Pitfalls of using microalbuminuria as a screening tool to identify subjects with increased risk for kidney and cardiovascular disease

Results of the Unreferred Renal Insufficiency (URI) study and the Early Renal Impairment and Cardiovascular Assessment in Belgium study (ERICABEL).

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# TABLE OF CONTENTS

**LIST OF ABBREVIATIONS**..................................................................................................................5

**CHAPTER 1: INTRODUCTION**.............................................................................................................7
1.1 Preface .................................................................................................................................................9
1.2 Normal renal handling of albuminuria ..............................................................................................11
1.3 Mechanisms of tubular and glomerular albuminuria ........................................................................13
1.4 Albuminuria as a manifestation of chronic kidney disease ...............................................................18
1.5 Albuminuria as a manifestation of cardiovascular disease ..............................................................21
1.6 The prevalence of albuminuria ...........................................................................................................25
1.7 How to measure albuminuria .............................................................................................................27
1.8 Outline and aims of the thesis ...........................................................................................................30
1.9 Participants and methods ....................................................................................................................31
1.10 References .......................................................................................................................................33

**CHAPTER 2: RESULTS**..........................................................................................................................43
2.1 Screening for cardiovascular and renal markers in unselected subjects: results from URI trial ..........43
2.1.1 Towards a rational screening strategy for albuminuria ................................................................45
2.1.2 Microalbuminuria is more consistent in presence of risk factors .................................................65
2.1.3 Should screening of renal markers be recommended in a working population ......................78
2.2 Screening for kidney disease in selected hypertensive subjects: results from the ERICABEL study ....96
2.2.1 Statin use and the presence of microalbuminuria ......................................................................97

**CHAPTER 3: GENERAL DISCUSSION AND CONCLUSIONS**..............................................................115
3.1 The prevalence of microalbuminuria and macroalbuminuria, and the associations with traditional cardiovascular risk factors in a general population .....................................................118
3.2 Should we use microalbuminuria as a screening tool to identify subjects with unrecognized cardiovascular risk factors in a presumably healthy population ...........................................120
3.3. Conditions which could lead to false positive test results of microalbuminuria .......... 121
3.4. Can we prevent ESRD by screening for microalbuminuria in a general population........ 123
   3.4.1. The disease should be an obvious burden for the individual and the community 
in terms of death, poor quality of life and socio-economic factors including 
health care costs ........................................................................................................ 124
   3.4.2. The natural course of disease should be well-known and the disease should go 
through an initial latent stage or be determined by risk factors, which can be 
detected by appropriate tests .................................................................................... 125
   3.4.3. A suitable test is highly sensitive and specific for the disease as well as being 
acceptable to the person screened ........................................................................... 126
   3.4.4. Screening followed by diagnosis and interventions in an early stage of the disease 
should provide a better prognosis than intervention after spontaneously sought 
treatment .................................................................................................................. 127
   3.4.5. Adequate treatment or other intervention possibilities are indispensable. 
Adequacy is determined both by proven medical effect and ethical and legal 
acceptability ................................................................................................................. 128
   3.4.6. The cost of case-finding should be economically balanced in relation to possible 
expenditure on medical care as a whole ....................................................................... 129
   3.4.7. Case-finding should be a continuing process and the interval of testing should be 
known ......................................................................................................................... 130
3.5  Conclusions ........................................................................................................... 132
3.6  References ............................................................................................................ 135
3.7  Conclusies ............................................................................................................. 143
3.8  References ............................................................................................................ 146
Dankwoord .................................................................................................................. 149
Curriculum Vitae ........................................................................................................ 151
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR</td>
<td>urinary albumin creatinine ratio (mg/g)</td>
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<tr>
<td>ACE-I</td>
<td>angiotensin converting enzyme inhibitor</td>
</tr>
<tr>
<td>ADA</td>
<td>American Diabetes Association</td>
</tr>
<tr>
<td>ADVANCE</td>
<td>Preterax and Diamicron Modified Released Control Education study</td>
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<tr>
<td>AER</td>
<td>albumin excretion rate (mg/24 h)</td>
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<tr>
<td>AKI</td>
<td>acute kidney injury</td>
</tr>
<tr>
<td>ARB</td>
<td>angiotensin receptor blocker</td>
</tr>
<tr>
<td>Aus Diab Kidney study</td>
<td>Australian Diabetes, Obesity and Lifestyle Study</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>BP</td>
<td>blood pressure</td>
</tr>
<tr>
<td>CGA</td>
<td>CKD classification: Cause, Glomerular filtration rate, Albuminuria</td>
</tr>
<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
</tr>
<tr>
<td>CKD</td>
<td>chronic kidney disease</td>
</tr>
<tr>
<td>CKD-EPI</td>
<td>Chronic Kidney Disease Epidemiology Collaboration</td>
</tr>
<tr>
<td>DALY</td>
<td>disability adjusted life year</td>
</tr>
<tr>
<td>DM</td>
<td>diabetes mellitus</td>
</tr>
<tr>
<td>EPIC-Norfolk</td>
<td>European Prospective Investigation into Cancer in Norfolk</td>
</tr>
<tr>
<td>ERA-EDTA</td>
<td>European Renal Association-European Dialysis Transplantation Association</td>
</tr>
<tr>
<td>EPICABEL</td>
<td>Early Renal Impairment and Cardiovascular Assessment in Belgium study</td>
</tr>
<tr>
<td>ESRD</td>
<td>end stage renal disease</td>
</tr>
<tr>
<td>GDP</td>
<td>gross domestic product</td>
</tr>
<tr>
<td>GFR</td>
<td>glomerular filtration rate</td>
</tr>
<tr>
<td>HMG-CoA reductase</td>
<td>3-hydroxy-3-methylglutaryl-CoA reductase</td>
</tr>
<tr>
<td>HOPE</td>
<td>Heart Outcomes and Prevention Evaluation Study</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>HUNT</td>
<td>Nord-Trøndelag Health Study</td>
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<tr>
<td>HT</td>
<td>hypertension</td>
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<tr>
<td>IDMS</td>
<td>isotope dilution mass spectrometry</td>
</tr>
<tr>
<td>IFCC</td>
<td>International Federation of Clinical Chemistry</td>
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<tr>
<td>IGT</td>
<td>impaired glucose tolerance</td>
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<tr>
<td>IMAU</td>
<td>intermittent microalbuminuria</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>K/DOQI</td>
<td>Kidney Disease Outcomes Quality Initiative</td>
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<tr>
<td>KDIGO</td>
<td>Kidney Disease Improving Global Outcomes</td>
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<tr>
<td>KEAPS</td>
<td>Kidney Evaluation and Awareness Program in Sheffield</td>
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<tr>
<td>NCEP</td>
<td>National Cholesterol Education Program</td>
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<tr>
<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
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<tr>
<td>NKF</td>
<td>National Kidney Foundation</td>
</tr>
<tr>
<td>NPHS1/2</td>
<td>congenital nephrotic syndrome of Finnish type</td>
</tr>
<tr>
<td>MAU</td>
<td>microalbuminuria</td>
</tr>
<tr>
<td>MDRD</td>
<td>Modification of Diet in Renal Disease</td>
</tr>
<tr>
<td>OCRL-1</td>
<td>oculocerebrorenal locus 1</td>
</tr>
<tr>
<td>ONTARGET</td>
<td>Ongoing Telmisartan Alone and in combination with Ramipril Global Endpoint Trial</td>
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<tr>
<td>PCER</td>
<td>physicochemical exposure risk</td>
</tr>
<tr>
<td>PCR</td>
<td>protein creatinine ratio</td>
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<tr>
<td>PMAU</td>
<td>persistent microalbuminuria</td>
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<tr>
<td>PolNef</td>
<td>Early detection of chronic kidney disease in Poland</td>
</tr>
<tr>
<td>PREVEND</td>
<td>Prevention of Renal and Vascular End Stage Disease Study Group</td>
</tr>
<tr>
<td>QALY</td>
<td>Quality Adjusted Life Year</td>
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<tr>
<td>RAAS</td>
<td>renin angiotensin aldosterone system</td>
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<tr>
<td>RCT</td>
<td>randomized control trial</td>
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<tr>
<td>RENAAL</td>
<td>Reduction of Endpoints in NIDDM with Angiotensin II Antagonist Losartan</td>
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<tr>
<td>ROADMAP</td>
<td>Randomized Olmesartan and Diabetes Microalbuminuria Prevention</td>
</tr>
<tr>
<td>RRT</td>
<td>renal replacement therapy</td>
</tr>
<tr>
<td>SGLT-2</td>
<td>sodium glucose co-transporter 2</td>
</tr>
<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
</tr>
<tr>
<td>TRPC</td>
<td>transient receptor potential canonical</td>
</tr>
<tr>
<td>UAC</td>
<td>urinary albumin concentration (mg/l)</td>
</tr>
<tr>
<td>USRDS</td>
<td>United States Renal Data System</td>
</tr>
<tr>
<td>URIS</td>
<td>Unreferred Renal Insufficiency Study</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>Zo-1</td>
<td>Zona Occludens 1</td>
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CHAPTER 1: INTRODUCTION
1.1 Preface

Around 20-25% of the Belgian population has hypertension and 5% has diabetes mellitus (1, 2). Patients with diabetes mellitus and/or hypertension have an increased risk for chronic kidney disease (CKD), yet only a small proportion of these patients progresses to end stage renal disease (ESRD) to a degree that renal replacement therapy (RRT) in the form of dialysis or kidney transplantation is required (3, 4). The prevalence of RRT was 1200 per million persons in Belgium at 31 December 2012 (5). Asymptomatic microalbuminuria might precede the development of ESRD by years (6, 7). Timely detection of microalbuminuria in patients with diabetes and hypertension, followed by administration of renal protective agents, might reduce the number of patients with ESRD (8). Consequently, the American Diabetes Association (ADA) recommends annual screening for microalbuminuria in diabetes patients (1). Yet, in only one fifth of the Belgian RRT patients, renal failure was caused by diabetes (5). Additionally, microalbuminuria is common in the general population and an independent predictor of cardiovascular and renal outcome (7, 9). It has been suggested to organize mass screening using microalbuminuria to identify subjects with increased risk for cardiovascular and renal events (3, 10, 11). Testing for microalbuminuria is easy to perform and does not cause inconvenience for the tested person. In the present thesis, we measured cardiovascular risk factors and albuminuria in a Belgian working cohort (URI: unreferred renal insufficiency study) and in a Belgian hypertensive cohort (ERICABEL: Early Renal impairment and Cardiovascular Assessment in Belgium study). We investigated which parameters are associated with microalbuminuria in the URI and ERICABEL study and whether screening for microalbuminuria is useful to identify persons with cardiovascular and renal disease.
Figure 1: normal renal handling of albumin

Figure 1a. Normal glomerulus and proximal tubule. Figure 1b. The glomerular filtration barrier comprises the innermost fenestrated glomerular endothelial cells, glomerular basement membrane and the outermost podocytes. The endothelial cells synthesize a negatively charged glycocalyx that forms a plug in the fenestrae. Podocytes attach to the outermost aspect of the glomerular filtration barrier by foot processes, between which there is a protein layer comprising the size barrier slit diaphragm. Figure 1c. Proximal tubular cell. Filtered albumin is taken up by the megalin/cubilin mediated endocytosis of proximal tubular cells, is internalized by endosomes, and degradated by the lysozymes. Adapted from (12).
1.2 Normal renal handling of albuminuria

Under normal conditions, the glomerular filtration barrier is nearly impermeable for albumin. The histologic components of the 'glomerular filtration barrier' include the endothelial surface layer of glomerular fenestrated capillaries; the glomerular basement membrane and the slit diaphragm (fig. 1b). Albumin filtration is restricted by the negatively charged endothelial glycocalyx and the glomerular basement membrane, and by the fine pores of the slit diaphragm (13-15). The slit diaphragm is a dynamic junction between podocyte foot processes (16). These foot processes form a scaffolding around the capillary loops that are anchored to the glomerular basement membrane via α3β1 integrin and α-/β-dystroglycans (17). Although the diameter of the glomerular slit pores is slightly smaller than the diameter of albumin (3.5 versus 3.6 nm), some albumin molecules might pass through the slit diaphragm by the variation in diameter of the slit-pores and the shape of albumin (14, 18). The flux of albumin across the glomerular filter is driven by different forces: convection, diffusion and electrokinetic effects (19). Experimental research in normal rats shows that the glomerular-sieving coefficient of albumin is 0.00062 (14). As the filtration of albumin in humans is considered to be small too, the amount of albumin filtered per day is significant because the glomerular filtration rate is so high (filtered albumin = glomerular filtration rate x plasma albumin x glomerular sieving coefficient of albumin: thus daily filtered albumin = 144 L/day (100 mL/min x 60 min. x 24h) x 37 g/l x 0.00062 = 3.3 g/day) (14). Most part of the filtered albumin (fig. 1a) is reabsorbed by the proximal tubular cells (fig. 1c). The tubular uptake of albumin is mediated by megalin and cubilin. Cubilin was originally identified as the intestinal intrinsic factor-B12 receptor and megalin is a member of the LDL receptor family (20).
Figure 2a shows a normally functioning proximal tubular cell with uptake of albumin, including carrier albumin for hormones, calcium, folate and vitamin A, D, B₁₂ (20). Following uptake and degradation of albumin into albumin fragments, the compound is metabolized and/or returned to the circulation (20).

**Figure 2: Endocytic receptors in the proximal tubular renal cells**

- **a:** Normal receptor mediated uptake of filtered proteins
- **b:** Loss of receptor function leading to tubular proteinuria
- **c:** Tubular protein overload
- **d:** Shedding of receptors

![Diagram of endocytic receptors](image)

**Figure 2a:** Megalin and/or cubilin normally mediate the uptake of filtered proteins, including carrier protein for vitamins and hormones. Following uptake and degradation of protein, the compound is metabolized and/or returned to the circulation. **2b:** Loss of functional receptor expression, as observed in rare inherited disorders, leads to defective uptake and tubular proteinuria with the possible loss of important nutrients and associated deficiencies. **2c:** Tubular protein overload, leads to increased tubular uptake of proteins causing tubular cell apoptosis and change of function with activation of proinflammatory and profibrotic mediators. **2d:** Shedding of receptors, as may be observed in diabetes, leads to increased urinary excretion of receptors and possible tubular cell dysfunction. Adapted from (20).
1.3 Mechanisms of tubular and glomerular albuminuria

Albuminuria occurs by dysfunction of the glomerular filtration barrier and/or of the receptor mediated endocytosis process of the proximal tubular cells. Figure 2b shows that dysfunction of proximal tubular receptors results in albuminuria and loss of nutrients (20). Whether this mechanism could lead to progressive CKD in humans has not been investigated yet. Mutations of the megalin encoding gene occur in rare genetic disorders, such as Donnai-Barrow and Facio-Oculo-Acustico-Renal Syndrome, characterized by multiple developmental anomalies and asymptomatic tubular albuminuria (20). Mutations of the cubulin encoding gene occur in Imerslund-Gräsbeck disease, characterized by asymptomatic tubular albuminuria and megaloblastic anemia due to intestinal and tubular vitamin B$_{12}$ malabsorption (20-22). A missense variant (SNP) in the cubulin gene is associated with tubular albuminuria in the general and diabetic populations (23). The presence of tubular albuminuria in Dent’s disease is related to genetic mutations (CLCN5 or OCRL1) causing abnormal endosomal and lysosomal trafficking in the proximal tubular cells (24, 25). The tubular dysfunction in Dent’s disease is associated with Fanconi syndrome, albuminuria, hypercalciuria, nephrocalcinosis, nephrolithiasis and renal failure (26).

Increased protein synthesis or glomerular albumin leakage might result in albuminuria as tubular albumin receptor mediated endocytosis is oversaturated (figure 2c). Tubular overflow proteinuria might be an expression of a hematological disorder, yet it might also occur by exposure of toxic agents, such as Chinese herbs, mercury or cadmium (27, 28). Both cadmium and mercury increase the liver synthesis of metallothionein. This heavy metal binding low molecular weight protein is freely filtered and is excreted by the kidney as the tubular reabsorption is saturated (29). Conceivably, albuminuria protects the body, including the kidneys against the accumulation of toxic heavy metals. However, in case of glomerular induced nephrotic range albuminuria, receptor mediated endocytosis acts as a biological Trojan horse, by reabsorption of toxic fatty acids and lipoproteins bound to albumin, leading to activation of pro-inflammatory and pro-fibrotic mediators, tubular cell apoptosis, interstitial inflammation, fibrosis and nephron loss (20, 30-32). These receptors could be a potential target for therapy. Adenoviral delivery of antisense RNA leading to partial knockdown of cubilin was shown to protect against Adriamycine-induced glomerulosclerosis and tubulointerstitial renal damage in rats despite resulting in higher levels of...
albuminuria (33). In patients with nephrotic range proteinuria, statins might abrogate the toxic effects of modified albumin by inhibiting protein uptake via inhibition of HMG-CoA reductase and reduced prenylation of proteins involved in the endocytosis of the proximal tubular cell (34-37), while in subjects with no albuminuria, statins induce tubular albuminuria by the same mechanism (38-40).

Figure 2d shows that a reduction of megalin expression on the proximal tubular cells and the coinciding increase of urinary megalin excretion, results in albuminuria. Shedding of the receptors has been observed in diabetic patients, yet the underlying mechanism remains to be elucidated (41). Additionally, diabetic patients with poor glycemic control have a higher proportion of glycated albumin in urine than in plasma, which has been attributed to lower efficiency of tubular uptake of modified glycated albumin (42). Reduction of albuminuria is observed after correcting hyperglycemia (43). In vitro, megalin expression is reduced by activation of tubular angiotensin II type 1A receptors (44). In diabetic rats, angiotensin II blockade restores megalin expression associated with a decrease in albuminuria (45).

Angiotensin II plays a key role in renal hemodynamic and molecular mechanisms which lead to albuminuria. Angiotensin II decreases renal blood flow and increases glomerular filtration for albumin by inducing vasoconstriction of the efferent glomerular arterioles (46). Besides, angiotensin II upregulates the expression of sodium-glucose cotransporter-2 (SGLT-2) in the proximal tubules of patients with diabetes (47). As sodium reabsorption increases, the concentration of sodium in the distal tubules decreases (47). This results in an inhibition of the tubuloglomerular feedback that causes hyperfiltration by vasodilatation of afferent arterioles and rising intraglomerular pressure (47). Interestingly, in adults with obesity and essential hypertension glomerular hyperfiltration is also associated with increased sodium reabsorption in the proximal tubules of the kidney (48). Additionally, direct release of angiotensin II, insulin, prostaglandins, glucagon and growth hormones increases the intraglomerular pressure (46). These hemodynamic disturbances lead to shedding of the endothelial surface layer of the glomerular fenestrated capillaries and subsequent leakage of albumin into the sub-podocyte space (15, 49). This consequently exposes the glomerular basal membrane and the podocytes to the deleterious effects of modified albumin, resulting in thickening of basal membrane and retraction of podocyte foot processes that lead to increased glomerular permeability for albumin (50).
The accompanying structural abnormalities, such as glomerular endothelial injury, foot process detachment and podocyte loss are more marked in diabetic patients with macroalbuminuria than in those with microalbuminuria or normoalbuminuria (51, 52). Traditionally, the presence of microalbuminuria in diabetic patients is considered as a sign of early nephropathy. In the eighties, Mogensen et.al. published retrospective data (figure 3) of 20 type 1 diabetic males who had poor metabolic control and hyperfiltration, and who subsequently developed microalbuminuria, overt nephropathy and decline of renal function over years (53-56).

**Figure 3**

Figure 3 Landmark publication of the natural course of diabetic nephropathy in 20 male patients with insulin dependent diabetes mellitus (GFR measured with iothalamate). Poor metabolic control is associated with GFR > 150 mL/min. After 7-14 years of diabetes, microalbuminuria develops, followed by clinically overt nephropathy. After 18 years of diabetes blood pressure rises, GFR starts to decline and ESRD is reached after 25 years. Adapted from (56).
This hyperfiltration theory has been universally accepted as the natural cause of diabetic nephropathy, and extended to individuals with impaired glucose tolerance, metabolic syndrome and hypertension since glomerular hyperfiltration in these conditions also predicts de novo microalbuminuria (56-60). For this reason, it is discussed whether a screening of microalbuminuria in the general population to detect subjects with early nephropathy should be recommended (11). Microalbuminuria is not only an early predictor for overt nephropathy, it predicts independently also cardiovascular disease (CVD) and mortality (61, 62). Moreover, whether the presence of microalbuminuria inevitably results in overt nephropathy is uncertain, especially nowadays more and more patients are treated for their underlying risk factors (4, 63).

In primary kidney disease the pathophysiologic course of proteinuric might be different. Mutations in molecular signaling pathways of the slit diaphragm or in podocyte genes have been implicated in the susceptibility for idiopathic focal and segmental glomerulosclerosis, minimal change nephropathy and membranous glomerulopathy (16, 64, 65). The sudden onset of overt nephropathy in non-diabetic glomerular diseases, is conceivably not preceded by the presence of longterm microalbuminuria in most cases. A mass screening for microalbuminuria will consequently not be effective to detect these relatively rare disorders. Studies of podocyte proteins, including nephrin (NPHS1), podocin (NPHS2), zona occludens-1 (ZO-1), α-actinin-4, CD2 associated protein and transient receptor potential canonical (TRPC) channels, and their mutation analysis have shed light on the pathogenesis of glomerular kidney disease and manifestation of albuminuria and emphasized the critical role of the podocyte and the slit diaphragm in maintaining the function of the glomerular barrier (17, 50). Beside genetic factors, also drugs, immunologic mechanisms, and bacterial toxins might induce the expression of podocyte co-stimulatory molecule B7-1 (also termed CD 80), leading to foot process effacement and albuminuria (17, 66). It is unclear why molecule B7-1 is expressed on the podocyte, as it is normally expressed on antigen presenting cells and B-cells (67). It is suggested that the disruption of the glomerular filter by the expression of molecule B7-1 may help the innate immune system in clearing the circulation from harmful agents through transient urinary albumin loss (68). Noteworthy, the onset of nephrotic syndrome in minimal change nephropathy is often preceded by an infection or allergic reaction (69). However, it is unknown whether screening for albuminuria in presence of infectious or allergic symptoms would be effective, since the pathophysiologic mechanisms in these diseases are entirely different from those leading to albuminuria.
in metabolic disorders, including diabetes, hypertension and obesity. The main focus in this thesis will be on cardiovascular risk factors, as the prevalence of these risk factors was high in the populations we investigated.
1.4 Albuminuria as a manifestation of chronic kidney disease

To identify patients at risk for progressive kidney disease, the National Kidney Foundation’s - Kidney Disease Outcomes Quality Initiative (NKF-K/DOQI) developed a definition and classification for CKD that is updated by the Kidney Disease Improving Global Outcomes (KDIGO) CKD recommendations in 2013 (70, 71). CKD is defined as abnormalities of kidney structure or function, present for more than 3 months, with implications for health (71). The CGA staging system classifies subjects with CKD based on the cause of CKD (C), renal dysfunction, manifested by reduced glomerular filtration rate (G) and kidney damage, manifested by presence of persistent albuminuria (A). Based on this subdivision, table 1 shows the risk for progressive CKD (71).

<table>
<thead>
<tr>
<th>GFR categories (ml/min/1.73m²)</th>
<th>ACR: urinary albumin creatinine ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 ≥ 90</td>
<td>Normal  Low  High</td>
</tr>
<tr>
<td>G2 60-89</td>
<td>Normal  Low  High</td>
</tr>
<tr>
<td>G3a 45-59</td>
<td>Low  High  Very high</td>
</tr>
<tr>
<td>G3b 30-44</td>
<td>High  Very high  Very high</td>
</tr>
<tr>
<td>G4 15-29</td>
<td>Very high  Very high  Very high</td>
</tr>
<tr>
<td>G5 &lt;15 or RRT</td>
<td>Very high  Very high  Very high</td>
</tr>
</tbody>
</table>

ACR: urinary albumin creatinine ratio

Table 1. Classification of chronic kidney disease (71)

GFR: glomerular filtration rate, adapted from (71).

Notice that the respective misnomers “normoalbuminuria”, “microalbuminuria” and “macroalbuminuria” have been replaced by “normal to mildly increased albuminuria”, “moderately increased albuminuria” (CKD stage A2) and “severely increased albuminuria” (CKD stage A3). Throughout this manuscript, we will continue to use the terminology “normoalbuminuria”, “microalbuminuria” and macroalbuminuria” as several of the publications
included in the present thesis were submitted before the publication of the CGA staging system at January 2013 (71).

The prevalence of CKD in the general population is estimated around 10-15% (72, 73). The ageing of the population along with the growing global prevalence of diabetes, obesity and other chronic non communicable diseases has led to a corresponding worldwide increase in prevalence of CKD (74). The prevalence of all stages CKD is 35% in elderly (age > 60 years), 40% in diabetes, 23% in hypertension, 17% in obesity and 41% in CVD, as estimated by the USRDS in 2013 (73). Yet, the majority of CKD patients is identified by ACR of 30-300 mg/g and/or reduced estimated GFR of 30-60 ml/min/1.73m² while a minority (10%) has developed an ACR higher than 300mg/g and/or an estimated GFR lower than 30 ml/min/1.73m² (73). Only 10% of the latter subgroup progresses to a degree that RTT is required (75). Consequently, the incidence rate for RRT is around 10-30 per 100,000 person-years and the prevalence of RRT in the general population is around 0.1% (3, 72).

It is debatable whether an uniform CKD definition is valid and appropriate for implementing patients into renal care (76, 77). One point of discussion is the physiological reduction of kidney function with increasing age and consequently, whether a reduction of GFR of a certain degree has the same pathophysiological meaning throughout different age strata (72, 73, 77). A substantial part of patients with an eGFR < 60 ml/min/1.73m² did not have preceding albuminuria and do not develop progressive CKD (78, 79).

Another concern is whether it is appropriate to include subjects with microalbuminuria in the definition of CKD, as microalbuminuria is frequently associated with cardiovascular risk factors (80). In addition, fluctuations in microalbuminuria occur commonly by temporary physiological responses after strenuous exercise, meal, fever, extreme cold or standing (43, 81-84). For this reason, repeated measurements of albuminuria should be obtained, and consequently persistent microalbuminuria is required for diagnosing CKD stage 1 and 2. As most trials do not include follow-up examinations, CKD stage 1 and 2 could not be identified directly. Therefore, the prevalence rates of microalbuminuria were assumed to be 51% and 75% for persons with eGFRs > 90 and, 60-90 ml/min/1.73m², respectively (85). This persistence ratio to define microalbuminuria acts as self-fulfilling prophecy to establish microalbuminuria as marker for progressive CKD, whereas macroalbuminuria has a higher specificity to detect renal damage and
decline of renal function (52, 86). Of note, diabetic patients with ACR > 3000 mg/g had an 8-fold higher risk for ESRD than those with ACR < 1500 mg/g (figure 4).

**Figure 4**

![Figure 4](image-url)  

**Figure 4.** Effect of baseline albuminuria on ESRD in the RENAAL study. Adjusted for baseline serum creatinine, blood pressure, HbA1c, age and ethnicity. Adapted from (87).
1.5 **Albuminuria as a manifestation of cardiovascular disease**

Figure 5 shows the relation between CKD, cardiovascular risk factors, CVD and mortality. Patients with primary kidney lesions develop cardiovascular lesions, and inversely patients with cardiovascular diseases are more prone to develop kidney dysfunction (88-91). This reciprocal relationship between kidney disease and cardiovascular disease accelerates both general and renal vascular damage and progression of kidney disease and mortality.

**Figure 5**

![Diagram showing the relation between Ageing, Hypertension, Diabetes, CVD, CKD, and mortality](image)

**Figure 5.** Ageing, hypertension and diabetes are the main causes of cardiovascular disease (CVD) and chronic kidney disease (CKD). CVD can be both a cause or a consequence of CKD both leading to increased risk for mortality.
In the 19th century, James Goodhart observed albuminuria in sedentary and unhealthy subjects, “bulky men or women complain of a certain amount of ill health, and it is found that they eat and drink to much, they take no exercise, very possibly have gouty antecedents, they have a congested state of the capillaries of the face, short breath, and are fat and unhealthy looking. The urine of such as these is often of high specific gravity, and contains little albumen. Give these people periodical purges, diet them, and make them live altogether more according to natural law, and albumen disappears” (92). Nowadays, one third of the US population are identified with “metabolic syndrome” clustering high blood pressure, abnormal lipid status, high glycaemia and/or large waist circumference (93-95). It is suggested that the Western sedentary lifestyle is an important cause of the metabolic syndrome (96). The metabolic syndrome is considered to be a pre-diabetes condition with increased cardiovascular risk (96). Cardiovascular risk factors trigger oxidative stress that upregulates the expression of inflammatory cytokines and induces endothelial dysfunction, leading to concomitant kidney damage that might manifest as microalbuminuria (50, 97-102). In a cross-sectional study of hypertensive patients, those with microalbuminuria combined to inflammation had more obesity, metabolic syndrome, cardiac hypertrophy and smokers, while patients with microalbuminuria in absence of inflammation had no increased cardiovascular risk factors (102). However, prospective studies show that higher levels of albuminuria are an independent prognostic predictor for cardiovascular morbidity and mortality in patients with diabetes, metabolic syndrome, vascular disease, hypertension and even in the general population (62, 103-108). The increased relative risk for CVD according to microalbuminuria is approximately the same in different cohorts (106, 108-111), but the absolute risk for CVD is much higher in diabetic versus non-diabetic cohorts (table 2). These findings are important, as they indicate that a screening program will be more successful targeting specific groups (such as diabetics and hypertensives). The panels of figure 6 shows Kaplan-Meier curves for free survival of CVD according to microalbuminuria in different populations. Figure 6a shows that slightly elevated levels of urinary albumin are already associated with CVD. Figure 6c show that diabetic populations have a higher absolute risk for CVD than general cohorts (figure 6a and 6b).
Table 2: Relative risk and absolute risk for adverse outcomes according to microalbuminuria (MAU) vs. no MAU in different studies.

<table>
<thead>
<tr>
<th>Study:</th>
<th>Relative risk according to MAU vs. no MAU</th>
<th>Absolute risk (per 1000 person years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Framingham (n=1,568)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Cardiovascular disease</td>
<td>2.9</td>
<td>1.6-5.4</td>
</tr>
<tr>
<td>-All-cause mortality</td>
<td>1.8</td>
<td>1.0-3.2</td>
</tr>
<tr>
<td>EPIC (n=22,368)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Cardiovascular disease</td>
<td>1.7</td>
<td>1.2-1.9</td>
</tr>
<tr>
<td>-All-cause mortality</td>
<td>1.5</td>
<td>1.2-1.8</td>
</tr>
<tr>
<td>-Cardiovascular mortality</td>
<td>2.0</td>
<td>1.6-2.7</td>
</tr>
<tr>
<td>CCHS (n=1,734)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Cardiovascular disease</td>
<td>2.0</td>
<td>1.4-2.9</td>
</tr>
<tr>
<td>-All-cause mortality</td>
<td>1.5</td>
<td>1.2-1.8</td>
</tr>
<tr>
<td>ADVANCE (n=10,640)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Cardiovascular disease</td>
<td>1.5</td>
<td>1.1-1.9</td>
</tr>
<tr>
<td>-Cardiovascular mortality</td>
<td>1.7</td>
<td>1.2-2.6</td>
</tr>
<tr>
<td>Diabetes (n=199)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Cardiovascular disease</td>
<td>2.2</td>
<td>2.1-2.5</td>
</tr>
<tr>
<td>-All-cause mortality</td>
<td>2.5</td>
<td>0.7-8.4</td>
</tr>
</tbody>
</table>

*Framingham*(106): General population, no diabetes, no hypertension, no previous CVD at baseline, high ACR ≥ 3.9 mg/g in men and ≥ 7.5 mg/g in women.
*EPIC*(108): General population, no previous CVD, 2% diabetes, 11% hypertension, high ACR > 20 mg/g.
*CCHS*(109): Copenhagen City Heart Study III: 100% hypertensive patients without previous CVD, 4% diabetes, high UAE > 6.9 mg/day.
*ADVANCE*(110): Diabetic patients, history of previous CVD 32%, high ACR ≥ 30mg/g.
*Diabetes*(111): Diabetic patients without macroalbuminuria, high UAE; 30-300 mg/d.
Figure 6. Kaplan-Meier survival curves for CVD in population-based cohorts (Framingham: fig. 6a. and EPIC: fig. 6b) and in type 2 diabetic cohort (fig. 6c) according to baseline ACR.

Median ACR = 3.9 mg/g in men and = 7.5 mg/g in women
1.6 The prevalence of albuminuria

The prevalence of microalbuminuria and macroalbuminuria ranged from 6 to 12% and 0.6 to 1.3% respectively, according to population cohorts (9, 72, 85, 112-115).

Table 3. Clinical studies reporting the prevalence of albuminuria in the general population

<table>
<thead>
<tr>
<th>Study</th>
<th>HUNT II</th>
<th>EPIC</th>
<th>KEAPS</th>
<th>PolNef</th>
<th>PREVEND</th>
<th>NHANES</th>
<th>AusDiab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Country</td>
<td>Norway</td>
<td>UK</td>
<td>UK</td>
<td>Poland</td>
<td>Netherlands</td>
<td>US</td>
<td>Australia</td>
</tr>
<tr>
<td>Invited</td>
<td>92,703</td>
<td>77,630</td>
<td>2,199</td>
<td>9,700</td>
<td>85,421</td>
<td>All</td>
<td>?</td>
</tr>
<tr>
<td>Responded</td>
<td>65,181</td>
<td>25,112</td>
<td>1,208</td>
<td>2,501</td>
<td>40,856</td>
<td>13,233</td>
<td>11,247</td>
</tr>
<tr>
<td>Age</td>
<td>&gt; 20</td>
<td>40-79</td>
<td>&gt; 18</td>
<td>&gt; 18</td>
<td>28-75</td>
<td>&gt; 20</td>
<td>?</td>
</tr>
<tr>
<td>THT</td>
<td>11</td>
<td>14.4</td>
<td>16.7</td>
<td>13.1</td>
<td>11.2</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3.4</td>
<td>2.3</td>
<td>4.4</td>
<td>6.6</td>
<td>2.6</td>
<td>6.8</td>
<td>7</td>
</tr>
<tr>
<td>CVD</td>
<td>8</td>
<td>9.8</td>
<td>11.6</td>
<td>?</td>
<td>3.6</td>
<td>15</td>
<td>0.8</td>
</tr>
<tr>
<td>Micro</td>
<td>7.1</td>
<td>11.8</td>
<td>7.1</td>
<td>11.9</td>
<td>7.2</td>
<td>8.2</td>
<td>6.0</td>
</tr>
<tr>
<td>Macro</td>
<td>0.8</td>
<td>0.9</td>
<td>0.7</td>
<td>1.3</td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threshold</td>
<td>17-250/25-355**</td>
<td>20-200*/&gt; 21/&gt; 30**</td>
<td>&gt; 20°</td>
<td>20-200°</td>
<td>30-300*</td>
<td>30-300*</td>
<td></td>
</tr>
<tr>
<td>Collection</td>
<td>morning°°</td>
<td>random</td>
<td>morning</td>
<td>random</td>
<td>morning</td>
<td>random</td>
<td>morning</td>
</tr>
</tbody>
</table>

Table 3 shows the numbers of subjects who were invited and responded, year of enrollment, age of inclusion, THT: prevalence of treated hypertension (%), prevalence diabetes (%), CVD: prevalence of cardiovascular disease, prevalence of micro= microalbuminuria and macro= macroalbuminuria (%), Threshold to define microalbuminuria: by (*): ACR = urinary albumin creatinine ratio (mg/g), by (°): UAE = urinary albumin excretion (mg/l) or by (**): ACR gender specific thresholds: male/female. Collection: how urine specimens were collected: first morning or random. (°°) in 9,598 subjects 3 first morning urine specimens were collected.
The prevalence of micro- or macro-albuminuria varied in these cohorts as a result of the different proportions of subjects with CVD, definitions of microalbuminuria and macroalbuminuria, age range and timing of urinary sample collection (table 3). According to the NHANES cohort, the prevalence of albuminuria was 34.2% in diabetes (microalbuminuria 28.1%, macroalbuminuria 6.1%), 14.5% in non-diabetic hypertension (microalbuminuria 12.8%, macroalbuminuria 1.7%), and 5.1% in non-diabetes non-hypertension (microalbuminuria 4.8%, macroalbuminuria 0.3%) (116). Yet, the prevalence of albuminuria (micro- and macro-albuminuria) varied also markedly between cohorts in the presence of diabetes (6-39%), hypertension (4-47%) and CVD (15-59%) (6, 117).
1.7 How to measure albuminuria

Clinical guidelines on how to measure albuminuria are not uniform with regard to patient preparation, timing of urine samples, units of reporting, thresholds to define micro- or macro-albuminuria and the methods which are used to measure urinary albumin and creatinine (42). Overnight urinary albumin excretion is considered as the golden standard, but is impractical in clinical settings and in epidemiologic studies. A practical alternative is measurement of urinary albumin creatinine ratio (ACR) in milligrams albumin per gram creatinine as the units of measure, in early or first morning urine samples (42, 118). Yet, albuminuria is commonly measured in random urine samples due to practical reasons. However, a higher within-subjects biological variability for ACR has been reported with random urine samples compared with first morning urine samples (119).

Preparation of the patient. Measuring albuminuria during circumstances of pyrexia, exposure to cold, gout attacks, glycosuria, anemia, hematuria, urinary tract infections, alcohol excess, heart failure, medication, after exercise and mental anxiety should be interpreted with caution as all these conditions might affect the level of albuminuria (92, 120). To exclude postural albuminuria, measuring albuminuria should be performed in a first morning sample, particularly in relatively young subjects (42).

Molecular forms of albumin. Albumin is the most abundant plasma protein, serving multiple functions as a carrier of metabolites (long-chain fatty acids, bilirubin...), ions (calcium...), hormones (thyroxin), organic molecules, vitamins and drugs, as an acid/base buffer, as antioxidant and by supporting the oncotic pressure and volume of the blood (121). Albumin can be degraded into albumin fragments of 0.5-5kD by time, proximal tubular cells (cubilin-megalin), proteolysis during passage through the urinary tract, oxidation in the urine and during specimen storage (42). Mutations of the gene encoding albumin (<1/1000) might affect renal albumin clearance, producing different proportions of modified albumin in urine compared to serum (122).

Methods for measuring urine albumin. Urinary albumin can be quantified with immunochemical methods, such as radioimmunoassay (RIA), immunoturbidimetry and immunonephelometry (42, 123). These routine methods use either polyclonal or monoclonal antibodies. Immunoassays with polyclonal antisera react with many modified albumins whereas immunoassays with monoclonal
antisera can only react with immunoreactively intact albumin (42). However, current
immunoassays have a considerable inter-assay coefficient of variation. Additionally, the
variability between-laboratory appears to be tremendous (124). Size exclusion high performance
liquid chromatography (HPLC) measures both immunoreactive and immunounreactive intact
albumin (125). Albuminuria can also be measured semiquantitatively with point of care methods
by colorimetric test strips (“dipsticks”), such as HemoCue Albumin 201 system (HemoCue®),
Clinitek Microalbumin Reagent strips (Bayer Health Care®) and the Chemstrip Micral Test strips
(Roche Diagnostic®) with a good sensitivity and specificity for urinary albumin excretion (24
hours)(126). Interestingly, a quantitative dye-binding based test strip analysis seems to be reliable
to detect microalbuminuria (127). Additionally, this test might be suitable to perform in a mass
screening as it is very cheap (2 Eurocent per test)(128). The National Kidney Disease Education
Program-International Federation of Clinical Chemistry (IFCC) Working Group is establishing
standardization of albumin measurements (42, 129).

Which threshold for microalbuminuria should be used. Microalbuminuria has been introduced as
a surrogate marker for early diabetic nephropathy based on a small retrospective study of 63
diabetic patients; seven of 8 patients with overnight albuminuria between 30-140 µg/min
developed overt nephropathy after 14 years (130). Another small study, defined early diabetic
nephropathy as an overnight urine albumin excretion between 30-140 µg/min, which was
predictive for the development of clinical proteinuria (131). These values are considered to be
approximately equivalent to an ACR of 20-100 mg/g in a random untimed urine sample. KDIGO
recommends to use a threshold for AER of 30-300 mg/24 hours (ACR of 30-300 mg/g) to
indicate microalbuminuria (71). This threshold is based on the “normal” values of the distribution
of ACR in apparently healthy young subjects and on the increased risk of all cause mortality,
cardiovascular mortality, acute kidney injury (AKI) and CKD progression once ACR exceeds 30
mg/g (6, 117). Also, the Scientific Advisory Board on the prevention of diabetic nephropathy of
the US National Kidney Foundation recommends to use an ACR risk-based cut off above 30
mg/g in random urine samples (132). This threshold can however be debated, as albuminuria is a
continuous parameter that over its entire range is related to cardiovascular events, even with
values below the threshold (133). In addition, such a cut-off of 30 mg/g does not give weight to
the prognostic values of substantially higher degrees of albuminuria (macroalbuminuria), which
carry a much higher risk to be associated with rapid decline of renal function than these relatively
modest values of 30 mg/g (86). Beside the amount of urinary albumin concentration, ACR depends also on muscle mass as it is measured in 24 hour creatinine collection. Consequently, ACR in females, elderly and in patients with muscle wasting overestimates true AER. Warram et. al. found a lower threshold in men (ACR ≥ 17 mg/g) than in women (ACR ≥ 25 mg/g) with serial specimens in 1,613 patients with diabetes mellitus type 1. These gender specific thresholds corresponded to the 95th percentiles of distribution of the ACR in 218 healthy control subjects (134). Higher values of ACR in women than in men are caused by the lower muscle mass and thus the lower urinary creatinine excretion. For that reason, we decided to use gender specific thresholds as proposed by Warram et. al., as these values are equivalent to an albumin excretion rate of 30 µg/min in men and 31 µg/min in women. As a matter of fact, thresholds of approximately 30 µg/min were also the ones used in the first studies (130, 131).
1.8 Outline and aims of the thesis

The presence of microalbuminuria is considered to be an independent indicator for cardiovascular and/or kidney disease in general populations (7, 88, 135). Microalbuminuria seems to be a suitable screening tool in persons with diabetes, as treatment of microalbuminuria delays the development of overt nephropathy (136). Although, screening for microalbuminuria in a general population, followed by treatment did not improve renal and cardiovascular outcome (137, 138). One reason for this disappointed result might be that microalbuminuria is an inappropriate test to select persons with cardiovascular and renal risk. This thesis discusses the pitfalls of using microalbuminuria to identify subjects with cardiovascular and renal events.

The aims of this thesis are:

1) To evaluate the prevalence of microalbuminuria and the associations with traditional cardiovascular risk factors in a general population.

2) To evaluate whether we can use microalbuminuria as a screening tool to identify subjects with unrecognized cardiovascular risk factors in a presumably healthy population.

3) To evaluate which conditions could lead to false positive test results of microalbuminuria.

4) Whether the risk of ESRD could be reduced by screening for microalbuminuria according to the literature.
1.9 Participants and methods

The Unreferred Renal Insufficiency (URI) cohort and Early Renal Impairment and Cardiovascular Assessment in Belgium (ERICABEL) cohort were applied for this thesis.

The URI study is a non-interventional prospective study that included 1,486 Caucasian workers who presented at a routine yearly occupational check-up between January 2007 and December 2009. The first study; “towards a rational screening strategy for albuminuria”, excluded subjects with hematuria, leucocyturia, known comorbidities as diabetes, cardiovascular disease, renal disease, and on antihypertensive drugs and lipid lowering drugs, leaving a cross-sectional cohort of 1,191 apparently healthy workers with a mean age of 38 ± 10 years. The second study, “microalbuminuria is more consistent in presence of risk factors” included 341 workers of the URI cohort who underwent at least two consecutive occupational heath care examinations. Subjects with macroalbuminuria and those with incomplete data were excluded, leaving a prospective cohort of 239 subjects with known and unknown cardiovascular risk factors. The third study “screening for kidney disease in a presumed healthy working population” included all screened workers with known and unknown cardiovascular risk factors. The presence of hematuria and subjects with incomplete data were exclusion criteria for further analysis, leaving a cross-sectional cohort of 1,398 workers.

Description of procedures: All subjects were investigated during their yearly check up by their occupational physician. Body weight was recorded to the nearest 0.1 kg and height was measured to the nearest centimeter. Waist circumference was measured by trained nurses following recommendations by WHO(139). Body mass index (BMI) was calculated as body weight in kg divided by height² (kg/m²). Blood pressure and resting heart rate were measured in sitting position by a calibrated electronic device (OMRON®). A questionnaire about current cigarette smoking, physical activity and prescribed medication was taken by an occupational physician in each participant. A random blood and urine spot specimen was collected and analyzed on the same day in one central laboratory (no frozen samples). Urinary albumin was measured by an immune turbidimetric method with an inter-assay coefficient of variation of 11.2% at a mean level of 82 mg/L and an inter-assay coefficient of variation of 4.9% at a mean level of 580 mg/l. Serum creatinine was analyzed by a colorimetric assay (compensated Jaffe reaction), calibrated by Isotope Dilution Mass Spectrometry (IDMS), with an inter-assay coefficient of variation of
1.75% at a mean level of 104 µmol/l (Roche). Serum cystatin C was determined by nephelometry with an inter-assay coefficient of variation of 5.1% at a mean level of 0.95 mg/l (Siemens DII). Serum CRP was measured with an immune turbidimetric method with an inter-assay coefficient of variation of 4.6% at a mean level of 3.2 mg/l and inter-assay coefficient of variation of 2.5% at a mean level of 5.5 mg/l.

The ERICABEL study included patients with hypertension, aged 40-70 years. We used the baseline data of the ERICABEL cohort, a non-interventional epidemiological study with a follow-up of 5 years that included 1,076 Caucasian patients with hypertension, defined as systolic blood pressure ≥140 mmHg and/or intake of at least one antihypertensive drug, recruited by 96 general practitioners, between 2006 and 2007, in Belgium. Of the 1076 patients included in this cross-sectional study, 420 patients had a missing value for at least one of the variables under investigation. Multiple imputation techniques were used to account for the missing data, using 20 imputations (140). All characteristics and outcome (MAU) were simultaneously used in the imputation model. The imputation was done using the R function aregImpute from the Hmisc package (141).

**Description of procedures:** Each participating primary care physician was asked to include 10 consecutive hypertensive patients aged between 40-70 years, in a 1:1 sex ratio. The eligible persons were evaluated at baseline and if eligible, sociodemographic information (age, sex, race, and education level), personal and family medical history, smoking status and medication use were collected prospectively in an online database. Body weight was recorded to the nearest 0.1 Kg and height was measured to the nearest centimeter. Body mass index (BMI) was calculated as body weight in Kg divided by height² (kg/m²). Blood pressure was measured according to the WHO criteria with a calibrated Omron HEM-907 device (average of 2 measurements, sitting, with 5 minutes in between). All these measurements were done by the primary care physician. After exclusion of a urinary infection or hematuria (negative Combur® test), MAU was screened by a Micral® dipstick test. MAU was considered present if measured albuminuria was ≥ 20mg/l on a morning midstream urine sample. Blood sampling was performed by the general practitioner in fasting patients.
1.10 References


62. Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS, de Jong PE, Coresh J, Gansevoort RT. Association of estimated glomerular filtration rate and albuminuria with all-cause and


CHAPTER 2: RESULTS

2.1. Screening for cardiovascular and renal markers in unselected subjects: results from URI trial.
2.1.1 Towards a rational screening strategy for albuminuria: Results from the Unreferred Renal Insufficiency Trial

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CHAPTER 2: Results

Abstract.

Background. There remains debate about the screening strategies for albuminuria. This study evaluated whether a screening strategy in an apparently healthy population based on basic clinical and biochemical parameters could be more effective than a strategy where screening for albuminuria is performed unselectively.

Methodology/Principal Findings. The Unreferred Renal Insufficiency (URI) Study is a cross-sectional study on the prevalence of metabolic risk factors in Belgian workers, volunteering to be screened during a routine yearly occupational check-up. Subjects (n=295) with treated hypertension, known diabetes, treated dyslipidaemia, cardiovascular and renal disease were excluded. Among 1,191 apparently healthy subjects, 23% had unknown hypertension, 13% had impaired glucose tolerance, 15.4% had normoalbuminuria, 4.2% had microalbuminuria and 0.4% had macroalbuminuria. Subjects with resting heart rate ≥85 bpm, plasma glucose ≥5.6 mmol/L and blood pressure ≥140/90mmHg were associated with albuminuria of any degree. A strategy where only subjects with at least one of these risk factors (n=431) were screened for albuminuria, would identify all subjects with macroalbuminuria (5/5), 64% of those with microalbuminuria (32/50), and less than half of those with normoalbuminuria (81/183). An alternative strategy whereby subjects were first screened for presence of albuminuria, and additional cardiovascular risk factors were only measured in subjects positive for albuminuria (n=238), would identify only 27% (118/431) of the subjects with additional and potentially modifiable cardiovascular risk factors. On the other hand, half of the subjects in this study with albuminuria (120/238, of which 102 had normoalbuminuria), had no additional cardiovascular risk factor at all.

Conclusions. Screening an apparently healthy population directly for albuminuria will result in a high percentage of false positives, mostly measured in the normal range. Screening for microalbuminuria and macroalbuminuria based on presence of additional, potentially modifiable risk factors appears to be more beneficial.
Introduction
The number of patients with end stage renal disease (ESRD) in need of renal replacement therapy has dramatically increased over the last decades (1). A substantial part of this increase is attributable to the rising prevalence of diabetes and/or hypertension (2-4). Microalbuminuria seems to be an important predictor of progressive renal disease and end stage renal disease in diabetic and hypertensive subjects (5, 6). It has been demonstrated that in these populations, preventive measures can delay the evolution to macroalbuminuria and potentially also the progression to renal failure (7, 8). Consequently, it is advised to screen these high risk subjects with diabetes and hypertension, in order to identify and eventually treat those at risk for progressive renal disease. Extending this line of reasoning, some authors advocate to screen also low risk groups, or even the general population, arguing that most persons with albuminuria and/or reduced eGFR (<60 ml/min/1.73m²) are asymptomatic (9). Van der Velde et al e.g. found that 45% of subjects with microalbuminuria were younger than 55 years and had no hypertension or diabetes (9). It is debated whether such a large scale screening project should be advocated (10, 11). Concerns are not only the cost of screening itself, but more importantly, the risk and the cost of treating false positive subjects (12). In the study by van der Velde et al, out of 40,854 subjects screened, 7.8% (n=3,200) had at least microalbuminuria, but only 45 of those developed ESRD over a 9 year follow up period (9). In addition, effective screening presumes that an intervention to alter the course of the disease is available (13), which is not the case if the subject only has albuminuria and no other modifiable risk factor. It would therefore be very useful to develop a screening strategy based on additional, modifiable, risk factors to enhance the yield of screening without having too much false positives, and to make interventions possible.

In this study, we used data collected in an apparently healthy working population, aged 17 to 65 years, to evaluate which easily obtainable parameters or combinations of them are associated with albuminuria, and whether using these risk factors can be of help to increase the effectiveness of a screening program.
Methods

Objectives: The primary aim was to determine the prevalence of different levels of albuminuria, some (cardiovascular) risk factors and their associations, to develop a rational screening strategy for albuminuria in the healthy population.

Participants: The Unreferred Renal Insufficiency (URI) is a cross sectional study that included only Caucasian workers (n=1,486) who presented at a routine yearly occupational check-up between January 2007 and December 2009, in Belgium. The presence of more than 100 leukocytes/μl (2.2%), and/or more than 50 erythrocytes/μl (2.4%) in the urinary sediment were considered as confounders for reliable measurement of urinary albumin; these subjects and subjects with incomplete data (1.8%) were consequently excluded from further analysis. Subjects with known comorbid conditions or risk factors of which it is well established that they could affect albuminuria, such as diabetes (1.2%), cardiovascular disease (0.3%), renal disease (0.4%), subjects on antihypertensive drugs (8.3%) and on lipid lowering drugs (3.3%) were also excluded, leaving a cohort of 1,191 apparently healthy subjects for analysis.

Description of procedures: All subjects were investigated during their yearly check up by their occupational physician. Body weight was recorded to the nearest 0.1 kg and height was measured to the nearest centimeter. Waist circumference was measured by trained nurses following recommendations by WHO (14). Body mass index (BMI) was calculated as body weight in kg divided by height² (kg/m²). Blood pressure and resting heart rate were measured in sitting position by a calibrated electronic device (OMRON®). A questionnaire about current cigarette smoking, physical activity and prescribed medication was taken by an occupational physician in each participant. A random blood and urine spot specimen was collected and analyzed on the same day in one central laboratory (no frozen samples). Urinary albumin was measured by an immune turbidimetric method with an inter-assay coefficient of variation of 11.2% at a mean level of 82 mg/L and an inter-assay coefficient of variation of 4.9% at a mean level of 580 mg/l. Serum creatinine was analyzed by a colorimetric assay (compensated Jaffe reaction), calibrated by Isotope Dilution Mass Spectrometry (IDMS), with an inter-assay coefficient of variation of 1.75% at a mean level of 104 μmol/l (Roche). Serum CRP was measured with an immune turbidimetric method with an inter-assay coefficient of variation of 4.6% at a mean level of 3.2 mg/l and inter-assay coefficient of variation of 2.5% at a mean level of 5.5 mg/l.
Definitions: Gender specific urinary albumin creatinine ratio (uACR) cutoff values as proposed by Warram et al. were used because men have a higher urinary excretion of creatinine than women due to higher muscle mass. Normoalbuminuria was defined as uACR: 0.6-1.8 mg/mmol (5-16 mg/g) in men and uACR 0.8-2.7 mg/mmol (7-24 mg/g) in women, microalbuminuria was defined as uACR 1.9-27 mg/mmol (17-249 mg/g) in men and uACR 2.8-39 mg/mmol (25-354 mg/g) in women, macroalbuminuria was defined as uACR ≥ 28 mg/mmol (250 mg/g) in men and uACR ≥ 40 mg/mmol (355 mg/g) in women (15). The Modification of Diet in Renal disease (MDRD) equation was used to assess the estimated GFR: 

\[ eGFR = 175 \times \text{standardized } S_{cr}^{-1.154} \times \text{age}^{-0.203} \times 1.212 \times 0.742 \times \frac{1}{\text{if male}} \times 0.97 \times \frac{1}{\text{if female}} \]

Impaired glucose tolerance (IGT) was defined as a plasma glucose level ≥ 5.6 mmol/L (17). Hypertension was defined as diastolic blood pressure ≥ 90 mmHg and/or systolic blood pressure ≥140 mmHg. Obesity was defined as BMI > 30 kg/m². Abdominal adiposity was defined according the National Cholesterol Education Program (NCEP) criteria: waist circumference >102 cm in men and >88 cm in women. Hypercholesterolemia was defined as serum total cholesterol >6.5 mmol/L.

Ethics: A written informed consent was obtained from all participants. The Ethics Committee of University Hospital Ghent approved the study (2006-038).

Statistical methods: SPSS 15.0 was used for all calculations. Results are presented as percentages or as mean ± standard deviation. The baseline characteristics of groups were compared by use of ANOVA: post-hoc Scheffé test (continuous variables), Kruskal-Wallis (continuous variable with a skewed distribution) and a Chi-square test (categorical variables). Ordinal regression analysis was performed to select the associated risk factors with different categories of albuminuria, in a random selection of 50% of the subjects. The significant continuous variables were dichotomized. These risk factors were validated in the other 50% of the sample. The prevalence and test characteristics, of normo-, micro- and macroalbuminuria were calculated, if at least one of these risk factors was present. The sensitivity was defined as the number of subjects with true-positive test results divided by the total number of subjects with albuminuria. The specificity was defined as the number of true-negative test results divided by the total number of subjects without albuminuria. The positive predictive value was defined as the number of true-positive test results divided by the total number of positive test results. The negative predictive value was defined as the number of true negative test results divided by the total number of negative test.
results. The positive likelihood ratio was defined as sensitivity divided by 1-specificity; the negative likelihood ratio was defined as 1-sensitivity divided by specificity.
Results

Our cohort (n=1,191) of apparently healthy subjects, after excluding those with treated hypertension, treated dyslipidaemia, known diabetes, cardiovascular or renal disease, still had a high prevalence of unknown hypertension, dyslipidaemia and impaired glucose metabolism.

Table 1. Basic characteristics and metabolic risk factors of 1,191 apparently healthy subjects.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>998</td>
<td>83.8</td>
</tr>
<tr>
<td>Unknown hypertension</td>
<td>279</td>
<td>23.4</td>
</tr>
<tr>
<td>Unknown hypercholesterolemia</td>
<td>86</td>
<td>7.2</td>
</tr>
<tr>
<td>Unknown IGT/diabetes</td>
<td>150</td>
<td>13.3</td>
</tr>
<tr>
<td>Obesity (BMI&gt;30kg/m²)</td>
<td>160</td>
<td>13.5</td>
</tr>
<tr>
<td>Abdominal adiposity (%)</td>
<td>168</td>
<td>16.0</td>
</tr>
<tr>
<td>No physical activity</td>
<td>491</td>
<td>49.2</td>
</tr>
<tr>
<td>Current smoking</td>
<td>370</td>
<td>32.4</td>
</tr>
<tr>
<td>Normoalbuminuria</td>
<td>183</td>
<td>15.4</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>50</td>
<td>4.2</td>
</tr>
<tr>
<td>Macroalbuminuria</td>
<td>5</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Unknown impaired glucose tolerance (IGT)/diabetes: plasma glucose ≥ 5.6 mmol/L.
Hypercholesterolemia: serum cholesterol >6.5 mmol/L. Hypertension: RR ≥140 and/or 90 mmHg.

Table 1 shows the basic characteristics and cardiovascular risk factors of the cohort.
Almost all subjects (98.9%) had an estimated GFR higher than 60 ml/min/1.73m² and none had an estimated GFR lower than 50 ml/min/1.73m². As expected, the cohort was rather young (age 38.3 ± 9.7 years, range 17-64). Albumin was detected in the urine of one fifth. The large majority of albuminuric subjects had normoalbuminuria, fewer subjects had microalbuminuria and macroalbuminuria was only rarely observed (table 1).
Table 2 shows the distribution of some measured clinical and biochemical parameters in subgroups according to different levels of albuminuria.

**Table 2.** Metabolic risk factors in subjects with different level of albuminuria.

<table>
<thead>
<tr>
<th>Albuminuria</th>
<th>no</th>
<th>Normo-</th>
<th>Micro-</th>
<th>Macro-</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=1,191 (%)</td>
<td>953</td>
<td>183</td>
<td>50</td>
<td>5</td>
</tr>
<tr>
<td>Age (years)</td>
<td>38.2±9.6</td>
<td>38.7±10.1</td>
<td>38.8±10.7</td>
<td>39.0±9.1</td>
</tr>
<tr>
<td>Men (%)</td>
<td>799 (83.8)</td>
<td>147 (80.3)</td>
<td>47 (94)</td>
<td>5 (100)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>126.9±13.8</td>
<td>129.8±14.1</td>
<td>132.6±18.3*</td>
<td>142.6±11.2</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>77.4±9.9</td>
<td>79.9±10.6*</td>
<td>80.8±13.2</td>
<td>90±8.9*</td>
</tr>
<tr>
<td>Unknown hypertension</td>
<td>199 (20.9)</td>
<td>55(30.1)*</td>
<td>20 (40)**</td>
<td>5(100)**</td>
</tr>
<tr>
<td>Resting heart rate</td>
<td>69.5±10.3</td>
<td>72.6±11.3**</td>
<td>75.9±16.1**</td>
<td>78.8±3.7</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.0±1.0</td>
<td>5.1±1.0</td>
<td>4.9±1.1</td>
<td>7.0±1.0**</td>
</tr>
<tr>
<td>Plasma glucose (mmol/L)</td>
<td>4.8±0.7</td>
<td>5.0±1.2*</td>
<td>5.0±0.9</td>
<td>5.2±1.1</td>
</tr>
<tr>
<td>Unknown IGT/diabetes (%)</td>
<td>103 (11.5)</td>
<td>34(19.3)*</td>
<td>12(24)*</td>
<td>1(20)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.9±3.9</td>
<td>25.5±4.3</td>
<td>25.3±5.3</td>
<td>27.5±2.7</td>
</tr>
<tr>
<td>Abdominal adiposity (%)</td>
<td>135 (16.1)</td>
<td>21 (12.7)</td>
<td>11 (25.6)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Serum uric acid (µmol/L)</td>
<td>315±71</td>
<td>315±77</td>
<td>339±77</td>
<td>482±119**</td>
</tr>
<tr>
<td>White blood cell count (10⁹/L)</td>
<td>7.0±1.9</td>
<td>7.2±1.9</td>
<td>7.9±2.3**</td>
<td>8.0±1.8</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>2.2±4.8</td>
<td>2.5±4.2</td>
<td>4.1±1.0</td>
<td>3.4±3.6</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>283 (31)</td>
<td>64 (35.6)</td>
<td>20 (40)</td>
<td>3 (60)</td>
</tr>
</tbody>
</table>

Unknown impaired glucose tolerance (IGT)/diabetes: plasma glucose ≥ 5.6 mmol/L. Hypertension: RR ≥140 and/or 90 mmHg. *p<0.05, **p<0.01 versus no albuminuria.

In a randomly selected cohort (n=599), a multivariate ordinal regression model selected resting heart rate, plasma glucose and hypertension as significant independent predictors for presence of albuminuria at any degree (table 3).
Table 3. Multivariate regression analyses to predict different categories of albuminuria

<table>
<thead>
<tr>
<th>Random sample n=599, R²=0.07</th>
<th>E</th>
<th>St error</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting heart rate (beats per min)</td>
<td>0.026</td>
<td>0.009</td>
<td>0.006</td>
</tr>
<tr>
<td>Plasma glucose (mmol/L)</td>
<td>0.341</td>
<td>0.141</td>
<td>0.015</td>
</tr>
<tr>
<td>Unknown Hypertension</td>
<td>0.487</td>
<td>0.237</td>
<td>0.040</td>
</tr>
<tr>
<td>White blood cell count</td>
<td>0.079</td>
<td>0.053</td>
<td>0.132</td>
</tr>
<tr>
<td>Serum uric acid (µmol/L)</td>
<td>0.001</td>
<td>0.001</td>
<td>0.464</td>
</tr>
<tr>
<td>Total serum cholesterol (mmol/L)</td>
<td>-0.106</td>
<td>0.104</td>
<td>0.310</td>
</tr>
</tbody>
</table>

The continuous variables were dichotomized (0 or 1) as follows: resting heart rate ≥ 85 bpm (cutoff level according to the 90th percentile); plasma glucose: ≥ 5.6 mmol/L (impaired glucose tolerance). Consequently, the prevalence of normoalbuminuria, microalbuminuria and macroalbuminuria in this randomly selected cohort, was higher in subjects with at least one risk factor (n=206) than in subjects with no risk factors (n=393), respectively: 21.4 vs 14.1% (n=44 vs 58); 8.7 vs 3.1% (n=18 vs 12) and 1.5 vs 0% (n=3 vs 0), p<0.001. Our risk assessment was validated in the other randomly selected cohort (n=592). The prevalence of normoalbuminuria, microalbuminuria and macroalbuminuria in this validation cohort, was also higher in subjects with at least one risk factor (n=225) than in subjects with no risk factors (n=367), respectively: 16.4 vs 12% (n=37 vs 44); 6.2 vs 1.6% (n=14 vs 6) and 0.9 vs 0% (n=2 vs 0), p=0.001. Table 4 shows the test characteristics for subjects with at least one risk factor, to identify normoalbuminuria, microalbuminuria and macroalbuminuria in both randomly selected cohorts.
Table 4. Test characteristics to identify normo-, micro- and macroalbuminuria with at least one risk factor in randomly selected populations and in the complete “healthy” population.

<table>
<thead>
<tr>
<th>Population</th>
<th>albuminuria</th>
<th>sensitivity</th>
<th>specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>LR+</th>
<th>LR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>random cohort</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=599</td>
<td>Normo-</td>
<td>43</td>
<td>70</td>
<td>24</td>
<td>85</td>
<td>1.4</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Micro-</td>
<td>60</td>
<td>70</td>
<td>11</td>
<td>96</td>
<td>2.0</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Macro-</td>
<td>100</td>
<td>70</td>
<td>2</td>
<td>100</td>
<td>3.3</td>
<td>≈0</td>
</tr>
<tr>
<td>validation cohort</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=592</td>
<td>Normo-</td>
<td>46</td>
<td>65</td>
<td>18</td>
<td>88</td>
<td>1.3</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Micro-</td>
<td>70</td>
<td>65</td>
<td>8</td>
<td>98</td>
<td>2.0</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Macro-</td>
<td>100</td>
<td>65</td>
<td>1</td>
<td>100</td>
<td>2.9</td>
<td>≈0</td>
</tr>
<tr>
<td>complete population</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=1191</td>
<td>Normo-</td>
<td>44</td>
<td>67</td>
<td>21</td>
<td>86</td>
<td>1.3</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Micro-</td>
<td>64</td>
<td>67</td>
<td>9</td>
<td>97</td>
<td>1.9</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Macro-</td>
<td>100</td>
<td>67</td>
<td>1</td>
<td>100</td>
<td>3.0</td>
<td>≈0</td>
</tr>
</tbody>
</table>

PPV: positive predictive value; NPV: negative predictive value; LR+: positive likelihood and LR-: negative likelihood ratio

Because our risk assessment fitted quite well in both cohorts, we applied it in the complete population. The prevalence of normoalbuminuria, microalbuminuria and macroalbuminuria in the complete population, was higher in subjects with at least one risk factor (n=431) than in subjects with no risk factors (n=760), respectively; 18.8 vs 13.4% (n=81 vs 102); 7.4 vs 2.4% (n=32 vs 18) and 1.2 vs 0% (n=5 vs 0), p<0.001 (figure 1).
Figure 1. The prevalence of albuminuria in subjects with at least one vs. none risk factors

![Bar chart showing the prevalence of albuminuria in subjects with at least one vs. none risk factors.](image)

We evaluated two strategies for screening albuminuria in our population, one strategy where a set of additional risk factors were screened as first line, with later screening for albuminuria only in subjects with at least one of those additional risk factors and an alternative strategy where albuminuria was screened as first line, and additional risk factors were measured only in those with albuminuria.

A strategy where only subjects with at least one modifiable risk factor (n=431) were screened for albuminuria, would identify all subjects with macroalbuminuria (5/5), 64% of subjects with microalbuminuria (32/50), but less than half of those with normoalbuminuria (81/183), table 4 shows the corresponding likelihoods and predictive values.

An alternative strategy whereby subjects were first screened for presence of albuminuria, and additional cardiovascular risk factors were only measured in subjects positive for albuminuria (n=238), would identify only 27% (118/431) of the subjects with additional and potentially modifiable cardiovascular risk factors. On the other hand, half of the subjects in this study with albuminuria (120/238, of which 102 had normal range albuminuria), had no additional cardiovascular risk factor at all.
Table 5 shows that subjects with no modifiable risk factor had also lower levels of risk factors which were not included in our screening model.

**Table 5.** Cardiometabolic profile in subjects with none versus one or more risk factors.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>None</th>
<th>≥ 1</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>760</td>
<td>431</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>37.0 ± 9.4</td>
<td>40.6 ± 9.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male gender (%)</td>
<td>604 (79.5)</td>
<td>394 (91.48)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Resting heart rate (bpm)</td>
<td>67.7 ± 8.3</td>
<td>74.9 ± 13.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>121.9 ± 10.4</td>
<td>137.9 ± 14.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>74.4 ± 8.3</td>
<td>84.3 ± 10.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma glucose (mmol/L)</td>
<td>4.6 ± 0.5</td>
<td>5.3 ± 1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>White blood cell count (10*10⁹/L)</td>
<td>6.9 ± 1.9</td>
<td>7.3 ± 1.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index (kg²/m)</td>
<td>25.2 ± 3.6</td>
<td>26.9 ± 4.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Abdominal obesity (%)</td>
<td>72 (10.8)</td>
<td>96 (24.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Obesity &gt;30 kg/m² (%)</td>
<td>71 (9.4)</td>
<td>89 (20.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.9 ± 0.9</td>
<td>5.3 ± 1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum uric acid (µmol/L)</td>
<td>305 ± 70</td>
<td>336 ± 77</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>2.0 ± 3.4</td>
<td>3.0 ± 7.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current smoking</td>
<td>225 (30.7)</td>
<td>145 (34.9)</td>
<td>0.130</td>
</tr>
<tr>
<td>No physical activity</td>
<td>282 (44.3)</td>
<td>209 (57.9)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Discussion

Our data indicate that albuminuria, unknown hypertension and impaired glucose metabolism are quite prevalent findings in an apparently healthy population. When present, albuminuria was mostly measured in the normal range, and was frequently found in subjects without other modifiable risk factors, making its relevance as a predictor of outcome questionable. A screening strategy for albuminuria starting from assessment of simple and easy to obtain risk factors, such as resting heart rate, blood pressure and plasma glucose level identified subjects at risk for micro- and macroalbuminuria in a more effective way than a strategy of screening a healthy population for albuminuria alone.

There is little debate that screening for albuminuria should be performed in patients with diabetes and/or hypertension, where early intervention can slow down deterioration of renal function. Whether it should also be performed in the general population remains ambiguous (12, 18, 19). There is heated debate whether screening the healthy population for presence of albuminuria is fulfilling all conditions requested to define a successful screening program (13).

A first request is that the screened factor either relates to an important health risk, or is prevalent in the population. In our healthy population, the prevalence of albuminuria was 20%. However, many cases had normoalbuminuria without additional risk factors. Those in favor of screening the general population argue that an urinary albumin excretion > 5µg/min is related with increased risk of cardiovascular morbidity and mortality (20-22). In some of these studies, an adjustment for presence of hypertension and diabetes was performed, indicating that albuminuria is an independent risk factor on top of diabetes and hypertension, but it is not clear whether the increased risk was also present in those with normal blood pressure and glucose levels (21). In the PREVEND cohort, hypertension and diabetes were not actually assessed objectively, but were based on “self declared” status (23). In our study, the prevalence of unknown hypertension and impaired glucose levels was high, even after excluding subjects with known risk factors, so most likely also in the PREVEND cohort a substantial part of “false negatives” for hypertension and impaired glucose tolerance are present. Accordingly, the definition of “healthy population” in the PREVEND cohort is probably not correct, making also the recommendation to screen the healthy population incorrect, as most likely a substantial number of subjects in this “healthy cohort” would have hypertension or elevated glucose levels if these would have been measured.
In other studies restricted to non-diabetic and non-hypertensive subjects, although the increased risk for renal disease in those with microalbuminuria seems dramatic, the absolute risk remains low, with less than 0.1% of persons with microalbuminuria ending up on renal replacement therapy, or 0.6% developing cardiovascular disease over an 8 year period (9, 24). In the PREVEND study, the relation between albuminuria and decline of renal function in subjects without known risk factors, was only observed in those individuals with macroalbuminuria (9). As mentioned, in our study, all subjects with macroalbuminuria would be detected if only subjects with more than one risk factor were screened, as none of the subjects without risk factors (the truly healthy population) had macroalbuminuria. In our cohort, the prevalence of microalbuminuria, in subjects without risk factors, was 2.4%. A similar figure was found in a New Zealand study (2.0%) in subjects without diabetes, impaired glucose tolerance, hypertension or dyslipidaemia (25). In the Copenhagen City Heart Study, the prevalence of microalbuminuria was also 2.0% in subjects without any feature of the metabolic syndrome, and these subjects had no increased risk for cardiovascular disease and death, suggesting that microalbuminuria by itself might not be an independent determinant of outcome without presence of associated risk factors (26). It can be that this microalbuminuria is the equivalent of “exertional”(27) or “orthostatic” albuminuria.

Another request for a screening program to be meaningful and effective, is that risk factors should be modifiable. As a prospective trial to test the hypothesis that medical management of microalbuminuria affects patient-centered events independent of blood pressure reduction is still lacking (28), screening for microalbuminuria in subjects without measured additional risk factors, appears not to be justified from a general health care perspective.

In our cohort of apparently healthy subjects, the likelihood of having albuminuria was related to the well established and potentially modifiable risk factors blood pressure and plasma glucose level, but also to resting heart rate. Two thirds of our cohort had none of these risk factors. Table 5 shows that those subjects had also much lower levels of other cardiovascular risk factors not included in our risk score. Nevertheless, 13.4% of these subjects had normoalbuminuria, but none had macroalbuminuria. Most likely, these subjects have thus a very low absolute risk for cardiovascular or renal disease, and the potential benefit of a treatment should be considered very low. Consequently, a strategy of screening only in those with at least one risk factor would miss only few potentially relevant cases, at the same time avoiding many “false positives”, who
compose nearly 50% of albuminuric subjects when unrestricted screening for albuminuria is performed first. However, testing for albuminuria in subjects with additional cardiovascular risk factors is warranted, as a more aggressive treatment can be defended in subjects with additional risk factors in presence of albuminuria compared to those with risk factors but without albuminuria.

There is substantial evidence that reduction of blood pressure, decreases the progression of renal disease and reduces cardiovascular events (29-31). Disturbed glucose metabolism, as indicated by increased plasma glucose levels, is a condition with increased risk for the development of overt diabetes. Life style modification, and the use of drugs such as metformin and acarbose can slow down progression to overt diabetes and/or cardiovascular disease in these subjects (32). The association of resting heart rate with albuminuria was previously mentioned (33, 34). An explanation could be that a high resting heart rate may cause mechanical stress that might contribute to renal endothelial dysfunction leading to albuminuria (35). Previous reports mentioned that tachycardia as a sign of sympathetic overactivity, is an independent risk factor for chronic kidney disease, cardiovascular and noncardiovascular mortality, even in an apparently healthy population (34, 36-38). Reduction of sympathetic overactivity by regular physical activity and smoking cessation appear to be beneficial in this patient group (39). Carvedilol appears to reduce proteinuria to a higher degree than expected by the blood pressure lowering effect alone in patients with hypertension, but it is unclear whether this effect is due to the reduction in heart rate modification or to genuine metabolic effects (40).

A strength of this study is the nearly 100% participation rate of a relatively young and apparently healthy occupational population. A limitation is that we only measured urinary albumin to creatinine ratio at one occasion, guidelines recommend to have at least two positive albumin to creatinine ratio’s in three consecutive first morning urine samples before labeling a person with microalbuminuria(41). Furthermore, the prevalence of microalbuminuria was underestimated if albumin to creatinine ratio was measured in morning urine samples (42), while an overestimation was observed if random samples were obtained (43).Thus, the association between subjects with albuminuria and additional risk factors could be confounded by measuring the urinary albumin to creatinine ratio at only one random occasion.
In conclusion, our data provide evidence to support the concept that screening for albuminuria should only be performed in subjects with additional and potentially modifiable risk factors, and that this strategy is more beneficial than screening the general population. We identified 3 parameters that are easy and cheap to obtain: blood pressure, plasma glucose and resting heart rate to identify subjects in whom further assessment of presence of albuminuria might be relevant.

Acknowledgment We thank all concerned employees of Adhesia (Occupational Heath Care, Ghent, Belgium) and the recruited participants who made this study possible.
CHAPTER 2: Results

References


CHAPTER 2: Results


CHAPTER 2: Results


2.1.2. Microalbuminuria is more consistent in presence of cardiovascular risk factors: Results from the Unreferred Renal Insufficiency Trial

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Available at http://www.ncbi.nlm.nih.gov/pubmed/22865595
Abstract

**Background.** The use of microalbuminuria (MAU) to screen for cardiovascular and renal risk might be hampered by its intermittent character. This prospective observational study assessed traditional risk factors in presumed healthy workers with intermittent MAU (IMAU) compared to persistent MAU (PMAU).

**Methods.** A cohort of 239 Belgian workers underwent at least two consecutive occupational check-ups with a median time of 12 months. Hypertension (HT) was defined as blood pressure ≥ 140/90 mmHg. Impaired glucose tolerance (IGT) was defined as plasma glucose ≥ 5.6 mmol/L. MAU was defined as urinary albumin to creatinine ratio of 17-249 mg/g in men and 25-354 mg/g in women. Workers with IMAU had one positive MAU and workers with PMAU had two positive MAU during follow up.

**Results.** The mean age of this mainly male (95%) cohort was 41± 9 years. The prevalence of persistent risk factors (HT and/or IGT) was higher in workers with PMAU than without MAU (8/12[67%] vs. 55/210[26%], p= 0.005) while workers with IMAU had no increased risk (5/17[29%]). The prevalence of PMAU was higher in workers with vs. without persistent risk factors (8/68= 12% vs. 4/171= 2%, p= 0.005) while the prevalence of IMAU was the same (5/68= 7% vs. 12/171= 7%, p= 0.93). The reproducibility of initial MAU at consecutive visits was higher in workers with vs. without persistent risk factors (8/9[89%] vs.4/11[36%], p= 0.03).

**Conclusions.** The use of MAU as a first step screening strategy in an occupational health care setting is hampered by false positives and low sensitivity to identify subjects with cardiovascular and renal risk. **Trial registration** 2006 NCT00365911
Introduction

There is still debate whether a large scale screening starting from MAU (microalbuminuria) may identify more accurately subjects at risk for cardiovascular and renal events than measuring simple clinical index factors (1-4). Supporters of direct screening for MAU substantiate that the presence of asymptomatic MAU can be measured easily in a simple urine spot whereas opponents substantiate that the use of MAU might be hampered by its intermittent character, leading to false positives (5-7). False positive measurements of MAU are provoked by temporary inflammation, exercise, stature, diet, urinary tract infection, hematuria and medication (8, 9). The HUNT study data revealed that subjects with persistent MAU (PMAU) had an increased mortality whereas subjects with intermittent MAU (IMAU) had no increased risk (10). Nevertheless, many outcome studies including MAU as a risk marker include only single measurements, most likely for practical reasons (11-13). The primary aim of this study is to assess the prevalence of traditional cardiovascular risk factors in workers with IMAU as compared to PMAU to asses whether IMAU or PMAU are related to enhanced cardiovascular risk.
Subjects and methods

The Unreferred Renal Insufficiency study is a non-interventional observational study that included 1486 voluntary Caucasian workers in Belgium (14). Some of these subjects (n= 341) underwent consecutive occupational check-ups between January 2007 and December 2009. Subjects with urinary albumin creatinine ratio (ACR) > 300 mg/g (n=1) and subjects with missing values of interest were excluded, leaving a cohort of 239 “presumed healthy” workers with at least two measurements of ACR, blood pressure and plasma glucose. This small subcohort (n=239) had approximately the same prevalence of hypertension and/or impaired glucose tolerance as the original population (38 vs 39%).

All participants were investigated annually by their occupational physician. At every investigation, body weight, height and waist circumference was measured by trained nurses following WHO recommendations. Body mass index (BMI) was calculated as body weight in kg divided by height$^2$ (kg/m²). Blood pressure and resting heart rate were measured in sitting position by a calibrated electronic device (OMRON®) and a questionnaire about current cigarette smoking, physical activity and prescribed medication was performed by a physician in each participant. In addition, a random blood and urine spot specimen was collected and analyzed on the same day in one laboratory, at every examination, to mimic a routine screening procedure. Urinary albumin was measured by an immune turbid metric method with an inter-assay coefficient of variation of 11.2% at a mean level of 82 mg/L and an inter-assay coefficient of variation of 4.9% at a mean level of 580 mg/L. Serum creatinine was analyzed by a colorimetric assay (compensated Jaffe reaction), calibrated by Isotope Dilution Mass Spectrometry, with an inter-assay coefficient of variation of 1.75% at a mean level of 104 µmol/L. Serum CRP was measured with an immune turbid metric method with an inter-assay coefficient of variation of 4.6% at a mean level of 3.2 mg/L and inter-assay coefficient of variation of 2.5% at a mean level of 5.5 mg/L.

Definitions: MAU was defined as ACR between 17-249 mg/g in men and 25-354 mg/g in women as proposed by Warram et al. (15). Subjects with only one positive MAU were determined as IMAU and those with at least two positive measurements were labeled as PMAU. Hypertension (HT) was defined as BP ≥ 140/90 mmHg. Persistent hypertension (PHT) was defined as at least two measurements were positive. Less than half of the subjects (n=10) treated with
antihypertensive agents (n=26) had PHT. Impaired glucose tolerance (IGT) was defined as plasma glucose ≥ 5.6 mmol/L. Persistent IGT (PIGT) was defined as at least two positive measurements. All subject who were treated for diabetes (n=6) had PIGT. The Modification of Diet in Renal disease (MDRD) equation was used to assess the estimated GFR:

\[
eGFR = 175 \times S_{cr}^{-1.154} \times \text{age}^{-0.203} \times 1.212 \times 0.742 \text{ [if female]} \]

Abdominal adiposity was defined according the National Cholesterol Education Program criteria: waist circumference >102 cm in men and >88 cm in women (17). The Framingham risk score was calculated in every subject (18). Institutional Review Board (IRB)/Ethics Committee approval was obtained at University Hospital Ghent (2006-038). This study was in adherence with the Declaration of Helsinki A written informed consent was obtained from all participants.

Statistical methods: SPSS Statistics 19 was used for all calculations. Results are presented as percentages or as mean ± standard deviation. The baseline characteristics of groups were compared by use of ANOVA: post-hoc Scheffé test (continuous variables), Kruskal-Wallis (continuous variable with a skewed distribution) or a Chi-square test (categorical variables).
Results

We followed 239 workers during annual consecutive clinical occupational check-ups with a median follow up of 12 months (range 8-35 months). Three examinations were performed on 38 subjects. In 220 of the 239 workers (92%) the time between the initial examination and the confirmation test was within 3-13 months and in 20 subjects this time interval was within 14-26 months. The mean age of this mainly male cohort (95%) was 41.1 ± 9.3 years, range 21-61 years. All workers had an estimated GFR higher than 60 ml/min/1.73m². At the first visit, 72 of 239 workers had hypertension (30%), 33 had impaired glucose tolerance (14%), 90 had hypertension and/or impaired glucose tolerance (38%) and 20 had MAU (8%) which was reproducible in 50 (70%), 23 (70%), 68 (76%) and 12 (60%), respectively, at subsequent visits (table 1).

Table 1. Reproducibility of microalbuminuria (MAU), hypertension (HT), impaired glucose tolerance (IGT) and HT and/or IGT at initial testing and during follow up.

<table>
<thead>
<tr>
<th>Initial test (n)</th>
<th>Follow-up (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAU</td>
</tr>
<tr>
<td>MAU</td>
<td>20</td>
</tr>
<tr>
<td>No MAU</td>
<td>219</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>HT</td>
<td>72</td>
</tr>
<tr>
<td>No HT</td>
<td>167</td>
</tr>
<tr>
<td></td>
<td>IGT</td>
</tr>
<tr>
<td>IGT</td>
<td>33</td>
</tr>
<tr>
<td>No IGT</td>
<td>206</td>
</tr>
<tr>
<td></td>
<td>HT and/or IGT</td>
</tr>
<tr>
<td>HT and/or IGT</td>
<td>90</td>
</tr>
<tr>
<td>No risk</td>
<td>149</td>
</tr>
</tbody>
</table>

HT = hypertension; IGT = impaired glucose tolerance; MAU is microalbuminuria. MAU is defined as ACR 17-249 mg/g in men and 25-354 mg/g in women. HT was defined as BP ≥140/90 mmHg. IGT was defined as plasma glucose ≥5.6 mmol/L.
Table 2. Cardiovascular risk factors in subjects with no microalbuminuria (MAU), intermittent MAU (IMAU) and persistent MAU (PMAU).

<table>
<thead>
<tr>
<th></th>
<th>No MAU</th>
<th>IMAU</th>
<th>PMAU</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>210</td>
<td>17 (7)</td>
<td>12 (5)</td>
<td></td>
</tr>
<tr>
<td>Male gender (%)</td>
<td>200 (95)</td>
<td>15 (88)</td>
<td>12 (100)</td>
<td>0.22</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>41± 9</td>
<td>40± 12</td>
<td>45± 11</td>
<td>0.32</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>70± 10</td>
<td>74± 16</td>
<td>72± 12</td>
<td>0.24</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>131± 14</td>
<td>129± 17</td>
<td>147± 30**°°</td>
<td>0.002</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>80± 10</td>
<td>76± 13</td>
<td>91± 16**°°</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.4± 3.6</td>
<td>25.9± 5.5</td>
<td>29.1± 7.6</td>
<td>0.09</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.2± 0.9</td>
<td>5.1± 1.2</td>
<td>5.5± 1.0</td>
<td>0.58</td>
</tr>
<tr>
<td>C-Reactive protein (mg/L)</td>
<td>1.9± 2.4</td>
<td>3.0± 2.8</td>
<td>3.9± 5.8</td>
<td>0.03</td>
</tr>
<tr>
<td>WBC count (10⁹/L)</td>
<td>7.0± 2.0</td>
<td>7.9± 1.5</td>
<td>7.4± 1.9</td>
<td>0.18</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.0± 1.4</td>
<td>5.1± 1.2</td>
<td>6.7± 4.4*</td>
<td>0.05</td>
</tr>
<tr>
<td>PHT and/or PIGT (%)</td>
<td>55 (26)</td>
<td>5 (29)</td>
<td>8 (67)*°</td>
<td>0.01</td>
</tr>
<tr>
<td>Adiposity (%)</td>
<td>37 (18)</td>
<td>3 (18)</td>
<td>4 (36)</td>
<td>0.32</td>
</tr>
<tr>
<td>Framingham score (%)</td>
<td>7± 5</td>
<td>7± 5</td>
<td>13± 11**°°</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>68 (32)</td>
<td>6 (35)</td>
<td>4 (33)</td>
<td>0.97</td>
</tr>
<tr>
<td>No physical activity (%)</td>
<td>81 (39)</td>
<td>6 (37)</td>
<td>9 (75)*</td>
<td>0.05</td>
</tr>
</tbody>
</table>

MAU is defined as ACR 17-249 mg/g in men and 25-354 mg/g in women, SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index, WBC: white blood cell count, PHT: persistent hypertension was defined as BP ≥140/90 mmHg in at least two occasions, PIGT was defined as plasma glucose ≥5.6 mmol/L in at least two occasions, adiposity defined by waist circumference >88 cm in women and >102 cm in men.

* p < 0.05, **p<0.001 vs no albuminuria, °p<0.05, °°p< 0.005 vs IMAU

Approximately one quarter (n=68) of the 239 workers had persistent hypertension and/or impaired glucose tolerance and only 5% of the workers (n=12) had MAU confirmed during follow up.

Workers with PMAU had higher mean blood pressure, mean plasma glucose, Framingham risk score and higher frequency of inactivity than workers without MAU, while workers with IMAU had no increased cardiovascular risk (table 2).

The prevalence of persistent HT and/or IGT was higher in workers with PMAU (8/12 [67%]) than in those with IMAU (5/17 [29%], p= 0.05) or without MAU (55/210 [26%], p= 0.005, table...
2). The prevalence of PMAU was higher in workers with risk factors (HT and/or IGT) than in workers without persistent risk factors (8/68 = 12% vs. 4/171 = 2%, \( p = 0.005 \)) while the prevalence of IMAU was the same in workers with risk factors (HT and/or IGT) vs. those without persistent risk factors (5/68 = 7% vs. 12/171 = 7%, \( p = 0.93 \)). Workers with risk factors (HT and/or IGT) had a higher reproducibility of initial MAU than workers without persistent risk factors (8/9 [89%] vs. 4/11 [36%] \( p = 0.03 \), table 3).

**Table 3.** Reproducibility of microalbuminuria (MAU) at initial testing and during follow-up in workers with and without persistent hypertension (PHT) and/or persistent impaired glucose tolerance (PIGT).

<table>
<thead>
<tr>
<th></th>
<th>Initial test (n)</th>
<th>Follow-up (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAU</td>
<td>No MAU</td>
</tr>
<tr>
<td>With PHT and/or PIGT (n=68)</td>
<td>9</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>MAU</td>
<td>No MAU</td>
</tr>
<tr>
<td>Without PHT and/or PIGT (n=171)</td>
<td>MAU</td>
<td>No MAU</td>
</tr>
</tbody>
</table>

MAU is defined as ACR 17-249 mg/g in men and 25-354 mg/g in women, PHT: persistent hypertension was defined as BP \( \geq 140/90 \) mmHg in at least two occasions; PIGT was defined as plasma glucose \( \geq 5.6 \) mmol/L in at least two occasions.
Discussion

Our data indicate that in subjects with initial MAU, the presence of MAU should be confirmed, to establish it as a renal and cardiovascular risk marker in an occupational screening programme. Screening for modifiable risk factors, such as blood pressure and plasma glucose, will identify proportionally more subjects who could benefit from available therapies than screening for MAU (14). We now add that individuals with intermittent MAU have approximately the same percentage of cardiovascular risk factors than subjects without MAU, and that subjects with persistent MAU have higher cardiovascular risk (19). As a consequence, screening for cardiovascular and/or renal risk in an occupational population should commence from traditional risk factors rather than from MAU as a first step. In addition, this study suggests that MAU is more consistent in presence of cardiovascular risk factors; thus, screening for MAU in subjects with persistent risk factors might be more effective than screening a presumed healthy population for MAU. Of note, subjects with cardiovascular risk factors are more likely to be screened for MAU as a surrogate marker for cardiovascular and renal outcome (20, 21).

According to our data, subjects with intermittent MAU were not associated with increased established cardiovascular risk factors and thus should be considered as “false positives” in a screening programme that uses MAU as first step to identify subjects with cardiovascular and renal risk. Besides the low reproducibility of MAU in our population, the use of MAU was further hampered by a low sensitivity to detect traditional risk factors. Only 13% (9/68) of the cases with persistent HT and/or IGT were identified when MAU was used as a first step to screen for cardiovascular and renal risk (table 3). The PREVEND trial also observed a low sensitivity of 12% to detect at least one traditional risk factor but the authors argued that the 10 years cardiovascular disease incidence in hypertensive subjects in absence of MAU is sufficiently low so as not to require treatment (22). However this claim is inconsistent with PREVEND data that revealed that half of the subjects on renal replacement therapy and 77% of cardiovascular deaths, had no MAU when they were screened (23, 24). Moreover, medical management in diabetes and/or hypertension even without influencing MAU can slow down the clinical course of progressive CKD, end stage renal disease, cardiovascular morbidity and mortality, whereas it is unproven whether making MAU as such disappear without influencing other risk factors would have a benefit on outcomes (25-27). When a screening program to detect cardiovascular and renal risk starts from MAU another question also arises: how to handle subjects with MAU in the
absence of cardiovascular risk factors. According to our data, more than half of the subjects (n=11) with initial MAU had no persistent cardiovascular risk factors. These subjects could be considered as having masked or premature cardiovascular risk; however, MAU was only confirmed in four subjects (36%). Some prospective trials showed that such subjects have a higher risk of developing de novo hypertension and diabetes (11, 12, 28). However, it is unclear whether preventive steps focusing on MAU would be effective to postpone this risk. More likely, there is a high probability that such subjects would pay a price, that is, the side effects of life-long medication and medical intimidation (1, 7).

Unfortunately, this small sample of young, white and mainly male subjects who were screened in a working environment, is not entirely representative of the global population. However, as the association between cardiovascular risk factors and MAU is weaker in women, the reproducibility of MAU would have been even lower if a similar percentage of women and men had been recruited (29, 30). Another limitation is that we could not exclude exercise-induced MAU in this cohort as MAU was measured during working time. MAU might fluctuate more in random urine samples than in early morning urine samples. Participants with only one positive MAU in at least two examinations, were considered as false positive. Indeed this study confirmed that at least two consecutive MAU measurements are necessary to identify subjects with an increased risk for cardiovascular and renal disease.

In conclusion, a first line screening for MAU in an occupational health care setting as a whole as an index for renal and cardiovascular risk appears inefficient because of low reproducibility of MAU and low sensitivity to detect traditional cardiovascular risk factors. Screening for MAU in subjects with modifiable risk factors might be more effective, because MAU seems to be more consistent in these subjects than in those without risk factors.

Acknowledgments

We thank all concerned employees of Adhesia (Occupational Health Care, Ghent, Belgium) and the recruited participants who made this study possible.
CHAPTER 2: Results

References


2.1.3. Should screening of renal markers be recommended in a working population?

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Abstract

Introduction. It is debated whether the general population should be screened for kidney disease. This study evaluated whether screening of albuminuria and estimated glomerular filtration rate (eGFR) in a working population should be recommended to detect subjects with CKD.

Methods. The Unreferred Renal Insufficiency (URI) study is a cross-sectional study in 1,398 workers aged 17-65. Markers of cardiovascular and renal disease were measured. Cardiovascular risk (CVR) was defined by hypertension (n= 416), diabetes (n= 45), dyslipidemia (n= 159) and/or history of a cardiovascular event (n= 10).

Results. In our population, 5% of the workers had microalbuminuria, 0.5% had macroalbuminuria and < 0.1% had eGFR < 60 ml/min/1.73m². All workers with an eGFR < 60 ml/min/1.73m² and/or macroalbuminuria (8/8) had at least one cardiovascular risk factor whereas this was the case in only half of workers with microalbuminuria (36/73, p= 0.007). In workers without cardiovascular risk factors, the presence of microalbuminuria was associated with low body mass index (BMI, p < 0.001) or physiochemical exposure risk (p < 0.001).

Conclusions. Screening of renal markers in a working population, identified only a few subjects with an eGFR < 60 ml/min/1.73m² or macroalbuminuria. Although microalbuminuria was more prevalent, it might not necessarily indicate kidney disease, as it may have a completely different meanings depending of the phenotype of the screened subjects. Besides underlying cardiovascular risk factors, microalbuminuria was also associated with low BMI in absence of any risk factor, suggesting presence of benign postural proteinuria. In addition, microalbuminuria also seemed to be related to physicochemical exposure. In view of the impossibility to further analyse this finding in the present study, the meaning of this observation needs to be further investigated.
**Introduction**

Chronic kidney disease (CKD) is increasingly considered as a prevalent and important condition (1). Subjects with CKD are identified by presence urinary albumin creatinine ratio (ACR) $> 30$ mg/g and/or an estimated glomerular filtration rate (GFR) $< 60$ ml per minute per $1.73m^2$ of body surface area (2). Several epidemiologic studies suggested that CKD is an independent risk factor for acute kidney injury, need for renal replacement therapy, cardiovascular events, all cause mortality and increased health care costs (3-7). However, the thesis that screening for CKD would improve clinical outcome is not established by interventional studies, and is thus still a matter of debate (8-10). A systematic review on the effect of screening programs in the general population, demonstrated that such programs detect large numbers of subjects with an eGFR $< 60$ ml/min/1.73m² and ACR $> 30$ mg/g, but that these subjects have a lower relative risk for progressive kidney disease than patients in a clinical setting (11). Hence, there is lot of debate whether the general population should be screened for CKD, even if it is considered as an important prevalent health problem (8, 9, 11). Misclassification of CKD frequently occurs in subjects with microalbuminuria, as a consequence of the high intra-individual variability of albuminuria (8). Moreover, in many subjects microalbuminuria tends to regress to normal levels, especially nowadays more and more patients are treated for their underlying risk factors (12). The current study investigated whether screening of renal markers should be recommended in a working population to detect CKD.
Methods

Aims: This study was performed to investigate whether screening for renal markers, such as microalbuminuria or macroalbuminuria and eGFR < 60 ml/min/1.73m², is useful to detect CKD in an unselected working population.

Participants: The Unreferred Renal Insufficiency (URI) study is a cross sectional evaluation including only Caucasian workers (n=1486) who presented at a routine annual occupational check-up between January 2007 and December 2009, in Belgium. The presence of more than 100 erythrocytes/µl in the urinary sediment was considered as a confounder for reliable measurements of urinary albumin; subjects showing this degree of hematuria (n=17) and subjects with incomplete data (n=71) were excluded from further analysis, leaving a cohort of 1,398 subjects. In our study, 351 (25%) workers were exposed to physicochemical hazard, such as silica, heavy metals, solvents.

Ethics: A written informed consent was obtained from all participants. The Ethics Committee of University Hospital Ghent approved the study (2006-038) (13).

Description of procedures

All subjects were investigated during their yearly check up by their occupational physician. Body weight was recorded to the nearest 0.1 kg and height was measured to the nearest centimeter. Waist circumference was measured by trained nurses following recommendations by WHO. Body mass index (BMI) was calculated as body weight in kg divided by height² (kg/m²). Blood pressure and resting heart rate were measured in sitting position by a calibrated electronic device (OMRON®). A questionnaire about current cigarette smoking, physical activity and prescribed medication was taken by an occupational physician in each participant. A random blood and urine spot specimen was collected and analyzed on the same day in one central laboratory (no frozen samples). Urinary albumin was measured by an immune turbidimetric method with an inter-assay coefficient of variation of 11.2% at a mean level of 82 mg/L and an inter-assay coefficient of variation of 4.9% at a mean level of 580 mg/l. Serum creatinine was analyzed by a colorimetric assay (compensated Jaffe reaction), calibrated by Isotope Dilution Mass Spectrometry (IDMS), with an inter-assay coefficient of variation of 1.75% at a mean level of 104 µmol/l (Roche). Serum CRP was measured with an immune turbidimetric method with an
inter-assay coefficient of variation of 4.6% at a mean level of 3.2 mg/l and inter-assay coefficient of variation of 2.5% at a mean level of 5.5 mg/l (13).

Definitions: Gender specific urinary albumin creatinine ratio (ACR) cut-off values as proposed by Warram et al. were used. Microalbuminuria was defined as ACR 17-250 in men and ACR 25-355 mg/g in women, macroalbuminuria as ACR > 250 in men and ACR > 355 mg/g in women (14). GFR was estimated by the combined creatinine-cystatin C based CKD-EPI equation (15). Abdominal adiposity was defined according to the National Cholesterol Education Program (NCEP) criteria as a waist circumference > 102 cm in men and > 88 cm in women (16). Hypertension was defined as blood pressure ≥140/90 or anti-hypertensive treatment. Diabetes was defined as plasma glucose ≥ 126 mg/dl or glucose lowering treatment (17). Dyslipidemia was defined as serum cholesterol ≥ 250 mg/dl or lipid lowering treatment.

Statistical methods: SPSS statistics 22 was used for all calculations. Results are presented as percentages or as mean ± standard deviation. The baseline characteristics of more than 2 groups were compared by use of ANOVA: post-hoc Scheffé test (continuous variables), Kruskal-Wallis (continuous variable with a skewed distribution). The baseline characteristics of two groups were compared by the use of t-test for continuous variables, Mann-Whitney for continuous variables with a skewed distribution and Chi-square test for categorical variables. Multivariate logistic regression analysis was performed for the association of microalbuminuria with other parameters. Subjects were classified by < 10th, 10-50th, 50th-90th and >90th percentile of BMI in presence of physicochemical exposure risk (PCER), CVR or no risk factor (table 3, figure 1). Subjects were classified according to median of age (17-39, 40-65 years) (table 4).
Results

In this population of 1,398 workers we found 416 subjects (30%) with hypertension (119 treated), 45 (3%) with diabetes (15 treated), 159 (11%) with dyslipidemia (44 treated) and 10 (0.7%) with a history of cardiovascular event. Since sometimes some of these risk factors coincided in the same patient, the total number of subjects with one or more cardiovascular risk factors (CVR) was 512 (37%), of whom only 151 were treated for at least one risk factor, resulting in 361 (26%) of this population having at least one untreated risk factor. In this cohort, 5% (n= 73) had microalbuminuria, 0.5% (n= 7) had macroalbuminuria and 0.1% (n= 1) had eGFR< 60 ml per minute per 1.73m² of body surface area in presence of microalbuminuria. Workers with vs. without cardiovascular risk factors had higher prevalence of microalbuminuria (7.0 vs. 4.2%, p = 0.001) and macroalbuminuria (1.4 vs. 0, p = 0.001). The worker with an eGFR < 60 ml/min/1.73m² and all workers with macroalbuminuria (8/8) had at least one cardiovascular risk factor whereas this was the case in only half of workers with microalbuminuria (36/73, p= 0.007). Only, 15 of these 36 workers with microalbuminuria (42%) and one of 7 (14%) workers with macroalbuminuria were treated for diabetes, hypertension and/or dyslipidemia.

In the overall cohort, workers with versus without physicochemical exposure risk (PCER) had a higher prevalence of microalbuminuria (7.4 vs. 4.6%, p = 0.04), but not a higher percentage of CVR (36 vs. 37%, p = 0.61). As cardiovascular risk factors confound the association between microalbuminuria and PCER, we split our cohort in workers with and without CVR. As expected, the workers with CVR had more additional risk factors than those without CVR (table 1). In the population with CVR, workers with vs. without PCER were only more likely to be male. In the population without CVR, workers with PCER (n= 226) had a higher prevalence of microalbuminuria (p <0.001), were younger (p < 0.001) and were more likely to be male (p< 0.001) than those without any risk (n = 660) (table1).
Table 1. Characteristics according to without vs. with physicochemical exposure risk (PCER) in workers without vs. with cardiovascular risk (CVR).

<table>
<thead>
<tr>
<th></th>
<th>No CVR (n=886)</th>
<th>CVR (n=512)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No PCER</td>
<td>PCER</td>
<td>No PCER</td>
</tr>
<tr>
<td>N</td>
<td>660</td>
<td>226</td>
<td>387</td>
</tr>
<tr>
<td>Age (years)</td>
<td>38 ± 10</td>
<td>36 ± 9°</td>
<td>44 ± 10</td>
</tr>
<tr>
<td>Female (%)</td>
<td>183 (28)</td>
<td>12 (5)°</td>
<td>57 (15)</td>
</tr>
<tr>
<td>ACR (mg/g)</td>
<td>4 (3–5)</td>
<td>5 (3–6)</td>
<td>13 (4–22)</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>18 (2.7)</td>
<td>19 (8.4)°</td>
<td>29 (7.5)</td>
</tr>
<tr>
<td>Macrolalbuminuria</td>
<td>0</td>
<td>0</td>
<td>5 (1.3)</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>199 (31)</td>
<td>73 (36)</td>
<td>118 (31)</td>
</tr>
<tr>
<td>Adiposity (%)</td>
<td>69 (13)</td>
<td>18 (8)</td>
<td>115 (33)</td>
</tr>
<tr>
<td>Inactive (%)</td>
<td>245 (44)</td>
<td>98 (51)</td>
<td>192 (59)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.1 ± 3.7</td>
<td>25.2 ± 3.2</td>
<td>27.9 ± 4.72</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>122 ± 11</td>
<td>123 ± 10</td>
<td>139 ± 16</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>74 ± 9</td>
<td>75 ± 8</td>
<td>86 ± 11</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>69 ± 9</td>
<td>68 ± 10</td>
<td>73 ± 12</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>85 ± 12</td>
<td>85 ± 12</td>
<td>94 ± 27</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>186 ± 31</td>
<td>186 ± 32</td>
<td>216 ± 45</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>2.2 ± 4.0</td>
<td>2.2 ± 4.2</td>
<td>2.9 ± 7.0</td>
</tr>
</tbody>
</table>

CVR: cardiovascular risk defined by hypertension, diabetes, dyslipidemia and/or cardiovascular event.
ACR (mg/g): urinary albumin creatinine ratio (95% CI of the mean) Microalbuminuria (MAU) is defined as UACR 17-250 in men and 25-355 mg/g in women. Adiposity: waist circumference > 102 cm in men and > 88 cm in women. BMI: body mass index. SBP: systolic blood pressure. DBP: diastolic blood pressure, CRP: C-reactive protein.
°p < 0.01 PCER vs. no PCER.
### Table 2. Characteristics according to absence or presence of microalbuminuria (MAU) in workers without vs. with physicochemical exposure risk (PCER) in absence vs. presence of cardiovascular risk (CVR)

<table>
<thead>
<tr>
<th></th>
<th>No CVR (n=886)</th>
<th>No PCER (n=660)</th>
<th>PCER (n=226)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No MAU</td>
<td>MAU</td>
<td>P</td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>642</td>
<td>18 (3)</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>38 ± 9</td>
<td>37 ± 11</td>
<td>0.59</td>
</tr>
<tr>
<td><strong>Female (%)</strong></td>
<td>178 (28)</td>
<td>5 (28)</td>
<td>0.40</td>
</tr>
<tr>
<td><strong>Smoking (%)</strong></td>
<td>193 (31)</td>
<td>6 (33)</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Adiposity (%)</strong></td>
<td>68 (13)</td>
<td>1 (8)</td>
<td>0.60</td>
</tr>
<tr>
<td><strong>Inactive (%)</strong></td>
<td>236 (44)</td>
<td>9 (53)</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>Low BMI &lt;10th per.</strong></td>
<td>62 (10)</td>
<td>10 (56)</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>25.2 ± 3.7</td>
<td>21.8 ± 3.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>122 ± 11</td>
<td>120 ± 12</td>
<td>0.53</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>74 ± 9</td>
<td>70 ± 11</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Heart rate (bpm)</strong></td>
<td>69 ± 9</td>
<td>71 ± 15</td>
<td>0.57</td>
</tr>
<tr>
<td><strong>Glucose (mg/dl)</strong></td>
<td>85 ± 12</td>
<td>87 ± 11</td>
<td>0.40</td>
</tr>
<tr>
<td><strong>Cholesterol (mg/dl)</strong></td>
<td>186 ± 31</td>
<td>177 ± 31</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>CRP (mg/l)</strong></td>
<td>2.2 ± 3.4</td>
<td>4.4 ± 1.4</td>
<td>0.48</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>CVR (n =505)</th>
<th>No PCER (n=382)</th>
<th>PCER (n=123)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No MAU</td>
<td>MAU</td>
<td>P</td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>353</td>
<td>29 (7)</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>44 ± 10</td>
<td>44 ± 11</td>
<td>0.88</td>
</tr>
<tr>
<td><strong>Female (%)</strong></td>
<td>52 (15)</td>
<td>4 (14)</td>
<td>0.58</td>
</tr>
<tr>
<td><strong>Smoking (%)</strong></td>
<td>102 (30)</td>
<td>12(43)</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Adiposity (%)</strong></td>
<td>97 (31)</td>
<td>15 (60)</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Inactive (%)</strong></td>
<td>170 (59)</td>
<td>15(60)</td>
<td>0.926</td>
</tr>
<tr>
<td><strong>Low BMI &lt;10th per.</strong></td>
<td>30 (9)</td>
<td>1 (4)</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>High BMI &gt;90th per.</strong></td>
<td>30 (9)</td>
<td>6 (21)</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>27.7 ± 4.6</td>
<td>30.0 ± 5.3</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>138 ± 16</td>
<td>146 ± 19</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>85 ± 11</td>
<td>90 ± 11</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Heart rate (bpm)</strong></td>
<td>72 ± 11</td>
<td>81 ± 14</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Glucose (mg/dl)</strong></td>
<td>93 ± 21</td>
<td>110 ± 64</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>Cholesterol (mg/dl)</strong></td>
<td>215 ± 45</td>
<td>216 ± 40</td>
<td>0.89</td>
</tr>
<tr>
<td><strong>CRP (mg/l)</strong></td>
<td>2.8 ± 6.6</td>
<td>6.2 ± 11.7</td>
<td>0.02</td>
</tr>
</tbody>
</table>

CVR: cardiovascular risk defined by hypertension, diabetes, dyslipidemia and/or cardiovascular events. Microalbuminuria (MAU) is defined as UACR 17-250 in men and 25-355 mg/g in women. Adiposity: waist circumference >102 in men and >88 cm in women. BMI: body mass index. SBP: systolic blood pressure. DBP: diastolic blood pressure, CRP: C-reactive protein.
In the population without CVR, workers with microalbuminuria had a higher percentage of low BMI (< 10th percentile of BMI; 38 vs. 12%, \( p < 0.001 \)) and PCER (51 vs. 25%, \( p < 0.001 \)) as compared to those without microalbuminuria. In the population without CVR in presence of PCER (table 2), workers with microalbuminuria had a higher heart rate \( (p= 0.04) \), lower BMI \( (p=0.04) \) but no higher percentage of low BMI \( (21 \text{ vs. } 9\%, \ p = 0.18) \) as compared to those without microalbuminuria. In the population without CVR and low BMI (table 3), workers with microalbuminuria had no higher percentage of PCER than those without microalbuminuria \( (29 \text{ vs. } 23\%, \ p = 0.62) \). In the population without any risk (table 2), workers with microalbuminuria had a higher percentage of low BMI \( (56 \text{ vs. } 10\%, \ p< 0.001) \) as compared to those without microalbuminuria. After a multivariate adjustment, high heart rate \( (p = 0.02) \) was retained as the only significant predictor for microalbuminuria in workers with PCER, whereas low BMI \( (p < 0.001) \) was retained as the only significant predictor for microalbuminuria in workers without any risk.

In the population with CVR (table 2), workers with microalbuminuria had a higher blood pressure, heart rate and CRP as compared to those without microalbuminuria, independent of PCER. After multivariate adjustment, high heart rate \( (p = 0.02) \) and CRP \( (p = 0.02) \) were retained as significant predictors for microalbuminuria in workers without PCER, whereas older age was retained as a significant predictor for microalbuminuria in workers with PCER.

Figure 1 shows the prevalence of microalbuminuria in lean \( (< 10\text{th} \text{ percentile of BMI}) \), normal \( (\text{between } 10-50\text{th} \text{ percentile of BMI}) \), overweight \( (\text{between } 50-90\text{th} \text{ percentile of BMI}) \) and obese workers \( (> 90\text{th} \text{ percentile of BMI}) \) according to no risk, PCER and CVR. In presence of CVR, overweight or obese workers had a higher prevalence of microalbuminuria than normal or lean workers \( (p < 0.05) \), whereas in absence of risk factors lean workers had a higher prevalence of microalbuminuria than workers with BMI > 10th percentile \( (p < 0.001, \text{table 3}) \). In the population without CVR, microalbuminuria was not affected by different BMI groups in workers with PCER (table 3).
**Table 3.** Prevalence of microalbuminuria (MAU) according to percentile of BMI in workers with no risk, with physicochemical exposure risk (PCER) and cardiovascular risk (CVR).

<table>
<thead>
<tr>
<th></th>
<th>BMI &lt; 10\textsuperscript{th} percentile</th>
<th>BMI 10-50\textsuperscript{th} percentile</th>
<th>BMI 50-90\textsuperscript{th} percentile</th>
<th>BMI &gt; 90\textsuperscript{th} percentile</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No risk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=658)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>72</td>
<td>284</td>
<td>235</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>34 ± 9</td>
<td>37 ± 10\textsuperscript{*}</td>
<td>40 ± 9\textsuperscript{**\circ}</td>
<td>38 ± 10\textsuperscript{*}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MAU</td>
<td>10 (13.9)</td>
<td>5 (1.8)\textsuperscript{**}</td>
<td>3 (1.3)\textsuperscript{**}</td>
<td>0\textsuperscript{**}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>PCER</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n= 226)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>22</td>
<td>86</td>
<td>104</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>30 ± 7</td>
<td>35 ± 9</td>
<td>38 ± 9\textsuperscript{*}</td>
<td>33 ± 9</td>
<td>0.002</td>
</tr>
<tr>
<td>MAU</td>
<td>4 (18.2)</td>
<td>9 (10.5)</td>
<td>5 (4.8)</td>
<td>1 (7.1)</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>CVR</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=503)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>40</td>
<td>234</td>
<td>179</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>38 ± 12</td>
<td>43 ± 10</td>
<td>45 ± 9\textsuperscript{*}</td>
<td>46 ± 9\textsuperscript{*}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MAU</td>
<td>2 (5)</td>
<td>10 (4.3)</td>
<td>16 (9)\textsuperscript{\circ}</td>
<td>7 (14)\textsuperscript{\circ}</td>
<td>0.01</td>
</tr>
</tbody>
</table>

CVR is defined by hypertension, diabetes, dyslipidemia and/or cardiovascular event. Microalbuminuria is defined as UACR 17-250 in men and 25-355 mg/g in women. BMI = body mass index (kg/m\textsuperscript{2}). *p<0.05, **p<0.001 vs. BMI ≤ 10\textsuperscript{th} percentile, °p = 0.01-0.05 vs. BMI 10-50\textsuperscript{th} percentile.
**Figure 1.** Prevalence of albuminuria in lean (BMI <10\textsuperscript{th} percentile), normal (BMI between 10-50\textsuperscript{th} percentile), overweight (BMI between 50-90\textsuperscript{th} percentile) and obese workers (BMI > 90\textsuperscript{th} percentile) in absence of risk factors, in presence of physicochemical exposure risk (PCER) and presence of cardiovascular risk factors (CVR).

Microalbuminuria is defined as UACR17-250 in men and 25-355 mg/g in women. CVR risk is defined by hypertension, diabetes, dyslipidemia and/or cardiovascular event. BMI (body mass index).
The prevalence of CVR increased above the age of 40, from 24 to 49% (p < 0.001). We showed that age is an effect modifier; the presence of microalbuminuria was associated with CVR and PCER in workers above the age of 40 while it was associated with low BMI and PCER in workers under the age of 40 years (table 4).

Table 4. Mean BMI (body mass index), the number of workers with cardiovascular risk factors (CVR) and of physicochemical exposure risk (PCER) according to median of age in absence or presence of microalbuminuria (MAU).

<table>
<thead>
<tr>
<th>Age</th>
<th>No MAU</th>
<th>MAU</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n</td>
<td>BMI</td>
</tr>
<tr>
<td>17-39</td>
<td>682</td>
<td>649</td>
<td>25.3 ± 3.9</td>
</tr>
<tr>
<td>40-65</td>
<td>705</td>
<td>666</td>
<td>26.9 ± 4.2</td>
</tr>
<tr>
<td>CVR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCER</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-39</td>
<td>511</td>
<td>487</td>
<td>134 (28)</td>
</tr>
<tr>
<td>40-65</td>
<td>358</td>
<td>345</td>
<td>73 (21)</td>
</tr>
</tbody>
</table>

CVR: cardiovascular risk defined by hypertension, diabetes, dyslipidemia and/or cardiovascular events. Microalbuminuria (MAU) is defined as UACR 17-250 in men and 25-355 mg/g in women. BMI: body mass index in kg/m².
Discussion

Screening for renal markers in a working population containing 1,398 workers, identified only one subject with an eGFR < 60 ml/min/1.73m² (n= 1) and a few subjects with macroalbuminuria (n= 7), all of whom had at least one traditional cardiovascular risk factor. More workers with microalbuminuria were detected, but half of these had no modifiable traditional cardiovascular risk factor (37/73). Microalbuminuria in these subjects was associated with low BMI or PCER. Young workers had a lower BMI in presence vs. absence of microalbuminuria. Conceivably, a substantial number of young workers had postural proteinuria and might be mislabeled as having a risk for cardiovascular and/or renal disease. Whether the presence of microalbuminuria in workers with PCER is related to early reversible kidney disease might be considered as potentially possible but remains to be proven. On the other hand, only a limited number of patients with traditional cardiac risk factors did already receive treatment for that risk factor. Screening for renal markers in a healthy population might thus identify patients without modifiable risk factors, whereas screening for and treating traditional cardiac risk factors in this group might be more appropriate (13).

The presence of microalbuminuria in the general population is independently considered as a renal and cardiovascular risk marker (18, 19). However, whether this simple marker can be easily used to start uniform treatment to improve renal and cardiovascular outcome remains uncertain (10, 20). Our data showed that the presence of microalbuminuria has completely different meanings according to the population concerned. Microalbuminuria is associated with CVR or PCER in workers above the age of 40, while it associated with low BMI or PCER in workers under the age of 40. Consequently, a substantial number of young, healthy and lean workers with microalbuminuria might have postural proteinuria. It has been suggested that postural albuminuria commonly occurs in healthy lean adolescents (21, 22). Microalbuminuria might develop in normal subjects by a physiological response after strenuous exercise or upon standing (23, 24). Postural albuminuria might be a sign of renal congestion (25), due to the compression of the left renal vein between the superior mesenteric artery and the abdominal aorta (nutcracker syndrome) (26). Lean subjects are more likely to develop postural proteinuria as the left renal vein is at higher risk to be compressed in the space between the aorta and the superior mesenterial artery which is smaller than on the right side (27). Adolescents with postural proteinuria have an
excellent outcome (28). Mostly the problem disappears beyond the age of 30, pointing to an irrelevant condition, rather than a risk factor (29).

Although it might be useful to know the potentially toxic conditions in a working population, in our database we had not registered the type of work nor measured toxic agents. However, we classified our patients into a subgroup with and without PCER. According to the literature, subjects who are exposed to heavy metals, such as cadmium, mercury might develop tubular overflow microalbuminuria (30-32). But, whether this microalbuminuria is an indicator for kidney disease remains unknown. In our study, no worker with PCER had macroalbuminuria. Thus, it seems unlikely that the presence of microalbuminuria in workers with PCER might contribute to the development of progressive CKD. However, microalbuminuria was associated with high heart rate in workers with PCER. Whether this might be related to CKD or CVD is unknown. This finding underlines the need of prospective studies of the long-term effects of toxic agents on markers of outcome in CKD and CVD.

One third of the workers had one or more traditional cardiovascular risk factor. Most of these were however untreated. In terms of prevention and cost-effectiveness, it would thus be more effective to screen for and treat traditional cardiovascular risk factors, rather than screening of renal markers. However, as the prevalence of renal markers was much higher in those with traditional cardiovascular risk factors, this patient group should be explored for presence of chronic kidney disease, of course one of the problems is that most of these risk factors are undetected or at least untreated. In this case the screening physician may remain unaware of the patient’s condition, and not recognize the direct reason to do the appropriate screening test for CKD. Therefore, traditional risk factors as fasting hyperglycemia and hypertension may be seen as important part of basic screening that is often neglected.

A strength of this study is that we not only used a “self-declared” status of diabetes, hypertension and dyslipidemia, but that we also measured plasma glucose, blood pressure and cholesterol, to identify subjects with unknown traditional risk factors. A limitation is that all parameters in this cohort were measured at one random occasion. Microalbuminuria might fluctuate more in random urine samples than in early morning urine samples (33, 34).

We conclude that direct screening of renal markers is not useful in a working population, as half of the subjects with microalbuminuria had no modifiable cardiovascular risk factors. The
presence of microalbuminuria in these workers might not be indicative for progressive CKD as none of them had macroalbuminuria. The presence of microalbuminuria in these mainly young workers was associated with low BMI or PCER. Consequently, young, healthy and lean workers with microalbuminuria might have postural proteinuria and thus an irrelevant condition, rather than that it would display a risk factor. The occurrence of microalbuminuria in workers with PCER was unrelated to CVR and should be further investigated. Most likely intervention in this population may engender medical frustration, unnecessary anxiety, harm, and worries in those affected and excess of costs to society, employers and individuals. To reduce these false positive results, a mass screening strategy to identify cardiovascular disease by using microalbuminuria seems to make sense only when limiting inclusion to subjects above the age of 40. However, screening for traditional cardiovascular risk factors identifies more subjects at risk for cardiovascular and renal disease than screening for microalbuminuria for potential preventive intervention (13).

**Acknowledgment** We thank all concerned employees of Adhesia (Occupational Heath Care, Ghent, Belgium) and the recruited participants who made this study possible.
References


2.2. Screening for kidney disease in selected hypertensive subjects: results from the ERICABEL trial: a non interventional epidemiological cohort study.
2.2.1. Statin use and the presence of microalbuminuria.

Arjan van der Tol¹, Wim Van Biesen¹, Steven Van Laecke¹, Kris Bogaerts², Koen De Lombaert³, Hans Warrinnier³ and Raymond Vanholder¹.

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² Interuniversity centre for biostatistics and statistical bioinformatics, Leuven, Belgium.
³ F. Hoffman-La Roche LTD, Brussels, Belgium.

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Abstract

**Background.** Microalbuminuria (MAU) is considered as a predictor or marker of cardiovascular and renal events. Statins are widely prescribed to reduce cardiovascular risk and to slow down progression of kidney disease. But statins may also generate tubular MAU. The current observational study evaluated the impact of statin use on the interpretation of MAU as a predictor or marker of cardiovascular or renal disease.

**Methodology/Principal Findings.** We used cross-sectional data of ERICABEL, a cohort with 1,076 hypertensive patients. MAU was defined as albuminuria ≥ 20mg/l. A propensity score was created to correct for “bias by indication” to receive a statin. As expected, subjects using statins vs. no statins had more cardiovascular risk factors, pointing to bias by indication. Statin users were more likely to have MAU (OR: 2.01, 95% CI: 1.34-3.01). The association between statin use and MAU remained significant after adjusting for the propensity to receive a statin based on cardiovascular risk factors (OR: 1.82, 95% CI: 1.14-2.91). Next to statin use, only diabetes (OR: 1.92, 95% CI: 1.00-3.66) and smoking (OR: 1.49, 95% CI: 0.99-2.26) were associated with MAU.

**Conclusions.** Use of statins is independently associated with MAU, even after adjusting for bias by indication to receive a statin. In the hypothesis that this MAU is of tubular origin, statin use can result in incorrect labeling of subjects as having a predictor or marker of cardiovascular or renal risk. In addition, statin use affected the association of established cardiovascular risk factors with MAU, blurring the interpretation of multivariable analyses.
CHAPTER 2: Results

Introduction

Microalbuminuria (MAU) is considered as a predictor or marker of cardiovascular morbidity and mortality, particularly in patients with other risk factors [1-4], and as a surrogate for early kidney damage especially in subjects with diabetes and hypertension [5,6]. Statins are frequently prescribed in patients with hypertension, diabetes and metabolic syndrome, to reduce cardiovascular morbidity and mortality [7]. Statins can reduce existent proteinuria [8-10] through a positive impact on endothelial dysfunction. In contrast, there is in vitro [11,12] and in vivo [13-16] evidence that statins are associated with de novo albuminuria and proteinuria. It is of importance to establish the association between statin use and MAU to correctly interpret presence of MAU as a predictor or marker of cardiovascular or renal disease in observational trials with a mixed population of subjects taking and not taking a statin. If statin use is associated with MAU, there is a risk of incorrect labeling of subjects as having a predictor or marker of cardiovascular or renal risk. The current study evaluated the association between statin use and MAU, and used a propensity score analysis to adjust for bias by indication. For this goal, we used the baseline data of the early renal impairment and cardiovascular assessment in Belgium (ERICABEL) trial, a prospective cohort of hypertensive patients followed by primary care physicians created to evaluate the impact of metabolic syndrome on cardiovascular and renal endpoints.
CHAPTER 2: Results

Methods

Objectives. The primary aim of the ERICABEL study was to determine the effect of metabolic risk factors on the evolution of renal function and cardiovascular outcome over 5 years, in patients aged between 40 and 70 years with diagnosed hypertension, and followed by their primary care physician. The current analysis was designed to evaluate 1° the association between statin treatment and MAU and 2° the impact of statin treatment on the interpretation of the association between individual cardiovascular risk factors and MAU in epidemiological studies.

Participants. We used the baseline data of the ERICABEL cohort, a non-interventional epidemiological study with a follow up of 5 years that included 1,076 Caucasian patients with hypertension, defined as systolic blood pressure ≥140mmHg and/or intake of at least one antihypertensive drug, recruited by 96 general practitioners, between 2006 and 2007, in Belgium. Of the 1076 patients included in this cross-sectional study, 420 patients had a missing value for at least one of the variables under investigation (see appendix table S1 for detailed list). Multiple imputation techniques were used to account for the missing data, using 20 imputations [17]. All characteristics and outcome (MAU) were simultaneously used in the imputation model. The imputation was done using the R function aregImpute from the Hmisc package [18].

Description of procedures. Each participating primary care physician was asked to include 10 consecutive hypertensive patients aged between 40-70 years, in a 1:1 sex ratio. The eligible persons were evaluated at baseline and if eligible, sociodemographic information (age, sex, race, and education level), personal and family medical history, smoking status and medication use were collected prospectively in an online database. Body weight was recorded to the nearest 0.1 Kg and height was measured to the nearest centimeter. Body mass index (BMI) was calculated as body weight in Kg divided by height² (kg/m²). Blood pressure was measured according to the WHO criteria with a calibrated Omron HEM-907 device (average of 2 measurements, sitting, with 5 minutes in between). All these measurements were done by the primary care physician. After exclusion of a urinary infection or hematuria (negative Combur® test), MAU was screened by a Micral® dipstick test. MAU was considered present if measured albuminuria was ≥ 20mg/l on a morning midstream urine sample. Blood sampling was performed by the general practitioner in fasting patients.
Definitions. The metabolic syndrome was defined as three or more of the following criteria, according to the National Cholesterol Education Program Third Adult Treatment Panel guidelines ATP III criteria [19]: elevated blood pressure \( \geq 130/85 \) mmHg and/or antihypertensive medication (by definition 100% in this cohort), (2) high plasma triglycerides (> 1.7 mmol/l), low HDL cholesterol (< 1.0 mmol/l in men and < 1.3 mmol/l in women), (4) abdominal adiposity (waist circumference > 102/88 cm men/women) and/or impaired glucose tolerance (IGT) (fasting plasma glucose \( \geq 6.1 \) mmol/l and/or known diabetes).

Ethics. The study was approved by an independent ethics committee review board, protocol number: ML 19208. A written informed consent was obtained from all participants.

Statistical methods. All analyses have been performed using SAS software (SAS software, version 9.2 of the SAS System for Windows. Copyright © 2002 SAS Institute Inc.) or R Version 2.12.0 (R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, 2009) [20]. MAU was considered as a dichotomic variable. Continuous variables were described by their mean, standard deviation, median and interquartile range. Categorical variables were summarised by frequencies and percentages.

A propensity score for statin use was created to correct for “bias by indication”. Propensity score analysis is a well established method to adjust for confounding by indication in observational trials [21,22]. Primarily, for the statistical analyses, 20 imputed samples were created. In a second step, within each of the 20 samples separately, a propensity model was constructed and the resulting propensity score was calculated for each of these patients. The propensity model included the following variables that were deemed to be possibly related to statin use: age, gender, BMI, waist circumference, SBP, previous CV event, CRP, fasting glucose, diabetes, serum uric acid, HDL and LDL cholesterol, triglycerides, use of angiotensin converting enzyme inhibitor/angiotensin receptor blocker (ACE-I/ARB) and smoking. Continuous variables in the model were included using restricted cubic splines: each continuous variable was included in the model using 3 dummy variables, called \( \text{var1} \), \( \text{var2} \) and \( \text{var3} \). For the first 5 imputed samples, a histogram of the propensity scores was presented by statin use (see appendix, table S2). In addition, in order to check the ability of the propensity scores to balance the two statin groups for baseline characteristics, tables were presented for the first 5 imputed samples, comparing the baseline characteristics between the groups (see appendix, table S3). In this way, patients with the
same “likelihood” or “propensity” to receive a statin (i.e. in this setting mainly with comparable cardiovascular risk factors), but in one case taking and in the other case not taking a statin, were compared for presence of MAU.

For comparison of continuous variables, ANOVA was used, adjusted for propensity scores, whereas for binary variables, logistic regression analyses, also adjusted for propensity scores, were employed. Logistic regression analyses were used to assess the association between statin use and MAU using the “GENMOD” procedure in SAS. The associations were assessed in each of the 20 imputation samples separately and the results were combined using the SAS procedure “MIANALYZE”. The following logistic regression models were used: 1°: Univariable model only including statin use; 2° a model including statin use and propensity scores (using a restricted cubic spline); 3° a model including statin use, propensity scores and all relevant variables mentioned above. Since the linearity assumption was deemed appropriate for all continuous variables in the model (p> 0.1 for the assessment of linearity in the full model), the final model only included linear terms for all variables.
CHAPTER 2: Results

Results

The baseline characteristics of the population are provided in tables 1 and 2.

Table 1. Baseline characteristics (categorical variables)

<table>
<thead>
<tr>
<th>parameter</th>
<th>No statins (N=724)</th>
<th>Statins (N=332)</th>
<th>Statin use unknown (N=20)</th>
<th>Total (N=1076)</th>
<th>P*</th>
<th>P§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>384/724 (53%)</td>
<td>132/332 (39.8%)</td>
<td>7/19 (36.8%)</td>
<td>523/1075</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>MS</td>
<td>239/624 (38.3%)</td>
<td>172/299 (57.5%)</td>
<td>2/4 (50.0%)</td>
<td>413/927</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>98/700 (14.0%)</td>
<td>104/327 (31.8%)</td>
<td>2/5 (40.0%)</td>
<td>204/828</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Smoker</td>
<td>249/698 (35.7%)</td>
<td>123/325 (37.9%)</td>
<td>3/5 (60.0%)</td>
<td>375/653</td>
<td>0.438</td>
<td>0.501</td>
</tr>
<tr>
<td>MAU</td>
<td>68/529 (12.9%)</td>
<td>60/248 (24.2%)</td>
<td>0/4 (0.0%)</td>
<td>128/653</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ACE-ARB</td>
<td>358/724 (49.5%)</td>
<td>232/332 (69.9%)</td>
<td></td>
<td>590/1056</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CV event</td>
<td>38/693 (5.5%)</td>
<td>77/325 (23.7%)</td>
<td>1/5 (20.0%)</td>
<td>116/1023</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

MS: metabolic syndrome, MAU: microalbuminuria, ACE-I/ARB: angiotensin converting enzyme inhibitor /angiotensin receptor blocker, CV event: cardiovascular event
p*: p values between all three groups; p§: p value between users vs. non statin users

There was an equal distribution in gender (51.3% males) in the overall cohort. There was a high prevalence of metabolic syndrome (44.5%), diabetes (19.8%), current smokers (36.5%) and MAU (16.4%) in the overall cohort. ACE-I and/or ARB were the most commonly prescribed antihypertensive agents (55.9%). History of a cardiovascular event was recorded in 11.3% of the patients. Mean age of the cohort was 57.5 ± 7.5 years. One third (30.8%) of the patients used a statin.
**Chapter 2: Results**

Table 2. Baseline characteristics (continuous variables)

<table>
<thead>
<tr>
<th>Patient Characteristic</th>
<th>Statistic</th>
<th>No statin</th>
<th>Statin</th>
<th>Statin use unknown</th>
<th>Total</th>
<th>P*</th>
<th>P§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td>56.8±7.6</td>
<td>59.1±7.1</td>
<td>58.2±6.0</td>
<td>57.5±7.5</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>Mean±SD</td>
<td>29.3±5.4</td>
<td>30.6±5.5</td>
<td>30.5±4.4</td>
<td>29.7±5.4</td>
<td>0.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>28.5</td>
<td>29.7</td>
<td>31.7</td>
<td>28.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>689</td>
<td>326</td>
<td>5</td>
<td>1075</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>Mean±SD</td>
<td>144±15</td>
<td>143±16</td>
<td>149±11</td>
<td>143±16</td>
<td>0.527</td>
<td>0.394</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>142.5</td>
<td>140.0</td>
<td>152.0</td>
<td>142.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>704</td>
<td>331</td>
<td>5</td>
<td>1040</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>Mean±SD</td>
<td>84±10</td>
<td>83±10</td>
<td>87±6</td>
<td>84±10</td>
<td>0.054</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>84.5</td>
<td>82.0</td>
<td>86.5</td>
<td>83.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>704</td>
<td>331</td>
<td>5</td>
<td>1040</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>Mean±SD</td>
<td>5.6±2.9</td>
<td>6.3±2.1</td>
<td>6.8±1.4</td>
<td>5.9±2.7</td>
<td>0.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>5.2</td>
<td>5.7</td>
<td>6.9</td>
<td>5.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>644</td>
<td>311</td>
<td>4</td>
<td>959</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uric acid (µmol/l)</td>
<td>Mean±SD</td>
<td>339±89</td>
<td>357±83</td>
<td>291±83</td>
<td>345±89</td>
<td>0.008</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>333</td>
<td>357</td>
<td>286</td>
<td>339</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>629</td>
<td>306</td>
<td>4</td>
<td>939</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>Mean±SD</td>
<td>1.6±1.1</td>
<td>1.9±1.1</td>
<td>2.0±1.7</td>
<td>1.7±1.1</td>
<td>0.011</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>1.3</td>
<td>1.6</td>
<td>1.4</td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>638</td>
<td>316</td>
<td>4</td>
<td>958</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-Cholesterol (mmol/l)</td>
<td>Mean±SD</td>
<td>3.2±0.8</td>
<td>2.7±1.0</td>
<td>2.9±0.8</td>
<td>3.0±0.9</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>3.2</td>
<td>2.6</td>
<td>3.2</td>
<td>3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>628</td>
<td>312</td>
<td>4</td>
<td>944</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-Cholesterol (mmol/l)</td>
<td>Mean±SD</td>
<td>1.5±0.5</td>
<td>1.4±0.4</td>
<td>1.3±0.4</td>
<td>1.5±0.5</td>
<td>0.004</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>1.4</td>
<td>1.3</td>
<td>1.4</td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>637</td>
<td>315</td>
<td>4</td>
<td>956</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>Mean±SD</td>
<td>0.5±0.8</td>
<td>0.4±0.5</td>
<td>0.4±0.4</td>
<td>0.5±0.7</td>
<td>0.148</td>
<td>0.055</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>0.3</td>
<td>0.2</td>
<td>0.4</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>608</td>
<td>291</td>
<td>4</td>
<td>903</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BMI: body mass index, SBP: systolic blood pressure, DBP diastolic blood pressure, CRP: C-reactive protein. p*: p values between all three groups; p§: p value between users vs. non statin users
## Table 3. Association between statin use and microalbuminuria (MAU)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Logistic regression model for presence of MAU</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>A. Univariable association</strong></td>
<td>Statin use</td>
<td>2.01</td>
<td>(1.34;3.01)</td>
</tr>
<tr>
<td><strong>B. Association adjusted for propensity score</strong></td>
<td>Statin use</td>
<td>1.82</td>
<td>(1.14; 2.91)</td>
</tr>
<tr>
<td></td>
<td>Pscore$</td>
<td>.</td>
<td></td>
</tr>
<tr>
<td><strong>C. Association fully adjusted</strong></td>
<td>Statin use</td>
<td>1.90</td>
<td>(1.15; 3.11)</td>
</tr>
<tr>
<td></td>
<td>Pscore</td>
<td>7.92</td>
<td>(0.18; 357.3)</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>1.02</td>
<td>(0.91; 1.14)</td>
</tr>
<tr>
<td></td>
<td>Diabetes</td>
<td>1.92</td>
<td>(1.004; 3.66)</td>
</tr>
<tr>
<td></td>
<td>Smoker</td>
<td>1.49</td>
<td>(0.99; 2.26)</td>
</tr>
<tr>
<td></td>
<td>CV event</td>
<td>1.47</td>
<td>(0.92; 2.34)</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>1.03</td>
<td>(0.86; 1.24)</td>
</tr>
<tr>
<td></td>
<td>Mean BP</td>
<td>0.96</td>
<td>(0.89; 1.04)</td>
</tr>
<tr>
<td></td>
<td>Fasting glucose</td>
<td>0.97</td>
<td>(0.91; 1.04)</td>
</tr>
<tr>
<td></td>
<td>Uric acid</td>
<td>0.89</td>
<td>(0.50; 1.50)</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
<td>0.99</td>
<td>(0.98; 1.01)</td>
</tr>
<tr>
<td></td>
<td>Cholesterol</td>
<td>1.00</td>
<td>(0.98; 1.02)</td>
</tr>
<tr>
<td></td>
<td>CRP</td>
<td>1.49</td>
<td>(0.01; 359.73)</td>
</tr>
</tbody>
</table>

$ Pscore= propensity score; The propensity score was fit using a restricted cubic spline. ACE-I/ARB: angiotensin converting enzyme inhibitor/angiotensin receptor blocker, CV event: cardiovascular event, BMI: body mass index, BP: Blood pressure, CRP: C-reactive protein.
In univariable analysis, statin users were more likely to be male (p< 0.001), had a higher frequency of metabolic syndrome (p< 0.001), diabetes type 2 (p< 0.001), MAU (p< 0.001), ACE-i/ARB treatment (p< 0.001), and previous cardiovascular events (p< 0.001), were older (p< 0.001), had larger BMI (p< 0.001), lower diastolic blood pressure (p= 0.02), higher fasting glucose (p< 0.001), serum uric acid (p= 0.004), and triglycerides (p= 0.003), had lower levels of LDL (p< 0.001) and HDL cholesterol (p= 0.001) compared to patients not taking statins (tables 1 and 2). The univariable odds ratio of MAU in patients using vs. not using a statin was 2.01 (95% CI: 1.34-3.01, p= 0.0009, table 3A).

After multivariable analysis including the propensity score for statin use, the odds of MAU was still significantly higher in patients taking a statin (OR: 1.82, 95% CI: 1.14-2.91, p= 0.01, table 3B). When all other variables were forced into the model, use of statin still was independently associated with a higher odds of MAU (OR: 1.90, 95% CI: 1.15-3.11, p= 0.01, table 3C). Next to statin use, only diabetes (OR: 1.92, 95% CI: 1.00-3.66, p= 0.05) and smoking (OR: 1.49, 95% CI: 0.99-2.26, p= 0.06) were independently associated with MAU after adjusting for the likelihood of receiving a statin, suggesting that prescription of a statin overrides the association between cardiovascular risk factors, MAU and creates collinearity by acting as a surrogate.
**Figure S1.** Flow chart of hypotheses to explain the observation of higher prevalence of MAU in statin users

- Observation: higher prevalence of MAU in patients taking vs. not taking a statin: OR 2.0 (95% CI: 1.3-3.0)
  - Bias by indication?
  - Propensity score analysis
    - Still higher prevalence of MAU in patients taking vs. not taking a statin OR 1.82 (95% CI 1.14-2.91)
      - Statins induce MAU
        - Residual confounding
          - Incorrect identification of risk factors for MAU
          - Incorrect adjustment for comorbid conditions in multivariable analysis
        - Tubular MAU
          - Incorrect labeling of subject as having a cardiovascular or renal risk factor
        - Glomerular MAU
          - Incorrect labeling when glomerular MAU, but independent of endothelial dysfunction
          - Correct labeling when glomerular MAU due to endothelial dysfunction
Discussion

Our data create concern on the use of MAU as a predictor or marker of cardiovascular or renal disease in cohorts with patients using statins. Statin use apparently could blur the interpretation of MAU by two potential mechanisms: 1° higher prevalence of MAU in patients using a statin, even after correction for bias by indication and 2° masking of cardiovascular risk factors in multivariable analyses, as statin use behaves as a surrogate for these markers. In epidemiological studies evaluating the association between cardiovascular risk factors and MAU, statin use can induce incorrect labeling of patients as having a cardiovascular or renal risk factor, and interpretation of other risk factors for cardiovascular or renal disease can be confounded by the way the use of statins is handled in the analysis. In this cross-sectional analysis, we observed a two-fold higher prevalence of MAU in subjects who use vs. those who do not use a statin. However, part of this association (figure S1) can be attributed to bias by indication, as patients are often prescribed statins because they have cardiovascular risk factors which are by themselves associated with enhanced risk for MAU. Indeed, we observed a higher prevalence of cardiovascular risk factors in patients taking vs. not taking a statin in our study. We tried to exclude this bias by indication by the use of a propensity score analysis. Adjusting for the propensity score allows to analyze the difference in occurrence of MAU between patients with a comparable propensity to receive a statin, while one group does whereas the other does not receive the drug. The technique of propensity score is well established to address confounding and bias by indication in observational studies [23,24]. However, this increased odds ratio remained present even after correcting for the fact that statins are usually prescribed in patients with cardiovascular risk factors which by themselves are associated with MAU, using the robust technique of propensity score. This observation can either be due to residual or unmeasured confounding or there can really be an induction of MAU by statin use (figure S1). Our data stress that statin use confounds the impact of the individual risk factors on MAU, as statin use behaves as a surrogate for presence of cardiovascular risk factors. As a consequence, in studies where MAU is either used as a marker or as a surrogate endpoint, the association between outcomes and certain cardiovascular risk factors can be blurred, and this in an unpredictable and variable fashion, depending upon the prevalence of statin use in the cohort. On the other hand, if statins really induce MAU, theoretically it can be both of glomerular or of tubular origin (figure S1). We did not find any publication, either human, animal or in vitro, indicating that statin associated
proteinuria is of glomerular origin, but at least three in vitro or animal studies demonstrated that statins do inhibit tubular reabsorption of filtered albumin and in this way could generate MAU in a dose-dependent manner and in absence of cytotoxicity [11,12,25]. There is also growing evidence that also in other conditions MAU can be the consequence of tubular dysfunction, even in presence of an entirely intact glomerulus [26,27]. One epidemiological study in humans also coined statin induced proteinuria as being tubular in origin, and even demonstrated a dose-effect relation with rosuvastatin [28]. This would explain why statins fail to consistently result in reduction of MAU in subjects with low grade MAU, or why higher vs. lower doses of statin fail to further reduce MAU [29,30], as the beneficial effect of statins on the glomerular MAU is counterbalanced by the induction of tubular MAU. It is very unlikely that statin induced MAU is associated with an increased cardiovascular risk, but its impact on the functional capacity and the morphological integrity of the kidneys is unknown. Even when the tubular albuminuria induced by statins is harmless [31], it interferes with the implication of MAU as predictor or marker of cardiovascular and renal risk by incorrectly labeling a subject as having a risk factor. Of note, this would imply that the prognostic impact of glomerular MAU (so not induced by statin use) in populations with a high prevalence of statin use, would be underestimated. The hypothesis that statin induced MAU is tubular in origin would also fit with the favorable effect of statins on cardiovascular disease and on progression of renal disease, as these effects are related to the reduction of glomerular MAU associated with the improvement of endothelial dysfunction [32,33].

**Limitations.** This cross-sectional study could not prove a causal connection between statin use and de novo MAU. Using the technique of propensity score we achieved “pseudo-randomization”, which in fact obviates the drawbacks imposed by the method of the study, but which is not free from unmeasured biases [24]. Unfortunately, although dose dependency and a higher frequency of more potent inhibitors of HMG coA reductase could add further strength to the association with MAU, the prescribed daily dose and type of statin were as per protocol not registered in our study. Another limitation of our study is that MAU was measured in a single morning urine, whereas guidelines recommend to have at least two positive MAU in three consecutive first morning urine samples before labeling a person as having MAU [34]. However, in view of the mechanism of inhibition of tubular endocytosis, it is unlikely that statin associated MAU would disappear by repeated testing, unless the statin would be stopped temporarily to
confirm the diagnosis. Second, intermittent MAU should not be considered as a predictor or marker of cardiovascular or renal risk, as it is not linked to endothelial dysfunction [35]. In line with this, we demonstrated in another cohort, that patients with intermittent MAU have far less cardiovascular risk factors as compared to patients with persisting MAU [36]. Our study shows that, in addition to the problems caused by single vs. multiple sampling, the use of a statin can also lead to an in incorrect labeling of subject as having MAU.

The strength of this study is that it reflects routine clinical practice in hypertensive patients. To the best of our knowledge, this is the first study pointing to an independent association between statin use and MAU, even after correction for bias by indication by the use of a propensity score, underlining the potential consequences of confounding induced by statin use on the interpretation of MAU as a predictor or marker of cardiovascular or renal disease in epidemiological trials.

According to our data, statins are independently associated with an increased prevalence of MAU, even after correction for bias by indication. As this MAU is most likely of tubular origin, it is uncertain and rather unlikely whether it has the same prognostic impact for renal and cardiovascular disease as endothelial dysfunction induced glomerular MAU. As such, it can lead to incorrect labeling of subjects as having a cardiovascular risk factor.
Acknowledgments


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CHAPTER 3: GENERAL DISCUSSION AND CONCLUSIONS
In this thesis we discuss whether cardiovascular risk factors and kidney damage could be screened by measuring albuminuria. Our results are based on two prospective studies, one study was performed in workers (URI study) and another study was performed in hypertensive subjects (ERICABEL study). In what follows we will discuss the main questions raised at the start of this thesis.
3.1. The prevalence of microalbuminuria and the associations with traditional cardiovascular risk factors in a general population.

In the URI study (n = 1,398), the prevalence of microalbuminuria was 5.2 and 0.5%. Only a minority of the workers with microalbuminuria (14/73 = 19%) had known diabetes and/or hypertension. In a Dutch population cohort (PREVEND), also a minority of the subjects with microalbuminuria (644/2,918 = 22%) had self-reported diabetes and/or hypertension (1). After exclusion of these known risk factors, the PREVEND trial reported a higher prevalence of microalbuminuria than the URI study (6.6 vs. 4.2%, p < 0.001), conceivably as a consequence of higher mean age (49.5 ± 12.9 y vs. 38.3 ± 9.7 y, p < 0.001), higher prevalence of smokers (42 vs. 31%, p < 0.001) and differences in methods to define microalbuminuria; urinary albumin concentration (UAC): 20-200 mg/l in the PREVEND study vs. urinary albumin creatinin ratio (ACR): 17-249 in men 25-354 mg/g in women in the URI study (2). The PREVEND study concluded that the presence of microalbuminuria predicts cardiovascular morbidity and mortality, even in subjects without recognized cardiovascular risk factors (2, 3). However, this association might be confounded by inflammation, unrecognized cardiovascular risk factors and ‘borderline’ levels of cardiovascular risk factors, such as prehypertension or impaired glucose tolerance (4-6).

In this thesis, we described in chapter 2.1.1 based on data from the URI study, that ACR in apparently healthy workers was positively associated with unrecognized hypertension (≥ 140/90 mmHg), plasma glucose (≥100 mg/dl) and resting heart rate (≥ 85 bpm). The prevalence of these risk factors was high (36%) in this presumably healthy population. Moreover, as described in chapter 2.1.3, one third of the workers of the overall URI population had at least one traditional cardiovascular risk factor (hypertension, diabetes and/or dyslipidemia) of whom only one third was known and treated. In the PREVEND study (n = 40,856) cardiovascular risk factors were only measured in selected subjects with UAC > 10 mg/l (n= 6,000) and in a randomly selected control group of the total study population with UAC < 10 mg/l (n = 2,592). Microalbuminuria as defined by UAE > 30 mg/24h was only confirmed in 933 subjects (7). Two thirds of these subjects (633/933) had hypertension, diabetes and/or a cardiovascular history, and one third (300/933) was considered to have isolated microalbuminuria. Nevertheless, the subjects with isolated microalbuminuria had associated additional cardiovascular risk factors, such as age, male gender, smoking, obesity and dyslipidemia, ‘borderline’ levels of cardiovascular risk factors, and
also an increased risk to develop diabetes, hypertension and cardiovascular diseases (7). Thus, persistent microalbuminuria is rather a reflection of widespread vascular damage that is linked to associated risk factors than an independent risk factor (8). In the URI study (chapter 2.1.1), two thirds of the workers with microalbuminuria had associated cardiovascular risk factors. We hypothesized that a subject with microalbuminuria in absence of any cardiovascular risk factor could be considered as a false positive. The prevalence of these false positive results was 2.4%. Similar figures were found in a New Zealand study (2.0%) and in the Copenhagen City Heart Study (2%) (9, 10). In subjects without any feature of the metabolic syndrome, the presence of microalbuminuria was not associated with increased risk for cardiovascular disease and death, suggesting that microalbuminuria by itself might not be an independent determinant of outcome (10).
3.2. Should we use microalbuminuria as a screening tool to identify subjects with unrecognized cardiovascular risk factors in a presumably healthy population?

The authors of the PREVEND trial suggest that screening for albuminuria improves the detection of unrecognized renal and cardiovascular risk factors (1, 11, 12). When this approach had been implemented in the URI cohort, only 9% (37/431) of the workers with at least one unrecognized risk factor would have been identified (chapter 2.1.1). The sensitivity of microalbuminuria to detect a subject with at least one cardiovascular risk factor was low (12%) in the PREVEND study as well, leaving most subjects (88%) with modifiable cardiovascular risk factors untreated (12). According to the authors of the PREVEND trial, hypertensive patients without microalbuminuria would not benefit from blood pressure lowering agents as the risk reduction for cardiovascular events would be too low. It was estimated that more hypertensive patients without vs. with microalbuminuria need to be treated (111 vs. 8) to prevent one cardiovascular event (13). Yet, no RCT exists to test the hypothesis that medical management of microalbuminuria reduces cardiovascular or renal events independent of blood pressure reduction (14). According to us, the ‘PREVEND’ approach might offer prospects for secondary prevention in high risk patients with microalbuminuria while more subjects would be (earlier) identified by screening for cardiovascular risk factors with more potential possibilities to reduce cardiovascular risk (chapter 2.1.1). The efficiency of interventions in patients with diabetes and/or hypertension is also practically more easy to assess by measuring the plasma glucose and blood pressure levels than it would be by controlling urinary albumin. Additionally, the interpretation of microalbuminuria is hampered by the fluctuations which commonly occur.
3.3. **Conditions which could lead to false positive test results of microalbuminuria**

Another problem of using microalbuminuria as a screening tool is the high number of false positive results. Biological variation and temporary conditions such as changes in blood pressure, plasma glucose, fever, urinary tract infections, hematuria, exposure to cold and physical exercise might result in false positive results (chapter 1). Microalbuminuria fluctuates more in random urine samples than in early morning urine samples (15). Conceivably, the URI trial included a substantial number of false positive test results as urine was collected randomly. The exact number of false positive subjects in our trial is unknown as we did not perform an overnight or 24-h urine collection in all subjects.

In the URI study, we used gender specific cut-off values (ACR: 17-249 for males and 25-354 mg/g for females) in an urine spot to indicate microalbuminuria, because men have a higher urinary excretion of creatinine than women due to higher muscle mass (16). According to the PREVEND trial, the sensitivity, specificity and positive predictive value of these gender specific cut-off values in an urinary spot, to predict microalbuminuria in subsequent 24-hours urine collection was 65, 98 and 65%, respectively (17). However, the PREVEND trial used a lower threshold to define microalbuminuria UAC > 10 mg/l during the prescreening, consequently more subjects with microalbuminuria were identified (increased sensitivity) but simultaneously also more subjects with a false positive result were included.

As described in chapter 2.1.2, within the URI study, a prospective analysis was designed to evaluate the reproducibility of microalbuminuria. Of the overall URI population, 341 subjects underwent consecutive occupational check-ups. Only 60% of the initially microalbuminuric subjects tested positive for microalbuminuria at a subsequent visit. The reproducibility of initial microalbuminuria was higher in subjects with vs. without traditional risk factors (89 vs. 36%, p = 0.03). In addition, subjects with persistent microalbuminuria had a higher cardiovascular risk score than subjects with intermittent or without microalbuminuria. These findings corroborate the data from the HUNT study that showed that intermittent microalbuminuria in presumably healthy subjects might simply reflect biological variation, whereas persistent microalbuminuria more likely explains a cardiovascular risk and/or disease (18). We assumed in chapter 2.1.1 that
approximately 1 of 3 detected workers had a false positive test result, as one third of the workers with microalbuminuria had no cardiovascular risk factor. In workers younger than 40 years, the presence of microalbuminuria was not associated with cardiovascular risk factors (chapter 2.1.3). In this group, the presence of microalbuminuria was associated with low BMI or with physicochemical exposure risk (PCER). Conceivably, a substantial number of young workers had postural proteinuria and might be mislabeled as having a risk for cardiovascular and/or renal disease (19-21). The meaning of microalbuminuria in workers with PCER is unknown. According to the literature, exposure to some heavy metals is associated to reversible tubular overflow albuminuria by increased liver synthesis of metallothionein (22-24).

Another condition that affects urinary albumin excretion is use of statins. In chapter 2.2.1 we showed that statin use is associated with microalbuminuria, even after adjusting for bias by indication to receive a statin. Other studies confirmed that statin use generates tubular albuminuria hence affecting the total excreted albumin (25-27). The effect of statins was maximal after 3 hours and disappears after 24 hours in healthy subjects (27). The ERICABEL study did not evaluate the timing of statin intake. The finding of an increased urinary α1-microglobulin/albumin ratio might help to distinguish statin induced tubular albuminuria from glomerular albuminuria (27). This might help to anticipate incorrect labeling of persons with an increased risk for a cardiovascular or renal disease. In the ERICABEL study no marker for tubular albuminuria was measured. Thus, whether microalbuminuria could be implemented in a mass screening program is uncertain as it might have different underlying causes, and prognostic meanings.
3.4. Can we prevent ESRD by screening for microalbuminuria in a general population?

The central idea of screening for albuminuria is early disease detection followed by adequate treatment to reduce the risk of the disease or progression. KDIGO recommends to screen for albuminuria in selected patients with hypertension, diabetes, and/or cardiovascular disease (28). However, some authors advocate to screen for albuminuria in the general population as many persons are not aware of the presence of cardiovascular risk factors (1). The criteria to select a disease for screening have been reported by Wilson and Jungner and the Council of Europe and are summarized in table 1 (29, 30). We discuss by means of these criteria whether a population wide albuminuria screening followed by appropriate treatment might lead to benefits in terms of prevention of end stage renal disease (ESRD).

Table 1. The Wilson-Jungner criteria (29) and recommendations of the Council of Europe (30)

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<table>
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<tr>
<td>1</td>
<td>The disease should be an obvious burden for the individual and the community in terms of death, poor quality of life and socio-economic factors including health care costs.</td>
</tr>
<tr>
<td>2</td>
<td>The natural course of the disease should be well known and the disease should go through an initial latent stage or be determined by risk factors, which can be detected by appropriate tests.</td>
</tr>
<tr>
<td>3</td>
<td>A suitable test is highly sensitive and specific for the disease as well as being acceptable to the person screened.</td>
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<tr>
<td>4</td>
<td>Screening followed by diagnosis and interventions in an early stage of the disease should provide a better prognosis than intervention after spontaneously sought treatment.</td>
</tr>
<tr>
<td>5</td>
<td>Adequate treatment or other intervention possibilities are indispensable. Adequacy is determined both by proven medical effect and ethical and legal acceptability.</td>
</tr>
<tr>
<td>6</td>
<td>The cost of case-finding should be economically balanced in relation to possible expenditure on medical care as a whole.</td>
</tr>
<tr>
<td>7</td>
<td>Case-finding should be a continuing process and the interval of testing should be known.</td>
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CHAPTER 3: General discussion and conclusions

3.4.1 ‘The disease should be an obvious burden for the individual and the community in terms of death, poor quality of life and socio-economic factors including health care costs’.

Although this condition seems to be fulfilled for ESRD (31-33), the incidence of ESRD might be too low to justify a population screening for albuminuria. In the PREVEND study (n= 40,854) only 13 subjects reached ESRD while 568 subjects experienced a CVD event in a follow-up period of 7 years (34). The burden of disease for the community can be measured by disability adjusted life year (DALY). DALYs are the sum of years of life lost due to premature death and years lived with disability (prevalence of a disease sequel multiplied by the disability weight for that sequel), compared to the standard life expectancy in full health (35). In the developed countries, the major part of the DALYs in the general population is lost by cardiovascular diseases whereas a negligible part is lost by ESRD (36). Conceivably, population-based screening for albuminuria is only useful if CVD would be detected early and prevented.
3.4.2 ‘The natural course of disease should be well-known and the disease should go through an initial latent stage or be determined by risk factors, which can be detected by appropriate tests’.

The precursor stage of diabetic kidney disease is detected by a sustained presence of microalbuminuria, a long preclinical phase before the development of overt nephropathy (37, 38). Consequently, one third of the diabetic patients have microalbuminuria (39). Prospective long term studies observed that 40-60% of the diabetic patients with microalbuminuria regressed to lower level of albuminuria spontaneously, or by improvement of glycaemia and blood pressure control, while only 20-30% of microalbuminuric diabetic patients progressed to a higher level of albuminuria (40-42). A major concern of using microalbuminuria as an early indicator for nephropathy is that this potentially causal relation is confounded by hematuria, age, gender, essential hypertension, hyperglycemia, obesity, inflammation, atherosclerosis and drugs (43-46). The presence of microalbuminuria is also frequently found in hypertensive subjects, even in the absence of diabetes (39). The prevalence of microalbuminuria was 16% in our Belgian hypertensive cohort (ERICABEL). Large epidemiological studies in general and high risk populations showed that microalbuminuria was independently associated with an increased likelihood of renal events and mortality (47, 48). However, it seems unlikely that microalbuminuria is part of the causal pathway for overt nephropathy in non-diabetic subjects, as the presence of microalbuminuria might be rather a sign of systemic endothelial dysfunction or underlying systemic disease (atherosclerosis, cancer, CVD...) than a (first) manifestation of kidney disease (49). The positive predictive value of microalbuminuria to find a subject with a primary kidney disease will be low as primary kidney diseases are rare. Moreover, in patients with a primary kidney disease the occurrence of microalbuminuria will progress rapidly to macroalbuminuria whereas diabetic patients with microalbuminuria slowly progress to macroalbuminuria. Additionally, the causes of renal failure in RRT patients differ widely: diabetes (18.3%), glomerulosclerosis (17.8%), polycystic kidney disease (9.6%), pyelonephritis (7.6%), renovascular disease (7.6%), hypertension (6.8%), miscellaneous (23%) and unknown (9%) in Belgium (50). Thus, unselected screening for microalbuminuria does not fulfill the second request, as the natural course of diabetic microalbuminuria could not extrapolated to other kidney diseases, where the initial latent stage is too short or absent.
3.4.3 ‘A suitable test is highly sensitive and specific for the disease as well as being acceptable to the person screened’.

Subjects with microalbuminuria have approximately a twofold relative risk for CVD or ESRD, but the absolute risk is 40 fold higher for CVD than for ESRD (34, 51, 52). According to the literature, the diagnostic performance of microalbuminuria to predict RRT is not satisfied, especially the positive predictive value is too low (0.8%) (1). According to the results of the PREVEND study, subjects with microalbuminuria in absence of cardiovascular risk factors had a normal decline of renal function over time whereas subjects with macroalbuminuria had a rapid decline of renal function (1). Collecting urine samples to measure albuminuria is clinically acceptable for the general population (1, 53). However, a person with a positive test result should be screened for a second time to confirm the diagnosis of microalbuminuria, whereas in subjects with macroalbuminuria a second urine sample is not necessary as fluctuations in this range of albuminuria are less prominent. Thus, screening for macroalbuminuria is more appropriate than screening for microalbuminuria to predict ESRD.
3.4.4 ‘Screening followed by diagnosis and interventions in an early stage of the disease should provide a better prognosis than intervention after spontaneously sought treatment’.

There is no robust evidence to suggest that interventions that directly target microalbuminuria result in a reduction of ESRD and CVD (14, 54). A randomized controlled trial (PREVEND-IT) showed that detecting subjects with microalbuminuria in the general population, followed by the initiation of an ACE-I vs. placebo was associated with a reduction in baseline albuminuria but this was not translated in a significant reduction of renal and cardiovascular events (55). In contrast, trials in patients with diabetes and/or hypertension who were treated with ACE-I vs. placebo notably showed a reduction of albuminuria, and a decrease of cardiovascular events and mortality (42, 56-62), although these trials are confounded by a reduction of blood pressure. Only in post hoc analyses, a reduction in baseline albuminuria has been associated to a reduction in cardiovascular morbidity and mortality, independent of blood pressure (63-66). In addition, reducing albuminuria is not always associated with improvement of clinical outcome. The ONTARGET trial showed that ARB + ACE-I vs. ACE-I or ARB reduce blood pressure and albuminuria but not the clinical outcome in patients with cardiovascular risk factors (67). The ROADMAP trial showed that an ARB on top of antihypertensive medication in 4,447 diabetes patients reduced blood pressure and albuminuria, but was associated with a higher cardiovascular mortality (15 vs. 3 patients, p = 0.01) (60). No evidence exists that protective treatment in patients with microalbuminuria avoids new cases of ESRD whereas ACE-I or ARB in patients with macroalbuminuria showed a 25% reduction of ESRD (68-75). All these data suggest that mainly screening for macroalbuminuria (and not microalbuminuria) followed by treatment could be effective to reduce ESRD.
CHAPTER 3: General discussion and conclusions

3.4.5 ‘Adequate treatment or other intervention possibilities are indispensable. Adequacy is determined both by proven medical effect and ethical and legal acceptability’.

Despite the use of ACE-I or ARB, some patients might develop macroalbuminuria and/or CKD stage 4 or 5. These patients should be timely referred to renal care to delay the progress to ESRD and to detect and manage associated conditions, such as anemia, hypertension, dyslipidaemia, hyperkaliemia, hyperphosphatemia, bone mineral disorders, cardiovascular disease and mental health disturbances. The availability of renal care (including RRT) depends on macro-economic parameters, such as gross domestic product (GDP) per capita, the percentage of GDP spent on health care and dialysis reimbursement (76). In countries spending less on health care the need for renal care is not being met, whereas in countries spending more on health care the need for renal care is being created by patients who survive long enough to develop ESRD (76).

Paradoxically, a rise of RRT patients is expected by introducing interventions that reduce cardiovascular mortality (77). Each 1-year increase in life expectancy at 60 years is associated with a 12% increase in RRT incidence (76). In Belgium, half of the patients who start with RRT is older than 75 (50). In the elderly population, the life expectancy is quite limited after the initiation of RRT, many experience loss of functional status and independence, much of the time gained will be spent in a health care setting, and one third of these elderly discontinue maintenance dialysis prior to death (78). This shows that chronic dialysis should not be proposed to patients with a short life expectancy as a necessity but as a choice, and that conservative (nondialytic) care is a legitimate option. In clinical practice, initiation and maintenance of chronic dialysis is encouraged in patients even when the treatment burden outweighs the medical and psychosocial benefits (79). The most important reason for this malpractice is that many nephrologists do not feel comfortable talking with patients about end-of-life treatment preferences (78). The real challenge in nephrology with regards to the ageing problem is to force back the increasing incidence of RRT in adults above the age of 75. We need more therapeutic options and/or better guidelines to delay RRT in CKD patients, particularly in elderly.
3.4.6 *The cost of case-finding should be economically balanced in relation to possible expenditure on medical care as a whole*.

Screening followed by diagnosis and intervention at an early reversible stage of disease should be cost-effective, which means that screening plus intervention should result in a more positive socioeconomic balance than not screening and following the traditional approach. The costs of screening contain not only the expenditure for the screening test, but also those of treating because of false positive screening results and not treating because of false negatives (80). False positive screening tests lead to increased medical visits with further tests, increased health care costs and unnecessary treatment with in some cases adverse effects that further increase cost (80). False negative test results provide false reassurance about the absence of disease and possibly longer waiting before installing appropriate treatment which again may increase costs. Even, a “truly positive test” has a psychological cost for the patient that comes from being labeled earlier in the natural history of the process than would have occurred without screening (80). According to the literature, screening for albuminuria (microalbuminuria and macroalbuminuria) in the general population is not cost-effective, unless selectively directed toward high risk groups or conducted at a ten-year interval (81-83). In addition, the beneficial effect of screening is to large extent based on a delay in onset of ESRD or death. This delay is clearly illustrated in a modeling analysis in patients with diabetes and hypertension that showed an increase of ESRD-free survival from 10.8 to 11.2 years per patient (5 months) and a life expectancy from 10.7 to 11.0 years per patient (4 months) by screening for albuminuria (microalbuminuria and macroalbuminuria) followed by optimized treatment versus no screening (84). However, these modeling studies need to be confirmed in an RCT, whereby the outcome of subjects who are invited for albuminuria screening should be compared to subjects who are not screened. In addition, an RCT obviates a potentially prognostic selection bias that occurs in a population-based screening program as volunteers are more likely to improve their health than those who refuse to be screened (80). However, an RCT to justify screening might fail by contamination. Contamination is the effect of opportunistic case-finding that commonly occurs in those who were randomized not to be screened (the control group). The latter effect was one of the main reasons why screening programs for diabetes and prostate cancer have failed (85-90). We suggest that an RCT to justify screening for CKD might fail as well, as nowadays more and more patients are already treated with ACE-I or ARB, irrespective of the presence of microalbuminuria.
3.4.7 ‘Case-finding should be a continuing process and the interval of testing should be known’.

A benchmark of US$50,000/QALY is often used for judging the cost-effectiveness of interventions (91). Figure 1 shows that only in diabetic patients annual screening vs. no screening is cost-effective. Decreasing the frequency of screening for microalbuminuria in patients with diabetes or hypertension has little impact on QALYs or ESRD incidence while simultaneously reducing the costs (82). As the lowest cost-effectiveness ratios were achieved when screening was started in persons between 50 and 60 years old (figure 1), it was hypothesized that screening for albuminuria (microalbuminuria and macroalbuminuria) beginning at the age of 50 years with an interval of 2 years, was cost-effective in hypertensive patients (82). Screening for albuminuria in subjects with neither diabetes nor hypertension is supposed to be cost-effective if it is started at the age of 50 years only with an interval of 10 years (81, 82). By using such long interval for testing albuminuria, patients with rapidly progressive kidney disease (such as glomerulonephritis) would be hard to identify while subjects with slow disease progression (such as diabetes) would be detected more frequently (length-bias sampling).
Figure 1. Cost-effectiveness of universal annual albuminuria screening beginning at different ages relative to no screening for the full population and subgroups with diabetes or hypertension, adapted from (82).

Whether population screening for microalbuminuria to detect and treat new cases of hypertension, diabetes and CVD might improve outcome is uncertain.
3.5 Conclusions

This thesis explores the pitfalls of screening for microalbuminuria to detect persons with cardiovascular and renal risk in an apparently healthy working (URI) and in a hypertensive (ERICABEL) cohort.

A conducted microalbuminuria screening to detect subjects with cardiovascular and renal risk in the URI population was not effective for two reasons. Firstly, only a small proportion (9%) of the unrecognized cardiovascular risk factors was detected. When traditional cardiovascular risk factors are taken into account to select candidates for screening, more subjects (36%) were detected with possibilities to improve cardiovascular and renal outcome than was the case from a population-based screening for microalbuminuria. Second, as the causes of microalbuminuria are widely divergent, not all subjects with microalbuminuria had at least one cardiovascular risk factor. In addition, the underlying condition of microalbuminuria determines the clinical outcome. This is confirmed in the literature; subjects with orthostatic microalbuminuria have an excellent prognosis, whereas diabetic patients with microalbuminuria have a high risk for overt nephropathy (92, 93). In the cross-sectional URI study, healthy young workers with microalbuminuria had a low BMI, suggesting orthostatic proteinuria. The presence of microalbuminuria was also associated in workers with physicochemical exposure risk. A longitudinal study is necessary to investigate the prognostic value of microalbuminuria in workers with physicochemical exposure risk. Furthermore, a longitudinal study is lacking that observes the outcomes in microalbuminuric subjects with different underlying conditions. Conceivably, interventions in persons with microalbuminuria in absence of cardiovascular risk factors engender medical frustration, unnecessary anxiety, harm, worries and lead to excess of costs, whereas interventions in persons with microalbuminuria in presence of modifiable cardiovascular risk factors may improve clinical outcome.

Microalbuminuria was associated with low BMI and physicochemical exposure risk in workers under the age of 40, whereas microalbuminuria was associated with cardiovascular risk factors in workers above the age of 40. Thus, if a mass screening for microalbuminuria to detect new cardiovascular or renal risk factors is conducted, it should start above the age of 40. Moreover, there is no direct evidence that treatment of microalbuminuria reduces cardiovascular disease.
Screening for microalbuminurie might become useful as new therapeutic options become available to prevent CVD.

According to the literature, the absolute risk to develop ESRD in patients with microalbuminuria seems to be too low to justify a population-based screening for albuminuria (48).

The predictive value of micro- or macroalbuminuria to develop ESRD increases dramatically by selecting subjects with an estimated GFR lower than 60 ml/min/1.73m² (94). It was estimated that 1.4% of a population (with an estimated GFR lower than 60 ml/min/1.73m² and micro- or macroalbuminuria) had an increased risk for ESRD (94). As the prevalence of reduced GFR increases with age, screening of renal markers to identify those with increased risk for ESRD has been suggested to be performed in subjects who are older than 55 years (94). However, the URI population was relatively young and we identified only one subject with microalbuminuria in presence of an estimated GFR lower than 60 ml/min/1.73m². Thus, screening for microalbuminuria in a relatively young population might not be useful to identify subjects with increased risk for ESRD, as almost all subjects had an estimated GFR higher than 60 ml/min/1.73m².

According to the URI study, screening for microalbuminuria is more useful in adults with vs. without cardiovascular risk factors to detect CKD. Screening for albuminuria in diabetic patients is considered to be effective as treatment of an ACE-I or ARB reduces the level of albuminuria and the number of ESRD in those with macroalbuminuria. However, screening is less effective as more and more patients are already treated with ACE-I or ARB, irrespectively of the presence of albuminuria (95). In addition, false positive test results might also occur in presence of cardiovascular risk factors. In the ERICABEL cohort we observed that statin use was associated with microalbuminuria in hypertensive patients, very likely related to the characteristics of statins to induce tubular albuminuria by inhibition of albumin reabsorption. The presence of microalbuminuria in statin users can lead to incorrect labeling of subjects as having an increased risk for overt nephropathy. We need more prospective studies to investigate the prognostic value of microalbuminuria in patients who are treated with statins.

The URI study showed that only persistent microalbuminuria is associated with increased cardiovascular risk. Microalbuminuria should be confirmed in a subsequent urine sample, as a
single measurement could be false positive due to a temporary condition. According to KDIGO, all subjects with microalbuminuria for longer than 3 months have CKD stage A2. Consequently, the prevalence of CKD (A2) is overestimated in most cohorts as mostly one single measurement of microalbuminuria is performed. Moreover, microalbuminuria in half of diabetic patients disappears according longitudinal studies. We showed that fluctuations of microalbuminuria commonly occur in persons without cardiovascular risk factors. The fluctuations of microalbuminuria generate a substantial bias if microalbuminuria is used as a screening tool or as a surrogate for renal or cardiovascular outcome. In the ERICABEL cohort, the presence of microalbuminuria fluctuated in hypertensive patients during a follow-up of 5 years. Thus, doubts could also arise about the diagnostic performance of using microalbuminuria in this population.

A rapid decline of renal function is more frequently observed in subjects with macroalbuminuria than in presence of no albuminuria or microalbuminuria (1, 75). Thus, screening for progressive kidney disease might be more effective by identifying subjects with maceralbuminuria (late or overt nephropathy) than by identifying subjects with microalbuminuria (early or potential nephropathy). Currently, no hard evidence exists whether screening for albuminuria is cost-effective in the general population, even by using a ten-year screening interval. Such mass screening might not be feasible as a substantial number of subjects may develop overt nephropathy over a short period of time. Whether a population screening program that measures albuminuria and estimates GFR (by serum creatinine and/or cystatin C) in subjects who are older than 55 years could reduce the incidence of ESRD should be studied in a RCT.
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CHAPTER 3: General discussion and conclusions


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138


CHAPTER 3: General discussion and conclusions


3.7 Conclusies

Deze thesis omvat twee studies waarbij het opsporen van cardiovasculaire en renale risicofactoren met behulp van microalbuminurie ter discussie staat. In de URI studie werden arbeiders geïncludeerd door arbeidsgeneeskundigen en in de ERICABEL studie werden hypertensie patiënten geïncludeerd door huisartsen.

Een screeningsprogramma om arbeiders op te sporen met cardiovasculaire en renale risicofactoren met behulp van een bepaling van albumine in een urinestaal leek niet zinvol wegens de volgende redenen. Ten eerste, slechts een klein deel (9%) van de personen met ongekende cardiovasculaire risicofactoren werd opgespoord met behulp van detectie van microalbuminurie. Door traditionele cardiovasculaire risicofactoren (bloeddruk, hartslag, bloedglucosespiegel) direct op te sporen, konden veel meer personen (36%) worden gedetecteerd met meer mogelijkheden om nier-, hart- en vaatziekten te voorkomen dan door het opsporen van microalbuminurie alleen. Ten tweede, zijn er ook andere onderliggende oorzaken van microalbuminurie dan louter cardiovasculair risicofactoren. Bijvoorbeeld, de aanwezigheid van orthostatische albuminurie heeft een goede prognose, daar waar de aanwezigheid van albuminurie bij diabetici een hoog risico voor macroalbuminurie en vervolgens nierfalen heeft (1, 2). Arbeiders die blootgesteld werden aan fysisch-chemische agentia hadden een hogere prevalentie van microalbuminurie, onafhankelijk van hun cardiovasculaire risicofactoren. Om de prognostische waarde van microalbuminurie bij arbeiders met fysisch-chemische blootstelling te investigeren is een longitudinale studie nodig. Eveneens ontbreekt er een studie waarbij de prognose van personen met de verschillende onderliggende oorzaken van microalbuminurie met elkaar worden vergeleken. Mogelijks zullen interventies bij personen met microalbuminurie in afwezigheid van cardiovasculaire risicofactoren aanleiding geven tot frustratie, angst, psychologische schade, neveneffecten van onnodig behandelingen en toename van medische kosten, terwijl interventies bij patiënten met microalbuminurie in aanwezigheid van behandelbare risicofactoren aanleiding zouden kunnen geven tot een verbetering van de klinische prognose.

De aanwezigheid van microalbuminurie was geassocieerd met een mager gestalte (orthostatische albuminurie) en aan fysisch-chemische blootstelling in arbeiders jonger dan 40 jaar, terwijl de aanwezigheid van microalbuminurie was geassocieerd met cardiovasculaire risicofactoren in arbeiders ouder dan 40 jaar. Een populatiescreening voor microalbuminurie om cardiovasculaire
en renale risicofactoren op te sporen lijkt dus pas zinvol vanaf het 40ste levensjaar. Doch, er is geen rechtstreeks bewijs dat het opsporen en vervolgens behandelen van microalbuminurie geassocieerd is met een significante daling van hart- en vaatziekten (4, 5). Het opsporen van microalbuminurie zou zinvol kunnen worden als nieuwe behandelingen ter beschikking worden gesteld om hart- en vaatziekten te voorkomen. Alhoewel, personen met microalbuminurie een verhoogd relatief risico vertonen om nierfalen te ontwikkelen, is het absolute risico te laag om een populatiescreening te verantwoorden. De predictieve waarde van microalbuminurie of macroalbuminurie om nierfalen te ontwikkelen stijgt aanzienlijk bij personen met een geschatte eGFR < 60 ml/min/1.73m² (3). Omdat de prevalentie van een geschatte GFR < 60 ml/min/1.73m² toeneemt met de leeftijd, zou een screening om personen met een verhoogd risico op ESRD te identificeren zinvol kunnen zijn vanaf een bepaalde leeftijd (bv. 55 jaar). In de URI populatie, waar bijna iedereen een geschatte GFR > 60 ml/min/1.73m² had (jonge arbeiders populatie) is de predictieve waarde van microalbuminurie om nierfalen te ontwikkelen dus veel te zwak.

Volgens de URI studie is het opsporen van microalbuminurie effectiever bij arbeiders met versus zonder cardiovasculaire risicofactoren om bijvoorbeeld nierschade te kunnen detecteren. Het opsporen van microalbuminurie in aanwezigheid van risicofactoren kan invloed hebben op de behandeling. Bij patiënten met diabetes is het opsporen van microalbuminurie zinvol omdat een behandeling met ACE-I of ARB, de incidentie van macroalbuminurie en vervolgens nierfalen reduceert (1). Alhoewel, het opsporen van microalbuminurie momenteel minder efficiënt is omdat meer en meer patiënten reeds worden behandeld met een ACE-I of ARB, onafhankelijk van de aanwezigheid van albuminurie (5). De afwezigheid van microalbuminurie bij patiënten die behandeld worden met een ACE-I sluit nierschade niet uit, terwijl de aanwezigheid van microalbuminurie in patiënten die behandeld worden met een statine niet altijd geassocieerd is met nierschade. De ERICABEL cohorte observeerde dat het gebruik van een statine geassocieerd was met microalbuminurie. Mogelijk doordat statines tubulaire proteinurie kunnen genereren door inhibtitie van tubulaire reabsorptie. De aanwezigheid van microalbuminurie in patiënten die een statine gebruiken zou aanleiding kunnen geven tot het incorrect vaststellen van een nierziekte. Meer prospectieve studies zijn nodig om de prognostische waarde van microalbuminurie in patiënten die worden behandeld met een statine in kaart te brengen.
De URI studie toonde dat enkel personen met persisterend microalbuminurie geassocieerd waren aan een verhoogd cardiovasculair risico. De aanwezigheid van microalbuminurie dient dus te worden bevestigd in een nieuw urinestaal, omdat tijdelijke omstandigheden (zoals fysische inspanning, koorts, stress e.a.) aanleiding kunnen geven tot een vals positieve meting. Volgens KDIGO, heeft iedereen met microalbuminurie dat langer dan 3 maanden persisteert CKD stadium A2. Hieruit volgt dat de prevalentie van CKD stadium A2 in cohorten overschat wordt wanneer slechts éénmalig microalbuminurie wordt gemeten. Bovendien, zal bij de helft van de diabetici met microalbuminurie “deze aandoening” verdwijnen in functie van de tijd (6). In de URI studie werd bij personen zonder risicofactoren frequent fluctuerende microalbuminurie waargenomen. Het fluctuerende karakter van microalbuminurie werd ook geobserveerd in de ERICABEL cohort (nog niet gepubliceerd).

Een vermindering van de nierfunctie wordt voornamelijk gezien bij personen met macroalbuminurie terwijl dit minder het geval is bij personen met microalbuminurie. Hieruit, zou men kunnen concluderen dat het opsporen van personen met macroalbuminurie effectiever is om nierinsufficiëntie te detecteren dan het opsporen van personen met microalbuminurie. Echter de kosten van een massascreening voor albuminurie worden hoger geschat dan de baten. De screeningskosten kunnen worden gereduceerd door een screeningsinterval van 10 jaar te hanteren. Echter, hierdoor zullen personen die macroalbuminurie ontwikkelen op enkele jaren niet gedetecteerd kunnen worden. Of een screeningsprogramma aanleiding zou kunnen geven om de incidentie van ESRD te reduceren door bepaling van albuminurie en een schatting van de GFR (door serumcreatinine en/of Cystatine C te meten) in personen ouder dan bv. 55 jaar dient in de toekomst onderzocht te worden.
CHAPTER 3: General discussion and conclusions

3.8 Referenties


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Arjan 2014
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Yogyakarta earthquake (Java May 2006)
    Chicuan earthquake (Chengdu May 2008)
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4 SCIENTIFIC PUBLICATIONS

ARTICLES IN INTERNATIONAL JOURNALS WITH REFEREE SYSTEM


ARTICLES IN NATIONAL JOURNALS


5 ABSTRACTS


Van der Tol A, Van Biesen W, De Groote G, Vermeiren F, Eeckhaut K, Vanholder R. Do women with CKD stage 3 as estimated by MDRD really have CKD? M272. World Congress of Nephrology 2009, Milan, Italy


