Selected Topics in Pediatric Hematology/Oncology
“COMPLEMENTOLOGY”
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Grand Rounds
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OBJECTIVES
- Recognize implications of complement pathway diseases
- Signs and symptoms of PNH and aHUS
- Complications of PNH and aHUS
- Recognized appropriate diagnostic testing for PNH and aHUS
- Understand treatment options in PNH and aHUS
- Provide a little entertainment
- Shamelessly plug the UTHSCSA Pediatric Hematology Consultation Service

Classically Different Topics
…but not so much
Both are diseases of complement dysfunction

PNH
- Rare, acquired, disease of the blood in children and young adults
- Characterized by hemolytic anemia, thrombocytopathy, impaired bone marrow function, and up to 5% risk of developing leukemia
- PNH is closely related to aplastic anemia
  - 30% of newly diagnosed cases of PNH evolve from aplastic anemia
  - The risk developing PNH after treatment for aplastic anemia with immunosuppressive therapy (anti-thymocyte globulin and cyclosporine) is approximately 20 to 30%.
- The median survival after diagnosis is 10 years

aHUS
- Rare disease of the kidney in children and young adults
- Characterized by hemolytic anemia, thrombocytopenia and acute renal failure
- Distinctly different illness from HUS caused by particular strains of the bacterium 0157 E.coli producing Shiga toxins
- Evidence that aHUS is a genetic disorder
- Chronic condition with predilection for repeated attacks of the disorder
- Develop chronic serious complications such as kidney failure

“I can live for two months on a good compliment”

Complement Function
The Good
- Part of the humoral immune system “guarding” the intravascular space against bacterial invasion
- 3 pathways
  - Classical Pathway
  - Lectin Pathway
  - Alternative Pathway
- “Always on”
- Regulatory proteins

The Bad
- Dysregulation of the complement system can lead to wide spread cellular injury often associated with hematologic and renal diseases
- (aHUS, dense deposit disease)
- Infections
  - HIV, Streptococcus pneumoniae
- Chemotherapeutic agents
- Autoimmune disorders
  - SLE, scleroderma
- Pregnancy
  - Pre-eclampsia, HELLP

The Complement Pathway
The Complement System: Always On, Strongly Amplified, Dependent on Natural Regulators

- A vital component of the natural protective immune system
- Always "on" to allow rapid immune response
- Simple triggers, including common infections and trauma, lead to rapid complement amplification
- Rapid amplification leads to powerful and destructive immune reactions
- Natural inhibitors of complement keep amplification in check and prevent uncontrolled complement activation

Discoveries began with the work of Thomas Hale Ham and Louis Pillemer in the late 1930's suggesting a novel antibody-independent mechanism for complement activation. The Ham acid hemolysis test was the up until recently the standard by which PNH was diagnosed.

Paroxysmal Nocturnal Hemoglobinuria

- Prevalence: 15.9 / million
- Diagnosed at all Ages
- Median age early 30's
- Acquired disease of hematopoetic stem cell
- Progressive disease
- Uncontrolled complement activation underlies the morbidities and mortality
- 5 year mortality: 35%

Clonal expansion of these “PNH stem cells” leads to affected progeny

CD59
- Prevents formation and augments instability of the C3 convertases, attenuating the complement cascade
- Forms a defensive shield for RBCs from complement-mediated lysis
- Inhibits the assembly of the membrane attack complex

The Defect in PNH

The Somatic Mutation of the PIG A Gene Prevents All GPI Anchored Proteins from Binding to Cell Surface

Paroxysmal Nocturnal Hemoglobinuria

...myths and legends

- It’s not paroxysmal
  - Even in the absence of symptoms, destructive progression of hemolysis is ongoing
- It’s not nocturnal
  - Hemolysis in PNH is subtle and constant, 24 hours a day
- Hemoglobinuria is a less commonly seen complication
  - ¾ patients present without hemoglobinuria
Historically Viewed as a Hemolytic Anemia

Normal red blood cells are protected from complement attack by a shield of terminal complement inhibitors. Without this protective complement inhibitor shield, PNH red blood cells are destroyed.

The peripheral blood of patients with PNH is a mosaic of normal and abnormal cells:
- PNH III cells are completely deficient in GPI-APs
- PNH II cells are partially (~90%) deficient
- PNH I cells express GPI-APs at normal density

93% of Patients With PNH Have Peripheral Blood Abnormalities

Anemia: Hemoglobin <12.0 g/L
Neutropenia: ANC <1.5×10^9/L
Thrombocytopenia: Platelet count <1.5×10^11/L

Characteristics of Thrombosis in PNH
- Can occur at typical and atypical sites:
  - DVT or PE most common
  - Budd-Chiari and dermal
- Both venous and arterial sites:
  - 39% of TE events occur at arterial sites
- Abdominal pain is a predictor of TE:
  - 3.6-fold increased risk
- Incidence of TE is elevated even in patients receiving anticoagulant therapy:
  - Clinical thrombosis evident in PNH patients with:
    - Minimal hemolysis
    - No transfusion history
    - Smaller clone size

Signs and Symptoms

Delays in diagnosis range from 1 to more than 10 years.

Clinical Signs or Symptoms
- Thrombosis: 40%
- Dyspnea: 66%
- Pulmonary Hypertension: 47%
- Chronic Kidney Disease: 64%
- Abdominal Pain: 57%
- Anemia: 88%
- Fatigue, impaired QOL: 96%
- Dysphagia: 47%
- Erectile Dysfunction: 47%

In patients with history of TE, there is a 7-fold increased risk of mortality.

Classification and Testing for PNH

<table>
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<tr>
<th>Condition</th>
<th>Description</th>
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<tbody>
<tr>
<td>PNH III</td>
<td>Complete deficiency of GPI-APs</td>
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<tr>
<td>PNH II</td>
<td>Partial deficiency (~90%) of GPI-APs</td>
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<tr>
<td>PNH I</td>
<td>Normal expression of GPI-APs</td>
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</table>

ICCS

Suggestions for PNH Testing by ICCS PNH Guidelines

Clinical indications for PNH testing

- Evidence of unexplained hemolysis with an anemia
- Non-thrombocytopenic purpura
- Asymmetrical, or unexplained, splenomegaly
- Thrombotic, or associated thrombocytopenia
- Other acquiredGlomerulonephritis, non-thrombocytopenic hemolytic anemia

Therapeutic implications

- Unexplained anemia (Budd-Chiari syndrome)
  - Other underlying causes: renal, vascular, splenic
- Central nervous system
- Dermatologic
- Other signs of accompanying hemolytic anemia (see above)
- ISS unexplained thrombocytopenia

Percentages of bone marrow failure

- Granulocytic failure
  - Unexplained agranulocytosis or hypogranulocytosis
  - Refractory cytopenia with multilineage dysplasia
  - Other cytopenias of idiopathic etiology following aplastic anemia

Why Look Beyond RBCs for PNH?

- Granulocytes provide more accurate representation of PNH clone size
- PNH population in marrow failure syndromes may not otherwise be identified
  - 40% of the MDS and AA in children
- Percentages of PNH RBCs may be affected by
  - Hemolysis
  - Blood transfusion

Important to Monitor Granulocytes and RBCs Over Time

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<tr>
<th>Date</th>
<th>Gran clone</th>
<th>RBC clone</th>
<th>CD24-Granulocytes</th>
<th>FLAER-GPI Anchor Marker</th>
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PNH Clone Expanded in <1 Year

PNH illustrates a model of disease states related to targets of complement. What about true regulatory dysfunction of the complement system?

Summary

- PNH may be more common than you might think
- Delays in diagnosis range from 1 to more than 10 years
- Test high-risk patients for PNH
- Reliable testing and reporting procedures matter
  - Granulocyte analysis in all cases
  - PNH testing on RBCs alone is not adequate
  - Adding quantitative results to report forms is essential
- With the advent of treatment options for PNH, there is a compelling reason to identify patients

**Atypical Hemolytic Uremic Syndrome**

- Dysregulation and/or excessive activation of the alternative pathway of complement plays a pivotal role in the pathogenesis of aHUS
  - can be both familial (<20%) and sporadic
- Inactivating mutations in genes encoding complement regulators (factor H, factor I, and membrane cofactor protein)
- Gain-of-function mutations in genes encoding the complement activators (C3 and factor B) have been described
- Mutations in the gene encoding thrombomodulin have also been described in aHUS.

**Chronic Uncontrolled Complement Activation Causes Platelet, Endothelial, Leukocyte/Monocyte Activation Leading to Inflammation and Systemic Small Vessel Occlusion**

**Early Diagnosis of aHUS: Challenges**

- aHUS can manifest with early signs and symptoms that are non-specific
- Often diagnosed late when organ damage already occurred
- Clinical presentation can be similar to other systemic thrombotic microangiopathies (TMA)
- Historically limited interest to differentiate aHUS from severe ADAMTS13 deficiency (TTP) as no specific management for aHUS
- aHUS is a rare disease and thus unfamiliar to many care takers:
  - Leads to lack of clinical suspicion
  - Perception that diagnosis requires identification of genetic mutation

**Genetic Loss of Natural Inhibitors Leads to Chronic Uncontrolled Complement Activation**

- Complement-mediated thrombotic microangiopathy (TMA)
- Sudden death with vital organ damage
- Chronic progressive course with premature mortality
- 1 year mortality/dialysis/permanent renal damage >50% despite PEPI
- Manifests at all ages

**Differential Diagnosis for Thrombotic Microangiopathies (TMAs)**

- Thrombocytopenia
  - Platelet count <150,000
  - >30% Decreased form based on
- Microangiopathic Hemolytic Anemia
  - Decreased Hgb/Hct
  - Hematocrit decrement

- Plus One or More of the Following:

  - Neurological Symptoms
  - Renal Impairment
  - Gastrointestinal Symptoms

- Evaluate ADAMTS13 Activity and Shiga-toxin/EHEC Test

  - ADAMTS13 Activity
  - Shiga-toxin/EHEC Positive

- TTP
- aHUS
- STEC-HUS

Diagnostic Testing Recommended in patients presenting with clinical features of aHUS

- Serum levels of C3, C4, factor H, and factor I
- Measurement of MCP expression on PMBCs by FACS
- Mutation screening of CFH, CFI, CD46, CFB, C3, and THBD
- Screening for genomic disorders affecting CFH and CFHRs 1-5
- Screening for factor H autoantibodies
- Measurement of ADAMTS13 activity

- aHUS: Diagnosis Does Not Require Identification of a Genetic Mutation
  - Genetic mutation cannot be identified in 30%-50% of patients with aHUS
  - Absence of identifiable genetic mutations should not rule out aHUS

Current Therapeutic Options

- Plasma exchange and/or plasma infusions
  - “removal/replacement of bad humors”
- Immunosuppressive agents +/- plasma exchange
  - Corticosteroids, azathioprine, mycophenolate mofetil
  - Rituximab (anti-CD20)
- Renal Transplantation
- Anti-C5 monoclonal antibody (eculizumab)

...But do they truly affect the underlying physiology?

Genetic Abnormalities and Clinical Outcomes

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<tr>
<td>THBD</td>
<td>Factor H</td>
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Eculizumab

Greenbaum L et al
- 17 pts enrolled >12 yrs despite >4 PE/PIT sessions
- 5 pts required dialysis at baseline
- All pts had platelet normalization at wk 26
- 4/5 pts became dialysis free
- No progression of disease to require new dialysis

Licht C et al
- 20 pts enrolled >12 yrs
- 7 pts improved >1 CKD level from baseline by week 26
- 9 pts improved >1 CKD level from baseline by week 60
- No progression of disease or new dialysis requirement

aHUS is Not an Acute Disease

- Due to a genetic deficiency of complement regulators, aHUS is a permanent, ongoing, life-long disease of systemic, complement-mediated TMA
- Complement-mediated TMA results in organ damage:
  - Sudden and catastrophic – leads to rapid loss of vital organs and sudden death
  - Progressive worsening of vital organ function – leads to vital organ failure and pre-mature death

aHUS is Not Only a Renal Disease as it Often Results in Damage or Failure of Other Vital Organs

- Cardiovascular
  - Myocardial infarction
  - Thrombosis
  - Endocarditis
  - Arterial vasculopathy
- Renal
  - Dialysis, transplant
- Pulmonary
  - Dyspnea
  - Pulmonary oedema
  - Pulmonary embolism
- Blood
  - Neutropenia
  - Complement depletion
  - Thrombosis
- CNS
  - Confusion
  - Seizures
  - Strokes
  - Encephalopathy
- Gastrointestinal
  - Vomiting
  - Acute pancreatitis
  - Colitis, diarrhoea
  - Liver necrosis
- Immunological
  - Fatigue
  - Hypertension
  - Reduced mobility

aHUS: Advancing the Understanding of Disease

- aHUS affects all ages and is not only a pediatric disease
- aHUS is not an acute but chronic, genetic & life-long disease
- Diagnosis does NOT require identifiable genetic mutation
- aHUS is not only a renal disease but affects all organs
- Plasma exchange/infusion has not been proven in well-controlled trials to be safe or effective
  - Leaves >50% of patients with death, dialysis or permanent renal damage within 1 year of diagnosis

Eculizumab

- Anti-C5 monoclonal antibody
  - Binds to the complement protein C5 inhibiting its cleavage to C5a and C5b preventing the formation of the membrane attack complex (MAC), C5b-9
- Biologic immunosuppressant
  - Patients must receive meningococcal vaccine 2 weeks prior to infusion
- Requires infusions every 1-2 weeks
  - Typically in aHUS lifelong therapy may be indicated. PNH patients may consider tailored therapy based on symptoms and evidence of clonal population
  - Eculizumab is expensive (~$400,000/year in the United States)
- Investigational uses in cold agglutinin disease and antibody mediated kidney transplant rejection