Starring: Anna Halpern MD, Sioban Keel MD, and Emily Stevens MD PHD; November 7, 2014
25 yo F transferred from OSH with 3 months of intermittent fever, rash, joint pain, headaches, and recent onset of pancytopenia
HPI

- Healthy young woman until 3 months ago when she developed fevers to 104
- Then developed ecchymotic rash on extremities, prolonged menses, headaches associated with nausea/vomiting, and migrating arthralgias
- Denied unusual exposures
- Meds: Only Ibuprofen and Tylenol prn. Denied supplements, OTC medications, natural medications
Relevant History

- Social history: Born in China, lived in Hong Kong from birth until age of 14 when settled in US. Mother and father live abroad. Lives with family friends in Kent. She is an student at UW
- No travel, camping, pets, TB exposure, or sexual contact
- Denied any alcohol, drug use
- FH: twin sister who died at 7 months from leukemia, possibly ALL
Outside Hospital FUO Workup

- Extensive ID workup neg: blood cx and CSF cx negative, parvovirus, HIV-1/HIV-2, TB, Hepatitis A, B, C, CMV, EBV, Treponemal Abs, stool cx, O&P, giardia, histo, coccidio, malaria
- Autoimmune workup neg: CK, Anti-CC2, RF, ESR, CRP, ANA, C3, C4, ESR, CRP all normal
- Heme: CBC, bleeding time, VWF levels and function, coags, F8, LDH, uric acid, SPEP, folate, B12, iron, ferritin were all normal
- Imaging
  - TEE did not show any vegetations
  - EGD: no gastritis
  - MRI brain: normal
  - CT CAP: normal

- Skin biopsy: neutrophilic dermatitis w/o vasculitis
- 3 weeks prior with normal CBC: BM biopsy → normocellular marrow at 50% cellularity with trilineage hematopoiesis, reactive plasmacytosis that was polyclonal and negative flow. AFB neg.
Current Presentation

- Transferred to our hospital for new pancytopenia that developed acutely over the past week
- Ongoing fevers 3-4 times weekly, mostly at night, and 20lb wt loss in 3 months
- No night sweats, adenopathy, early satiety
- Does have ongoing rash, arthralgias, migraines
- Denies new exposures or medications
Exam

Exam:
- Afebrile, normal vital signs, well appearing
- No lymphadenopathy, splenomegaly
- Ecchymotic rash on upper extremities, no other rash
- Otherwise normal, no joint abnormalities
ANC nadir 30; Reticulocyte Count 6 (0.2%)
Bone marrow biopsy: markedly hypocellular marrow (10-15%) with myeloid hypoplasia and no significant dysplastic features, normal megakaryocytes, no infiltrates, granulomas, or lymphocyte populations

- Flow neg for abnormal B/T-cell populations
- 2.2% blasts by flow
- 46, XY [20], IFISH MDS panel neg
- PB PNH flow: no GPI-deficient granulocyte monocyte or erythrocyte population
Differential:

Idiopathic aplastic anemia

Hypoplastic MDS

Marrow Insult: drugs, virus, toxin
Aplastic Anemia

- Thought most likely idiopathic aplastic anemia
  - Prodrome of FUO, rash, joint pains was acknowledged to be unusual
  - Did start neupogen given ongoing fevers

- Camitta Criteria (Blood 1976) for severe aplastic anemia
  - At least 2+: granulocytes <500, plts <20, reticulocytes <1% and bone marrow had to be markedly hypocellular <25% (or moderately hypoplastic (25-50%) with <30% remaining cells hematopoietic origin)
Idiopathic Acquired Aplastic Anemia

- Defined by co-existence of pancytopenia and unexplained reduced marrow hematopoietic cellularity with no major dysplastic signs and replacement by fat cells
- Immune-mediated disorder in which oligoclonal cytotoxic T cells target and destroy hematopoietic progenitor cells
- Usually “idiopathic” but rarely associated with seronegative hepatitis (documented in 5-10% pts with acquired AA) or other autoimmune disease (5->25% in studies, more common in patients dx >55)
Idiopathic Aplastic Anemia- Clinical Picture

- Clinically: usually just pancytopenia, no enlarged lymph nodes or hepatosplenomegaly
  - Reticulocytopenia, macrocytosis, preserved lymphocyte count
- Cytogenetics abnormalities present in about 12% (Rovo et al, BMT 2013), which may come and go during course of disease or with immune suppression
  - Most frequent abnormalities are trisomy 8, trisomy 6, 5q-, anomalies of chromosome 7 and 13
- To diagnose must exclude other reasonable entities, sometimes repeat marrow exams are needed
Idiopathic Aplastic Anemia - Diagnosis

- Must rule out: marrow toxins, hypoplastic MDS, hypoplastic AML/ALL, large granular lymphocytosis, PNH
  - Drugs, virus, toxin: mostly ruled out by labs and history
  - Hypoplastic MDS: differences in bone marrow

- Also should always rule out bone marrow failure disorders such as Fanconia Anemia and telomere length disorders (such as Dyskeratosis Congenita)
MDS vs Aplastic Anemia

| Table 3. Main diagnostic characteristics of aplastic anemia and hypoplastic myelodysplastic syndromes |
|---------------------------------------------------------------|---------------------------------------------|
| **Aplastic anemia**                                           | **Hypoplastic MDS**                         |
| Cytopenia                                                    | Present                                      |
| BM cellularity                                               | Aplastic (<10% cellularity) or hypocellular |
| BM hematopoiesis                                             | Present in nest, ‘hot spots’                 |
| Erythropoiesis                                               | Typically decreased                          |
| Myelopoiesis                                                 | Decreased or absent                          |
| Megakaryopoiesis                                             | Present                                      |
| Dysplasia                                                    | Present                                      |
| Erythropoiesis                                               | Possible                                     |
| Myelopoiesis                                                 | Normal morphology                            |
| Megakaryopoiesis                                             | Possible                                     |
| Blasts                                                       | Absent                                       |
| CD34+ or CD117+ immunohistochemistry                         | Nearly absent                                |
| Marrow fibrosis                                              | Absent                                       |
| Karyotype                                                    | Clonal abnormality possible (about 12%)      |
| PNH clone                                                    | Frequent                                     |
| Splenomegaly at diagnosis                                    | Absent                                       |
|                                                             | Variable                                     |
|                                                             | Normal or increased                          |
|                                                             | Possible                                     |
|                                                             | -7/del(7q)                                   |
|                                                             | 5/del(5q)                                    |
|                                                             | Unusual                                      |
|                                                             | Possible                                     |

MDS: Dysplasia present (especially of megakaryopoiesis), blasts, increased CD34 and CD117 positive cells on IHC

(Rovo et al, BMT 2014)
Bone Marrow Failure Disorders
Inherited Bone Marrow Failure Syndromes

- Diverse group of genetic disorders that ultimately result in the loss of blood production
- Characterized by bone marrow failure, congenital anomalies, and increased risk of malignant disease
- Representative diseases are Fanconi Anemia (FA) and Dyskeratosis Congenita, Diamond-Blackfan anemia
- Genetic lesions responsible for these diseases and their manifestations have now been characterized
BMF: Fanconi Anemia

- Autosomal recessive disease that is the most frequent inherited cause of aplastic anemia
- Characterized by congenital abnormalities (short stature, endocrine abnormalities, skin/eyes/kidney abnormality), development disability
- Increased propensity for cancer, most often AML and 90% develop bone marrow failure by age 40
FA Pathophysiology

- Result of a genetic defect in a cluster of proteins responsible for DNA repair
- At least 16 genes identified as playing a causative role
  - All gene products function in common processes to maintain genomic stability and repair DNA interstrand cross-links
- Diagnosed by chromosome fragility testing
  - Treat cultures of peripheral blood lymphocytes with mitomycin C and diepoxynbutane to determine if increased breakage
FA and BMF

• Most pts with FA will develop BMF, culminating in pancytopenia
• Blood counts normal at birth, median age 7 develop thrombocytopenia then leukopenia, then all 3 lineages
• It is the DNA damage to hematopoietic stem cells that ultimately limits the regeneration of hematopoiesis
  ○ Bone marrow is one of the most sensitive tissues in the body to DNA damage
  ○ Genetic instability within the FA-deficient HSCs results in the progressive loss of this cell population and genesis of neoplastic clones
• But what is the source of this endogenous DNA damage?
Endogenous Genotoxins

- New evidence, mainly in FA murine models, suggests that endogenous aldehydes are the source of DNA damage.
- Small aldehydes are ubiquitous in the environment (exogenous source such as alcohol) and are an endogenous by-product of cellular metabolism.
- They are highly reactive, forming DNA adducts in vivo/vitro.
- FA-deficient cells have been shown to be hypersensitive to small aldehydes, which cause double-strand breaks and chromosomal aberrations.
- Garaycoechea et al (Nature 2012) showed that the combined inactivation of aldehyde detoxification and the FA DNA-repair pathway in mice resulted in persistent damage to HSCs with a phenotype similar to aplastic anemia.
Ald2/Fancd2 -/- Mouse Model

Garaycoachea et al (Nature 2012)
Aldehyde as a Genotoxin

- HSCs possess a high level of aldehyde dehydrogenase activity and appear especially sensitive to aldehyde-mediated toxicity
- A large percentage of Southeast Asian populations express a dominant-negative allele of the aldehyde-catalyzing enzyme, aldehyde dehydrogenase 2 (ALDH2)
- This ALD2 variant is associated with accelerated BMF in Japanese FA patients (Hira et al, Blood 2013)
- May provide a therapeutic target in the future
BMF: Telomere Disorders

- Telomeres are repeat sequences at the ends of chromosomes that are protective of chromosomal structures.
- Telomeres inevitably shorten with every cell cycle, which has been hypothesized to be fundamental to normal senescence of cells, tissues, and organisms.
- Molecular mechanisms have evolved to maintain the length and protective function of telomeres.
Telomerase and Shelterin Complex

- Shelterin is a collection of DNA-binding proteins that cover and protect telomeres.
- Telomerase is the enzyme responsible for telomere elongation.
- TERT is a reverse transcriptase enzyme that uses an RNA molecule (TERC) as the template to elongate the 3' ends of telomeres.
- Inherited mutations in these genes can cause a range of human diseases, in a diverse array of tissues, including the hematopoietic tissue, lung and liver.

Townseley, Blood 2014
Dykeratosis Congenita (DC)

- Dyskeratosis congenita is the prototype of telomere disease
- It is characterized by bone marrow failure, mucocutaneous abnormalities, pulmonary fibrosis, liver cirrhosis, and increased susceptibility to cancer, including acute myeloid leukemia and SCC of the tongue
- Triad: lacy reticular rash, dystrophic nails, oral leukoplakia

- Genetically heterogeneous with AR, AD and X-linked mutations of different genes of the telomerase or shelterin complex
DC and Telomeropathies- Diagnosis

- Can be diagnosed by measuring average telomere lengths of chromosomes within peripheral blood leukocytes
- Standardly terminal restriction fragment length analysis by Southern blot, or now more commonly quantitative PCR and standardized flow-FISH compared with age-matched controls
- Can also screen for gene mutations but more time consuming and usually not done prospectively

Telomere Length Analysis
Idiopathic Aplastic Anemia: Therapy
Therapy in Brief

- Historically: androgens have hematologic improvement (HI) rates at high as 50% (Townsley et al, Blood 2014)

- Immunosuppressive therapy (IST): (Young et al, Blood 2006)
  - ATG plus cyclosporine $\rightarrow$ hematologic improvement in 60-70% pts
  - Relapses occur in 1/3 of responders
  - 10-15% clonal evolution: manifest late as myelodysplasia

- Allogeneic Stem Cell transplant: only curative option

- TPO-mimetics now being explored
Telomeres and IST

- Telomere length can be useful prognostically in AA and can predict response to IST
- In a single institution analysis of 183 patients with severe AA treated from 2000-2008 with IST:
  - Measured pre-treatment telomere length prospectively
  - No relationship between telomere length and hematologic response
  - Telomere length was associated with relapse, clonal evolution and mortality (multivariate analysis)**
  - When evaluated as a continuous variable, telomere length inversely correlated with probability of hematologic relapse
  - Survival differed between those in the first vs second-fourth quartile of telomere length: 66% vs 84% at 6 years (p=.008)

(Scheinberg et al, JAMA 2010)
(Scheinberg et al, JAMA 2010)
Patient Course

- WBC improved dramatically on neupogen, able to go home
- Chromosomal fragility testing on PB lymphocytes and telomere lengths were not supportive of Fanconi Anemia or Dyskeratosis congenita
- Came back to clinic for a few weeks after discontinuation of neupogen with normal counts
Re-presented one week later with fevers x 3 days, diarrhea, vomiting, dehydration, low counts:

- Denied any new medications/exposures
- None of the above symptoms noted in the hospital
- Despite 24 hours of IVF, she said she hadn’t yet urinated to give a urine sample for fevers
- Finally nurse caught her urinating, and the sample was obtained . . .
The urine never lies . . .

- Urine toxicology
  - Positive for: Secobarbital, Glutethimide (Doriden)
- Secobarbital = barbiturate, death with dignity
- Glutethimide is a sedative-hypnotic used to potentiate the high of codeine syrup
- Detective Emily Stevens received a call from Washington State Toxicology Lab
  - These drugs have recently made a comeback in Washington State after being featured in the film The Wolf of Wall Street
- Case reports have associated them with pancytopenia and bone marrow aplasia
- Patient referred to psychiatry for follow up

(*Focosi et al Acta Haematol 2208; Ng, Chemical Research in Toxicology 2013)
"When it comes to quaaludes....."
Internet Availability
Take Home Points

- In the diagnosis of idiopathic aplastic anemia, always rule out FA/telomere disorders and well as potential exogenous marrow toxins.
- There is emerging evidence suggesting the genotoxin causing DNA damage in hematopoietic stems cells in FA is endogenous aldehydes.
- Telomeropathies can affect multiple organ systems causing fibrosis or fatty replacement of the marrow, liver and lungs and can be diagnosed via PB telomere length analysis.
- The urine never lies.
References

Thanks

- To Emily Stevens for her detective work!
- For Sioban Keel for her support with the whole case
Telomeres: Instigator or Bystander?

- These authors argue that telomere attrition is not simply a biomarker for more severe disease but rather a clear mechanism for destabilization of the genome
  - Evidence from in vitro and animal experiments shows that critical shortening of telomeres causes chromosome instability, tumor formation and cancer progression
  - Clinically, telomere length has been associated with human cancer such as the increased frequency of tongue cancer and leukemia in dyskeratosis congenita
  - Solid tumors evidence as well: in Barrett’s Esophagus, there is increased risk of developing esophageal adenocarcinoma in people with shorter leukocyte telomere length at presentation
    (Risques et al, Gastroenterology 2008)