PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

Rare but Real

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Outline

- Discus symptoms and complications of PNH
- Discuss pathophysiology of PNH
- Identify clinical categories of PNH
- Identify high risk patients
- Discuss laboratory diagnosis for PNH
- Overview of management of PNH

Paroxysmal Nocturnal Hemoglobinuria (PNH)

- Rare benign clonal acquired hematopoietic stem-cell (HSC) disorder
- Somatic mutation of X-linked phosphatidylinositol glycan class A (*PIGA*) gene
- Can arise *de novo* or in the setting of acquired bone marrow (BM) failure
- Product of *PIGA* gene is required for synthesis of anchor protein that ties other proteins to the cell surface known as glycosylphosphatidylinositol (GPI-anchor)
- Two GPI-anchored proteins (CD55&CD59) normally function as complement regulatory proteins

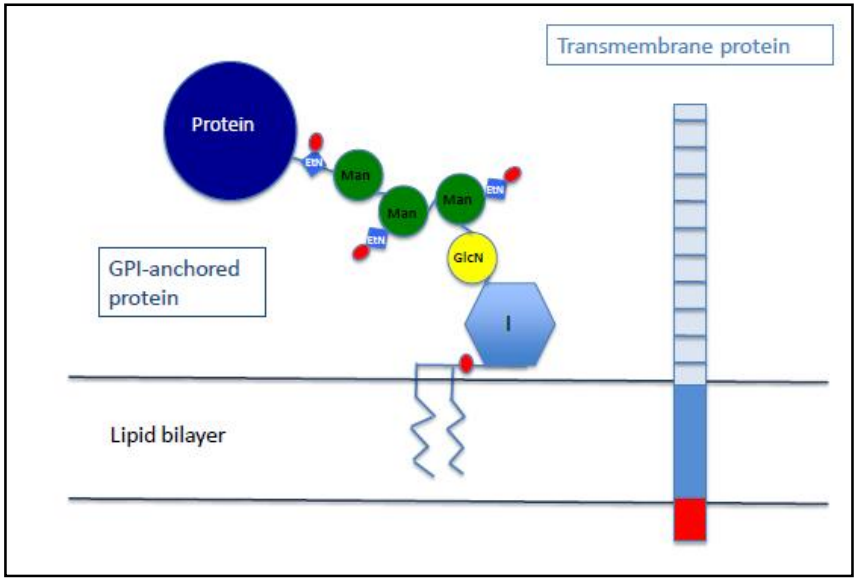
CD59

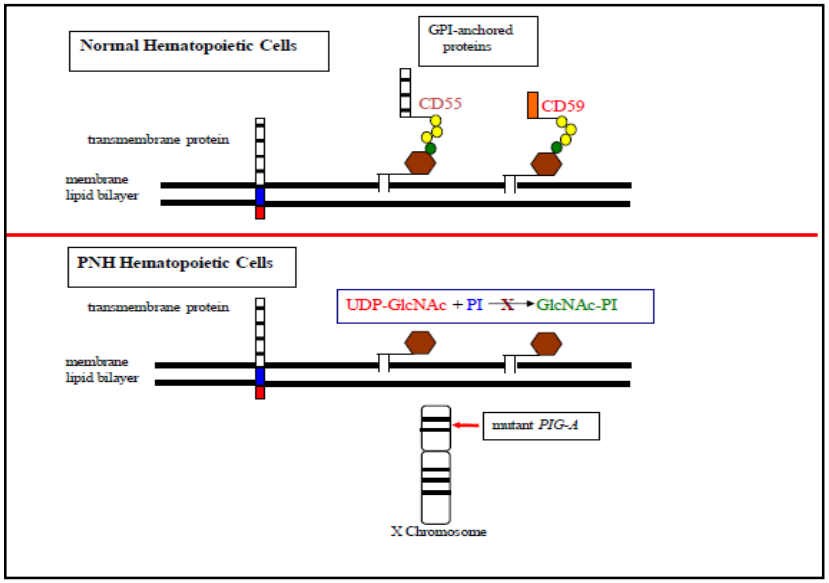
- Membrane inhibitor of reactive lysis (MIRL)
- Forms defensive shield for RBCs
- Inhibits the assembly of the membrane attack complex

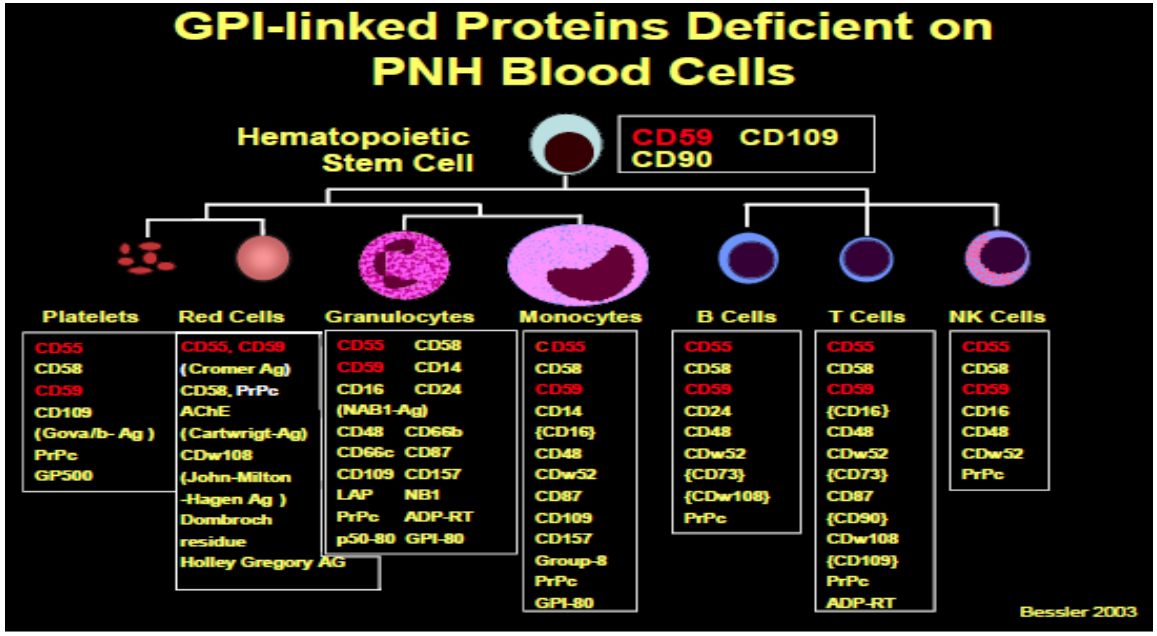
CD55

- Decay accelerating factor (DAF)
- Prevents formation and augments instability of C3 convertase

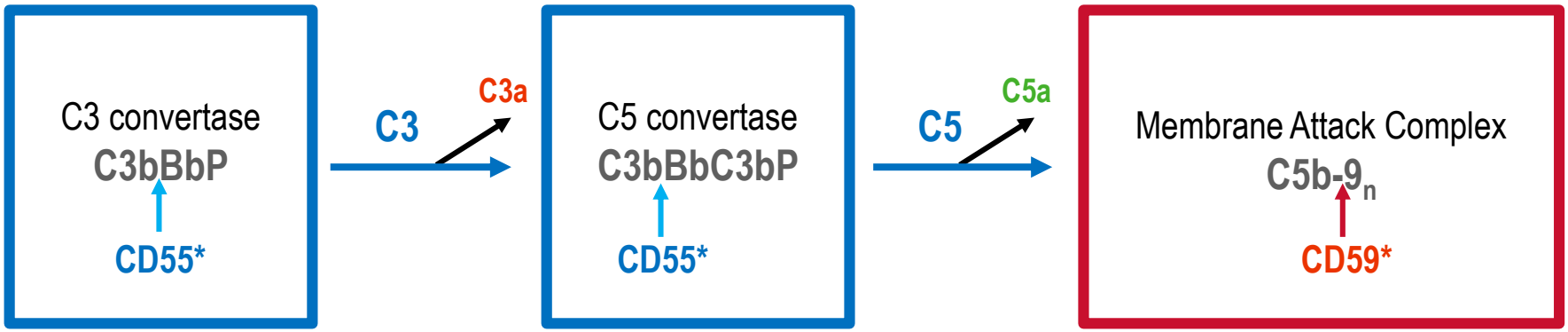
PNH


Transmembrane protein
Protein
EtN
Man
Man
Man
EtN
EtN
GlcN
I
GPI-anchored protein
Lipid bilayer


Normal Hematopoietic Cells
GPI-anchored proteins
CD55
CD59
transmembrane protein
membrane lipid bilayer
PNH Hematopoietic Cells
transmembrane protein
UDP-GlcNAc + PI
GlcNAc-PI
membrane lipid bilayer
mutant PIG-A
X Chromosome


GPI-linked Proteins Deficient on PNH Blood Cells
Hematopoietic Stem Cell
CD59 CD109 CD90
Platelets
CD55
CD58
CD59
CD109
(Gova/b- Ag)
PrPc
GP500
Red Cells
CD55, CD59
(Cromer Ag)
CD58, PrPc
AChE
(Cartwrigt-Ag)
CDw108
(John-Milton -Hagen Ag)
Dombroch residue
Holley Gregory AG
Granulocytes
CD55 CD58
CD59 CD14
CD16 CD24
(NAB1-Ag)
CD48 CD66b
CD66c CD87
CD109 CD157
LAP NB1
PrPc ADP-RT
p50-80 GPI-80
Monocytes
CD55
CD58
CD59
CD14
{CD16}
CD48
CDw52
CD87
CD109
CD157
Group-8
PrPc
GPI-80
B Cells
CD55
CD58
CD59
CD24
CD48
CDw52
{CD73}
{CDw108}
PrPc
T Cells
CD55
CD58
CD59
{CD16}
CD48
CDw52
{CD73}
CD87
{CD90}
CDw108
{CD109}
PrPc
ADP-RT
NK Cells
CD55
CD58
CD59
CD16
CD48
CDw52
PrPc
Bessler 2003

Alternative Pathway of Complement



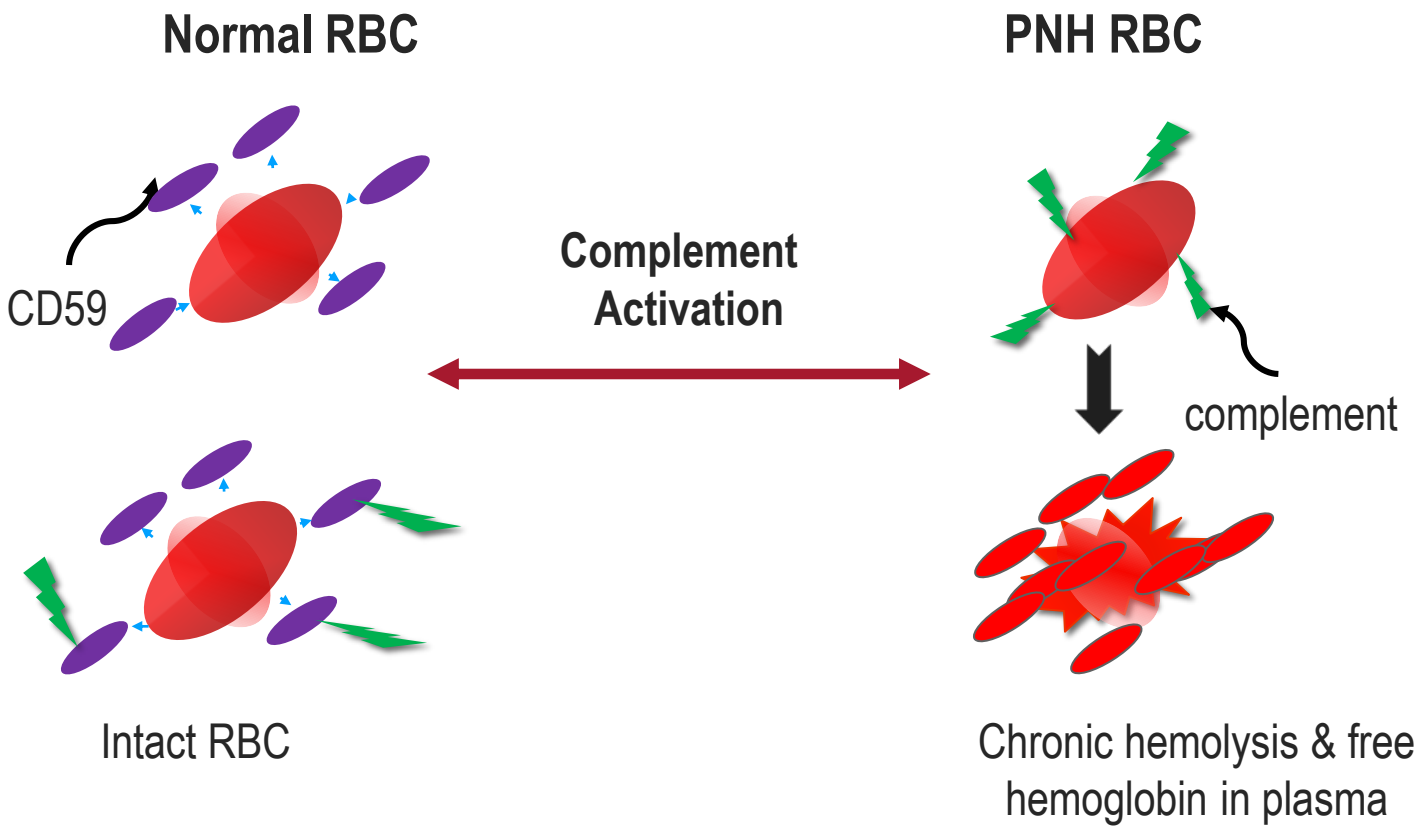
*GPI-anchored complement regulatory proteins deficient in PNH

Charles J. Parker Hematology 2011;2011:21–29

Paroxysmal Nocturnal Hemoglobinuria (PNH)

- Deficiency can be partial or complete
- Seen in WBCs and RBCs
- Characterized by continuous destruction of PNH RBCs due to vulnerability to complement mediated lysis

RBCs Susceptible to Lysis by Terminal Complement Activation



PNH

Paroxysmal

Destructive progressive ongoing hemolysis even in the absence of symptoms

Nocturnal

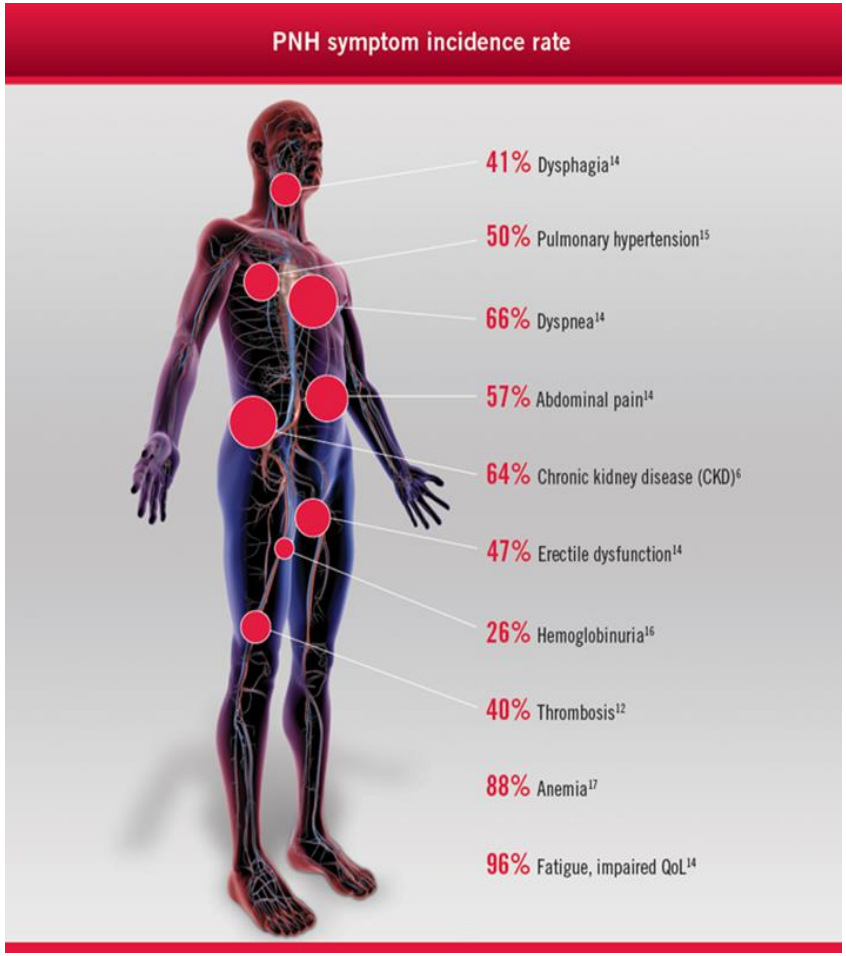
Hemolysis in PNH is subtle and constant 24 hours a day

Hemoglobinuria

Is a less commonly seen complication ~ 75% of patients present without hemoglobinuria

Clinical Manifestations of PNH

- Fatigue, impaired quality of life
- Anemia
- Dyspnea
- Chronic kidney disease
- Abdominal pain
- Pulmonary hypertension
- Erectile dysfunction
- Dysphagia
- Thrombosis
- Hemoglobinuria
- Bone marrow failure



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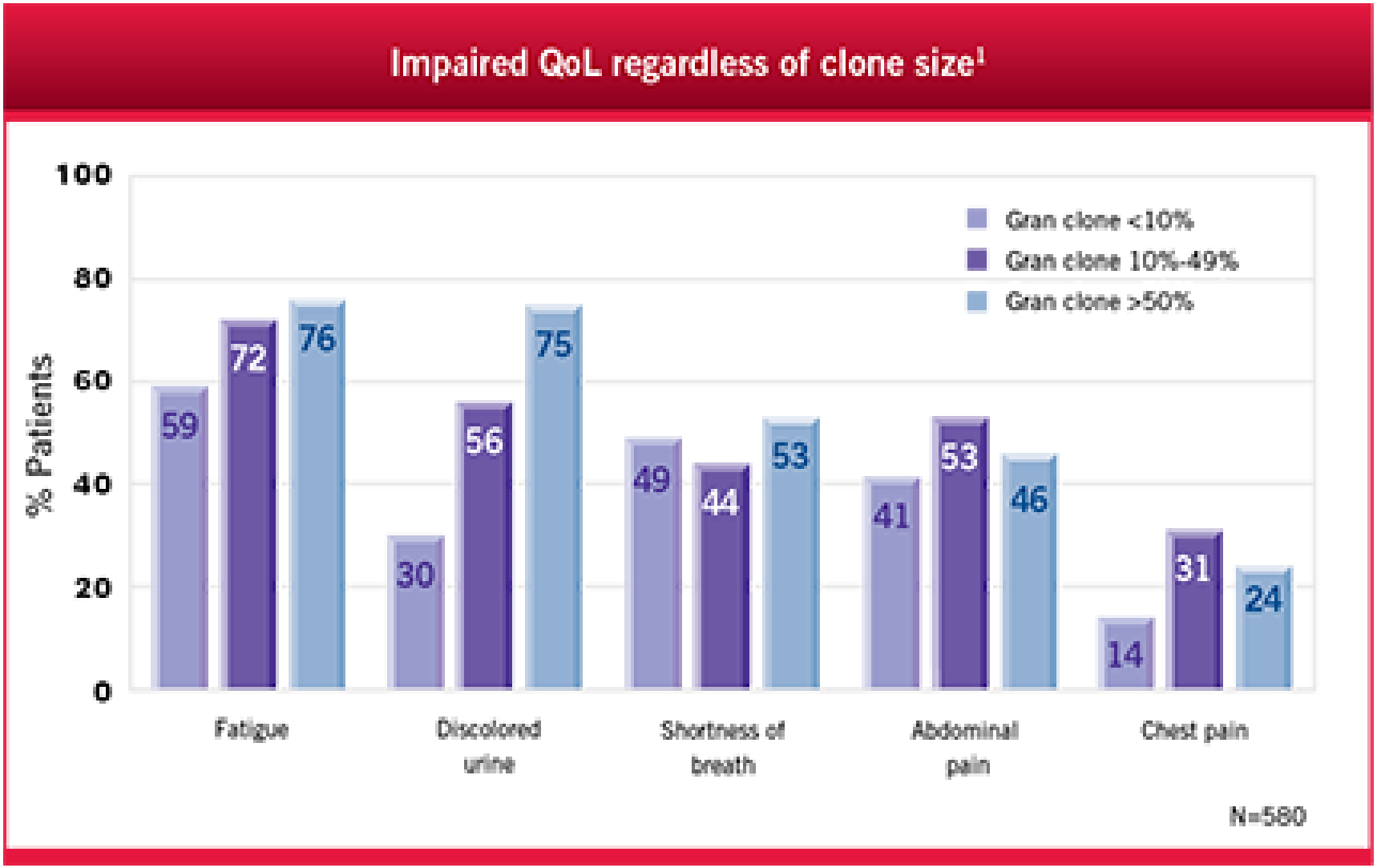
PNH easy to miss. Impossible to ignore



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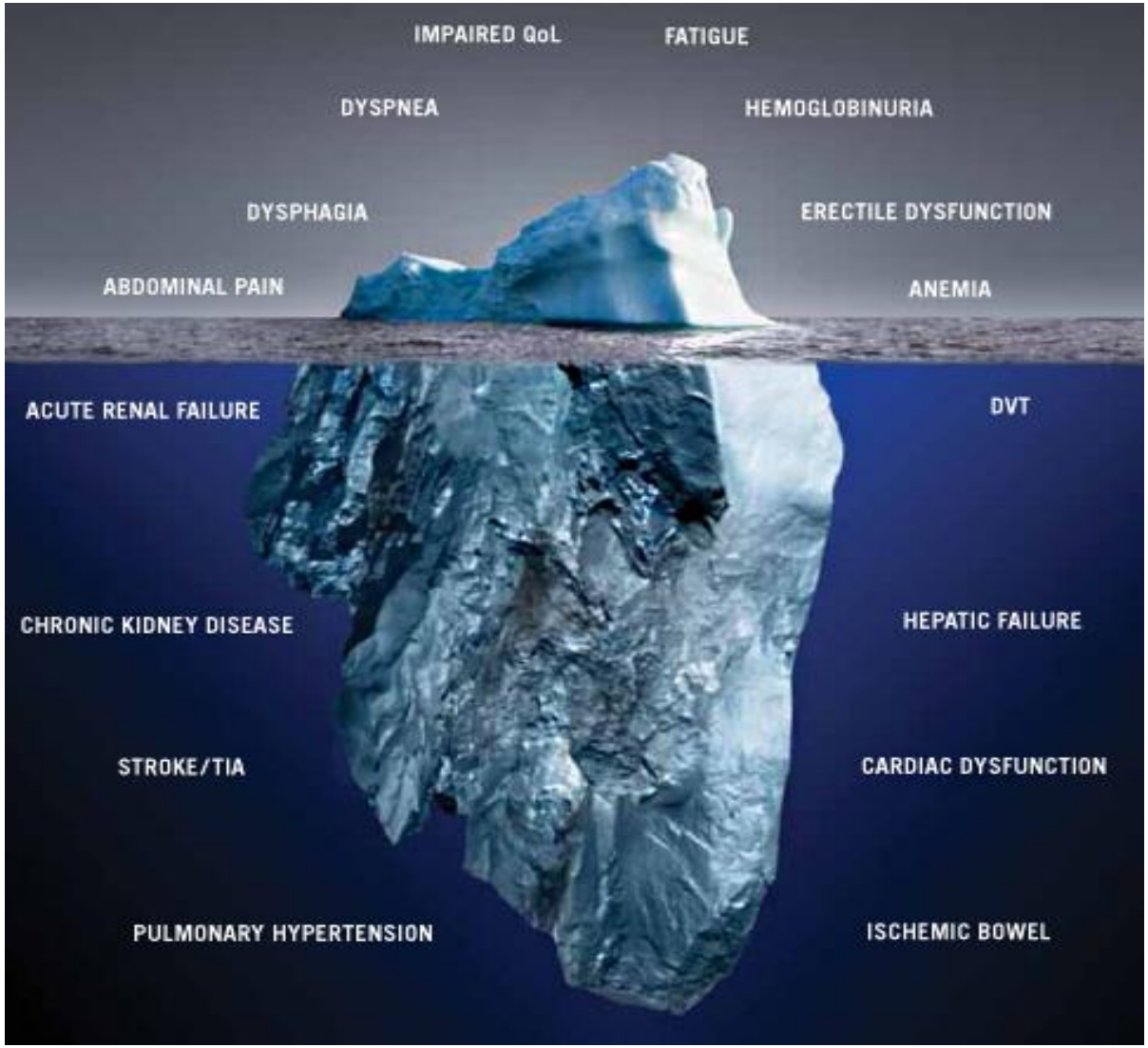
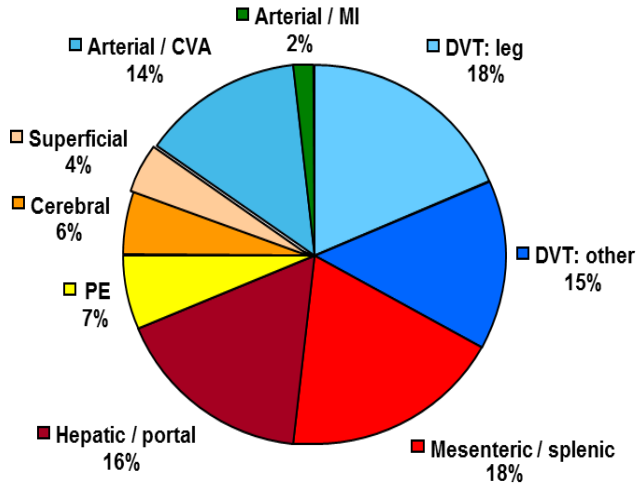
what you can't see can hurt you the most..



Pathophysiology of PNH

- **Hemolysis**
  - Complement activation
- **Chronic kidney disease**
  - Toxicity of free hemoglobin and iron with extensive hemoglobin deposition
- **Esophageal spasm, abdominal pain, pulmonary hypertension, fatigue and smooth muscle dystonia**
  - Nitric oxide scavenging
- **Thrombosis**
  - Nitric oxide depletion
  - Disrupted fibrinolysis
  - Disrupted tissue factor inhibitor pathway
- **Bone marrow failure**
  - PNH is closely related to bone marrow failure syndromes
  - Mutant HSC exhibit a survival advantage and tend to expand leading to hemolysis



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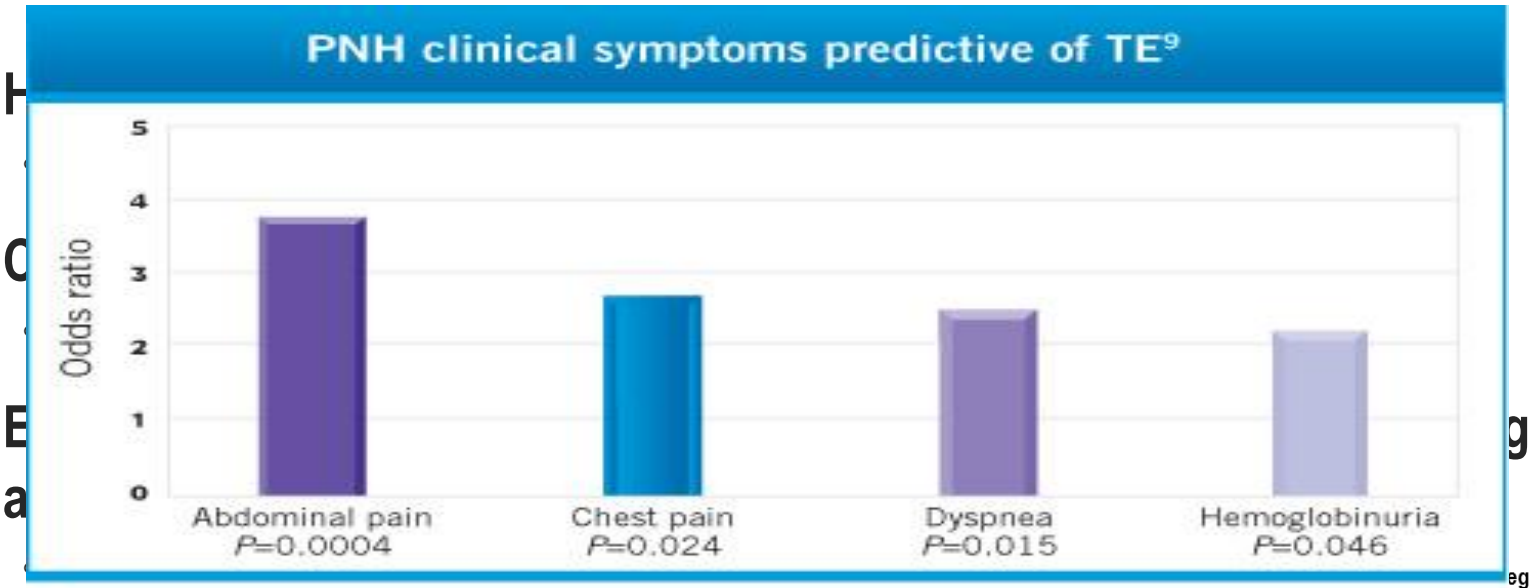

- H
- C
- E gue a

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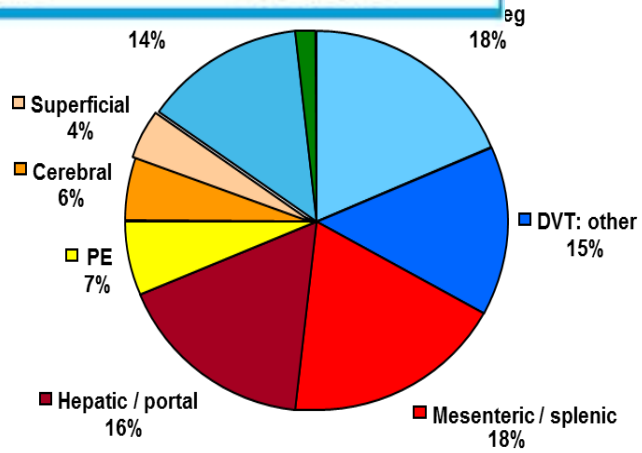

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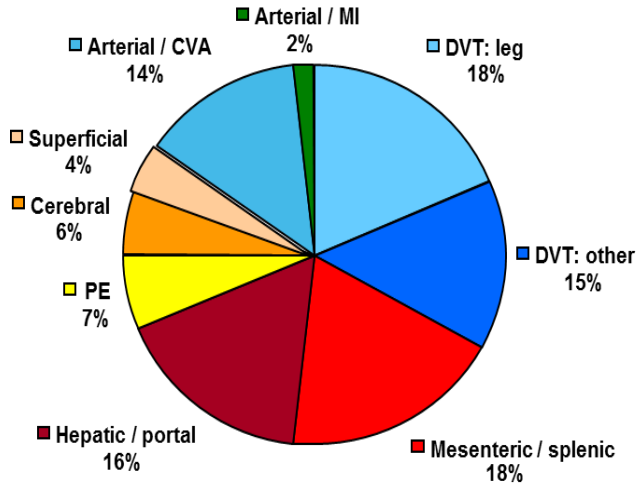


Clinical Categories of PNH

1. **Classical PNH, includes hemolytic and thrombotic patients**
   - Marked hemolysis
   - Hemoglobinuria
   - Lactate dehydrogenase (LDH)
   - Normal BM with erythroid hyperplasia
   - PNH clone >50%

2. **PNH in the setting of BM failure syndromes**
   - Mild hemolysis
   - Minimal abnormality in biochemical markers of hemolysis
   - BM shows the concomitant BM failure
   - PNH clone usually small (<10%)

3. **Subclinical PNH**
   - No clinical or biochemical evidence of intravascular hemolysis
   - BM shows concomitant BM failure
   - Small PNH clone (<1%)

Early Diagnosis is Essential for Improved Patient Management and Prognosis

International Clinical Cytometry Society (ICCS) Guidelines and International PNH Interest Group (IPIG) recommend evaluation of high risk patients:

- Coombs negative hemolytic anemia (22.7%)
- Hemoglobinuria (18.9%)
- Aplastic anemia (AA) (26.3%)
- Refractory anemia-myelodysplastic syndrome (RA-MDS) (17%)
- Unexplained venous or arterial thrombosis (1.4%)
- Unexplained cytopenia (5.7%)

PNH Clone in Patients with AA

- Aplastic anemia: disease where the BM stops making RBCs, WBCs, and platelets
- PNH clone present in 40-50% of patients with severe AA
- PNH clone size in patients with AA may increase rapidly and unpredictably
- Presence of PNH clone in severe AA is associated with low morbidity and mortality, and reported to be predictive of response to immunosuppressive therapy
- Clone size often decreases after immunosuppressive therapy

PNH Clone in Patients with MDS

- Myelodysplastic syndrome: group of diverse BM disorders where BM does not produce enough healthy blood cells
- More than 1 out of 18 patients with MDS have PNH clone
- Most studies showed that PNH clones were only present in patients with RA-MDS
- PNH clone in other categories of MDS has been reported in limited number of studies
- RA-MDS patients with detectable PNH clone have more indolent clinical course

Diagnosis of PNH

- Sucrose hemolysis test and Ham test, both show increased sensitivity of PNH RBCs to complement-mediated hemolysis under standard conditions (lows specificity and sensitivity, miss small populations)

- Complement lysis sensitivity, measures the amount of hemolysis at different concentrations of complement (laborious, difficult to standardize, miss small populations)

- Mutation analysis can provide final confirmation, however mutations are common in normal individuals which are usually polyclonal and occur in differentiated progenitors

Diagnosis of PNH

- Flow-cytometry performed on peripheral blood is the established method of choice for the diagnosis and monitoring of PNH

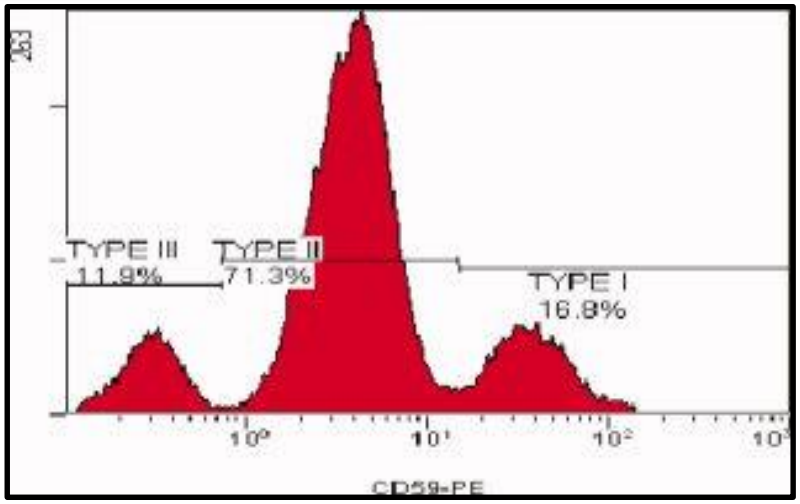
- Both RBCs and WBCs should be tested
  - WBC PNH clone can sometimes be detected in the absence of a RBC clone
  - A significant RBC PNH clone is always associated with a WBC clone
  - Explained by the fact that RBC clone size may be affected by hemolysis or transfusion

RBC Analysis

- Identify and quantify cells lacking expression of CD59 or CD55 (Type III)
- Identify and quantify cells partially deficient (Type II) if present
- Glycophorin-A (CD235a): lineage marker used to gate on RBCs
- CD59 is superior over CD55

RBC Analysis

A



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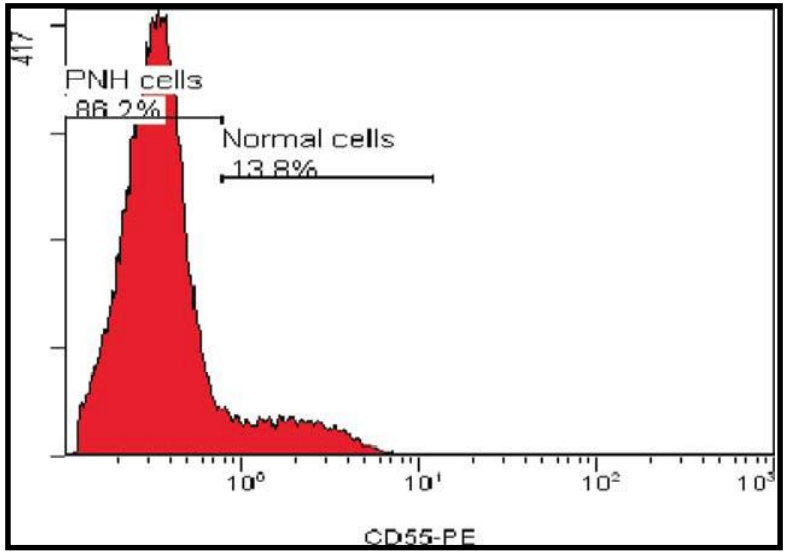

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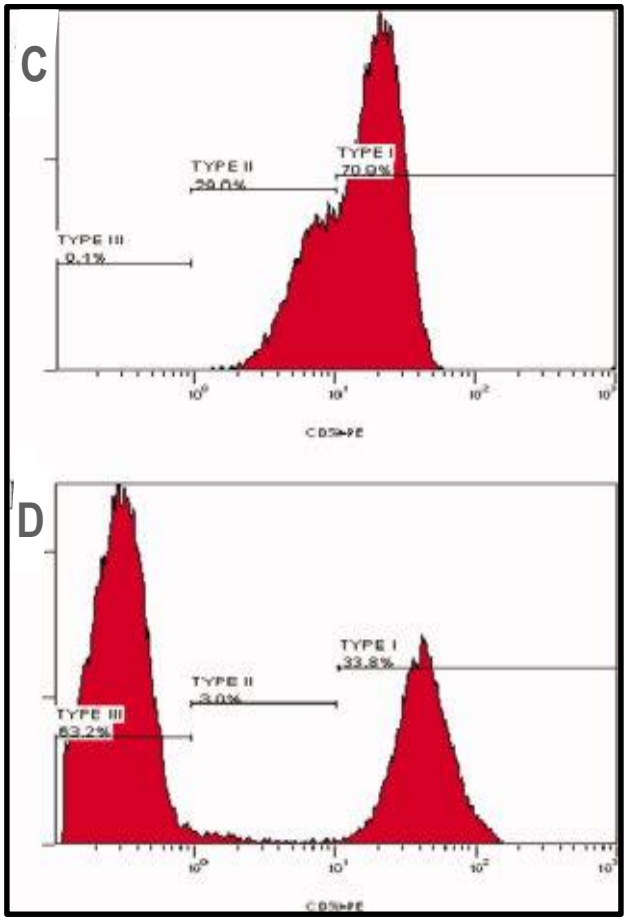

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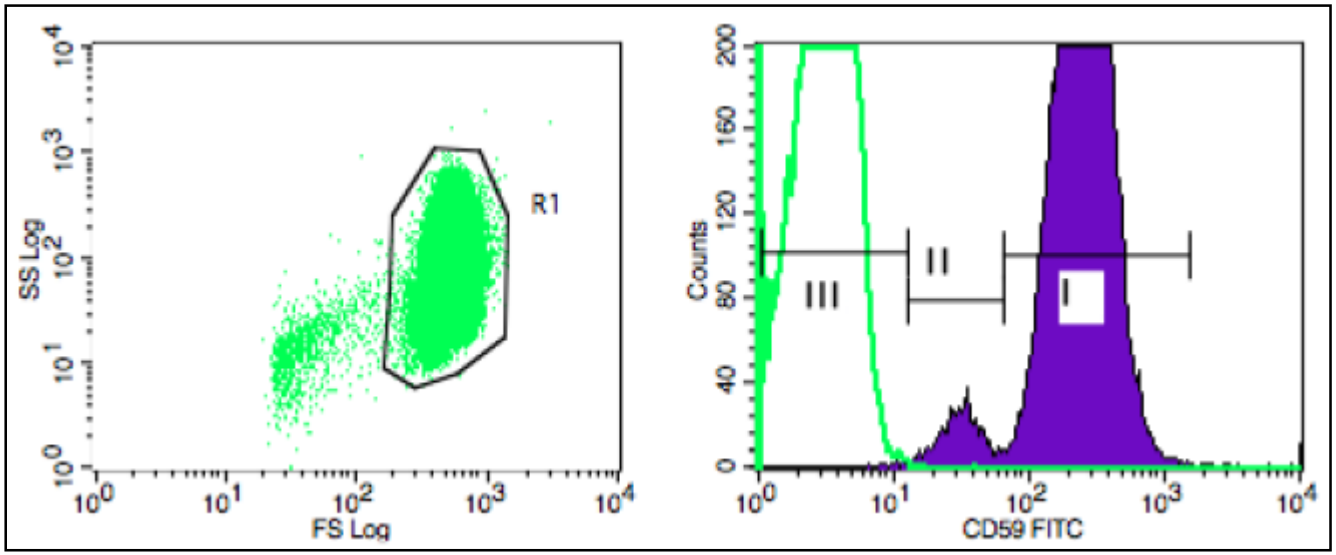
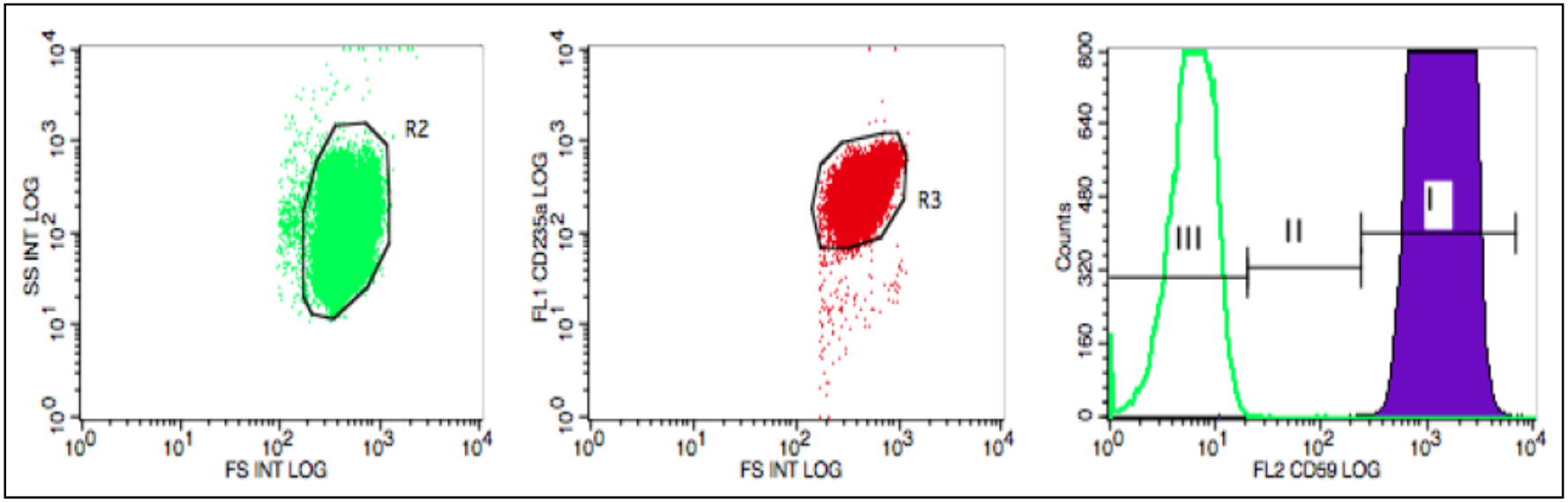
RBC Analysis

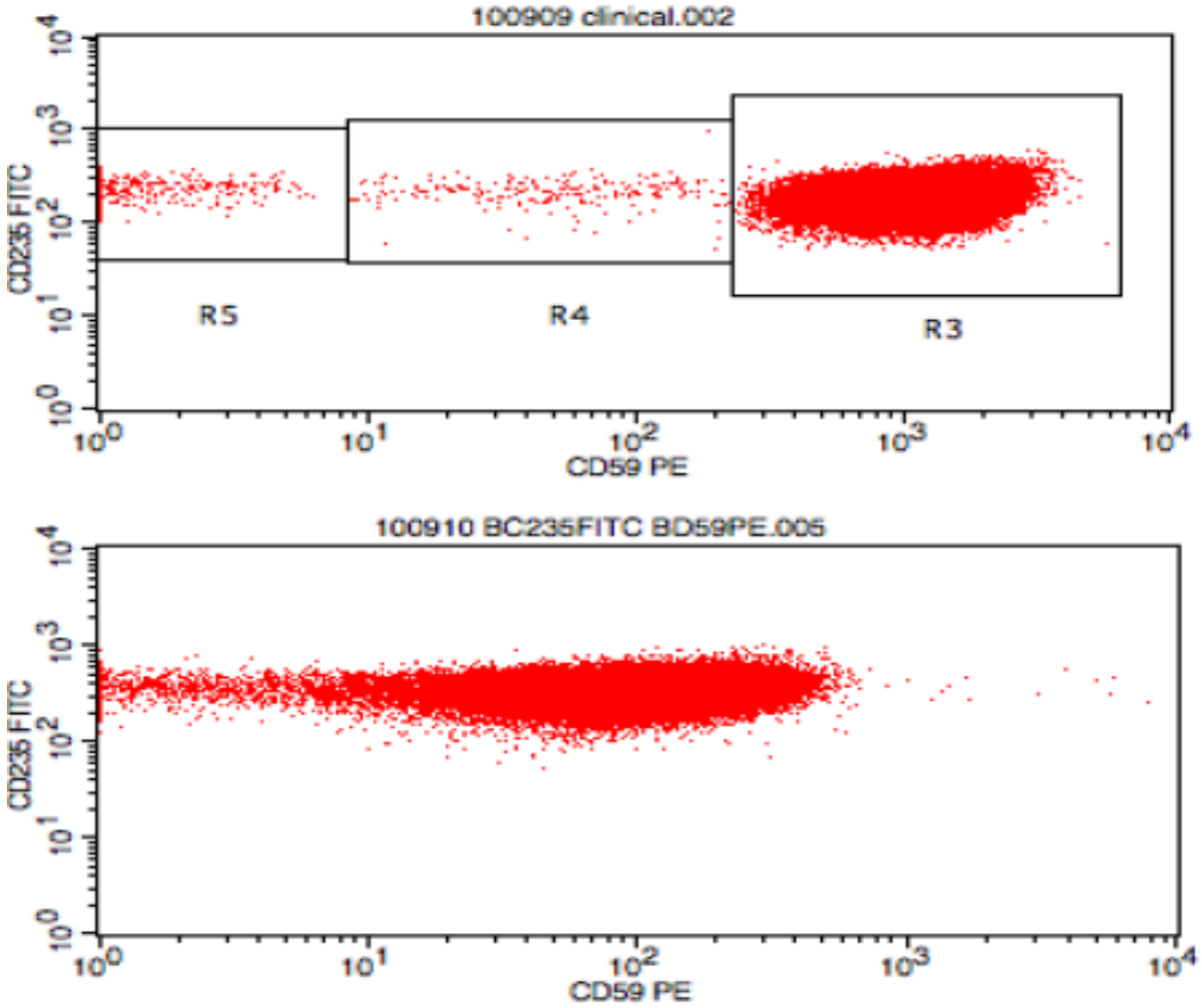


Type 1=95.2
Type II=4.72
Type III =0.02%



Sutherland DR et al. *Cytometry Part* B 2012;82B:195–208

RBC Analysis



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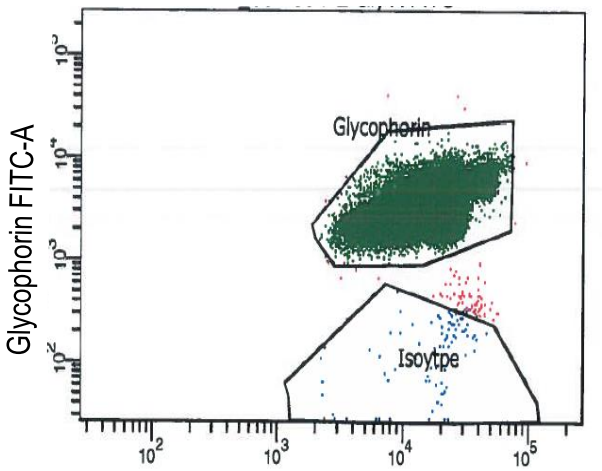
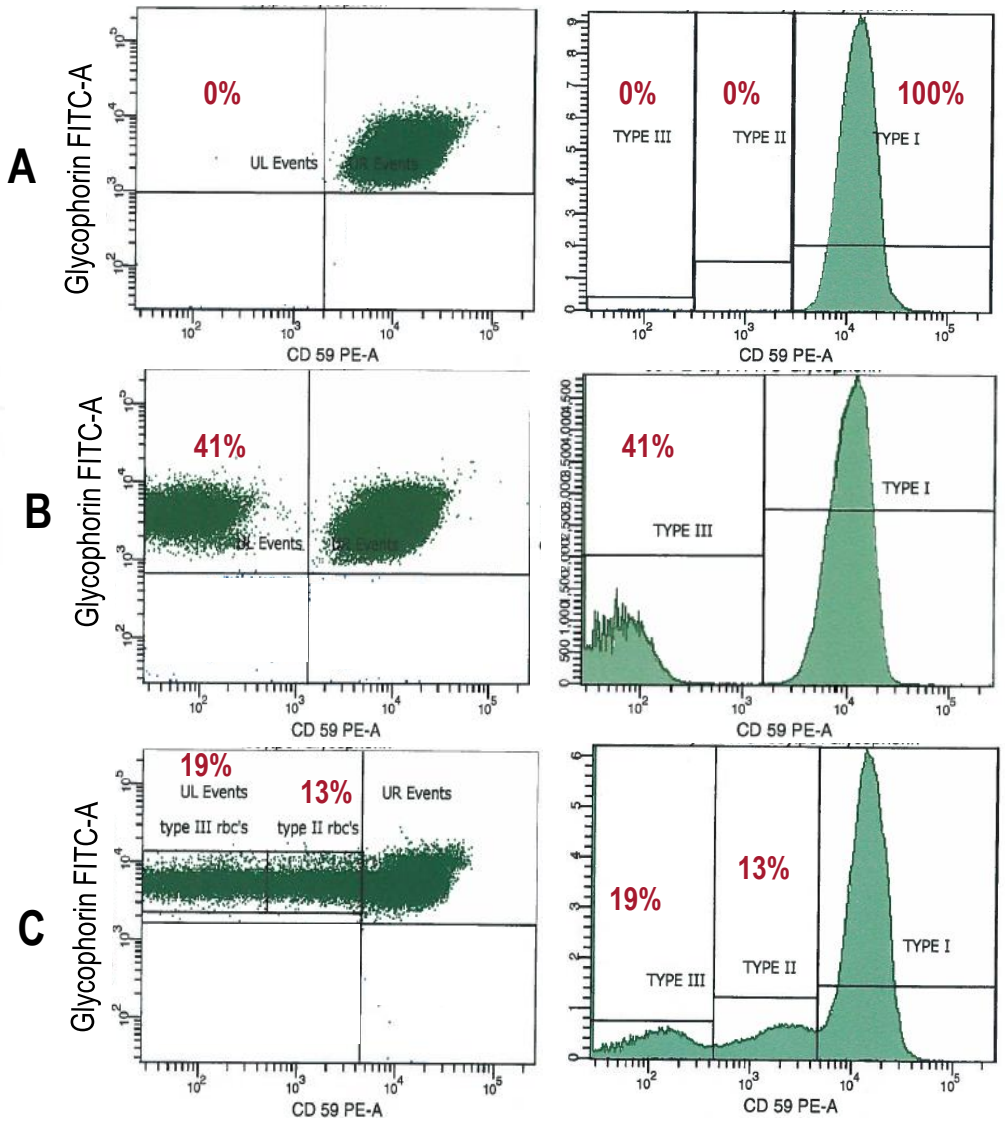
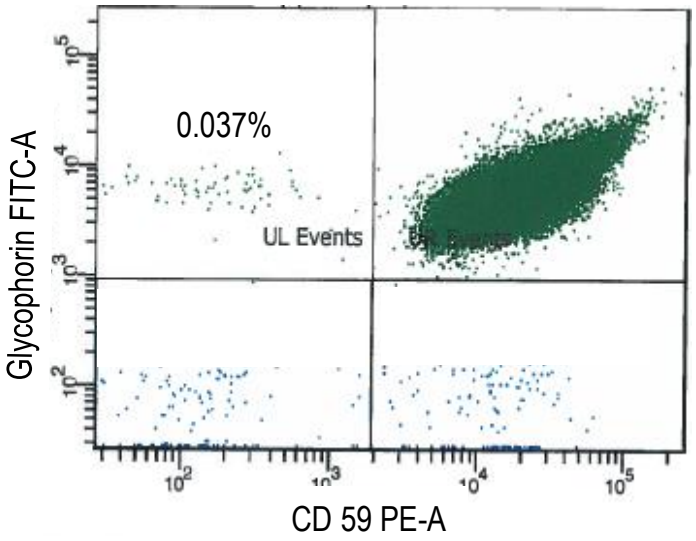
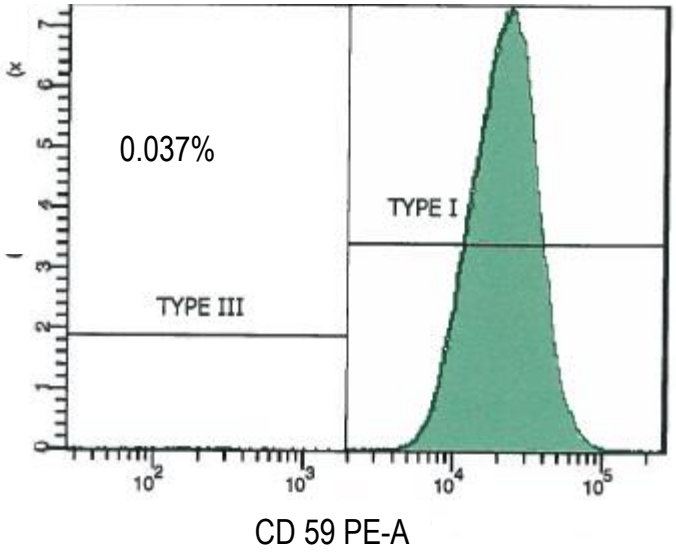




High Sensitivity RBC Analysis

- Count more RBCs for small PNH clone
- Sensitivity of 0.005 % for RBCs is achievable





WBC Analysis

Granulocytes

- Most commonly used to assess PNH clone size
- Occasionally Type II granulocytes can be detected

Monocytes

- Analyzed to confirm the granulocyte PNH clone
- Monocytes clone size often higher than granulocyte clone
- Sensitivity and precision is lower due to lower cell number
- Occasionally Type II can be detected

WBC Analysis

- **Lineage specific gating for higher sensitivity**
  - CD15 to gate on granulocytes
  - CD64 or CD33 to gate on monocytes

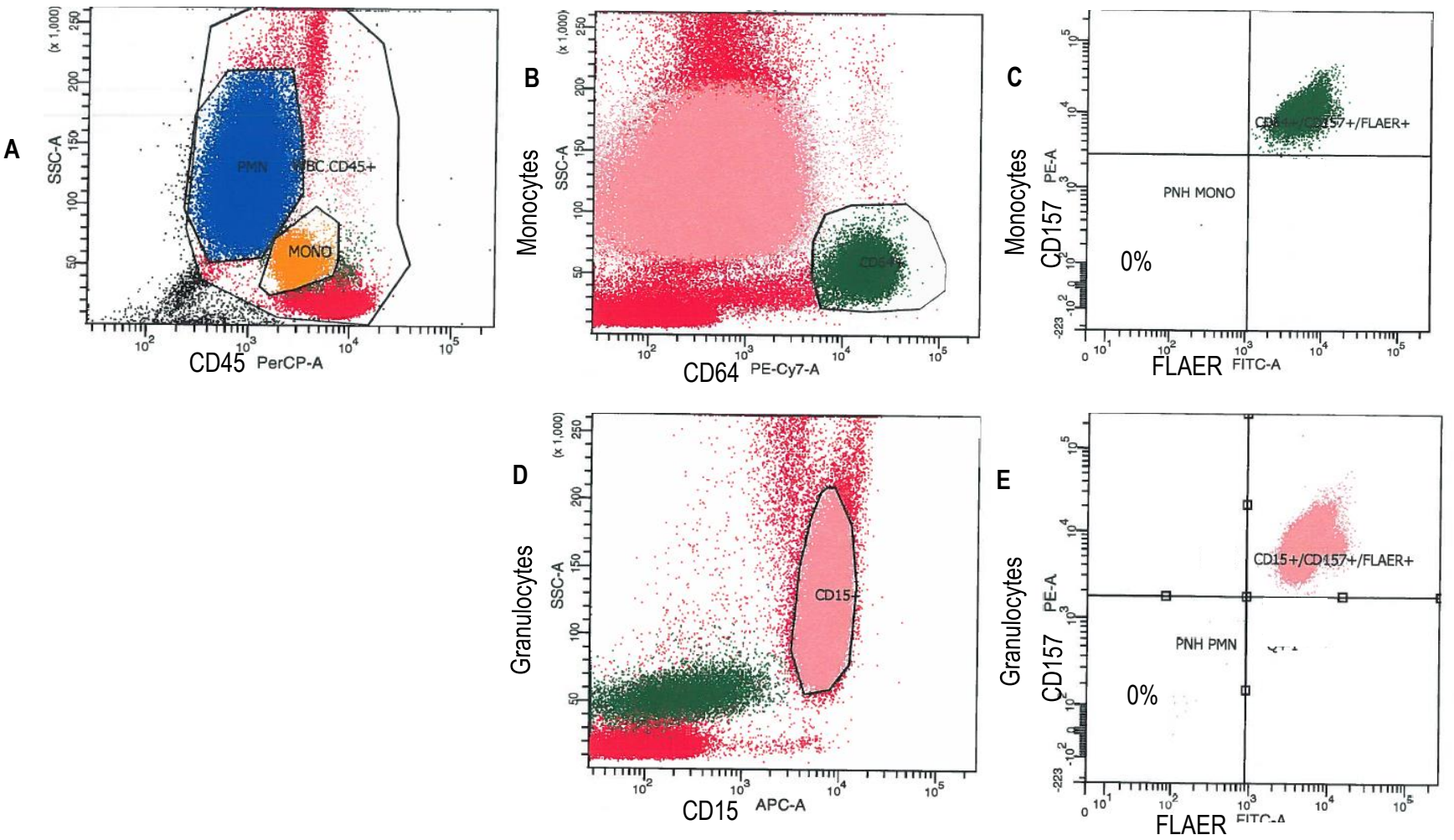
- **Assess two GPI linked proteins on each cell population**
  - CD24 or CD157 and FLAER are evaluated on granulocytes
  - CD14 or CD157 and FLAER are evaluated on monocytes

- FLAER (fluorescein-labeled pro-aerolysin) is a flourochrome conjugated inactive bacterially derived channel forming protein; binds specifically to GPI anchors

High Sensitivity WBC Analysis

- Useful for the diagnosis of subclinical PNH associated with BM failure disorders
- Not needed for the diagnosis of classic PNH
- Sensitivity of 0.01% for WBC is achievable
- Acquisition of sufficient events, evaluation of multiple parameters and assessing frequency of PNH cells in normal samples are critical to limit false positives

WBC Analysis



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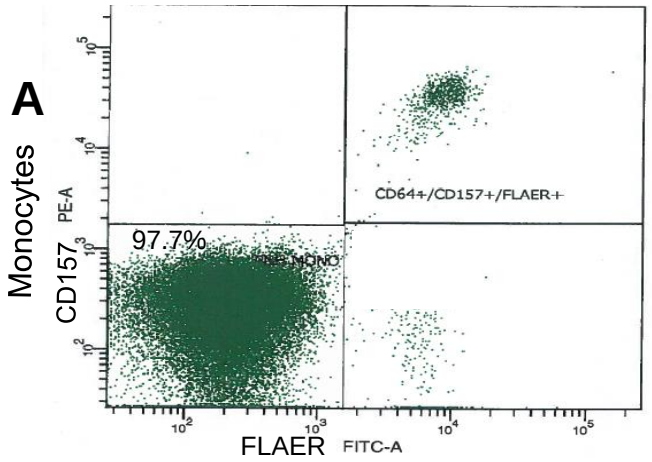

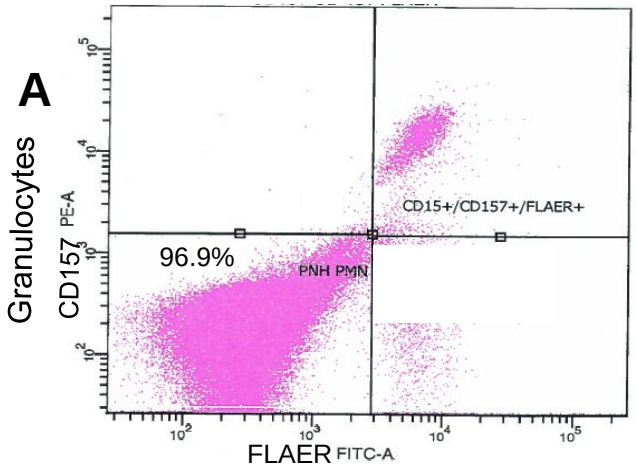

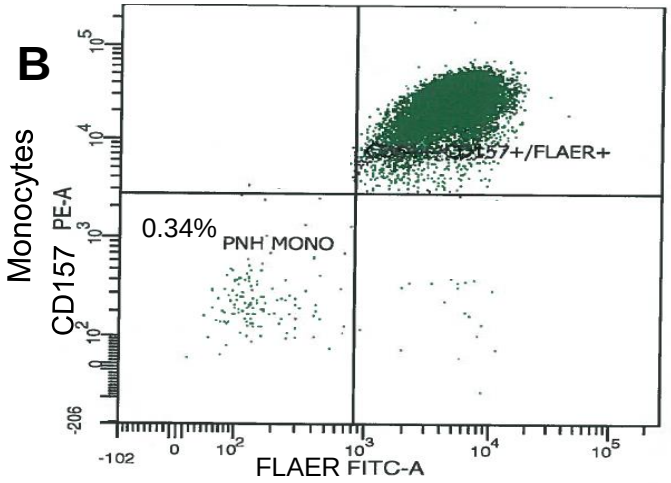

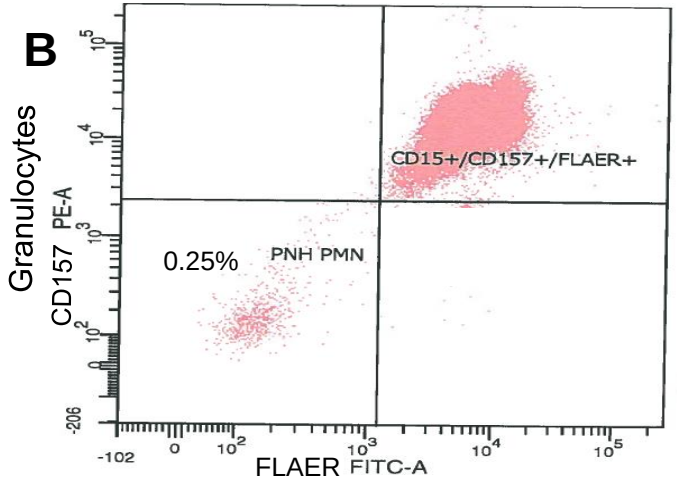

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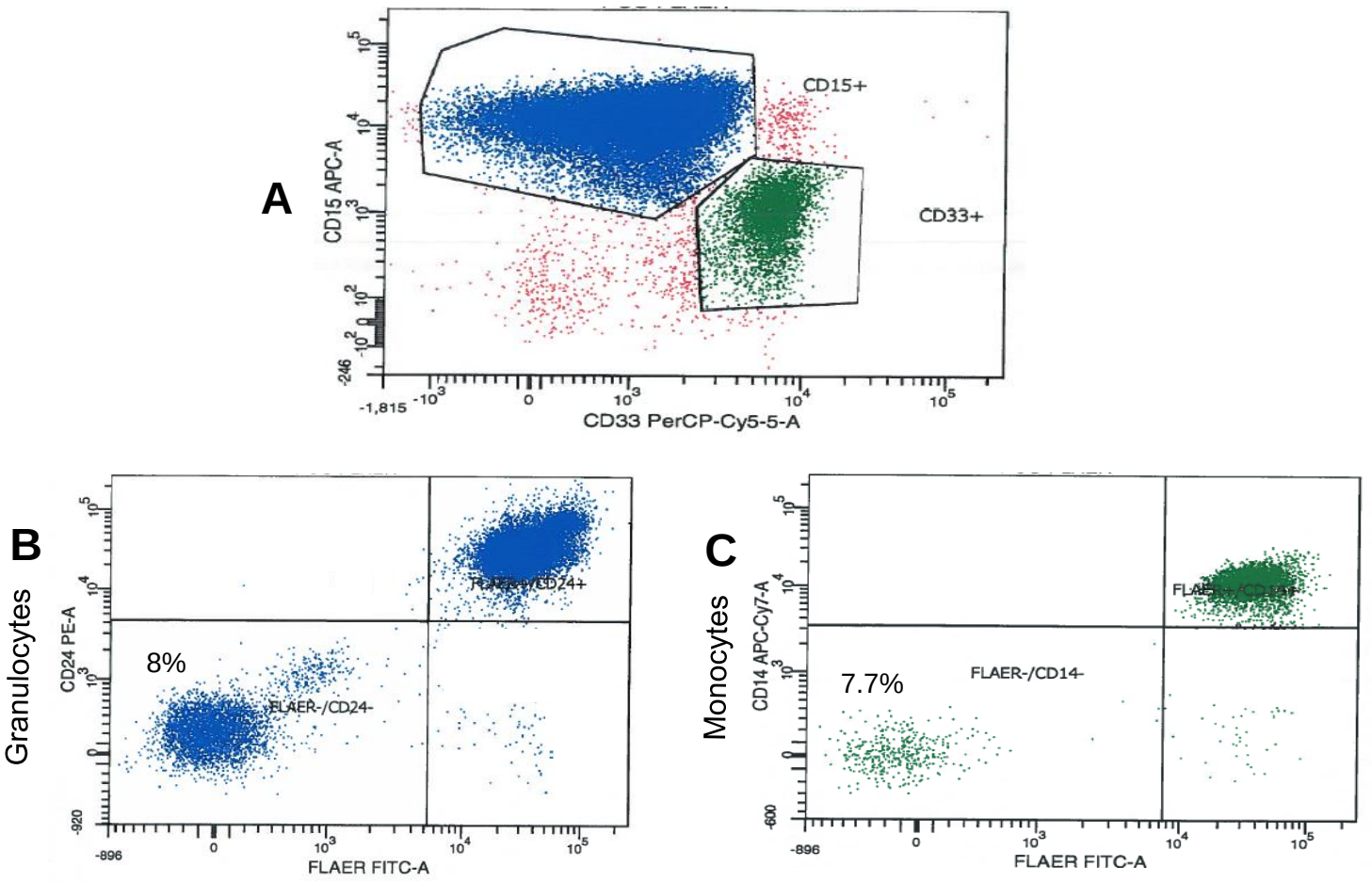
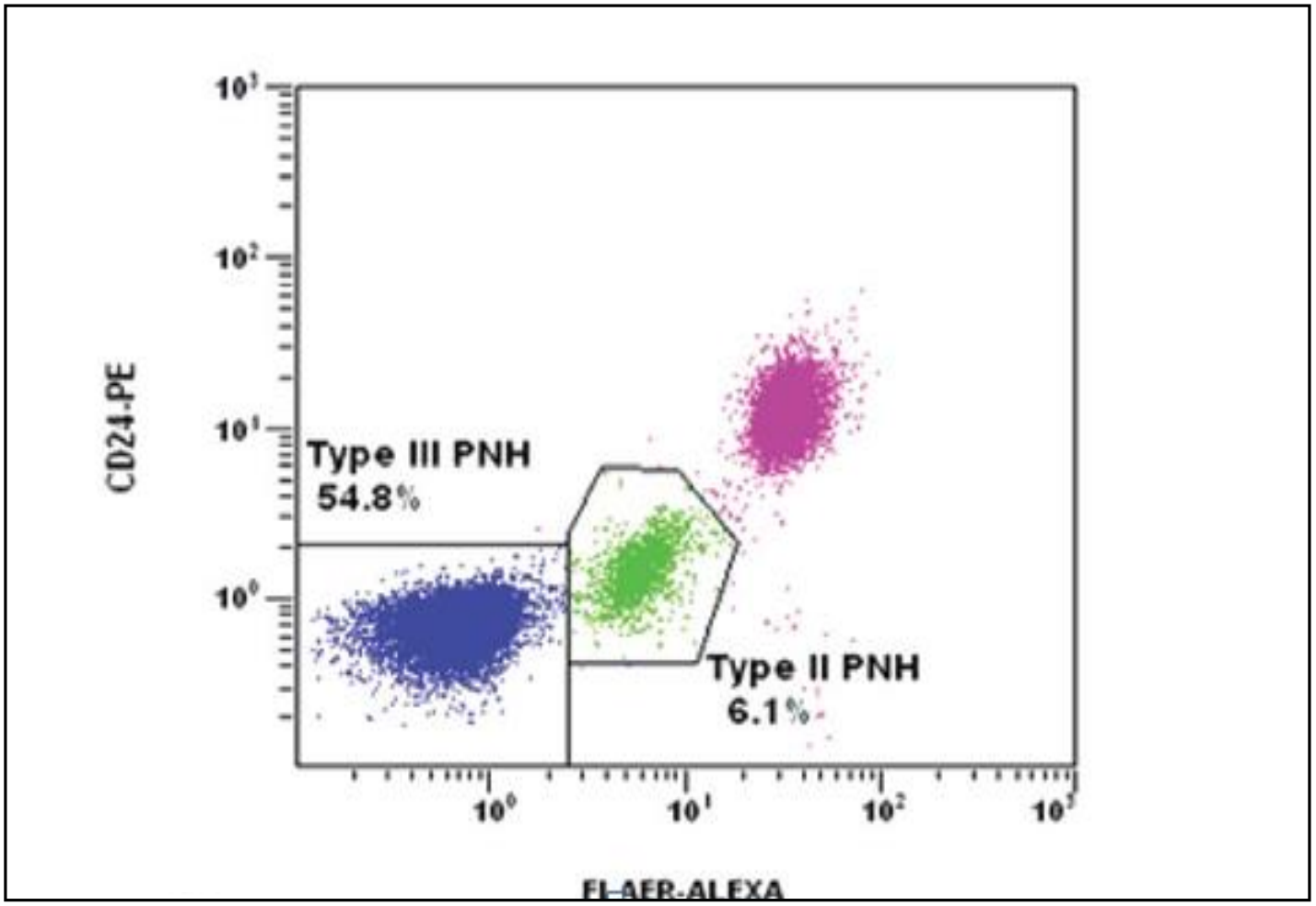
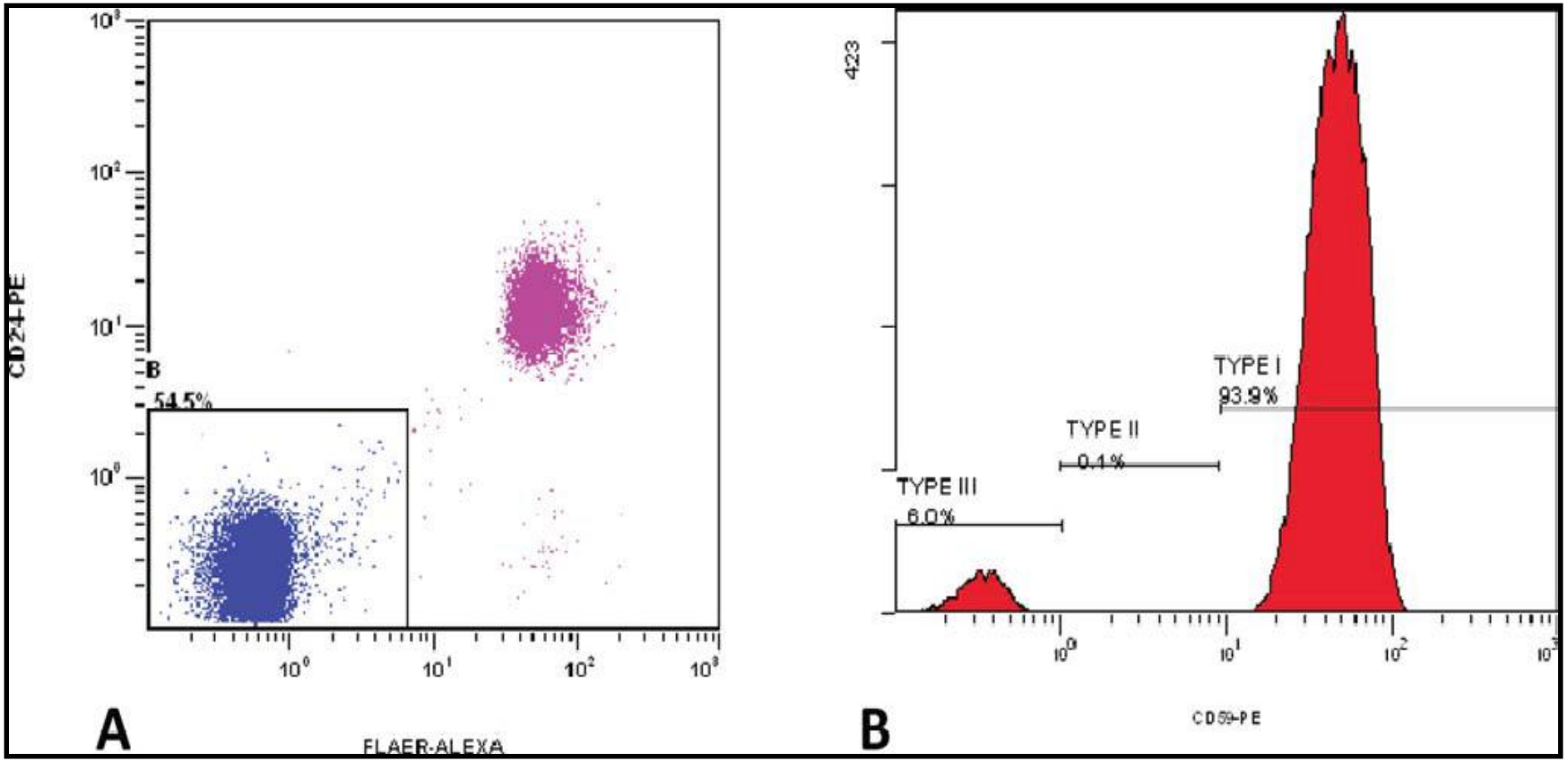


WBC Analysis



Michael J. Borowitz et al. Cytometry Part B (Clinical Cytometry) 78B:211–230 (2010)

WBC/RBC Analysis



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Treatment of PNH

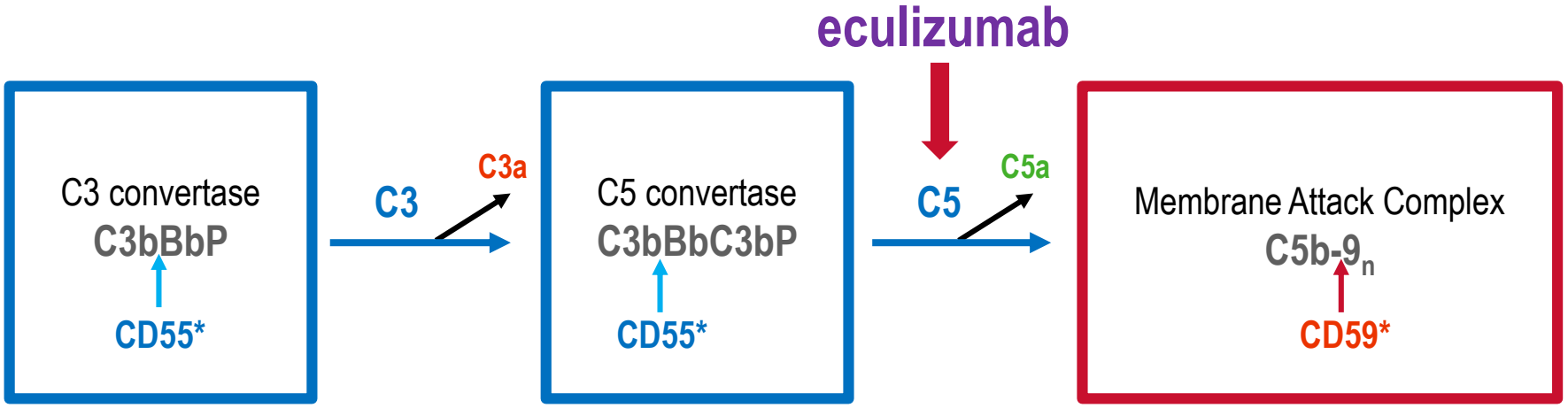
- Folic acid supplementation and supportive care for patients with minimal symptoms
- Blood transfusion
- Steroids
- Prophylactic anticoagulation: not been proven to decrease risk of thrombosis
- Allogenic BM transplantation
- Eculizumab (Soliris)

Treatment of PNH

Eculizumab

- Humanized monoclonal antibody that effectively blocks complement activation at C5 that inhibits terminal complement activation
- Stops hemolysis and related effects
- Markedly reduces transfusion requirements
- Patients with subclinical clones are not candidates for treatment with eculizumab
- Blocking the terminal portion of complement predisposes to *Neisseria*
- Expensive
- Must be given IV every 12-14 days

Alternative Pathway of Complement



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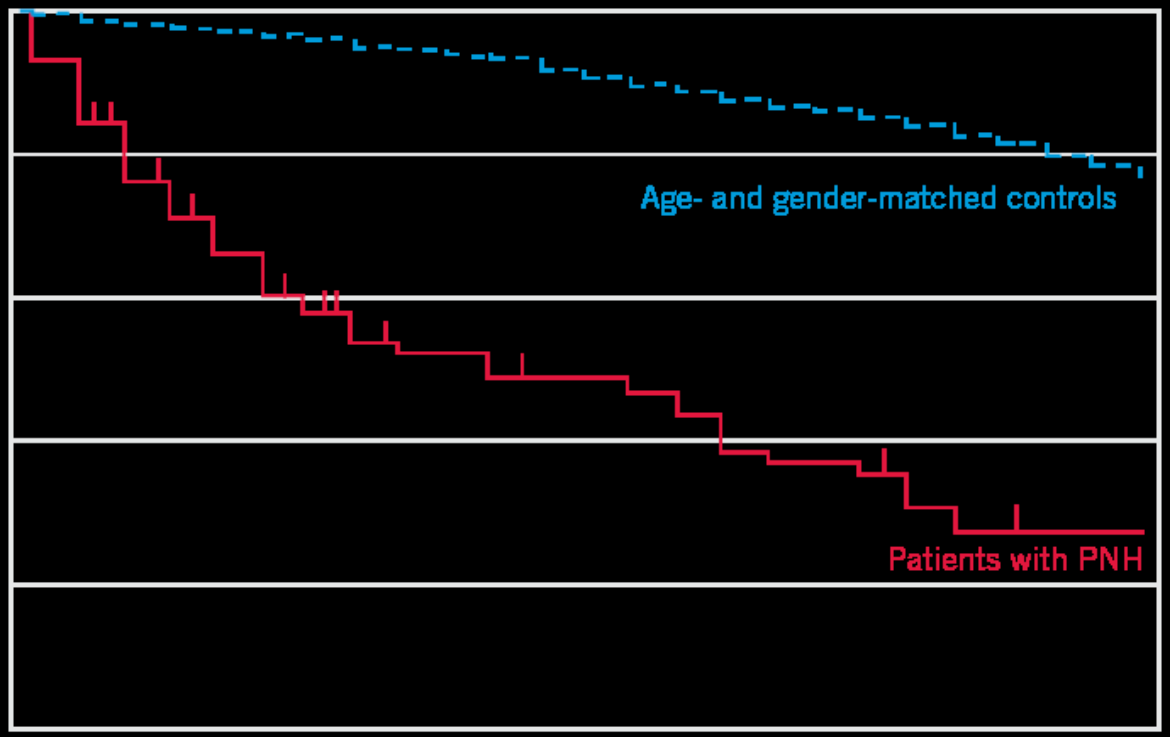
Charles J. Parker Hematology 2011;2011:21–29

Treatment of PNH

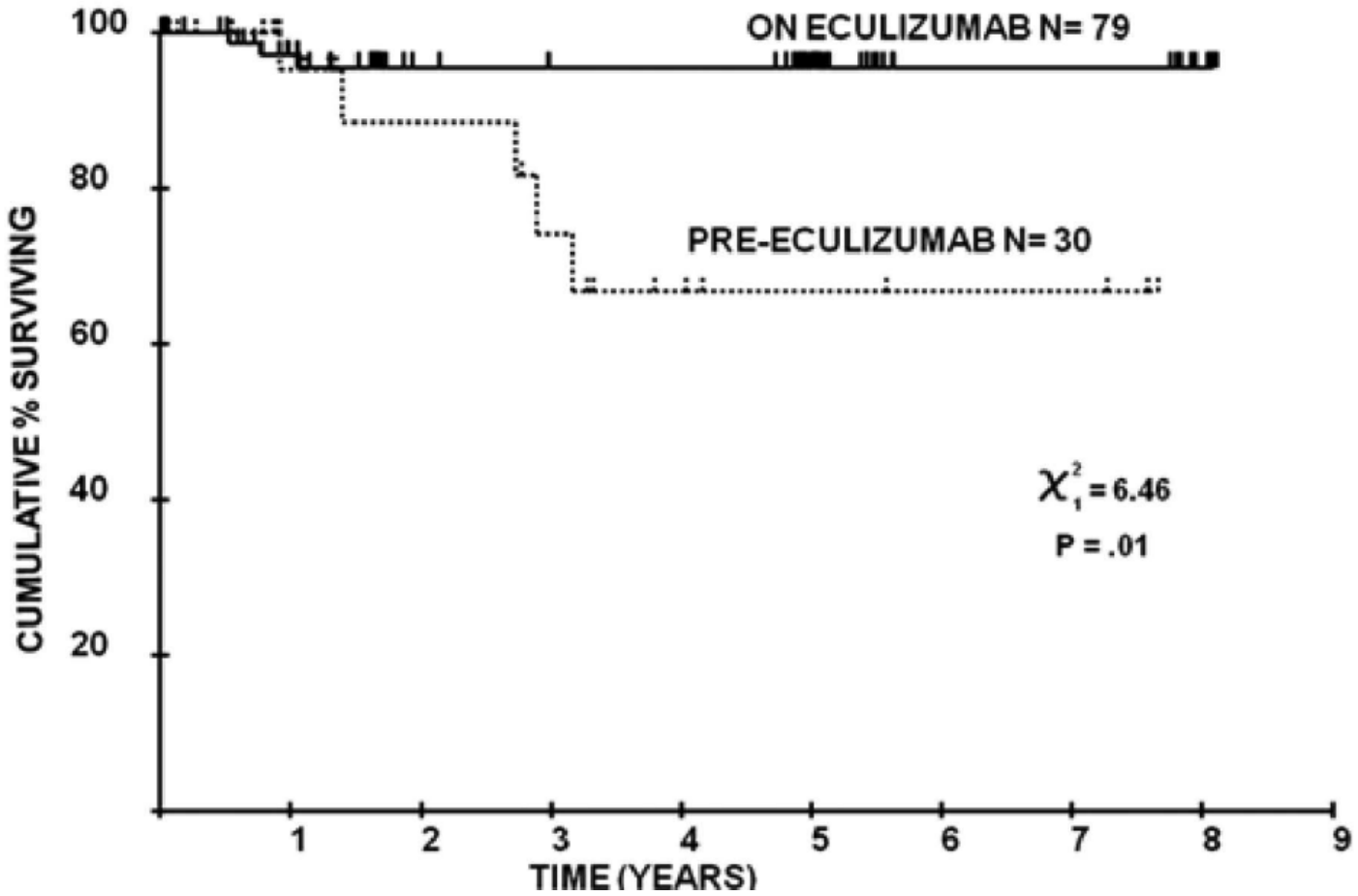
Survival of PNH patients receiving best historical care[1]



35%
of PNH patients die within 5 years of diagnosis despite best historical care[1]

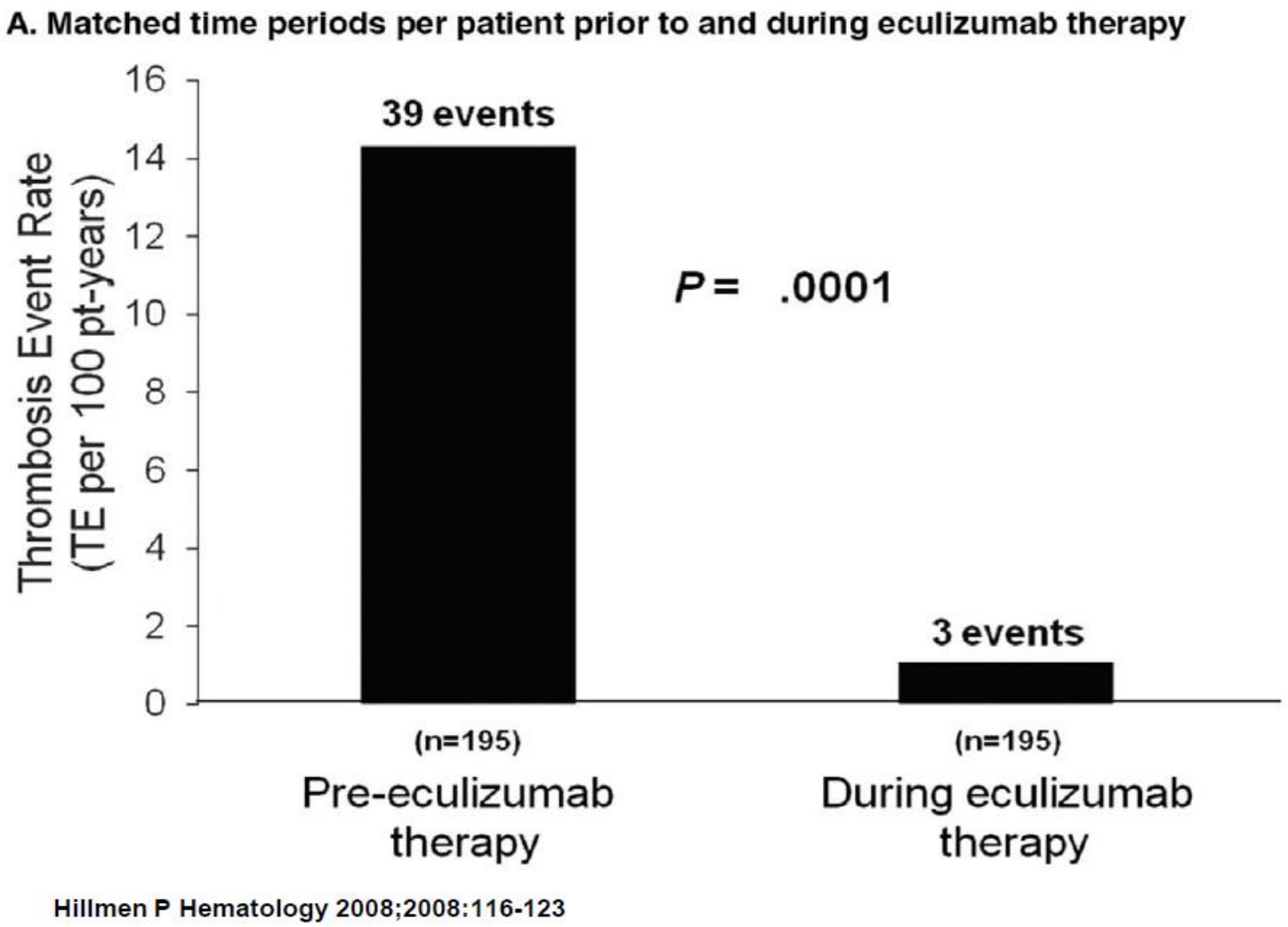
Treatment of PNH

Overall Survival of Patients Before and After Eculizumab



Kelly RJ et al. Blood 2011;117(25): 6786

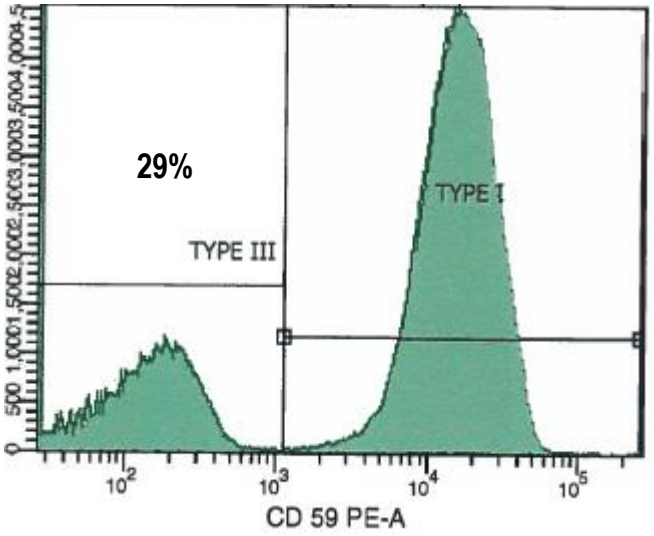
Treatment of PNH

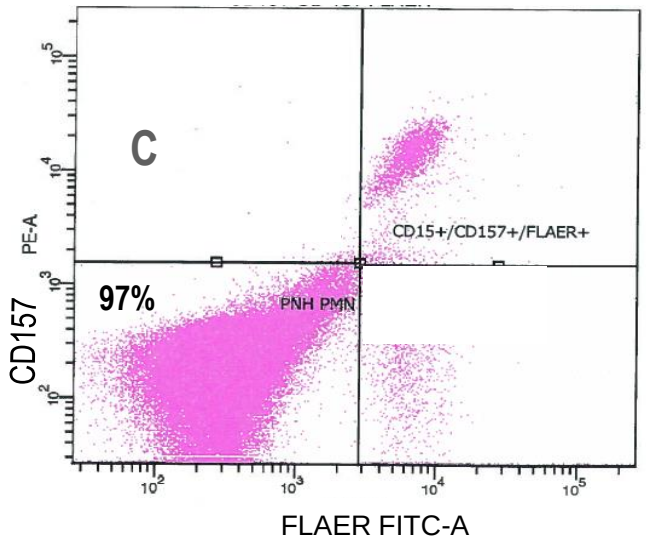


Hillmen P Hematology 2008;2008:116-123

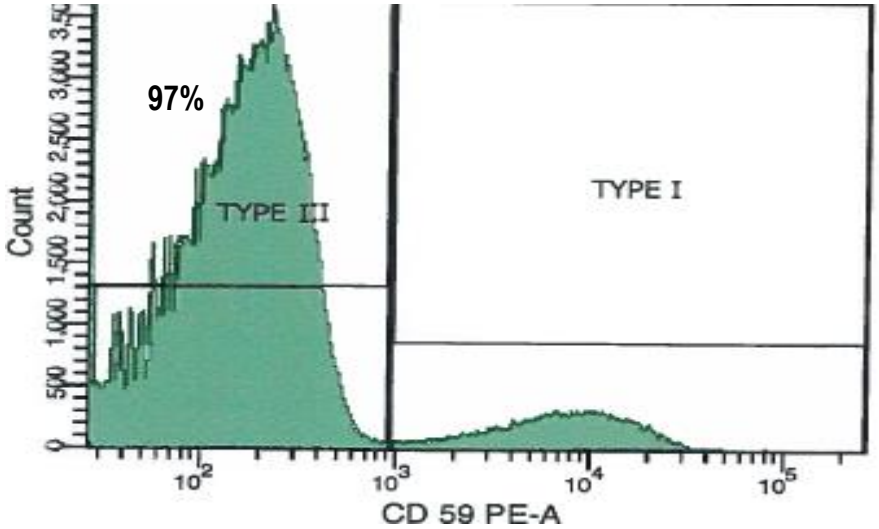
RBC PNH Clone Size in a Patient Treated with Eculizumab

A





B

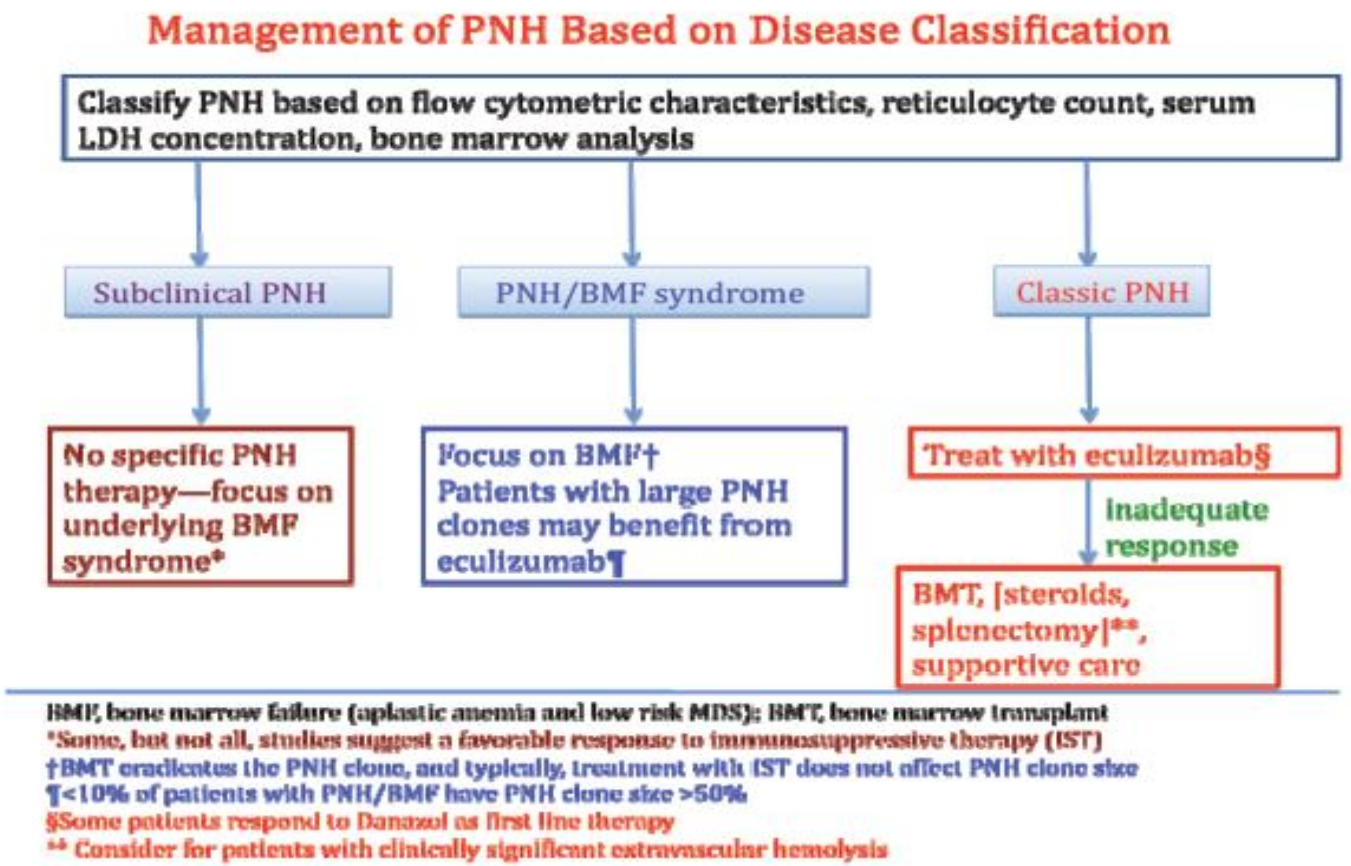


Follow-up Testing

- Patients with established diagnosis of PNH should monitor the size of the PNH clone at regular intervals (if disease is stable, annual monitoring is sufficient)
- Any changes in clinical or hematologic parameter requires more frequent monitoring whether these show worsening or improvement
- Small clones in AA should be monitored because of the risk of developing hemolytic PNH
- Monitoring of RBC PNH clone is useful for assessing response to eculizumab therapy

Clone size	<0.1%	0.11-1.0%	1.01-10%	10.01-100%
Recommended Follow up	6-12 months	3-6 months	3-6 months	As indicated

Treatment algorithm based on disease classification



Charles J. Parker Hematology 2011;2011:21–29

Conclusion

- PNH is a clonal hematopoietic stem cell disease
- Mutation in PIG-A gene resulting in complement mediated hemolysis
- Clinically presents with : anemia, thrombosis, cytopenia, renal failure, pains, and impaired quality of life
- Diagnosis: Flow Cytometry for RBCs and WBCs
  (↓ GPI-anchored proteins)
- Treatment: Eculizumab (Ab C5)

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Thank you! | Questions?