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Using Biomarkers in ANCA-associated Vasculitis

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Relevant Financial Disclosures

Sharon A. Chung, MD MAS

- I have nothing to disclose.
Outline

- ANCA
  - Disease activity
  - Prognosis and treatment response
- CD19
  - Predictor for relapse
  - Guide to treatment
- Future directions: Gene expression profiling
The perpetual controversy regarding ANCA…
Do ANCA titers reflect disease activity?

- Prospective, observational cohort study ancillary to RCT of 180 pts with active GPA (WGET)
  - Prospectively followed with serial ANCA titers

- Anti-PR3 levels weakly associated with disease activity
- 40% of pts had relapse within 1 year of increase in anti-PR3 levels

Finkielman JD et al., Ann Intern Med 2007
ANCA and disease activity

- Meta-analysis of 9 articles investigating ANCA levels and disease activity

- Comparison for MI
  - Increased CK: LR+ 22
  - Positive troponin I: LR+ ~13

- "Modestly predictive"

Tomasson G et al., Rheumatology 2012
ANCA, disease type, and disease activity

- 166 MPO+ or PR3+ patients with AAV
  - 104 with renal involvement, 62 without renal involvement
  - Mean f/u 49 ± 33 months, 18 ± 14 MPO/PR3 assessments

ANCA epitopes

- 25 epitopes recognized by anti-MPO antibodies in 87 samples
- Patients and healthy subjects produced multiple anti-MPO antibodies
- Antibodies to some epitopes seen only during active disease
- Epitope spreading also seen in anti-PR3 antibodies

Roth et al. J Clin Invest 2013
ANCA and prognosis

- Retrospective long-term outcomes of 148 AAV pts enrolled in RCT of oral vs. IV pulse cyclophosphamide (CYCLOPS)
  - Median follow-up 4.3 years

![Graph showing risk of relapse dependent on limb and ANCA status]

- Anti-PR3+ more likely to relapse, independent of treatment with daily oral or pulse cyclophosphamide

Harper L et al., Ann Rheum Dis 2012
ANCA and prognosis

Time to relapse by ANCA type

- MPO
- PR3

Probability of Remaining in Complete Remission

Days from Complete Remission to Relapse

P < 0.01

MPO (N=52)
PR3 (N=94)

Overall, P < 0.01
MPO, P = 0.34
PR3, P = 0.71

Time to relapse by ANCA type and treatment arm

- CYC
- MPO
- RTX
- CYC
- PR3
- RTX

Specks U et al. NEJM 2013
ANCA type and treatment response

- Post-hoc analysis of RAVE (Rituximab vs. Cyclophosphamide, n=197)

<table>
<thead>
<tr>
<th></th>
<th>PR3-AAV</th>
<th>MPO-AAV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RTX (n=66)</td>
<td>CYC/AZA (n=65)</td>
</tr>
<tr>
<td>CR at 6 months</td>
<td>43 (65)</td>
<td>31 (48)</td>
</tr>
<tr>
<td>CR at 12 months</td>
<td>31 (47)</td>
<td>21 (32)</td>
</tr>
<tr>
<td>CR at 18 months</td>
<td>24 (36)</td>
<td>19 (29)</td>
</tr>
</tbody>
</table>

- Higher remission rate with RTX in PR3+ individuals at 6 months

ANCA and treatment response

- RTX efficacy higher in PR3+ with relapsing disease

**Table 3** Treatment response among patients with PR3-AAV who received RTX versus patients with PR3-AAV who received CYC/AZA

<table>
<thead>
<tr>
<th></th>
<th>OR*</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients with PR3-AAV (n=131)†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR at 6 months</td>
<td>2.11</td>
<td>1.04 to 4.30</td>
<td>0.04</td>
</tr>
<tr>
<td>CR at 12 months</td>
<td>1.96</td>
<td>0.95 to 4.05</td>
<td>0.07</td>
</tr>
<tr>
<td>CR at 18 months</td>
<td>1.44</td>
<td>0.68 to 3.05</td>
<td>0.34</td>
</tr>
<tr>
<td>Patients with PR3-AAV with relapsing disease at baseline (n=81)‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR at 6 months</td>
<td>3.57</td>
<td>1.43 to 8.93</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CR at 12 months</td>
<td>4.32</td>
<td>1.53 to 12.15</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CR at 18 months</td>
<td>3.06</td>
<td>1.05 to 8.97</td>
<td>0.04</td>
</tr>
</tbody>
</table>

ANCA: Summary

- No clear association between ANCA titers and disease activity:
  - Disease type—renal vs. non-renal?
  - Targeting different epitopes—pathogenic vs. non-pathogenic?
  - Different assays?
  - Would not increase immunosuppression based on ANCA titers alone

- PR3+ associated with increased relapse rate (worse prognosis)

- PR3+ may predict better response to rituximab therapy—needs to be studied prospectively
CD19

- Present on essentially all B-cells except for plasma cells

Burmester GR et al. Nat Rev Rheumatol 2013
Can CD19+ B cells predict relapse?

- **RAVE 18 month followup:**
  - **RTX:** 76 in complete remission → 24 flared
    - CD19+ B cells detected in 21/24 (88%)
    - # days detected before relapse: mean 80.2 (range 1-286)
    - 3 limited relapses in absence of CD19+ B cells
  - **CYC:** 70 in complete remission → 17 flared after B cell depletion
    - CD19+ B cells detected in 11/17 (65%)
    - # days detected before relapse: mean 102 (range 0-259)
    - 2/6 relapses in absence of CD19+ B cells were severe

Specks U et al. NEJM 2013
Can CD19+ B cells guide RTX dosing?

MAINRITSAN2:

- 162 AAV patients (117 GPA, 45 MPA) with active disease → remission induction → complete remission → randomized to:
  - RTX 500 mg IV days 0 and 15, then month 6, 12, and 18
  - RTX 500 mg IV day 0, and then re-dosed if CD19+ B cells became detectable or ANCA titers increased (q3 months)

- Endpoint: # relapses at 28 months

<table>
<thead>
<tr>
<th></th>
<th>Experimental (n=81)</th>
<th>Scheduled (n=81)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flare rate</td>
<td>14 (17.3%)</td>
<td>8 (10%)</td>
</tr>
<tr>
<td>Median infusions, (IQR)</td>
<td>3 (2-4)</td>
<td>5 (5-5)</td>
</tr>
</tbody>
</table>

P=0.20

Under powered?

Charles P et al. 2016 ACR Annual Meeting Abstract 16L
CD19: Summary

- Relapses can occur during B cell depletion
  - Can be severe

- Role of CD19 (and ANCA titers) to guide rituximab dosing
  - Await additional details of MAINRITSAN2 trial
    - MAINRITSAN3: duration of rituximab therapy
Future possibilities: Gene expression

- CD8+ T cell gene expression signature at baseline associated with AAV prognosis
- Also observed in SLE
- Allows concurrent analysis of multiple genes instead of single gene (or protein)

McKinney EF et al. Nat Med 2010
Future possibilities: Gene expression

- Whole blood RNA sequencing from RAVE baseline samples
- 2,436 transcripts differentially expressed
- 11 gene composite score
  - 1 unit increase associated with not responding (OR 2.13, P=0.01)
  - Independent of age, BVAS/WG, ANC, Hgb, prednisone use

Table 2. Differences in the expression of the 11 genes that make up the granulocyte multigene composite score in nonresponders versus responders

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Gene symbol</th>
<th>Fold change</th>
<th>Likelihood ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloperoxidase</td>
<td>MPO</td>
<td>2.42</td>
<td>14.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proteinase 3</td>
<td>PR3</td>
<td>2.65</td>
<td>10.33</td>
<td>0.001</td>
</tr>
<tr>
<td>Elastase, neutrophil expressed</td>
<td>ELANE</td>
<td>2.43</td>
<td>11.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bactericidal/permeability-increasing protein</td>
<td>BPI</td>
<td>2.20</td>
<td>12.61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Azurocidin 1</td>
<td>AZU1</td>
<td>2.64</td>
<td>12.78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cathepsin G</td>
<td>CTSG</td>
<td>2.46</td>
<td>12.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>LTF</td>
<td>1.81</td>
<td>6.93</td>
<td>0.008</td>
</tr>
<tr>
<td>Defensin A3</td>
<td>DEFA3</td>
<td>2.15</td>
<td>9.21</td>
<td>0.002</td>
</tr>
<tr>
<td>Defensin A4</td>
<td>DEFA4</td>
<td>2.56</td>
<td>13.48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lipocalin 2</td>
<td>LCN2</td>
<td>1.93</td>
<td>8.73</td>
<td>0.003</td>
</tr>
<tr>
<td>Cathelicidin antimicrobial peptide</td>
<td>CAMP</td>
<td>1.66</td>
<td>6.76</td>
<td>0.009</td>
</tr>
</tbody>
</table>

77 responders, 35 non-responders

Grayson PC et al. Arth Rheumatol 2015
Summary

- **ANCA**
  - Utility to indicate disease activity remains controversial
  - May provide information for prognosis and treatment response
  - Remaining questions: assay type and epitope specificity

- **CD19**
  - Patients flare in absence of detectable CD19+ B cells in peripheral blood
  - Need additional data to determine utility for dosing

- Future biomarkers likely will be composite of multiple gene/protein markers, instead of single candidate markers
Thank you!

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