Neutrophil gelatinase-associated lipocalin as a new biomarker in diagnosis of renal diseases

Lipokalina związana z żelatynazą neutrofili - nowy biomarker w diagnostyce chorób nerek

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Summary

With regard to insufficient sensitivity of diagnostic markers used so far for evaluation of renal function, the search for new markers of renal failure is needed, which may enable the early diagnosis of renal disease and commencing the proper therapy. Neutrophil gelatinase-associated lipocalin (NGAL) is a newly discovered biomarker of renal failure, that beside of traditional indices seems to be useful in the diagnosis of acute and chronic forms of this pathology. Determination of lipocalin in the urine, which is a biological material routinely used in laboratory diagnostics may be useful in the clinical practice.

Streszczenie

Ze względu na niewystarczającą czułość dotychczas stosowanych, do oceny czynności nerek, parametrów diagnostycznych wciąż poszukuje się nowych markerów uszkodzenia nerek, które umożliwiałyby wczesne rozpoznanie choroby i włączenie odpowiedniego leczenia. Lipokalina związana z żelatynazą neutrofili (NGAL) to nowoodkryty wczesny biomarker uszkodzenia nerek, który, obok tradycyjnych wskaźników, może znaleźć zastosowanie w diagnostyce ostrej i przewlekłej niewydolności nerek. Jej oznaczanie w moczku, który jest materiałem biologicznym stosowanym w rutynowej diagnostyce laboratoryjnej, może okazać się przydatne w praktyce klinicznej.

Key words: neutrophil gelatinase-associated lipocalin (NGAL), renal diseases

Słowa kluczowe: lipokalina związana z żelatynazą neutrofili (NGAL), choroby nerek

Abbreviations:
AKI - acute kidney injury
CCM – Critical Care Medicine
CIN - contrast induced nephropathy
CKD - chronic kidney disease
CPB – cardiopulmonary bypass
GFR – glomerular filtration rate
KIM-1 - kidney injury molecule-1
MMP-9 - matrix metalloproteinase-9
NF-kB – nuclear factor kappa B cells
NGAL - neutrophil gelatinase-associated lipocalin
NSAIDs - nonsteroidal anti-inflammatory drugs
RIFLE - Risk, Injury, Failure, Loss, End-stage kidney disease

The diagnosis of kidney dysfunction using serum creatinine along with radiological and histological examination of the kidneys is often insufficient due to the low accuracy or high invasiveness. Commonly used serum creatinine assay is not a sensitive indicator of impaired renal filtration and depends on extrarenal factors such as muscle mass, sex, age and diet. Taking into account the limited diagnostic value of serum creatinine rise in early stages of acute renal failure, and difficulties in the proper daily urine collection to assess creatinine clearance therefore is a need for new markers of kidney failure. The concentration of such marker in the blood or urine should change already in preclinical phase of the disease. It will allow the early diagnosis and inclusion of appropriate treatment of this pathology. New early markers of renal failure, with higher sensitivity and specificity, include
Neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1, acting as a KIM-1 and interleukin 18 (IL-18). They are described as “renal troponins” [1,2,3]. Neutrophil gelatinase-associated lipocalin NGAL named also human neutrophil lipocalin (HNL) is a secretory protein belonging to the family of lipocalins, present in neutrophil granules [1,4]. This glycoprotein is also known in mice as lipocalin 2, oncogene protein 24p33 or urocalcin, and in rats as neu-related-protein or 25 kDa α2-microglobulin-related protein [4]. It has been demonstrated that the human NGAL consists of a single disulphide-bridged polypeptide chain containing 178 amino-acid residues with a molecular mass of 22 kDa, which due to glycosylation may increase to 25 kDa. Isoelectric point of this glycoprotein is at pH= 8,4 [1]. Activation of neutrophils leads to release of NGAL from the granules. It is secreted as a monomer, and also in low concentration as a dimer. It may also occur in complex with type IV collagenase, called gelatinase B or matrix metalloproteinase-9 to form heterodimer of mass 125 kDa (Fig. 1) [4]. Like other lipocalins, NGAL has a β-barrel structure with a hydrophobic calyx. The common feature of this family proteins is the structure-dependent ability to bind and transport small lipophilic molecules, such as free fatty acids, retinoids, arachidonic acid and steroids [5]. It has been shown that many lipocalins possess specific surface receptors, but NGAL receptor has not yet been identified [4]. However, the existence of bacterial ligands for NGAL was reported by Goetz et al [5,6]. It has been shown that these ligands for NGAL are catecholate-ferric siderophores – small iron-binding molecules which are synthesized by bacteria [5,6].

**Physiological function of NGAL**

Although NGAL has been identified over a decade ago, the physiological function of this protein is still poorly understood. Human NGAL was originally isolated from the supernatant of activated neutrophils. Initially it was considered as a marker of infection, since elevated levels of this protein were observed in inflammatory and acute bacterial infections. It has been demonstrated that NGAL exerts a bacteriostatic effect by destroying siderophores, and therefore may be involved in control of inflammatory processes in the cells [5]. Moreover, regardless of the infectious conditions, increased concentrations of NGAL were found in asthma, chronic obstructive pulmonary disease, as well as adenomas, adenocarcinomas of the breast and urothelial carcinomas [4,7]. Moreover, this protein is present in the trachea, stomach, colon, and proximal renal tubules [7]. The presence of NGAL has been demonstrated in many other human tissues but at very low levels. They however significantly increased in impaired epithelial cells, including kidney. This increase was mediated by transcription factors, including NF-κB that affected cell survival and proliferation [2,5]. NGAL activates the formation of nephrinos in early stage of kidney development, through its protective effect [8]. Due to the low molecular mass and resistance to degradation NGAL can be easily secreted by the cells of the thick part of ascending arm of the Henle Loop and collecting tubules and excreted in the urine, both in free and MMP-9 complexed form [4]. The concentration of NGAL in the urine correlates well with the concentration in the blood, what makes it a useful marker in the diagnosis of kidney diseases [1].

**NGAL in early diagnosis of acute kidney injury**

Acute kidney injury – AKI – the new term appearing in the literature to replace “acute renal failure” is a common complication after cardiac surgery, kidney transplantation, cardiogenic and septic shock, after the administration of X-ray contrast or chemotherapeutic agents such as some antibiotics or NSAIDs. It has been shown that AKI develops in approximately 7% of hospitalized patients and up to 30-50% of patients after heart surgery [9]. In among 5.7% of patients requiring dialysis, mortality rate is as high as 60-80%. It demonstrates that acute renal failure is an independent risk factor for death [9,10]. Despite the well documented importance of modified RIFLE classification for AKI, including changes in serum creatinine, there is still a need for a marker of higher sensitivity, which would be helpful in early diagnosis of acute kidney injury [2].

The preclinical studies demonstrated that NGAL is an easily detectable protein in the blood or urine after activation of nephrotoxic factor [5,9,11]. It made it a putative non-invasive marker in the diagnosis of AKI. Study of Bennett et al. [12] demonstrated that urine NGAL is an early indicator of acute renal damage in patients after cardiac surgery. In 51% of the 196 children who developed acute renal failure after cardiac surgery, after 2 hours, a 15-fold increase of NGAL concentrations in the urine was observed and up to 25-fold after 4 and 6 hours [12]. For comparison, serum creatinine has increased as late as 2-3 days after cardiopulmonary bypass [12]. This was also confirmed by studies of Mishra et al [13]. Other prospective studies conducted in children after cardiac surgery showed a significant increase in the NGAL concentration in the urine and plasma 2-6 hours after surgery, while the increase in serum creatinine was observed only after several days [14]. It indicates that urine NGAL can serve as an early marker for ischemic renal damage in children after the surgery [4].

Figure 1. Structural form of NGAL [according to 4].
Also, in adult patients who developed acute renal failure after heart surgery, defined as an increase in serum creatinine, an increase of NGAL in urine was found after 1-3 hours after surgery [14].

Numerous data demonstrate the existence of correlation between early postoperative NGAL concentrations in the urine or plasma and the severity of acute renal failure, the duration of patient’s hospitalization, the need for dialysis and increased mortality [14]. Therefore, the concentration of NGAL in the urine 2 hours after surgery correlates with the severity and duration of acute renal failure [12,13].

NGAL seems to be an independent predictive indicator in the development of acute renal failure [14]. Many studies demonstrated that the concentration of NGAL significantly correlated with the acute pathological states. The increase of its concentration was observed in patients with ischemic renal injury often leading to acute renal failure, acute tubular necrosis, or acute tubulo-interstitial nephropathy [4]. It is worth to emphasize that patients with ischemic heart disease frequently exhibit different degrees of renal dysfunction due to accompanying diseases such as diabetes, hypertension and congestive heart failure, despite normal serum creatinine [15]. Due to the increase of NGAL concentration preceding that of serum creatinine, and no technical problems in its determination it seems to be a promising new biomarker for the early diagnosis of acute renal injury [14].

The concentration of NGAL in serum and urine increases in patients undergoing coronary angiography due to coronary artery disease [15] and 4-8 hours after intervention, respectively. A significant correlation between NGAL and other markers of kidney function: cystatin C, GFR and serum creatinine has been observed. It suggests that NGAL is a useful marker of acute renal failure in patients after coronary angiography [15]. Similar correlations have been observed by Mishra et al [13] and Devarajan [9]. It should be stressed that this NGAL increase is transient and lasts up to 8 hours [15,13]. It is compatible with concept that NGAL is released to circulation by neutrophiles activated during coronarography [15].

Many other disorders of kidney function are also associated with increased concentrations of NGAL in plasma or urine [4]. It is suggested that NGAL may be also an early biomarker of contrast induced nephropathy (CIN), which is frequent in exposed patients with chronic kidney disease, diabetes, impaired fasting glucose or heart failure [16].

NGAL in chronic renal failure

Chronic kidney disease (CKD) represents a serious risk of cardiovascular complications and death. It is estimated that the incidence of CKD in the U.S. is as high as 16%, whereas in Poland the incidence of CKD in the general population is equal to 10% [3].

The determination of NGAL in the urine may be also an indicator of chronic kidney disease (CKD) [2]. Bolignano et al [18] proposed the use of NGAL in clinical practice as a predictive factor of worsening renal function in patients with chronic renal failure. In patients with CKD the increases of NGAL in the urine correlated with respective alterations in serum creatinine, GFR and proteinuria [9]. Furthermore Mitsnefes et al [19] found a significant correlation between serum NGAL and cystatin C levels. Due to the fact that NGAL is a sensitive marker of renal damage [20], its determination in chronic kidney disease with proteinuria allows to assess the degree of kidney damage. It rises the possibility using this protein for early identification and monitoring of patients with CKD. It has been demonstrated that even a single determination of NGAL in the urine, but not serum creatinine, may be helpful in differentiating patients with acute renal failure and chronic kidney disease [21]. NGAL is also a marker in the pathological process leading to the polycystic kidney disease or glomerulonephritis [14,18].

It seems that NGAL may also be an early predictive marker of disease activity in patients with lupus nephritis [3,22]. Suzuki et al [22] observed a significant NGAL increase in plasma and urine of children with SLE compared with juvenile
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Table II.
NGAL as a biomarker of acute kidney injury [according to 5].

<table>
<thead>
<tr>
<th>Significance of NGAL</th>
<th>References</th>
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<tr>
<td>specific marker of acute kidney injury (AKI)</td>
<td>5, 10</td>
</tr>
<tr>
<td>differentiation of AKI types (pre-renal azotemia and intrinsic AKI)</td>
<td>21, 9</td>
</tr>
<tr>
<td>sensitive marker in early and established diagnosis</td>
<td>5, 9</td>
</tr>
<tr>
<td>increases proportionally to damage or kidney function loss</td>
<td>5, 14</td>
</tr>
<tr>
<td>detected for development of kidney failure</td>
<td>5</td>
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<tr>
<td>available as automated and standardized assay</td>
<td>12, 5, 9, 14</td>
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idiopathic arthritis and healthy children groups. Rubinstein et al [23] found a significant relationship between NGAL concentration in the urine and exacerbation of lupus nephritis. Due to poor sensitivity of previously used parameters assessing renal function, the search for new diagnostic markers of kidney damage is currently the subject of numerous studies. Neutrophil gelatinase-associated lipocalin (NGAL) seems to be an early biomarker of severe acute and chronic kidney pathologies. Its determination in the urine may be very useful in clinical practice (Table II).

References

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