Serum Proteins Reflecting Inflammation, Injury, and Repair As Biomarkers of Disease Activity in ANCA-Associated Vasculitis

For the RAVE/ITN Study Group
ACR Annual Scientific Meeting, 2011

Disclosure

• Drs. Monach and Merkel have served as consultants for Genentech, which provided:
  – Partial funding for RAVE clinical trial
  – No funding for the current study

Background

• After initial diagnosis and treatment of ANCA-associated vasculitis (AAV), disease course is highly variable

• Current activity of disease is not always apparent clinically
  – ANCA titers, ESR, CRP: useful but not sufficient to guide treatment

• Additional tests to distinguish active AAV from remission are needed

Objectives

• Primary
  – Identify serum proteins that distinguish active AAV from remission

• Secondary
  – Distinguish active AAV from healthy controls
  – Distinguish mildly active AAV from remission
  – Correlations between marker levels
  – Combine markers to better distinguish active AAV from remission

Study Design - Samples

• Source of Samples
  – Rituximab in ANCA-Associated Vasculitis clinical trial (RAVE)
    • RCT of rituximab vs. cyclophosphamide / azathioprine
    • All subjects had severe AAV at screening (BVAS/WG ≥ 3)
    • 75% GPA (Wegener’s), 25% MPA
    – Healthy controls, Boston University (n=68)

• Choice of Samples / Time Points
  – 186 subjects at screening (of 197 total)
  – 162 subjects also at month 6
    • 137 in remission (BVAS/WG = 0)
    • 25 with mildly active disease (median BVAS/WG = 1)

Study Design – Marker Selection

• 28 serum proteins in a custom microarray
  – Chosen from list of ~100 individually validated “sandwich” immunoassays
  – Chosen to reflect diverse processes
  – 17 markers studied previously by other groups, 11 not previously reported

• ESR and CRP
  – Available for all visits
### Study Design - Markers

- **Cytokines**
  - G-CSF
  - GM-CSF
  - IFN
  - IL-6
  - IL-15
  - IL-18
  - Osteopontin
- **Damage and Repair**
  - ACE
  - bFGF
  - KIM-1
  - MMP-3
  - NGF
  - PDGF-AB
  - TIMP-1
- **Chemokines**
  - CXCL13 / BCA-1
  - CXCL8 / IL-8
  - CXCL10 / IP-10
  - CCL5 / RANTES
  - CCL17 / TARC
- **Soluble Receptors**
  - IL-18BP
  - sIL-2R
  - sIL-6R
  - sTNF-RII
- **Inflammation and Vascular Injury**
  - Clusterin
  - CRP
  - ESR
  - ICAM-1
  - NGAL / Lipocalin-2
  - PAI-1
  - VCAM-1

*Underlined* = not previously reported

### Analysis - Primary

- **Primary objective**: severe active AAV vs. remission in the same subjects (n=137)
  - Difference between marker level at screening (active) and remission, Signed Rank test
  - Receiver operating characteristic (ROC) curve analysis
    - Area under the ROC curve = AUC
    - Optimal cut-point = maximum sum of sensitivity and specificity (Youden index)

### Analysis - Secondary

- **Secondary objectives**
  - Severe active AAV (n=186) vs. healthy controls (n=68) (Wilcoxon Rank Sum)
  - Milder active AAV (n=25) vs. remission at month 6 (Wilcoxon Rank Sum)
  - Correlation: Spearman coefficients
  - Combination of markers: Logistic regression
    - Outcome: active AAV vs remission (n=137)
    - Predictors: log-transformed markers, forward or backward selection (yielded same results)

### Results

- **Cytokines**
  - G-CSF
  - GM-CSF
  - IFN
  - IL-6
  - IL-15
  - IL-18
  - Osteopontin
- **Damage and Repair**
  - ACE
  - bFGF
  - KIM-1
  - MMP-3
  - NGF
  - PDGF-AB
  - TIMP-1
- **Chemokines**
  - CXCL13 / BCA-1
  - CXCL8 / IL-8
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- **Soluble Receptors**
  - IL-18BP
  - sIL-2R
  - sIL-6R
  - sTNF-RII
- **Inflammation and Vascular Injury**
  - Clusterin
  - CRP
  - ESR
  - ICAM-1
  - NGAL / Lipocalin-2
  - PAI-1
  - VCAM-1

*Red* = Active > Remission in the same subjects (primary objective)

*Italicized* = Active > Controls

#### ROC Analysis

- **“Best” Markers (AUC > 0.7)**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Active AAV vs. Remission</th>
<th>Mild AAV vs. Remission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marker</td>
<td>AUC</td>
<td>Sensitivity</td>
</tr>
<tr>
<td>ACE</td>
<td>0.75</td>
<td>75%</td>
</tr>
<tr>
<td>BCA-1</td>
<td>0.86</td>
<td>77%</td>
</tr>
<tr>
<td>ESR</td>
<td>0.78</td>
<td>68%</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>0.72</td>
<td>60%</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.75</td>
<td>75%</td>
</tr>
<tr>
<td>IL-15</td>
<td>0.74</td>
<td>58%</td>
</tr>
<tr>
<td>IL-18BP</td>
<td>0.74</td>
<td>54%</td>
</tr>
<tr>
<td>KIM-1</td>
<td>0.76</td>
<td>72%</td>
</tr>
<tr>
<td>MMP3</td>
<td>0.69</td>
<td>92%</td>
</tr>
<tr>
<td>NGAL</td>
<td>0.73</td>
<td>74%</td>
</tr>
<tr>
<td>NGF</td>
<td>0.74</td>
<td>67%</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>0.83</td>
<td>76%</td>
</tr>
</tbody>
</table>

* AUC = area under the ROC curve, maximum = 1.0, random = 0.5
“Best” markers: marker values

<table>
<thead>
<tr>
<th>Marker</th>
<th>Units</th>
<th>Screening Median (25%; 75%)</th>
<th>Month 6 Remission Median (25%; 75%)</th>
<th>Controls Median (25%; 75%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE</td>
<td>ng/ml</td>
<td>N = 137</td>
<td>N = 137</td>
<td>N = 68</td>
</tr>
<tr>
<td>BCA-1/CXCL13</td>
<td>pg/ml</td>
<td>70 (74.2;489)</td>
<td>32.0 (18.2,55.6)*</td>
<td>29.6 (19.8,44.8)*</td>
</tr>
<tr>
<td>ESR</td>
<td>mm/hr</td>
<td>37 (16.60)</td>
<td>14.7 (7.22)*</td>
<td>ND</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>pg/ml</td>
<td>21.6 (7.65;109)</td>
<td>5.68 (2.60,15.5)*</td>
<td>2.87 (2.18,4.31)*</td>
</tr>
<tr>
<td>IL-6</td>
<td>pg/ml</td>
<td>21.6 (7.65;109)</td>
<td>5.68 (2.60,15.5)*</td>
<td>2.87 (2.18,4.31)*</td>
</tr>
<tr>
<td>IL-15</td>
<td>pg/ml</td>
<td>116 (21.6;768)*</td>
<td>14.6 (6.11,55.1)*</td>
<td>13.9 (6.11,46.5)*</td>
</tr>
<tr>
<td>KIM-1</td>
<td>pg/ml</td>
<td>242 (73.4;744)</td>
<td>45.6 (17.2,127)*</td>
<td>19.8 (6.08,128)*</td>
</tr>
<tr>
<td>MMP3</td>
<td>ng/ml</td>
<td>38.6 (46.8;1145)</td>
<td>13.8 (11.3,29.1)*</td>
<td>10.2 (5.02,15.3)*</td>
</tr>
<tr>
<td>NGAL</td>
<td>ng/ml</td>
<td>271 (135;370)</td>
<td>2.48 (1.25,4.32)*</td>
<td>2.11 (0.77,4.53)*</td>
</tr>
<tr>
<td>NGF</td>
<td>pg/ml</td>
<td>9.11 (3.15;37.0)</td>
<td>2.48 (1.25,4.32)*</td>
<td>2.11 (0.77,4.53)*</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>ng/ml</td>
<td>477 (302;862)</td>
<td>166 (125,233)*</td>
<td>117 (65.4,163)*</td>
</tr>
</tbody>
</table>

* P<0.0001 by Signed Rank test. † P=0.0001-0.001. ‡ P=0.001-0.05.

Selected Biomarkers, ROC curves

Comparisons:
- Active (screening) vs remission at month 6 (red)
- Active (screening) vs healthy controls (blue)
- Mildly active at month 6 vs. remission at month 6 (green)

Correlations Between Markers

- Weak correlation of CRP and ESR with other markers: r < 0.3
- Weak correlations of CXCL13 / BCA, MMP-3, TIMP-1 with each other: r ≤ 0.2
- Group of highly (r > 0.5) correlated markers: CXCL10 / IP-10, GM-CSF, IFNγ, IL-6, IL-8, IL-15, IL-18, IL-18BP, KIM-1, NGFβ, sIL-2R

Multiple Markers to Distinguish Active AAV from Remission

<table>
<thead>
<tr>
<th>Marker*</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariable</td>
</tr>
<tr>
<td>ACE</td>
<td>0.23 (0.15 – 0.35)</td>
</tr>
<tr>
<td>ESR</td>
<td>2.01 (1.61 – 2.51)</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>1.37 (1.25 – 1.51)</td>
</tr>
<tr>
<td>MMP3</td>
<td>4.03 (2.97 – 5.47)</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>3.17 (2.36 – 4.28)</td>
</tr>
</tbody>
</table>

* Data used: 137 subjects with one active and one remission sample each
- Modeled by logistic regression:
  - Marker levels as predictors (AUC > 0.7), active/remission as outcome
  - Odds ratios per 2-fold increase in marker level
- AUC of 5-marker model = 0.96 (but only 0.78 for milder active disease)

Conclusions

- CXCL13 / BCA-1: novel marker of active AAV
- Confirmation of MMP-3 and TIMP-1 as promising markers of active AAV
- Weak correlations among these markers and ESR and CRP indicate that models based on multiple markers will be useful
- Distinguishing mild / limited disease from remission remains challenging

Future Directions

- Testing of markers longitudinally in AAV
  - Validation of association with active AAV
  - Prediction of future flare or remission
- Comparison to other inflammatory diseases and to infectious diseases
  - Including other vasculitides
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