

# The Monomer is the Major Form of NGAL in Urine and Plasma in Severe Sepsis

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## Background

Neutrophil gelatinase-associated lipocalin (NGAL) is a marker of acute kidney injury (AKI), but is also released from neutrophils as part of the inflammatory response and on phagocytosis of bacteria. It has been reported that the injured kidney releases NGAL in its monomer form. In contrast, neutrophils have been claimed to release NGAL primarily as homodimer, i.e. two NGAL monomers linked by a disulfide bridge<sup>1</sup>. Patients with severe sepsis have both an elevated release of NGAL from neutrophils and a high risk of AKI. We have measured the levels of NGAL monomer and homodimer in urine and plasma samples from patients suffering from severe sepsis.

## Methods

For quantifying the two main molecular forms of NGAL in clinical samples, we developed sandwich ELISAs specific for NGAL monomer and homodimer, respectively, as well as an ELISA for “total NGAL” (monomer + homodimer). These assays use mouse monoclonal antibodies raised against recombinant human NGAL. The specificities of the ELISAs were verified by means of recombinant human monomer and homodimer standards as well as native NGAL in fractions from gel filtration (molecular size exclusion chromatography) of EDTA plasma.

NGAL monomer and homodimer were measured in sets of urine and EDTA plasma samples from 151 ICU patients suffering from severe sepsis<sup>2</sup> with the specific ELISAs and compared with NGAL measured with a fully automated NGAL assay, The NGAL Test™, BioPorto Diagnostics A/S, Denmark. AKI was defined as a 50% increase in plasma creatinine levels.

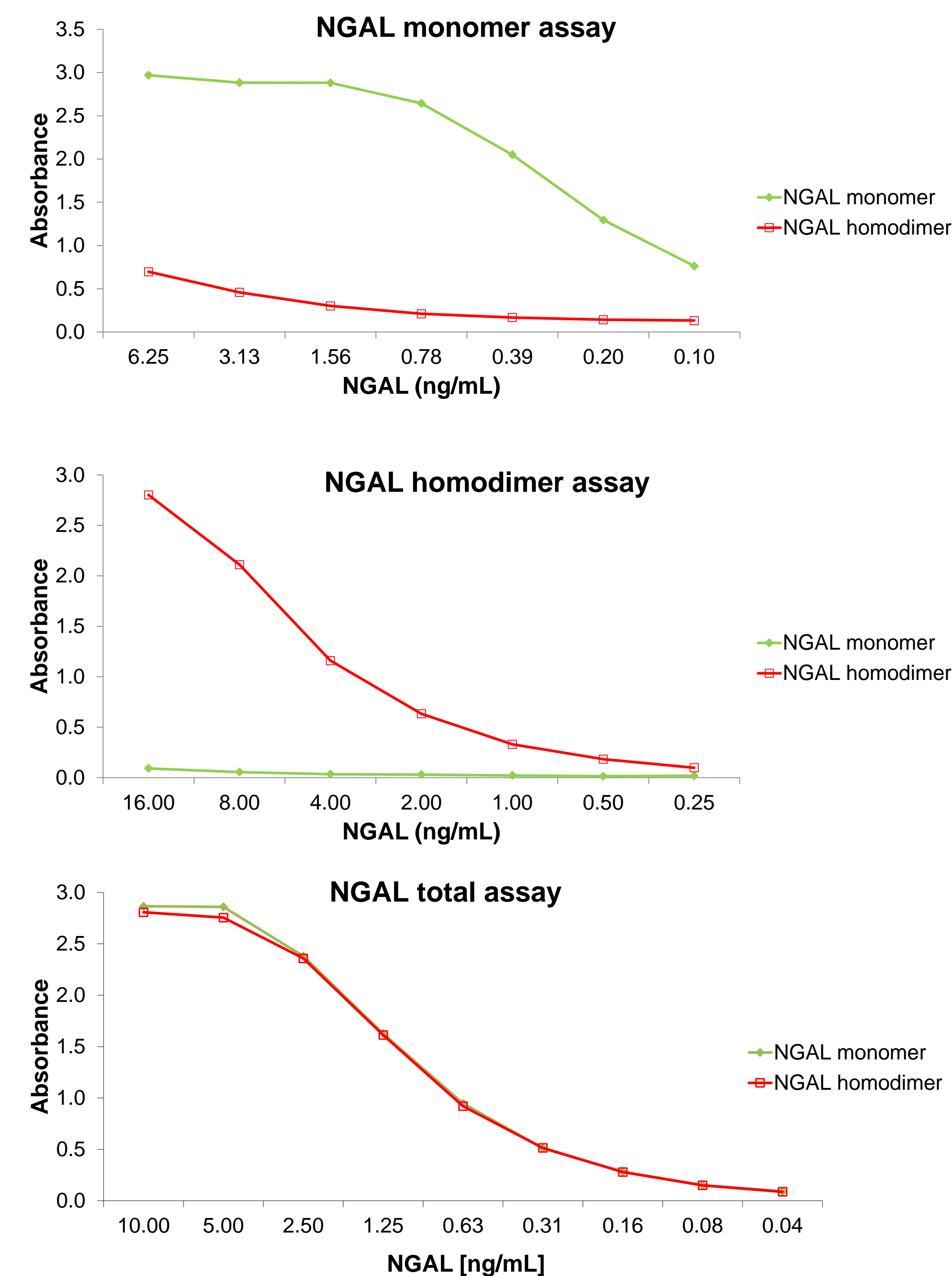
## Results

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).

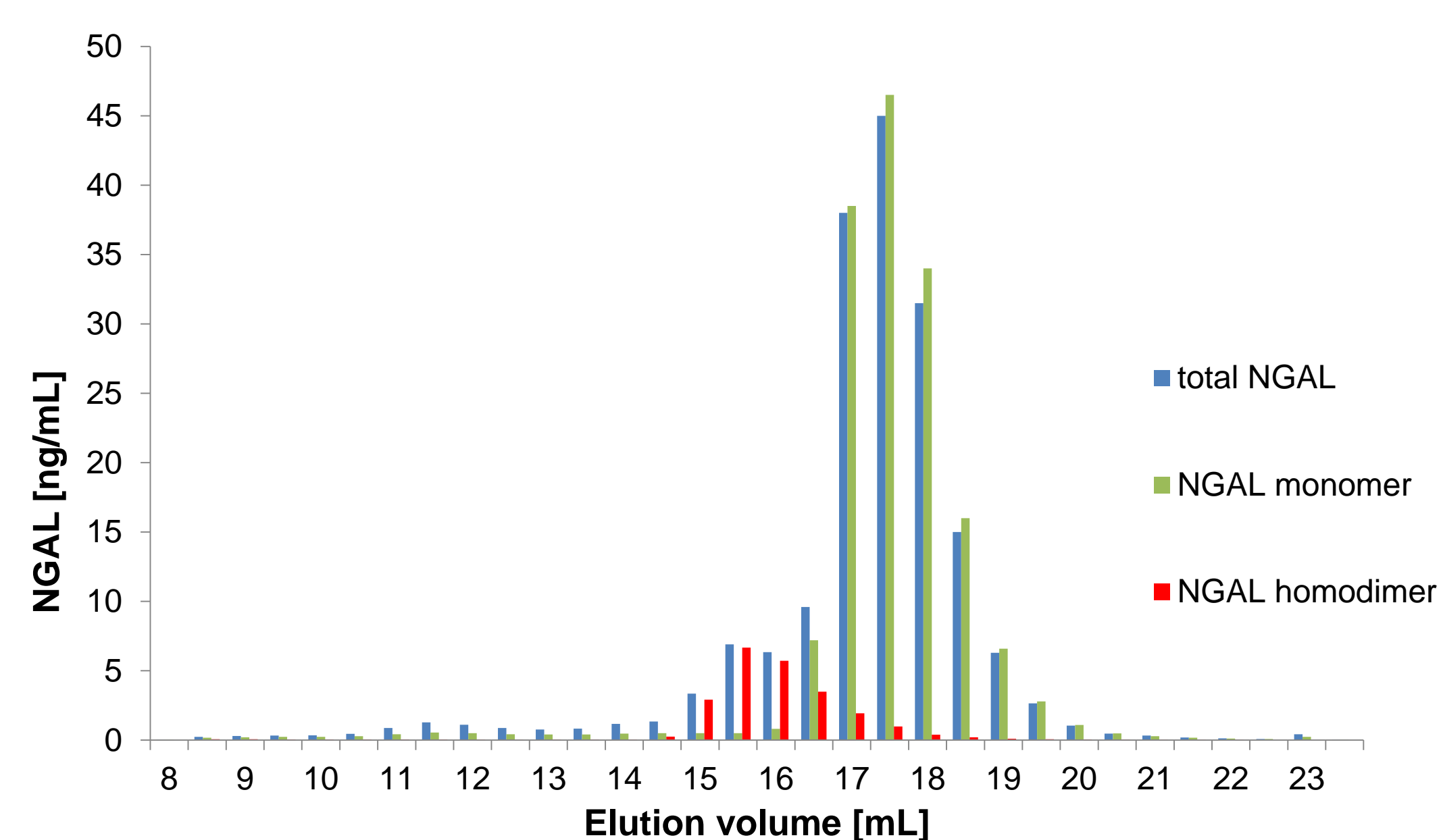
Gel filtration of a plasma sample from a patient suffering from severe sepsis revealed a major peak of NGAL monomer and a minor peak of NGAL homodimer (Figure 2). The NGAL homodimer content was 12% of the “total NGAL” in this sample.

Analysis of the NGAL forms in urine and plasma from 151 ICU patients with severe sepsis showed that NGAL monomer was the major form in both sample types (Table 1). The median NGAL homodimer content was 8.7 ng/mL in urine and 30.6 ng/mL in plasma, corresponding to 1.2% and 6.0%, respectively, of “total NGAL”. The median NGAL monomer content was 374 ng/mL in urine and 485 ng/mL in plasma. Results obtained with The NGAL Test™ showed a very good agreement with NGAL monomer concentrations.

83 of the 151 patients developed AKI. NGAL monomer and NGAL measured with The NGAL Test™ in urine and plasma performed equally well in diagnosing AKI (AuROC 0.75 and 0.74, respectively, in plasma, and 0.78 and 0.78 in urine; Table 1 and Figure 3). For NGAL homodimer, the AuROC values were lower (0.70 in urine and 0.59 in plasma).



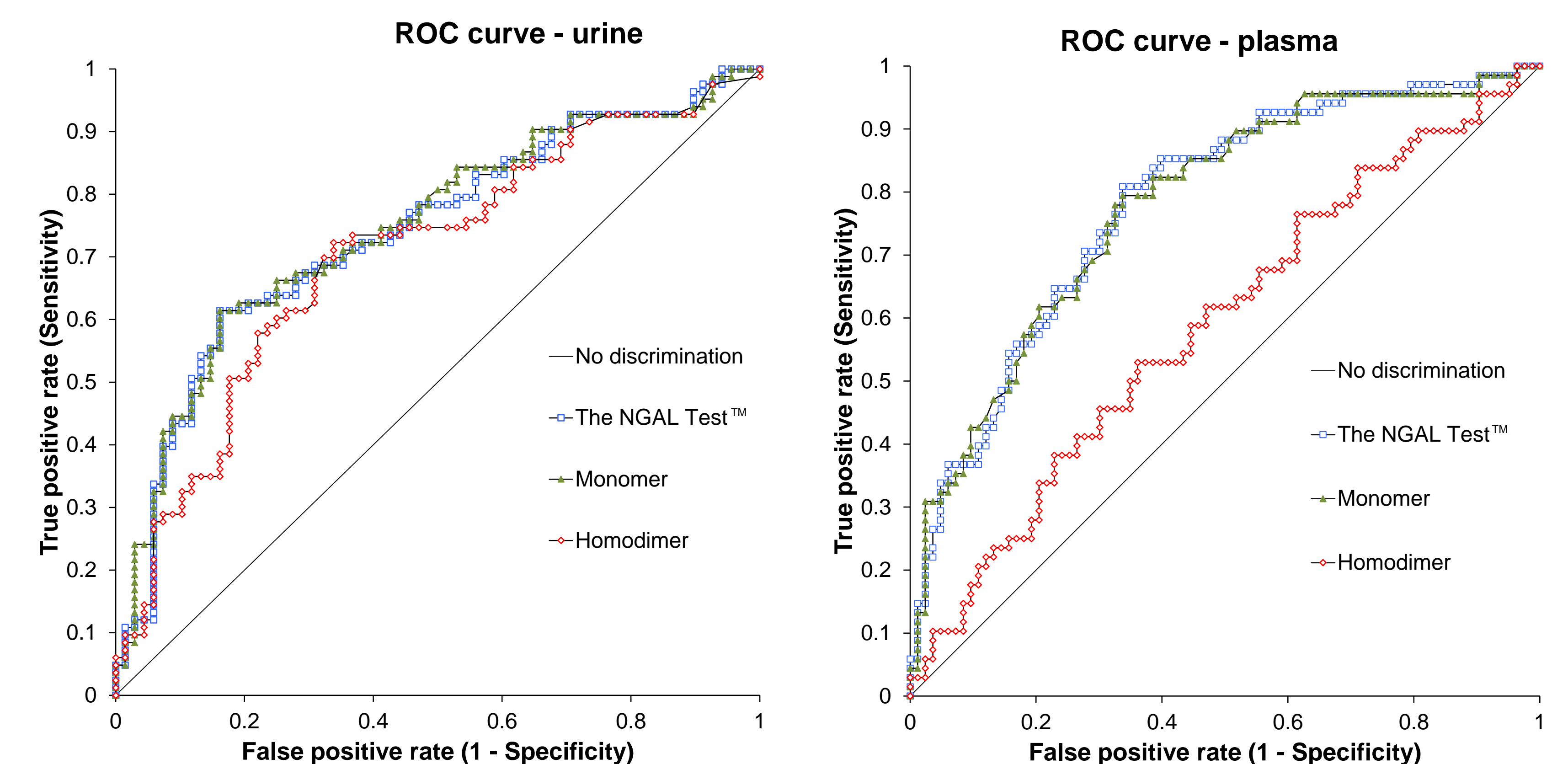
**Figure 1.** Specificity of ELISAs for monomer, homodimer and “total” NGAL tested with recombinant NGAL monomer and homodimer.



**Figure 2.** Levels of monomer, homodimer and “total” NGAL in fractions from gel filtration of a 10-fold diluted plasma from a patient suffering from severe sepsis. NGAL was measured with the molecular form-specific ELISAs.

		Median	25 <sup>th</sup> and 75 <sup>th</sup> percentiles	AuROC	95% confidence interval
		ng/mL	ng/mL		
Urine	Homodimer	8.7	1.2 – 31.8	0.70	0.62 – 0.79
	[% of “total” NGAL]	[1.2%]	[1.0% – 2.6%]		
	Monomer	374	82 – 1917	0.75	0.67 – 0.82
	The NGAL Test™	374	81 – 2082	0.74	0.66 – 0.82
Plasma	Homodimer	30.6	17.2 – 50.6	0.59	0.50 – 0.68
	[% of “total” NGAL]	[6.0%]	[3.3% – 11.3%]		
	Monomer	470	238 – 853	0.78	0.70 – 0.85
	The NGAL Test™	485	247 – 839	0.78	0.71 – 0.85

**Table 1.** Molecular forms of NGAL in urine and plasma from 151 ICU patients with severe sepsis. The area under receiver operating characteristic (AuROC) curve reflects the performance of the different NGAL forms in diagnosing AKI.



**Figure 3.** Receiver operating characteristic (ROC) curves for the diagnosis of AKI with different NGAL assays in urine and plasma from patients suffering from severe sepsis. See Table 1 for AuROC values. The level of NGAL homodimer in 42% of the urine samples was below the detection limit and the highest possible homodimer concentration, i.e. 0.2% of the “total” NGAL concentration, was used. This could make the performance of the homodimer values look better than if measured low values had been available for use.

## Conclusions

The monomer is the major form of NGAL found in urine and plasma from patients suffering from severe sepsis. 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 in samples from patients suffering from severe sepsis.

<sup>1</sup>Cai et al (2010) Clin J Am Soc Nephrol 5:2229-2235

<sup>2</sup>Perner et al (2011) Trials 12: 24