Cold Agglutinin Disease

Sigbjørn Berentsen, MD, PhD
Department of Medicine, Haugesund Hospital, Haugesund,
and Clinical Institute 2, University of Bergen, Bergen,
Norway

UK Myeloma Forum Spring Day:
The Immunology of Myeloma and Paraproteinaemias
London: 19th March, 2014
Disclosures

No financial or other conflicts of interest
82 year old woman, admitted March 2013

- Previously healthy; mild hypertension
- 2010 (GP): Transient anemia (Hgb 10.2 g/dL) related to febrile infection; no definitive diagnosis

Recent history

- Dec 2012: Fatigue, patient suspected anemia
- March 2013: GP: Hgb 6.4 g/dL → hospital
- Hgb 6.5 g/dL. MCV 102. Reticulocytes 198 x 10⁹/L
- WBC, diff.count, PLT: Normal. CRP 28 mg/L
- Ferritin 1599 μg/L; normal transferrin saturation
- Creatinine/B12/Folic acid/TSH/T4: Normal
- LDH 657 U/L, bilirubin 66 μmol/L, haptoglobin.<0.1 g/L
Case report, *cont.d*...

- Polyspecific DAT positive (+++)
- Monospecific DAT
  - Positive (++++) for C3d
  - Negative for IgG, IgA, IgM and C3c

**Autoimmune hemolytic anemia**

DAT pattern strongly indicative of cold antibody type
Autoimmune hemolytic anemia

**Warm-antibody AIHA (~75%)**
- Primary
- Secondary or associated

**Cold-antibody AIHA (~25%)**
- Primary chronic cold agglutinin disease (CAD) (~15% of AIHA)
- Secondary cold agglutinin syndrome (CAS)
  - Acute, infection-associated CAS
  - CAS secondary to malignancy
- Paroxysmal cold hemoglobinuria (PCH) (<<< 1%)

*Mixed warm and cold-antibody AIHA (< 1%)*
Cold-antibody AIHA: Diagnostic algorithm

Case report, cont.d...

- Cold agglutinin titer 2048
- s-C3 1.5, s-C4 0.08
- IgM 3.3 g/L. Monoclonal IgMκ

Flow cytometry, BM
- Small, extra B-cell population
- Cellular κ/λ-ratio 4.0

BM biopsy
- CAD-associated B-cell lymphoproliferative disease (20%) with some features similar to lymphoplasmacytic lymphoma (LPL)
Case report, cont.d...

Diagnosis:

Primary chronic cold agglutinin disease (CAD),

classical findings
Monoclonal serum protein in 84 patients with primary CAD

- Monoclonal IgM 71
- Monoclonal IgG 3
- Monoclonal IgA 3
- “Biclonal” IgM and IgG 2

*Light chain restriction:*
κ: 74  λ: 2  Data not available: 3

Berentsen & al. Haematologica 2006; 91: 460-6
Flow cytometry, 
bone marrow aspirates

- Data from 40 patients
- Median cellular $\kappa/\lambda$ ratio = 7.8 (0.9 – 186)
- Cellular $\kappa/\lambda$ ratio > 3.5 in 36 patients (90%)

Berentsen & al. Haematologica 2006; 91: 460-6
## Underlying clonal disorder

*Mayo Clinic historical data, N=69*

<table>
<thead>
<tr>
<th>Hematologic diagnosis</th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGUS</td>
<td>42</td>
<td>61</td>
</tr>
<tr>
<td>Macroglobulinemia</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Unspecified lymphoproliferative disorder</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Other lymphoma</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>CLL</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Cutaneous T-cell lymphoma</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

## Bone marrow histology

Norwegian population based, cross-sectional study, N=66

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal / reactive lymphocytosis</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Irregular lymphoid hyperplasia</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Non-Hodgkin B-cell lymphoma</td>
<td>50</td>
<td>76</td>
</tr>
<tr>
<td>Lymphoplasmacytic lymphoma</td>
<td>33</td>
<td>50</td>
</tr>
<tr>
<td>Marginal zone lymphoma</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Small lymphocytic B-cell lymphoma / CLL</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Clonal lymphocytosis / other small B-cell lymphoma</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>66</td>
<td>100</td>
</tr>
</tbody>
</table>

Berentsen & al. Haematologica 2006; 91: 460-6
CAD in WM / WM in CAD: Established view (2010)

Waldenström’s macroglobulinemia

Chronic cold agglutinin disease

**WM with CAD:**
Up to 50% of ‘primary’ CAD
3-5% of WM

Based on data reported by:
Berentsen et al. Haematologica 2006; 91: 460-466
Stone et al. Semin Oncol 2003; 30: 318-3
CAD in WM / WM in CAD: Established view (2010)

Waldenström’s macroglobulinemia

Chronic cold agglutinin disease

**WM with CAD:**
Up to 50% of ‘primary’ CAD
3-5% of WM

…but is this model correct?
MYD88 L265P-mutation

- Described 2012
  - 49 of 54 patients with WM
  - 3 of 3 with non-secretory LPL
  - Not detected in healthy individuals
  - Rare in other B-cell lymphoma, myeloma and IgM-MGUS
    

- Subsequently described even in some patients with
  - IgM-MGUS
  - Diffuse large B cell lymphoma
  - Splenic marginal zone lymphoma
    
Typical bone marrow histology findings in CAD

Randen et al. Haematologica 2014; 99(3): 497-504
Recent histopathology study

- 54 patients with clinically well-characterized CAD
- Histological re-evaluation of bone marrow (all patients) and spleen specimens (two patients)
- Fresh frozen bone marrow from 15 patients

- Systematic re-evaluation of morphology
- Immunostaining with CD20, PAX-5, Bcl-6, Bcl-10, CD3, CD138, IgK, IgL, IgM, IgD, CD38, CD31, cyclin D1, MUM1, XBP-1, Blimp-1
- Re-evaluation of flow cytometry data
- Molecular clonality analysis and sequence analysis of monoclonal gene rearrangements

Randen et al. Haematologica 2014; 99(3): 497-504
Histopathology study: conclusions

- CAD has a homogeneous morphologic picture different from LPL and similar to bone marrow involvement by MZL.
- The lymphoid cells in CAD have an immunophenotype similar to MZL.
- However, none of the patients had extramedullary MZL.
- Molecular analysis shows *IGHV4-34* gene usage with a variable number of mutations.
- MYD88 L265P mutation is *not* present.

- *It seems that CAD-associated bone marrow disorder is a distinct clonal lymphoproliferative disease.*

Randen et al. Haematologica 2014; 99(3): 497-504
Cold agglutinins

can be characterized by their –

- titer
- thermal amplitude
- Ig class
- Specificity (in CAD):
  - anti-I
  - rarely anti-Pr, anti-P or others

**Correct handling of samples is essential**

Blood specimens **MUST** be kept at 37-38°C from sampling until serum has been removed from the clot
Cold agglutinin  Cryoglobulin
Cold hemagglutination
Cold hemagglutination

Courtesy of Prof. Geir E. Tjønnfjord, Oslo University Hospital
Complement-mediated hemolysis in CA-AIHA

Berentsen S et al. Hematology 2007; 12: 361–70
Primary CAD: Clinical features

- Hemolytic anemia
  - Median Hgb 9.0 g/dL
  - Lower tertile 8.0 g/dL
  - Transfusion dependency at some time (50%)
  - Hemoglobinuria (15%)

- Cold-induced circulatory symptoms (90%)

- Exacerbation in febrile illness (~75%)

Berentsen S et al. Haematologica 2006; 91: 460–6
Acute phase hemolysis in CAD

• Our case description: “2010 (GP): Transient anemia (Hgb 10.2 g/dL) related to febrile infection”

“Paradoxical” hemolysis in CAD:

• ~75% of CAD patients experience exacerbations in –
  - febrile infections
  - major trauma or major surgery

• Continuous complement consumption in steady state CAD
  - C4 often undetectable
  - Low C4 levels seem rate-limiting for hemolysis

• C4 levels increase following acute phase reaction

→ Exacerbation of hemolytic anemia
Therapy

• Not all patients require pharmacological therapy.
  – Drug treatment (percentages of patients)
    • 73% (Berentsen et al. 2006)
    • 82% (Swiecicki et al. 2013)

• Corticosteroids / unspec. immunosuppr.: ineffective
  (Dacie 1992, Berentsen et al. 2006, Swiecicki et al. 2013)

• Documented therapies / response rates
  – Rituximab monotherapy / ~50% (median duration ~1 year)
    (Berentsen et al. 2004, Schöllkopf et al. 2006)
  – Fludarabine-rituximab / 75% (Berentsen et al. 2010)

• Possible candidate therapies
  – Bendamustine-rituximab (ongoing study + 1 case report)
  – Bortezomib-based regimens (ongoing study + case reports)
  – Eculizumab (2 case reports)
  – New complement blocking agents
### Fludarabine-rituximab response data, N=29

<table>
<thead>
<tr>
<th>Response level</th>
<th>Frequencies</th>
<th>Increase in hemoglobin level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>CR</td>
<td>6</td>
<td>21</td>
</tr>
<tr>
<td>PR</td>
<td>16</td>
<td>55</td>
</tr>
<tr>
<td>NR</td>
<td>7</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>100</td>
</tr>
</tbody>
</table>

Berentsen et al. Blood 2010; 116: 3180-4
FR: Estimated response duration

Berentsen et al. Blood 2010; 116: 3180-4
FR for CAD: toxicity

Short-term hematologic toxicity:

Grade III-IV: 12 41%
Grade IV: 4 14%

No therapy-related deaths

Risk of long-term toxicity may be a concern (not investigated)
Complement blocking agents in CAD??

**Anti-C1s (TNT003)**
[Panicker 2013; preclinical]

**Anti-C5 (eculizumab)**
[Röth 2009; case report]
TNT003: An anti-complement C1s moAb

TNT003 inhibits CAD autoantibody-mediated hemolysis

Panicker S & al.
55th Annual Meeting of ASH, 2013: Paper 64043
Case report, *cont.d*...

Which therapy should our patient receive?
Conclusions (1)

- The pathogenesis in CAD is entirely different from that in polyclonal autoimmune cytopenias

- CAD (cold agglutinin disease) is a well-defined clinico-pathological entity
  - Distinct from secondary cold agglutinin syndrome (CAS)
  - Probably a specific clonal lymphoproliferative disorder, distinct from LPL and MZL
Conclusions (2)

- Studies of pathogenesis have explained –
  - the cold-induced circulatory symptoms,
  - the hemolytic anemia,
  - the exacerbations in febrile illness or following surgery,
  - the lack of effect of splenectomy,
  - the resistance to many therapies;

- and have provided a clue to efficient new therapies
  - which should still be further improved:
  - Patients with CAD requiring therapy should be included in prospective trials
Thanks to...

Major cooperation partners:

Geir E. Tjønnfjord
Ulla Randen
Ruth Langholm
Jan Delabie
Elling Ulvestad

Oslo University Hospital/
University of Oslo

Haukeland University Hospital/
University of Bergen

Co-investigators in multicenter studies:

Henrik Hjorth-Hansen
Anne Marita Vågan
Waleed Ghanima
Bjørn Tore Gjertsen
Robert Brudevold
Fuad Victor Shammas
Jon Hjalmar Sørbø

...and many others

Others: Tom Eirik Mollnes

University of North Norway /University of Oslo
Revealing the landscape...

Thank you for your attention