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Review article
Pregledni članak

THE IMPORTANCE OF KIM-1 DETERMINATION IN TISSUE AND URINE OF PATIENTS WITH DIFFERENT KIDNEY DISEASES

ZNAČAJ ODREĐIVANJA KIM-1 U TKIVU I URINU BOLESNIKA SA RAZLIČITIM BOLESTIMA BUBREGA

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Summary: There is an urgent need for early renal biomarkers for the monitoring of proximal tubular injury because tubulointerstitial disease accompanies many processes leading to chronic and end stage kidney disease. One of these is kidney injury molecule-1 (KIM-1) a new specific histological biomarker for diagnosing early tubular injury from renal biopsies but also in urine. This transmembrane tubular protein with unknown function is undetectable in normal kidneys, but is the hallmark of virtually all proteinuric, toxic and ischaemic kidney diseases. Recent data revealed its possible pathophysiological role in modulating tubular damage and repair. This review is focused on the structural and biochemical aspects of KIM-1, its expression pattern and its pathophysiological role in renal disease. Also, the prognostic value of KIM-1 in relation to urinary protein excretion will be discussed, as well the potential of KIM-1, as the biomarker of renal damage, as a predictor of renal function decline and its perspectives for monitoring therapy response.

Keywords: kidney injury molecule (KIM-1), biomarker, tubular damage marker

Kratak sadržaj: U poslednje vreme je postala očigledna potreba za novim bubrežnim biomarkerima za monitoring oštećenja proksimalnih tubula jer je pokazano da promene u tubulointersticijumu značajno doprinose progresiji hronične bubrežne slabosti i vode ka terminalnoj fazi bolesti. Jedan od njih je i kidney injury molecule-1 (KIM-1), novi, specifičan biomarker za dijagnozu ranih tubulskih oštećenja koji se određuje u tkivu bubrega ali i u urinu bolesnika. Ovaj transmembranski tubulski protein sa nepoznatom funkcijom se ne detektuje u zdravim bubrezima, ali je obeležje skoro svih proteinuričnih, toksičnih i ishemičnih bolesti bubrega. Nedavna istraživanja su ukazala na njegovu eventualnu patofiziološku ulogu u moduliranju tubulskog oštećenja i reparaciji. U ovom preglednom članku biće predstavljen strukturalni i biohemijski aspekt KIM-1, njegova ekspresija i patofiziološka uloga u bubrežnim bolestima. Takođe će biti razmatrana i njegova prognostička uloga u odnosu na proteinuriju kao i uloga biomarkera bubrežnog oštećenja i prediktora smanjenja bubrežne funkcije ali i perspektive za monitoring odgovora na terapiju.

Ključne reči: marker oštećenja bubrega (KIM-1), biomarker, marker oštećenja tubula

Introduction

The standard methods of assessing renal function have kept the measurement of serum blood urea nitrogen and creatinine, biomarkers that are insensitive and nonspecific, especially in the setting of acute kidney injury (AKI). It is also important to recognize that changes in serum creatinine and blood urea nitrogen concentrations primarily reflect functional changes in filtration capacity and are not true 'injury markers'. There is a crucial need for better biomarkers of AKI for its timely diagnosis, for the prediction of severity and outcome and for the monitoring of proximal tubule injury in AKI but also for progression in chronic kidney disease (CKD) (1).

As is well known, a number of comorbidities are associated with CKD and prognosis is poor because many patients experience disease progression to end stage renal disease (2). The mechanisms of injury underlying this progression are blurred, but the decline in renal function is associated with the degree

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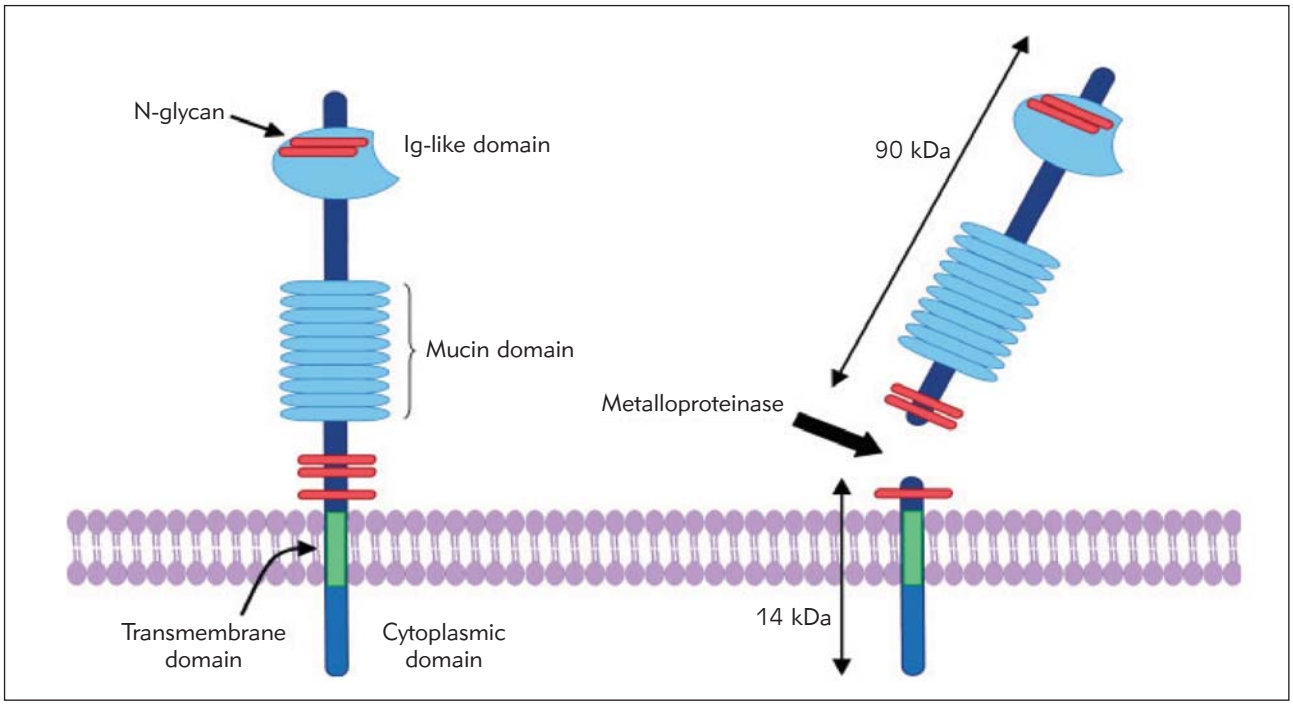

Figure 1 Structure of KIM-1 (7).

of proteinuria and with histological findings of glomerulosclerosis and interstitial fibrosis (3, 4). Proteinuria is not only a marker of renal damage, but ultrafiltrated proteins are injurious to the kidney, thereby contributing to tubulointerstitial (TIN) damage (5, 6). As TIN damage is an important mediator and a common pathway to end-stage renal disease, a sensitive tissue marker of tubular injury, which can be used to identify or confirm the presence of epithelial cell injury even when morphological changes are minimal, would be helpful in the evaluation of biopsy material (7).

During the last decade some studies have pointed out kidney injury molecule-1 (KIM-1), a recently revealed transmembrane protein, as a marker for proximal tubular injury, the hallmark of virtually all proteinuric, toxic and ischaemic renal diseases. Recently, much attention has been paid to its possible pathophysiological role in modulating tubular damage and repair (8, 9).

Ongoing text will try to explain the structure and expression of KIM-1, its possible functional role in the kidney, as well as whether it could be a potential marker and predictor of kidney disease, and the significance of early detection of kidney injury and monitoring of therapy response.

Structure of KIM-1

KIM-1 is a type-1 transmembrane protein (presented in *Figure 1*) with an extracellular domain that consists of a signal peptide, an Ig domain and a mucin domain. Also, there is one transmembrane domain and a short intracellular domain with at least one important tyrosine phosphorylation domain. The protein can be cleaved by a metalloproteinase, after which the ectodomain (90 kDa) appears in the urine, leaving a 14 kDa membrane-bound fragment that is tyrosine-phosphorylated (Tyr-P) (7).

KIM-1 expression

KIM-1 is non-detectable in normal kidneys, but strong KIM-1 induction has been shown in animal models of ischaemic (8), toxic (10) and proteinuric (11) renal disease. Tubular KIM-1 expression was also observed in human renal biopsies after ischaemic or toxic acute tubulus necrosis (12, 13), in tubular cells adjacent to renal carcinoma cells (14) as well as in allograft nephropathy (15).

The KIM-1 ectodomain can be cleaved and detected in urine; previously, it was shown that the cleaved KIM-1 ectodomain could be quantified and related to the extent of renal damage in experimental renal disease (12, 13) as well in human renal disease (16, 17).

Van Timmeren et al. (16) recently showed that the majority of KIM-1-positive tubules in various human renal diseases are of proximal origin, as was identified by double labelling studies with the marker for proximal tubules – aquaporin-1. Also, recent studies revealed that KIM-1 is localized in the apical membrane of dilated tubules in acute and chronic

tubular injury (9, 16). Localization of KIM-1 expression appears to be related to the susceptibility of specific tubular segments to different types of injury (18). In ischaemic injury, KIM-1 expression is most prominent in the S3 segment in the corticomedullary region, well known as the most susceptible to ischaemia-induced injury. KIM-1 expression is also prominent in the mid-cortical and superficial tubules in renal disease models, where the primary insult is not predominantly directed to the S3 segment (e.g. in proteinuria-induced renal damage, folic acid-induced renal injury and polycystic kidney disease) (17–19).

Also, van Timmeran et al. (16) pointed out that tubular KIM-1 expression is related to TIN damage and inflammation. Double labelling immunohistochemistry in various experimental and human renal diseases has revealed that KIM-1-positive tubules are associated with aggregates of macrophages and areas with increased expression of α-smooth muscle actin (α-SMA), a marker of myofibroblast transformation. This indicates the presence of prefibrotic changes. Also, osteopontin, a tubular-derived protein involved in chemotaxis and repair (20), as well as vimentin, an intermediate filament involved in tubular dedifferentiation, showed a relationship with KIM-1 in most of the tubules in human renal disease, indicating that KIM-1-positive tubular cells have a dedifferentiated phenotype (16).

Functional role of KIM-1 in the kidney

Recently, the potential functional role of KIM-1 in the kidney was discovered.

Tubular epithelial cells become active/injured upon different forms of renal injury (hypoxia/ischaemia or toxins) and will express KIM-1 at the apical membrane. Metalloproteinases can slice KIM-1 into a soluble part and a short membrane-bound fragment while the tubular epithelial cells will produce various proinflammatory cytokines and chemokines. These will draw inflammatory cells to the renal interstitium and initiate interactions with interstitial fibroblasts. After that, with ongoing injury, the tubular epithelial cells can undergo programmed cell death (apoptosis). Apoptotic bodies express phosphatidylserine on their surfaces. KIM-1-expressing tubular epithelial cells can bind to surface-specific epitopes on the apoptotic bodies, specifically to phosphatidylserine, and can phagocytose dying cells and other debris from the tubular lumen. Activation and proliferation of fibroblasts and myofibroblasts leads to excessive synthesis of extracellular matrix (ECM) and eventually to fibrosis (7, 8).

There is still disagreement about the function of KIM-1: is it actively regulating the inflammation process or is its expression just a response to damage, attempted recovery and/or repair? Currently, it can only be considered whether tubular KIM-1 expression is actively involved in the process of repair and/or damage or is just a result of ischaemia, proteinuria or renal fibrosis (7).

Also, the function of soluble KIM-1 in the urine is not yet clarified. Soluble, shedded KIM-1 may form a protective layer on the proximal tubular cells, thereby protecting them from protein casts that are formed within the lumen. It remains to be elucidated whether inhibiting its release or neutralizing its activity in the urine is beneficial or harmful (21).

KIM-1 as a marker of kidney disease

No other organs express KIM-1 to a degree that would influence renal excretion of KIM-1 (1) so it seems to be a sensitive and selective biomarker of injured proximal tubular cells. Van Timmerman et al. (11) and Krammer et al. (18) in experimental but also van Timmerman et al. (16) and Vaidya et al. (12) in human renal disease discovered that elevated urinary (shedded) KIM-1 levels are strongly related to tubular KIM-1 expression.

Only 30 μL urine is needed for measurements and since the KIM-1 ectodomain is stable at room temperature, KIM-1 can be quantified in 24 h urines. The microsphere-based Luminex xMAP technology with polyclonal antibodies raised against the human KIM-1 ectodomain is widely used for measuring urinary KIM-1 excretion. Wandres et al. (22) in the post hoc analysis of a randomized controlled trial found that the lower limit of detection for this assay is 4 pg/mL, mean urinary KIM-1 excretion in control subjects is 58 $\pm$ 8.0 ng/day while, in contrast, in untreated patients with non-diabetic proteinuria (mean proteinuria 3.8 g/day), KIM-1 excretion is 1706 $\pm$ 498 ng/day. This microbead technique is an adaptation of the previously described sandwich ELISA assay and urinary KIM-1 measured by this ELISA assay (13). Also, very recently KIM-1 dipsticks (RenaStick) were developed as a rapid diagnostic assay for kidney damage, providing sensitive and accurate detection of KIM-1 (23). However, more extensive validation studies are needed to confirm the utility of these dipsticks.

In experimental settings amelioration of renal damage with renoprotective involvement reduces renal KIM-1 expression in rodents (24). So these data reflect the reversibility of early tubular injury. Also, in the human population antiproteinuric treatment reduces urinary KIM-1 excretion in non-diabetic proteinuric patients with well-preserved and stable kidney function (22).

KIM-1 as a predictor of kidney disease

More important is that recent studies in the human population revealed that KIM-1 not only functions as a marker, but also has predictive value for

AKI (25), CKD (16, 22) as well as prognostic significance in transplant recipients (15).

Liangos et al. (25) showed that urinary KIM-1 is predictive for adverse clinical outcomes in a cohort of 201 hospitalized patients with acute kidney injury. Patients within the highest KIM-1 quartile have a 3.2-fold higher odds ratio for dialysis or hospital death compared to patients within the lowest quartile.

In CKD it was recently made known that progressive renal function decline during follow-up in human proteinuric disease is strongly associated with urinary levels of another marker of tubular cell damage, neutrophil gelatinase-associated lipocalin (26). So far, long-term follow-up data on the prognostic significance of urinary KIM-1 in human renal disease are lacking.

In transplant recipients Zhnag et al. (15) found that renal KIM-1 expression is more sensitive than histology for detecting early tubular injury in human allografts. Positive KIM-1 staining in proximal tubules significantly correlates with the severity of the injury, as was measured by deterioration of allograft function. KIM-1 expression level in transplant biopsies may indicate the potential for improvement of kidney function, since higher KIM-1 expression predicts a better outcome, with better serum creatinine, over 18 months (15). On the other hand, van Timmeren at al. (27) in a long-term follow-up study pointed out that higher urinary KIM-1 excretion was predictive of worse renal prognosis and graft loss in a cohort of 145 renal transplant recipients, which was independent of creatinine clearance, donor age and, in particular, proteinuria.

Recently, Han et al. (28) have suggested that urinary 'biomarker panels' might be better in predicting tubular injury than a single urinary biomarker.

Significance of early detection of kidney injury and monitoring of therapy response

As mentioned before, in both the acute and chronic disease as well as during renoprotective treatment there is a critical need for the early detection and monitoring of kidney injury. Early detection of chronic kidney disease may have more encouraging outcomes, since renoprotective intervention can take place at an earlier stage of kidney disease, when renal function decline has not yet started. This implies the need for simple, non-invasive and specific biomarkers to monitor the pathophysiological processes occurring within the kidney. In clinical practice, serum creatinine, 24 h urinary creatinine excretion and estimating glomerular filtration rate (GFR) with creatinine-based formulae are widely used to detect chronic kidney disease and its progression. However, urinary KIM-1 levels may have better prognostic value in predicting outcome than serum creatinine and urine output in AKI (25). KIM-1 may reflect even slight tubular damage caused by proteinuria and ischaemia and possibly detect progressive chronic renal damage before a decline in renal function, similar to other urinary tubular proteins or markers of reduced tubular protein reabsorption, such as N-acetyl-β-D-glucosaminidase, α1-microglobulin or β2-microglobulin (29, 30).

Proteinuria generally reflects glomerular damage but the long-term renal outcome is determined by the severity of TIN injury in the majority of kidney diseases. So, there is a close pathophysiological relationship between proteinuria and TIN damage. The severity of pretreatment TIN damage predicts a blunted response to renoprotective intervention, with a worse long-term renal outcome, which can be ameliorated by intensified treatment (31). Thus, possible sensitive biomarkers in clinical practice that explicitly reflect the severity of this pretreatment TIN renal damage may identify the patients who need intensified renoprotective treatment (32). Experimental data in rats show that, in spite of a reduction in proteinuria, pronounced progression of renal interstitial damage can be present (33). Therefore, the therapy response to proteinuria and renal interstitial damage can dissociate, suggesting that biomarkers for tubulointerstitial damage could be valuable as prognostic markers, either independently or, more likely, in combination with proteinuria.

KIM-1 is only expressed in the areas of TIN damage and tubular dedifferentiation and probably has an important role in the clearance of apoptotic (tubular) cells, and urinary KIM-1 levels reflect renal expression. This molecule is therefore a very promising candidate for non-invasive monitoring of this important pathophysiological process. The decrease in urinary KIM-1 suggests that TIN damage is ameliorated by antiproteinuric intervention (22). To establish the prognostic impact of KIM-1 relative to proteinuria, future long-term studies should investigate whether glomerular (proteinuria) and interstitial markers (KIM-1) have independent prognostic impact and consequently could provide independent treatment targets. If so, it might be useful to test whether targeting treatment on KIM-1, in addition to proteinuria, can improve outcome in progressive renal function loss (7).

However, for future use in clinical practice, next to targeting treatment on proteinuria and KIM-1, other biomarkers will be needed to predict therapy response and renal outcome more precisely. We will probably need 'biomarker panels' for this purpose, as was also suggested by Han et al. (28).

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

References

1. Bonventre JV. Kidney injury molecule-1 (KIM-1): a urinary biomarker and much more. Nephrol Dial Transplant 2009; 24 (11): 3265–8.
2. Kronenberg F. Emerging risk factors and markers of chronic kidney disease progression. Nature reviews, Nephrology 2009; 5: 677–89.
3. Zandi-Nejad K, Eddy AA, Glassock RJ, Brenner BM. Why is proteinuria an ominous biomarker of progressive kidney disease? Kidney Int Suppl 2004; S76–S89.
4. Rodriguez-Iturbe B, Johnson RJ, Herrera-Acosta J. Tubulointerstitial damage and progression of renal failure. Kidney Int Suppl 2005; S82–S86.
5. Remuzzi G, Benigni A, Remuzzi A. Mechanisms of progression and regression of renal lesions of chronic nephropathies and diabetes. J Clin Invest 2006; 116: 288–96.
6. Nangaku M. Mechanisms of tubulointerstitial injury in the kidney: final common pathways to end-stage renal failure. Intern Med 2004; 43: 9–17.
7. Waanders F, Van Timmeren MM, Stegeman CA, Bakker S, Van Goor H. Kidney injury molecule-1 in renal disease. J Pathol 2010; 220: 7–16.
8. Ichimura T, Bonventre JV, Bailly V, et al. Kidney injury molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. J Biol Chem 1998; 273: 4135–42.
9. Ichimura T, Asseldonk EJ, Humphreys BD, Gunaratnam L,Duffield JS, Bonventre JV. Kidney injury molecule-1 is a phosphatidylserine receptor that confers a phagocytic phenotype on epithelial cells. J Clin Invest 2008; 118 (5): 1657–68.
10. Ichimura T, Hung CC, Yang SA, Stevens JL, Bonventre JV. Kidney injury molecule-1 (Kim-1): a tissue and urinary biomarker for nephrotoxicant-induced renal injury. Am J Physiol Renal Physiol 2003; 286: F552–F563.
11. Van Timmeren MM, Bakker SJ, Vaidya VS, Bailly V, Schuurs TA, Damman J, et al. Tubular kidney injury molecule-1 in protein-overload nephropathy. Am J Physiol Renal Physiol 2006; 291: F456–F464.
12. Vaidya VS, Ramirez V, Ichimura T, Bobadilla NA, Bonventre JV. Urinary kidney injury molecule-1: a sensitive quantitative biomarker for early detection of kidney tubular injury. Am J Physiol Renal Physiol 2006; 290: F517–F529.
13. Han WK, Bailly V, Abichandani R, Thadhani R, Bonventre JV. Kidney injury molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. Kidney Int 2002; 62: 237–44.
14. Han WK, Alinani A, Wu CL, Michaelson D, Loda M, McGovern FJ, et al. Human kidney injury molecule-1 is a tissue and urinary tumor marker of renal cell carcinoma. J Am Soc Nephrol 2005; 16: 1126–34.
15. Zhang PL, Rothblum LI, Han WK, et al. Kidney injury molecule-1 expression in transplant biopsies is a sensitive measure of cell injury. Kidney Int 2008; 73: 608–614.
16. van Timmeren M, Bakker SJL, Vaidya VS, Bailly V, Schuurs TA, Damman J, Stegeman CA, Bonventre JV, van Goor H. Tubular kidney injury molecule-1 (KIM-1) in human renal disease. J Pathol 2007; 212: 209–17.
17. Ichimura T, Hung CC, Yang SA, Stevens JL, Bonventre JV. Kidney injury molecule-1: a tissue and urinary biomarker for nephrotoxicant-induced renal injury. Am J Physiol Renal Physiol 2004; 286 (3): F552–563.
18. Kramer AB, van Timmeren MM, Schuurs TA, Vaidya VS, Bonventre JV, Van Goor H, Navis G. Reduction of proteinuria in adriamycin-induced nephropathy is associated with reduction of renal kidney injury molecule (KIM-1) over time. Am J Physiol Renal Physiol 2009; 296 (5): F1136–F1145.
19. Kuehn EW, Park KM, Somlo S, Bonventre JV. Kidney injury molecule-1 expression in murine polycystic kidney disease. Am J Physiol Renal Physiol 2002; 283 (6): F1326–1336.
20. van Timmeren MM, Bakker SJ, Vaidya VS, Bailly V, Schuurs, Damman J, et al. Tubular kidney injury molecule-1 in protein-overload nephropathy. Am J Physiol Renal Physiol 2006; 291 (2): F456–464.
21. Bailly V, Zhang Z, Meier W, Cate R, Sanicola M, Bonventre JV. Shedding of kidney injury molecule-1, a putative adhesion protein involved in renal regeneration. J Biol Chem 2002; 277 (42): 39739–48.
22. Waanders F, Vaidya VS, Van Goor H, Leuvenink H, Damman K, Hamming I, et al. Effect of renin–angiotensin–aldosterone system inhibition, dietary sodium restriction, and/or diuretics on urinary kidney injury molecule 1 excretion in nondiabetic proteinuric kidney disease: a post hoc analysis of a randomized controlled trial. Am J Kidney Dis 2009; 53 (1): 16–25.
23. Vaidya VS, Ford GM, Waikar SS, Wang Y, Clement MB, Ramirez V, et al. A rapid urine test for early detection of kidney injury. Kidney Int 2009; 76 (1): 108–14.
24. De Borst MH, van Timmeren MM, Vaidya VS, De Boer RA, Van Dalen MB, Kramer AB, et al. Induction of kidney injury molecule-1 in homozygous Ren2 rats is attenuated by blockade of the renin–angiotensin system or p38 MAP kinase. Am J Physiol Renal Physiol 2007; 292 (1): F313–320.
25. Liangos O, Perianayagam MC, Vaidya VS, Han WK, Wald R, Tighiouart H, et al. Urinary N-acetyl-β-D-glucosaminidase activity and kidney injury molecule-1 level are associated with adverse outcomes in acute renal failure. J Am Soc Nephrol 2007; 18 (3): 904–12.
26. Bolignano D, Lacquaniti A, Coppolino G, Donato V, Campo S, Fazio MR, et al. Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. Clin J Am Soc Nephrol 2009; 4 (2): 337–44.
27. Van Timmeren MM, Vaidya VS, Van Ree RM, Oterdoom LH, deVries AP, Gans ROB, et al. High urinary excretion of kidney injury molecule-1 is an independent predictor of graft loss in renal transplant recipients. Transplantation 2007; 84 (12): 1625–30.

28. Han WK, Wagener G, Zhu Y, Wang S, Lee HT. Urinary biomarkers in the early detection of acute kidney injury after cardiac surgery. Clin J Am Soc Nephrol 2009; 4 (5): 873–82.

29. Nickolas TL, Barasch J, Devarajan P. Biomarkers in acute and chronic kidney disease. Curr Opin Nephrol Hypertens 2008; 17 (2): 127–32.

30. Ležaić V. Determination of biomarkers in serum and urine and their significance in dignostics kidney disorders. Journal of Medical Biochemistry 2010; 29: 288–97.

31. Kramer AB, Laverman GD, Van Goor H, Navis G. Interindividual differences in anti-proteinuric response to ACEi in established adriamycin nephrotic rats are predicted by pretreatment renal damage. J Pathol 2003; 201 (1): 160–7.

32. Perico N, Cattaneo D, Remuzzi G. Kidney injury molecule 1: in search of biomarkers of chronic tubulointerstitial damage and disease progression. Am J Kidney Dis 2009; 53 (1): 1–4.

33. Hamming I, Navis G, Kocks MJ, Flan Goor H. ACE inhibition has adverse renal effects during dietary sodium restriction in proteinuric and healthy rats. J Pathol 2006; 209 (1): 129–39.

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