NGAL as a marker of renal injury

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NGAL: how useful is the new marker of kidney damage?

NGAL (neutrophil gelatinase-associated lipocalin) has recently generated great interest as an early marker of renal injury. However, like many other endogenous biomarker molecules, it is not produced by just one cell type and different pathologies in different tissues can all provoke responses. Results must be interpreted with due regard to concurrent conditions in the individual patient in order to make optimal use of this sensitive marker.

by Dr L. O. Uttenthal

Investigations on the performance of neutrophil gelatinase-associated lipocalin (NGAL) as an early marker of renal injury have certainly not stood still since my short introductory article in CLI [1]. Papers on NGAL are appearing at a rate of over two a week, and the main emphasis is indeed on its role as a marker of renal injury. However, other aspects of this fascinating molecule have not been ignored. Its pathophysiology and its role as a marker in adenocarcinomas are receiving attention, as are its roles in inflammation, atheroma and thrombi, as well as what may be its main function: a binder and transporter of siderophore iron.

NGAL as a marker of renal injury

Several studies have confirmed and consolidated the usefulness of NGAL as an early marker of renal injury, and other studies are underway.

Cardiac procedures

Pediatric cardiopulmonary bypass operations

The first important study [2] was carried out in children undergoing cardiopulmonary bypass. Results showed that urinary NGAL concentrations above 50 ng/mL, measured two hours after surgery, were highly predictive of a subsequent increase in serum creatinine of 50% or more over basal values. This was a "clean" study, as these were children undergoing elective cardiac surgery who were as healthy as their cardiac abnormality allowed.

Adult cardiopulmonary bypass operations

A similarly conceived study in adult cardiac surgery patients has recently been reported [3]. This study showed a marked rise in urinary NGAL even in the first post-operative sample that was taken. Interestingly, a notable rise was also seen in patients who did not subsequently develop a serum creatinine rise of 50% or more. However, in those patients in whom there was such a creatinine rise, the urinary NGAL level also continued to rise, peaking at three hours post-operatively, while the level fell in the other patients. The urine levels observed were such that any cutoff values diagnostic of subsequent acute renal dysfunc-

tion (ARD) at one hour or three hours after surgery would be many times greater than those observed in the paediatric cases [2].

Many adult patients presenting for cardiopulmonary bypass surgery will have a variety of other disorders, such as inflammatory conditions, which are associated with moderate elevations of NGAL. Furthermore, NGAL is also expressed in the arterial endothelium and smooth muscle associated with atherosclerotic plaque, as well as in macrophages within the plaque [4]. It is possible, therefore, that some NGAL may be released from these sources during cardiac surgery and may be rapidly excreted by the kidneys once normal circulation is restored. However, it is also possible that most of the "immediate" release of NGAL into the urine is of renal origin. In that case, it could be postulated that the kidney's NGAL response is so rapid and sensitive that even a minor ischaemic renal injury can easily be detected, even though the kidney will quickly recover without giving rise to the functional impairment leading to a 50% rise in serum creatinine.

A potential cause of renal injury in cardiopulmonary bypass operations is the use of large doses of aprotinin as an antifibrinolytic agent to reduce blood loss [5]. It will be interesting to assess the degree to which the use of aprotinin correlates with NGAL responses and subsequent ARD.

Percutaneous coronary interventions

NGAL as a marker of renal injury has also been studied in patients subjected to percutaneous coronary interventions (PCI) with coronary angiography [6, 7]. Here, the chief risk for the kidneys is thought to be the radiographic contrast agents used in this procedure. A significant (but modest) rise in serum NGAL was seen in the first sample taken at two hours after PCI; this peaked at four hours, while the rise in urine NGAL peaked at eight hours. Multivariate analysis was performed with serum creatinine, which did not rise significantly during the 48 hours of the study, as well as with serum cystatin C, which peaked at 24 hours. None of the patients developed ARD. This is an interesting study of potential subclinical renal injury from the contrast agent, in which NGAL was clearly the earliest responding marker. However, if the rise in NGAL was really due to nephrotoxic renal injury, it is surprising that the serum response pre-empted the urinary response. It should be noted that both the mean serum level and the mean urinary NGAL level were elevated above normal basal levels even before PCI. NGAL is present in the arteries and atheromatous plaque visualised in this study, and raised plasma NGAL levels have previously been observed in patients with risk factors for atherosclerosis [8]. It is possible, therefore, that the early rise in serum NGAL level may have been due to a direct effect of PCI on the cannulated arteries.

Rapidity of the NGAL response

The fact that the post-operative samples in [3] were taken at various times after the start of the bypass, raises the question of just how rapid the renal NGAL response to injury is. In published clinical studies, the first sample has been taken either one or two hours after the commencement or conclusion of the potentially injurious procedure. The fact that very substantial rises in NGAL level are seen after one hour suggests that significant rises might also be observed after a shorter time of 30 or even 15 minutes. This question remains to be formally addressed. However, if the kidney is capable of mounting an NGAL response in very much under an hour, it casts doubt on whether the initiation of the response is entirely due to the upregulation of transcription and translation. Such a rapid response would imply that pre-synthesised NGAL is being released by a secretory mechanism. While protein expression of NGAL by the kidney is modest in its normal state, the NGAL that appears in kidney tubule cells after an injury (cisplatin nephrotoxicity) shows a punctate appearance when viewed by a light microscope following immunohistochemistry [9], strongly suggesting localisation in specific secretory granules. This is also in line with the presence of a typical signal peptide in the NGAL precursor. These questions require answers, and many other details regarding the secretion of NGAL from the injured tubule cell require elucidation.
Renal transplantation
NGAL determination will probably also have a part to play in renal transplantation. The correlation of urinary and serum NGAL values with subsequent graft function has been studied in 30 brain-dead kidney donors [10]. A very clear predictive value for the urinary NGAL level was found, such that levels below 65 ng/mL were associated with immediate function of both grafts, levels above 150 ng/mL were associated with delayed or primary non-function of both grafts. Serum levels showed a similar trend. Early measurement of urinary NGAL in the recipient also has predictive value, high levels predicting delayed graft function [11]. After graft biopsy a strong correlation is observed between NGAL immunostaining intensity and cold ischaemia time, peak post-operative serum creatinine and delayed graft function [12].

Cритically ill patients
Unselected patients
A prime concern when assessing the value of NGAL as a marker of renal injury is that the marker should also be functional in patients with a variety of serious concomitant conditions that not only put patients at high risk of renal injury, but also lead to the independent elevation of NGAL from non-renal sources. Such conditions include sepsis and serious local infections, in which NGAL is released from neutrophils, serious chest infections, in which there may be further NGAL release from the respiratory epithelium, as well as a variety of adenocarcinomas that also express NGAL. In studies of “unselected” critically ill patients, it should always be borne in mind that NGAL is released from inflamed or malignantly transformed epithelia. For the time being, the status of the NGAL-MMP-9 complex in the diagnosis of acute renal injury is uncertain, and it would be prudent to regard the free NGAL and the NGAL-MMP-9 complex as two independent markers that are likely to show different response pattern in different pathologies. It is also far from clear how the linkage of NGAL to MMP-9 in humans relates to NGAL’s important function as a binder and carrier of siderophore iron. This linkage has an inhibitory effect on MMP-9 autodegradation and thus tends to enhance the proteolytic effect of the enzyme [18], an effect that has been considered important in studies on the pathology of atheromatous plaque and the effects of mural thrombus on the wall of aortic aneurysms.

Special cases
Urinary NGAL level has also been studied in children with diarrhea-associated haemolytic-uraemic syndrome [14]. Here a cutoff value of 200 ng/mL was found, and children with lower levels within the first five days of hospitalisation were much less likely to require dialysis. This provides further illustration of the fact that cutoff values for the optimal diagnostic or prognostic use of NGAL as a marker of kidney damage are very dependent on the patient population being studied.

Chronic kidney disease
While the chief diagnostic role of NGAL is considered to be as a marker for acute renal injury, studies are also being carried out on its behaviour in chronic renal disorders. These include some very different pathologies, and it is evident that these will have very different effects on NGAL levels, and that the stage of the particular pathology will also be significant. It is therefore hardly to be expected that particular NGAL levels could be applied to any generalised diagnostic or prognostic evaluation of undifferentiated chronic renal failure. Nevertheless, in patients with childhood-onset systemic lupus erythematosus (SLE) it has been found that urinary NGAL levels were moderately to strongly correlated with renal disease activity and renal damage, but not with extra-renal disease activity or damage [15]. Children with chronic kidney disease in stages 2-4 had serum NGAL levels that correlated significantly, if not highly so, with glomerular filtration rate (GFR), and at lower values of GFR, serum NGAL level correlated rather better than cystatin C level [16]. However, whether this correlation found in a group of patients with heterogeneous renal conditions can be used to assess individual patients will depend on more detailed studies being carried out on patients with chronic kidney diseases of defined aetiology.

Renal and extra-renal NGAL: free NGAL and NGAL-MMP-9 complex
It is apparent from some of the studies described that NGAL is far from being produced exclusively by the injured kidney. Different levels of extra-renal secretion of NGAL in concomitant conditions influence the cutoffs that have to be employed to ensure that NGAL levels are optimally diagnostic of renal injury. NGAL of extra-renal origin continues to receive attention, both with respect to its role in neutrophil activity and inflammation as well as in relation to its expression in certain cancers. In renal tubule cells and neutrophil precursors, as well as in other cells including epithelial cells that have undergone malignant transformation, NGAL may be co-expressed with gelatinase B, also called matrix metaloproteinase-9 (MMP-9). Human NGAL has an extra cysteine residue that permits covalent linkage to MMP-9, a feature that has given NGAL its present name, but this residue is absent in rats and mice, so that covalent NGAL-MMP-9 complexes cannot occur in these animals [17]. The relationship between free NGAL and NGAL-MMP-9 complex is by no means a regular one, and this relationship requires further study both in relation to renal injury and to extra-renal NGAL release from inflamed or malignantly transformed epithelia. For the time being, the status of the NGAL-MMP-9 complex in the diagnosis of acute renal injury is uncertain, and it would be prudent to regard the free NGAL and the NGAL-MMP-9 complex as two independent markers that are likely to show different response pattern in different pathologies.

Conclusions
In the current phase of investigation, NGAL is still being evaluated as a renal marker, and levels are thus being compared with other renal markers, especially the traditional functional marker, serum creatinine, and the more recent marker of GFR, serum cystatin

Figure 1. The molecular structure of NGAL.
C. The trouble with these comparisons is that raised NGAL of renal origin is a direct response to tubule cell injury, while the others are functional markers that may, after a period of time, reveal the effect of such an injury on the accumulation of creatinine and cystatin C in the blood. Serum creatinine is the physician’s old, cheap and unreliable friend; despite repeated warnings in textbooks and reviews, it is sometimes forgotten just how variable the rate of creatinine release into the blood may be in patients with very different dietary habits and muscle mass, and how rapidly levels may change in critical inflammatory and infectious diseases [19]. Thus many of the currently accepted definitions of ARD, or the RIFLE criteria for acute renal injury, which are based on absolute and/or relative rises in serum creatinine, have little hope of providing an accurate diagnosis of ARD in seriously ill patients where the process of the creatinine production is so variable. Cystatin C may be a better indicator of glomerular filtration because its supply is less variable. Nevertheless, it takes time for cystatin C to accumulate in the blood after a renal injury. Assessments of NGAL as a marker of acute renal injury in comparison to these markers, or clinical criteria based on them, are thus comparisons of different entities, destined to give unclear answers. Such studies must be performed with due care, in order to follow levels of these markers over an adequate period of time and allow for the slow response of the functional markers.

If it were possible to measure NGAL exclusively of renal origin, it would probably be one of the best markers of renal tubule cell damage that could be conceived. In urine, NGAL shows a 10,000-fold concentration rise from normal levels in cases of renal injury; in plasma the maximum rise is about 100-fold. This makes NGAL potentially a very sensitive marker of different degrees of renal injury. However, the lower end of this wide dynamic range represents NGAL increases from extra-renal sources, so it will be the diagnostician’s privilege and responsibility to take this into account.

References
2. Mishra J et al. Neutrophil gelatinase-associated lipoca-