

New insights into urinary proteins as markers of cardiovascular risk in hypertension

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More than 20 years ago, the Framingham Study demonstrated that an increase in urinary proteins was associated with a high risk of cardiovascular events, both coronary heart disease and stroke [1]. Not until recently has the importance of urinary albumin excretion (UAE) as a prognostic marker in hypertension been recognized. Initially, information came from cross-sectional studies that demonstrated a clustering of cardiovascular risk factors and organ damage associated with an increase in UAE [2–5]. Subsequently, from follow-up studies, a given value of UAE measured at the beginning was associated with total mortality, and with cardiovascular mortality or morbidity over time [6–8]. Furthermore, the level of albuminuria during antihypertensive treatment with either losartan- or atenolol-based therapy was closely related to cardiovascular risk during treatment, implying that changes in albuminuria translate to changes in risk [9]. Consequently, UAE is presently the subject of intense research. Moreover, microalbuminuria assessment is now recommended in a risk stratification strategy for hypertension management [10,11] because it offers valuable information that is easy to obtain at low cost.

In this issue of the journal, Schrader *et al.* [12] analysed the prognostic value of urinary proteins with respect to the occurrence of cardiovascular and cerebrovascular events and all-cause mortality in a large cohort of hypertensives during an angiotensin-converting enzyme-based treatment over an average of 42.5 months. In addition, the authors assessed the treatment-induced reduction of urinary proteins and their impact on the outcome of events. Besides confirming the prognostic value of urinary proteins with respect to the risk of developing cardiovascular events and total mortality, their study introduces other points that deserve comment, including the significance of tubular proteinuria in hypertension as a marker of cardiovascular risk, and whether or not a reduction in urinary protein is followed by a reduction in risk.

Tubular proteinuria in hypertension

The name tubular proteinuria refers to the excretion of low-molecular weight proteins that are freely and regularly filtered in the glomeruli and undergo a process of reabsorption by high-capacity receptor-mediated endocytosis in the proximal tubule [13]. β_2 -microglobulin and α_1 -microglobulin are among the low-molecular weight proteins that are used as markers of tubular dysfunction. Although α_1 -microglobulin is more stable than β_2 -microglobulin at low pH [14], α_1 -microglobulin excretion has some relation to glomerular selectivity, which partly limits the capacity to separate the glomerular or tubular origin of the α_1 -microglobulin measurements [15].

At the molecular level, two multiligand endocytic receptors, megalin and cubilin, appear to be largely responsible for the proximal tubular reabsorption of proteins [16,17]. The two receptors apparently collaborate in the uptake of ligands and, following binding to the receptors at the apical membrane, the ligands are internalized into coated vesicles and subsequently into early and late endosomes. Whereas the receptors are recycled to the apical membranes, the ligands are transferred to lysosomes for degradation of the protein. The tubular protein can result from defects in glomerular selectivity with an increase in the amount of proteins in the ultrafiltrate, from extrarenal overload proteinuria or from defects in the tubular cells themselves [16]. In glomerular abnormalities and in overload proteinuria, although the capacity of the proximal tubule for reabsorption is very high, an overload of protein due to competitive events for receptor binding may result in the excretion of not only the abnormal proteins, but also of other proteins than those normally reabsorbed and processed in the proximal tubule.

Hypertension-induced renal damage involves not only glomerular structures, but also tubulointerstitial ones [18,19]. The consequences of high blood pressure in the glomeruli, and the resulting increment in the passage of albumin leading to an increase in urinary albumin excretion, even in small amounts, have been studied to a great extent [20]. However, the tubulointerstitial changes have received less attention because these can only be assessed in experimental models or in biopsy specimens. An increase in blood pressure could result in increased transmission of pressure to the glomeruli and to the postglomerular vasculature, particularly in the juxta-medullary region. Although the glomerular structures are able to handle the elevated pressures due to the presence of smooth muscle-like mesangial cells and podocytes, the

postglomerular capillary network is less adapted to protect itself from increased pressure. An increment in peritubular capillary pressure results in morphologic changes and the recruitment of muscle-like myofibroblasts that encircle the peritubular capillaries. Moreover, there are functional changes, such as the loss of endothelial nitric oxide synthase in endothelial cells [21]. These changes could lead to permanent damage, with a significant reduction in the number of peritubular capillaries, and this rarefaction phenomenon has been reported in renal biopsies from essential hypertension [22].

The presence of tubular proteinuria and urinary albumin excretion overlaps not only in the study of Schrader *et al.* [12], which was carried out in hypertensives, but also in a recent report in type 2 diabetics [23]. Both studies have relevant data in common: (i) the higher the urinary albumin excretion level, the higher the α_1 -microglobulin excretion level; (ii) the prevalence of α_1 -microglobulin excretion is higher in the subjects with microalbuminuria and/or proteinuria; and (iii) a non-negligible percentage of subjects (approximately 15%) with 'normal' urinary albumin excretion had high α_1 -microglobulin excretion. Consequently, some questions arise from these findings. What does tubular proteinuria mean in these patients? Does tubular proteinuria indicate relevant tubulointerstitial damage that leads to abnormal handling of proteins in the proximal tubule? Is tubular proteinuria a consequence of the increased filtered albumin in the glomeruli or is the microalbuminuria, in part, a consequence of tubular dysfunction?

There are plentiful data in favour of the hypothesis that tubular proteinuria is parallel to and may depend on the increased filtration of albumin in this hypertensive population. To begin with, there is a close relationship between urinary albumin excretion and tubular proteinuria in the above-mentioned studies [12,23]; the higher the urinary albumin, the higher the α_1 -microglobulin excretion. Second, the reduction of tubular proteinuria is in the same proportion as that for albumin due to antihypertensive treatment [12]. A significant reduction of albumin excretion during antihypertensive treatment is mainly produced by decreasing the high blood pressure-induced haemodynamic overload, and it is less marked when structural damage is present. The impact of haemodynamic forces on the proximal tubular cells is less direct than it is on the glomeruli, and tubular structural damage is not expected to be reduced by antihypertensive treatment. Third, tubular proteinuria has a lower risk for cardiovascular events than microalbuminuria or the combination of tubular proteinuria plus microalbuminuria [12].

If we assume that tubular proteinuria is dependent on urinary albumin in hypertension, how can we explain the

presence of tubular proteinuria in the absence of microalbuminuria? Two potential explanations, not mutually exclusive, can be offered. One is related to the threshold for defining microalbuminuria and the other is related to the presence of non-immunoreactive albumin in the urine of these patients.

Microalbuminuria is defined as an UAE of 30–300 mg/24 h (or equivalent amounts) using timed-overnight or spot urine samples. The definition comes from studies that have established its value as a marker of the risk of developing nephropathy in diabetic subjects [24]. When the potential prognostic value of microalbuminuria on cardiovascular disease was assessed in both diabetic and non-diabetic populations, the threshold value pointing to an increment of risk was largely below the UAE value of 30 mg/24 h, regardless of the population studied [25]. Therefore, many of the 'normoalbuminurics' in the study by Schrader *et al.* [12] would have had urinary albumin in the highest range of 'normality' and demonstrated high tubular protein excretion even though they were considered to be normoalbuminuric.

Recently, stress has been proposed to explain the presence of a non-negligible amount of complete molecules of albumin that are not recognized by immunological methods, but are by high-performance liquid chromatography [26]. Although the clinical significance of this non-immunoreactive albumin is not fully understood, the proportion of non-immunoreactive over immunoreactive albumin depends on the total amount of albumin in urine; the lower the amount, the higher the proportion [27]. It is plausible that subjects with isolated tubular proteinuria have a non-negligible amount of non-immunoreactive albumin that may interfere with the reabsorption of tubular protein, such as immunoreactive albumin.

Changes in urinary proteins and cardiovascular risk in hypertension

A key point in considering UAE and/or urinary proteins as an intermediate endpoint is the demonstration that a reduction in urinary proteins is followed by a significant reduction in cardiovascular and/or renal events. To date, only one study, which was not initially designed to test the hypothesis, has been published [9]. The Losartan Intervention For Endpoint reduction in hypertension (LIFE) [8,9], a double-blind study of hypertensives with left ventricular hypertrophy in which subjects were randomized in parallel arms to losartan or atenolol, is the largest ever study with microalbuminuria assessment carried out upon entry and at yearly intervals during study follow-up. Although the study is limited by UAE assessment being performed using a yearly unique spot urine sample, the large number of subjects analysed partly overcomes these limitations. The study has amply demonstrated that the rate of the primary

composite cardiovascular endpoint of cardiovascular death, fatal or non-fatal stroke, and fatal or non-fatal myocardial infarction, increases by four- to five-fold from the lowest to the highest decile of albumin/creatinine ratio [9].

In the study by Schrader *et al.* [12], the authors provide information about the changes in urinary proteins during follow-up and the relationship with the outcome of cardiovascular events. However, the information provided was limited because it was presented as qualitative changes (from normal to normal, from normal to pathological, from pathological to normal and from pathological to pathological) and no quantitative analysis with urinary protein values was performed. Whatever the case, normalization of any pathological urinary protein during treatment was associated with a trend to fewer cardiovascular events compared with persisting proteinuria. Conversely, newly-developed proteinuria was associated with a trend to increase events. Taken together, these two studies suggest that in-treatment levels of albumin are closely related to the risk of a subsequent cardiovascular event.

Future studies employing an appropriate design and analysis are required to better understand the nature and significance of the different type of urinary proteins [28] and to acknowledge microalbuminuria as an intermediate endpoint [29].

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