Urinary Biomarkers and Patient Outcome in Chronic Kidney Disease and Atherosclerotic Heart Disease: The value of IgM-uria and IgG-uria

Tofik, Rafid

2014

Urinary Biomarkers and Patient Outcome in Chronic Kidney Disease and Atherosclerotic Heart Disease:

The value of IgM-uria and IgG-uria

Rafid Tofik

LUND
UNIVERSITY

DOCTORAL DISSERTATION
by due permission of the Faculty of Medicine, Lund University, Sweden.

To be defended at Segerfalksalen, BMC, Sölvegatan 17, Lund, on 27 September 2014 at 10 am.

Faculty opponent

Professor Bengt Lindholm, Karolinska Institutet

Stockholm, Sweden

Organization	Document name
LUND UNIVERSITY	DOCTORAL DISSERTATION
Department of Nephrology, Clinical Sciences, Lund	Date of issue: 2014-08-23
Author(s): Rafid Tofik	Sponsoring organization

Title and subtitle

Urinary Biomarkers and Patient Outcome in Chronic Kidney Disease and Atherosclerotic Heart Disease: The value of IgM-uria and IgG-uria

Abstract

Risk stratification of patients with chronic kidney disease and atherosclerotic heart disease is crucial. Microalbuminuria (MA) is associated with an increased risk of kidney and cardiovascular (CV) death, especially in patients with diabetes. However, MA in many instances is not sensitive for disease outcome; for instance, not all diabetic patients with albuminuria progress to kidney failure, and not all patients with diabetic kidney disease (DKD) have albuminuria. Furthermore, albuminuria is not essentially associated with endothelial dysfunction or glomerular lesions in non-diabetic patients. Urinary excretion of larger proteins, such as IgM and IgG would better reflect glomerular dysfunction and vascular endothelial damage. In this thesis we aimed to evaluate the value of IgG-uria and IgM-uria in predicting kidney and CV outcome in patients with diabetes, glomerulonephritis and atherosclerotic heart disease.

In study I, 139 patients with type-1 diabetes, and in study II, 106 patients with type-2 diabetes, were followed at the diabetes outpatient clinic, for an average of 18 and 5 years, respectively. Type-1 & -2 diabetic patients had about a 3-fold increase in CV and renal mortality (HR = 2.7, $p = 0.004$, and HR = 3.6, $p < 0.001$, respectively) if they had an increased urine IgM excretion, even when adjusted to the degree of albuminuria. In study III, 178 consecutive patients who presented with acute chest pain to the emergency department of Lund University Hospital were followed for up to 2 years. Patients with acute coronary syndrome had significantly higher baseline IgM-uria than those with non-specific chest pain. Chest pain patients with IgM-uria at time of presentation had a 3-fold higher risk for the occurrence of subsequent major CV event (HR = 3.3, $p = 0.001$). In study IV, 189 patients with proteinuric primary glomerulonephritis were followed at the nephrology outpatient clinic for an average of 8 years. Patients with an increased urine IgG excretion had an increased risk for end-stage kidney disease (ESKD), even when adjusted for the degree of albuminuria and kidney function (HR = 5.9, $p = 0.001$).

In conclusion, IgM-uria could be a useful biomarker for kidney and cardiovascular risk assessment of patients with diabetes and atherosclerotic heart disease. IgM-uria could imply an extensive atherosclerotic vascular disease. IgG-uria is helpful in identifying patients with glomerulonephritis at increased risk for disease progression to ESKD.

Key words: Proteinuria, Albuminuria, urine IgM, urine IgG, glomerulonephritis, chronic kidney disease, atherosclerosis, cardiovascular disease, diabetic kidney disease.

Classification system and/or index terms (if any)

Supplementary bibliographical information	Language
Lund University, Faculty of Medicine Doctoral Dissertation Series 2014:105	
ISSN and key title 1652-8220	ISBN 978-91-7619-034-0
Recipient's notes	Number of pages 113 / Price
	Security classification

Urinary Biomarkers and Patient Outcome in Chronic Kidney Disease and Atherosclerotic Heart Disease:

The value of IgM-uria and IgG-uria

Rafid Tofik

LUND
UNIVERSITY

Cover illustration: Schematic cartoon demonstrating the glomerular filtration barrier, small pores, large pores and shunt-pathway.

Lund University, Faculty of Medicine
ISBN 978-91-7619-034-0
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2014

To mother and father
my wonderful family
my brother and sister

Contents

Abstract

Risk stratification of patients with chronic kidney disease and atherosclerotic heart disease is crucial. Microalbuminuria (MA) is associated with an increased risk of kidney and cardiovascular (CV) death, especially in patients with diabetes. However, MA in many instances is not sensitive for disease outcome; for instance, not all diabetic patients with albuminuria progress to kidney failure, and not all patients with diabetic kidney disease (DKD) have albuminuria. Furthermore, albuminuria is not essentially associated with endothelial dysfunction or glomerular lesions in non-diabetic patients. Urinary excretion of larger proteins, such as IgM and IgG would better reflect glomerular dysfunction and vascular endothelial damage. In this thesis we aimed to evaluate the value of IgG-uria and IgM-uria in predicting kidney and CV outcome in patients with diabetes, glomerulonephritis and atherosclerotic heart disease.

In study I, 139 patients with type-1 diabetes, and in study II, 106 patients with type-2 diabetes, were followed at the diabetes outpatient clinic, for an average of 18 and 5 years, respectively. Type-1 & -2 diabetic patients had about a 3-fold increase in CV and renal mortality (HR = 2.7, $p = 0.004$, and HR = 3.6, $p < 0.001$, respectively) if they had an increased urine IgM excretion, even when adjusted to the degree of albuminuria. In study III, 178 consecutive patients who presented with acute chest pain to the emergency department of Lund University Hospital were followed for up to 2 years. Patients with acute coronary syndrome had significantly higher baseline IgM-uria than those with non-specific chest pain. Chest pain patients with IgM-uria at time of presentation had a 3-fold higher risk for the occurrence of subsequent major CV event (HR = 3.3, $p = 0.001$). In study IV, 189 patients with proteinuric primary glomerulonephritis were followed at the nephrology outpatient clinic for an average of 8 years. Patients with an increased urine IgG excretion had an increased risk for end-stage kidney disease (ESKD), even when adjusted for the degree of albuminuria and kidney function (HR = 5.9, $p = 0.001$).

In conclusion, IgM-uria could be a useful biomarker for kidney and cardiovascular risk assessment of patients with diabetes and atherosclerotic heart disease. IgM-uria could imply an extensive atherosclerotic vascular disease. IgG-uria is helpful in identifying patients with glomerulonephritis at increased risk for disease progression to ESKD.

List of Publications

This thesis is based on the following publications:

I. **Tofik R**, Torffvit O, Rippe B, Bakoush O. Increased urine IgM excretion predicts cardiovascular events in patients with type 1 diabetes nephropathy. BMC Med. 2009 Aug 4;7:39. doi: 10.1186/1741-7015-7-39.

II. **Tofik R**, Torffvit O, Rippe B, Bakoush O. Urine IgM-excretion as a prognostic marker for progression of type 2 diabetic nephropathy. Scand J ClinLabInvest.2011.Apr;71(2):123-8.doi:10.3109/00365513.2010.542828. Epub 2010 Dec 7.

III. **Tofik R**, Ekelund U, Torffvit O, Swärd P, Rippe B, Bakoush O. Increased urinary IgM excretion in patients with chest pain due to coronary artery disease. BMC Cardiovasc Disord. 2013 Sep 13;13:72. doi: 10.1186/1471-2261-13-72.

IV. **Tofik R**, Aziz R, Reda A, Rippe B, Bakoush O. The value of IgG-uria in predicting renal failure in idiopathic glomerular diseases. A long-term follow-up study. Scand J Clin Lab Invest. 2011 Apr;71(2):123-8. doi: 10.3109/00365513.2010.542828. Epub 2010 Dec 7.

Abbreviations

ACR	Albumin creatinine ratio
AGE	Advanced glycation end products
Ang II	Angiotensin II
Apo CIII	Apolipoprotein CIII
AT1R	Angiotensin type 1 receptors
CKD	Chronic Kidney Disease
CV	Cardiovascular
DM	Diabetes Mellitus
DKD	Diabetic Kidney Disease
ESKD	End-stage Kidney Disease
FSGS	Focal segmental glomerulosclerosis
GBM	Glomerular basement membrane
GFB	Glomerular filtration barrier
GFR	Glomerular filtration rate
GN	Glomerulonephritis
GTPase	Guanosine triphosphatases
H^+-ATPase	Proton-adenosine triphosphatase
HMW	High molecular weight
HPSG	Heparan sulphate proteoglycans
hsCRP	High sensitive C-reactive protein
IgAN	Immunoglobulin A nephropathy
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHD	Ischemic heart disease

IL-1β	Interleukin-1beta
kDa	Kilodalton
KDOQI	Kidney Disease Outcomes Quality Initiative
LDL	Low-density lipoprotein
LMW	Low molecular weight
LPL	Lipoprotein lipase
MA	Microalbuminuria
MCN	Minimal change nephropathy
MN	Membranous nephropathy
NADPH	Nicotinamide adenine dinucleotide phosphate
NO	Nitric Oxide
PKC	Protein kinase C
Protein HC	Protein α1-microglobulin
Rac1	Ras-related C3 botulinum toxin substrate 1
RAGE	Receptor of advanced glycation end products
RAS	Renin-angiotensin system
RhoA	Ras homolog gene A
ROS	Reactive oxygen species
TGF-β	Transforming growth factor beta
TNF-α	Tumor necrosis factor-alpha
TNT	Troponin T
VEGF	Vascular endothelial growth factor
Å	Ångström

Aims Of The Thesis

1. STUDY I: To study the prognostic value of IgM-uria in predicting the cardiovascular and renal outcomes in patients with type-1 DM.

2. STUDY II: To study the prognostic value of IgM-uria in patients with type-2 diabetes in comparison to albuminuria.

3. STUDY III: To explore the association of IgM-uria and acute coronary syndrome in patients with acute chest pain, and its impact in predicting subsequent major cardiovascular events.

4. STUDY IV: To study the prognostic value of IgG-uria in comparison to albuminuria in patients with idiopathic glomerular disease.

INTRODUCTION

Chronic kidney disease (CKD), diabetes mellitus (DM) and ischemic heart disease (IHD) have been considered to be a major global health problems because of rising incidence and prevalence, high financial costs and fatal outcomes [1-3]. Patients with such chronic diseases are highly vulnerable to cardiovascular (CV) complications and premature death [4]. Fortunately, preventive medical management is available for patients at risk. However, in many cases it is still difficult to assess the individual risk for subsequent cardiovascular complications and outcomes [5]. Therefore, there is a constant need for new better markers to early identify risk groups, for immediate preventive management of complications.

Albuminuria is a well-established urinary biomarker for the diagnosis and prognostic evaluation of patients with kidney diseases. It is associated with generalized vascular endothelial dysfunction and worse cardiovascular outcome [6-9]. The risk of kidney and CV disease is already high in patients with urine albumin concentrations lying at the "upper normal limit" [10]. However, not all patients with albuminuria will progress to kidney failure and not all patients with kidney disease develop albuminuria [11, 12]. Albuminuria *per se* could be due to other factors than glomerular injury, such as proximal tubular dysfunction [13], or due to other non-pathological conditions such as physical exercise [14-19] Recently, some experimental and epidemiological studies have pointed out a better correlation between increased urine concentrations of high molecular weight proteins and the diagnosis and prognostic evaluation of kidney disease [20-24]. In this thesis we investigated the significance of urine concentrations of IgM and IgG in predicting the risk of end-stage kidney disease and cardiovascular death in longitudinal cohorts of patients with glomerular diseases, diabetes mellitus, and coronary artery disease.

The Kidneys

Each human being has two kidneys, located retroperitoneally inside the abdomen and each kidney is supplied by a renal artery. Total blood flow to the kidneys is about 1100 ml/min, or about 22% of the cardiac output. The “nephron” is the smallest functional unit in the kidney. Each kidney consists of approximately one million nephrons. The nephron contains a “glomerulus” through which the blood filtered, and “tubules” in which the filtered fluid, the primary urine, is converted to the final urine. Several tubules drain into a larger collecting duct. The glomerulus is made up by a capsule-like structure called the “Bowman’s capsule” in which a network of small anastomosing glomerular capillary vessels covered by epithelial cells invaginates. The primary urine is collected in the “Bowman’s space” before it runs further into the proximal tubule (figure 1).

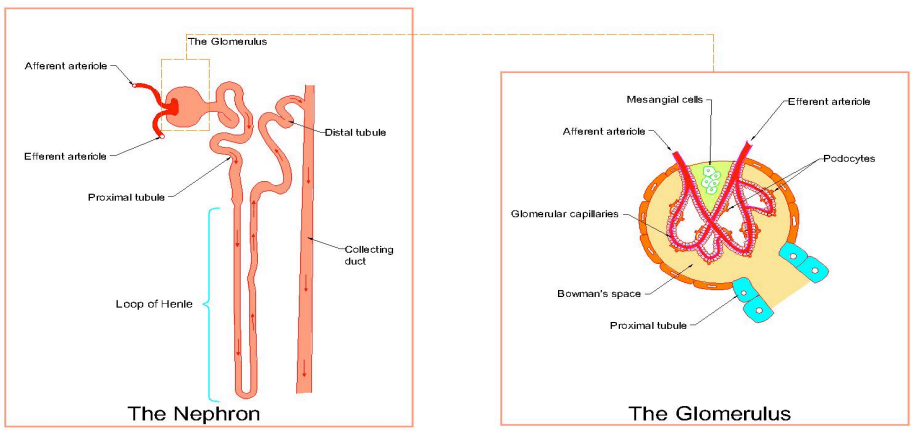


Figure 1. The structure of the nephron and the glomerulus.

Both kidneys filter about 180 liter of plasma a day. At the tubular system of the nephron, about 99% of the primary urine (which is normally devoid of proteins and cellular elements but has similar composition as plasma) is reabsorbed to maintain fluid, electrolyte and acid-base balance, resulting in the final urine in the collecting ducts.

Besides their main and most important function in filtering plasma, eliminating waste products from the filtrate and returning back vital substances to the circulation; the kidneys play an important role in many other vital physiological functions. They regulate water and electrolyte balance, acid-base balance, arterial blood pressure, erythrocyte homeostasis and vitamin D3-production.

The glomerular filtration barrier

The glomerular filtration barrier (GFB) consists of three sequential layers (figure 2). The first layer is the endothelial cell layer that lines the capillary from the inside. It is perforated by relatively large holes (about 60 – 90 nm in diameter), called *fenestrae.* This layer is endowed with a negatively charged glycocalyx that consists of glycosaminoglycans and proteoglycans [25-27]. The second layer is the glomerular basement membrane (GBM). It is a meshwork of collagen IV and laminin, cross-linked with negatively charged sulfated glycoproteins and proteoglycans [27]. The third outermost layer, is the epithelial layer which consists of highly specialized contractile cells, the podocytes, with its interdigitating foot processes that embrace the glomerular capillaries. The foot processes contain F-actin filaments and non-muscle myosin. The gaps between the foot processes, the *filtration slits,* are interconnected by a thin membranous structure (40 nm wide), the *slit diaphragm,* through which the glomerular filtrate percolates. The slits are made up by a number of adherence and signaling proteins, mainly nephrin, podocin and Neph1, that play a critical role in maintaining of the structural integrity of the podocyte cytoskeleton, and thereby, the whole filtration barrier [28]. The podocytes are also covered by negatively charged glycoproteins [29, 30]. All these three layers continuously interact with each other mechanically and biochemically.

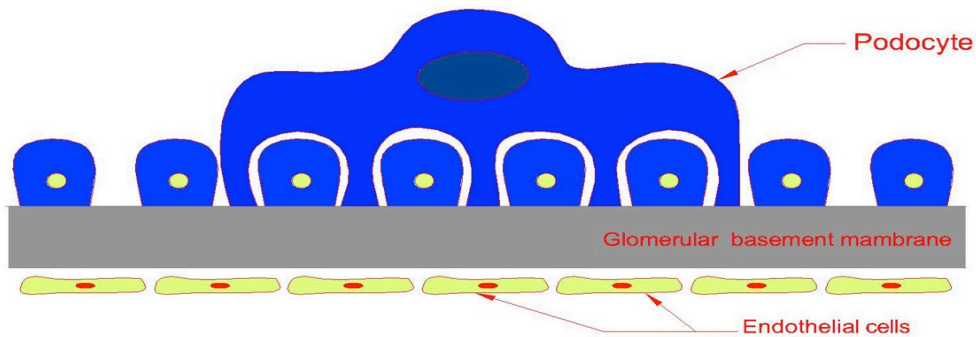


Figure 2. A schematic drawing of the structure of the glomerular filtration barrier.

Until now it is still controversial which part of the GFB is representing the major filtration barrier to macromolecules. However, dysfunction of any of the above mentioned layers of the GFB, and possibly, changes in the hemodynamic state, may result in proteinuria [29, 31]. The GFB has a highly dynamic sieving capacity. Its permeability to large molecules, like albumin, can rapidly and dynamically change in response to different physiological and pathophysiological conditions such as, systemic inflammation, trauma, acute hyperglycemia, increased plasma levels of ANP (atrial natriuretic peptide) and increased plasma levels of angiotensin II [13, 15, 32-36].

Transport theories for protein sieving across the glomerular filtration barrier

The permeability (sieving coefficients) of the GFB to various molecules decreases with increasing size of the molecule. Normally, for larger proteins, like albumin (molecular radius ~36 Å), only tiny amounts are filtered across the glomerulus (one molecule out of 10,000) [29], and more than 97% of the filtered albumin is normally reabsorbed by the proximal tubules [13, 32]. For very large proteins, such as IgM (molecular radius 120 Å), these molecules normally do not pass the GFB, except in case of severe damage of the GFB. The transport of proteins through the GFB is not only restricted by size-selectivity but also, to some extent, by charge-selectivity of the filtration barrier and size, shape and charge of the permeating molecule, in addition to glomerular plasma flow and pressure [20, 37, 38]. A widely used model for transport of proteins across the GFB is the ”two-pore with a shunt” model [39-42]. According to this model, the majority of the pores are of radius 37.5 Å and, in addition, there is a very small number (1×10^{-6} of the small pore number) of large pores of radius 110 Å [37, 38, 43]. The small pore population is affected by negative charge, which makes the barrier more impermeable to negatively charged macromolecules of the same size. Thus, the negatively charged native albumin molecule of molecular radius 36 Å is totally excluded from the negatively charged small pore radius 37.5 Å because of charge-selectivity [13]. At normal ionic strength approximately 20% of the Debye length (i.e. 20% of 8 Å) will functionally add on to the albumin radius and has to be subtracted from the small pore radius to account for the impact of the negative charge-selectivity of the GFB [29, 44]. Thereby, albumin and larger proteins are confined to large pores (by convection) for their passage across the GFB [37, 38, 43]. In addition to these pores there may be large sporadic physiological ”defects” or ”shunts” in the GFB. These shunts are large enough to allow transport of larger proteins like IgM and even red blood cells [37, 45, 46]. The normal glomerular sieving coefficient to red blood cells is only ~ 10^{-9}.

Another widely used model of the GFB permeability is the (negative charged) fiber-matrix model with or without the presence of large pores [40]. This model is mechanistically and mathematically not very different from the two-pore model, but is somewhat less intuitive.

Proteinuria

The excreted urinary proteins can be classified according to the molecular size of the filtered proteins into low molecular weight (LMW) proteinuria (molecular weight 10 - 44 kDa), intermediate (MW 44 - 90 kDa) and high molecular weight (HMW) proteinuria (MW > 90 kDa). Proteinuria can also be classified according to the site of damage in the nephron, into ”*glomerular proteinuria”,* where larger proteins pass through a defect in the GFB (size- or charge-selectivity defect); or ”*tubular proteinuria”* when smaller proteins filtered across the GFB cannot be reabsorbed by a defect in the proximal tubules. “*Overload proteinuria*” refers to overproduction of proteins in the plasma to be filtered in large amounts that exceed the tubular capability for reabsorption. In glomerular diseases, the major protein leaked to the urine is plasma albumin, and in general, proteinuria refers often to albuminuria, unless specifically stated otherwise (e.g. Bence-Jones proteinuria). Glomerular proteinuria could be of “selective” type, when mainly albumin is seen in the urine; and of “non-selective” type, when larger proteins (such as IgG, IgM and α-macroglobulin) are also seen in significant amount with albumin in the urine.

A daily urinary albumin excretion of < 30 mg/day or a urine albumin/creatinine ratio (ACR) < 3 mg/mmol, is referred to as “normoalbuminuria”. A low grade increase in the urine albumin excretion is called “microalbuminuria” which refers to urinary albumin excretion range between 30-300 mg/day or ACR 3-30 mg/mmol. Albuminuria over 300 mg/day or ACR > 30 mg/mmol is defined as “macroalbuminuria”. “Nephrotic range proteinuria” usually refers to urinary albumin excretion > 3 g/day or ACR > 300 mg/mmol.

Protein reabsorption in the proximal tubules

The reabsorption of protein molecules in the initial part of the proximal tubules occurs by binding to the brush-border apical membrane receptor (megalin and cubulin) and a receptor-mediated endocytosis [47]. The apical endocytosis apparatus is mainly located in the first two segments of the proximal tubule. Filtered protein molecules bind to megalin and cubulin receptors at the apical membrane, forming apical clathrin-coated pits. These pits release from the membrane to form clathrin-coated vesicles. After losing the clathrin-coat, the protein-megalin/cubulin ligands start to dissociate, by vesicular acidification via H^+-ATPase, into uncoated acidic endosomes, and the megalin and cubulin recycle back to the apical membrane via dense apical tubules. The endosomes are then taken up by lysosomes for degradation of the protein molecules into amino-acids, to be returned back to the circulation [48-51] (figure 3). The time until protein molecules are taken up by lysosomes may take up to 15 minutes and for albumin degradation to amino acids up to 120 minutes [52]. In tracer studies there is a linear accumulation of radiolabeled proteins (albumin) in the proximal tubules during ~ 0 – 8 min, which is used in tissue-uptake studies of protein transport across the GFB [38]. Normally the tubular reabsorption of protein is almost complete (~ 98%). However, the tubular maximum for protein reabsorption is rapidly exceeded in states of increased proteinuria. Hence, proteinuria in glomerular disease is a result of both glomerular size-selectivity defects and (partial) saturation of tubular reabsorption.

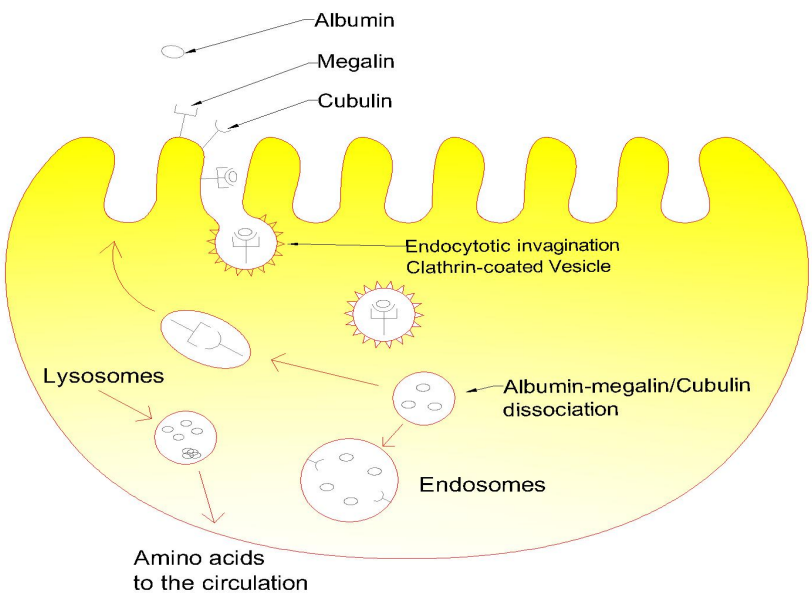


Figure 3. A schematic drawing of cellular albumin reabsorption in the proximal tubule.

Proteinuria and cardiovascular disease

Patients with proteinuria, with or without diabetes, carry an increased risk for cardiovascular mortality [53]. The degree of urinary albumin excretion seems to follow a linear relationship with the risk of cardiovascular disease (CVD) [54]. Even a small increase in urine albumin excretion (at 1 mg/mmol), with or without other risk factors, such as DM and hypertension, is associated with higher risk for CVD and mortality [55, 56]. Albuminuria could reflect a generalized vascular endothelial dysfunction of small vessels (retinal, glomerular) and large vessels simultaneously [57-62]. Thus, albuminuria and CVD might be initiated by the same pathophysiological process of vascular endothelial dysfunction [57]. Albuminuria predicts CV mortality independently from traditional CV risk factors (diabetes, hypertension, dyslipidemia, smoking) and non-traditional CV risk factors (elevated plasma levels of C-reactive protein, interleukin-6, fibrinogen and white blood cells), [53, 54, 63].

Low grade albuminuria is associated with an increased CV death in patients with ischemic heart disease and myocardial infarction [64-66]. It is associated with the diagnosis of left ventricular hypertrophy, cerebrovascular insult and a 3-fold increased risk for heart failure [67-70].

Urinary biomarkers

Albumin is the main plasma protein, which produced by the liver (concentration in serum 34 - 45 g/L). Albumin is a compact, stable but flexible protein. It is ellipsoid (140 Å x 40 Å) in shape and comprised of 609 amino acids. The molecular weight is 69 kDa and the molecular radius is ~36 Å. Native albumin has a net negative charge of ~ -20. Albumin, by its abundance and negative charge, accounts for about 75% of the plasma oncotic pressure. In addition, it functions as an antioxidant and is immunomodulatory. It plays an important role in acid-base buffering and transport of molecules and ions, such as fatty acids, steroids and vitamins. Approximately 4% of the albumin pool, of which 50% is intravascular and 50% is extravascular (interstitial), is metabolized per day. The half-life of albumin is thus about 16-17 days [71, 72].

Immunoglobulin M (IgM) is a pentamer of IgG with a molecular weight of 950 kDa, and a molecular radius of ~120 Å, forming the largest antibody isotype molecule. It accounts for about 5-10% of all antibodies in the body. The normal plasma concentration is 0.5-4 g/L. There are two types of IgM, the natural IgM, which is the natural antibody produced predominantly by the B1 cells, and the antibody-mediated IgM [73, 74]. IgM has a high binding affinity to target antigens.

Immunoglobulin G (IgG) is a monomer with a molecular weight of 150 kDa, and a molecular radius of 55 Å. The normal plasma concentration is 5-15 g/L. IgG accounts for about 80% of all antibodies in the body. It predominates in secondary immunity response against infectious microorganisms. Differing in the number of disulphide bonds, there are four subclasses of human IgG namely IgG_1 (65%), IgG_2 (25%), IgG_3 (6%), and IgG_4 (4%) of different isoelectric points [75]. The IgG_4 is known to be negatively charged, while the IgG_2 is neutral.

α1-microglobulin (protein HC) is a small globular glycoprotein with charge heterogeneity and a molecular weight of 26 kDa [76]. The mean plasma concentration is 44 mg/L [77]. The liver is the main site for synthesis. Protein HC has a strong cell protective effect by its antioxidant nature, in addition to its immunoregulatory properties [78, 79]. Increased urinary excretion of protein HC is a good indicator for renal proximal tubular dysfunction [80, 81].

Chronic Kidney Disease (CKD)

Chronic kidney disease (CDK) is defined as presence of kidney damage, proteinuria, or decreased glomerular filtration rate (GFR). The GFR is the amount of filtered plasma through the glomerular filtration barrier in milliliters per minute and $1.73m^2$ surface area ($mL/min/1.73m^2$). Thus, CKD diagnosed when there is a decline in GFR, presence of albuminuria or presence of structural kidney damage. Independent of traditional risk factors, progression of CKD is associated with unpleasant outcomes like kidney failure, kidney replacement therapy, and premature cardiovascular death [82]. Even in the early CKD stages there is an increasing incidence of CV mortality [83, 84].

The causes of CKD are numerous. Diabetes mellitus (DM), glomerulonephritis and hypertension, are the main causes [85-87]. Other causes may include; interstitial nephritis, genetic disorders (polycystic kidney disease, Alport syndrome), chronic heart failure, liver cirrhosis or systemic infections (HIV infection). Individuals of age > 60 years, or chronic use of non-steroidal anti-inflammatory drugs are at increased risk for CKD [2].

The clinical staging of the CKD is mainly based on the measured or estimated GFR levels (table 1) [2, 88].

Table 1. The clinical staging of CKD according to the KDOQI.

Staging	GFR mL/min/1.73m^2	Severity
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	End-stage kidney disease

Prevalence and incidence of CKD

The prevalence of CKD in general population in the US and in Europe is about 10% of adults [88, 89]. The prevalence of early stages of the CKD disease (CKD1-4, 3.3%, 3.0%, 4.3% and 0.2% respectively) is 100 times higher than the prevalence of end-stage kidney disease (G5 0.1%) [2]. The incidence of CKD increases with age. The annual incidence in middle aged is about 1%. Women have higher incidence of CKD but lower risk for kidney failure than men. African Americans have higher risk for kidney failure [90-92].

CKD and occurrence of cardiovascular disease (CVD)

Patients with CKD have high prevalence of ischemic heart disease, peripheral vascular disease, cerebrovascular disease, hypertension and cardiomyopathy. Therefore, patients with CKD are at higher risk to die of CVD than to develop end-stage kidney disease (ESKD) [93, 94]. Once they reach ESKD, the CV mortality increases by 10 to 30 times, mainly due to acute myocardial infarction and heart failure [94, 95].

Glomerulonephritis

Glomerulonephritis is an immune-mediated inflammation within the glomerulus, and is characterized by an increased glomerular filtration of different plasma proteins (glomerular proteinuria) [96, 97]. The glomerulonephritis (GN) is classified according to etiology (primary or secondary), clinical presentation (asymptomatic, acute, rapidly progressive or chronic) and according to histopathology (proliferative or non-proliferative, diffuse or focal etc.). GN represents a major cause of ESKD worldwide. However, the clinical course and outcome is highly variable [98]. The main burden of GN is the progression to chronic kidney disease with the accompanied risks of premature CV death, end-stage kidney disease, dialysis and renal transplantation.

Epidemiology

Glomerulonephritis in general is more common in male than female. IgA-nephropathy (IgAN), focal segmental glomerulosclerosis (FSGS) membranous nephropathy, and small vessel vasculitis are the most commonly diagnosed in adults, while minimal change nephropathy (MCN) is the most commonly diagnosed in children [99]. The incidence rate of primary GN worldwide varies between 0.2 and 2.5/100.000 population/year depending on the type of GN. It is 0.6/100.000/year for MCN, 0.8/100.000/year for FSGS, 1.2/100.000/year for membranous nephropathy and 2.5/100.000/year for IgAN [100]. However, in southern Sweden (Skåne district), the incidence rate for IgA is much higher, and accounts for about 4/100.000/year (*the Swedish Renal Registry, www.snroline.se*).

Types of Glomerulonephritis

The common types of glomerulonephritis are discussed briefly in Table 2.

Table 2. The causes, clinical symptoms and complications of the common types of glomerulonephritis.

	IgAN	**FSGS**	**MCN**	**MN**
Cause	Mesangial deposition of polymeric IgA1	Podocytes injury, cause still unknown	Effacement of podocytes foot processes, unknown pathology	Immune deposits of IgG and complement in the glomerular basement membrane.
Symptoms	- 50-60% macroscopic hematuria - 30% asymptomatic microscopic hematuria and proteinuria - 5-10% nephrotic or nephritic syndrome.	- Clinical presentation as in nephrotic syndrome.	- Mainly children - Abrupt onset nephrotic syndrome	- Mainly nephrotic syndrome in Caucasians.
Clinical course	- 20-40% develop ESKD	- > 50% develop ESKD.	- 40% spontaneous remission. - ESKD is rare	- 1/3 spontaneous remission. - 1/3 sustained symptoms. - 1/3 ESKD.
References	[98, 101]	[102]	[103]	[104]

IgAN = IgA-nephropathy; FSGS = focal segmental glomerulosclerosis; MCN = minimal change nephropathy; MN = membranous nephropathy; ESKD = end-stage kidney disease.

Diabetes Mellitus

Diabetes is a multi-systemic disease characterized by hyperglycemia and is resulting from defects in insulin secretion or action or both [105]. Diabetes mellitus is associated with multi-organ damage and dysfunction and premature mortality due to ischemic heart disease, and cerebrovascular disease [106]. According to the WHO the world prevalence of diabetes mellitus among adults (aged 20-79 years) estimated to increase from 6.4 % in 2010 to 7.7 % in 2030, and the number of adult patients with DM is expected to rise up to 439 million by 2030 [107]. This increment is predominantly due to the global increase in obesity, aging, stressful life conditions, smoking and decreased physical activity [108]. In developed countries the majority of people with DM are > 64 years of age, while in developing countries the majority are between 45-64 years of age. Men have higher prevalence for DM than women and the prevalence also varies among different races and ethnics [109].

Classification

According to the American Diabetes Association 2011 [105], DM is classified into four types,

1. Type-1 DM, is an immune-mediated disease where autoantibodies attack insulin-producing β-cells in the pancreas leading to hyperglycemia due to insufficient insulin hormone production. Patients require immediate insulin-replacement therapy. This type accounts for only 5-10% of patients with DM, and it does mainly affect children and adolescents.

2. Type-2 DM, is associated with increased insulin resistance in the cells, while insulin production remains unaffected initially. Type-2 DM accounts for about 90% of all diabetics and it affects mainly middle-aged adults and older. Most patients in this type are obese.

3. Other specific types, like genetic defects in insulin action, pancreatic disease, endocrinopathies (Cushing´s syndrome, Acromegaly), drug induced, infections, uncommon (Stiff-man syndrome).

4. Other genetic syndromes sometimes associated with diabetes, like Down´s syndrome

Cardiovascular disease and diabetes

Diabetic patients have nearly twice the mortality of the general population, because of prevalent cardiovascular disease, coronary artery disease, cerebrovascular disease and peripheral vascular disease [110]. Furthermore, diabetes is the main cause of blindness, lower leg amputation and stroke in adults [3, 111, 112]. There is a strong correlation between diabetes and the known well established markers of atherosclerosis (hyperglycemia, hyperlipidemia and low-grade inflammation) [113]. Furthermore the occurrence of persistent proteinuria in diabetic patients considerably increases the risk for cardiovascular death [114].

Diabetic Kidney Disease

DKD is a long-term kidney and vascular complication of diabetes mellitus (DM), and is manifested clinically by persistent albuminuria (> 30 mg/24h or a spot-urine albumin creatinine ratio > 3 mg/mmol), elevated blood pressure and progressive decline in kidney function. About one-third of the patients who have had DM over 20 years develop persistent microalbuminuria, even when diabetes is controlled [115-117], and about 15% of newly diagnosed type-2 DM have evidence of microalbuminuria [118]. DKD is a leading cause of end-stage kidney disease (ESKD), where about half of the patients on kidney replacement therapy are diabetics [119-122]. DKD is associated with many-fold increase in cardiovascular (CV) and all cause mortality [123]. In type-2 DKD, the clinical course and the kidney structural changes are more heterogeneous, and are usually influenced by aging, atherosclerosis and hypertension. [124-126]. DKD is more prevalent among Americans and Asians than Caucasians [127].

Clinically, the DKD can be classified according to urinary albumin excretion into two stages, incipient nephropathy characterized by low grade albuminuria of 3-29 mg/mmol, (previously called microalbuminuria), and established nephropathy characterized by high grade albuminuria of > 30 mg/mmol (previously called macroalbuminuria) [117]. Histologically, the glomerular lesions have been recently classified into 4 classes; Class 1: Thickening of the glomerular basement membrane (GBM); Class 2: Mesangial expansion (a. Mild, b. Severe); Class 3: Nodular sclerosis (Kimmelstiel-Wilson lesions); and Class 4: advanced diabetic glomerular sclerosis [128].

The Pathophysiology of DKD

During the course of diabetes, the body undergoes several metabolic derangements due to chronic hyperglycemia and increased insulin resistance. Diabetic kidney damage is believed to occur via different metabolic pathways, represented by the non-enzymatic glycation of proteins and the formation of advanced glycation end products (AGE), generation of reactive oxygen species (ROS), activation of protein kinase C (PKC), abnormal polyol metabolism, and activation of renin-angiotensin system (RAS). [129-131]. The receptors for AGE (RAGE) are upregulated in the diabetic kidney, mainly in podocytes and mesangial cells [132]. The stimulation of AGE-RAGE axis elicits oxidative stress generation, by increasing ROS and decreasing endothelial nitric oxide (NO) production [133]. ROS lead to inflammation (mediated by macrophages and cytokines) and fibrogenic stimulation (mediated mainly by transforming growth factor β (TGF-β) and vascular endothelial growth factor (VEGF)) at the glomerulus, resulting in glomerulosclerosis and tubulointerstitial fibrosis [134]. The hyperglycemia-induced activation of PKC is also important in the ROS generation and activation of the TGF-β and VEGF [135]. The activation of the local RAS leads to the production of angiotensin II (Ang II) which in turn increases glomerular capillary pressure and stimulates renal fibrosis through Ang II type1 receptors (AT1R) and stimulation of growth factors, such as TGF-β [136]. Finally, alteration of the polyol metabolism at the cellular level is assumed to increase the mitochondrial ROS generation [137]. All these metabolic alterations cause vascular wall inflammation, fibrosis, atherosclerosis and thrombosis [138, 139].

The early functional and structural alterations of the diabetic kidney include glomerular hyperperfusion, hypertrophy of the nephron and gross renal enlargement [140]. In the very early stages of DKD, albuminuria is mainly attributed to a defect in the albumin reabsorption in the proximal tubules because of decreased expression of megalin receptors and deficient endocytosis of albumin. The loss of charge- and size-selectivity of the glomerular filtration barrier increases the glomerular permeability for albumin [13, 51, 141, 142]. The loss of size-selectivity is due to vascular endothelial dysfunction, thickening of the glomerular basement membrane and loss of podocytes [143].

Atherosclerosis

Atherosclerosis is a chronic inflammatory disease leading to coronary, cardiovascular and peripheral vascular disease [144, 145]. It is characterized by endothelial dysfunction caused by different risk factors, such as high blood cholesterol, smoking, hyperglycemia, and high blood pressure. This results in atherogenesis, an accumulation of calcium and low-density lipoprotein cholesterol (LDL-cholesterol) inside the vessels wall. Atherogenesis triggers the inflammatory cells in the vessel wall leading to the release of cytokines and growth factors resulting in atherosclerotic plaque formation. The atherosclerotic plaque is made of a fatty core (also called necrotic core) and is covered by fibrotic cap of smooth muscles. These fatty plaques may promote thrombosis and finally may rupture inside the vessel wall leading to acute vascular incident like acute myocardial infarction and stroke.

The Pathophysiology of Atherosclerosis

The normal human blood vessel wall consists of three layers: 1. The tunica intima, which is the inner layer in contact with the blood. It consists of a monolayer of endothelial cells overlying a basement membrane. 2. The tunica media, which is the middle layer. It contains circular smooth muscle fibers and an extracellular matrix. 3. The adventitia, which is the outermost layer of connective tissue. It contains nerve endings, microvessels and mast cells.

During the process of atherogenesis, the vascular endothelium is subjected to oxidative stress and free radical formation, which is aggravated by the presence of e.g. hypertension and hyperglycemia. The LDL molecules find their way inside the vessel wall through the damaged endothelium where they undergo modification (oxidation and lipolysis) [146]. They attach to the endothelium by lipoprotein lipase (LPL) and heparan sulphate proteoglycans (HPSG). The oxidized lipoprotein and local angiotensin II (Ang II) stimulate the vascular wall cells to produce pro-inflammatory cytokines [147]. Apolipoprotein CIII (Apo CIII) acts as a pro-inflammatory mediator. It increases the LDL binding to the endothelium via Toll-like receptors [148-150]. LDL particles start to aggregate to form lipid droplets. At the same time the endothelial cells express adhesion molecules that capture the white blood cells (the monocytes) from the blood stream. Chemoattractant mediators mediate the migration of the monocytes inside the tunica intima. Once migrated inside the vessel wall, the monocytes differentiate into macrophages. The macrophages start to take up modified LDL droplets by scavenger receptors and form larger cells called ”foam cells”. Due to

their large size, the foam cells will be entrapped inside the intima. These cells have the ability to release pro-inflammatory mediators such as Interleukin-1beta (IL-1β), interferon-γ and tumor necrosis factor (TNF-α) that aggravate the inflammatory process [151, 152]. The accumulation of foam cells forms the fatty core of atheroma [153, 154]. Ang II acts as a pro-inflammatory mediator in the vascular endothelium, and plays a pivotal role in the pathogenesis of atherosclerosis [147, 155, 156]. Ang II exerts its effect mainly by stimulation of type 1 receptors (AT1R), which are upregulated during inflammation [157]. Ang II induces vascular wall smooth muscle hypertrophy and fibrosis by promoting the production of cytokines and reactive oxygen species (ROS) [147, 158]. In the kidney, Ang II has the ability to stimulate NADPH (nicotinamide adenine dinucleotide phosphate)-oxidase, which activates ROS generation [159]. ROS triggers several intracellular downstream calcium ion mediated signaling pathways in the glomerular filtration barrier, which via further downstream steps activate the small guanosine triphosphatases (GTPases) (Rac1 and RhoA), that are responsible for podocyte and endothelial cell contraction, inflammation, migration, growth, apoptosis, fibrosis and proteinuria [160-163]. The perivascular adipocytes in patients with metabolic syndrome play a role in the process of atherosclerosis through the secretion of vasoactive substances, such as Ang II and endothelin-1 and the pro-inflammatory cytokines that mediate chronic inflammation and other adipokines, such as leptin, that stimulate smooth muscle cell proliferation and migration [164, 165]. The perivascular adipocytes also produce a number of anti-inflammatory adipokines, such as adiponectin [166, 167]. Adiponectin inhibits NADPH-oxidase and modulates oxidative stress [168]. Urinary excretion of adiponectin has shown to be correlated to vascular wall inflammation, atherosclerosis and the degree of albuminuria [169, 170].

MATERIALS AND METHODS

An overview of patient characteristics in studies I - IV is presented in Table 3. All the studies were approved by the Regional Ethical Committee of Lund University, and all the recruited patients gave informed consent.

Table 3. Summary of the baseline and follow-up characteristics, inclusion and exclusion criteria of studies I - IV.

	Study I	**Study II**	**Study III**	**Study IV**
Number, (male/female)	139 (79 / 60)	106 (74 / 32)	178 (101 / 77)	189 (113 / 76)
Age, median yrs. (range)	35 (18 - 80)	67 (33 - 85)	69 (39 - 98)	48 (17 - 89)
Recruitment period	1984 - 2003	1992 - 2004	1st Sept. - 31th Dec. 2010	1993 - 2004
Place of recruitment	Diabetes out-patient clinic, LUH	Diabetes out-patient clinic, LUH	Emergency Department, LUH	Nephrology Department, LUH
End of follow-up (year)	2007	2009	2012	2009
Follow-up time, (range)	18 yrs. (1 – 22 yrs.)	5 yrs. (6 mo. – 13 yrs.)	16 mo. (13 – 20 mo.)	8 yrs. (1 mo. – 15 yrs.)

	Study I	Study II	Study III	Study IV
Inclusion criteria	Type-1 diabetes	Type-2 diabetes	Acute chest pain	Idiopathic GN
Exclusion criteria	GN, systemic disease, kidney failure	GN, systemic disease, kidney failure	Infection, thromboembolism malignancy, kidney failure	DKD, Systemic disease, Kidney failure
End point	CV death & ESKD	CV death & ESKD	CV death	ESKD
Tested urine biomarker	Urine IgM	Urine IgM	Urine IgM	Urine IgG
Type of study	Observational, Prospective	Observational, Prospective	Observational, Prospective	Observational, Prospective,

LUH = Lund University Hospital; DKD = Diabetic kidney disease; GN = Glomerulonephritis; CV = Cardiovascular; ESKD = End-stage kidney disease.

Study I

139 patients with type-1 diabetes mellitus with regular follow-up at the diabetes outpatient clinic, Lund (Skåne) University Hospital, were recruited to the study. The patients' cohort was divided into subgroups according to urinary albumin excretion; patients with normoalbuminuria (n = 44, 15 female), patients with microalbuminuria (n = 46, 21 female) and patients with macroalbuminuria (n = 49, 24 female). The cohort was also divided into subgroups according to the urine IgM concentration; patients with low IgM-uria (n = 71, 22 female), and patients with high IgM-uria (n = 68, 38 female). The level of albuminuria and IgM-uria was confirmed by the average value of at least 2 out of 3 consecutive morning spot-urine samples. The patients' disease course was recorded until death, start of kidney replacement therapy or until the end of the study at 2007. The average follow up time was 18 years.

Study II

106 patients with type-2 diabetes mellitus at regular follow-up at the diabetic out-patient clinic, Lund (Skåne) University Hospital, were recruited to the study. The patients cohort was divided according to urine albumin concentration into; normoalbuminuria (n = 14, 7 female), microalbuminuria (n = 26, 7 female) and macroalbuminuria (n = 66, 18 female); and according to urine IgM concentration into; low IgM-uria (n = 51, 8 female) and high IgM-uria (n = 55, 24 female). The level of albuminuria and IgM-uria was confirmed by the average of at least 2 out of 3 consecutive morning spot-urine samples. The patients' disease course was recorded till death, start of renal replacement therapy or end of the study at 2009. The average follow up time was 5 years.

Study III

In this case-control study, 178 consecutive patients presenting with chest pain suggestive of acute coronary syndrome (ACS), at the Emergency Department at Lund (Skåne) University Hospital, were recruited during 53 daytime shifts in the period between September 1st and December 31th 2012. Patients were initially assessed by the attending physician with history, physical examination, electrocardiogram (ECG) and blood tests, including high sensitive plasma troponin T (TNT). The final diagnosis was retrieved from the discharge records of the patients. Urine proteins (albumin, IgG, IgM), plasma troponin, plasma hsCRP were taken at time of admission to the emergency department. The patients were followed until death, occurrence of major cardiovascular event or end of the study at 2012.

The patients were divided into 3 groups according to final diagnosis. 58 (23 female) patients had ACS. 55 (19 female) patients had stable angina (SA), which was defined as patients with previous history of CV event with no emerging ECG changes and normal TNT levels. And 65 (35 female) patients had non-specific chest pain with no history of CV event, normal ECG and TNT levels.

Study IV

189 patients with biopsy-verified proteinuric chronic GN and relatively normal kidney function (S. creatinine < 150 µmol/l) (table 4), were recruited to the study. These patients were participants of an investigation program for glomerular diseases conducted at the Nephrology department, Lund (Skåne) University Hospital. Urinary and blood samples were collected in the early morning of the day of kidney biopsy. The patient cohort was divided into subgroups according to the urine concentrations of IgG, protein HC and albumin (table 5). The patients' disease course was recorded up to death, start of kidney replacement therapy, or end of the study at 2009. The average follow-up time was 8 years.

Table 4. The types and numbers of glomerular diseases.

GN type	Number
Mesangio-proliferative	75
Minimal change nephropathy	32
IgA-nephropathy	31
Nephrosclerosis	24
Membranous GN	21
FSGS	6

Table 5. Patients were divided according to quartiles of urine concentrations of IgG, protein HC and albumin.

Quartile	IgG- uria *(mg/mmol)*	HC-uria *(mg/mmol)*	Albuminuria *(mg/mmol)*
Q1	< 1.8	< 0.6	< 20
Q2	1.8 - 5	0.6 - 1.2	20 - 109
Q3	5 - 14	1.2 - 2.8	110 - 279
Q4	> 14	> 2.8	> 280

Q = Quartile; IgG = immunoglobulin G; protein HC = α1-microglobulin

Patient follow-up

The included patients in the studies were followed prospectively. Patients' clinical assessment, laboratory results and final diagnosis were obtained from the patients' hospital records. Cardiovascular death was defined as all deaths where unequivocal non-CV death was established. End-stage kidney disease (ESKD) was defined as the start of kidney replacement therapy. Major CV events were defined as any subsequent event of acute myocardial infarction (AMI), unstable angina, stroke, acute heart failure due to ischemic heart disease or CV death during the follow-up time. The cause of death was obtained from the National Death Registry at the Swedish Board of Health and Welfare and the patients' hospital records [171].

Laboratory analysis

The urine and plasma proteins (albumin, IgG and creatinine) were analyzed at the Central Clinical Chemistry Laboratory at the University Hospital in Lund. Serum and urinary creatinine concentrations were analyzed by creatinine-amidino-hydrolase (KODAK EKTACHEM analyzer, Instrument Kodak, NY, USA). Urinary albumin and IgG concentrations were measured on fresh urine samples using immunoturbidimetry using a Cobas Mira S system (Roche Inc.) and monospecific rabbit antisera obtained from Dako (Copenhagen, Denmark) [172]. The urine samples were stored at -20°C until analyses of urine IgM concentrations [45, 173]. Urine IgM was measured by ELISA method described in detail elsewhere [37, 45, 174, 175].

Glomerular filtration rate

The glomerular filtration rate (GFR) was estimated by Lund-Malmö (LM) equation and MDRD (Modification of Diet in Renal Disease) equation [176-179].

The MDRD equation:

$$eGFR = \{186.3 \times (\text{serum creatinine mg/dl})^{-1.154} \times (\text{age})^{-0.203} (\times 0.742 \text{ if female}).$$

The Lund-Malmö (LM) equation for Patients with plasma creatinine (pCr) < 150 mmol/l:

$$eGFR = e^{4.62-0.0112\times pCr-0.0124\times age+0.339\times \ln(age)-0.226(\text{if female})}$$

The Lund-Malmö (LM) equation for patients with plasma creatinine (pCr) > 150 mmol/l:

$$eGFR = e^{8.17-0.0005\times pCr-1.07\times \ln(pCr)\times age+0.339\times \ln(age)-0.2226 (\text{if female})}$$

Statistical analysis

Data were presented as median (range) or mean ± SE as appropriate. The statistical comparison was performed when appropriate by; Kruskal-Wallis H test, Mann-Whitney U test, Fisher´s exact test and Pearson Chi-squared (χ^2) test. A p-value < 0.05 and a 95% Confidence Interval (CI) and were applied as thresholds of statistical significance. The Cox-regression analysis was used to estimate the hazard ratio (HR) for the occurrence of outcome. The Kaplan-Meier survival analysis and the Log Rank test were used to assess the difference in survival between subgroups.

RESULTS

Study I and II

Patients with type-2 diabetes mellitus were older, and had lower kidney function and higher baseline albuminuria and IgM-uria than those with type-1 DM (table 6). However, irrespective of the level of albuminuria, an increased urine IgM excretion in patients with type-1 or type-2 diabetes was associated with a significantly lower kidney and patient survival compared to those with low urine IgM excretion (table 7).

Out of 139 patients with type-1 diabetes, 32 (18 female) patients died of cardiovascular (CV) disease and 20 (9 female) patients reached ESKD during the 18 years follow-up time. Patients with either micro- or macroalbuminuria and high IgM-uria had a 2.7 fold increased risk for CV and renal mortality, compared to those with low IgM-uria (figure 4a). The same findings were observed in patients with type-2 diabetes mellitus (n = 106), where the composite outcome for CV mortality and ESKD was 4.1 folds higher in patients with either micro- or macroalbuminuria and high IgM-uria, compared to those with low IgM-uria (figure 4b, table 7).

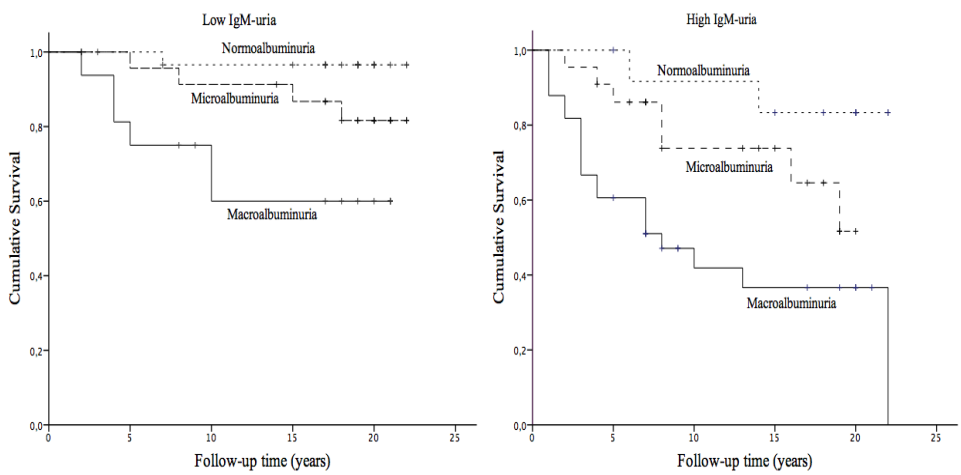


Figure 4a. The composite outcome of renal and cardiovascular death by albuminuria and urine IgM-uria in 139 patients with type-1 DM. The relative risk for microalbuminuria and high IgM-uria RR=2.8, and the relative risk for macroalbuminuria and high IgM-uria RR=2.5.

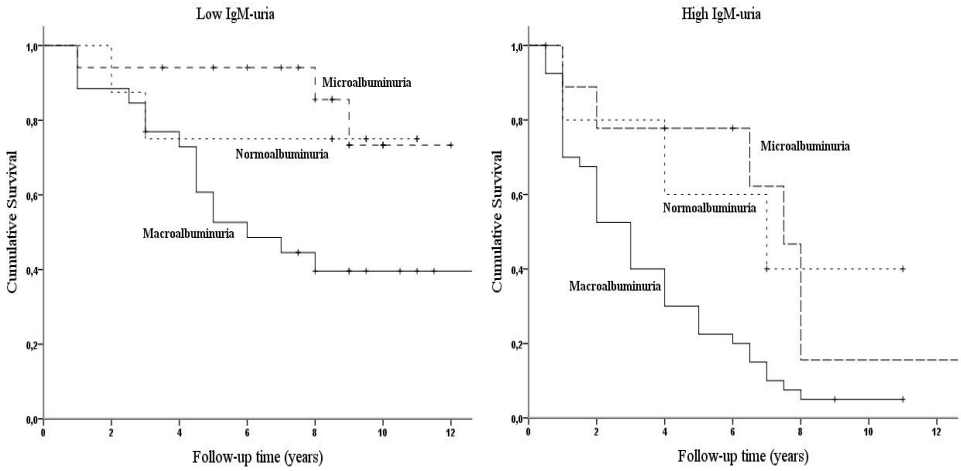


Figure 4b. The composite outcome of renal and cardiovascular death by albuminuria and IgM-uria in 106 patients with type-2 DM. The relative risk for microalbuminuria and high IgM-uria RR=5.5, and the relative risk for macroalbuminuria and high IgM-uria RR=2.7.

Table 6, illustrates the baseline characteristics of the studied patients of type-1 and -2 diabetes in studies I, and II.

Table 6. Comparison of the baseline characteristics of patients with type-1 and type-2 DM according to the degree of IgM-uria (low and high). Values are presented as median (range).

	Type-1 DM		**Type-2 DM**	
IgM group	*Low IgM*	*High IgM*	*Low IgM*	*High IgM*
Age, yrs.	35 (18 - 74)	37.5 (20 - 80)	66 (33 - 79)	68 (44 - 85)
Duration, yrs.	15 (1 - 65)	24.5 (1 - 67)	10 (1 - 35)	14 (1 - 46)
S. creatinine, *mmol/l*	78 (54 - 216)	86 (42 - 486)	81 (40 - 293)	140 (53 - 325)
ACR, *mg/mmol*	3.6 (0.23 - 268)	21.3 (0.26 - 640)	23.8 (0.7 - 543)	154 (0.7 - 1026)
MCR, *mg/mmol*	0.005 (0.0017 - 0.0091)	0.0158 (0.0095 - 0.363)	0.009 (0.0048 - 0.0147)	0.0293 (0.0151 - 0.631)
MAP, *mmHg*	93 (80 - 120)	100 (78 - 133)	106.7 (80 - 131)	110 (83 - 150)
eGFR, *ml/min* × *1.73m*^{2}	88 (27 - 141)	67 (9 - 144)	86.7 (19.5 - 157)	39.4 (12 - 111)
HbA1c, *%*	8.3 (4.5 - 13.4)	9 (5.5 - 13.2)	6.7 (3.9 - 10)	7.2 (4.5 - 14.9)

ACR = urine albumin/creatinine ratio; MCR = urine IgM/creatinine ratio; MAP = mean arterial pressure; eGFR = estimated glomerular filtration rate; HbA1c = Glycosylated Hemoglobin Test.

In both type-1 and -2 diabetic patients, those with high IgM-uria had a higher risk for renal and CV mortality than those with low IgM-uria, irrespective to the level of albuminuria (table 7).

Table 7. The rate of renal and cardiovascular death (combined event) per 100 patients-year, in patients with type-1 and type-2 diabetes, according to degree of albuminuria and IgM-uria.

		Type1 Combined event per 100 patient-year	Relative Risk (RR)	**Type2** Combined event per 100 patient-year	Relative Risk (RR)
Normoalbuminuria	Low IgM-uria	0.19	4.7	3.2	3
	High IgM-uria	0.9		9.8	
Microalbuminuria	Low IgM-uria	0.95	2.8	2.3	5.5
	High IgM-uria	2.7		12.3	
Macroalbuminuria	Low IgM-uria	2.9	2.5	9.4	2.7
	High IgM-uria	7.3		19.0	

Study III

Patients with acute coronary syndrome (ACS) at time of presentation to the emergency department had significantly higher baseline urinary albumin and IgM levels than those with non-specific chest pain (p = 0.001, p = 0.029, *respectively*) (figure 5a). Out of 178 patients, 5 (1 female) patients died of CV cause and 40 (19 female) patients suffered a subsequent major CV event during a ~2 years follow-up. The risk for occurrence of subsequent major CV event was predicted by IgM-uria independently of albuminuria, where as chest pain patients with high IgM-uria had a 3 fold increase in risk for future CV event compared to those with low IgM-uria (RR = 3.3, p = 0.001) (figure 5b).

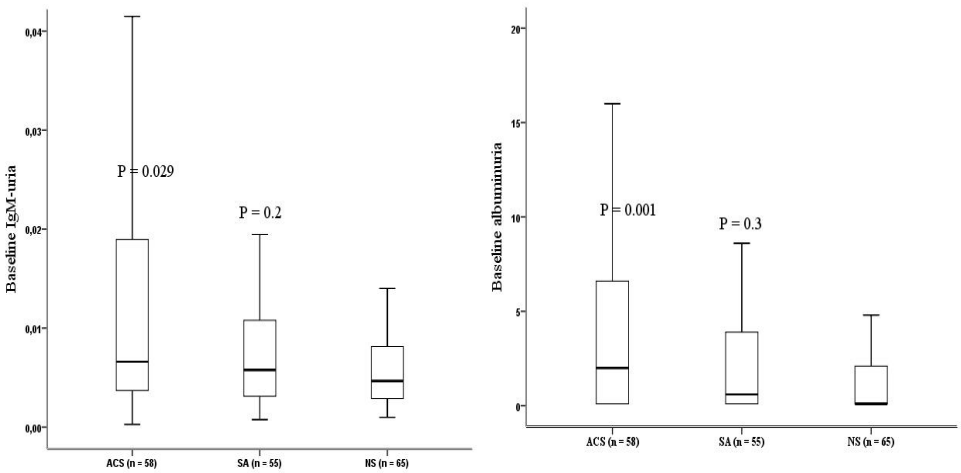
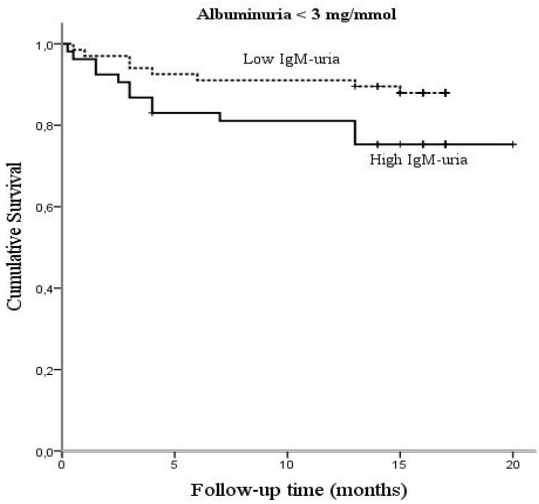


Figure 5a. The pattern of proteinuria in 178 patients diagnosed with acute coronary syndrome (ACS), Stable angina (SA), or non-specific chest pain (NS).



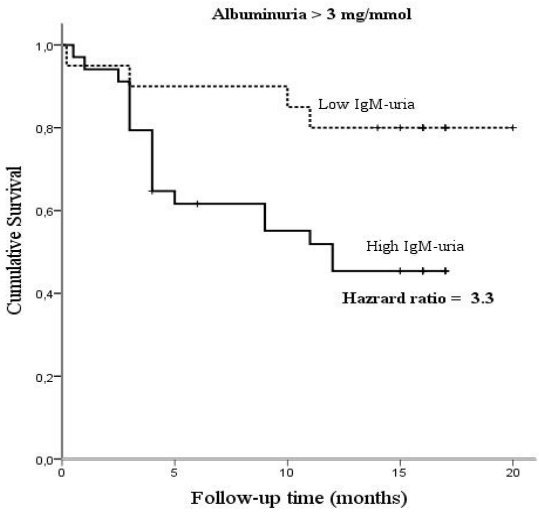


Figure 5b. IgM-uria and cardiovascular outcome in 178 patients with acute chest pain.

Study IV

In this study we found that an increased baseline urine IgG excretion in patients with proteinuric glomerular disease was associated with an increased risk for ESKD, independently of the level of albuminuria (HR = 5.9, $p < 0.001$). IgG-uria concentration of > 5 mg/mmol was the best cutoff level that identified glomerulonephritis patients at increased risk for ESKD (figure 6a). Also patients with a baseline urine protein HC of > 2.8 mg/mmol had 2.9 fold risk for ESKD (figure 6b). There was no significant difference in risk for ESKD with different levels of albuminuria (figure 6c).

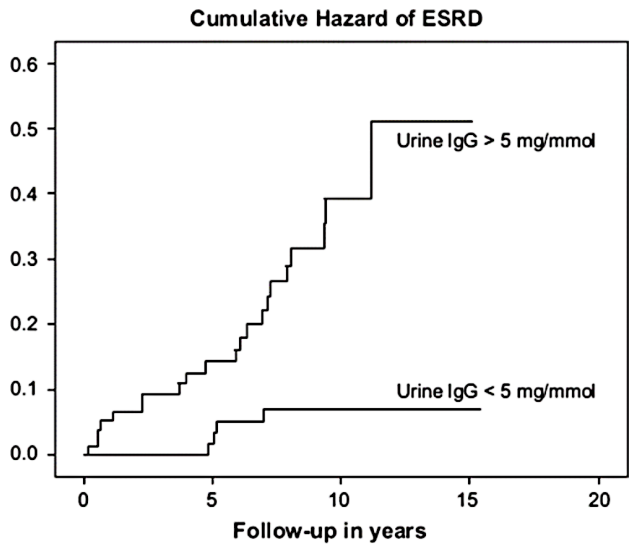


Figure 6a. Cumulative risk of end-stage kidney disease (ESKD) according to a cut-off level of IgG-uria (less than *vs.* greater than 5 *mg/mmol*). Relative Risk ~ 6, Log-rank test < 0.001.

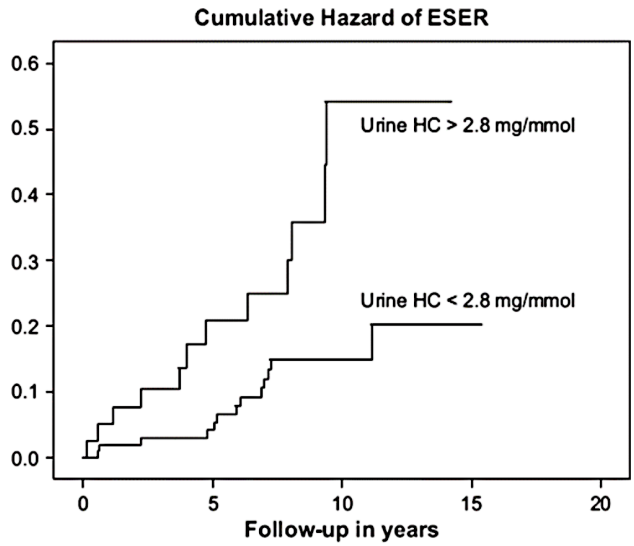


Figure 6b. Cumulative risk of end-stage kidney disease (ESKD) according to a cut-off level of HC-uria (less than *vs*. greater than 2.8 mg/mmol). Relative Risk ~ 3, Log-rank test = 0.006.

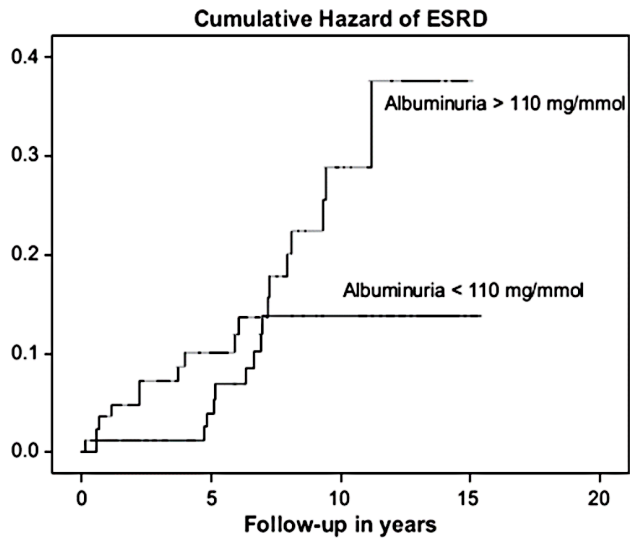


Figure 6c. Cumulative risk for end-stage kidney disease (ESKD) according to cut-off level of albuminuria (less than *vs.* greater than 110 mg/mmol). Log-rank test = 0.07.

DISCUSSION

In studies I and II, we found that patients with type-1 and type-2 diabetes with an increased urine IgM excretion (high IgM-uria) are at higher risk of kidney and cardiovascular complications than those with low IgM-uria, irrespective of the degree of albuminuria. The ability of albuminuria in predicting disease outcome has been questioned in many studies, e.g. up to 30% of diabetic patients with diabetic kidney disease (DKD) have a normal urine albumin excretion, despite decline in kidney function and progressive vascular endothelial damage [12, 180]. Furthermore, not all DKD patients with microalbuminuria progress to macroalbuminuria, and some of them may regress to the normal range of urinary albumin excretion [181]. An increased urine IgM excretion in normoalbuminuric diabetic patients, has also been recently reported in another American study by Gohda et al [182]. Thus, albuminuria might be a less sensitive marker of glomerular kidney disease progression. This fact is of enormous concern, since in routine clinical practice, albuminuria is used for diagnosis and risk stratification of patients with DKD. We believe that, by including IgM-uria in the management protocols of patients with type-1 or type-2 diabetes mellitus might further improve the sensitivity of the routine clinical diagnostic and prognostic tools [20, 21, 23, 183]. The finding of higher urine IgM excretion in patients with type-2 diabetes compared to type-1 diabetes could be due to the difference in age and pathophysiological mechanisms of DKD in both types of diabetes [124-126, 184, 185]. While chronic hyperglycemia is the main driving mechanism for DKD in type-1 DM, the metabolic syndrome of obesity, hypertension, dyslipidemia, and hyperglycemia is the main driving element in the pathology of DKD in type-2 diabetes [186-188].

Markers of general inflammation, such as pro-inflammatory cytokines, high sensitive C-reactive protein (hsCRP) and albuminuria are also associated with cardiovascular disease [189-194]. However, in recent large cohort studies the clinical value of these markers in estimation of the CV risk in healthy and diabetic patients is doubtful [195, 196]. Besides, albuminuria may or may not reflect the degree of vascular endothelial dysfunction in healthy individuals with coronary artery disease [197]. In study III, IgM-uria was found to be associated with increased risk for the occurrence of subsequent CV events in patients presenting to the emergency department (ED) with acute chest pain. The extent of association between IgM-uria and the degree of vascular wall inflammation and atherosclerosis need to be studied. However, the data from our research group,

strongly suggest that the glomerular passage of large molecules, in the size of IgM is mainly occurred through large shunts or defects in the glomerular filtration barrier (GFB). Thus, the presence of IgM-uria conceivably reflects severe size-selectivity dysfunction of the glomerular filtration barrier, secondary to generalized atherosclerosis and systemic inflammation. This is further strengthened by the fact that (study III) patients with acute coronary syndrome (ACS) and IgM-uria have significantly elevated levels of hsCRP, and troponin T, reflecting systemic inflammation, associated with atherosclerosis and coronary artery disease [194, 198], [199]. At the kidney level, atherosclerosis is associated with elevated glomerular vascular wall resistance, resulting in glomerular ischemia, and as a consequence, a markedly increased population of highly unselective glomerular shunt-pathways [21, 32, 200, 201]. This could explain the association of IgM-uria with cardiovascular mortality in diabetic patients and in patients with coronary artery disease (figure 7).

Urine IgM analysis is a simple non-invasive urine test that could be included in the general clinical setting, together with urine albumin creatinine ratio (ACR), in risk stratification of patients with DKD and patients with atherosclerotic heart disease. This could help in early introduction of multifactorial cardiovascular preventive treatment strategy to improve the overall cardiovascular risk profile and patients' outcome (figure 8).

In study IV; patients with idiopathic proteinuric glomerulonephritis with an increased urine IgG excretion at time of diagnosis are found to be more likely to progress to kidney failure than patients with only high degree of albuminuria [20-24, 202]. The cut-off level of IgG-uria > 5 mg/mmol was determined to best predict the risk of kidney failure. An increased urinary excretion of IgG (molecular radius 55 Å) implies loss in size selectivity of the glomerular filter and severe glomerular damage, while pure albuminuria may occur if the small pore radius is increased or charge-selectivity of the glomerular filter is lost, as is proposed to be the case in minimal change nephropathy. This condition is not associated with progressive kidney damage [203]. This further strengthened by the association of IgG-uria with occurrence of tubulo-interstitial fibrosis and an increased urine protein HC concentration [22]. Protein HC is a low-molecular weight (LMW) protein that passes "unrestricted" through the glomerulus and is normally reabsorbed by the proximal tubules. Thus, an increased urine protein HC concentration may reflect tubulo-interstitial damage secondary to unselective proteinuria. [24, 204, 205].

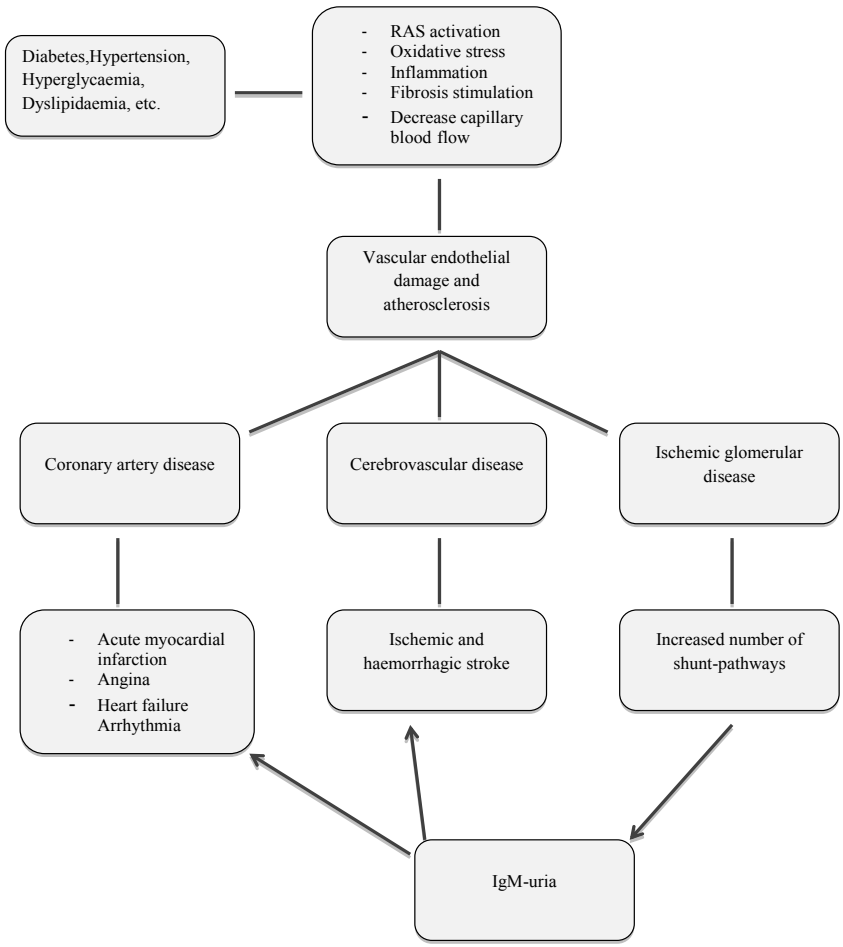


Figure 7. A simple flowchart representing the pathophysiology of IgM-uria and its association with cardiovascular disease.

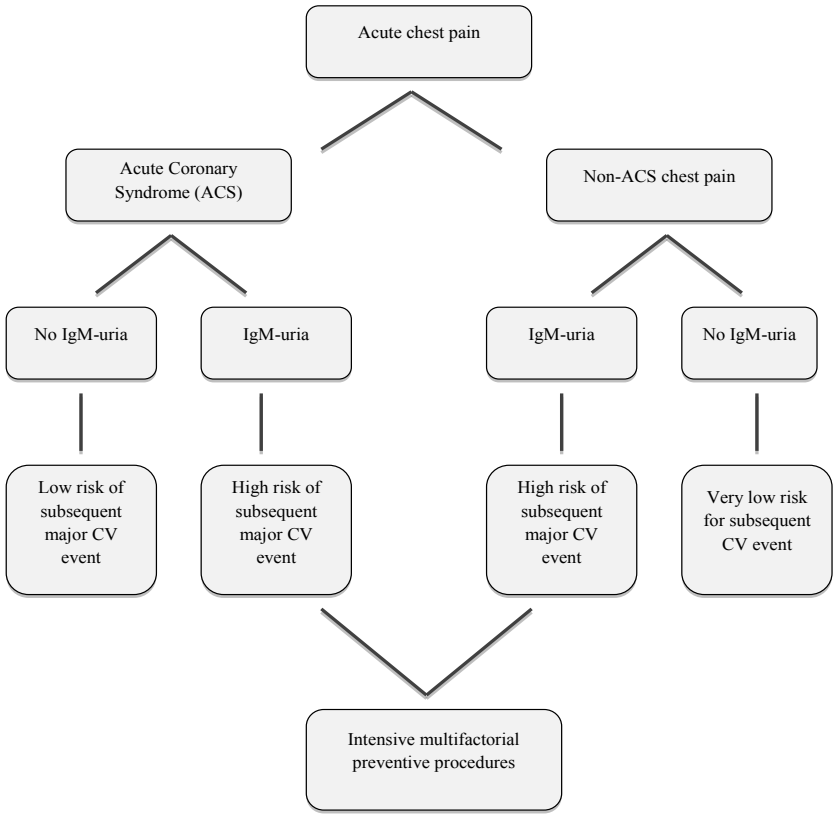


Figure 8. A proposal for the use of IgM-uria in the risk assessment of patients with acute chest pain.

CONCLUSIONS

- IgM-uria is associated with an increased risk for cardiovascular and end-stage kidney disease in both type-1 and type-2 diabetes mellitus.

- IgM-uria in patients with chest pain is associated with an increased risk of occurrence of cardiovascular events.

- Adding IgM-uria in the risk stratification of diabetic and chest pain patients could improve disease outcome by promoting early initiation of cardiovascular disease preventive treatment.

- IgG-uria could be helpful in risk stratification of patients with proteinuric glomerulonephritis for early identification and management of patients at high risk for kidney disease progression.

Further perspectives

The studies in this thesis are a continuation of previously presented data on the impact of high molecular weight proteinuria in patients with chronic kidney disease. Our study results report for the first time the potential value of IgM-uria in the risk assessment for worse kidney and cardiovascular outcomes in patients with diabetes and coronary artery disease. However, the results have raised many questions that need to be further investigated. We need to better understand the mechanisms of the association of IgM-uria with atherosclerotic and cardiovascular disease. Therefore, studying the correlation between IgM-uria, and pro-inflammatory cytokines, such as interleukins, gamma-interferon and tumor necrosis factor would be of interest. Besides, we also intend to study the correlation between IgM-uria and anti-inflammatory adipokines (adiponectin), which was recently shown to be correlated to poor kidney function in diabetic patients.

Populärvetenskaplig sammanfattning (Swedish Summary)

Diabetes mellitus, kronisk njursjukdom samt aterosklerotisk hjärtsjukdom utgör de vanligaste orsakerna till hjärt-kärlsjukdom och för tidig dödlighet. Dessa sjukdomar har blivit ett stort världsomfattande hälsoproblem med ökande prevalens och incidens. Tidig identifiering av patienter som ligger i riskzonen för komplikationer, framförallt hjärt-kärl komplikationer, torde vara av stort värde for både patienter och samhälle. Därför är behovet av att finna nya markörer för hjärt-kärlsjukdom ytterst angeläget.

Det är sedan tidigare känt att även en lätt förhöjd utsöndring av äggvita i urinen, s.k. mikroalbuminuri, är en oberoende riskfaktor för njursjukdom, ateroskleros och dödlighet i hjärt-kärlsjukdom. Dock, mikroalbuminuri kan förekomma vid såväl olika sjukdomstillstånd (såsom diabetes, högt blodtryck, övervikt, etc.), som en rad olika icke sjukliga tillstånd (såsom vid fysisk ansträngning, trauma, graviditet, etc.). Dessutom, en stor del av patienterna med diabetisk njursjukdom, trots befintlig diabetisk njurskada, har ingen uttalad albuminuri. Senaste experimentella och epidemiologiska studier har visat ett samband mellan utsöndringen av mycket stora proteiner, som IgG och IgM, i urinen med ökad risk för njursjukdom. I denna avhandling har vi studerat betydelsen av utsöndringen av dessa stora proteiner (IgG och IgM) i urinen vid vanligt förekommande sjukdomstillstånd, såsom diabetes, glomerulonefrit och ischemic hjärtsjukdom, och dess samband med hjärt-kärl dödlighet och njursvikt.

I studie I och II har vi undersökt 139 patienter med typ-1, *respektive* 106 patienter med typ-2 diabetes som har början till njurskada, vid njurmottagningen på universitetssjukhuset i Lund. Medianuppföljningstiden var 18 år *respektive* 5år. De som hade en hög grad av IgM-uri vid studiestart, oavsett grad av albuminuri, hade en signifikant högre risk (ca 3-4 gånger högre) att avlida av komplikationer som hjärtinfarkt, hjärtstillestånd samt att utveckla njursvikt under uppföljningstiden.

I studie III, undersöktes sambandet mellan urin-IgM och förekomsten av större hjärt-kärl händelser, såsom akut hjärtinfarkt, angina pectoris, akut hjärtsvikt och stroke. Vi inkluderade 178 patienter som sökte akutmottagningen vid universitetssjukhuset i Lund för akut bröstsmärta. Vid ankomsten till akutmottagningen, efter patientens samtycke, togs urin- och blodprover samt EKG. Proverna analyserades för urinproteiner (albumin och IgM) samt plasma troponin T (hjärtinfarkt markör) och högsensitiv snabbsänka (hsCRP). Studien visade att patienter som hade akut kranskärlssjukdom vid ankomsten till akutmottagningen hade signifikant högre grad av IgM-uri jämfört med de som hade icke-specifik bröstsmärta. Dessutom, dem med hög IgM-uri vid ankomsten, oavsett grad av albuminuri, hade 3 gånger högre risk inom ca 2 år för att dö i hjärtsjukdom eller utveckla en större hjärt-kärl händelse.

I studie IV, studerades 189 patienter med biopsi-verifierad primär glomerulonefrit (kronisk inflammation i njurnefroner, som kan leda till njursvikt och dialys). Patienterna utgjorde en del av en större kohort som genomgått ett utredningsprogram för glomerulär sjukdom på njurmottagningen vid universitetssjukhuset i Lund. Medianuppföljningstiden var 8år. Patienter med hög grad av IgG-uri vid studiestart hade en signifikant högre risk att utveckla njursvikt, oberoende av grad av albuminuri. Resultatet visade att urin-IgG på > 5 mg/mmol var det bästa cut-off värdet för att identifiera glomerulonefrit-patienter med risk för njursvikt.

Njurens minsta funktionella enhet nefronet, består av ett kärlnystan (glomerulus) som producerar urin genom filtration av blodplasma genom kapillärväggen (den glomerulära filtrationsbarriären). Den glomerulära filtrationsbarriären består av 3 lager: endotelcellerna (finns närmast blodet), det glomerulära basalmembranet samt podocytcellerna med sina fotutskott (omsluter kapillärerna). Alla tre lager samspelar för att filtret ska vara tätt. För stora proteiner, som albumin (radie 36 Å), är det endast mycket små mängder som passerar ut (1 av 10 000 albuminmolekyler). Ännu större proteiner, som IgM (radius 120 Å), kan passera filtret endast via mycket stora hål (läckor), som föreligger vid en svår skada i filtret. Därför kan en skada i ett eller flera av dessa lager (som till exempel vid kärlförkalkningar eller inflammation) leda till att stora proteiner kan komma att filtreras ut i urinen.

Förekomst av IgG/IgM i urinen i stora mängder speglar således en betydligt större skada i glomerulära filtret. Då våra studieresultat har visat att IgM i urinen hos diabetiker och kranskärlsjuka är starkt förknippat med ökad risk för njur- och hjärt-kärlsjukdomar, torde urin-IgM vara en markör för en underliggande ateroskleros och kronisk kärlinflammation. Ateroskleros och inflammation i sin tur orsakar minskat blodflöde, så kallad ischemi, som medför ökat antal mycket stora hål (läckor) i det glomerulära filtret och därmed IgM-uri. Svåra glomerulonefriter

orsakar också stora skador i det glomerulära filtret vilket leder till ökat antal mycket stora porer. Vi tror att graden av IgG-uri (och med all sannolikhet IgM-uri) kan reflektera svår glomerulärskada, och därmed njursvikt. Dessa fynd ger oss en möjlighet att hitta patienter som riskerar att utveckla komplikationer tidigare än vad som är möjligt idag.

Konklusion: Högriskpatienter som vi kan identifiera med hjälp av IgG och IgM i urinen, bör genomgå intensivare uppföljning och behandling, för att på så sätt minska risken för framtida skador på hjärta, blodkärl och njurar. I praktiken innebär detta en ny approach för omhändertagandet av denna patientgrupp.

Grants

This work was supported by the Swedish government (ALF grants); The Swedish Medical Research Council (Vetenskapsrådet, grant #08285); Kungliga Fysiografiska Sällskapet I Lund; Läkarsällskapet I Lund and the Swedish Heart and Lung Foundation (Hjärt-Lungfonden); We appreciate and greatly acknowledge all support.

Acknowledgments

I would like to take the opportunity to thank everyone who helped me during this interesting journey. They have all made it possible for me to commence and complete this enormous task. I am sincerely grateful to my principal tutor, Associate Professor Dr. **Omran Bakoush**, for introducing me to the research environment and patiently taking me through this difficult task of educational research. I am so deeply humbled by his gracious support and positive attitude. I am thankful for all the time and extra hours he spent in supervising me, taking care of me and encouraging me. This work would not been accomplished without him. I would also like to acknowledge and commend Professor Dr. **Bengt Rippe** for his continuous and generous guidance and support. I appreciate his outstanding enthusiastic spirit and his enormous knowledge. Thanks for being so nice and ever ready to share your time.

I would also express my sincere gratitude to;
Associate Professor Dr. **Ole Torffvitt**, my co-author, for introducing me to this research, using his laboratory facilities for IgM analysis and for valuable revision of manuscripts.

Associate Professor Dr. **Ulf Ekelund**, my colleague and co-author, for giving me the chance to present my research at the emergency department, helping me with the needed preparations for study IV and for constructive feedback and revision of manuscripts.

Dr. **Per Swärd**, my colleague and co-author, for fantastic co-operation, sharing ideas and stimulatory discussions.

Anna Rippe and **Josefin Axelsson** for introducing me to the animal laboratory and being so nice to me. Thank you for unforgettable funny moments during coffee breaks.

Kersting Wihlborg for professional secretary help. Thank you for letting me feel welcomed every time I knocked the door.

Pia Myllenberg for excellent secretary help.

Åsa Pettersson for helping me with the IgM analyses and taking care of the samples.
My colleagues, nurses and staff of the **Emergency Department in Lund,** for fantastic help and superb cooperation. Special thanks to my previous managers at the ED specifically, Associate Professor Dr. **Hans Friberg**, Dr. **Ann-Kathrin Demény**, **Margaretha Grabe-Andersson** and Dr. **Jan Ljunghusen** for their support.

The nurses and the staff of the Acute Medical Ward **(MAVA)** and the Cardiac Care Unite **(HIA)**, for fantastic help in sample collection.

Nurse **Mona** and nurse **Margareta** at the Kidney Out-patient Clinic in Lund, for their exceptional hard work in collection and storage of the samples.

The personnel at **Media Tryck**, Lund University, for wonderful edition of my posters and this thesis.

My friends and colleagues, **Mohaned Hameed**, **Dalia Rhman**, **Wathik Alsalim**, **Ali Salloom** and **Muhammad Kadum** for standing by my side in times of need.

Last but not least, I need to honor the most precious persons in my life, my **parents**, my wife **Noor** and children **Selma** and the "**coming angel"**, my brother **Mohammad** and sister **Rasha** for their endless love, encouragement, support and praying for me. Thank you for your understanding and patience. You made my life worth living. Thank you Rasha for the nice drawings in the thesis. Thank you Noor and Selma for providing me all the support I needed and for providing me all the requirements for this work to be accomplished. Thanks for being there for me.

References

1. Smith SC, Jr., Chen D, Collins A, Harold JG, Jessup M, Josephson S, Logstrup S, Sacco RL, Vardas PE, Wood DA *et al*: **Moving from political declaration to action on reducing the global burden of cardiovascular diseases: a statement from the Global Cardiovascular Disease Taskforce**. *Journal of the American College of Cardiology* 2013, **62**(22):2151-2153.
2. Levey AS, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW, Hogg RJ, Perrone RD, Lau J, Eknoyan G *et al*: **National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification**. *Annals of internal medicine* 2003, **139**(2):137-147.
3. Norhammar A, Schenck-Gustafsson K: **Type 2 diabetes and cardiovascular disease in women**. *Diabetologia* 2013, **56**(1):1-9.
4. Yusuf S, Reddy S, Ounpuu S, Anand S: **Global burden of cardiovascular diseases: part I: general considerations, the epidemiologic transition, risk factors, and impact of urbanization**. *Circulation* 2001, **104**(22):2746-2753.
5. Ekelund U, Nilsson HJ, Frigyesi A, Torffvit O: **Patients with suspected acute coronary syndrome in a university hospital emergency department: an observational study**. *BMC emergency medicine* 2002, **2**(1):1.
6. Gansevoort RT, Correa-Rotter R, Hemmelgarn BR, Jafar TH, Heerspink HJ, Mann JF, Matsushita K, Wen CP: **Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention**. *Lancet* 2013, **382**(9889):339-352.
7. Toyama T, Furuichi K, Ninomiya T, Shimizu M, Hara A, Iwata Y, Kaneko S, Wada T: **The impacts of albuminuria and low eGFR on the risk of cardiovascular death, all-cause mortality, and renal events in diabetic patients: meta-analysis**. *PloS one* 2013, **8**(8):e71810.
8. Yun L, Xu R, Zhang L, Li G, Huang S, Yao Y, Li J: **The role of microalbuminuria in arterial endothelial dysfunction in hypertensive patients with carotid plaques**. *International heart journal* 2014, **55**(2):153-159.
9. Siddiqi FS, Advani A: **Endothelial-podocyte crosstalk: the missing link between endothelial dysfunction and albuminuria in diabetes**. *Diabetes* 2013, **62**(11):3647-3655.
10. Ruggenenti P, Remuzzi G: **Time to abandon microalbuminuria?** *Kidney international* 2006, **70**(7):1214-1222.
11. Cohen-Bucay A, Viswanathan G: **Urinary markers of glomerular injury in diabetic nephropathy**. *International journal of nephrology* 2012, **2012**:146987.
12. Jha JC, Jandeleit-Dahm KA, Cooper ME: **New insights into the use of biomarkers of diabetic nephropathy**. *Advances in chronic kidney disease* 2014, **21**(3):318-326.

13. Rippe C, Rippe A, Torffvit O, Rippe B: **Size and charge selectivity of the glomerular filter in early experimental diabetes in rats**. *Am J Physiol Renal Physiol* 2007, **293**(5):F1533-1538.
14. Kornhauser C, Malacara JM, Macias-Cervantes MH, Rivera-Cisneros AE: **Effect of exercise intensity on albuminuria in adolescents with Type 1 diabetes mellitus**. *Diabetic medicine : a journal of the British Diabetic Association* 2012, **29**(1):70-73.
15. Axelsson J, Mahmutovic I, Rippe A, Rippe B: **Loss of size selectivity of the glomerular filtration barrier in rats following laparotomy and muscle trauma**. *Am J Physiol Renal Physiol* 2009, **297**(3):F577-582.
16. Nergelius G, Vinge E, Grubb A, Lidgren L: **Renal impairment after hip or knee arthroplasty. Urinary excretion of protein markers studied in 59 patients**. *Acta orthopaedica Scandinavica* 1997, **68**(1):34-40.
17. Gosling P, Sanghera K, Dickson G: **Generalized vascular permeability and pulmonary function in patients following serious trauma**. *The Journal of trauma* 1994, **36**(4):477-481.
18. Gosling P, Shearman CP, Gwynn BR, Simms MH, Bainbridge ET: **Microproteinuria: response to operation**. *British medical journal* 1988, **296**(6618):338-339.
19. Vlachou E, Gosling P, Moiemen NS: **Microalbuminuria: a marker of endothelial dysfunction in thermal injury**. *Burns : journal of the International Society for Burn Injuries* 2006, **32**(8):1009-1016.
20. Bakoush O, Torffvit O, Rippe B, Tencer J: **High proteinuria selectivity index based upon IgM is a strong predictor of poor renal survival in glomerular diseases**. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 2001, **16**(7):1357-1363.
21. Bakoush O, Segelmark M, Torffvit O, Ohlsson S, Tencer J: **Urine IgM excretion predicts outcome in ANCA-associated renal vasculitis**. *Nephrol Dial Transplant* 2006, **21**(5):1263-1269.
22. Bakoush O, Grubb A, Rippe B, Tencer J: **Urine excretion of protein HC in proteinuric glomerular diseases correlates to urine IgG but not to albuminuria**. *Kidney international* 2001, **60**(5):1904-1909.
23. Bakoush O, Torffvit O, Rippe B, Tencer J: **Renal function in proteinuric glomerular diseases correlates to the changes in urine IgM excretion but not to the changes in the degree of albuminuria**. *Clinical nephrology* 2003, **59**(5):345-352.
24. Bazzi C, D'Amico G: **The urinary excretion of IgG and alpha1-microglobulin predicts renal outcome and identifies patients deserving treatment in membranous nephropathy**. *Kidney international* 2002, **61**(6):2276.
25. Rostgaard J, Qvortrup K: **Electron microscopic demonstrations of filamentous molecular sieve plugs in capillary fenestrae**. *Microvascular research* 1997, **53**(1):1-13.
26. Pries AR, Secomb TW, Gaehtgens P: **The endothelial surface layer**. *Pflugers Archiv : European journal of physiology* 2000, **440**(5):653-666.
27. Jeansson M, Haraldsson B: **Morphological and functional evidence for an important role of the endothelial cell glycocalyx in the glomerular barrier**. *American journal of physiology Renal physiology* 2006, **290**(1):F111-116.

28. Arif E, Rathore YS, Kumari B, Ashish F, Wong HN, Holzman LB, Nihalani D: **Slit diaphragm protein Neph1 and its signaling: a novel therapeutic target for protection of podocytes against glomerular injury**. *The Journal of biological chemistry* 2014, **289**(14):9502-9518.
29. Haraldsson B, Nystrom J, Deen WM: **Properties of the glomerular barrier and mechanisms of proteinuria**. *Physiol Rev* 2008, **88**(2):451-487.
30. Cheng H, Harris RC: **The glomerulus--a view from the outside--the podocyte**. *The international journal of biochemistry & cell biology* 2010, **42**(9):1380-1387.
31. Jarad G, Miner JH: **Update on the glomerular filtration barrier**. *Current opinion in nephrology and hypertension* 2009, **18**(3):226-232.
32. Rippe C, Rippe A, Larsson A, Asgeirsson D, Rippe B: **Nature of glomerular capillary permeability changes following acute renal ischemia-reperfusion injury in rats**. *Am J Physiol Renal Physiol* 2006, **291**(6):F1362-1368.
33. Axelsson J, Rippe A, Venturoli D, Sward P, Rippe B: **Effects of early endotoxemia and dextran-induced anaphylaxis on the size selectivity of the glomerular filtration barrier in rats**. *American journal of physiology Renal physiology* 2009, **296**(2):F242-248.
34. Axelsson J, Rippe A, Rippe B: **Acute hyperglycemia induces rapid, reversible increases in glomerular permeability in nondiabetic rats**. *American journal of physiology Renal physiology* 2010, **298**(6):F1306-1312.
35. Axelsson J, Rippe A, Rippe B: **Transient and sustained increases in glomerular permeability following ANP infusion in rats**. *American journal of physiology Renal physiology* 2011, **300**(1):F24-30.
36. Axelsson J, Rippe A, Oberg CM, Rippe B: **Rapid, dynamic changes in glomerular permeability to macromolecules during systemic angiotensin II (ANG II) infusion in rats**. *Am J Physiol Renal Physiol* 2012, **303**(6):F790-799.
37. Tencer J, Frick IM, Oquist BW, Alm P, Rippe B: **Size-selectivity of the glomerular barrier to high molecular weight proteins: upper size limitations of shunt pathways**. *Kidney international* 1998, **53**(3):709-715.
38. Lund U, Rippe A, Venturoli D, Tenstad O, Grubb A, Rippe B: **Glomerular filtration rate dependence of sieving of albumin and some neutral proteins in rat kidneys**. *Am J Physiol Renal Physiol* 2003, **284**(6):F1226-1234.
39. Oberg CM, Rippe B: **A distributed two-pore model: theoretical implications and practical application to the glomerular sieving of Ficoll**. *American journal of physiology Renal physiology* 2014, **306**(8):F844-854.
40. Rippe B, Davies S: **Permeability of peritoneal and glomerular capillaries: what are the differences according to pore theory?** *Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis* 2011, **31**(3):249-258.
41. Deen WM, Bridges CR, Brenner BM, Myers BD: **Heteroporous model of glomerular size selectivity: application to normal and nephrotic humans**. *The American journal of physiology* 1985, **249**(3 Pt 2):F374-389.
42. Ohlson M, Sorensson J, Haraldsson B: **Glomerular size and charge selectivity in the rat as revealed by FITC-ficoll and albumin**. *American journal of physiology Renal physiology* 2000, **279**(1):F84-91.
43. Rippe B, Haraldsson B: **Transport of macromolecules across microvascular walls: the two-pore theory**. *Physiological reviews* 1994, **74**(1):163-219.
44. Oberg CM, Rippe B: **Quantification of the electrostatic properties of the glomerular filtration barrier modeled as a charged fiber matrix separating**

anionic from neutral Ficoll. *American journal of physiology Renal physiology* 2013, **304**(6):F781-787.
45. Tencer J, Torffvit O, Thysell H, Rippe B, Grubb A: **Proteinuria selectivity index based upon alpha 2-macroglobulin or IgM is superior to the IgG based index in differentiating glomerular diseases. Technical note**. *Kidney Int* 1998, **54**(6):2098-2105.
46. Schurek HJ, Neumann KH, Flohr H, Zeh M, Stolte H: **The physiological and pathophysiological basis of glomerular permeability for plasma proteins and erythrocytes**. *European journal of clinical chemistry and clinical biochemistry : journal of the Forum of European Clinical Chemistry Societies* 1992, **30**(10):627-633.
47. Saito A, Takeda T, Hama H, Oyama Y, Hosaka K, Tanuma A, Kaseda R, Ueno M, Nishi S, Ogasawara S *et al*: **Role of megalin, a proximal tubular endocytic receptor, in the pathogenesis of diabetic and metabolic syndrome-related nephropathies: protein metabolic overload hypothesis**. *Nephrology* 2005, **10 Suppl**:S26-31.
48. Christensen EI, Birn H, Storm T, Weyer K, Nielsen R: **Endocytic receptors in the renal proximal tubule**. *Physiology* 2012, **27**(4):223-236.
49. Christensen EI, Birn H, Verroust P, Moestrup SK: **Membrane receptors for endocytosis in the renal proximal tubule**. *International review of cytology* 1998, **180**:237-284.
50. Birn H, Fyfe JC, Jacobsen C, Mounier F, Verroust PJ, Orskov H, Willnow TE, Moestrup SK, Christensen EI: **Cubilin is an albumin binding protein important for renal tubular albumin reabsorption**. *The Journal of clinical investigation* 2000, **105**(10):1353-1361.
51. Tojo A, Kinugasa S: **Mechanisms of glomerular albumin filtration and tubular reabsorption**. *International journal of nephrology* 2012, **2012**:481520.
52. Gekle M: **Renal tubule albumin transport**. *Annual review of physiology* 2005, **67**:573-594.
53. Gerstein HC, Mann JF, Yi Q, Zinman B, Dinneen SF, Hoogwerf B, Halle JP, Young J, Rashkow A, Joyce C *et al*: **Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals**. *JAMA : the journal of the American Medical Association* 2001, **286**(4):421-426.
54. Karalliedde J, Viberti G: **Microalbuminuria and cardiovascular risk**. *American journal of hypertension* 2004, **17**(10):986-993.
55. Klausen K, Borch-Johnsen K, Feldt-Rasmussen B, Jensen G, Clausen P, Scharling H, Appleyard M, Jensen JS: **Very low levels of microalbuminuria are associated with increased risk of coronary heart disease and death independently of renal function, hypertension, and diabetes**. *Circulation* 2004, **110**(1):32-35.
56. Arnlov J, Evans JC, Meigs JB, Wang TJ, Fox CS, Levy D, Benjamin EJ, D'Agostino RB, Vasan RS: **Low-grade albuminuria and incidence of cardiovascular disease events in nonhypertensive and nondiabetic individuals: the Framingham Heart Study**. *Circulation* 2005, **112**(7):969-975.
57. Stehouwer CD, Smulders YM: **Microalbuminuria and risk for cardiovascular disease: Analysis of potential mechanisms**. *Journal of the American Society of Nephrology : JASN* 2006, **17**(8):2106-2111.

58. Xu B, Dai M, Li M, Sun K, Zhang J, Wang T, Sun J, Chen Y, Xu M, Lu J *et al*: **Low-grade albuminuria is associated with peripheral artery disease in Chinese diabetic patients**. *Atherosclerosis* 2014, **232**(2):285-288.
59. Choi SW, Yun WJ, Kim HY, Lee YH, Kweon SS, Rhee JA, Choi JS, Shin MH: **Association between albuminuria, carotid atherosclerosis, arterial stiffness, and peripheral arterial disease in Korean type 2 diabetic patients**. *Kidney & blood pressure research* 2010, **33**(2):111-118.
60. Liu CS, Pi-Sunyer FX, Li CI, Davidson LE, Li TC, Chen W, Lin CC, Huang CY, Lin WY: **Albuminuria is strongly associated with arterial stiffness, especially in diabetic or hypertensive subjects--a population-based study (Taichung Community Health Study, TCHS)**. *Atherosclerosis* 2010, **211**(1):315-321.
61. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A: **Albuminuria reflects widespread vascular damage. The Steno hypothesis**. *Diabetologia* 1989, **32**(4):219-226.
62. Parving HH, Nielsen FS, Bang LE, Smidt UM, Svendsen TL, Chen JW, Gall MA, Rossing P: **Macro-microangiopathy and endothelial dysfunction in NIDDM patients with and without diabetic nephropathy**. *Diabetologia* 1996, **39**(12):1590-1597.
63. Diercks GF, van Boven AJ, Hillege HL, Janssen WM, Kors JA, de Jong PE, Grobbee DE, Crijns HJ, van Gilst WH: **Microalbuminuria is independently associated with ischaemic electrocardiographic abnormalities in a large non-diabetic population. The PREVEND (Prevention of REnal and Vascular ENdstage Disease) study**. *European heart journal* 2000, **21**(23):1922-1927.
64. Jafar TH, Qadri Z, Hashmi S: **Prevalence of microalbuminuria and associated electrocardiographic abnormalities in an Indo-Asian population**. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 2009, **24**(7):2111-2116.
65. Gosling P, Hughes EA, Reynolds TM, Fox JP: **Microalbuminuria is an early response following acute myocardial infarction**. *European heart journal* 1991, **12**(4):508-513.
66. Berton G, Cordiano R, Mazzuco S, Katz E, De Toni R, Palatini P: **Albumin excretion in acute myocardial infarction: a guide for long-term prognosis**. *American heart journal* 2008, **156**(4):760-768.
67. Wachtell K, Olsen MH, Dahlof B, Devereux RB, Kjeldsen SE, Nieminen MS, Okin PM, Papademetriou V, Mogensen CE, Borch-Johnsen K *et al*: **Microalbuminuria in hypertensive patients with electrocardiographic left ventricular hypertrophy: the LIFE study**. *Journal of hypertension* 2002, **20**(3):405-412.
68. Liu JE, Robbins DC, Palmieri V, Bella JN, Roman MJ, Fabsitz R, Howard BV, Welty TK, Lee ET, Devereux RB: **Association of albuminuria with systolic and diastolic left ventricular dysfunction in type 2 diabetes: the Strong Heart Study**. *Journal of the American College of Cardiology* 2003, **41**(11):2022-2028.
69. Sander D, Weimar C, Bramlage P, Brandt T, Rosin L, Siebler M: **Microalbuminuria indicates long-term vascular risk in patients after acute stroke undergoing in-patient rehabilitation**. *BMC neurology* 2012, **12**:102.
70. Cho BH, Kim JT, Chang J, Choi KH, Park MS, Cho KH: **Prediction of hemorrhagic transformation in acute ischaemic stroke by micro- and macroalbuminuria after intravenous thrombolysis**. *European journal of*

neurology : the official journal of the European Federation of Neurological Societies 2013, **20**(8):1145-1152.
71. Garcia-Martinez R, Caraceni P, Bernardi M, Gines P, Arroyo V, Jalan R: **Albumin: pathophysiologic basis of its role in the treatment of cirrhosis and its complications**. *Hepatology* 2013, **58**(5):1836-1846.
72. Quinlan GJ, Martin GS, Evans TW: **Albumin: biochemical properties and therapeutic potential**. *Hepatology* 2005, **41**(6):1211-1219.
73. Boes M: **Role of natural and immune IgM antibodies in immune responses**. *Molecular immunology* 2000, **37**(18):1141-1149.
74. Ilag LL: **Immunoglobulin M as a vaccine adjuvant**. *Medical hypotheses* 2011, **77**(4):473-478.
75. Ladwig PM, Barnidge DR, Snyder MR, Katzmann JA, Murray DL: **Quantification of Serum IgG Subclasses by Use of Subclass-Specific Tryptic Peptides and Liquid Chromatography-Tandem Mass Spectrometry**. *Clinical chemistry* 2014.
76. Grubb AO, Lopez C, Tejler L, Mendez E: **Isolation of human complex-forming glycoprotein, heterogeneous in charge (protein HC), and its IgA complex from plasma. Physiochemical and immunochemical properties, normal plasma concentration**. *The Journal of biological chemistry* 1983, **258**(23):14698-14707.
77. Takagi K, Kin K, Itoh Y, Enomoto H, Kawai T: **Human alpha 1-microglobulin levels in various body fluids**. *Journal of clinical pathology* 1980, **33**(8):786-791.
78. Olsson MG, Allhorn M, Bulow L, Hansson SR, Ley D, Olsson ML, Schmidtchen A, Akerstrom B: **Pathological conditions involving extracellular hemoglobin: molecular mechanisms, clinical significance, and novel therapeutic opportunities for alpha(1)-microglobulin**. *Antioxidants & redox signaling* 2012, **17**(5):813-846.
79. Sverrisson K, Axelsson J, Rippe A, Gram M, Akerstrom B, Hansson SR, Rippe B: **Extracellular fetal hemoglobin induces increases in glomerular permeability: inhibition with alpha1-microglobulin and tempol**. *American journal of physiology Renal physiology* 2014, **306**(4):F442-448.
80. Tsukahara H, Hiraoka M, Kuriyama M, Haruki S, Nakamura K, Suehiro F, Sudo M: **Evaluation of proximal tubular function in preterm infants by urinary alpha 1-microglobulin**. *Acta paediatrica Japonica; Overseas edition* 1993, **35**(2):127-129.
81. Mantur M, Kemona H, Dabrowska M, Dabrowska J, Sobolewski S, Prokopowicz J: **alpha1-microglobulin as a marker of proximal tubular damage in urinary tract infection in children**. *Clinical nephrology* 2000, **53**(4):283-287.
82. Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, McCullough PA, Kasiske BL, Kelepouris E, Klag MJ *et al*: **Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention**. *Circulation* 2003, **108**(17):2154-2169.
83. Herzog CA, Asinger RW, Berger AK, Charytan DM, Diez J, Hart RG, Eckardt KU, Kasiske BL, McCullough PA, Passman RS *et al*: **Cardiovascular disease in chronic kidney disease. A clinical update from Kidney Disease: Improving Global Outcomes (KDIGO)**. *Kidney international* 2011, **80**(6):572-586.

84. Barylski M, Nikfar S, Mikhailidis DP, Toth PP, Salari P, Ray KK, Pencina MJ, Rizzo M, Rysz J, Abdollahi M *et al*: **Statins decrease all-cause mortality only in CKD patients not requiring dialysis therapy--a meta-analysis of 11 randomized controlled trials involving 21,295 participants**. *Pharmacological research : the official journal of the Italian Pharmacological Society* 2013, **72**:35-44.
85. Cao Y, Li W, Yang G, Liu Y, Li X: **Diabetes and hypertension have become leading causes of CKD in Chinese elderly patients: a comparison between 1990-1991 and 2009-2010**. *International urology and nephrology* 2012, **44**(4):1269-1276.
86. Goderis G, Van Pottelbergh G, Truyers C, Van Casteren V, De Clercq E, Van Den Broeke C, Buntinx F: **Long-term evolution of renal function in patients with type 2 diabetes mellitus: a registry-based retrospective cohort study**. *BMJ open* 2013, **3**(12):e004029.
87. Stanton RC: **Clinical challenges in diagnosis and management of diabetic kidney disease**. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2014, **63**(2 Suppl 2):S3-21.
88. Eckardt KU, Coresh J, Devuyst O, Johnson RJ, Kottgen A, Levey AS, Levin A: **Evolving importance of kidney disease: from subspecialty to global health burden**. *Lancet* 2013, **382**(9887):158-169.
89. Coresh J, Selvin E, Stevens LA, Manzi J, Kusek JW, Eggers P, Van Lente F, Levey AS: **Prevalence of chronic kidney disease in the United States**. *JAMA : the journal of the American Medical Association* 2007, **298**(17):2038-2047.
90. Bash LD, Coresh J, Kottgen A, Parekh RS, Fulop T, Wang Y, Astor BC: **Defining incident chronic kidney disease in the research setting: The ARIC Study**. *American journal of epidemiology* 2009, **170**(4):414-424.
91. Grams ME, Chow EK, Segev DL, Coresh J: **Lifetime incidence of CKD stages 3-5 in the United States**. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2013, **62**(2):245-252.
92. Peralta CA, Katz R, DeBoer I, Ix J, Sarnak M, Kramer H, Siscovick D, Shea S, Szklo M, Shlipak M: **Racial and ethnic differences in kidney function decline among persons without chronic kidney disease**. *Journal of the American Society of Nephrology : JASN* 2011, **22**(7):1327-1334.
93. Levey AS, Beto JA, Coronado BE, Eknoyan G, Foley RN, Kasiske BL, Klag MJ, Mailloux LU, Manske CL, Meyer KB *et al*: **Controlling the epidemic of cardiovascular disease in chronic renal disease: what do we know? What do we need to learn? Where do we go from here? National Kidney Foundation Task Force on Cardiovascular Disease**. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 1998, **32**(5):853-906.
94. Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, McCullough PA, Kasiske BL, Kelepouris E, Klag MJ *et al*: **Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention**. *Hypertension* 2003, **42**(5):1050-1065.
95. Herzog CA, Ma JZ, Collins AJ: **Poor long-term survival after acute myocardial infarction among patients on long-term dialysis**. *The New England journal of medicine* 1998, **339**(12):799-805.

96. Chadban SJ, Atkins RC: **Glomerulonephritis**. *Lancet* 2005, **365**(9473):1797-1806.
97. Stewart JH, McCredie MR, Williams SM, Jager KJ, Trpeski L, McDonald SP, Group EIS: **Trends in incidence of treated end-stage renal disease, overall and by primary renal disease, in persons aged 20-64 years in Europe, Canada and the Asia-Pacific region, 1998-2002**. *Nephrology* 2007, **12**(5):520-527.
98. Caliskan Y, Kiryluk K: **Novel biomarkers in glomerular disease**. *Advances in chronic kidney disease* 2014, **21**(2):205-216.
99. Briganti EM, Dowling J, Finlay M, Hill PA, Jones CL, Kincaid-Smith PS, Sinclair R, McNeil JJ, Atkins RC: **The incidence of biopsy-proven glomerulonephritis in Australia**. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 2001, **16**(7):1364-1367.
100. McGrogan A, Franssen CF, de Vries CS: **The incidence of primary glomerulonephritis worldwide: a systematic review of the literature**. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 2011, **26**(2):414-430.
101. Yu HH, Chiang BL: **Diagnosis and classification of IgA nephropathy**. *Autoimmunity reviews* 2014, **13**(4-5):556-559.
102. Sakamoto K, Ueno T, Kobayashi N, Hara S, Takashima Y, Pastan I, Matsusaka T, Nagata M: **The direction and role of phenotypic transition between podocytes and parietal epithelial cells in focal segmental glomerulosclerosis**. *American journal of physiology Renal physiology* 2014, **306**(1):F98-F104.
103. Mok CC, Cheung TT, Lo WH: **Minimal mesangial lupus nephritis: a systematic review**. *Scandinavian journal of rheumatology* 2010, **39**(3):181-189.
104. Kerjaschki D: **Pathomechanisms and molecular basis of membranous glomerulopathy**. *Lancet* 2004, **364**(9441):1194-1196.
105. American Diabetes A: **Diagnosis and classification of diabetes mellitus**. *Diabetes care* 2011, **34 Suppl 1**:S62-69.
106. Alberti KG, Zimmet PZ: **Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation**. *Diabetic medicine : a journal of the British Diabetic Association* 1998, **15**(7):539-553.
107. Shaw JE, Sicree RA, Zimmet PZ: **Global estimates of the prevalence of diabetes for 2010 and 2030**. *Diabetes research and clinical practice* 2010, **87**(1):4-14.
108. Jones CA, Krolewski AS, Rogus J, Xue JL, Collins A, Warram JH: **Epidemic of end-stage renal disease in people with diabetes in the United States population: do we know the cause?** *Kidney international* 2005, **67**(5):1684-1691.
109. Wild S, Roglic G, Green A, Sicree R, King H: **Global prevalence of diabetes: estimates for the year 2000 and projections for 2030**. *Diabetes care* 2004, **27**(5):1047-1053.
110. Kleinman JC, Donahue RP, Harris MI, Finucane FF, Madans JH, Brock DB: **Mortality among diabetics in a national sample**. *American journal of epidemiology* 1988, **128**(2):389-401.
111. Gregg EW, Gu Q, Williams D, de Rekeneire N, Cheng YJ, Geiss L, Engelgau M: **Prevalence of lower extremity diseases associated with normal glucose levels,**

impaired fasting glucose, and diabetes among U.S. adults aged 40 or older. *Diabetes research and clinical practice* 2007, **77**(3):485-488.
112. Morrish NJ, Wang SL, Stevens LK, Fuller JH, Keen H: **Mortality and causes of death in the WHO Multinational Study of Vascular Disease in Diabetes**. *Diabetologia* 2001, **44 Suppl 2**:S14-21.
113. Pacilli A, De Cosmo S, Trischitta V, Bacci S: **Role of relationship between HbA1c, fibrinogen and HDL-cholesterol on cardiovascular disease in patients with type 2 diabetes mellitus**. *Atherosclerosis* 2013, **228**(1):247-248.
114. Borch-Johnsen K, Kreiner S: **Proteinuria: value as predictor of cardiovascular mortality in insulin dependent diabetes mellitus**. *British medical journal* 1987, **294**(6588):1651-1654.
115. Dahlquist G, Stattin EL, Rudberg S: **Urinary albumin excretion rate and glomerular filtration rate in the prediction of diabetic nephropathy; a long-term follow-up study of childhood onset type-1 diabetic patients**. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 2001, **16**(7):1382-1386.
116. Raile K, Galler A, Hofer S, Herbst A, Dunstheimer D, Busch P, Holl RW: **Diabetic nephropathy in 27,805 children, adolescents, and adults with type 1 diabetes: effect of diabetes duration, A1C, hypertension, dyslipidemia, diabetes onset, and sex**. *Diabetes care* 2007, **30**(10):2523-2528.
117. Molitch ME, DeFronzo RA, Franz MJ, Keane WF, Mogensen CE, Parving HH, Steffes MW, American Diabetes A: **Nephropathy in diabetes**. *Diabetes care* 2004, **27 Suppl 1**:S79-83.
118. Adler AI, Stevens RJ, Manley SE, Bilous RW, Cull CA, Holman RR, Ukpds G: **Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64)**. *Kidney international* 2003, **63**(1):225-232.
119. Williams ME: **Diabetic CKD/ESRD 2010: a progress report?** *Seminars in dialysis* 2010, **23**(2):129-133.
120. Ritz E, Zeng XX, Rychlik I: **Clinical manifestation and natural history of diabetic nephropathy**. *Contributions to nephrology* 2011, **170**:19-27.
121. Williams ME, Lacson E, Jr., Teng M, Ofsthun N, Lazarus JM: **Hemodialyzed type I and type II diabetic patients in the US: Characteristics, glycemic control, and survival**. *Kidney international* 2006, **70**(8):1503-1509.
122. Centers for Disease C, Prevention: **Incidence of end-stage renal disease attributed to diabetes among persons with diagnosed diabetes --- United States and Puerto Rico, 1996-2007**. *MMWR Morbidity and mortality weekly report* 2010, **59**(42):1361-1366.
123. Groop PH, Thomas MC, Moran JL, Waden J, Thorn LM, Makinen VP, Rosengard-Barlund M, Saraheimo M, Hietala K, Heikkila O *et al*: **The presence and severity of chronic kidney disease predicts all-cause mortality in type 1 diabetes**. *Diabetes* 2009, **58**(7):1651-1658.
124. Fioretto P, Caramori ML, Mauer M: **The kidney in diabetes: dynamic pathways of injury and repair. The Camillo Golgi Lecture 2007**. *Diabetologia* 2008, **51**(8):1347-1355.
125. Bakoush O, Tencer J, Tapia J, Rippe B, Torffvit O: **Higher urinary IgM excretion in type 2 diabetic nephropathy compared to type 1 diabetic nephropathy**. *Kidney international* 2002, **61**(1):203-208.

126. Roshan B, Stanton RC: **A story of microalbuminuria and diabetic nephropathy**. *Journal of nephropathology* 2013, **2**(4):234-240.
127. Young BA, Maynard C, Boyko EJ: **Racial differences in diabetic nephropathy, cardiovascular disease, and mortality in a national population of veterans**. *Diabetes care* 2003, **26**(8):2392-2399.
128. Tervaert TW, Mooyaart AL, Amann K, Cohen AH, Cook HT, Drachenberg CB, Ferrario F, Fogo AB, Haas M, de Heer E *et al*: **Pathologic classification of diabetic nephropathy**. *Journal of the American Society of Nephrology : JASN* 2010, **21**(4):556-563.
129. Garud MS, Kulkarni YA: **Hyperglycemia to Nephropathy via Transforming Growth Factor Beta**. *Current diabetes reviews* 2014, **10**(3):182-189.
130. Yamagishi S, Matsui T: **Advanced glycation end products, oxidative stress and diabetic nephropathy**. *Oxidative medicine and cellular longevity* 2010, **3**(2):101-108.
131. Giacco F, Brownlee M: **Oxidative stress and diabetic complications**. *Circulation research* 2010, **107**(9):1058-1070.
132. Wendt TM, Tanji N, Guo J, Kislinger TR, Qu W, Lu Y, Bucciarelli LG, Rong LL, Moser B, Markowitz GS *et al*: **RAGE drives the development of glomerulosclerosis and implicates podocyte activation in the pathogenesis of diabetic nephropathy**. *The American journal of pathology* 2003, **162**(4):1123-1137.
133. Yamagishi S, Nakamura K, Matsui T: **Regulation of advanced glycation end product (AGE)-receptor (RAGE) system by PPAR-gamma agonists and its implication in cardiovascular disease**. *Pharmacological research : the official journal of the Italian Pharmacological Society* 2009, **60**(3):174-178.
134. Raj DS, Choudhury D, Welbourne TC, Levi M: **Advanced glycation end products: a Nephrologist's perspective**. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2000, **35**(3):365-380.
135. Kizub IV, Klymenko KI, Soloviev AI: **Protein kinase C in enhanced vascular tone in diabetes mellitus**. *International journal of cardiology* 2014, **174**(2):230-242.
136. Kamiyama M, Urushihara M, Morikawa T, Konishi Y, Imanishi M, Nishiyama A, Kobori H: **Oxidative stress/angiotensinogen/renin-angiotensin system axis in patients with diabetic nephropathy**. *International journal of molecular sciences* 2013, **14**(11):23045-23062.
137. Forbes JM, Coughlan MT, Cooper ME: **Oxidative stress as a major culprit in kidney disease in diabetes**. *Diabetes* 2008, **57**(6):1446-1454.
138. Yamagishi S, Imaizumi T: **Diabetic vascular complications: pathophysiology, biochemical basis and potential therapeutic strategy**. *Current pharmaceutical design* 2005, **11**(18):2279-2299.
139. Rahbar S, Figarola JL: **Novel inhibitors of advanced glycation endproducts**. *Archives of biochemistry and biophysics* 2003, **419**(1):63-79.
140. Bak M, Thomsen K, Christiansen T, Flyvbjerg A: **Renal enlargement precedes renal hyperfiltration in early experimental diabetes in rats**. *Journal of the American Society of Nephrology : JASN* 2000, **11**(7):1287-1292.
141. Deckert T, Feldt-Rasmussen B, Djurup R, Deckert M: **Glomerular size and charge selectivity in insulin-dependent diabetes mellitus**. *Kidney international* 1988, **33**(1):100-106.

142. Sward P, Rippe B: **Acute and sustained actions of hyperglycaemia on endothelial and glomerular barrier permeability**. *Acta physiologica* 2012, **204**(3):294-307.
143. Wolf G, Chen S, Ziyadeh FN: **From the periphery of the glomerular capillary wall toward the center of disease: podocyte injury comes of age in diabetic nephropathy**. *Diabetes* 2005, **54**(6):1626-1634.
144. Libby P, Okamoto Y, Rocha VZ, Folco E: **Inflammation in atherosclerosis: transition from theory to practice**. *Circulation journal : official journal of the Japanese Circulation Society* 2010, **74**(2):213-220.
145. Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ: **Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data**. *Lancet* 2006, **367**(9524):1747-1757.
146. Apostolov EO, Ok E, Burns S, Nawaz S, Savenka A, Shah S, Basnakian AG: **Carbamylated-oxidized LDL: proatherosclerotic effects on endothelial cells and macrophages**. *Journal of atherosclerosis and thrombosis* 2013, **20**(12):878-892.
147. Kranzhofer R, Schmidt J, Pfeiffer CA, Hagl S, Libby P, Kubler W: **Angiotensin induces inflammatory activation of human vascular smooth muscle cells**. *Arteriosclerosis, thrombosis, and vascular biology* 1999, **19**(7):1623-1629.
148. Olin-Lewis K, Krauss RM, La Belle M, Blanche PJ, Barrett PH, Wight TN, Chait A: **ApoC-III content of apoB-containing lipoproteins is associated with binding to the vascular proteoglycan biglycan**. *Journal of lipid research* 2002, **43**(11):1969-1977.
149. Cole JE, Kassiteridi C, Monaco C: **Toll-like receptors in atherosclerosis: a 'Pandora's box' of advances and controversies**. *Trends in pharmacological sciences* 2013, **34**(11):629-636.
150. Curtiss LK, Black AS, Bonnet DJ, Tobias PS: **Atherosclerosis induced by endogenous and exogenous toll-like receptor (TLR)1 or TLR6 agonists**. *Journal of lipid research* 2012, **53**(10):2126-2132.
151. Bjorkbacka H, Fredrikson GN, Nilsson J: **Emerging biomarkers and intervention targets for immune-modulation of atherosclerosis - a review of the experimental evidence**. *Atherosclerosis* 2013, **227**(1):9-17.
152. Libby P: **Inflammation in atherosclerosis**. *Arteriosclerosis, thrombosis, and vascular biology* 2012, **32**(9):2045-2051.
153. Galkina E, Ley K: **Immune and inflammatory mechanisms of atherosclerosis (*)**. *Annual review of immunology* 2009, **27**:165-197.
154. Libby P, Ridker PM, Hansson GK: **Progress and challenges in translating the biology of atherosclerosis**. *Nature* 2011, **473**(7347):317-325.
155. Lee D, Lee KH, Park H, Kim SH, Jin T, Cho S, Chung JH, Lim S, Park S: **The effect of soluble RAGE on inhibition of angiotensin II-mediated atherosclerosis in apolipoprotein E deficient mice**. *PloS one* 2013, **8**(8):e69669.
156. Qi G, Jia L, Li Y, Bian Y, Cheng J, Li H, Xiao C, Du J: **Angiotensin II infusion-induced inflammation, monocytic fibroblast precursor infiltration, and cardiac fibrosis are pressure dependent**. *Cardiovascular toxicology* 2011, **11**(2):157-167.
157. Wang X, Khaidakov M, Ding Z, Mitra S, Lu J, Liu S, Mehta JL: **Cross-talk between inflammation and angiotensin II: studies based on direct**

transfection of cardiomyocytes with AT1R and AT2R cDNA. *Experimental biology and medicine* 2012, **237**(12):1394-1401.
158. Abadir PM, Walston JD, Carey RM, Siragy HM: **Angiotensin II Type-2 receptors modulate inflammation through signal transducer and activator of transcription proteins 3 phosphorylation and TNFalpha production**. *Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research* 2011, **31**(6):471-474.
159. Garrido AM, Griendling KK: **NADPH oxidases and angiotensin II receptor signaling**. *Molecular and cellular endocrinology* 2009, **302**(2):148-158.
160. Spindler V, Schlegel N, Waschke J: **Role of GTPases in control of microvascular permeability**. *Cardiovascular research* 2010, **87**(2):243-253.
161. Sonneveld R, Ferre S, Hoenderop JG, Dijkman HB, Berden JH, Bindels RJ, Wetzels JF, van der Vlag J, Nijenhuis T: **Vitamin D down-regulates TRPC6 expression in podocyte injury and proteinuric glomerular disease**. *The American journal of pathology* 2013, **182**(4):1196-1204.
162. Greka A, Mundel P: **Balancing calcium signals through TRPC5 and TRPC6 in podocytes**. *Journal of the American Society of Nephrology : JASN* 2011, **22**(11):1969-1980.
163. Axelsson J, Rippe A, Sverrisson K, Rippe B: **Scavengers of reactive oxygen species, paracalcitol, RhoA, and Rac-1 inhibitors and tacrolimus inhibit angiotensin II-induced actions on glomerular permeability**. *American journal of physiology Renal physiology* 2013, **305**(3):F237-243.
164. Timar R, Timar B, Degeratu D, Serafinceanu C, Oancea C: **Metabolic syndrome, adiponectin and proinflammatory status in patients with type 1 diabetes mellitus**. *The Journal of international medical research* 2014.
165. Li FY, Cheng KK, Lam KS, Vanhoutte PM, Xu A: **Cross-talk between adipose tissue and vasculature: role of adiponectin**. *Acta physiologica* 2011, **203**(1):167-180.
166. Rajsheker S, Manka D, Blomkalns AL, Chatterjee TK, Stoll LL, Weintraub NL: **Crosstalk between perivascular adipose tissue and blood vessels**. *Current opinion in pharmacology* 2010, **10**(2):191-196.
167. Ohashi K, Ouchi N, Matsuzawa Y: **Anti-inflammatory and anti-atherogenic properties of adiponectin**. *Biochimie* 2012, **94**(10):2137-2142.
168. Sharma K, Ramachandrarao S, Qiu G, Usui HK, Zhu Y, Dunn SR, Ouedraogo R, Hough K, McCue P, Chan L *et al*: **Adiponectin regulates albuminuria and podocyte function in mice**. *The Journal of clinical investigation* 2008, **118**(5):1645-1656.
169. Kopf S, Oikonomou D, von Eynatten M, Kieser M, Zdunek D, Hess G, Morcos M, Forsblom C, Bierhaus A, Groop PH *et al*: **Urinary excretion of high molecular weight adiponectin is an independent predictor of decline of renal function in type 2 diabetes**. *Acta diabetologica* 2014, **51**(3):479-489.
170. Jorsal A, Petersen EH, Tarnow L, Hess G, Zdunek D, Frystyk J, Flyvbjerg A, Lajer M, Rossing P: **Urinary adiponectin excretion rises with increasing albuminuria in type 1 diabetes**. *Journal of diabetes and its complications* 2013, **27**(6):604-608.
171. Hammar N, Alfredsson L, Rosen M, Spetz CL, Kahan T, Ysberg AS: **A national record linkage to study acute myocardial infarction incidence and case fatality in Sweden**. *International journal of epidemiology* 2001, **30 Suppl 1**:S30-34.

172. Tencer J, Thysell H, Grubb A: **Analysis of proteinuria: reference limits for urine excretion of albumin, protein HC, immunoglobulin G, kappa- and lambda-immunoreactivity, orosomucoid and alpha 1-antitrypsin**. *Scandinavian journal of clinical and laboratory investigation* 1996, **56**(8):691-700.
173. Tencer J, Thysell H, Andersson K, Grubb A: **Long-term stability of albumin, protein HC, immunoglobulin G, kappa- and lambda-chain-immunoreactivity, orosomucoid and alpha 1-antitrypsin in urine stored at -20 degrees C**. *Scandinavian journal of urology and nephrology* 1997, **31**(1):67-71.
174. Torffvit O, Rippe B: **Size and charge selectivity of the glomerular filter in patients with insulin-dependent diabetes mellitus: urinary immunoglobulins and glycosaminoglycans**. *Nephron* 1999, **83**(4):301-307.
175. Ohlsson S, Bakoush O, Tencer J, Torffvit O, Segelmark M: **Monocyte chemoattractant protein 1 is a prognostic marker in ANCA-associated small vessel vasculitis**. *Mediators of inflammation* 2009, **2009**:584916.
176. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D: **A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group**. *Annals of internal medicine* 1999, **130**(6):461-470.
177. Nyman U, Grubb A, Larsson A, Hansson LO, Flodin M, Nordin G, Lindstrom V, Bjork J: **The revised Lund-Malmo GFR estimating equation outperforms MDRD and CKD-EPI across GFR, age and BMI intervals in a large Swedish population**. *Clinical chemistry and laboratory medicine : CCLM / FESCC* 2014, **52**(6):815-824.
178. Matsushita K, Tonelli M, Lloyd A, Levey AS, Coresh J, Hemmelgarn BR, Alberta Kidney Disease N: **Clinical risk implications of the CKD Epidemiology Collaboration (CKD-EPI) equation compared with the Modification of Diet in Renal Disease (MDRD) Study equation for estimated GFR**. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2012, **60**(2):241-249.
179. Nyman HA, Dowling TC, Hudson JQ, Peter WL, Joy MS, Nolin TD: **Comparative evaluation of the Cockcroft-Gault Equation and the Modification of Diet in Renal Disease (MDRD) study equation for drug dosing: an opinion of the Nephrology Practice and Research Network of the American College of Clinical Pharmacy**. *Pharmacotherapy* 2011, **31**(11):1130-1144.
180. Marshall SM: **Natural history and clinical characteristics of CKD in type 1 and type 2 diabetes mellitus**. *Advances in chronic kidney disease* 2014, **21**(3):267-272.
181. Caramori ML, Fioretto P, Mauer M: **The need for early predictors of diabetic nephropathy risk: is albumin excretion rate sufficient?** *Diabetes* 2000, **49**(9):1399-1408.
182. Gohda T, Walker WH, Wolkow P, Lee JE, Warram JH, Krolewski AS, Niewczas MA: **Elevated urinary excretion of immunoglobulins in nonproteinuric patients with type 1 diabetes**. *American journal of physiology Renal physiology* 2012, **303**(1):F157-162.
183. Torffvit O, Kalani M, Apelqvist J, Eliasson B, Eriksson JW, Brismar K, Jorneskog G: **Increased Urine IgM and IgG(2) Levels, Indicating Decreased**

Glomerular Size Selectivity, Are Not Affected by Dalteparin Therapy in Patients with Type 2 Diabetes. *Biochemistry research international* 2012, **2012**:480529.

184. Gambara V, Mecca G, Remuzzi G, Bertani T: **Heterogeneous nature of renal lesions in type II diabetes**. *Journal of the American Society of Nephrology : JASN* 1993, **3**(8):1458-1466.
185. Moriya T, Ohno S, Hirasawa Y, Tanaka K, Fujita Y: **Detailed glomerular ultrastructure in Japanese type 2 diabetic patients by the quick-freezing and deep-etching method**. *Diabetes research and clinical practice* 2008, **80**(3):424-431.
186. Steinke JM, Mauer M, International Diabetic Nephropathy Study G: **Lessons learned from studies of the natural history of diabetic nephropathy in young type 1 diabetic patients**. *Pediatric endocrinology reviews : PER* 2008, **5 Suppl 4**:958-963.
187. Drummond K, Mauer M, International Diabetic Nephropathy Study G: **The early natural history of nephropathy in type 1 diabetes: II. Early renal structural changes in type 1 diabetes**. *Diabetes* 2002, **51**(5):1580-1587.
188. Altemtam N, Russell J, El Nahas M: **A study of the natural history of diabetic kidney disease (DKD)**. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 2012, **27**(5):1847-1854.
189. Lehrke M, Millington SC, Lefterova M, Cumaranatunge RG, Szapary P, Wilensky R, Rader DJ, Lazar MA, Reilly MP: **CXCL16 is a marker of inflammation, atherosclerosis, and acute coronary syndromes in humans**. *Journal of the American College of Cardiology* 2007, **49**(4):442-449.
190. Brunetti ND, Munno I, Pellegrino PL, Ruggero V, Correale M, De Gennaro L, Cuculo A, Campanale EG, Di Biase M: **Inflammatory cytokines imbalance in the very early phase of acute coronary syndrome: correlations with angiographic findings and in-hospital events**. *Inflammation* 2011, **34**(1):58-66.
191. Emerging Risk Factors C, Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, Collins R, Danesh J: **C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis**. *Lancet* 2010, **375**(9709):132-140.
192. Chan D, Ng LL: **Biomarkers in acute myocardial infarction**. *BMC medicine* 2010, **8**:34.
193. Tedgui A, Mallat Z: **Cytokines in atherosclerosis: pathogenic and regulatory pathways**. *Physiological reviews* 2006, **86**(2):515-581.
194. Kuo HK, Al Snih S, Kuo YF, Raji MA: **Chronic inflammation, albuminuria, and functional disability in older adults with cardiovascular disease: the National Health and Nutrition Examination Survey, 1999-2008**. *Atherosclerosis* 2012, **222**(2):502-508.
195. Schottker B, Herder C, Rothenbacher D, Roden M, Kolb H, Muller H, Brenner H: **Proinflammatory cytokines, adiponectin, and increased risk of primary cardiovascular events in diabetic patients with or without renal dysfunction: results from the ESTHER study**. *Diabetes care* 2013, **36**(6):1703-1711.
196. Emerging Risk Factors C, Kaptoge S, Di Angelantonio E, Pennells L, Wood AM, White IR, Gao P, Walker M, Thompson A, Sarwar N *et al*: **C-reactive protein, fibrinogen, and cardiovascular disease prediction**. *The New England journal of medicine* 2012, **367**(14):1310-1320.

197. Duran M, Kalay N, Ardic I, Yarlioglues M, Kayaalti F, Yilmaz Y, Akpek M, Kaya MG, Topsakal R: **Microalbuminuria is not associated with endothelial dysfunction and coronary atherosclerosis in patients with acute coronary syndromes**. *Renal failure* 2010, **32**(6):659-665.
198. Sezer M, Akdeniz C, Aslanger E, Kaplan A, Yilmaz A, Guz G, Umman B, Bugra Z, Umman S: **Role of C-reactive protein in determining microvascular function in patients with non-ST-segment elevation acute coronary syndrome undergoing percutaneous coronary intervention**. *The American journal of cardiology* 2013, **111**(12):1734-1738.
199. Lindahl B, Diderholm E, Lagerqvist B, Venge P, Wallentin L, Investigators FI: **Mechanisms behind the prognostic value of troponin T in unstable coronary artery disease: a FRISC II substudy**. *Journal of the American College of Cardiology* 2001, **38**(4):979-986.
200. Bakoush O, Tencer J, Torffvit O, Tenstad O, Skogvall I, Rippe B: **Increased glomerular albumin permeability in old spontaneously hypertensive rats**. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 2004, **19**(7):1724-1731.
201. Diamond JR: **Analogous pathobiologic mechanisms in glomerulosclerosis and atherosclerosis**. *Kidney international Supplement* 1991, **31**:S29-34.
202. Deegens JK, Wetzels JF: **Fractional excretion of high- and low-molecular weight proteins and outcome in primary focal segmental glomerulosclerosis**. *Clinical nephrology* 2007, **68**(4):201-208.
203. Polanco N, Gutierrez E, Covarsi A, Ariza F, Carreno A, Vigil A, Baltar J, Fernandez-Fresnedo G, Martin C, Pons S *et al*: **Spontaneous remission of nephrotic syndrome in idiopathic membranous nephropathy**. *Journal of the American Society of Nephrology : JASN* 2010, **21**(4):697-704.
204. Bazzi C, Petrini C, Rizza V, Arrigo G, Beltrame A, Pisano L, D'Amico G: **Urinary excretion of IgG and alpha(1)-microglobulin predicts clinical course better than extent of proteinuria in membranous nephropathy**. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2001, **38**(2):240-248.
205. Holdt-Lehmann B, Lehmann A, Korten G, Nagel H, Nizze H, Schuff-Werner P: **Diagnostic value of urinary alanine aminopeptidase and N-acetyl-beta-D-glucosaminidase in comparison to alpha 1-microglobulin as a marker in evaluating tubular dysfunction in glomerulonephritis patients**. *Clinica chimica acta; international journal of clinical chemistry* 2000, **297**(1-2):93-102.