DEVELOPMENT OF A NEW ENZYME-LINKED IMMUNOSORBENT ASSAY KIT TO DETECT NGAL IN HUMAN SERUM AND ITS APPLICATION TO CHRONIC KIDNEY DISEASE

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INTRODUCTION

Neutrophil Gelatinase-Associated Lipocalin (NGAL) or Lipocalin-2 (LCN2) is a member of the lipocalin family of proteins which are known for the transportation of small hydrophobic ligands[1]. NGAL was originally discovered in the granules of neutrophils but has since been found in many other human tissues including breast, kidney and liver. NGAL itself has many functions, for instance sequestering of iron, prevention of bacterial growth, chemoattraction of neutrophils, reduction of oxidative stress and regulation of cancer cell survival[2]. It has been reported however that NGAL is highly upregulated upon kidney damage where levels can rise by ~ 10 fold in 3 hours depending on the type and severity of injury[3-5]. The early detection of biomarkers for kidney injury may improve the diagnosis of conditions such as chronic kidney disease (CKD) or acute kidney injury (AKI) and allow timely determination of treatment which may ultimately slow progression. The availability of tests enabling the detection of this protein represents an advantage in clinical research settings. This study aimed to develop a new enzyme-linked immunosorbent assay (ELISA) for the detection of NGAL in human serum.

METHODOLOGY

A colorimetric 2-step sandwich immunoassay was employed. The capture antibody was immobilised and stabilised on a 96-well microtitre plate surface. The analyte, if present in the sample, binds to the capture antibody and then a second antibody labelled with horseradish peroxidase binds to the analyte. Absorbances were read at 450nm. The signal is proportional to the concentration of the analyte in the sample. All assay kit reagents are ready to use. Recognition of NGAL was tested with analysis of 40 CKD samples (10 normal, 10 stage 1, 10 stage 2 and 10 stage 3). Statistical analyses were performed by Mann Whitney test (with bon ferroni correction) (Medcalc version 16.4.3).These samples were also measured on another commercially available ELISA and the results compared by linear regression analysis.

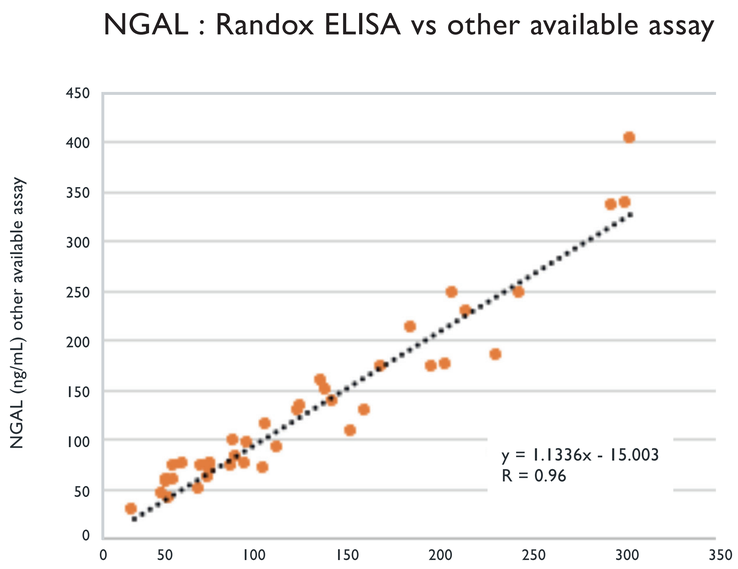
RESULTS

The assay exhibited a functional sensitivity of 20 ng/mL (measuring range of 0-2000 ng/mL, allowing for a 1 in 100 sample dilution).

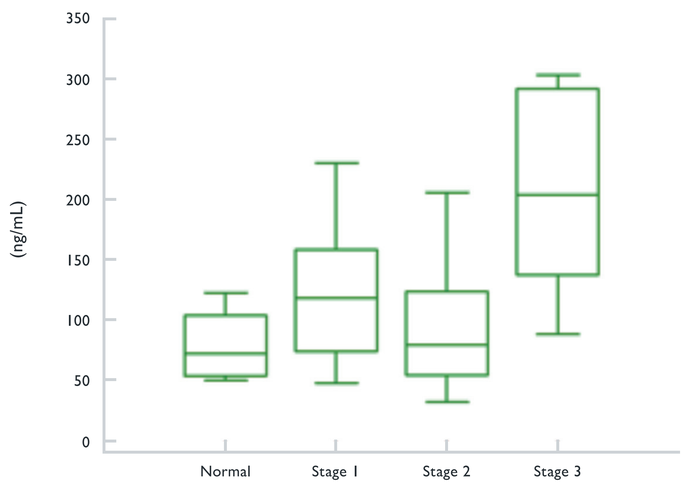
The intra-assay precision, expressed as %CV, was <10% (n=12) for different concentration levels.

Intra-assay precision (n=12)	
	CV (%)
Level 1	3.9
Level 2	2.6
Level 3	4.3
Level 4	3.7
Level 5	6.1
QC 1	8.9
QC 2	6.6

The correlation study showed, with 40 samples ranging from 32 to 302 ng/mL, a correlation coefficient of 0.96 and a slope of 1.1.



Concentrations of native NGAL from Stage 3 CKD serum samples (median 205ng/mL) were significantly elevated when compared to controls (median 72ng/mL), p=0.0030. Stage 1 and 2 samples were not significantly elevated when compared to controls.



14.056.690G, 14.052.690G, 14.066.690G

CONCLUSION

The results show applicability of the developed ELISA for the sensitive detection of NGAL in serum. NGAL is shown to be a useful biomarker for patients at stage 3 CKD. The assay presents all kit reagents ready to use and 48 samples can be measured in less than 3 hours. This assay is a useful analytical tool for clinical research studies.

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