

Immunochemical 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 identically.

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).

Gel 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).

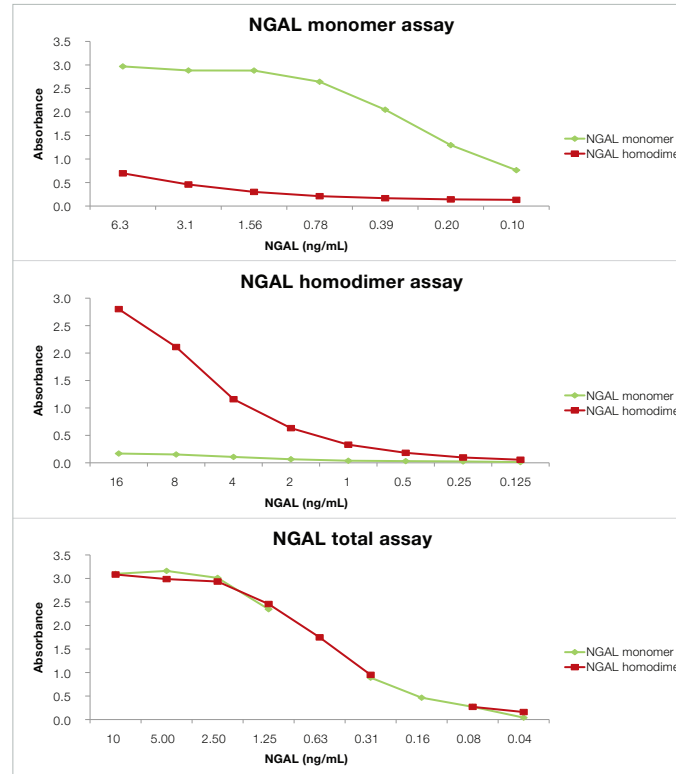


Figure 1. Specificity of ELISAs for monomer, homodimer and "total" NGAL tested with recombinant NGAL monomer and homodimer.

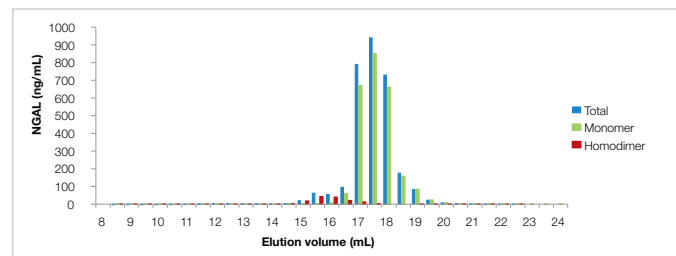


Figure 2. Levels of monomer, homodimer and "total" NGAL in fractions from gel filtration of urine sample #18.

Sample No.	The NGAL Test ng/mL	"Total" NGAL ELISA ng/mL	Monomer NGAL ELISA ng/mL	Homodimer NGAL ELISA ng/mL	Homodimer/"Total"	(Monomer + Homodimer)/"Total"
1	109	108	97	0.2	0.10%	90%
2	324	347	321	1.0	0.30%	93%
3	888	965	930	5.8	0.60%	97%
4	10	82	116	0.1	0.10%	141%
5	229	218	220	1.6	0.70%	102%
6	601	669	754	2.8	0.40%	113%
7	1512	1794	1726	13.1	0.70%	97%
8	44	41	37	1.8	4.30%	96%
9	443	440	511	3.0	0.70%	117%
10	48	45	41	0.4	0.80%	92%
11	989	992	1064	11.0	1.10%	108%
12	714	817	868	7.1	0.90%	107%
13	168	166	167	1.1	0.70%	101%
14	5647	6191	5431	86.3	1.40%	89%
15	431	450	475	2.4	0.50%	106%
16	1234	1405	1283	14.8	1.10%	92%
17	100	89	89	0.2	0.20%	99%
18	2044	1819	1653	143.1	7.90%	99%
19	1264	1277	1176	7.5	0.60%	93%
20	147	120	121	0.6	0.50%	101%
21	79	80	79	0.5	0.60%	99%
22	1036	1038	1044	11.4	1.10%	102%
23	1263	1252	990	14.0	1.10%	80%
24	124	125	110	0.7	0.60%	89%
25	685	839	988	3.9	0.50%	118%
26	763	835	857	12.2	1.50%	104%
27	99	85	82	<LLD		97%
28	1297	1361	1170	78.3	5.80%	92%
29	235	227	235	<LLD		103%
30	2158	2038	2078	<LLD		102%
31	2168	2222	1998	<LLD		90%
32	96	86	79	2.7	3.10%	95%
33	100	127	125	1.1	0.80%	99%
34	81	77	74	0.6	0.70%	98%
35	27	25	20	4.6	18.50%	99%
36	1106	1095	971	14.8	1.30%	90%
37	56	51	50	0.2	0.40%	98%
38	301	303	307	0.7	0.20%	102%
39	4730	4724	4491	6.1	0.10%	95%
40	42	43	44	0.4	1.00%	104%
Median	378	394	398	3	0.70%	99%
IQR	100 - 1138	88 - 1135	95 - 1049	1 - 11	0.5% - 1.1%	93% - 102%

Table 1. Molecular forms of NGAL in 40 urine samples from ICU patients. In 4 samples the NGAL homodimer level was below the detection limit (<LLD).

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.