NGAL, or neutrophil gelatinase-associated lipocalin, is a small, robust protein expressed by neutrophils and various epithelia, including the renal proximal tubules. While initially proposed as a marker for infections and certain adenocarcinomas, it is now apparent that its early and dramatic rise in urine after renal injury may make it a useful marker of such injury.

Neutrophil gelatinase-associated lipocalin, or NGAL [1], belongs to the lipocalin family of proteins. These are typically small secreted proteins characterised by their ability to bind small, hydrophobic molecules in a structurally conserved pocket formed by a β-pleated sheet, and to form macromolecular complexes. Many lipocalins also bind to specific cell-surface receptors, but so far no NGAL receptor has been identified. NGAL has many synonyms: it is also known as neutrophil lipocalin (NL or HNL for the human form) [2], lipocalin 2, oncogene protein 24p33 or urocrisin [4] in the mouse, and neu-related lipocalin [5] or 25 kDa α2-microglobulin-related protein [6] in the rat. Human NGAL consists of a single disulphide-bridged polypeptide chain of 178 amino-acid residues with a calculated molecular mass of 22 kDa [1], but glycosylation increases its apparent molecular mass to 25 kDa. In neutrophils and urine it occurs as a monomer, with a small percentage of dimer and trimer [Figures 1 and 2], and it also occurs as a complex with 92-kDa human neutrophil type IV collagenase, also called gelatinase B or matrix mettalloproteinase-9 (MMP-9) [1, 7].

Human NGAL was originally isolated from the supernatant of activated neutrophils [1], but it is also expressed at a low level in other tissues including the kidney, prostate and epithelia of the respiratory and alimentary tracts [8, 9]. It is strongly expressed in adenomas and inflamed epithelia of the bowel [10], adenocarcinomas of the breast [11] and urothelial carcinomas [12].

Because of its small molecular size and resistance to degradation, NGAL is readily excreted and detected in the urine, both in its free form and in complex with MMP-9. Urinary levels correlate with plasma or serum levels whatever the cause of increased NGAL production [own data], but particularly high urinary levels can be expected when it is released directly into the urine by the kidney tubules or by urothelial carcinomas. It is uncertain how far NGAL-MMP-9 complexes from sources remote from the urinary tract are excreted as such into the urine, or reform in the urine after independent excretion of NGAL and MMP-9 [7].

While the functions of NGAL are not fully understood, it appears to be upregulated in cells under "stress", e.g. from infection, inflammation, ischaemia or neoplastic transformation, or in tissues undergoing involution, such as the post-partum mouse uterus and mammary glands on weaning. In relation to a possible antibacterial role, NGAL binds enterobactin and other siderophores, depriving the microorganisms of Fe3+, an important nutritional requirement [13]. Its complex formation with MMP-9 appears to protect MMP-9 enzymatic activity from degradation [7]. The upregulation of NGAL in involuting tissues has led to the postulation of a role in apoptosis, but it appears more likely that NGAL is associated with a survival response [14]. This seems to be so in the kidney, where NGAL-siderophore-iron complex rescues the mouse kidney from ischaemic injury [15].

NGAL in inflammation or infection
NGAL is released from the secondary granules of activated neutrophils [1] and plasma levels rise in inflammatory or infective conditions, especially in bacterial infections [16]. Thus the level of NGAL in plasma or serum has been proposed as a marker of infection. However, as levels of NGAL may also be raised in neoplastic conditions and renal disorders independently of any infective process, this proposed application should be treated with caution. NGAL may also be raised in infections in patients with an uncountably low number of neutrophils due to leukaemia or treated leukaemia, showing that the source of the raised NGAL in infections is not only the neutrophils. Indeed, serum NGAL levels correlate very poorly with the neutrophil count in unselected critically ill patients [own data]. In view of the possible release of NGAL from the kidney (see below) when sepsis becomes severe enough to affect it, data on NGAL in sepsis should by reassessed to take this into account.

NGAL and the kidney
Even before NGAL had been isolated from human neutrophils, its mouse homologue 24p3 was known to be expressed by kidney cells and to undergo an early, dramatic upregulation (14- to 20-fold) in response to SV-40 viral infection [18]. A similar early and dramatic upregulation was later observed in rat proximal tubule cells after ischaemia-reperfusion injury [19], and raised plasma levels of NGAL were found to be strongly correlated with decreased renal function in patients with renal damage due to systemic vasculitis [20]. The results for renal ischaemia-reperfusion injury were subsequently confirmed and extended to nephrotoxic agents [21, 22, 23]. It has been suggested that urinary NGAL levels may serve as an early marker for ischaemic renal injury in children after cardiopulmonary bypass [24]. Raised urinary and serum NGAL levels have also been observed in patients with established renal failure [own data] and patients with functioning renal grafts also showed urinary levels that were sufficiently raised to be readily detectable by Western blotting [12]. It is therefore apparent that a large variety of renal disorders are associated with raised plasma and urinary levels of NGAL. While plasma and urinary NGAL levels are closely correlated in acute conditions, it is to be expected that urinary NGAL levels will be particularly high after ischaemic renal injury severe enough to result in acute renal failure, acute tubular necrosis or acute tubulo-interstitial nephropathy. However, the use of urinary NGAL as a potential marker for these conditions is...
subject to the proviso that the presence of concurrent conditions that are independently associated with raised NGAL levels are taken into account.

NGAL quantification
In many of the above studies on NGAL, the protein has been quantified by immunoblotting. However, some research groups have developed specific ELISAs, based on polyclonal and/or monoclonal antibodies to NGAL [16, 25]. The recent development of a commercial sandwich ELISA that measures NGAL in urine, plasma or serum [Figure 3] should make it easier for clinical investigators to assess the potential of this interesting molecule, either as a diagnostic marker for different pathologies, or as a marker of disease progression or response to treatment. Some NGAL may be released from neutrophils during the preparation of serum, making it generally preferable to use plasma, and urine should be centrifuged to remove neutrophils in cases of urinary tract infection.

NGAL as a potential diagnostic marker
It is apparent that a variety of independent pathologies are associated with raised levels of urinary or plasma NGAL. Therefore the finding of a raised level cannot be independently diagnostic of any one of these pathologies. Other information concerning the patient must be taken into account in order to assess the significance of the result. As other quite effective marker molecules are available to assess inflammatory and infective states (e.g. procalcitonin as a sepsis marker), and as more specific markers are available for many of the cancers in which NGAL is raised, it seems likely that the interest in NGAL will centre chiefly on its role as a marker of kidney damage, where its early and marked response to the insult (within 2 hours [24]) makes it one of the best markers hitherto studied. It is to be expected that serial rather than isolated single measurements of NGAL, whether in urine or plasma, will provide the most useful data from patients with several concurrent pathologies.

References

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Figure 2. The NGAL dimer, which occurs as a small % in neutrophils and urine.

Figure 3. The NGAL ELISA kit (Antibodyshop A/S) measures NGAL in urine, plasma or serum.