Neutrophil Gelatinase-Associated Lipocalin as a Biomarker of Disease Activity in Pediatric Lupus Nephritis

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By

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List of Abbreviations

24p3 : Mouse 24p3/uterocalin

A1M : α 1-Microglobulin

ACE : Angiotensin-Converting Enzyme

A-FABP : Adipocyte-Fatty Acid Binding Protein

AGP : Acid Glycoprotein

ANA : Antinuclear Antibody

APPs : Acute-Phase Proteins

ARBs : Angiotensin II Receptor Blockers

BBP : Bilin-Binding Protein

Blg : Beta Lactoglobulin

BLyS : B-Lymphocyte Stimulator

BMI : Body Mass Index

BUN : Blood Urea Nitrogen

C : Complement

CD : Cluster of Differentiation

CNS : Central Nervous System

DNA : Deoxyribonucleic Acid

dsDNA : Double stranded Deoxyribonucleic Acid

ELISA : Enzyme-Linked Immunosorbent Assay

ESR : Erythrocyte Sedimentation Rate

FABPs : Fatty-Acid-Binding Proteins

fMLP : formyl-Methionyl-Leucyl-Phenylalanine

GFR : Glomerular Filtration Rate

HS : Highly Significant

HSP : Henoch–Schönlein Purpura

Ig : Immunoglobulin

IL : Interleukin

ISN/RPS : International Society of Nephrology/Renal Pathology Society

IVCY : Intravenous Cyclophosphamide

kDa : kilo Dalton

LN : Lupus Nephritis

LPS : Lipopolysaccharide

LTB4 : Leukotriene B4

MMF : Mycophenolate Mofetil

MMP-9 : Matrix Metalloproteinase-9

n : Number

NGAL : Neutrophil Gelatinase Associated Lipocalin

NIH : National Institutes of Health

NS : Not significant

NSAIDS : Nonsteroidal Anti-Inflammatory Drugs

PAF : Platelet Activating Factor

PP14 : Pregnancy Protein 14

RA : Rheumatoid Arthritis

RBCs : Red Blood Cells

RBP : Retinol-Binding Protein

REA : Retinoic Acid

S : Significant

SCRs : Structurally Conserved Regions

SIP : Superinducible protein

SLE : Systemic Lupus Erythematosus

SLEDAI : Systemic Lupus Erythematosus Disease Activity Index

TNF : Tumor Necrosis Factor

WHO : World Health Organization

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Introduction

Renal involvement is one of the main determinants of poor prognosis of systemic lupus erythematosus (SLE) and is more frequently encountered in children than in adults with SLE ***(Hochberg, 1997)***. Currently available renal biomarkers, *i.e.* measures of the degree of SLE renal disease activity and severity, are too insensitive to allow for early identification of patients with active SLE nephritis, prohibiting timely initiation of therapy to avoid permanent renal damage ***(Ho et al., 2001)***. Randomized clinical trials in SLE are hindered by the lack of high-quality biomarkers to verify the effects of therapies within a short period of time ***(Schiffenbauer et al., 2004)***.

Neutrophil gelatinase-associated lipocalin (NGAL) is a member of the lipocalin family of proteins that has been extensively studied in acute kidney injury ***(Schmidt-Ott et al., 2007)***. NGAL is one of the most robustly expressed proteins in the kidney following ischemic or nephrotoxic injury in both animals ***(Supavekin et al., 2003)*** and humans ***(Devarajan, 2005)***. Importantly, a recent prospective pediatric study demonstrated that concentrations of NGAL in urine and plasma represent novel, sensitive, and specific biomarkers for

early identification of acute kidney injury following cardiac surgery ***(Mishra et al., 2005)***.

Aim of the Work

In this study, we hypothesized that serum NGAL change with renal disease activity. The purpose of this study was to assess the relationship of serum NGAL levels with disease activity in pediatric SLE with special emphasis on nephritis.

Neutrophil Gelatinase Associated Lipocalin

Structure:

Various mediators of inflammation, such as prostaglandins, platelet activating factor (PAF), leukotrienes, interleukin 8(IL-8), and granulocyte colony stimulating factor, are chemotactic for neutrophils, which are recruited to inflammatory foci, resulting in the augmentation and progression of the immune response (***Nielsen et al.,1996***).

Activated neutrophils release a variety of membrane-associated proteins and soluble compounds from intracellular granules that mediate neutrophil functions: endothelial adhesion, migration across basement membranes, and the phagocytosis and destruction of pathogenic microorganisms **(*Borregaard, 1997*)**.

*N*eutrophil *g*elatinase *a*ssociated *l*ipocalin (NGAL), a member of the lipocalin family, is released from neutrophil granules as a 25 kDa monomer, a 46 kDa disulfide-linked homodimer, and a disulfide-linked heterodimer with gelatinase B (matrix metalloproteinase 9) **(*Kjeldsen et al.,1993*).**

NGAL is expressed in immature neutrophil precursors **(*Bundgaard et al., 1994*)** and in epithelial cells during both

inflammation and neoplastic transformation (***Nielsen et al., 1996***). Both the murine (24p3) and the rat analogue of NGAL (*neu*-related lipocalin) were originally identified in screens for genes overexpressed during tumorigenesis **(*Stoesz and Gould, 1995*).** Lipocalins are a diverse family of small proteins that generally bind small, hydrophobic ligands but which can also bind soluble, extracellular macromolecules and specific cell-surface receptors **(*Flower, 1996*).**

The lipocalin family is a large, and ever expanding, group of proteins exhibiting great structural and functional variation, both within and between species **(*Flower, 1996*)**.

Lipocalins are typically small (160-180 residues in length), extracellular proteins sharing several common molecular recognition properties: the binding of small, principally hydrophobic molecules (such as retinol); binding to specific cell-surface receptors; and the formation of covalent and non-covalent complexes with other soluble macromolecules. Although they have been classified mainly as transport proteins, it is now clear that members of the lipocalin family fulfil a wide variety of different functions. Despite many common characteristics and common functions, membership of the lipocalin family has been defined largely on the basis of sequence, or structural, similarity and it has

grown to encompass a large corpus of proteins. Within this the lipocalins display unusually low levels of overall sequence conservation, with pairwise comparisons often falling below 20%, the nominal threshold for a reliable alignment. However, all lipocalins share sufficient similarity, in the form of short characteristic conserved sequence motifs, to form the basis of a useful definition of family membership **(*Flower et al.,1993*)**.

Most lipocalins share three characteristic conserved sequence motifs-the kernel lipocalins-while other more divergent family members-the outlier lipocalins-typically share only one or two. Lipocalins of known three-dimensional structure include a group of kernel lipocalins: retinol-binding protein (RBP) **(*Cowan et al.,1990*)**, beta lactoglobulin (Blg) **(*Brownlow et al., 1997*)**, insecticyanin **(*Holden et al., 1987*)**, bilin-binding protein (BBP) **(*Huber et al., 1987*)**, major urinary protein **(*Bocskei et al., 1992*)**, α_{2u}-globulin **(*Chaudhuri et al., 1999*)**, epididymal retinoic acid-binding protein **(*Newcomer, 1993*)**, and neutrophil lipocalin **(*Coles et al., 1999*).**

There are also a number of outlier lipocalin groups including odorant-binding protein **(*Spinelli et al., 1998*)**, Bos d2 allergen **(*Rouvinen et al., 1999*)**, nitrophorin **(*Andersen et al., 1998*)**, and a histamine binding protein from the tick

Rhipicephalus appendiculatus **(*Paesen et al., 1999*)**. The common structure of the lipocalin protein fold is now well-described **(*Flower, 1995*)**. The lipocalin fold is a highly symmetrical all-β structure dominated by a single eight-stranded antiparallel β sheet closed back on itself to form a continuously hydrogenbonded β barrel. In cross-section, this has a flattened or elliptical shape. The β-barrel encloses a ligand-binding site composed of both an internal cavity and an external loop scaffold. The diversity of cavity and scaffold gives rise to a variety of different binding modes each capable of accommodating ligands of different size, shape, and chemical character. The eight β-strands of the barrel, labelled A-H are linked by a succession of +1 connections, giving it the simplest possible β-sheet topology. The seven loops, labelled L1-L7, are all typical of short β-hairpins, except loop L1: this is a large Ω loop. Loop L1 forms a lid folded back to close partially the internal ligand-binding site found at this end of the barrel. Between strand H and the short terminal strand I is an α-helix; this is an everpresent feature of the lipocalin fold but is not conserved in its position relative to the axis of the β-barrel nor in its length. Previous work has analysed the conservation of sequence and structure in the lipocalin protein family **(*Bocskei et al., 1992*).**

These accounts show how the common core characteristic of the lipocalin fold is dominated by three large structurally conserved regions (SCRs): SCR1 (strand A and the 3_{10}-like helix preceding it), SCR2 (strands F and G, and loop L6 linking them), and SCR3 (strand H and adjoining residues). Other SCRs of the common core are small and can be neglected. The three principal SCRs each contain a sequence motif that is wholly, or partly, invariant. Together with three other distinct protein families: the fatty-acid-binding proteins (FABPs), avidins, metalloproteinase inhibitors (and the presently enigmatic triabin), the lipocalin family forms part of a larger structural superfamily: the calycins. This is an example of a `structural superfamily': a set of proteins with closely related three-dimensional structures that show no significant overall similarity at the sequence level. **(*Fuentes-Prior et al., 1997*).**

Unlike other lipocalins, NGAL shows no affinity for retinoic acid (REA) (***Pervaiz and Brew, 1987***) but is reported to bind the tripeptide *N*-formyl-Met-Leu-Phe (fMLP), a potent neutrophil chemoattractant (***Sengelov et al., 1994***), and possibly other lipophilic mediators of inflammation such as platelet activating factor (PAF), leukotriene B4 (LTB4), and lipopolysaccharide (LPS)(***Nielsen et al., 1996***). Through these