Immunoochemical Determination of Monomer, Homodimer and Total Neutrophil Gelatinase-Associated Lipocalin


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Background
NGAL (neutrophil gelatinase-associated lipocalin) released from the kidney in early acute kidney injury is reported to be chiefly in the monomer form, whereas NGAL released from neutrophils in inflammation contains a substantial amount of homodimer in addition to monomer. Immunochemical assays that can specifically measure monomer and homodimer forms of NGAL without the need for molecular size separation are therefore of interest for the rapid measurement of NGAL in different pathological conditions, as well as a “total” NGAL assay that measures monomer and homodimer forms simultaneously.

Methods
By pairwise testing of a large series of mouse monoclonal antibodies raised against recombinant human NGAL in sandwich ELISA, we have developed immunochemical assays specific for monomer and homodimer NGAL, as well as an assay for “total” NGAL. The specificities of the ELISAs were verified by means of recombinant human NGAL monomer and homodimer standards and peaks of native NGAL obtained by gel filtration (molecular size exclusion chromatography) of urine. The levels of the NGAL forms were measured in 40 urine samples from ICU patients. NGAL was also measured with a fully automated central laboratory particle-enhanced turbidimetric immunoassay, The NGAL Test™ (BioPorto Diagnostics A/S).

Results
The monomer NGAL assay cross-reacted <1% with homodimer NGAL and the homodimer NGAL assay cross-reacted <0.1% with monomer NGAL, while the “total” NGAL assay reacted equally with NGAL monomer and homodimer on a mass basis (Figure 1).

Get filtration of urine sample #18 revealed a major peak of NGAL monomer and a minor peak of NGAL homodimer (Figure 2). Analysis of the NGAL forms in 40 randomly selected urine samples showed that NGAL monomer is the major form. The median NGAL homodimer content is as little as 3 ng/mL (only 0.7% of “total” NGAL) and rarely exceeds 100 ng/mL (Table 1).

Conclusions
- ELISAs specific for monomer and homodimer forms of NGAL were developed, as well as an ELISA for “total” NGAL that measures monomer and homodimer equally on a mass basis.
- NGAL monomer is the major form of NGAL in urine from ICU patients, while the occurrence of NGAL homodimer is low and sporadic.
- The NGAL Test™, a particle-enhanced turbidimetric immunoassay for central laboratory equipment, gave results for urine samples that were comparable with those obtained by the ELISAs for monomer and “total” NGAL.
- The occasional presence of low levels of NGAL homodimer has little impact on the interpretation of results obtained with monomer-reactive, homodimer cross-reactive NGAL assays.