL) (ng/L)	49.4 (38.9–59.9)	HbA1c (%)	6.00 (5.94–6.07)
Normalized uNGAL (ng/mg creatinine)	59.3 (41.0–77.6)	***Liver function panel***	
Serum creatinine (µmol/l)	78.4 (76.9–80.0)	Alanine transaminase (ALT) (U/l)	31.9 (30.4–33.3)
eGFR (CKD-EPI) (ml/min/1.73m^2)	87.7 (86.3–89.0)	Aspartate transaminase (AST) (U/l)	28.9 (28.1–29.7)
Cystatin C (mg/l)	0.83 (0.81–0.85)	Gamma-glutamyl transpeptidase (GGT) (U/l)	40.5 (36.8–44.3)
Albumin in urine (mg/l)	34.4 (28.1–40.6)	Alkaline phosphatase (ALP) (U/l)	72.6 (71.1–74.1)
Albumin-creatinine ratio (ACR) (mg/g)	33.9 (27.0–40.9)	Total bilirubin (µmol/l)	10.3 (10.0–10.7)
Lipids		Albumin (g/l)	44.5 (44.3–44.7)
Total cholesterol (mmol/l)	5.53 (5.45–5.60)	***Hematology***	
HDL (mmol/l)	1.52 (1.50–1.55)	Red blood cell count (10^{12}/L)	4.67 (4.64–4.70)
Fasting LDL (mmol) [N=208]	3.34 (3.18–3.49)	Hemoglobin concentration (g/L)	138.9 (138.0–139.7)
Fasting triglycerides (mmol/l) [N=208]	1.57 (1.43–1.70)	Hematocrit (%)	43.6 (43.4–43.9)
Inflammation		Reticulocyte count (10^{12}/L)	0.049 (0.048–0.050)
Highly sensitive CRP (mg/l)	6.00 (5.94–6.07)	MCV (fl)	93.7 (93.3–94.1)
Heart disease		MCH (pg)	29.9 (29.7–30.0)
BNP (pg/ml)	57.3 (49.8–64.8)	MCHC (g/L)	318.7 (317.8–319.7)
Highly sensitive troponin (ng/l)	2.72 (2.20–3.24)	Hemoglobin concentration in reticulocytes (pg)	33.8 (33.6–33.9)
Nutritional parameters		Red cell distribution width (RDW) (%)	14.17 (14.09–14.24)
Vitamin B12 (pg/ml)	291.4 (275.7–307.0)	Platelet count (10^9/L)	250.8 (246.6–254.9)
Folic acid (mg/ml)	18.3 (17.7–19.0)	Mean platelet volume (MPV) (fl)	11.1 (11.0–11.2)
Ferritin (ng/ml)	137.0 (126.9–147.1)	Platelet distribution width (PDW) (%)	13.4 (13.2–13.5)
Electrolytes		White blood cell count (WBC) (10^9/L)	6.81 (6.68–6.94)
Sodium (mmol/l)	141.7 (141.5–141.9)	Neutrophil granulocytes, absolute count (10^9/L)	4.12 (4.01–4.24)
Potassium (mmol/l)	4.79 (4.72–4.86)	Eosinophil granulocytes, absolute count (10^9/L)	0.167 (0.159–0.175)
Chloride (mmol/l)	103.0 (102.8–103.3)	Basophil granulocytes, absolute count (10^9/L)	0.017 (0.014–0.019)
Calcium phosphate metabolism		Lymphocytes, absolute count (10^9/L)	2.01 (1.96–2.05)
Parathormone (PTH) (pmol/l)	7.24 (6.93–7.54)	Monocytes, absolute count (10^9/L)	0.47 (0.46–0.49)
Calcium (mmol/l)	2.40 (2.39–2.41)		
Inorganic phosphate (mmol/l)	1.62 (1.55–1.69)		

The median absolute uNGAL concentration was 21 [interquartile range (IQR) 11,42] ng/L, and the median normalized uNGAL concentration was 20 [IQR 11,44] ng/mg creatinine. The distribution of normalized uNGAL concentrations is shown in figure 3.1.. Among the investigated primary care

patients, 10.3% [95% confidence interval (CI) 8.4,12.2] exhibited values above 100 ng/L, a recently employed decision limit (Haase et al., 2009; Haase et al., 2011). This proportion decreased to 6.2% [95% CI 4.9,7.9] at a decision limit of 150 ng/L. A total of 589 patients exhibited evidence of kidney damage, as indicated by albuminuria >30mg/g creatinine and/or decreased eGFR <90 ml/min/1.73m^2, whereas 411 patients had normal renal laboratory parameters (Levey et al., 2011).

Figure 3. 1. Distribution of normalized uNGAL concentrations in the investigated cohort.



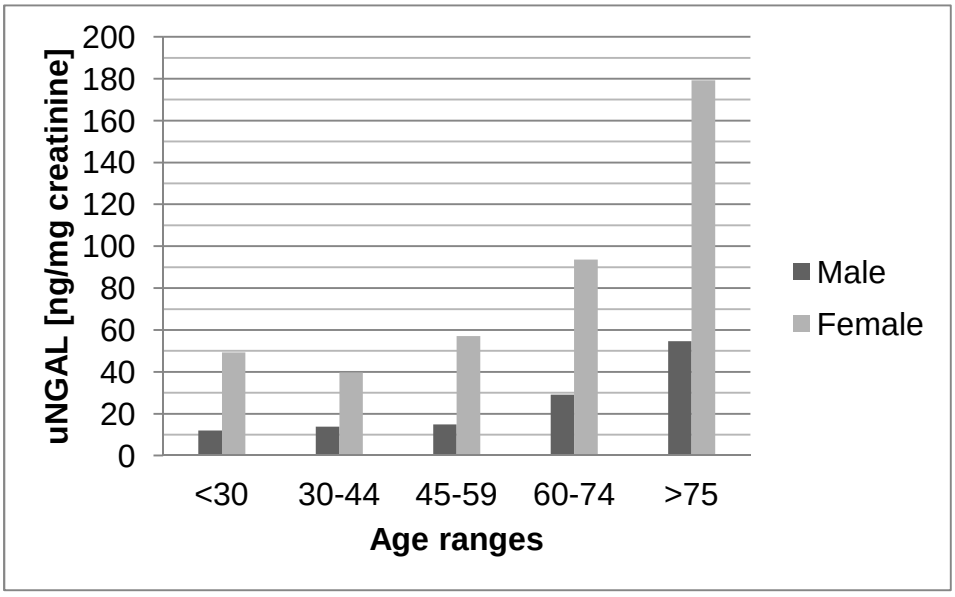
We also defined a non-healthy subgroup (n=798) with known cardiovascular disease, diabetes mellitus, kidney disease, malignant neoplastic disease, connective tissue disease and/or kidney damage as indicated by reduced eGFR<90 ml/min/1.73m^2 and/or albuminuria >30mg/g creatinine, similar to the approach reported in the Nijmegen Biomedical Study (Wetzels et al., 2007). In this subgroup, the prevalence of increased uNGAL>100 ng/L amounted 11.4% [95% CI 9.4,13.8], whereas the prevalence of increased uNGAL>150 ng/L was 6.4% [95% CI 4.9,8.3]. When looking at presumably healthy individuals being characterized by normal kidney markers (i.e. eGFR >90 ml/min/1.73m^2 and albuminuria <30mg/g creatinine) non-smoking status, an absence of known cardiovascular disease (i.e. history of coronary artery disease, stroke, peripheral vascular disease, arterial hypertension, heart failure), diabetes mellitus, kidney disease, malignant neoplastic disease,

and connective tissue disease reveals a subgroup of 202 individuals (Wetzels et al., 2007). This subgroup had significantly lower prevalences of patients with uNGAL >100 ng/L (5.9% [95% CI 3.5,10.1]; p=0.03) and patients with uNGAL >150 ng/L (1.5% [95% CI 0.5,4.3]; p=0.01) than the subgroup with non-healthy individuals.

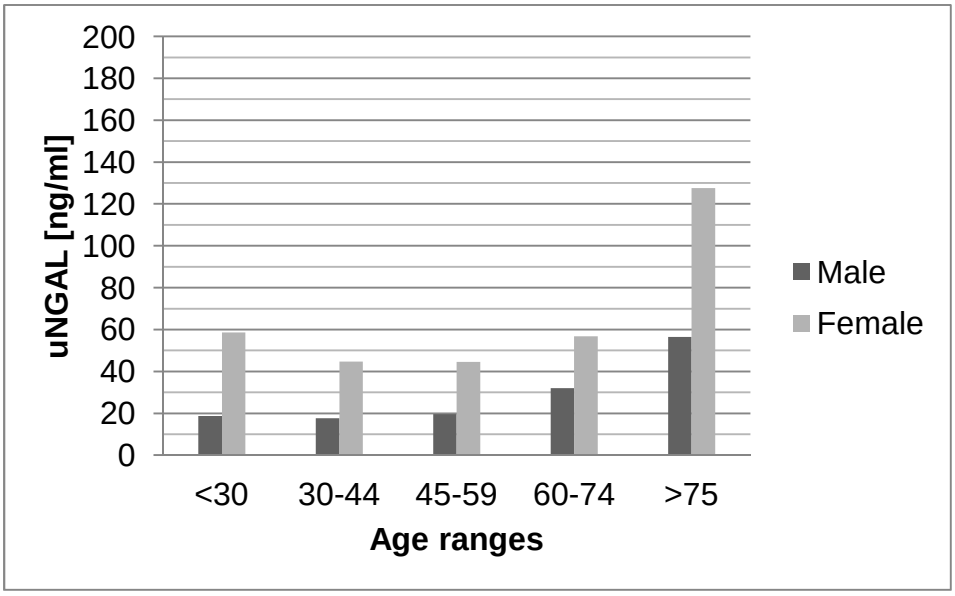
uNGAL levels were significantly correlated with age (ρ=0.18, p<0.001), heart rate (ρ=0.09, p=0.007), diastolic blood pressure (ρ=-0.14, p<0.001), mean arterial pressure (ρ=-0.09, p=0.005) and pulse pressure (ρ=0.07, p=0.03). Female patients had significantly higher uNGAL levels than did male patients (29 ng/mg creatinine [95% CI 15,65] vs. 13 ng/g creatinine [95% CI 8,22], p<0.001). In figure 3.2., uNGAL values are shown in both absolute concentrations and those relative to creatinine. We observed that the ratio between male and female uNGAL concentrations in each age stratum was significantly higher in the relative uNGAL than in the absolute uNGAL concentrations. Furthermore, it was evident that absolute and relative uNGAL concentrations increased with each age stratum. No associations were found between uNGAL and BMI, systolic blood pressure or language region in Switzerland.

Figure 3. 2. Stratification of mean uNGAL concentrations: a.) the concentrations normalized to creatinine; b.) the absolute concentrations. Each stratum shows significantly higher concentrations in female patients (p<0.001). As consequence of lower creatinine excretion in females, the difference is more pronounced in the normalized uNGAL values. Analogously, the increase of uNGAL concentrations with age is more pronounced in normalized concentrations. The more pronounced increase of normalized concentrations with age can also be attributed to a lower creatinine excretion occurring at older age (Perrone et al., 1992).

a.)



b.)



The correlations of uNGAL with various laboratory parameters are listed in table 3.3., demonstrating that the parameters of kidney disease, liver disease, inflammation, heart failure, glucose control, iron metabolism and calcium phosphate metabolism (Ca, PTH) are weakly correlated with uNGAL. Upon examining the hematologic parameters, we found that the red blood cell parameters and the counts of total white blood cells, platelets and phagocytic cells were correlated with uNGAL levels.

Table 3. 3. Spearman's correlation coefficients (ρ) between normalized uNGAL and risk factors/laboratory parameters.

Variable	ρ	P	Variable	ρ	P
Age	0.183	<0.001	***Calcium phosphate metabolism***		
BMI	-0.021	0.524	Parathormone	0.067	0.034
Systolic blood pressure	-0.022	0.490	Calcium	0.004	0.906
Diastolic blood pressure	-0.144	<0.001	Inorganic phosphate	0.120	<0.001
Mean arterial pressure	-0.091	0.005	***Diabetes mellitus***		
Pulse pressure	0.070	0.030	HbA1c	0.096	0.003
Heart rate	0.087	0.007	***Liver function panel***		
Kidney			Alanine transaminase	-0.198	<0.001
Serum creatinine	-0.183	<0.001	Aspartate transaminase	-0.171	<0.001
eGFR (CKD-EPI)	-0.163	<0.001	Gamma-glutamyl transpeptidase	-0.122	<0.001
Cystatin C	0.077	0.015	Alkaline phosphatase	0.117	<0.001
Urine albumin	0.038	0.236	Total bilirubin	-0.205	<0.001
Albumin-creatinine ratio (ACR)	0.271	<0.001	Albumin	-0.164	<0.001
Lipids			***Hematology***		
Total cholesterol	0.012	0.714	Red blood cell count	-0.293	<0.001
HDL	0.150	<0.001	Hemoglobin concentration	-0.347	<0.001
Fasting LDL (n=207)	-0.033	0.641	Hematocrit	-0.258	<0.001
Fasting triglycerides (n=207)	0.011	0.720	Reticulocyte count	-0.068	0.032
Inflammation			MCV	0.075	0.018
Highly sensitive CRP	0.131	<0.001	MCH	-0.073	0.023
Heart disease			MCHC	-0.182	<0.001
BNP	0.205	<0.001	Hemoglobin concentration in reticulocytes	-0.102	0.001
Highly sensitive troponin I	0.030	0.342	Red cell distribution width	0.161	<0.001
Nutritional parameters			Platelet count	0.147	<0.001
Vitamin B12	0.023	0.466	Mean platelet volume	-0.034	0.289
Folic acid	0.043	0.175	Platelet distribution width	-0.028	0.374
Ferritin	-0.211	<0.001	White blood cell count	0.065	0.040
Electrolytes			Neutrophil granulocytes, absolute count	0.074	0.021
Sodium	-0.023	0.473	Eosinophil granulocytes, absolute count	0.026	0.413
Potassium	0.049	0.120	Basophil granulocytes, absolute count	0.022	0.494
Chloride	-0.033	0.293	Lymphocytes, absolute count	-0.007	0.818
			Monocytes, absolute count	-0.106	0.001

After classifying patients according to the proposed KDIGO classification of chronic kidney disease, the mean uNGAL concentrations for all the different stages were calculated. As presented in table 3.4., with the exception of the G1 stage (eGFR >105 ml/min/1.73m^2), at a given eGFR stage, the uNGAL concentrations (after logarithmic transformation) increased significantly with increased albuminuria. Similarly, with the exception of the A1 stage <10 mg/mg, at a given albuminuria stage,

the log-transformed uNGAL concentrations increased significantly with decreasing eGFR. Interestingly, a total of 6.5% [95% CI 5.1,8.2] of the patients had no signs of kidney damage (i.e., eGFR >60 ml/min/1.73m^2 and albuminuria <30 mg/g creatinine) together with uNGAL levels >100 ng/L. This proportion declined to 2.8% [95% CI 1.9,4.0] when a cut-off of 150 ng/L was employed and to 2.0% [95% CI 1.3,3.1] with a cut-off of 200 ng/L.

Table 3. 4. Associations of uNGAL with the different stages of chronic kidney disease, as proposed in the KDIGO classification (Levey et al., 2011). With the exception of the A1 (<10 mg/g) and G1 (>105 ml/min/1.73m^2) stages, log-transformed uNGAL concentrations show a significantly increasing trend with decreasing eGFR and increasing albuminuria, respectively. The italic font indicates the p-value for tests for the trends in log-transformed uNGAL concentrations across each respective row and column. Different shading provides information on categories with increased risk for adverse outcomes, as described in the proposed KDIGO classification (Levey et al., 2011). Finally, bold italic font indicates the proportion of patients with an increased absolute uNGAL concentration >100 ng/ml together with the 95% confidence interval (CI).

			Albuminuria stage (mg/g creatinine)			
			A1 (< 10) mean±SD	A1 (10-29) mean±SD	A2-3 (30-1999) mean±SD	Total mean±SD
eGFR Stage (ml/min/1.73m^2)	G1 (>105)	uNGAL (ng/mg creat)	30.8±44.6	34.0±46.0	36.9±39.2	32.5±44.2
						p=0.151
		% >100ng/mL	***8.5 (6.8-10.2)***	***8.3 (6.6-10.0)***	***7.4 (5.8-9.0)***	***8.3 (6.6-10.0)***
	G1 (90-105)	uNGAL (ng/mg creat)	27.3±33.4	43.4±63.8	86.5±238.9	39.4±96.4
						p=0.001
		% >100ng/mL	***3.2 (2.1-4.3)***	***9.1 (7.3-10.9)***	***9.1 (7.3-10.9)***	***5.5 (4.1-6.9)***
	G2 (75-89)	uNGAL (ng/mg creat)	29.7±44.4	67.4±113.3	60.4±69.5	47.1±80.9
						p<0.001
		% >100ng/mL	***3.5 (2.4-4.6)***	***19.2 (16.8-21.6)***	***12.1 (10.1-14.1)***	***10.2 (8.3-12.1)***
	G2 (60-74)	uNGAL (ng/mg creat)	27.4±28.2	58.2±94.4	208.7±674.2	79.7±328.1
						p=0.003
		% >100ng/mL	***10.3 (8.4-12.2)***	***14.0 (11.8-16.2)***	***30.3 (27.5-33.1)***	***16.2 (13.9-18.5)***
	G3-5 (<60)	uNGAL (ng/mg creat)	37.6±50.0	95.0±152.5	296.7±1194.5	176.1±816.7
						p=0.016
		% >100ng/mL	***4.0 (2.8-5.2)***	***21.2 (18.7-23.7)***	***25.0 (22.3-27.7)***	***18.6 (16.2-21.0)***
	Total	uNGAL (ng/mg creat)	29.3±39.8	57.2±97.6	151.7±686.8	59.3±294.3
			p=0.459	*p=0.002*	*p=0.006*	*p<0.001*
		% >100ng/mL	***5.5 (4.1-6.9)***	***14.3 (12.1-16.5)***	***17.6 (15.2-20.0)***	***10.3 (8.4-12.2)***

Finally, logistic regression models were fitted to determine if the significant associations between the anthropometric measurements and laboratory parameters were independent of age and gender. A total of 103 patients had uNGAL levels above 100 ng/L. In the first model, the mean arterial blood pressure, heart rate, age and gender were entered into the model. In the second model, the kidney function parameters creatinine and albumin/creatinine ratio (ACR) as well as gender and age were entered. HDL, HbA1c, BNP, PTH, ferritin, phosphate, gender and age were entered into the third model. A fourth model included ALT, AST, γ-GT, alkaline phosphatase, bilirubin, albumin, gender and age. A fifth and final model included hemoglobin concentration, MCH, hemoglobin concentration in the reticulocyte, RDW, total WBC count, platelet count, gender and age. In all these models, only age, gender, CRP, WBC, ASAT, alkaline phosphatase and MCH remained significant. These models indicate that the most of the observed associations were due to the independent variables of age and gender.

3.5. DISCUSSION

This study showed that non-acutely ill patients already exhibit detectable uNGAL concentrations on routine primary care visits. Our results indicate that these concentrations increase with decreasing kidney function and increasing albuminuria. A substantial proportion of patients had elevated uNGAL concentrations of >100 ng/L with otherwise normal kidney tests (i.e., eGFR and ACR). Furthermore, the uNGAL level was observed to be associated with a variety of anthropometric measurements and laboratory parameters. Logistic regression modeling indicated that age and gender are important determinants of uNGAL concentration. In a recent review, Soni et al. stated that most studies on NGAL utilized small, homogeneous cohorts investigated in single-center settings (Soni et al., 2010). Our relatively large, heterogeneous cohort investigated in a multicenter setting can thus be regarded as a strength of the present investigation.

uNGAL is a protein composed of 178 amino acid residues and with a molecular weight of 25 kDa (Strong et al., 1998). Biologically, the main ligands for NGAL are siderophores, which are small iron-chelating molecules (Goetz et al., 2002). Dual actions for NGAL have been proposed: a) bacteriostasis by scavenging the iron needed for bacterial growth; and b) growth factor effects that modulate several cellular responses such as proliferation, apoptosis, and differentiation (Schmidt-Ott et al., 2007; Bolignano et al., 2009).

The finding that age and gender are critical determinants of uNGAL concentration is very interesting. Recently, a meta-analysis by Haase et al. reported that NGAL was substantially better at indicating acute kidney injury in children than in adults (Haase et al., 2009). We did not investigate children, and we did not investigate acutely ill patients. Nevertheless, the present work indicates that there is a variety of associations with uNGAL in stable adult patients and that most of these associations are

dependent on age and gender. It can thus be assumed that age confers disease states that bias uNGAL concentrations. A substantially lower occurrence of concomitant disease states in children may offer an explanation of why NGAL seems to perform better in children than in adults (Haase et al., 2009).

We showed that women have significantly higher normalized uNGAL (normalized to urinary creatinine) concentrations. This finding can be only partially explained by the fact that creatinine excretion in women is substantially lower than that in men (Perrone et al., 1992). A significant gender difference remains when examining only the absolute values. A similar phenomenon can be observed with older age (Perrone et al., 1992). Similarly, lower creatinine excretion with older age can only partially explain higher uNGAL levels in older age. To our knowledge, these gender and age differences have not previously been described, and the reasons for these differences remain unclear. We did not assess urinary leukocyte counts, which in urinary tract infections have been shown to correlate with uNGAL concentrations (Decavele et al., 2011). However, because only patients without acute diseases were included in our study, the influence of urinary tract infections, which is an acute disease occurring primarily in women, can be regarded as minor. Another explanation may be that during routine sampling conditions, the urine may have been contaminated by material originating from the anogenital tract. Together, our findings implicate the need for gender-specific and age-specific reference intervals and decision limits.

Furthermore, our results also illustrate the effect of gender-related differences in creatinine excretion, resulting in a more pronounced gender-specific difference in uNGAL concentrations. Because creatinine excretion is influenced not only by gender but also by a variety of other factors (e.g., muscle mass, age, diet, and muscle wasting) (Perrone et al., 1992), and because these factors frequently are encountered in intensive care settings (Wells et al., 1997; Erley et al., 2001; Bagshaw et al., 2008), some unknown amount of bias can be expected in situations where uNGAL testing is clinically indicated. For the accurate interpretation of test results, it is important to minimize such bias. The potential advantage of minimizing bias by accounting for urine dilution, as performed by normalization based on urinary creatinine, may be offset by the fact that a variety of non-renal factors influence creatinine excretion. Until further data are available, the normalization of uNGAL for urinary creatinine may be regarded as questionable (Waikar et al., 2010). Assessment of urine osmolality or urine conductivity could offer more accurate ways to account for urine dilution.

Damman et al. showed that uNGAL in patients with CKD is correlated with eGFR and albuminuria (Damman et al., 2008). The present study demonstrated that when albuminuria exceeds 10 mg/g creatinine and eGFR decreases to values below 105 ml/min/1.73m^2, uNGAL concentrations increase with decreasing eGFR and increasing albuminuria. This finding is particularly interesting because an increase in mean uNGAL concentrations mirrors the risk for developing complications of CKD, as shown in the proposed KDIGO classification of CKD (e.g., cardiovascular outcomes, death, or progression to end-stage renal disease).(Levey et al., 2011) uNGAL may thus represent a marker for

renal stress even in the absence of acute kidney injury (Haase et al., 2011; Paragas et al., 2011). Indeed, increased concentrations of uNGAL in patients with CKD have already been described as a marker for impaired kidney function and as a predictor of poor outcomes such as the progression of CKD (Mitsnefes et al., 2007; Bolignano et al., 2008; Bolignano et al., 2009).

James et al. described albuminuria and impaired eGFR as strong predictors for the development of AKI (James et al., 2010). Our investigation revealed a relatively large proportion of primary care patients with uNGAL concentrations >100 ng/L with an absence of indicators for kidney disease. We speculate that patients with normal eGFR and normal albuminuria but increased excretion of uNGAL are at high risk for the development of AKI. It may be worthwhile to investigate patients with this constellation prospectively to identify other patients at high risk for the development of AKI. It may be advisable to not only complement an eGFR assessment with that of albuminuria but, in some patients, also with a uNGAL assessment (Grams et al., 2010; Bydash et al., 2011; Thakar et al., 2011).

Non-renal conditions such as brain tumors, inflammatory bowel diseases, and breast cancer have been reported to present with increased NGAL concentrations (Fernandez et al., 2005; Manfredi et al., 2008; Smith et al., 2008; Poniatowski et al., 2009). The present study did not exclude patients suffering from these diseases. Nevertheless, the proportion of patients with tumors and inflammatory bowel disease was low. The nonspecificity of uNGAL for the development of AKI was also determined in patients presenting at an emergency department. In this setting, the prevalence of increased uNGAL was approximately 1%, which is somewhat lower than that observed in our investigation. Therefore, the uNGAL concentration in non-acutely ill primary care patients may be regarded to possess a limited specificity for predicting the development of AKI (Nickolas et al., 2008).

This study has several limitations. First, due to its cross-sectional nature, some patients may have developed AKI after their assessment, which could not be excluded. However, the frequency of elevated uNGAL concentrations seems much higher than what would be reasonably expected. Second, the study assessed only one uNGAL concentration. It can be assumed that a dynamic interpretation of at least two uNGAL concentrations would lead to an increase in the diagnostic specificity. Third, the investigated patients can be described as not having a clinical indication for the determination of uNGAL. However, before a patient undergoes cardiac surgery or receives a contrast media, he or she is often observed in a primary care setting. Hence, our study may indicate that a baseline uNGAL value should be determined before patients undergo procedures putting them at a higher risk for AKI, i.e., before acute kidney insult occurs. Fourth, eGFR is only an estimation of GFR. Regarding the performances of the MDRD and CKD-EPI equations, there is little doubt that these equations underestimate true measured GFR (mGFR), and so, induce an overestimation of CKD prevalence. We did not assess mGFR and hence the relationship between of CKD and uNGAL concentrations might be somewhat biased. In view of this, it has to be emphasized that so far (to the best of our knowledge) there are no studies available having comparing NGAL concentrations with

mGFR. Finally, the observed correlations are significant but are not strong. Therefore, they should not be overemphasized, and this information is not expected to play a major role in clinical practice. Nevertheless, our study points to the importance of age and gender as important determinants of the baseline levels for uNGAL. In summary, we believe that these limitations do not invalidate our findings.

3.6. CONCLUSION

Overall, our investigations identified age and gender as important determinants of uNGAL concentrations, and the determination of age- and gender-specific reference intervals or decision limits is suggested. Furthermore, we could demonstrate that the gender-specific and age-specific differences in uNGAL concentrations, when normalized to urinary creatinine, is more pronounced than in absolute uNGAL concentration, probably due to decreased creatinine excretion in females. This raises questions about the suitability of employing normalized values, especially in intensive care, where creatinine excretion is expected not only to be influenced by age and gender. Interestingly, uNGAL concentrations mirror the different stages of chronic kidney disease, and, consequently, the prognosis related to a respective CKD stage (Levey et al., 2011). Additionally, we identified a substantial proportion of patients with normal eGFR and normal albuminuria but increased uNGAL concentrations. Accordingly, uNGAL may be useful as a marker for renal stress, indicating a potentially increased risk for the development of AKI in these patients. However, these assumptions should be examined in further studies.

3.7. ACKNOWLEDGMENTS

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4. DISCUSSION

This Ph.D. thesis focuses on the epidemiology of CKD, on its diagnosis and on its relationship with AKI. In the first part of this work the prevalence of CKD in a Swiss primary care setting has been determined. The renal status of 1'000 patients recruited in seven of the 26 Swiss cantons, including all five Swiss cantons with university affiliated medical faculties (i.e. Basel, Bern, Geneva, Vaud, and Zurich), the largest canton in central Switzerland (Lucerne), and the Italian speaking canton of Ticino, has been evaluated. A CKD prevalence of 23% was assessed by using the most recent guidelines (KDIGO, 2013). The extrapolation to national level, adjusted for age and gender, revealed that about 11.4% of the Swiss population older than 15 years may suffer from CKD, having a substantially reduced eGFR (<60 ml/min/1.73m^2) and/or relevant proteinuria (ACR ≥30 mg/g).

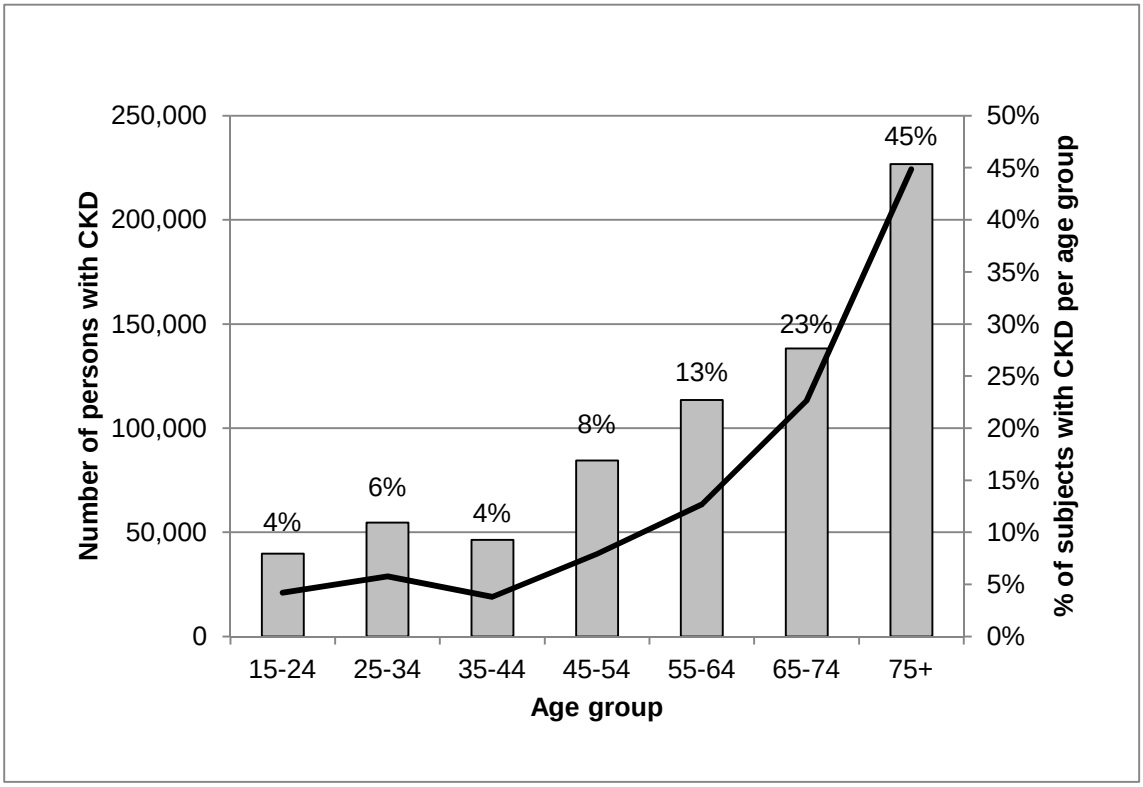
In the second part of this thesis the concentration of uNGAL in primary care has been investigated. The median absolute uNGAL level was 21 ng/L, and elevated uNGAL (>100 ng/L) together with normal kidney test results (i.e. normal eGFR and albuminuria) were found in 6.5% of all patients. Age and gender were indentified as important determinants of uNGAL concentrations: females and older subjects had significantly higher uNGAL concentrations. When examining the proposed KDIGO classification of CKD, uNGAL levels showed a positive relationship with different stages of CKD.

The main findings of the studies carried out during the present Ph.D. thesis are summarized and discussed in the following sections. Moreover, going beyond the publications represented in chapters 2 and 3, the relationship between NGAL and AKI as well as between AKI and CKD has been briefly reviewed. Finally, future research needs and recommendations based on the study results and on the actual scientific knowledge will be provided.

4.1. CHRONIC KIDNEY DISEASE PREVALENCE

The results of the first study suggest that the prevalence of impaired renal function and/or CKD in Switzerland is considerably high (Tomonaga et al., 2013). In the study sample, 23% of the patients fulfilled the criteria of CKD (i.e. eGFR <60 ml/min/1.73m^2 and/or ACR >30 mg/g), as defined by the KDIGO guidelines (KGIDO, 2013; Levey et al., 2011a). The extrapolation to the national level, adjusted for age and gender distribution, suggested that 11.4% of subjects older than 15 years (i.e. around 700'000 persons) in Switzerland may suffer from CKD. The prevalence of CKD seems to be around 4-6% in young adults (i.e. <45 years of age). Thereafter there is an age dependent increase of CKD prevalence: from 8% for subjects aged 45-54 years to 45% for subjects older than 75 years (Figure 4.1.). Every second individual who may have a CKD is older than 65 years of age, almost 30% are 45-65 years old, and 20% are younger than 45 years.

Figure 4. 1. Prevalence of chronic kidney disease in primary care.



The prevalence rates in the study sample were used to estimate the prevalence at primary care level. Overall, the prevalence of CKD in primary care has been estimated to be at 19%. Between 8% and 14% of the patients below 54 years of age visiting a general practitioner may suffer from a reduced renal function. Thereafter the percentage of patients who may suffer from CKD strongly increases with age: 19% for patients aged 55-64 years, 29% for patients aged 65-74 years, and 53% for patients older than 75 years.

Despite the difference in study design and study population, our study results are comparable with those of two recently published Swiss studies (Ponte et al., 2013; Forni et al., 2011).

In the CoLaus study, a Swiss population-based, cross-sectional study conducted in Lausanne in 2003-2006 the prevalence of CKD all stages was 10.0% (95% CI 9.2-10.8%) (Ponte et al., 2013). The sample for this estimation consisted of 5'921 non-stratified and randomly selected Caucasian participants, contacted and recruited by letter (participation rate was 41%). About 48% of the participants were males, and the mean age was 53 years. Concerning the sample characteristics, men reported a mean BMI of 26.6 Kg/m^2, 42.1% had hypertension, 9.3% diabetes, and 29.1% were active smokers. Women had a mean BMI of 25.1 Kg/m^2, 30.1% had hypertension, 4.1% diabetes, and 24.9% were smokers.

In the Swiss Survey on Salt, a prospective, nationwide survey conducted in 2010-2011, a prevalence of about 7.7% for CKD stage 3 or higher has been reported (Forni et al., 2011). The study population consisted of a random sample of 1'377 subjects, recruited using a list of randomly selected households from the major Swiss telecommunication company's home phone directory. For each household, one person was randomly selected and invited to participate in the study. The mean age was 47.3 years, with 51.2% females, a mean BMI of 25.1 kg/m^2, 25.6% prevalence of hypertension (32.3% and 19.1% for males and females, respectively), and 17.3% smokers.

In our study with 1'000 subjects recruited in a primary care setting, 57% were females, with a mean age of 56 years. Men had a mean BMI of 28 Kg/m^2, 38.6% had hypertension, 17.3% had diabetes, and 16.4% was smoking. Women had a mean BMI of 27 Kg/m^2, 29.1% had hypertension, 12.0% had diabetes, and 18.9% were smokers.

It is a real challenge to decide which study result may be more representative for the Swiss population. With the data collected by the Swiss FSO it has been estimated that in 2012 the mean age of the Swiss population was 41.3 years (47.7 years if only subject older than 15 years are included) (FSO, 2014a). The mean BMI was 24.7 Kg/m^2 (25.5 Kg/m^2 males, 23.8 Kg/m^2 females) (FSO, 2014b). Concerning the main co-morbidities hypertension and diabetes, the Swiss FSO reported that the prevalence of medically diagnosed high blood pressure in the Swiss population older than 15 years was around 21.8% (23.2% males, 20.5% females) in 2007 and 26.8% (28.0% males, 25.7 females) in 2012 (FSO, 2014c), whereas the prevalence of medically diagnosed diabetes in subjects older than 15 years was 4.5% (males 4.9%, females 4.2%) in 2007 and 4.7% (males 5.5%, females 3.9%) in 2012 (FSO, 2014d). In 2012, 28.2% of the population older than 15 years was smoking (32.4% males, 24.2% females) (FSO, 2014e). On the basis of these data and the study design, it may be argued that the most representative study is the Swiss Survey on Salt. Unfortunately, the estimation of CKD prevalence in this survey was exclusively based on eGFR, meaning that only subjects with at least CKD stage 3 have been taken into account. The CoLaus study, despite being Swiss population-based, cross-sectional study, included relatively old subjects with a high prevalence of hypertension. This might be due to selection effects: participants, who had to be 35-75 years old, were recruited per mail in a single city, with a participation rate of 41%.

The comparison with international literature published in the last five years shows a broad variation of CKD prevalence. Moreover, even if published recently, several studies used relatively old database (i.e. older than ten years). Depending on study design, study population and sampling year, the prevalence ranged from 2.6% for CKD stages 3-5 in 2007 in Finnland to 42% for CKD all stages in 2009-2010 in the UK (Juutilainen et al., 2012; Gifford et al., 2011). Despite the big range of data on CKD prevalence, the results of the most international studies were comparable with our data. For example, the NHANES and CDC reported, for the US population, prevalence rates of 13.1% in 1999-2004 and 15.0% in 2007-2012 respectively (Coresh et al., 2007; CDC, 2014). In China, the prevalence of CKD has been estimated between 10% and 16% (Zhang et al., 2012: 10.8%; Gu et al.,

2013: 12.5%; Jiang et al., 2010: 15.2%), whereas in Japan it ranges from 12.9% in 2005 to 19% in 2002 (Imai et al., 2009; Nagata et al., 2010). In the UK, Kearns et al. estimated a national prevalence of CKD stage 3-5 of 4.3% in 2009 (Kearns et al., 2013), whereas the prevalence in the Italian CAHRES and INCIPE studies were around 8% in 2011 and 13.2% in 2008 respectively (De Nicola et al., 2011; Gambaro et al., 2010). Table 4.1. summarises the estimated CKD prevalence reported in selected national and international studies. Different study populations, designs, and methodologies are probably the main reasons behind such large range of prevalence. Nevertheless, the central tendency towards prevalence rates between 10% and 15% is consistent with the results of our study.

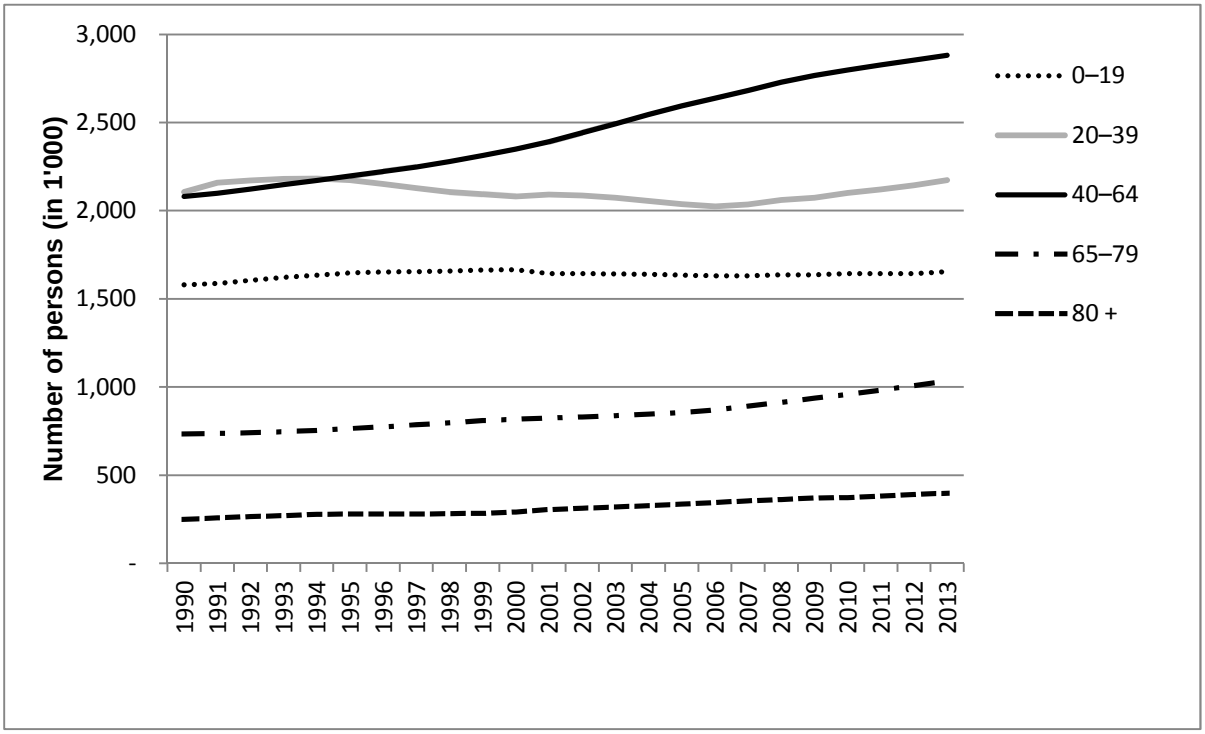
Table 4. 1. CKD prevalence in selected publications.

Author	Reference year	Country	Study type	CKD prevalence
Ponte et al., 2013	2003-2006	Switzerland	Population-based survey, >15 year	All stages: 10.0% Stage 3-5: 4.5%
Forni et al., 2011	2010-2011	Switzerland	Population-based sample, 35-75 years	Stages 3-5: 7.7%
Juutilainen et al., 2012	2007	Finnland	Population-based survey, 25-74 years	Stages 3-5: 2.6%
Gifford et al., 2011	2009-2010	UK	Population-based survey,> 18 years	All stages: 42.3% Stages 3-5: 11.8%
Kearns et al., 2013	2009	UK	Population-based survey, > 18 years	Stages 3-5: 4.3%
Glynn et al., 2009	unclear	Ireland	Primary care population, >50 years	All stages: 16.7%
De Nicola et al., 2011	2008	Italy	Population-based survey, 35-79 years	All stages: Males: 8.1% Females: 7.8% Stages 3-5: Males: 3.5% Females: 2.4%
Gambaro et al, 2010	2008	Italy	Random population sample, >40 years	Stages 1-4: 13.2%
Otero et al., 2010	2001	Spain	Random population sample, >20 years	Stages 3-5: 6.9%
Coresh et al., 2007	1999-2004	US	Population-based sample, >20 years	Stages 1-4: 13.1%
CDC, 2014	2007-2012	US	Population-based sample, >20 years	Stages 1-4: 15.0% Stages 3-4: 8.0%
Zhang et al., 2012	unclear	China	Population-based sample, adults	10.8%
Gu et al., 2013	2007-2009	China	Random population sample, >18 years	12.5%
Jiang et al., 2010	2006-2007	China	Population-based sample, >30 years	15.2%
Imai et al., 2009	2005	Japan	Population-based sample, >20 years	12.9%
Nagata et al., 2010	2002	Japan	Population-based sample, >40 years	19.0%

Whether it is more important to know the CKD prevalence at population level or at primary care level depends from the perspective. From an epidemiological point of view it is fundamental to know the total number and the distribution of renal problems in the whole population. The characteristics of the patients affected by CKD (in particular the age, gender, and CKD associated risk factors) may help to estimate future trends in CKD prevalence.

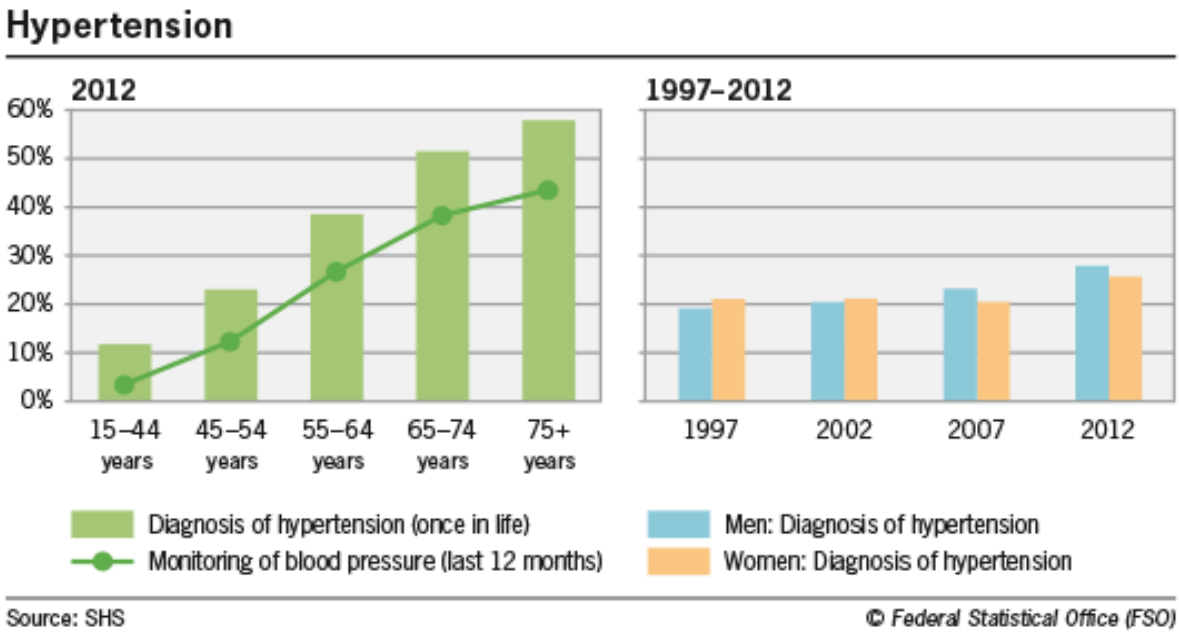
The statistics of the Swiss FSO concerning the three main risks for CKD, i.e. age, diabetes, and hypertension may give a hint about possible future trends. As illustrated in figure 4.2., in the past two decades the total number of persons living in Switzerland aged 0-19 years or 20-39 years remained almost constant (FSO, 2014a). In contrast, the groups including older subjects (40-64, 65-79, and more than 80 years) showed a constant increase. The age dependency ratio, defined as number of persons older than 65 years divided by the number of persons aged 20-64 years (ideally the labour force, i.e. the productive part of the population), was 23.5% in 1990 and 28.0% in 2012. The Swiss FSO estimates that it will reach 33.4% by 2020, 43.0% by 2030, and almost 50% by 2040.

Figure 4. 2. Age distribution in Switzerland, 1990-2013 (in 1'000).



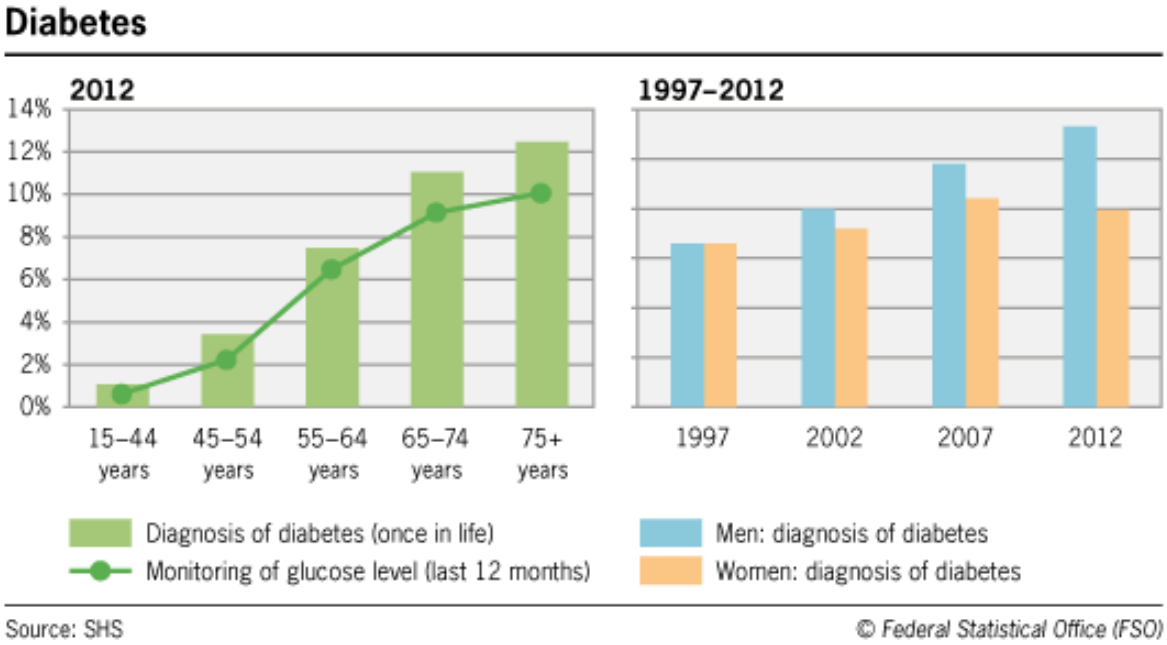
Concerning hypertension, the percentage of people already diagnosed with high blood pressure once during their lifetime increased by 7% within the last 15 years (FSO, 2014c). This increase was particularly noticeable among men and in general among persons aged 75 years and older (Figure 4.3.).

Figure 4. 3. Prevalence of hypertension in Switzerland.



Similarly, the percentage of people with diabetes increased since 1997, more strongly and more regularly among men than women (Figure 4.4.) (FSO, 2014d).

Figure 4. 4. Prevalence of diabetes in Switzerland.



Even assuming that the prevalence of hypertension and diabetes will reach a plateau and thus will stop increasing, the simple fact that the whole population is ageing suggests that the prevalence of renal problems may grow up in the next decades. For this reason, the introduction of preventive programs may be important to avoid or slow down the increase of CKD prevalence in the Swiss population.

From a healthcare perspective, it may be more valuable to know which percentage and what kind of patients visiting a GP may have a renal disease. In the Swiss health survey conducted in 2012, 70.9% of the males and 85.7% of the females older than 15 years in Switzerland reported at least one outpatient visits in the past 12 months. For older persons (aged 65 years or more), these percentages were higher than 86% for both genders (FSO, 2014f). With such high percentage of people visiting a physicians yearly it may be possible to perform opportunistic controls for patients at high risk, being aware of the age dependency of renal function and taking into account the major risk factors associated with CKD. As already mentioned in the introduction, there is actually no direct cure for CKD. Hence, early diagnosis, treatment of the underlying cause, and implementation of secondary preventive measures are fundamental for CKD patients in order to relieve symptoms, slow or prevent progression of the condition, and reduce the risk of developing related problems.

Primary prevention measures should per se aim to eliminate or reduce the risk factors of CKD. More specifically, this involves prevention strategies to reduce the incidence and prevalence of hypertension and diabetes, in order to reduce the number of subjects at high risk of developing CKD (e.g. healthy diet, regular physical activity, maintain healthy weight, manage stress, avoid tobacco smoke). Secondary prevention measures mainly consist of measures for early detection of CKD, allowing prompt interventions to prevent or delay CKD progression. In this optic, a regular control of the blood pressure, glucose levels, and renal function (i.e. of the eGFR and ACR) should be performed for persons at high risk. Treatment of CKD in early stages may include, besides patient education, ACE inhibitors or ARBs for blood pressure control, anti-diabetics for blood glucose control, and treatment of other co-morbidities.

4.2. NGAL AS MARKER OF CHRONIC KIDNEY DISEASE

This study showed that non-acutely ill patients already exhibit detectable uNGAL concentrations on routine primary care visits.

When comparing the subgroup of presumably healthy individuals to the subgroup of unhealthy subjects, a significantly lower prevalence of patients with uNGAL >100 ng/L was showed (5.9% [95% CI 3.5-10.1] versus 11.4% [95% CI 9.4-13.8]; p=0.03). Similarly, by using a uNGAL cut-off value of 150 ng/L, presumably healthy subjects reported lower prevalence if compared to unhealthy subjects

(1.5% [95% CI 0.5-4.3] versus 6.4% [95% CI 4.9-8.3]; p=0.01). Healthy individuals were characterized by normal kidney markers (i.e. eGFR >90 ml/min/1.73m^2 and albuminuria <30 mg/g creatinine), non-smoking status, an absence of known cardiovascular diseases (i.e. history of coronary artery disease, stroke, peripheral vascular disease, arterial hypertension, heart failure), diabetes mellitus, kidney disease, malignant neoplastic disease, and connective tissue disease.

uNGAL levels were significantly correlated with age (ρ=0.18, p<0.001), and female patients had significantly higher uNGAL levels than did male patients (29 ng/mg creatinine [95% CI 15-65] vs. 13 ng/g creatinine [95% CI 8-22], p<0.001). The logistic regression modeling confirmed that age and gender are important determinants of uNGAL concentration.

A recently published study conducted in 2011-2012 in Belgium and investigating the NGAL values in a sample of 338 randomly selected, non-smoking healthy volunteers aged 0-95 years, also reported significant gender effect on NGAL (p<0.0001) as well as significant correlation with age (p<0.0001) (Pennemans et al., 2013). The mean NGAL values for the age classes below 70 years, normalized for creatinine, ranged from 12.3 to 17.3 ng/mg creatinine in males and from 24.0 to 33.7 ng/mg creatinine in females. Similar results were reported in a study including 174 healthy subjects attending a routine health check clinic in Ireland (Cullen et al., 2012). The authors reported significant gender-related differences for NGAL (with women having higher concentration) as well as significant age-related differences for NGAL between age categories. The 95th percentile for NGAL was determined by 107 ng/ml.

Concerning the relationship with CKD, the results of the study included here indicate that the mean values of uNGAL and the percentage of patients with elevated uNGAL increase with decreasing kidney function and increasing albuminuria. Across eGFR stages, the mean uNGAL values raised from 32.5±44.2 ng/mg creatinine for stage G1 (>105 ml/min/1.73m^2) to 176.1±816.7 ng/mg creatinine for stages G3-5 (<60 ml/min/1.73m^2). At the same time, the percentage of patients with elevated uNGAL concentration (i.e. >100 ng/ml) raised from 8.3% [95% CI 6.6-10.0] to 18.6% [95% CI 16.2-21.0]. Similarily, across the ACR stages, the mean uNGAL values raised from 29.3±39.8 ng/mg creatinine for stage A1 (<10 mg/g) to 151.7±686.8 ng/mg creatinine for stage A2-3 (30-1999 mg/g). The percentage of patients with elevated uNGAL concentration (i.e. >100 ng/ml) raised from 5.5% [95% CI 4.1-6.9] to 17.6% [95% CI 15.2-20.0]. A substantial proportion of patients (6.5% [95% CI 5.1-8.2]) had elevated uNGAL concentrations of >100 ng/L with otherwise normal kidney tests (i.e., eGFR and ACR).

A study conducted in Netherlands showed that structural tubular damage, as measured by uNGAL concentrations, was highly prevalent in patients with chronic heart failure if compared to healthy controls (Damman et al., 2008). As in our study, both serum creatinine and eGFR were significantly associated with uNGAL levels. Similar results have been found in a Polish study including 92 non-diabetic adult patients with CKD stages 2-4 as well as in a sample of 45 US children with CKD stages

2-4 (Malyszko et al., 2008; Mitsnefes et al., 2007). In an Italian cohort study including 96 adults patients affected by non-terminal CKD (i.e. with eGFR >15 ml/min/1.73m^2), both serum and urinary NGAL were inversely, independently, and closely related to eGFR at baseline (Bolignano et al., 2009). After a median follow-up of 18.5 months, one third of the patients showed CKD progression, defined as doubling of baseline serum creatinine and/or onset of ESRD. The regression analysis showed that NGAL predicted CKD progression independently of other potential confounder (e.g. age and eGFR). The authors reported that an increase of 10 ng/ml of uNGAL was associated with a 3% increased risk of progression. In a more recent prospective observational cohort study performed in the UK and including 158 adult patients with CKD stage 3-4, the baseline creatinine-normalized uNGAL was associated with the combined endpoints of initiation of RRT or death, independent of conventional renal and cardiovascular risk factors (Smith et al., 2013). Higher baseline values were also independently predictive of rapid renal function decline (i.e. ≥5 ml/min/1.73m^2) over one year follow-up. Smith et al. concluded that creatinine-normalized uNGAL improves the prediction of risk of kidney disease progression in patients with established pre-dialysis CKD. Moreover, they also reported that adding the creatinine-normalized uNGAL to a multivariate model of conventional risk factors improved the risk estimates of disease progression also in patients with low-level proteinuria. This point may be of importance for patients experiencing a rapid decline in the eGFR, but in whom total urine protein excretion remains relatively normal (MacIsaac et al., 2014). The probably biggest study assessing the performance of uNGAL for outcome prediction has been performed in the US and included 3'386 patients with CKD in the Chronic Renal Insufficiency Cohort (CRIC) study (Liu et al., 2013). Over an average follow-up of 3.2 years, 689 patients showed CKD progression, defined as eGFR decrease by half or incident ESRD. Even after accounting for several CKD progression risk factors (i.e. eGFR, albuminuria), uNGAL remained a significant and independent risk factor, in particular in the first two follow-up years. However the authors concluded that adding uNGAL to a model including eGFR, proteinuria, and other risk factors, only modestly improved the prediction of outcome events. This last conclusion is basically in contrast with the observation of the UK study performed by Smith et al.. Review of the two studies reveals that they are barely comparable. Concerning the population characteristics, the US study included 3'386 patients, 47% were women, with a mean age of 58.2 years; about 42% of the participants were Caucasian, 44.5% had diabetes, and the mean BMI was 32.2 Kg/m^2; the mean follow-up was 3.2 years. In the UK study only 158 patients were included, 75% were males, with a mean age of 69 years; all participants were Caucasian, 25% had diabetes, and the mean BMI was 28.4 Kg/m^2; the follow-up was 2 years. Both studies were not originally designed for assessing the NGAL prediction power for CKD progression: the US study is based on the CRIC study, whereas the UK study is a sub-study of the Academic study. The US study included patients with CKD stages 2-4, and the Modification of Diet in Renal Disease study equation (MDRD) was used for the eGFR calculation. The English study included patients with CKD stages 3-4, and the eGFR was calculated with the CKD-EPI equation. Moreover, Liu et al. reported as limitation the fact that NGAL measurements were made on urine samples that were not rapidly processed after voiding: the patients were instructed to keep their 24-h urine sample

refrigerated at home up to one week before in-person visit. This suboptimal handling may have caused bias/error from protein degradation or other processes. In effect, the reported uNGAL concentrations in the US study seems to be lower than other studies of CKD cohorts (median 17.2 ng/ml, interquartile range 8.1-39.2 ng/ml). Finally, the definition of CKD progression and endpoints differed between the studies: in the US study, disease progression was defined as composite endpoint of incident ESRD or halving of eGFR (from baseline). In the UK study the primary composite endpoint was defined as death or initiation of RRT, whereas the secondary endpoint addressing rapid renal decline was a yearly eGFR decline of ≥5 ml/min/1.73m^2.

In summary, there is general agreement that NGAL is significantly correlated with serum creatinine and eGFR. Concerning its predictive potential for progressive CKD, there are discordant opinions. The fact that NGAL seems to mirror the risk for developing complications of CKD (e.g. cardiovascular outcomes, death or progression to ESRD), as shown in the KDIGO classification of CKD, suggests that it may be a good marker for CKD progression (Levey et al., 2011b). However it is not yet clear if adding a NGAL test to the classical prognostic factors for CKD (e.g. eGFR, albuminuria, age, gender, BMI, hypertension and diabetes) will substantially improve the prediction of outcome events in CKD patients.

An improved prediction of disease progression and clinical events may improve patient management in several ways. For example, high risk patients may be controlled more frequently and treated more intensively. For patients showing a particularly high probability of reaching ESRD, RRT might be planned in advance. Treating physiciansmay have more time to inform patients on the therapeutic options (hemodialysis, peritoneal dialysis, kidney transplantation). In case of hemodialysis, patients are minimally involved in the treatment procedures, but are required to adhere to specific schedules and travel to the dialysis unit. In contrast, peritoneal dialysis offers more independence (can be performed at home) and more flexible scheduling (requires less time, but has to be done every day of the week), but requires an active participation of the patient (the treatment is basically done by the patients himself, by a parent, or by a home nurse). Patients requiring dialysis but living far away from dialysis centres might opt for a peritoneal dialysis, or may simply decide to relocate to a more practical place. Patients requiring transplantation may have more time to find a possible donor.

Within well defined high risk CKD populations, specific studies may be conducted to better understand CKD itself. Many mechanisms and physical/molecular/genetic pathways leading to disease progression are still unknown.

As one of the most promising renal biomarkers, NGAL should be further investigated. Beside baseline NGAL values in the general population, the NGAL concentrations should be accurately investigated in well conducted, prospective studies including large and representative population samples (e.g. without exclusion of CKD patients with many co-morbidities). Possible associations between NGAL

and frequent co-morbidities in CKD patients (e.g. diabetes, hypertension, cardiovascular diseases, cancers, infections) should be investigated.

4.3. NGAL AS A MARKER OF ACUTE KIDNEY INJURY

Like creatinine and eGFR, NGAL seems to be associated with both AKI and CKD. NGAL is a protein expressed in renal tubular cells and neutrophils (Bolignano et al. 2008). In healthy people, NGAL is usually expressed at very low levels. In contrast, it is markedly induced in injured epithelial cells and increases dramatically following kidney damage (Devarajan et al. 2010). Early and reliable AKI detection is important in order to start with therapy as soon as possible. This may prevent irreversible changes in renal function and could improve patient outcomes.

The possible association between NGAL and CKD has already been discussed in the previous chapter. Following pages will give a short overview of the relationship of NGAL and AKI.

Both serum and urine NGAL were shown to be early biomarkers of AKI in several patient populations. Haase-Fielitz et al. have recently published a critical evaluation of the current status of NGAL as a marker of AKI (Haase-Fielitz et al., 2014). The authors analysed the results of 58 studies reporting on the use of NGAL for the early prediction and prognosis of AKI. The main results were divided in three major groups: NGAL for the prediction of AKI after cardiac surgery, NGAL for the prediction of AKI in critically ill patients, and NGAL for the prediction of AKI after kidney transplantation.

- NGAL for the prediction of AKI after cardiac surgery: NGAL, measured in 25 studies including in total more than 7'000 patients, showed a good predictive power for the subsequent development of AKI (Area under the curve (AUC) was 0.82 for uNGAL and 0.83 for serum NGAL). These results seem to be relatively robust, considering the fact that the comparability of the included studies may be questionable: many of them were single-centre studies with a limited sample size (<200 patients) and the AKI definition differed between studies. Moreover different NGAL cut-off values and different test devices were used.

- NGAL for the prediction of AKI in critically ill patients: the data of 22 studies including 8'500 patients admitted in emergency departments were analysed. Again, NGAL showed a good predictive power (AUC 0.80 for uNGAL and 0.79 for serum NGAL). One of the largest included studies showed in particular that when patients with eGFR <60 ml/min/1.73m^2 were excluded from the analyses, both plasma and urine NGAL (AUC 0.75±0.10 and 0.79±0.10 respectively) displayed diagnostic superiority over serum creatinine and eGFR (AUC 0.65±0.10 and 0.67±0.10 respectively) (De Geus et al. 2011). This suggests that the accuracy of NGAL in predicting AKI is highest in patients with normal renal function prior to acute illness. A multicentre prospective cohort study investigating several urinary biomarkers in 1'635 patients admitted to emergency

departments concluded that NGAL was the most useful biomarker (AUC 0.81 at a cut-off of 104 ng/ml) and that it was predictive of severity and duration of AKI (Nickolas et al., 2012).

- NGAL for the prediction of AKI after kidney transplantation: to investigate the prediction power of NGAL after kidney transplantation, 14 studies with in total 1'079 patients were analysed. The measurement of NGAL 6-12 hours after kidney transplantation showed a good predictive performance (AUC 0.87) for the prediction of delayed graft function. In a Finnish study conducted by Hollmen et al., uNGAL measured on the transplantation day identified patients with severe kidney injury and inferior long-term organ survival, predicting prolonged delayed graft function even in patients with good urine output and decreasing creatinine (Hollmen et al., 2011).

Two studies enrolling almost 4'000 cardiac surgical, critically ill, or emergency department patients analysed the prognostic power of the NGAL according to serum creatinine status (Nickolas et al., 2012; Haase et al., 2011). The authors reported that the NGAL concentrations complemented the information obtained by measuring the creatinine concentrations in diagnosing AKI and predicting prognosis. Patients with a "subclinical AKI", i.e. those with elevated NGAL but normal creatinine concentration had a 2-3 higher risk of death or need for RRT compared to patients with normal NGAL and normal creatinine concentrations.

Knowing which patients are at higher risk of death or RRT is important to ameliorate patient management by introducing early therapies and more regular controls.

4.4. CHRONIC KIDNEY DISEASE, ACUTE KIDNEY INJURY AND NGAL

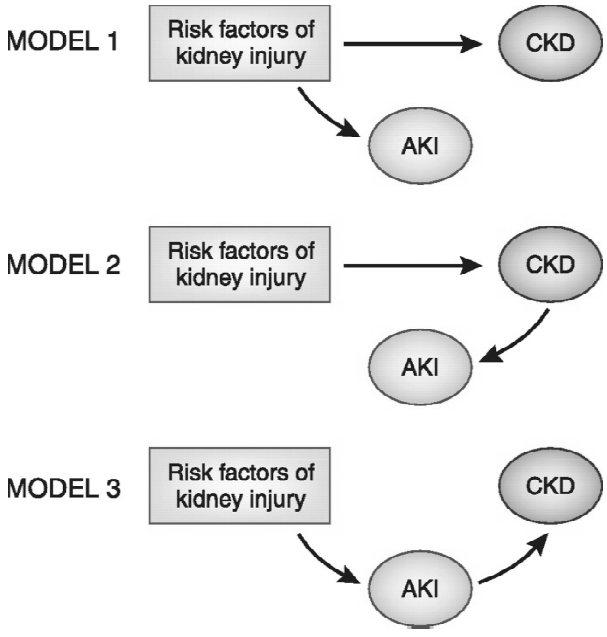
4.4.1. Chronic kidney disease and acute kidney injury: two distinct syndromes?

In the last decades, reduced renal function has been classified as two distinct syndromes: acute and chronic kidney failure (i.e. AKI and CKD). Both diseases are staged according to the serum creatinine concentration or the GFR. To facilitate the clinical approach to these diseases, separate conceptual models have been developed in the past years (Bellomo et al., 2012; Levey et al., 2012).

Several discussions have been raised on whether the existing epidemiological and clinical data are sufficient to establish a causality relationship between AKI and CKD (Rifkin et al., 2012; Hsu, 2012; Belayev et al., 2014). In a review published in 2012, Rifkin et al. tried to examine the existing evidence of causality (of AKI contributing to the development of CKD) by assessing several of the standard causality criteria proposed by Sir Austin Bradford Hill (Hill, 1965). Table 4.2. illustrates that for almost every criterion they found pros and cons supporting or not a causality relationship. Rifkin et al. suggested that there may be several hypothetical causal models of the AKI-CKD association, as illustrated in figure 4.5.. In the first model, several risk factors of kidney injury may lead to either AKI

or CKD. In the second model the risk factors firstly induce CKD, which consequently leads to AKI. In the third model an AKI precedes the development of CKD. Unfortunately, the temporal relationship between the two diseases is not yet completely understood. Some patients fully recover their renal function after an AKI, whereas some of them may develop a CKD and show a relatively fast progression to ESRD (Coca et al., 2012; Thakar et al., 2011). On the opposite hand, there is evidence that patients with CKD show higher risk of hospital admission with AKI (James et al., 2010a). Biologic plausibility, like temporality, is difficult to assess: the association between AKI and CKD may be direct or indirect as well as unidirectional or bidirectional. To test whether AKI really causes CKD, healthy individuals should be recruited and relevant kidney damage should be recorded. After recovery, patients should be followed-up for long-term to examine potential chronicity of kidney disease. Such study is probably unfeasible: the occurrence of relevant kidney damage in healthy individuals is rare, and an artificially inflicted kidney injury would of course be unethical. Case control design might be an option. However, case control studies bear a high risk of selection bias, i.e. they would need to be very carefully designed. On the other side, to test whether CKD really increases the risk of AKI, it would be necessary to perform a study taking into account all possible confounders and avoiding biases. CKD patients often show polymorbidity: it may be very difficult to confirm a causal relationship between CKD and AKI in the presence of cardiovascular diseases, diabetes, or other diseases.

Figure 4. 5. Three hypothetical causal models of the acute kidney injury - chronic kidney disease association.



AKI, acute kidney injury; CKD, chronic kidney disease (Rifkin et al., 2012).

Table 4. 2. Bradford Hill's considerations for causality inference between acute kidney injury and chronic kidney disease.

Benchmark	Definition/Comments	Application to AKI-CKD Association
1. Temporality[a]	The cause (exposure) must precede the effect (outcome)	Pro: AKI happens weeks, months, or years before CKD
		Con: Acute-on-chronic does not follow this rule
2. Strength of association	Stronger association may make causality more likely	Pro: Some studies indicate a strong association between AKI and future CKD
		Con: The reported strengths of the associations are not consistent
3. Biologic gradient (dose-response)	Greater exposure increases the incidence or magnitude of the effect	Pro: AKI severity or more frequent AKI episodes may be associated with a higher likelihood of CKD
		Con: Because patients with more severe forms of AKI are also sicker, the dose-response relationship may simply be detecting the residual confounding from severity of illness
4. Consistency	The association can be replicated in studies in different settings using different methods	Pro: Various forms of AKI in different clinical settings have been demonstrated to lead to CKD
		Con: Some forms of AKI (e.g., hemolytic-uremic syndrome) do not lead to future kidney disease for years to decades
5. Biologic plausibility	The association is consistent with known biologic or pathologic processes[b]	Pro: The tubular and glomerular injury may lead to permanent damage
		Con: Even non–acute tubular necrosis human AKI (e.g., prerenal azotemia) is associated with future risk for CKD
6. Experimentation	The putative effect can be altered (prevented or mitigated) by an experimental regimen	Pro: In some animal models, permanent injury after AKI has been shown
		Con: Some models show full recovery of AKI without renal dysfunction or fibrosis, particularly in younger animals
7. Specificity	A single cause produces the effect without other pathways	Pro: Prior AKI episodes can fully explain CKD incidence
		Con: AKI is only one of the correlates of CKD; many cases of CKD happen in patients who have never had an AKI, and many of the factors that are present in patients with AKI are the same risk factors for CKD
8. Biologic coherence	The association is consistent with the natural history of the disease or laboratory findings	Pro: A lower risk for CKD should occur as a result of preventing AKI
		Con: Natural history of CKD has little, if anything, to do with AKI
9. Analogy	The effect of similar factors may be considered in other populations or under different settings	Pro: AKI precedes CKD irrespective, as seen in animal models; acute myocardial infarction can lead to remodelling and progressive chronic heart failure
		Con: There is no analogy in other acute-leads-to-chronic setting, such pulmonary disease and liver disease

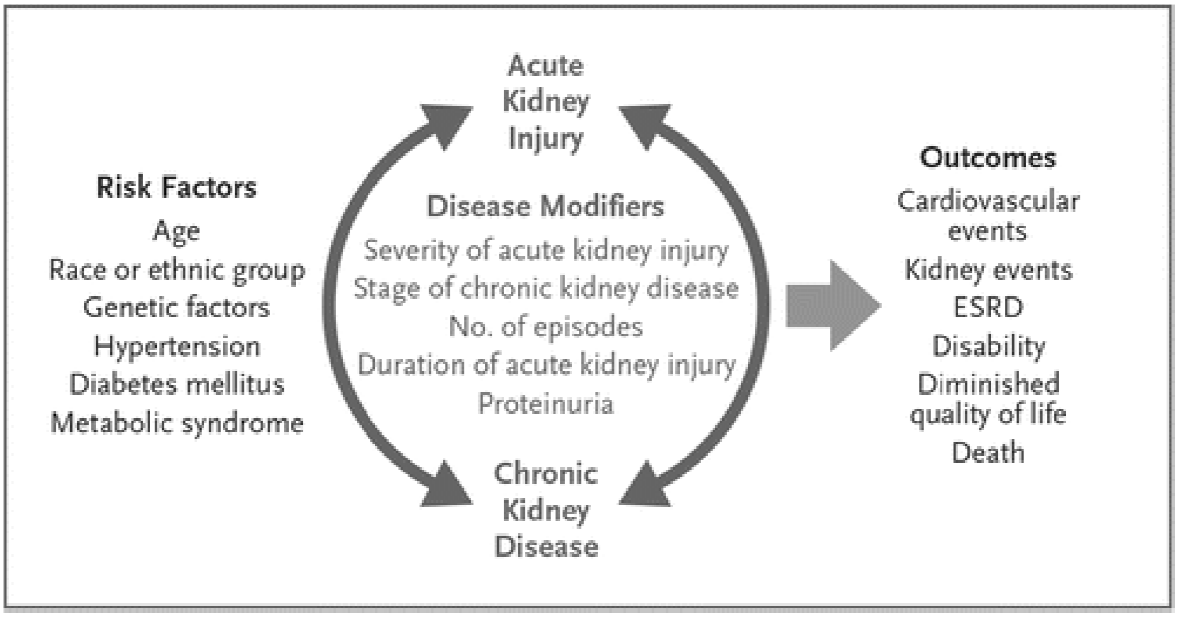
[a] Note that temporality is the only necessary (but not sufficient) condition of causality. [b] Studies that disagree with established understanding of biologic processes may force a re-evaluation of accepted beliefs. AKI, acute kidney injury; CKD, chronic kidney disease (Adapted from Rifkin et al., 2012).

4.4.2. Chronic kidney disease and acute kidney injury: two interconnected diseases

Contrasting the theories mentioned in the previous chapter, several studies have recently started to suggest that CKD and AKI may not be two distinct syndromes, but rather two closely interconnected conditions. Indeed, each condition can be considered as risk factors for the other, and both chronic and acute diseases are risk factors for cardiovascular diseases (Chawla et al, 2012).

As illustrated in figure 4.6., AKI and CKD share several risk factors (e.g. age, hypertension, diabetes, etc.) and may lead to similar outcomes (e.g. kidney events, ESRD, cardiovascular events, death, etc.) (Chawla et al, 2014). Moreover, between the two diseases there are several disease modifiers that may increase the risk of developing CKD after AKI, or vice versa.

Figure 4. 6. Acute kidney injury and chronic kidney disease as an interconnected syndrome.



Acute kidney injury and chronic kidney disease share common risk factors and disease modifiers. When acute kidney injury occurs without pre-existing kidney disease, chronic kidney disease still may develop. Conversely, the presence of chronic kidney disease is an important risk factor for the development of acute kidney injury. Either acute kidney injury or chronic kidney disease (and presumably their combination) is associated with an increased risk of death and may result in complications such as cardiovascular disease, progressive decreases in kidney function, diminished quality of life, and the development and progression of disability. End stage renal disease (ESRD) denotes end-stage renal disease (Reproduced with permission from Chawla et al., 2014, Copyright Massachusetts Medical Society).

Independently from the classical risk factors, recent studies suggest that the most important risk factor for AKI is a pre-existing CKD. On the other side, several observational studies have shown a strong reproducible association between AKI and the subsequent development of CKD (Chawla et al., 2011; Chawla et al., 2012; Coca et al., 2012; Ishani et al., 2009; James et al., 2010b; Wald et al., 2009). These studies have shown that a substantial proportion of AKI patients, including those without previous CKD, often recover some degree of renal function but then have a progression to advanced CKD stages, with increased long-term risk of ESRD and excess mortality. For example, in a cohort US study including 233'803 Medicare patients hospitalized in 2000, it has been calculated that the hazard ratio for developing ESRD was 41.2% (96% CI 35.6-49.1) for patients with both AKI and CKD compared to those without kidney disease (Ishani et al., 2009), 13.0% (95% CI 10.6-16.0) for patients with AKI and without previous CKD, and 8.4% (95% CI 7.4-9.6) for patients with CKD and without AKI. In a Canadian study including 19'022 adults undergoing coronary angiography, the effects of AKI in later long-term decline in kidney function over a period of 3 years have been investigated (James et al., 2010b). Compared to patients without AKI, the adjusted odds ratios of sustained loss of kidney function at 3 months after surgery were 4.74 (95% CI 3.92-5.74) for patients with mild AKI and 17.31 (95% CI 12.03-24.09) for those with moderate-to-severe AKI, if compared to patients without AKI. Concerning the long-term changes in renal function beyond 3 months following surgery, analysed among patients with eGFR <90 ml/min/1.73m^2, mild and moderate-to-severe AKI patients showed respectively adjusted odds ratios of 1.60 (95% CI 1.19-2.14) and 3.12 (95% CI 1.95-4.99), if compared to patients without AKI. In a systematic review and meta-analysis investigating the incidence of CKD and ESRD after AKI, the authors reported that patients with AKI had a higher risk for developing CKD (pooled adjusted hazard ratio 8.8, 95% CI 3.1-25.5), ESRD (pooled adjusted HR 3.1, 95% CI 1.9-5.0), and mortality (pooled adjusted HR 2.0, 95% CI 1.3-3.1) if compared to patients without AKI (Coca et al., 2012).

Clinically speaking, the relationship between CKD and AKI is important. The renal function of AKI patients should be regularly controlled for possible development or progression of CKD. On the other hand, knowing that a patient has a CKD should increase the awareness of clinicians (in particular in emergency departments, intensive care units, or surgery departments) concerning increasing risk of AKI.

4.4.3. NGAL as general biomarker of kidney disease

The studies investigating NGAL in CKD and/or AKI patients seems to support the interconnection of the two diseases. In fact, there is in general good evidence that NGAL significantly correlated with serum creatinine and eGFR, i.e. with the two mostly used marker for the diagnosis and staging of CKD and AKI. Several studies suggested that NGAL seems to be an independent biomarker with the promising potential to improve the diagnosis and to facilitate the identification of patients at high risk of CKD or AKI.

The evidence supporting NGAL as a valuable biomarker of CKD and AKI has been recently discussed in a review of Ronco et al. (Ronco et al., 2014). In summary, the studies performed until now suggest that NGAL may be useful alongside serum creatinine, urine output, and other biomarker in various, but specific, settings. There is evidence that NGAL is useful in predicting kidneys injuries in paediatric AKI, in stratifying and selecting patient for RRT, in assessing AKI in critically ill patients with sepsis, in guiding fluid resuscitation in severely burned patients, as early marker of delayed graft function in kidney transplantation, and as biomarker for cardiovascular disease and heart failure.

Since almost all studies on NGAL have been performed in specific populations and settings (e.g. patients with sepsis in intensive care unit, patients after cardiac surgery) it is still premature to implement it in routine clinical use.

4.5. PERSPECTIVES/RECOMMENDATIONS

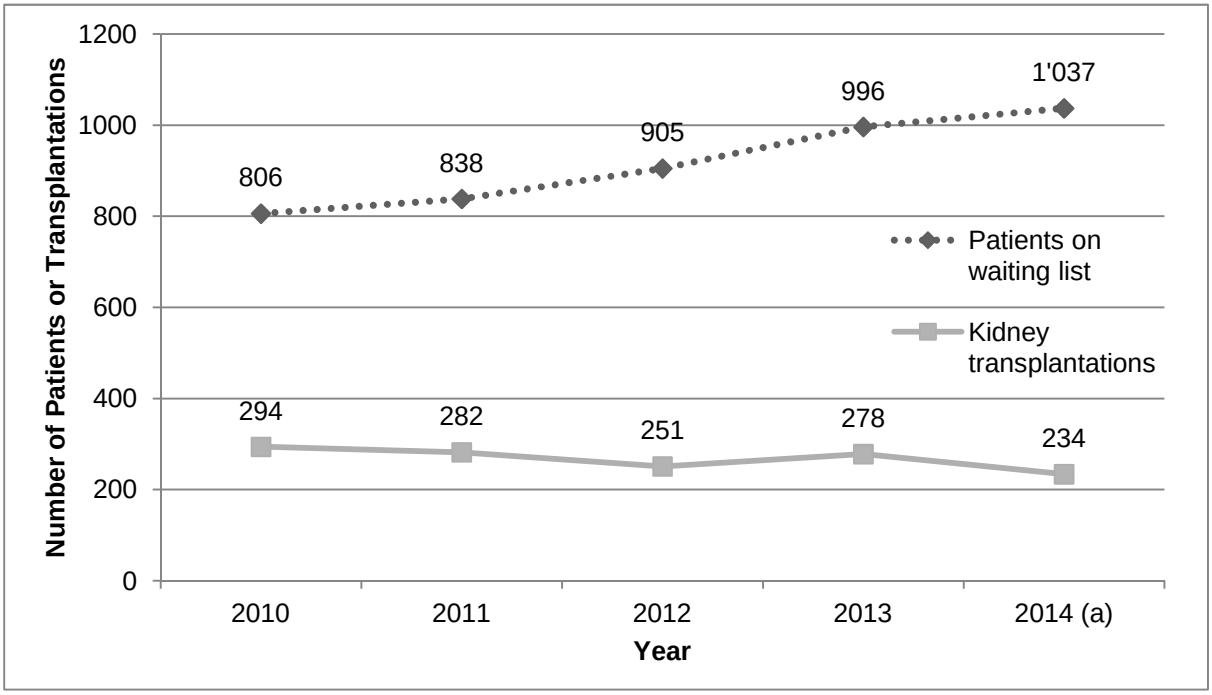
4.5.1. CKD prevalence: is it a real, solvable problem?

The results of this work suggest that the prevalence of CKD in Switzerland is high. The number of subjects with CKD presumably increased in the past decades, and will probably continue growing up in accordance/concomitance to the increasing prevalence of the major risk factors for renal diseases (e.g. older age, hypertension, diabetes, obesity, hypercholesterolemia). One of the major problems in CKD diagnosis and management is the fact that CKD often remains silent or asymptomatic, especially in early stages. As consequence, many CKD patients are identified only after reaching the most severe stage, i.e. ESRD, which usually requires dialysis or renal transplantation. This group of severely ill patients is basically comparable to the classical "tip of the iceberg".

Even if relatively few CKD patients actually reach ESRD (many patients die before for other diseases, like for example cardiovascular diseases or cancers), the representation of how many patients are waiting for or have received a renal transplantation in the last few years in Switzerland is worrying (figure 4.7.) (SwissTransplant, 2014). Between 2010 and 2014 (n.b. data available only until the 30. September), the number of patients waiting for a renal transplantation increased from 806 to 1'037

(+29%). However, during the same period, the number of available organs and performed transplantations remained stable between 250 and 300. Moreover, the mean waiting time before transplantation increased by almost one year (from 851 days in 2010 to up to 1'146 days in 2014). These numbers appear even more impressive if compared to those of other organ transplantations. For example, in 2013 there were 33 heart transplantations, with 59 patients on waiting list, and a mean waiting time of 312 days. The same year there were 109 liver transplantations, with 140 patients on waiting list, and a mean waiting period of 198 days. Kidney is by far the most requested and transplanted organ. However, the number of donators doesn't reflect the high request of organs.

Figure 4. 7. Trends in renal transplantation in Switzerland.



(a) Data only from 01.01.2014 until 30.09.2014. Source: SwissTransplant, 2014 - Schweizerische Nationale Stiftung für Organspenden und Transplantation.

Concerning the second RRT option, it has been estimated that there are actually around 3'500-4'000 patients in dialysis, with a prevalence increase of ca. 4% yearly (Steiger et al., 2012). As reported by the Swiss Society of Nephrology, there are actually 92 dialysis centers in Switzerland (Swiss Society of Nephrology, 2014). The majority of them are located in public or private hospitals, with relatively high capacities (i.e. > than 4'000 dialysis per year), other are specialized dialysis centers with usually lower capacity. The actual number of centers seems to be appropriate to treat around 4'000 patients per year. However, if the number of patients requiring RRT will increase, there may be several problems concerning availability of beds, devices, and trained staff.

It is evident that it is unrealistic and probably counterproductive to introduce a screening for renal problems for the whole Swiss population. However it may be possible to sensibilize the general practitioners. They may be able to opportunistically check the renal biomarker (in particular eGFR and ACR) for patients at high risk of CKD. Identified CKD patients may receive treatments for underlying causes (e.g. hypertension, diabetes), should undergo regular controls of their kidney function (e.g. yearly eGFR and ACR measurements), and should receive advice concerning lifestyle amelioration and possible outcomes in case of disease progression (i.e. RRT).

As already mentioned, renal problems are significantly associated with several highly prevalent diseases (e.g. hypertension, diabetes, hypercholesterolemia, obesity) or comportments (e.g. smoking or alcohol consumption). Therefore, the simplest and most effective way to limit the increase of CKD is prevention of its risk factors. In general, for healthy people it may be sufficient to have an healthy lifestyle, regular physical exercise, healthy diet (including adequate glucose, salt, lipid and alcohol consumption), and quit smoking in order to dramatically reduce their risk of developing CKD (and hypertension, diabetes, etc.). In case of already existing co-morbidities, in addition to lifestyle changes, it may be necessary to start a medical treatment (e.g. anti-diabetics, ACE inhibitors or ARB for high blood pressure). In cases of moderate to severe CKD, a referral to nephrological care might be helpful to optimize patient management. Compared to primary care physicians, nephrologists should be more informed about the actual standard treatments of CKD patients. Potential benefits of early referral might include the identification and treatment of causes of CKD, stabilisation of the renal filtration, and identification of factors affecting renal function (e.g. use of nephrotoxic drugs). A recently published Cochrane review showed reduced mortality and hospitalisation, better uptake of peritoneal dialysis, and earlier placement of arteriovenous fistulae for patients with CKD who were referred early to a nephrologist. Moreover, early referral was associated with better preparation and placement of dialysis access (Smart et al., 2014).

At present, it is still unclear which and how many patients with CKD will progress to ESRD requiring RRT. The probability of disease progression depends on various clinical (age, gender, co-morbidities, smoking), environmental (exposure to heavy metals, chemicals, nephrotoxic substances), and genetic factors (family history of CKD). However, it is reasonable to assume that interventions slowing or stopping disease progression by successfully treating the underlying disease are very important to avoid RRT and may have a greater impact if implemented earlier.

4.5.2. CKD awareness

It has already been mentioned that prevention plays a key role in CKD management. Besides having an healthy lifestyle and reducing the risk factors for CKD, the recognition of CKD in early stages is important in order to slow or prevent progression to severe and symptomatic stages by treating the underlying causes (e.g. hypertension, diabetes). Moreover, patients with more advanced stages might be referred to nephrology care in order optimize disease management.

In this optic, the major issue is probably the disease awareness of patients and clinicians. In a cross-sectional study collecting data from 2000-2009 for 26'213 subjects in the Kidney Early Evaluation Program (KEEP), a community-based screening program, only 10.0% of the participants were aware of suffering from CKD (Kurella Tamura et al., 2011). The proportion in awareness was particularly low for early CKD, with 5.1%, 6.3%, and 10.0% for stages 1 to 3, respectively. In contrast, almost 40% of the patients with CKD stage 4, and 60% of those with CKD stage 5 were aware of having renal disease.

Another US study assessed CKD awareness in 1'852 adults with eGFR <60 ml/min/1.73m^2 using data from the National Health and Nutrition Examination Surveys (NHANES 1999-2008) (Tuot et al., 2011). The analyses were stratified based on the additional number of clinical markers of CKD (i.e. albuminuria, hyperkalemia, hyperphosphatemia, anemia, acidosis, elevated blood nitrogen, uncontrolled hypertension). Only 4% of the patients with 0-1 clinical markers were aware of having CKD. The percentage increased to 10% for those with 2-4 clinical markers (adjusted OR compared to the first group was 1.9, p=0.04). Finally, 16% of the patients with 5-7 clinical markers were aware of their renal condition (adjusted OR 3.6, p=0.06). The authors suggested that the low percentage of awareness, even among patients with a great number of clinical markers, may reflect poor recognition of CKD by primary care clinicians.

In a study comparing diagnostic accuracy for CKD patients, the authors reported that primary care physicians and nephrologists correctly recognized the presence of CKD for 56% and 96% of the patients, respectively. (Boulware et al., 2006). Ineffective/unclear communication between test providers, clinicians, and patients may also be an issue affecting CKD awareness.

The CKD awareness is surprisingly low despite the fact that the awareness of its main risk factors, i.e. diabetes and hypertension, is relatively high. A US analysis published in 2010 reported that the hypertension awareness among a representative population sample of 42'856 adults aged older than 18 years recruited in the NHANES was around 80% (Egan et al., 2010). Similarly, another US study including 14'611 individuals aged at least 12 years reported that among subjects with diabetes 19% were undiagnosed (i.e. the diabetes awareness was 81%) (Cowie et al., 2010)

The level of awareness among clinicians and patients should therefore be improved to prevent CKD, as well as its progression and its consequences (e.g. cardiovascular problems).

4.5.3. NGAL limitations

Despite the relatively good number of publications and studies concerning NGAL, the analysis of this biomarker and the interpretation of the results show several limitations.

NGAL appears to be sensitive and specific in homogenous patient populations, i.e. in specific settings. In fact, the majority of the studies performed and published until now were single centre studies with relatively small samples of highly selected patients. Therefore the validity of the results for a broader spectrum of patients (i.e. the external validity) is not yet clear and should be further investigated.

Moreover depending on the setting, on the analysing technique (e.g. immunohistochemistry, western blot, mRNA expression, ELISA), and on the device used, different cut-off values have been proposed. A standardisation of the measurements and clear guidelines concerning cut-off values and interpretation of the test results are necessary to improve the comparability of the performed studies.

Several studies have shown that NGAL was associated with increasing age, decreasing eGFR, increasing ACR, increasing risk of CKD or AKI, and increasing mortality. However, to assess if its analysis may really improve patients management (including risk stratification, treatment intervention, outcomes), prospective randomized trials are still needed.

4.6. FINAL RECOMMENDATIONS

An increase in CKD awareness among the whole Swiss population is probably the first and most important step to improve CKD management on a large scale. A generally healthier lifestyle should reduce the risk of CKD, and an increased awareness of the main risk factors associated to CKD may lead to a earlier detection and treatment of renal dysfunctions. Patients with moderate to severe stages might be referred to a nephrologist in order to receive a optimized disease management, according to disease severity, patient characteristics, and underlying causes.

How to sensibilize the Swiss population in an effective way should be discussed taking into account the possible stakeholders (e.g. patients, clinicians, politicians, insurance companies, pharmaceutical industries). One already existing example of event organized to increase the awareness of renal diseases is the world kidney day. The world kidney day has been introduced in 2006 (on the 2nd Thursday in March) to raise the world global health awareness concerning renal and renal-associated diseases (http://www.worldkidneyday.org). The aims of this day are, for example, to encourage systematic screening of all patients with diabetes and hypertension for CKD, to encourage preventive behaviours, and to educate medical professionals about their key role. Every year a particular theme concerning renal diseases has been highlighted (e.g. "Are your Kidney OK?" in the first edition in 2006, "Protect your kidneys: Keep your pressure down" in 2009, "AKI" in 2013, or "CKD and aging" in

2014). For 2015 the slogan of the world kidney day will be "Kidney Health for All". On the website it is possible to find campaign materials (with media outlets with education about CKD, its risk factors, and its consequences) or event tips (e.g. "have a kidney educative class in your school", "organize a physical activity event", etc.). Since seven years Switzerland participate to this event. For example, between the 10th and the 22nd of March 2014 it was possible to visit 23 pharmacies in five Swiss Cantons (Zug, Schwyz, Obwalden, Nidwalden, and Uri) in order perform a screening test (for 15 instead of about 50 Swiss Francs). This action is obviously praiseworthy, however it is far away from being "at national level" (the population of the five mentioned Swiss Cantons represents less than 5% of the total Swiss population).

Another important aspect, besides general awareness of CKD, is the provider awareness of CKD guidelines. Clinicians should be particularly aware that older age, diabetes, and hypertension are the main risk factors of CKD. Therefore they should do specific tests to assess the renal function for patients at high risk.

Moreover, a correct and standardized interpretation of the test results is fundamental. In this optic, in the past years the KDOQI and thereafter the KDIGO guidelines have been created (KDOQI, 2012; KDIGO, 2013). Unfortunately the level of awareness of the Swiss physicians concerning these guidelines is unknown. In a US study investigating the CKD awareness among clinicians it has been reported that only about one third of the family practice physicians and general internists were aware of any issued guidelines regarding referral of patients with CKD (among nephrologists the percentage was 79%) (Boulware et al., 2006).

Finally, more high quality clinical studies are necessary to investigate the diagnostic and prognostic power of NGAL in CKD and AKI patients. The STARD (Standards for Reporting of Diagnostic Accuracy) criteria should be applied to plan and conduct multicentre, multi-marker, multi-time point, randomized prospective studies involving large samples and employing pertinent clinical end points and patient-relevant outcomes (STARD, 2008). Cost-benefit and cost-utility analyses should be performed in order to demonstrate that NGAL may have a positive impact on patient outcomes and on the costs of clinical care.

Moreover the lack of standardized measurements, the lack of specific cut-off values for NGAL, as well as lack of gold standard of CKD and AKI definitions strongly limit a widespread use of NGAL in clinical practice. Guidelines concerning NGAL measurements and interpretation may improve the quality and comparability of NGAL studies.

To conclude it is important to emphasize that, at present, it is still unclear which and how many patients with CKD will progress to ESRD requiring RRT. Therefore it is extremely difficult to assess whether more and more accurate controls for CKD in primary care will automatically lead to fewer cases of ESRD and RRT. As already mentioned, the probability of disease progression depends on

various clinical (age, gender, co-morbidities, smoking), environmental (exposure to heavy metals, chemicals, nephrotoxic substances), and genetic factors (family history of CKD). However, it is reasonable to assume that interventions slowing or stopping disease progression by successfully treating the underlying disease are important to avoid ESRD, and might have a greater impact if implemented earlier.

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5. CURRICULUM VITAE

PERSONAL BACKGROND

Name	Yuki TOMONAGA
Place / Date of birth	Locarno / 9 June 1981
Nationality	Swiss
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Civil status	Single
Languages	Italian: mother tongue German: fluent French: fluent English: good
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EDUCATION AND WORK EXPERIENCES

2010 - 2015	**Ph.D. student in Epidemiology at the Swiss Tropical and Public Health Institute** University of Basel, Switzerland Project: "The prevalence and identification of chronic kidney disease in Switzerland", Institute of Social and Preventive Medicine, University of Zurich, Switzerland (Prof. Dr. med. Thomas D. Szucs), Swiss Tropical and Public Health Institute, University of Basel, Switzerland (Prof. Marcel Tanner)
2010 - present	**Scientific associate, Institute of Social and Preventive Medicine** University of Zurich, Switzerland
2010 - present	**Assistant lecturer, Public Health Weiterbildung** University of Zurich and University of Basel, Switzerland B303.30.14: "Economic evaluation in public health"
2008 - present	**Assistant lecturer, Faculty of Science** University of Zurich, Switzerland BIO 410: "Research methodology for studies on human health and disease" BIO 418: "Clinical epidemiology and quantitative research in health care"
2013	**Summer School in Public Health Policy, Economics and Management, Swiss School of Public Health + (SSPH+)** Lugano, Switzerland Methodology and Practical Application of Economic Evaluation and HTA in Health Care
2013	**Courses at the ETH Zurich** Zurich, Switzerland 752-6105-00 S: Epidemiology and Prevention 752-6151-00 S : Health Concepts
2012	**Summer School in Public Health Policy, Economics and Management, Swiss School of Public Health + (SSPH+)** Lugano, Switzerland Chronic diseases: population-based approaches and public health management
2008 - 2009	**Scientific advisor of the European Centre for Disease Prevention and Control (ECDC)** Stockholm, Sweden

2007 - 2009	**University Professional of Advanced Studies in Pharmaceutical Medicine** European Center of Pharmaceutical Medicine (ECPM), University of Basel, Switzerland
2006 - 2007	**Master of Science in Human Biology** University of Zurich, Switzerland Master thesis title: “Clinical benefit of Point-of-Care testing of acute coronary syndromes, heart failure and thromboembolic events in primary care” done under the supervision of Prof. Dr. med. Thomas D. Szucs at the Institute of Social and Preventive Medicine, University of Zurich, Switzerland
2002 - 2005	**Bacherol of Science** University of Zurich, Switzerland Main formation in human biology, molecular biology and neurology
2001 - 2002	**Petty officer formation in the Swiss Army** Frauenfeld (TG) / Bure(JU), Switzerland
2000 - 2002	**Program of study Biochemistry** ETH Zurich, Switzerland
1996 - 2000	**High School (Maturity type A)** Liceo Cantonale di Locarno (TI), Switzerland
1992 - 1996	**Middle School** Losone (TI) , Switzerland
1987 - 1992	**Primary School** Solduno/Losone (TI) , Switzerland

6. APPENDIX

6.1. ONGOING PUBLICATIONS

Tomonaga Y, Szucs T, Risch L, Ambühl P. Parathyroid hormone, hyperparathyroidism and chronic kidney disease in primary care.

6.2. PUBLICATIONS

Kasenda B, Schandelmaier S, Sun X, von Elm E, You J, Blümle A, **Tomonaga Y**, Saccilotto R, Amstutz A, Bengough T, Meerpohl JJ, Stegert M, Olu KK, Tikkinen KA, Neumann I, Carrasco-Labra A, Faulhaber M, Mulla SM, Mertz D, Akl EA, Bassler D, Busse JW, Ferreira-González I, Lamontagne F, Nordmann A, Gloy V, Raatz H, Moja L, Rosenthal R, Ebrahim S, Vandvik PO, Johnston BC, Walter MA, Burnand B, Schwenkglenks M, Hemkens LG, Bucher HC, Guyatt GH, Briel M. Subgroup analyses in randomised controlled trials: cohort study on trial protocols and journal publications. *BMJ*. 2014 Aug 1;349:g4921. doi: 10.1136/bmj.g4921. PMID: 25085445

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6.3. POSTERS PRESENTATIONS

Tomonaga Y, Szucs TD, Risch L and Ambühl PM. Parathyroid Hormone, Hyperparathyroidism and Chronic Kidney Disease in Primary Care. Swiss Society of Nephrology, Interlaken, Switzerland, December 2013. *Swiss Medical Weekly* (2013) Supplementum 202, 16 S.

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Szucs TD, **Tomonaga Y**, Roediger A, Sazanov V and Ambegaonkar BM. Prevalence of multiple vs. isolated lipid disorders in a sample of swiss adults. Multiple Risk Factors in Cardiovascular Diseases, Venice, Italy, October 2008. *Journal of Clinical Lipidology* (2008), Supplement to Volume 2, No 5S, S42.

Hug M, **Tomonaga Y**, Gutzwiller F, Lüscher TF, Riesen WF, Diemand A, Schwenkglenks M and Szucs TD. Klinischer Nutzen der Point-of-Care kardialen Risikostratifizierung in der Grundversorgung. Schweizerische Gesellschaft für Allgemeinmedizin (SGAM), Zürich, Switzerland, September 2008.