Abstract

Background: Lupus nephritis is a major complication in systemic lupus erythematosus (SLE) patients, with significant morbidity and mortality rates. The lack of available specificity and sensitivity estimates of current laboratory tools for determining renal exacerbations has led to the search for alternative biomarkers that may contribute to nephritis and help predict kidney failure. The objective of this study was to investigate the positive prevalence and correlation between neutrophil gelatinase-associated lipocalin (NGAL) and other biomarkers associated with lupus nephritis in a cohort of suspected SLE (sSLE) patients.

Methods: Patient samples selected for evaluation had positive anti-dsDNA antibodies measured by ELISA (Aesku Diagnostics) and titers ≥10 by Crithidia luciliae immunofluorescence test (CLIFT, INOVA Diagnostics). High avidity dsDNA IgG antibodies (ELISA, INOVA Diagnostics), anti-C1q IgG antibodies (ELISA, INOVA Diagnostics), serum creatinine (Roche Diagnostics Modular P), as well as serum and urine NGAL (Bioporte Diagnostics on the Roche cobas e601) were determined in parallel. For these investigations, paired serum and urine samples from 71 sSLE patients (35-64 years of age; 52 female, 19 male) and 50 healthy adult controls (22-64 years of age; 33 female, 17 male) were evaluated.

Results: Comparison of sSLE to healthy controls showed significant differences for all markers evaluated (p values ≤ 0.005 by unpaired t-test). Elevated NGAL concentrations (>175 and >112 ng/mL for serum and urine, respectively) were found in 39% (range 28-46 ng/mL) and 42% (range 2-11 mg/mL) in healthy controls for serum and urine, respectively. The positive prevalence of elevated NGAL concentrations was comparable to serum creatinine (39%) and anti-C1q antibodies (33%), whereas high avidity dsDNA antibodies had a higher positive prevalence (55%) than NGAL. Additionally, the only significant association observed between NGAL and the other markers evaluated was for serum creatinine (Pearson correlation coefficient 0.425 and 0.451 for serum and urine NGAL, respectively). The overall combined concordance for all markers determined by Cronbach Alpha Coefficient was 0.415. This value was reduced when NGAL was removed from the combined concordance analysis (0.421 and 0.451 for serum and urine, respectively).

Conclusions: Neutrophil gelatinase-associated lipocalin (NGAL) is a small protein expressed in the neutrophils and certain epithelia, including the renal tubules. Under normal conditions, NGAL concentrations are low in serum and plasma, but rise sharply from basal concentrations in response to kidney injury to reach diagnostic concentrations within a very short period of time; as much as 24 hours or more prior to any significant rise in serum creatinine. This expression of NGAL in response to kidney injury has been reported in a variety of acute and chronic diseases, making it an ideal biomarker for the early detection of lupus nephritis (LN). LN is a major complication in systemic lupus erythematosus (SLE) with significant morbidity and mortality rates, and is clinical evident in 40-85% of these patients.

Introduction

Neutrophil gelatinase-associated lipocalin (NGAL) is a small protein expressed in the neutrophils and certain epithelia, including the renal tubules. Under normal conditions, NGAL concentrations are low in serum and plasma, but rise sharply from basal concentrations in response to kidney injury to reach diagnostic concentrations within a very short period of time; as much as 24 hours or more prior to any significant rise in serum creatinine. This expression of NGAL in response to kidney injury has been reported in a variety of acute and chronic diseases, making it an ideal biomarker for the early detection of lupus nephritis (LN). LN is a major complication in systemic lupus erythematosus (SLE) with significant morbidity and mortality rates, and is clinical evident in 40-85% of these patients.

Several biomarkers including antibodies against double-stranded DNA (anti-dsDNA) and C1q are believed to play a major role in the pathogenesis of LN. Rising titer of these antibodies and hypocomplementemia have been associated with the activity of this disease, allowing for the prediction of renal flare and/or damage. However, the lack of optimal and reliable estimates in the assessment of disease exacerbations has led to the search for alternative biomarkers in LN. In the case of LN, the use of organ-specific biomarker(s) may have more clinical relevance in identifying relapse or guiding treatment decisions.

The objective of this study was to investigate the positive prevalence and correlation between correlation between NGAL and other biomarkers associated with renal functions and/or disease pathogenesis in a cohort of suspected SLE (sSLE) patients.

Materials and Methods

• Sample size for evaluation from patients suspected of systemic lupus erythematosus (sSLE) based on positive anti-dsDNA antibody results by ELISA (Aesku Diagnostics) and titers ≥10 by Crithidia luciliae immunofluorescence test (CLIFT, INOVA Diagnostics).

• Paired serum and urine samples from 71 sSLE patients (35-64 years of age; 52 female, 19 male) and 50 healthy adult controls (22-64 years of age; 33 female, 17 male) were evaluated.

• Samples were evaluated using the following assays:
  - High avidity dsDNA IgG antibodies, serum (ELISA, INOVA Diagnostics)
  - Anti-C1q IgG antibodies, serum (ELISA, INOVA Diagnostics)
  - Serum creatinine, serum (Roche Diagnostics Modular P)
  - NGAL, serum and urine (Bioporte Diagnostics on the Roche cobas e601)

• Significant differences were observed when comparing sSLE patients to healthy controls for all sample types.

Conclusions

• Prevalence of elevated NGAL levels in sSLE patients was comparable to the positivity rate of other markers associated with renal involvement in SLE, except for high avidity dsDNA antibodies. The inclusion of NGAL thus contributes to the overall combined concordance of SLE renal markers evaluated. These findings suggest the usefulness of including NGAL, in conjunction with other biomarkers, for the evaluation and/or management of SLE.

Table 1. Data Summary of SLE Biomarkers

|        | Healthy Controls | sSLE
<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>Serum NGAL</td>
<td>Serum NGAL</td>
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<tr>
<td>High avidity dsDNA (U/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-75%</td>
<td>0.01-13</td>
<td>0.01-13</td>
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<tr>
<td>Range</td>
<td>0.00-13</td>
<td>0.00-13</td>
</tr>
<tr>
<td>Mean</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>SD</td>
<td>0.14</td>
<td>0.14</td>
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|        | Healthy Controls | sSLE
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<tbody>
<tr>
<td></td>
<td>Serum NGAL</td>
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<tr>
<td>Anti-C1q (U/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.243</td>
<td>-0.042</td>
<td>-0.042</td>
</tr>
<tr>
<td>0.233</td>
<td>-0.042</td>
<td>-0.042</td>
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<tr>
<td>0.030</td>
<td>-0.042</td>
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Table 2. Correlation between NGAL and other SLE Biomarkers

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<tr>
<td>Serum creatinine</td>
<td>0.433</td>
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<tr>
<td>Serum NGAL</td>
<td>-0.039</td>
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<tr>
<td>Urine NGAL</td>
<td>0.329</td>
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</table>

A comparison between NGAL and indicated biomarkers was estimated by Pearson Correlation Coefficient. A value of 0 represents ideal correlation.

Table 3. Correlation between NGAL and other SLE Biomarkers

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<td>Urine NGAL</td>
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A comparison between NGAL and indicated biomarkers was estimated by Pearson Correlation Coefficient. A value of 0 represents ideal correlation.

References


Acknowledgements

Support for this study was provided by the ARUP Institute for Clinical and Experimental Pathology. We gratefully acknowledge Bioporte Diagnostics and INOVA Diagnostics for providing reagent kits and Amantha Hanauer for sample collection and de-identification.

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