“Nitrated nucleosome levels in patients with systemic lupus erythematosus: associations with ethnicity, autoantibody status and disease activity”

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• Nothing to disclose.
Objectives

- Nitrated nucleosomes (NN) – a potential new blood test in SLE.

- Why is nitration important?

- Why are nucleosomes important?

- Which factors affect NN levels?
Nitration & Nitrosylation

Blood flow shearing forces

NO-dependent vasodilators

Low conc. NO
- Transcription factors
- Heme-containing enzymes
- Soluble GC (cGMP)

High conc. NO
- DNA deamination
- Nitration (Tyr-NO₂)
- Nitrosylation (Cys-NO)

Reactive O₂ species ('O₂')

L-arginine

Inflammation

cNOS

iNOS

NO

NITRATED NUCLEOSOMES

DNA

H1 histone

8-histone core

(Nucleosome basic structure)
Why Nucleosomes?

- Impaired apoptotic clearance plays a pivotal role in SLE pathogenesis.

- This leads to accumulation of nuclear debris, including nucleosomes which stimulates production of autoantibodies.

- Both nucleosomes and anti-nucleosome antibodies have been found in serum of patients with SLE and levels correlate with disease activity.

So, if both nitration and nucleosomes are important in SLE then levels of nitrated nucleosomes (NN) could be important.
Research questions

- Can we develop a new test to measure NN levels in patients with SLE?

- Do NN levels correlate with ethnicity and immunological profile?

- Do NN levels correlate with disease activity?
New Capture ELISA for NN

Results expressed in A.U. by comparison to the same standard positive control loaded on every plate.

- Anti-human IgG
- Anti-histone Ab
- Nitrated nucleosome (patient sera)
- ENZYME (HRP conjugate)
- Anti-nitrotyrosine Ab (streptavidin plate)
Methods:
- Longitudinal retrospective study
- 49 SLE patients (398 samples)
- Follow-up 7.4 years
- 40 healthy controls

SLE cohort characterization:
- 81% women (n = 39)
- Mean age 36 years (SD 13; min 13; max 17).
- 23 Caucasian/ 18 Afro-Caribbean/ 8 other ethnicities
Results

- Patients with SLE have significantly higher NN levels than the healthy controls ($p=0.012$).

- **Not every SLE patient has serum NN.**
  - 18 patients: no NN at any time-point.

- **BUT 65% do and the level varies widely over time.**
  - 31 patients: positive for NN which varied over time
    - Mean 32.; SD 62.2
    - Range: min 0; max 270.4

**So, which factors influence NN levels?**
NN levels correlate with ethnicity

![Graphs showing correlation between NN levels and ethnicity](image)

\( p = 0.0302 \)
NN correlate with ENA profile
The anti-Sm association dominates over ethnicity

\[ p = 0.024 \]

\[ p < 0.0001 \]
For most (375) samples we had corresponding disease activity measurement using BILAG.

<table>
<thead>
<tr>
<th>Category</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current activity</td>
<td>High (BILAG&gt;5) vs. Low</td>
</tr>
<tr>
<td>Activity over last two years</td>
<td>Persistently mod/high vs. persistently low</td>
</tr>
<tr>
<td>Anti-dsDNA</td>
<td>High (&gt;50IU/l) vs. Low</td>
</tr>
<tr>
<td>C3</td>
<td>Low (&lt;0.9g/l) vs. high</td>
</tr>
</tbody>
</table>
The future - immunological tests NN levels correlate with clinical and serological disease activity

<table>
<thead>
<tr>
<th></th>
<th>Number of samples (n)</th>
<th>Mean NN level (SD; min; max)</th>
<th>p-value (high vs. low)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sustained disease activity</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Persistently moderate-high activity</td>
<td>188</td>
<td>40.5 (51.7; 0; 270.4)</td>
<td>0.039</td>
</tr>
<tr>
<td>Persistently low activity</td>
<td>154</td>
<td>24.8 (72.3; 0; 244.9)</td>
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</tr>
<tr>
<td><strong>Current disease activity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BILAG&lt; 5</td>
<td>204</td>
<td>25.6 (57.33; 0; 248.6)</td>
<td>0.045</td>
</tr>
<tr>
<td>BILAG≥ 5</td>
<td>171</td>
<td>37.9 (69.39; 0; 270.4)</td>
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</tr>
<tr>
<td><strong>Anti-dsDNA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High ( &gt;50IU/ml)</td>
<td>171</td>
<td>29.0 (48.1; 0; 206.9)</td>
<td>0.035</td>
</tr>
<tr>
<td>Low ( ≤50IU/ml)</td>
<td>177</td>
<td>21.9 (38.6; 0; 206.9)</td>
<td></td>
</tr>
<tr>
<td><strong>C3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High ( ≥ 0.9g/l)</td>
<td>207</td>
<td>20.8 (40.6; 0; 206.9)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Low ( &lt;0.9g/l)</td>
<td>138</td>
<td>31.6 (47.0; 0; 206.9)</td>
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</tbody>
</table>
Different organ system flares may correlate differently with NN levels
Conclusions

• NN are present in 65% patients with SLE and vary over time.

• NN are higher in patients who are anti-Sm pos. and this is independent of ethnicity (confirmed by multivariate analysis).

• On univariate analysis: NN levels correlate with markers of disease activity.

• NN levels appear to be are higher in certain types of disease flare.

• Multivariate analysis is on going
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