Circulating calprotectin in autoimmune rheumatic diseases

Laura Martinez-Prat, Scientist, Autoimmunity R-Unit, Inova Diagnostics (Barcelona)
Workshop Autoinmunidad Congreso Laboratorio Clínico
Sevilla, 25 de Octubre de 2019
Disclosures

• Employee of Inova Diagnostics, R&D
Circulating calprotectin in rheumatic diseases

Contents

• Background
• Circulating calprotectin in autoimmune rheumatic disease
  • Overview
  • Rheumatoid arthritis
• The importance of pre-analytical sample processing
Background
Circulating calprotectin in rheumatic diseases

Background (I)

• Calprotectin = S100A8/S100A9 = MRP8/14 (Myeloid-Related Protein)

• Expression:
  • Normal physiological conditions: myeloid cells
  • High concentrations at local sites of inflammation or in the serum of patients with autoimmune inflammatory diseases
  • Strongly up-regulated in many tumors

• Function: regulation of inflammatory processes and immune response
  • Intracellular functions
  • Extracellular functions: proinflammatory, antimicrobial, oxidant-scavenging and apoptosis-inducing activities

Circulating calprotectin in rheumatic diseases
Background (II)

- Humoral secretion mechanism still to be understood
- Mainly produced by activated monocytes and neutrophils in the circulation and inflamed tissues
- Connection between inflammation and the adaptive immune response?

Circulating calprotectin in rheumatic diseases
Background (III)

- Fecal calprotectin used to detect intestinal inflammation (ulcerative colitis and Crohn disease) and as a biomarker for IBD
- Role in disease pathogenesis, diagnosis, prognosis, and monitoring of rheumatic diseases
Circulating calprotectin in autoimmune rheumatic diseases
Circulating calprotectin in rheumatic diseases
Overview (I)

### Circulating calprotectin in rheumatic diseases

**Overview (II)**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spondyloarthritis</strong></td>
<td>- Increased levels in synovial fluid and serum</td>
</tr>
<tr>
<td></td>
<td>- Expressed by keratinocytes and phagocytes in psoriatic lesions</td>
</tr>
<tr>
<td><strong>Juvenile idiopathic arthritis</strong></td>
<td>- Increased levels in serum and synovial tissue and fluid</td>
</tr>
<tr>
<td></td>
<td>- Predicts response to therapy and disease flares</td>
</tr>
<tr>
<td><strong>Systemic sclerosis</strong></td>
<td>- Increased levels in serum and bronchoalveolar lavage fluid in patients with severe lung fibrosis positively correlate with DA</td>
</tr>
<tr>
<td></td>
<td>- Increased levels in skin of patients with diffuse cutaneous SSc</td>
</tr>
<tr>
<td><strong>Sjögren syndrome</strong></td>
<td>- Increased levels in serum (higher than HI), saliva and salivary glands (marker of local inflammatory activity?)</td>
</tr>
<tr>
<td></td>
<td>- Serum levels correlated with some indices of disease activity</td>
</tr>
<tr>
<td><strong>SLE</strong></td>
<td>- Up-reg in kidneys and skin</td>
</tr>
<tr>
<td></td>
<td>- Highly upregulated in lupus nephritis; increased levels in the urine of LN patients positively correlate with DA</td>
</tr>
<tr>
<td></td>
<td>- Increased mRNA levels in neutrophils, monocytes and plasmacytoid DCs</td>
</tr>
<tr>
<td></td>
<td>- Low inflammatory activity of S100a9−/− mice in a murine lupus model</td>
</tr>
<tr>
<td><strong>Idiopathic inflammatory myopathies</strong></td>
<td>- Increased levels in serum</td>
</tr>
<tr>
<td></td>
<td>- Activates myoblasts</td>
</tr>
</tbody>
</table>

### Circulating calprotectin in rheumatic diseases

#### Overview (III)

<table>
<thead>
<tr>
<th><strong>Psoriatic arthritis</strong></th>
<th><strong>Adult-onset still’s disease</strong></th>
<th><strong>Gout</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Serum levels increased compared with HI</td>
<td>- Serum levels higher than in controls or other autoimmune disorders → diagnostic value?</td>
<td>- Role in pathogenesis?</td>
</tr>
<tr>
<td>- Associated with articular rather than skin involvement</td>
<td>- Positive correlation with laboratory biomarkers</td>
<td>- Serum levels ≥2 µg/ml in patients with active gout, correlated with synovial fluid levels</td>
</tr>
<tr>
<td>- Correlates with clinical measures (# involved joints, Ritchie Articular Index…)</td>
<td>- Associated with DAS and treatment response</td>
<td>- Correlation with DA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Osteoarthritis</strong></th>
<th><strong>Behçet’s disease</strong></th>
<th><strong>ANCA-associated Vasculitis</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Involved in and predictive of cartilage damage</td>
<td>- Serum levels higher than HI</td>
<td>- Serum levels correlated with DA but not with ANCA; increase is predictive of relapse with higher likelihood than ANCA</td>
</tr>
<tr>
<td>- At high levels in serum and also synovial fluid (up to 5–7 µg/ml); expression in synovium only if inflammation is present</td>
<td>- Uveitis and oral aphthous ulcerations potential sources</td>
<td>- Expressed in the kidneys of patients with AAV associated glomerulonephritis</td>
</tr>
</tbody>
</table>

---

Circulating calprotectin in rheumatoid arthritis (RA)
Circulating calprotectin in RA
Background (I)

- Most up-regulated protein in RA synovial tissue and fluid
  - Macrophages, PMNs, synovial fibroblasts, and chondrocytes
- Amplification of the inflammatory process, neutrophil and monocyte recruitment, cartilage destruction, and bone resorption
- Macrophages producing it present in crucial sites of joint destruction
  → Bone resorption

Ometto et al. Experimental Biology and Medicine 2017;242:859–873

DOI: 10.5772/54218
Circulating calprotectin in RA
Background (II)

- Potential of calprotectin as a biomarker in rheumatoid arthritis
Circulating calprotectin in RA Diagnosis (I)

• Limited diagnostic value
  • Higher levels in serum compared to HI or patients with other rheumatological disease
  • Associated with RF and ACPA but also observed in seronegative RA
  • High association with laboratory and ultrasonography markers of inflammation in RA patients

• High levels in RA synovial fluid
  • Might differentiate RA from OA and other inflammatory arthritides
  • Presence in RA but not in other disease controls by proteomic analysis

# Circulating calprotectin in RA Diagnosis (II)

<table>
<thead>
<tr>
<th>Study</th>
<th>Purpose of study</th>
<th>Cut-off</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grossi et al.</td>
<td>Diagnosis</td>
<td>0.9 µg/mL</td>
<td>95.3%</td>
<td>82.2%</td>
</tr>
<tr>
<td>Adel et al.</td>
<td>Diagnosis, DA</td>
<td>0.45 µg/mL *Diagnosis</td>
<td>75%</td>
<td>90%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.95 µg/mL *DA</td>
<td>80%</td>
<td>76%</td>
</tr>
<tr>
<td>Mansour et al.</td>
<td>Diagnosis, DA</td>
<td>0.939 µg/mL</td>
<td>88.6%</td>
<td>100%</td>
</tr>
<tr>
<td>Inciarte-Mundo et al.</td>
<td>Diagnosis, DA, ultrasound remission</td>
<td>2.47 µg/mL *DAS28 &gt; 2.6 as the reference variable</td>
<td>84%</td>
<td>87%</td>
</tr>
<tr>
<td>Hurnakova et al.</td>
<td>Ultrasound remission</td>
<td>3.33 µg/mL *DAS28 &gt; 3.2 as the reference variable</td>
<td>77.5%</td>
<td>80.9%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.7 µg/mL *DAS28-CRP as reference</td>
<td>71%</td>
<td>98%</td>
</tr>
</tbody>
</table>


**Effect of pre-analytical sample processing?**
Circulating calprotectin in RA
Prognosis (I) – Disease activity and severity

- Useful and reliable biomarker of disease activity and severity
  - Associations with DAS28-ESR, DAS28-CRP, SDAI, CDAI
  - Biomarker with the strongest association with both US and clinical DA assessments during 1 year of follow-up in established RA starting bDMARDs


Circulating calprotectin in RA
Prognosis (II) – Disease activity and severity

• Inflammatory marker
  • Significant associations with several inflammatory indices: CRP, ESR, serum amyloid A protein
  • When assessing disease activity in different stages of RA

• Neutrophil extracellular traps (NETs) contain calprotectin

## Circulating calprotectin in RA Prognosis (III) – CRP vs. Calprotectin

<table>
<thead>
<tr>
<th></th>
<th>C-reactive protein (CRP)</th>
<th>Calprotectin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Origin</strong></td>
<td>Liver cells; CRP is synthesized by the liver in response to factors released by macrophages</td>
<td>Neutrophils; unknown mechanism.</td>
</tr>
<tr>
<td><strong>Half-life</strong></td>
<td>18 hours</td>
<td>5 hours</td>
</tr>
<tr>
<td><strong>Protein characteristics</strong></td>
<td>100 kDa homo-pentamer (25.1kDa)</td>
<td>24 kDa hetero-dimer formed by S100A8 (10.8kDa) and S100A9 (13.2kDa).</td>
</tr>
<tr>
<td><strong>Increased in</strong></td>
<td>• Bone infection, or osteomyelitis</td>
<td>• In several autoimmune rheumatic diseases</td>
</tr>
<tr>
<td></td>
<td>• Arthritis flare-up</td>
<td>• In RA, associated with inflammation, synovitis, disease activity, joint damage…</td>
</tr>
<tr>
<td></td>
<td>• Inflammatory bowel disease</td>
<td>• Up-regulated in many tumors</td>
</tr>
<tr>
<td></td>
<td>• Tuberculosis</td>
<td>• Still under evaluation</td>
</tr>
<tr>
<td></td>
<td>• Lupus or another connective tissue disease or autoimmune disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Cancer, especially lymphoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Pneumonia</td>
<td></td>
</tr>
</tbody>
</table>
Circulating calprotectin in RA Prognosis (IV) – Calprotectin vs. CRP

- Calprotectin might be superior to CRP in predicting synovial inflammation and joint damage
  - Better performance than CRP and ESR
  - Better correlation with clinical indices (DAS28, SDAI…)
  - It may reflect inflammatory activity where CRP fails to do so
  - Superior to CRP for monitoring US-determined synovial inflammation
  - Unlike CRP and ESR, accurate biomarker for assessing DA and remission in patients receiving Tocilizumab

Potential inflammatory marker for autoimmunity

Circulating calprotectin in RA Prognosis (V) - Synovitis

Calprotectin is significantly associated with ultrasound (US) assessments of disease activity and US-detected synovitis.

<table>
<thead>
<tr>
<th></th>
<th>Sum GS score</th>
<th>Sum PD score</th>
<th>Assessors global VAS</th>
<th>DAS28</th>
<th>Swollen joint count (of 32)</th>
<th>Tender joint count (of 32)</th>
<th>Patients global VAS</th>
<th>Joint pain VAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calprotectin</td>
<td>0.39*</td>
<td>0.62*</td>
<td>0.60*</td>
<td>0.49*</td>
<td>0.47*</td>
<td>0.17*</td>
<td>0.27*</td>
<td>0.32*</td>
</tr>
<tr>
<td>S100A12</td>
<td>0.39*</td>
<td>0.42*</td>
<td>0.44*</td>
<td>0.35*</td>
<td>0.35*</td>
<td>0.14</td>
<td>0.22*</td>
<td>0.23*</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.42*</td>
<td>0.45*</td>
<td>0.53*</td>
<td>0.29*</td>
<td>0.41*</td>
<td>0.09</td>
<td>0.20*</td>
<td>0.24*</td>
</tr>
<tr>
<td>VEGF</td>
<td>0.28*</td>
<td>0.18*</td>
<td>0.14</td>
<td>0.10</td>
<td>0.13</td>
<td>-0.03</td>
<td>0.15</td>
<td>0.21*</td>
</tr>
<tr>
<td>ESR</td>
<td>0.19*</td>
<td>0.30*</td>
<td>0.46*</td>
<td>0.67*</td>
<td>0.22*</td>
<td>0.27*</td>
<td>0.28*</td>
<td>0.33*</td>
</tr>
<tr>
<td>CRP</td>
<td>0.41*</td>
<td>0.47*</td>
<td>0.64*</td>
<td>0.42*</td>
<td>0.30*</td>
<td>0.15</td>
<td>0.18*</td>
<td>0.24*</td>
</tr>
</tbody>
</table>

Abbreviations: Sum GS score Sum of greyscale scores on a 0–3 scale of 36 joints and four tendon sheaths, Sum PD score Sum power Doppler scores on a 0–3 scale of 36 joints and 4 tendon sheaths, VAS Visual analogue scale, DAS28 Disease Activity Score in 28 joints (including erythrocyte sedimentation rate), VEGF Vascular endothelial growth factor, IL-6 Interleukin 6, ESR Erythrocyte sedimentation rate, CRP C reactive protein

*P < 0.05
**P < 0.001


Calprotectin to discriminate Power Doppler US Synovitis

AUC = 0.8261
Circulating calprotectin in RA Prognosis (VI) – Joint damage

- Strongly and independently correlates with joint damage
- It correlates with radiographic scores and some evidence that baseline levels might be predictive of radiographic damage
- Independent predictor of clinical and radiographic joint damage after 10 years

Circulating calprotectin in RA
Response to treatment (I)

- A number of immunomodulators, such as TNF-a inhibitors, may reduce calprotectin expression
- Serum levels have shown a prompt and more marked response to inflammatory changes compared with classic inflammatory indices
- Sensitive biomarker for assessing treatment response

Nair et al. PLOS ONE 2016;11:e0152362.
Circulating calprotectin in RA
Response to treatment (II)

• Serum calprotectin levels are a strong predictor of response to biological treatments in patients with RA

Redefining Autoimmunity

Circulating calprotectin in RA
Response to treatment (III) – Precision Medicine

• Increasing numbers of studies showing a potential utility to predict treatment response
  • Predictor of treatment response for anti-TNF (better than CRP, ESR)
  • S100A9 as a biomarker of responsiveness to the Mtx/Etanercept combination

Nair et al. PLOS ONE 2016;11:e0152362.
Redefining Autoimmunity

• Potential for personalizing biological treatment and prediction of response in RA patients using calprotectin levels + clinical predictors

Circulating calprotectin in RA
Response to treatment (IV) – Precision Medicine

Algorithm for personalized treatment of RA patients indicated for biological treatment

Nair et al. PLOS ONE 2016;11:e0152362.
Circulating Calprotectin in RA
Potential therapeutic target

- S100A8 and/or S100A9 proposed as therapeutic targets
- Evidence that a number of immunomodulators, including TNF-α inhibitors or JAK/STAT inhibitors, may reduce calprotectin expression
- In an arthritis murine model, the blockade of S100A9 through a monoclonal antibody was effective
  - Role as a treatment target in RA and, possibly, in other immune-mediate arthritides?

The importance of pre-analytical sample processing
Circulating calprotectin in rheumatic diseases
Pre-analytics

• Coagulation may induce release of calprotectin from neutrophils
  • Calprotectin can be released from neutrophil granulocytes in blood *in vitro*
  • Pre-analytical sample processing is typically ignored or overlooked

• Importance of pre-analytical sample processing

• Lack of assay standardization between studies
  • Variability of results

Circulating calprotectin in rheumatic diseases
Pre-analytics

- Calprotectin measured in plasma showed the strongest associations with assessments of disease activity.
- EDTA-plasma (vs. serum) should preferably be used when evaluating disease activity in RA patients.

Conclusions
Circulating calprotectin in rheumatic diseases
Conclusions

- Circulating calprotectin levels are elevated in a number of autoimmune rheumatic diseases
- It has been proposed as the potential inflammatory marker for autoimmunity
- In rheumatoid arthritis:
  - Limited diagnostic value
  - Value as prognostic marker: association with disease activity, inflammation, synovitis, joint damage
  - It can help predict responsiveness to treatment
    - Precision Medicine
- Importance of pre-analytical sample processing
Circulating calprotectin in rheumatic diseases

Acknowledgments

• Inova colleagues
  • Roger Albesa
  • Emily Fitzgerald
  • David Lucia
  • Silvia Casas
  • Chelsea Bentow
  • Michael Mahler

• San Giovanni Di Dio Hospital, Florence
  • Maria Infantino’s group

• Werfen Iberia
Thank you!