A-TYPICAL CASE???

Heme School 2012
Amy DeZern, MD, MHS
Patient Presentation

- 72yo real estate broker from New Jersey
- Suffered a tick bite in Spring 2008
- Pancytopenia:
  - WBC 3500  ANC 1250--- No recurrent infections
  - Hb 11.9  MCV 102-- No PRBCs
  - Plts 87-- No bleeding
- Treated for 4 months with doxycycline without change
  - Lyme serologies negative
- Counts stably low
- Ongoing atypical lymphs

- Bone marrow biopsy performed
Biopsy 2008

- Hypercellular for age at 40-50%
- No significant or obvious dysplasia
- Myeloid predominance with mild granulocytic hyperplasia
- Flow without phenotypically abnormal population- no increase in CD34+ blasts
- Cytogenetics: 45X (-Y) in 5% (1/20)
  - Can be normal variant in elderly men

**COMMENTS:** An evolving MPD/MDS cannot be excluded. Accompanying smear shows atypical lymphocytes.
Diagnosis: “Early MDS”

• “Given all these findings with hypercellular marrow, pancytopenia, macrocytic anemia, everything points toward low-grade myelodysplastic syndrome”

• Watchful waiting and clinically well
  • Monthly CBCs x year- slow decrease
  • Consideration of Azacytidine
## IPSS Risk Stratification

<table>
<thead>
<tr>
<th>Prognostic Variable</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td><strong>Marrow blasts (%)</strong></td>
<td>&lt;5%</td>
</tr>
<tr>
<td><strong>Karyotype class</strong>*</td>
<td>Good</td>
</tr>
<tr>
<td><strong># of cytopenias</strong></td>
<td>0 or 1</td>
</tr>
</tbody>
</table>

* **Karyotypes**: Good = normal, -Y, del(5q) alone, del(20q) alone; Poor = chromosome 7 abnormalities or complex; Intermediate = other karyotypes

**Cytopenias**: Hb < 10 g/dL, ANC <1800/uL, platelets <100,000/uL

### Risk Groups

<table>
<thead>
<tr>
<th>IPSS</th>
<th>Low</th>
<th>Int-1</th>
<th>Int-2</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.5-1.0</td>
<td>1.5-2.0</td>
<td>2.5-3.5</td>
<td></td>
</tr>
</tbody>
</table>

Lower risk MDS (Low, Int-1) is associated with a median survival of 3.5-5.7 years

Patient was not deterred…

- Sought 2\textsuperscript{nd} opinion—arrived at JHH 18 months after initial diagnosis

- Pancytopenia:
  - WBC 2840 (37% P, 47% L) ANC 1060—No recurrent ID
  - Hb 10.7 MCV 105—No PRBCs
  - Plts 87—No bleeding

- Additional work up
  - TSH wnl
  - Ferritin 379
  - B\textbf{12} 398
  - Folate 498
  - CRP 0.1
  - Parvo PCR negative
  - PNH flow- negative
Additional Work Up

- Peripheral blood flow:
  - Subpopulation of T cells with CD3, dim CD5, CD56 and CD57 accounting for about 4-5% of total cells or about 10-15% of lymphocytes. These may represent large granular lymphocytes.

- T cell receptor rearrangement: POSITIVE

So… New diagnosis: LGL!!
History of LGL

• First mention in literature in 1982 in German journal *Blut* (translation: Blood)
  • LGL phenotype noted in cells found in post BMT patients with skin GVHD
• First classical description by Loughran et al 1985
  • Lymphocytosis, neutropenia, thrombocytopenia in 3 pts with splenomegaly
  • Multiple autoantibodies… RF, ANA +
  • Clonal marrow infiltration CD3+, CD8+
  • “Cytopenias appeared to be antibody mediated and not related to direct suppression of hematopoietic progenitors”

Classification

- 1989 FAB called it T-CLL
- 1990 MIC Study group renamed LGL leukemia
- 1994 REAL criteria
  - CD3- NK cell LGL
  - T cell LGL= distinct clonal entity- CD3+ T cells that undergo TCR gene rearrangement
- 1999 WHO criteria
  - T cell granular lymphocytic leukemia- a subgroup of mature peripheral T cell neoplasms
Large Granular Lymphocytes

- Just remember: “Normal LGLs” = 10-15% of peripheral blood mononuclear cells
- Circulate through blood in search of infected cells
- Make contact through receptor ligand and induce cell death
- LGLs then supposed to undergo apoptosis themselves but…
- Unchecked proliferation and cytotoxicity → results in autoimmunity or malignancy
- Then it’s OUR PROBLEM!

Leuk and Lymp December 2011; 52(12):2217-2225.
LGL function

- Searched for infected cells to induce apoptosis
- Two mechanisms
  1. Through contact of Fas ligand with its receptor (CD59R) on infected cell
  2. Through release of Perforin (and other cytotoxins) that makes pore in target cell and granzyme B released into it which cleaves/activated caspases → cell death
To truly make the diagnosis

Clinical context
- Splenomegaly
- Cytopenia
  - Neutropenia
  - Anemia
- Lymphocytosis
- Auto-immune conditions (RA)

Peripheral blood smears:
  - excess of LGL > 0.5 x 10^9/L

PB Immunophenotyping using flow cytometry (FCM)

If T cell subtype:
  - clonality assessment
    (using TCRγ PCR and/or
     Vβ FCM repertoire analysis)
If NK cell subtype:
  - Kir expression using FCM
Karyotype not mandatory

In case of LGL count < 0.5 x 10^9/L
  and appropriate clinical context
  Bone marrow aspirate and/or biopsy
  With histioimmunochemistry mandatory

Lamy T, Loughran T P Blood 2011;117:2764-2774
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- Splenomegaly
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In case of LGL count < 0.5 x 10^9/L
and appropriate clinical context

Bone marrow aspirate and/or biopsy
With histolimmunohistochemistry  mandatory
Associated with LGL

- Rheumatoid Arthritis
  - Felty’s
- Sjogren’s
- AIHA
- PRCA
- ITP
- Other benign cytopenias
- B cell lymphoproliferative d/o’s
- MDS
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- Splenomegaly
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In case of LGL count < 0.5x10^9/L
and appropriate clinical context
Bone marrow aspirate and/or biopsy
With histoimmunochemistry mandatory
LGL

- Abundant pale cytoplasm
- Azurophilic granules
- Larger cells with #s between 2-10E9 (normal 0.25E9)
- Dutch skirting
To truly make the diagnosis

**Clinical context**
- Splenomegaly
- Cytopenia
  - Neutropenia
  - Anemia
- Lymphocytosis
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Peripheral blood smears:
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- Bone marrow aspirate and/or biopsy
  - With histioimmunochemistry mandatory

Lamy T, Loughran T P. Blood 2011;117:2764-2774
Peripheral blood flow cytometry

- CD3- NK cells (Flow CD7, CD16, CD56) (NO TCR)

- CD3+ T cells (Flow dimCD5, CD8, CD57) (+TCR)
To truly make the diagnosis

Clinical context
- Splenomegaly
- Cytopenia
  - Neutropenia
  - Anemia
- Lymphocytosis
- Auto-immune conditions (RA)

Peripheral blood smears:
excess of LGL > 0.5 x 10^9/L

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Lamy T, Loughran T P Blood 2011;117:2764-2774
TCR (code 3472) (not T cell chimerism follow up, or CD4 T cell subsets)

- Needed to distinguish the LGL neoplasm from reactive lymphocytosis
- Establish clonality through gene rearrangement studies
- TCR on the T cell recognized antigens and undergoes rearrangement of the variable (V), diversity (D) and joining (J) gene segments during thymic development
- Done via Southern blot or PCR (done here)
  - Can be done on fresh, frozen or paraffin embedded tissue
To truly make the diagnosis

Clinical context
- Splenomegaly
- Cytopenia
  - Neutropenia
  - Anemia
- Lymphocytosis
- Auto-immune conditions (RA)

Peripheral blood smears:
- excess of LGL > 0.5 x 10^9/l

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Bone marrow aspirate and/or biopsy
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Lamy T, Loughran TP Blood 2011;117:2764-2774
Treatment

- **GOAL**: To avoid M and M of cytopenias
  - Infections
  - Transfusion needs
  - Concomittant systemic AI disease

- No standard of care due to lack of appropriate RCTs

- Always the option of watchful waiting
  - Must convince everyone (patient, referring MD, yourself) it is not about the numbers

- Steroids: can alleviate any B symptoms, improve cytopenias and augment other meds but do not produce durable remissions nor eliminate the clone

- IST regimens based on retrospective or prospective single institution studies
Immunosuppression

- Methotrexate - used first line by most since 1994

- Single study, prospective, uncontrolled, all failed prednisone
  - 5 of 10 pts treated with 10mg/m2 weekly had normalization of CBC
  - Median time to respond: 1 month
  - 50% of patients don’t respond
  - In those who respond, ~60% have disappearance of clone by TCR

<table>
<thead>
<tr>
<th>Patients</th>
<th>Indication for TR</th>
<th>Weekly dose</th>
<th>ORR</th>
<th>CHR</th>
<th>MR</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Neutropenia</td>
<td>10 mg</td>
<td>60</td>
<td>50</td>
<td>3/5</td>
</tr>
<tr>
<td>3</td>
<td>RA + infections</td>
<td>7.5 mg</td>
<td>100</td>
<td>100</td>
<td>2/3</td>
</tr>
<tr>
<td>8</td>
<td>Neutropenia</td>
<td>5–13.5 mg/m²</td>
<td>86</td>
<td>14</td>
<td>NR</td>
</tr>
<tr>
<td>62</td>
<td>Neutropenia, anemia, RA</td>
<td>7.5 mg/m²</td>
<td>55</td>
<td>21</td>
<td>NR</td>
</tr>
</tbody>
</table>

- If treat for > 5 year with low dose MTX - side effects: CNS toxicity, GI disturbances, hepatotoxicity

IST (cont)

- CsA 5-10mg/kg day
  - Single case report from *Blut* 1989
  - More prospective series
    - Trigger: neutropenia (many 2\textsuperscript{nd} and 3\textsuperscript{rd} line)
    - No correlation between CsA level and ANC response
    - Clones not eliminated
    - Nephrotoxicity, HTN

<table>
<thead>
<tr>
<th>Patients</th>
<th>CSA levels</th>
<th>ORR</th>
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<tbody>
<tr>
<td>5</td>
<td>250–400 ng/ml</td>
<td>100</td>
</tr>
<tr>
<td>23</td>
<td>Variable</td>
<td>78</td>
</tr>
<tr>
<td>25</td>
<td>250–400 ng/ml</td>
<td>56</td>
</tr>
<tr>
<td>24</td>
<td>Variable</td>
<td>21</td>
</tr>
</tbody>
</table>

- In French series, better for patients with primarily anemia
- HLA-DR4 predictive of response

*Blut*, 1989. Blood. **1998** May 1;91(9):3372-8; *Haematologica* 2010;95(9):1534-1541
Other options

- Oral Cytoxan 50-100 mg/ day
  - Best use in patient with PRCA 2/2 LGL
  - Largest series French: 32 pts treated 2nd line for neutropenia
  - ORR 66% (CHR in 47%)
  - Worry about tAML so use in first line setting debated

- Growth factors
  - Can at times be used to bridge during IST initiation (in case of NF) but not effective long term

- Splenectomy
  - Series of 15 pts, all with CHR but clones persisted

- Purine analogs
  - Fludarabine
  - Pentostatin (2/5 pts respond)

- Alemtuzumab
  - 30 mg QDAY x 6 weeks
  - 1/8 response

- Death from T LGL 6.5-36% in reported series

Haematologica 2010;95(9):1534-1541
Lamy T, Loughran T P Blood 2011;117:2764-2774
Back to heme clinic...2012 update (age 76y)

- Pancytopenia:
  - WBC 1910  ALC  630 ANC 1090--- No recurrent infections
  - Hb 8.7   MCV 111--  10 U PRBCs over 20 months (6 in last 6 months)
  - Plts 56-- No bleeding and on warfarin
- LDH 319
- Cr 1.0 (Has receive Aranesp x 4 without response)
- B12 155   MMA 228 (wnl)
  - (Oh, btw doc, I can’t feel my feet up to my socks x 2 years)
  - Smear with hypersegmented polys
JHH eval (after B12 repleted and still requiring PRBCs)

- PB Flow: 4% Large NK/T granular lymphocytes
- TCR: “Oligoclonal” with 4 peaks and NOT typical for LGL

- BMBx: hypercellular (80-90%) for age with myeloid predominance. The M:E ratio is markedly increased (>10:1). Megakaryocytes are relatively increased in number and atypical, many with hypolobated, hyperchromatic nuclei. No lymphoid aggregates are present. Blasts are not increased (0.6%)…COMMENT: “soft and nonspecific”

- Karyotype 19/20 (95%) –Y
  - Incidental finding in older males vs clonal abnormality in neoplasms
  - 75% cutoff point to consider the cells missing a Y chromosome to be a disease related clone

- BFUE 3.7 bursts/ E9 MMNC

Genes Chromosomes & Cancer 27:11-16,200
1. New marrow blast categories
   ---≤2, >2 - <5, 5 - 10, >10 - 30%

2. Refined cytogenetic abnormalities and risk groups
   ---16 (vs 6) specific abnormalities, 5 (vs 3) subgroups

3. Evaluation of depth of cytopenias
   ---clinically and statistically relevant cutpoints used

4. Inclusion of differentiating features
   ---Age, Performance Status, serum ferritin, LDH, Beta-2microglobulin

5. Prognostic model with 5 (vs 4) risk categories
   ---improved predictive power
IPSS- R… for MDS

• Cytogenetics:
  • **Very good**: -Y, del(11q)
  • Good: Normal, del(5q), del(12p), del(20q), double including del(5q)
  • Intermediate: del(7q), +8, +19, i(17q), any other single or double independent clones;
  • Poor: -7, inv(3)/t(3q)/del(3q), double including-7/del(7q), Complex: 3 abnormalities
  • Very poor: Complex > 3 abnormalities

Blood. 2012 Jun 27.Epub
# IPSS-R Risk Stratification

<table>
<thead>
<tr>
<th>Prognostic Variable</th>
<th>SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Cytogenetics</td>
<td>Very Good</td>
</tr>
<tr>
<td>BM Blast%</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Platelets</td>
<td>&gt;100</td>
</tr>
<tr>
<td>ANC</td>
<td>&gt;0.8</td>
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**IPSS-R Risk Stratification**

**Table 4. IPSS-R Prognostic Risk Categories/Scores**

<table>
<thead>
<tr>
<th>RISK CATEGORY</th>
<th>RISK SCORE</th>
</tr>
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<tbody>
<tr>
<td>Very Low</td>
<td>&lt;1.5</td>
</tr>
<tr>
<td>Low</td>
<td>&gt;1.5-3</td>
</tr>
<tr>
<td>Intermediate</td>
<td>&gt;3-4.5</td>
</tr>
<tr>
<td>High</td>
<td>&gt;4.5-6</td>
</tr>
<tr>
<td>Very High</td>
<td>&gt;6</td>
</tr>
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</table>

- Low: Median survival 5.3 (5.1-5.7) years
- Median time to 25% AML 10.8 (9.2-NR) years
MDS with LGL clone… not so atypical!
LGL and MDS

• 100 consecutive patients with cytopenia and primary diagnoses of MDS or T-LGL
  • 76 patients had MDS alone
  • 15 had T-LGL alone
  • 9 patients had T-LGL/MDS

• ~10% of patients with MDS have LGL clones in other series

• 28% of overlap patients respond to IST compared to the ~50-60% of LGL alone
  • (similar to MDS response rates to ATG/CsA)

Summary

- Firm criteria for diagnosing LGL
- First line therapy is IST with MTX
  - 50% CHR only
- R-IPSS- clearer but not marked changes
- LGL clones can be seen in MDS
- Substantial overlap to bone marrow failure syndromes
- Fluidity between diagnoses
  - Continued reassessment is key- can be more than one
- Treating one may unmask the other