# REVIEWS

Marian Klinger, Barbara Bernat

# Nephrotic syndrome – recent advances. Proteinuria as a manifestation and a mediator of progressive renal disease

## Zespół nerczycowy – najnowsze doniesienia. Białkomocz jako objaw i czynnik sprawczy postępujących chorób nerek

Department of Nephrology and Transplantation Medicine Wroclaw Medical University, Poland

## Abstract

Proteinuria is a common marker of glomerular injury and is connected with alterations of structure of filtration barrier. Podocyte proteins: nefrin, CD2-AP, podocin are pivotal elements in maintenance of proper glomerular filtration. The massive proteinuria is the cause of glomerular and interstitial fibrosis. Treatment with agents inhibiting the renin-angiotensin system retards the progressive course of chronic renal diseases (**Adv. Clin. Exp. Med. 2003, 12, 2, 179–183**).

**Key words:** proteinuria, glomerular filtration barrier, interstitial fibrosis, angiotensin II receptor antagonists, angiotensin converting enzyme inhibitors.

## Streszczenie

Białkomocz należy do głównych wskaźników uszkodzenia kłębuszków nerkowych. Białka komórek stopowatych: nefryna, CD2-AP i podocyna, są osiowymi elementami utrzymującymi prawidłową filtrację kłębuszkową. Masywny białkomocz powoduje włóknienie kłębuszków i tkanki śródmiąższowej. Stosowanie leków hamujących układ renina-angiotensyna opóźnia postęp przewlekłych chorób nerek (**Adv. Clin. Exp. Med. 2003, 12, 2, 179–183**).

**Słowa kluczowe:** białkomocz, bariera filtracyjna, włóknienie śródmiąższowe, antagoniści receptora angiotensyny II, inhibitory enzymu konwertującego.

Proteinuria is one of the hallmarks of the glomerular injury and dysfunction. It manifests as an increase in urinary excretion of different plasma proteins, namely albumin and immunoglobulins. The studies in humans and in experimental animals have been focused on finding components of filtration apparatus that damaged cause proteinuria.

Filtration through the glomerular capillary wall occurs along an extracellular pathway including the endothelial pores, the GBM and the slit diaphragm. All these components are quite permeable for water, ions and small solutes. The barrier function of glomerular capillary wall for macromolecules is selective for size, shape and charge. The size selectivity of the filtration barrier is in part established by the dense network of the GBM. The most restrictive structure appears to be the slit diaphragm. The charge selectivity results from the dense accumulation of negatively charged molecules throughout the entire depth of the filtration barrier, including the surface coat of endothelial cells, and the high content of negatively charged heparan sulfate proteoglicans in the GBM and glycocalyx enriched with sialoglycoproteins on the surface of visceral epithelial cells [1].

## Mechanisms of proteinuria

Functional studies in proteinuric states in humans have shown that usually there is a rela-

tively higher elevation of the clearance values for albumin as compared to IgG. It is seen especially in minimal change disease and in early stage of diabetic nephropathy.

The fact that there are always variable degrees of alteration in size-selectivity along with loss of charge-selectivity suggests that there are certain structural components of the filtration barrier that are commonly affected and which ultimately influence the values of both parameters of the fractional clearance at the same time [1].

## Glomerular basement membrane alterations

There is a wide variety of circulating cations that could localize in the GBM in various forms of glomerulonephritis and cause the neutralization of the GBM. The most prevalent ones include lisozyme and myeloperoxidase derived from macrophages and leucocytes and factor 4 derived from platelets. In addition, several cationic antigens and antibodies have been shown to bind with the GBM anionic sites and induce an immune injury, e.g. nucleosomes in lupus nephritis. The thickening of GBM is observed in membranous glomerulonephritis, diabetic nephropathy and Alport's syndrome. Also in the latter and in the thin basement membrane nephropathy the thin GBM is seen [2].

Degradation of the GBM components: the core-peptide of proteoglicans, type IV collagen, laminin, fibronectin [2] occur by the action of proteolytic enzymes, serine proteases such as elastase [3]. This is supported by the finding of increased urinary excretion of GBM, type IV collagen and laminin fragments in various types of glomerulonephritis. Preexposure of the GBM to reactive oxygen species (ROS) action increase their susceptibility to proteolytic degradation. ROS may cause distortion of GBM affecting podocyte-GBM connection [4, 5]. Moreover ROS induce granulocyte macrophage colony stimulating factor (GM-CSF) production by podocytes enhancing glomerular inflammation and glomerular injury [6].

## Visceral epithelial cell alterations

Accumulating evidence indicates that podocytes, with their primary and secondary foot processes, and the interpodocyte slit membranes are the final glomerular seal to prevent leakage of circulating plasma proteins.

The alterations in podocytes are seen almost universally in most proteinuric states [5, 7]. These include fusion and effacement of foot processes with focal areas of detachment from the GBM. The alterations in the podocyte foot processes with the loss of sialoglycoproteins, and podocyte detachment from the GBM are the critical events that lead to accentuated transglomerular passage of proteins, along with the loss of cell surface negative charge.

Podocytes in the adult are incapable of regenerative cell replication. The loss of podocytes for any reason can only be compensated for by hypertrophy of the remaining podocytes.

Loss of podocytes leads to the loss of separation of the tuft from Bowman's capsule resulting in a segmental synechia. Naked GBM areas allow access to the GBM by the parietal cells of Bowman's capsule. Thereby a gap in the parietal epithelium comes into existence, through which glomerular tuft structure come into direct communications with the interstitium. Tuft adhesion represents the first stage in the development of segmental glomerulosclerosis [8, 9].

Data have been presented that misdirected filtration through Bowman's capsule, with filtrate spreading along the corresponding tubule, might be an essential mechanism accounting for the extension of the glomerular damage to the tubule and the surrounding interstitium [10].

## Podocytopenia and disease severity in IgA nephropathy

IgA nephropathy is common form of glomerulonephritis and is characterized by mesangial deposition of IgA. Patients with severe renal dysfunction had a reduced number of podocytes per glomerulus. The degree of podocytopenia correlated with the extent of glomerular sclerosis and of impairment of permselectivity and GFR, with worsening injury below an apparent threshold podocyte number of about 250 cells per glomerulus. There were no corresponding correlations between these indices of injury and the number of mesangial and endothelial cells [11].

## Nephrin – a key component of the glomerular filtration barrier

Nephrin is a major protein in the slit diaphragm and lateral podocyte membranes. Congenital nephrotic syndrome of the Finnish type (CNF), a disease characterized by a fusion of podocyte foot processes and uncontrolled massive proteinu-

ria, is considered as a prototype disease disrupting the filtration barrier [12, 13]. The characteristic nephrin mutations in the CNF patients, induction of rapid proteinuria by *in vivo* injection of antinephrin antibodies and remarkable down regulation of nephrin-specific mRNA levels in experimental models, all suggests its crucial role in maintaining of the glomerular filter [14]. In spite of discreet changes in podocytes in minimal change disease it has been shown that granularization of nephrin corresponded to the degree of foot process effacement and the amount of proteinuria [15].

# CD2-associated protein (CD2AP) and podocin – the other major functional components of the glomerular filtration barrier

CD2AP molecule is apparently involved in the cell-to-cell interactions needed to activate the T cells of the immune system. When the CD2AP gene was knocked out in mice, it came out that--besides the defect of the immune system – the animals developed congenital nephrotic syndrome and died of the kidney disease [16]. Examining the animal's kidneys, it was found that that most of mice's podocytes no longer bore foot processes, and their slit diaphragms were largely missing. The researchers suggest that CD2AP anchors nephrin to the internal protein fibers that form the podocyte cytosceleton, thus helping form and stabilize the slit diaphragm [13, 17].

Podocin is the other important protein of the slit diaphragm. It interacts directly with nephrin and presumably links ion channels to the cytoskeleton [18]. Mutations in gene NPHS2 encoding podocin are responsible for familial and nonfamilial instances of focal segmental glomerulosclerosis (FSGS) [19] and as the consequence for part of cases of steroid-resistant nephrotic syndrome [20].

# Expression of nephrin in acquired human glomerular disease

Using immunoelectron microscopy it has been found that the expression of nephrin in GN (minimal change disease, membranous and membranoproliferative GN, IgA nephropathy, lupus nephritis, cryoglobulinemic nephritis) was decreased in the areas where the foot processes were affected, and comparable with that of normal controls where the foot process interspaces were preserved [21]. When compared to normal reactivity in glomeruli the antinephrin antibodies clearly showed down-regulation in membranous, membranoproliferative and IgA nephropathies, either globally or focally in only some capillary loops. With the 18C7 antibody (for CNF proteinuria-specific epitopes, with no reactivity of normal kidney tissue) glomerular positivity was found in 61 of 120 biopsies. The staining was always visceral with different degrees of intensity and diffusion [22].

These data suggest that unmasking or the novo expression of distinct glomerular proteins may be an important feature reflecting the pathophysiological events in these diseases with altered glomerular permeability, while only mild changes in the slit diaphragm protein nephrin appear to take place.

# Mechanisms of the interstitial fibrosis development in glomerulonephritis

The effect of the massive proteinuria for proximal tubular epithelial cells is the endocytosis of protein with consequent cell injury, organelle congestion, lysosomal swelling and rupture, eventually exposing cell cytoplasm and renal interstitium to the injurious effect of lysosomal enzymes [3, 31]. Urinary enzyme N-acetyl-ß-glucosaminidase (NAG) excretion correlates with tubular cells injury [23]. It has been shown the relationship between the amount of proteinuria, specially IgG and $\alpha$ 1microglobulin excretion and NAG excretion, which may be a marker of responsiveness to therapy [24].

Tubules express receptors for hormones and growth factors, which are filtered as part of non-selective proteinuria in a variety of glomerular diseases. Transforming growth factor-beta (TGF-$\beta$), insulin-like growth factor-I, hepatocyte growth factor are involved in the specific interactions with tubular receptors, and they stimulate the synthesis of collagen type I and IV by proximal tubular cells. They also activate tubular epithelial cells to the production of chemokines (such as monocyte chemoattractant protein-1 and Rantes), which may underlie mononuclear cell migration into the interstitium [4].

Interstitial fibrosis development also occurs in result of transdifferentiation of tubular epithelial cells into fibroblasts. The origin of fibroblasts and

myofibroblasts is still not clear: they can be derived from resident interstitial fibroblasts or from resident interstitial fibroblasts or from perivascular cells. They can also be attracted into the interstitium by the action of locally produced cytokines, particularly by PDGF. Alternatively, interstitial fibroblasts and myofibroblasts could be derived from tubular cells trough a process of transdifferentiation.

In experimental models new data were obtained about mechanisms of glomerular and interstitial scarring. The cyclic stretch of mesangial cells caused by intraglomerular hypertension activates the synthesis of at least two cytokines TGF-β and CTGF (connective tissue growth factor), which result the extracellular matrix accumulation and renal injury progression [25]. An increase in glomerular TGF-β mRNA was reported after the onset of hyperglycemia in diabetic rats [26].

# Therapeutic measures to reduce urinary protein excretion in patients with glomerular disease

The renoprotective effect of angiotensin converting enzyme inhibitors (ACEI) and angiotensin II receptor antagonists (ARA) is well established and they are now routinely used in chronic renal disease to reduce proteinuria and slow progression. ACEI and ARA may lower intraglomerular pressure by both decreasing systemic pressure and inducing efferent arteriolar vasodilatation. In addition, these agents may also inhibit the direct – or TGF- β1 – mediated angiotensin II enhancement of collagen deposition [25]. The early introduction of renin-angiotensin system inhibitors in chronic proteinuric nephropaties before the appearance of renal insufficiency should be preferable option [27]. The combined use of an ACE-I and an ARA would augment the beneficial influences of both classes of agents: ARA would provide a complete blocking of AT1 receptors of angiotensin II, not guaranteed by ACE-I, whereas the latter would contribute with an increased synthesis of bradykinin [28, 29].

In the last two meetings of The American Society of Nephrology more than 20 studies have analyzed the antiproteinuric effect of the combination ACE-I and ARA. Nearly all have found it significantly greater effect than the one observed with ACE-I or ARA, although the doses of these were increased when used alone. Interestingly there were no differences in blood pressure that could explain the greater antiproteinuric effect of the combination was observed.

In one of the latest study it has been shown that degree of proteinuria reduction depends on doses of ACE-I and its combination treatment with ARA. ACE-I-lisinopril doses well controlling the blood pressure (20 mg/day) reduced the proteinuria in 45%, but doubled caused 75% lowering of proteinuria. When ARA-losartan was added in doses 50–100 mg per day the 85% reduction of protein loss has been obtained [30].

## References

[1] **Kanwar Y. S., Liu Z. Z., Kashihara N., Wallner E. I.:** Current Status of the Structural and Functional Basis of Glomerular Filtration and Proteinuria. Seminars in Nephrology, 1991, 4, 390–413.

[2] **Miner J. H.:** Renal basement membrane components. Kidney Int. 1999, 56, 2016–2024.

[3] **Kuźniar J.:** The participation of the neutrophils in chronicity and progression of glomerulopathies. Wroclaw Medical University, Wrocław 1992.

[4] **Kriz W., Gretz N., Lemley K. V.:** Progression of glomerular diseases: Is the podocyte the culprit? Kidney Int.1998, 54, 687–697.

[5] **Topham P. S., Haydar S. A., Kuphal R., Lightfoot J. D., Salant D. J.:** Complement-mediated injury reversibly disrupts glomerular epithelial cell actin microfilaments and focal adhesions. Kidney Int. 1999, 55, 1763–1775.

[6] **Greiber S., Muller B., Daemish P., Pavenstadt H.:** Reactive Oxygen Species Gene Expression in Podocytes: Induction of Granulocytes Macrophage-Colony-Stimulating Factor. J. Am. Soc. Nephrol. 2002, 13, 86–95.

[7] **Pagtalunan M. E., Miller P. L., Jumping-Eagle S., Nelson R. G., Myers B. D., Rennke H. G., Coplom N. S., Sun L., Meyer T. W.:** Podocyte Loss and Progressive Glomerular Injury in Type II Diabetes. J. Clin. Invest. 1997, 99, 342–348.

[8] **Kriz W., Lemley K. V.:** The role of podocyte in glomerulosclerosis. Curr. Opin. Nephrol. Hyprtens. 1999, 8, 489–497.

[9] **Kerjaschki D.:** Caught flat-footed: podocyte damage and molecular based of focal glomerulosclerosis. J. Clin. Invest. 2002, 108, 1583–1587.

[10] **Abbate M., Zoja C., Corna D., Capitanio M., Bertani T., Remuzzi G.:** In progressive nephropathies, overload of tubular cells with filtered proteins translates glomerular permeability dysfunction into cellular signals of interstitial inflammation. J. Am. Soc. Nephrol. 1998, 9, 1213–1224.

[11] **Lemley K. V., Lafayette A., Safai G., Derby G., Blouch K., Squarer A., Myers B. D.:** Podocytopenia and disease severity in IgA nephropathy. Kidney Int. 2002, 61, 1475–1485.

[12] **Luimula P., Ahola H., Wang S. X., Solin M. L., Aaltonen P., Tikkanen I., Kerjaschki D., Holthofer H.:** Nephrin in experimental glomerular disease. Kidney Int. 2000, 58, 1461–1468.

[13] **Wickelgren I.:** First Components Found for Key Kidney Filter. Science 1999, 286, 225–226.

[14] **Holthofer H., Ahola H., Solin M. L., Wang S., Palmen T., Luimula P., Miettinen A., Kerjaschki D.:** Nephrin Localizes at the Podocyte Filtration Slit Area and Is Characteristically Spliced in the Human Kidney. Am. J. Pathol. 1999, 155, 1681–1687.

[15] **Wernerson A., Duner F., Pettersson E., Widholm S. M., Berg U., Ruotsaleinen V., Tryggvason K., Hultenby K., Soderberg M.:** Altered ultrastructural distribution of nephrin in minimal change nephrotic syndrome. Nephrol. Dial. Transplant. 2003, 18, 70–76.

[16] **Shih N. Y., Li J., Karpitskii V., Nguyen A., Kanagawa O., Miner J. H., Shaw A. S.:** Congenital nephrotic syndrome in mice lacking CD-2 associated protein. Science 1999, 286, 312–315.

[17] **Yuan H., Takeuchi E., Taylor G. A., Mclaughlin M., Brown D., Salant D.:** Nephrin Dissociates from Actin, and Its Expression Is Reduced in Early Experimental Membranous Nephropathy. J. Am. Soc. Nephrol. 2002, 13, 946–956.

[18] **Kaplan J., Pollak M. R.:** Familial focal segmentalglomerulosclerosis. Curr. Opin. Nephrol. Hyprtens. 2001, 10, 183–187.

[19] **Pollak M. R.:** Inherited Podocytopathies: FSGS and Nephrotic Syndrome from a Genetic Viewpoint. J. Am. Soc. Nephrol. 2002, 13, 3016–3023.

[20] **Frishberg Y., Rinat C., Megged O., Shapira E., Feinstein S., Raas-Rotschild A.:** Mutations in NPHS2 Encoding Podocin Are a Prevalent Cause of Steroid-Resistant Nephrotic Syndrome among Israeli-Arab Children. J. Am. Soc. Nephrol. 2002, 13, 400–405.

[21] **Huh W., Kim D.J., Kim M. K., Kim Y. G., Oh H. Y., Ruotsalainen V., Tryggvason K.:** Expression of nephrin in acquired human glomerular disease. Nephrol. Dial. Transplant. 2002, 17, 478–484.

[22] **Wang S. X., Rastaldi M. P., Patari A., Ahola H., Heikkila E., Holthofer H.:** Patterns of nephrin and a new proteinuria-associated protein expression in human renal diseases. Kidney Int. 2002, 61, 141–144.

[23] **Bazzi C., Petrini C., Rizza V., Arrigo G., Napodano P., Paparella M., D'Amico P.:** Urinary N-acetyl-$\beta$-glucosaminidase excretion is a marker of tubular cell dysfunction and a predictor of outcome in primary glomerulonephritis. Nephrol. Dial. Transplant. 2002, 17, 1890–1896.

[24] **Bazzi C., Rizza V., Petrini C., Arrigo G., Beltrame A.:** Urinary excretion of IgG and $\alpha_1$-microglobulin predicts clinical course better tham extent of proteinuria in membranous nephropathy. Am J Kidney Dis, 2001, 38, 240–248.

[25] **Cortes P., Riser B. L., Yee J., Narins R. G.:** Mechanical strain of glomerular mesangial cells in the pathogenesis of glomerulosclerosis: clinical implications. Nephrol. Dial. Transplant. 1999, 14, 1351–1354.

[26] **Flyvbjerg A., Gronbaek H., Bak M., Nielsen B., Christiansen T., Hill C., Logan A., Orskov H.:** Diabetic kidney disease: the role of growth factors. Nephrol. Dial. Transplant. 1998, 13, 1104–1107.

[27] **Hilgers K. F., Mann J. F.:** ACE Inhibitors versus AT1 Receptor Antagonists in Patients with Chronic Renal Disease. J. Am. Soc. Nephrol. 2002, 13, 1100–1108.

[28] **Gainer J. V., Morrow J. D., Loveland A., King D. J., Brown N. J.:** Effect of Bradykinin-Receptor Blockade on the Response to Angiotensin-Converting-Enzyme Inhibitor in Normotensive and Hypertensive Subjects. N. Engl. J. Med. 1998, 339, 1285–1292.

[29] **Chanstra J. P., Neau E., Drogoz P., Arevalo Gomez M. A., Lopez Novoa J. M., Calise D., Pecher C., Bader M., Girolami J. P., Bascands J. L.:** *In vivo* bradykinin B2 receptor activation reduces renal fibrosis. J. Clin. Invest. 2002, 110, 371–379.

[30] **Loverman G. D., Navis G., Henning R. H., De Jong P. E., De Zeeuw D.:** Dual renin-angiotensin system blockade at optimal doses of ptoteinuria. Kidney Int. 2002, 62, 1020–1025.

[31] **Remuzzi G., Rugenenti P., Begnini A.:** Understanding of nature of renal disease progression. Kidney Int. 1997, 51, 2–15.

## Address for correspondence:

Marian Klinger
Department of Nephrology and Transplantation Medicine
Wroclaw Medical University
Traugutta St. 57/59
50-417 Wrocław
Poland