

Albuminuria – chasing the message, not the messenger

Henrik Birn, Simon D. Roger

Department of Renal Medicine, Gosford Hospital, Gosford, NSW, Australia.

Received for publication: 25/03/2009
Accepted: 01/04/2009

Key-Words:

Albumin, angiotensin, chronic kidney disease, endocytosis, hypertension, megalin, proximal tubule.

emerging evidence for a significant role of renal proximal tubule cell function.

INTRODUCTION

The presence of abnormal amounts of albumin in the urine has evolved from a diagnostic marker in kidney disease into a prognostic marker important for establishing prognosis and treatment options not only in kidney disease, but also in a number of other conditions including diabetes and cardiovascular disease. Treatment of albuminuria is based on clinical trials that have established the benefit of blood pressure control and inhibition of the renin-angiotensin system. Despite greater understanding of the molecular mechanisms regulating urinary excretion of albumin, the fundamental changes that lead to increased albumin excretion in such a variety of different conditions are still poorly understood. This review will focus on some of the potential pathophysiological mechanisms involved emphasising the

IDENTIFYING ALBUMINURIA – WHAT SHOULD WE MEASURE?

The gold standard for measurement of albuminuria is a timed, usually 24-hour urine collection. The current cut-off values (Table 1) defining albuminuria and microalbuminuria has been challenged by several studies showing an increased risk of cardiovascular disease associated with even lower levels of urinary albumin excretion¹⁻³ also suggesting that there may not be a well-defined lower limit for the association between albumin excretion in the urine and cardiovascular risk. An albumin-creatinine ratio correlates well with 24-hour urine collections and may serve as a substitute for the diagnosis and quantitation of albuminuria⁴ and is recommended by most guidelines^{5,6}. A morning spot urine sample representing overnight urine production is a better estimate of 24-hour urine collection than a random sample⁷. Urine creatinine excretion is

Table 1

Cut-off values for urinary albumin excretion.^{5,4} The test is considered positive if elevated on two out of three occasions.

	24 h urine	Albumin/creatinine-ratio	Albumin/creatinine-ratio (male)	Albumin/creatinine-ratio (female)
Normal albumin-excretion	<30 mg/24 h (<20 µg/min)	<3.5 mg/mmol (<30 mg/g)	<2.5 mg/mmol (<20 mg/g)	<3.5 mg/mmol (<30 mg/g)
Micro-albuminuria	30 to 300 mg/24 h (20 to 200 µg/min)	3.5 to 35 mg/mmol (30 to 300 mg/g)	2.5 to 25 mg/mmol (20 to 200 mg/g)	3.5 to 35 mg/mmol (30 to 300 mg/g)
Albuminuria	>300 mg/24 h (>200 µg/min)	>35 mg/mmol (>300 mg/g)	>25 mg/mmol (>200 mg/g)	>35 mg/mmol (>300 mg/g)

dependent on a number of factors including sex, age, and muscle mass. Thus, when estimating 24-hour urine albumin excretion using albumin-creatinine ratio, the reference interval is sex- and age-dependent. While this has been documented in several studies many laboratories do not correct for this and so far there is limited documentation of clinical benefit from applying sex- and age-related reference intervals. Studies indicate that significant amounts of albumin fragments are excreted in the urine, possibly resulting from tubular degradation of filtered albumin, followed by luminal secretion of albumin fragments^{8,9}. Although the clinical implication of this has not been established, changes in the relative excretion of albumin fragments to intact albumin have been identified in diabetes^{10,11}, possibly reflecting changes in tubular function. This could indicate that measurements of urinary albumin fragments could provide further information about kidney dysfunction.

■ MECHANISM OF ALBUMINURIA – WHAT IS THE CONCERN?

Proteinuria has been a long standing indicator of kidney disease. In this setting proteinuria is believed to reflect a change in the balance between glomerular filtration and tubular reabsorption of protein either as a result of increased filtration, or decreased tubular reabsorption, or both. Endothelial dysfunction is associated with microalbuminuria and may in fact precede the development of the latter, suggesting that vascular changes play a role both in the development of microalbuminuria and associated cardiovascular risk¹². Albumin is the major plasma protein and constitutes about 75% of urinary protein in many cases of proteinuria¹³. The amount of albumin normally filtered in the glomerulus has been the focus of recent controversy. Several micropuncture studies have estimated the concentration of albumin in the ultrafiltrate between 1 and 50 µg/ml, corresponding to a filtered load of albumin between 170 mg and 9 g per 24-hour in humans¹⁴. A controversial study in rats based on two-photon confocal microscopy has suggested the normal filtration of very large amounts of albumin corresponding to >100 g of albumin per day in humans¹⁵. This, however, could not be confirmed by others using a similar technique¹⁶. Despite these controversies it is apparent that normal tubular reabsorption of albumin is crucial to prevent albuminuria

and filtered albumin is readily reabsorbed by efficient proximal tubule reabsorptive mechanisms^{17,18}. Receptor mediated endocytosis involving two large, multiligand receptors, megalin and cubilin, has been identified as the major mechanism responsible for the proximal tubule reabsorption of albumin¹⁹⁻²¹. This process involving the interaction between multiple membrane proteins constitutes a powerful apparatus for the uptake of a large number of different proteins^{17,22}. In addition to an essential role in tubular uptake of proteins, these receptors are important regulators of lysosomal activity²³ and possess intracellular signalling potential establishing a link between these receptors and changes in tubular function²⁴. Tubular dysfunction has been identified in a number of conditions associated with albuminuria, including early diabetes²⁵⁻²⁷. Furthermore, experimental studies have shown that albumin causes tubular changes eventually leading to the induction of inflammation, tubular epithelial-mesenchymal transformation and interstitial fibrosis¹⁸. Overload albuminuria in rats and mice results in interstitial inflammation and fibrosis and albumin exposure *in vitro* induces the expression of a number of inflammatory and fibrogenic mediators²⁸⁻³⁵ and causes apoptosis^{36,37} and fibrosis³⁸. The exact cascade of events leading to changes in proximal tubule phenotype has not been fully identified and may involve signalling pathways dependent and/or independent on the tubular endocytosis of albumin^{24,39,40}.

■ TREATMENT OF ALBUMINURIA – WHAT ARE WE TREATING?

Non-nephrotic proteinuria in general causes no symptoms and thus the aim of treatment is to prevent the associated renal and cardiovascular morbidity and mortality. There is a strong association between the reduction of albuminuria and attenuation of renal deterioration in patients with renal disease, almost irrespective of cause. Similarly there is strong clinical evidence that treatment of microalbuminuria in diabetes is associated with a decreased risk of morbidity. These clinical observations constitute the rationale for treating albuminuria in these patients. Despite the association between microalbuminuria and cardiovascular disease, there is currently no compelling clinical evidence for the specific treatment of microalbuminuria outside the

diabetic population. The primary aim of treatment in the microalbuminuric, non-diabetic population is to reduce overall cardiovascular risk by multiple interventions including lifestyle intervention, blood pressure control and treatment of dyslipidaemia.

Strict blood pressure control is a well recognised, important intervention in renal protection and reduction of albuminuria. This effect is observed with all anti-hypertensives, however, there is additional evidence that inhibition of the renin-angiotensin system (RAS) using ACE inhibitors and angiotensin II receptor blockers provides additional reduction of urinary albumin and preservation of renal function. Several studies have been performed to demonstrate the additional renoprotective effect of RAS blockade as compared to other anti-hypertensives both in diabetic and non-diabetic renal disease⁴¹. Although this position has been challenged by others^{42,43}, including the large ALLHAT trial⁴⁴, most guidelines recommend the use of ACE-inhibitors or angiotensin receptor blockers in the setting of significant albuminuria and hypertension or diabetes^{45,46}. Additional use of aldosterone blockade and HMGCo-ase inhibitors has been shown to reduce albuminuria in selected patients^{47,48}, but long term benefits are still to be established. While the effect of these drugs in part may be due to their effects on renal haemodynamics and glomerular autoregulation⁴⁹ there is experimental evidence to suggest additional direct and indirect effects of angiotensin II on proximal tubular handling of proteins. Proximal tubule cells express both the angiotensin II type 1 (AT-1) receptor and the AT-2 receptor⁵⁰. In rats as well as *in vitro* angiotensin II decreases expression of the albumin receptor megalin and reduces cellular albumin endocytosis^{51,52}. In rats with experimental diabetes decreased albumin endocytosis is restored by treatment with ACE-inhibitor and AT-1 receptor blocker⁵¹. *In vitro* studies suggest that stimulation of AT-2 receptors, but not AT-1 receptors may stimulate albumin uptake, suggesting that the balance between stimulation of the two angiotensin II receptors may be important for regulation of tubular albumin uptake⁵³. Thus, there is experimental evidence supporting direct, positive tubular effects of RAS blockade in addition to its established effects on blood pressure and renal haemodynamics. Whether these effects are important for the protection of renal function in proteinuric kidney disease remains to be established.

■ PERSPECTIVES

Albuminuria is an important indicator and prognostic factor in kidney disease and other conditions. The mechanisms of albuminuria include glomerular, tubular, and possibly vascular dysfunction. Several treatment strategies aimed at reduction in albuminuria has been established and shown to be effective in clinical studies, however, our understanding of the mechanism(s) by which this effect is mediated is still not fully elucidated. Whereas experimental studies to some extent allows for the separation of various pathophysiological mechanisms, this is difficult in the setting of human disease confounded not only by multiple coexisting disease mechanisms but also by the heterogeneity in disease presentation and background. A major challenge is to identify reliable markers of glomerular, vascular and tubular dysfunction which can be used to separate these in a clinical setting and facilitate the development and evaluation of targeted therapy.

Conflict of interest statement. None declared.

References

- Weir MR. Microalbuminuria and cardiovascular disease. *Clin J Am Soc Nephrol* 2007;2:581-590
- Klausen K, Borch-Johnsen K, Feldt-Rasmussen B, *et al*. Very low levels of microalbuminuria are associated with increased risk of coronary heart disease and death independently of renal function, hypertension, and diabetes. *Circulation* 2004;110:32-35
- Ruggenenti P, Remuzzi G. Time to abandon microalbuminuria? *Kidney Int* 2006;70:1214-1222
- Ewald B, Attia J. Which test to detect microalbuminuria in diabetic patients? A systematic review. *Aust Fam Physician* 2004;33:565-571
- Guideline Development Group members. Urinary albumin:creatinine and protein:creatinine ratios, and their relationship to 24-hour urinary protein. In: *Chronic Kidney Disease. National clinical guideline for early identification and management in adults in primary and secondary care*, London: Royal College of Physicians of London, 2008;41-47
- K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002;39:S1-266
- Witte EC, Lambers Heerspink HJ, de Zeeuw D, Bakker SJ, de Jong PE, Gansevoort R. First morning voids are more reliable than spot urine samples to assess microalbuminuria. *J Am Soc Nephrol* 2009;20:436-443
- Bakker SJ, Gansevoort RT, de Zeeuw D. Albuminuria: what can we expect from the determination of nonimmunoreactive albumin? *Curr Hypertens Rep* 2009;11:111-117
- Osicka TM, Panagiotopoulos S, Jerums G, Comper WD. Fractional clearance of albumin is influenced by its degradation during renal passage. *Clin Sci (Lond)* 1997;93:557-564
- Comper WD, Osicka TM, Jerums G. High prevalence of immuno-unreactive intact albumin in urine of diabetic patients. *Am J Kidney Dis* 2003;41:336-342
- Strong KJ, Osicka TM, Comper WD. Urinary-peptide excretion by patients with and volunteers without diabetes. *J Lab Clin Med* 2005;145:239-246

12. de Zeeuw D, Parving HH, Henning RH. Microalbuminuria as an early marker for cardiovascular disease. *J Am Soc Nephrol* 2006;17:2100-2105
13. Bottini PV, Ribeiro Alves MA, Garlipp CR. Electrophoretic pattern of concentrated urine: comparison between 24-hour collection and random samples. *Am J Kidney Dis* 2002;39:E2
14. Maack T. Renal handling of proteins and polypeptides. In: Windhager, EE, ed. *Handbook of Physiology. Renal Physiology*, New York: Oxford University Press, 1992;2039-2082
15. Russo LM, Sandoval RM, McKee M, *et al*. The normal kidney filters nephrotic levels of albumin retrieved by proximal tubule cells: retrieval is disrupted in nephrotic states. *Kidney Int* 2007;71:504-513
16. Tanner GA. Glomerular sieving coefficient of serum albumin in the rat: a two-photon microscopy study. *Am J Physiol Renal Physiol* 2009; In press.
17. Birn H, Christensen EI. Renal albumin absorption in physiology and pathology. *Kidney Int* 2006;69:440-449
18. Pollock CA, Poronnik P. Albumin transport and processing by the proximal tubule: physiology and pathophysiology. *Curr Opin Nephrol Hypertens* 2007;16:359-364
19. Birn H, Fyfe JC, Jacobsen C, *et al*. Cubilin is an albumin binding protein important for renal tubular albumin reabsorption. *J Clin Invest* 2000;105:1353-1361
20. Zhai XY, Nielsen R, Birn H, *et al*. Cubilin- and megalin-mediated uptake of albumin in cultured proximal tubule cells of opossum kidney. *Kidney Int* 2000;58:1523-1533
21. Christensen EI, Birn H. Renal handling of albumin in normal rat. *Kidney Int* 2000; 57:1207-1209
22. Christensen EI, Birn H. Megalin and cubilin: multifunctional endocytic receptors. *Nat Rev Mol Cell Biol* 2002;3:256-266
23. Nielsen R, Courtroy PJ, Jacobsen C, *et al*. Endocytosis provides a major alternative pathway for lysosomal biogenesis in kidney proximal tubular cells. *Proc Natl Acad Sci U S A* 2007;104:5407-5412
24. Biemesderfer D. Regulated intramembrane proteolysis of megalin: linking urinary protein and gene regulation in proximal tubule? *Kidney Int* 2006;69:1717-1721
25. Tojo A, Onozato ML, Ha H, Kurihara H, Sakai T, Goto A, Fujita T, Endou H. Reduced albumin reabsorption in the proximal tubule of early-stage diabetic rats. *Histochem Cell Biol* 2001;116:269-276
26. Russo LM, Sandoval RM, Campos SB, Molitoris BA, Comper WD, Brown D. Impaired tubular uptake explains albuminuria in early diabetic nephropathy. *J Am Soc Nephrol* 2009;20:489-494
27. Hryciw DH, Lee EM, Pollock CA, Poronnik P. Molecular changes in proximal tubule function in diabetes mellitus. *Clin Exp Pharmacol Physiol* 2004;31:372-379
28. Zoja C, Donadelli R, Colleoni S, *et al*. Protein overload stimulates RANTES production by proximal tubular cells depending on NF-kappa B activation. *Kidney Int* 1998;53:1608-1615
29. Zoja C, Morigi M, Figliuzzi M, *et al*. Proximal tubular cell synthesis and secretion of endothelin-1 on challenge with albumin and other proteins. *Am J Kidney Dis* 1995;26:934-941
30. Wang Y, Chen J, Chen L, Tay YC, Rangan GK, Harris DC. Induction of monocyte chemoattractant protein-1 in proximal tubule cells by urinary protein. *J Am Soc Nephrol* 1997;8:1537-1545
31. Drumm K, Bauer B, Freudinger R, Gekle M. Albumin induces NF-kappaB expression in human proximal tubule-derived cells (HKC-1). *Cell Physiol Biochem* 2002;12:187-196
32. Tang S, Leung JC, Abe K, *et al*. Albumin stimulates interleukin-8 expression in proximal tubular epithelial cells in vitro and in vivo. *J Clin Invest* 2003;111:515-527
33. Yard BA, Chorianopoulos E, Herr D, van der Woude FJ. Regulation of endothelin-1 and transforming growth factor-beta1 production in cultured proximal tubular cells by albumin and heparan sulphate glycosaminoglycans. *Nephrol Dial Transplant* 2001;16:1769-1775
34. Wohlfarth V, Drumm K, Mildenerger S, Freudinger R, Gekle M. Protein uptake disturbs collagen homeostasis in proximal tubule-derived cells. *Kidney Int* 2003;63:5103-5109
35. Donadelli R, Zanchi C, Morigi M, *et al*. Protein overload induces fractalkine upregulation in proximal tubular cells through nuclear factor kappaB- and p38 mitogen-activated protein kinase-dependent pathways. *J Am Soc Nephrol* 2003;14:2436-2446
36. Thomas ME, Brunskill NJ, Harris KP, *et al*. Proteinuria induces tubular cell turnover: A potential mechanism for tubular atrophy. *Kidney Int* 1999;55:890-898
37. Erkan E, De Leon M, Devarajan P. Albumin overload induces apoptosis in LLC-PK(1) cells. *Am J Physiol Renal Physiol* 2001;280:F1107-F1114
38. Zoja C, Benigni A, Remuzzi G. Cellular responses to protein overload: key event in renal disease progression. *Curr Opin Nephrol Hypertens* 2004;13:31-37
39. Baines RJ, Brunskill NJ. The molecular interactions between filtered proteins and proximal tubular cells in proteinuria. *Nephron Exp Nephrol* 2008;110:e67-e71
40. Motoyoshi Y, Matsusaka T, Saito A, *et al*. Megalin contributes to the early injury of proximal tubule cells during nonselective proteinuria. *Kidney Int* 2008;74:1262-1269
41. Wenzel RR. Renal protection in hypertensive patients: selection of antihypertensive therapy. *Drugs* 2005;65 Suppl 2:29-39:29-39
42. Casas JP, Chua W, Loukogeorgakis S, *et al*. Effect of inhibitors of the renin-angiotensin system and other antihypertensive drugs on renal outcomes: systematic review and meta-analysis. *Lancet* 2005;366:2026-2033
43. Esnault VL, Brown EA, Apetrei E, *et al*. The effects of amlodipine and enalapril on renal function in adults with hypertension and nondiabetic nephropathies: a 3-year, randomized, multicenter, double-blind, placebo-controlled study. *Clin Ther* 2008;30:482-498
44. Rahman M, Pressel S, Davis BR, *et al*. Renal outcomes in high-risk hypertensive patients treated with an angiotensin-converting enzyme inhibitor or a calcium channel blocker vs a diuretic: a report from the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). *Arch Intern Med* 2005;165:936-946
45. KDOQI. *Chronic Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease*. *Am J Kidney Dis* 2007;49:S12-S154
46. Harris D, Thomas M, Johnson D, Nicholls K, Gillin A. The CARL guidelines. Prevention of progression of kidney disease. *Nephrology (Carlton)* 2006;11:S2-S197
47. Bomback AS, Kshirsagar AV, Amamoo MA, Klemmer PJ. Change in proteinuria after adding aldosterone blockers to ACE inhibitors or angiotensin receptor blockers in CKD: a systematic review. *Am J Kidney Dis* 2008;51:199-211
48. Sandhu S, Wiebe N, Fried LF, Tonelli M. Statins for improving renal outcomes: a meta-analysis. *J Am Soc Nephrol* 2006;17:2006-2016
49. Bidani AK and Griffin KA. Pathophysiology of hypertensive renal damage: implications for therapy. *Hypertension* 2004;44:595-601.
50. Miyata N, Park F, Li XF, Cowley AW, Jr. Distribution of angiotensin AT1 and AT2 receptor subtypes in the rat kidney. *Am J Physiol* 1999;277:F437-F446
51. Tojo A, Onozato ML, Kurihara H, Sakai T, Goto A, Fujita T. Angiotensin II blockade restores albumin reabsorption in the proximal tubules of diabetic rats. *Hypertens Res* 2003;26:413-419
52. Hosojima M, Sato H, Yamamoto K, *et al*. Regulation of megalin expression in cultured proximal tubule cells by angiotensin II type 1A receptor- and insulin-mediated signaling cross talk. *Endocrinology* 2009;150:871-878
53. Benndorf RA, Krebs C, Hirsch-Hoffmann B, *et al*. Angiotensin II type 2 receptor deficiency aggravates renal injury and reduces survival in chronic kidney disease in mice. *Kidney Int* 2009; In press
54. de Jong PE, Curhan GC. Screening, monitoring, and treatment of albuminuria: Public health perspectives. *J Am Soc Nephrol* 2006;17:2120-2126

Correspondence to:

Dr Simon D. Roger
 Department of Renal Medicine, Gosford Hospital,
 Gosford 2250, NSW, Australia
 Email: sroger@nscchahs.health.nsw.gov.au