

Hematology Cases: Common, Complex, and Rare

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Research Focus: Cost-effectiveness, decision analysis, systems-based hematology



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Objectives

Use discussion of three cases to:

- Highlight the pathophysiology and treatment of atypical hemolytic uremic syndrome
- Discuss the approach to therapy of immune thrombocytopenia
- Review the diagnosis and treatment of hemophagocytic lymphohistiocytosis



Case 1

30-year-old woman with no significant past medical history

10 days prior to admission:

- mild URI
- 1-2 days of “loose stools” (non-bloody)

1-2 days prior to admission:

- progressive debilitating fatigue, exertional dyspnea, and some easy bruising



Labs

134	96	88	92
3.5	21	12.31	

9.60	4.0	111
	11.8	

15.0	32.0
	1.2

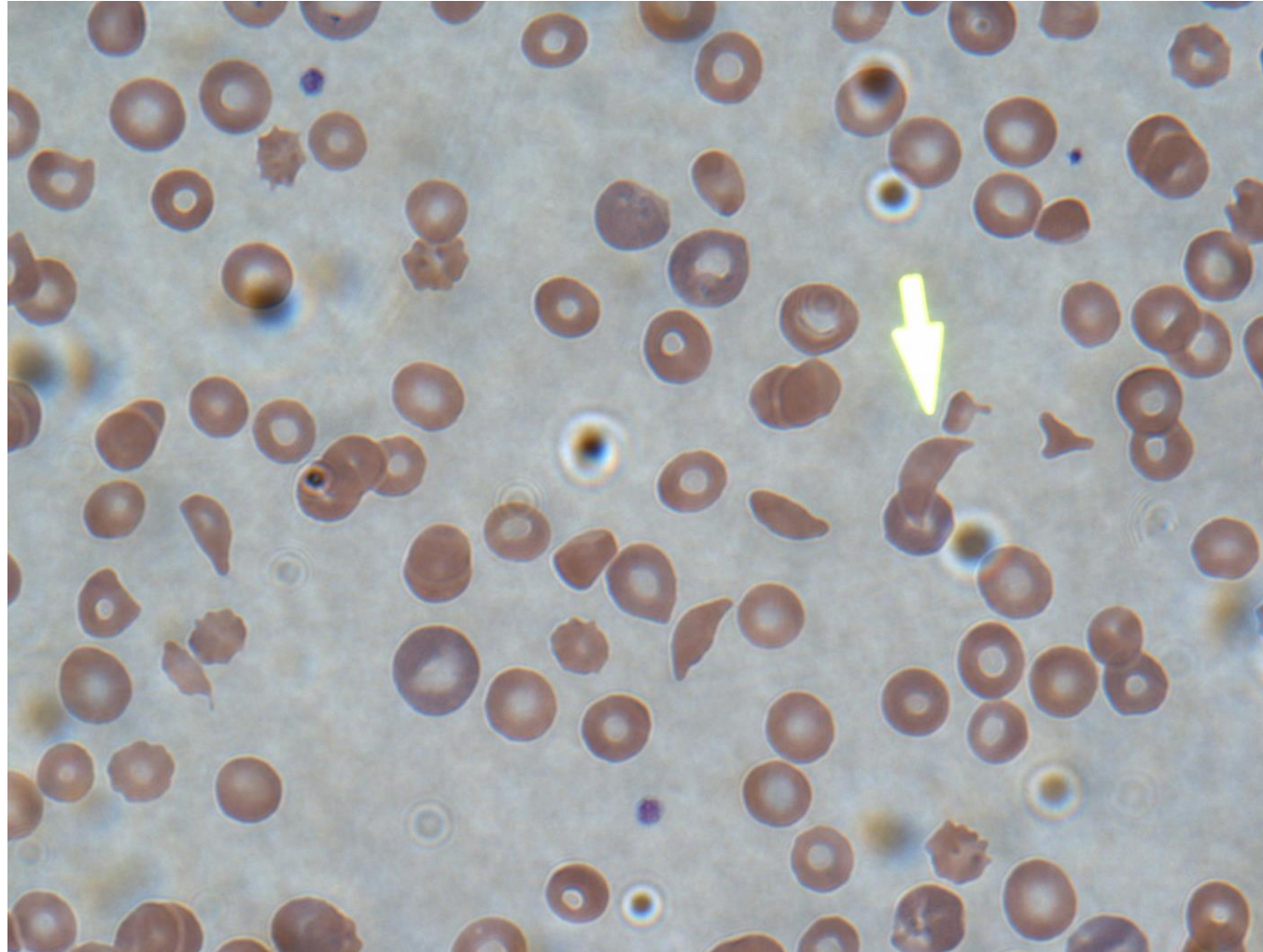
LDH: 1566
Tbili: 1.3
ALT/AST: 29/35
TP: 4.2
Alb: 3.0

U/A:
Glucose: negative
Bili: negative
Ketones: 1+
Blood: 3+
Protein: 3+
Nitrite: negative
Leuk Est: neg
RBC: 4-10
WBC: 0-4

hCG: negative
DAT: negative
C3: 79 (normal 90-180)
C4: 11 (10-40)
Stool culture negative;
including E. coli O157:H7



Peripheral Blood Smear



Laboratory features in TTP vs. HUS

VARIABLE†	NORMAL RANGE	NONFAMILIAL	
		ACUTE EPISODE	
		<i>TTP</i> (<i>n</i> = 24)	<i>HUS</i> (<i>n</i> = 13)
Platelets ($\times 10^{-3}/\text{mm}^3$)			
Median		17	47
Range	125–320	6–126	7–99
Hemoglobin (g/dl)			
Median		9.3	9.0
Range	13.5–16.8 (men) 12.1–15.4 (women)	5.4–14.0	6.9–9.7
Lactate dehydrogenase (U/liter)			
Median		925	990
Range	≤ 480	328–5935	315–5392
Bilirubin (mg/dl)			
Median		1.4	1.0
Range	≤ 1.1	0.1–9.6	0.4–24.4
Creatinine (mg/dl)			
Median		1.1	3.8
Range	≤ 1.3	0.6–2.8	0.4–10.1
von Willebrand factor antigen (U/ml)			
Median		2.08	2.70
Range	0.50–1.50	0.44–3.93	1.80–4.97



Atypical HUS: Overview

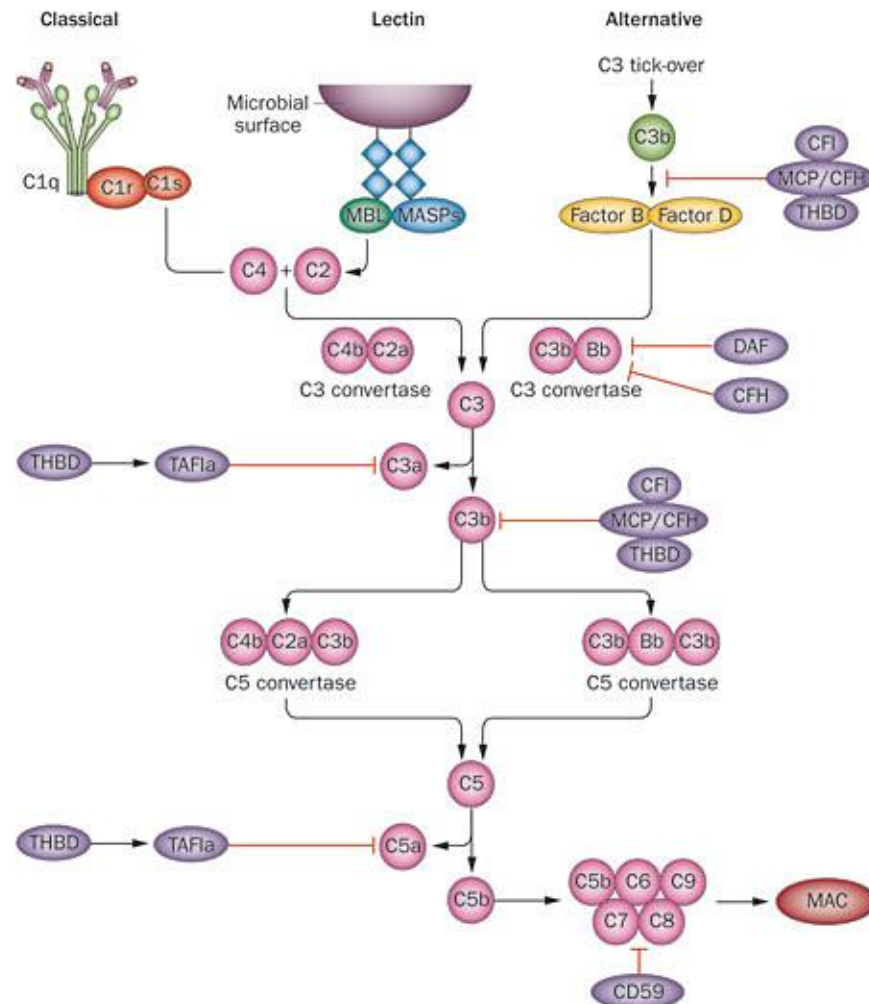
Arises through dysregulation of the alternative complement pathway
Multiple loss-of-function and gain-of-function mutations in multiple complement pathway genes are known to predispose

- Factor H, factor I, MCP, thrombomodulin, factor B, C3
- ~50% of patients have heterozygous mutations
- ~10% have more than one mutation
- Penetrance is 50%; additional genetic/environmental modulators likely to be at play

Renal involvement is predominant



aHUS: The Alternative complement pathway



Gene	Role of mutation	Frequency
CFH	Mutation results in a quantitative deficiency of protein or altered binding to C3b	23%
MCP	Mutant proteins have low C3b-binding capacity and therefore decreased cofactor activity	7%
CFI	Mutations induce a default of secretion of the protein or disrupt its cofactor activity altering degradation of C3b/C4b	4%
C3	Mutations interfere with binding of C3 to MCP and regulation by MCP or increased binding to CFB resulting in increased C3 convertase formation.	8%
CFB	Mutated proteins binds excessively to C3b and stabilize the C3 convertase, making it resistant to decay by CFH, enhancing formation of C5b-9 complexes and deposition of C3 fragments onto endothelial cell surfaces	1%
THBD	Mutated proteins are less effective at moderating CFI-mediated inactivation of C3b	5%
CFHR1/3	Associated with CFH Abs	6%
CFHR5	Unknown	Not reported
Fusion proteins	Results in nonfunctional CFH	Not reported
CFH Ab	Anti-CFH IgG binds to CFH and inhibits CFH binding to C3b and cell surfaces	3%
Unknown		52%

Treatment Prior to Eculizumab for aHUS

Plasma Exchange

- Recommended treatment prior to Eculizumab based on case series
- No RCTs. Loirat et al. (Ped Neph 1988) showed no benefit of plasma infusion, but did not distinguish HUS and atypical HUS

Transplantation

- High rate of recurrence with renal transplant alone unless MCP mutation.
- Case reports of liver and renal transplant shows proof of principle, but 2 cases with bad outcomes (Remuzzi et al. Lancet 2002)



Treatment of aHUS: Pre and Post Eculizumab

Pre-ECULIZUMAB therapy

Plasma infusion may treat patients w/ quantitative complement factor deficiencies.

Some complement proteins (i.e. MCP) are membrane bound and defects cannot be corrected by plasma therapy

2009 European Study Group guidelines: endorsement of plasmapheresis

With this “standard” treatment: ~60% initial response; 25% mortality; 50% CKD

ECULIZUMAB therapy

monoclonal antibody against C5

First-in-class terminal complement inhibitor

Approved for PNH in 2007; for aHUS in 2011

World’s most expensive drug - ~\$500,000 per year

Requires meningococcal vaccination pre-initiation +/- antibiotic prophylaxis

Much improved survival and better renal outcomes, including with ESRD



Treatment of aHUS: How long?

Originally suggested that lifelong therapy should be continued
....especially by the company that makes eculizumab

But...

Study of 17 patients with aHUS treated with eculizumab

Taken off eculizumab and monitored

13/17 patients did not require further therapy

Those that recurred were successfully treated

Blood 2017 130:368

Recent Study

55 patients, adults and children on eculizumab for aHUS

28 patients had complement-related protein mutations

13 relapsed, of which 11 went back into remission

Increased risk of relapse: complement mutations or high C5-9 in serum.

Blood 2021



Take Home Points: Case 1

TTP

- Hereditary (Upshaw-Schulman syndrome)-ADAMTS13 deficiency
- Acquired: Antibody to ADAMTS13
 - Idiopathic--usually IgG inhibitor to ADAMTS13
 - May be associated with pregnancy, malignancy, lupus
 - Response to plasma exchange in 90+%
 - Relapse rate 10-30%

HUS

- Shiga toxin producing E. Coli
- Seen almost entirely in children
- Excellent prognosis with no therapy

Atypical HUS

- Genetic mutations, and rarely antibodies that cause inappropriate activation of alternative complement cascade
- Grim prognosis in the pre-eculizumab era
- Prognosis with eculizumab still being defined



CASE 2

25 yo healthy woman

CC: rash on lower extremities

PE:

- skin: rash
- HEENT: hemorrhagic bullae in mouth
- Otherwise normal

Labs:

- WBC 3.9 with normal diff; Hct 35 with normal MCV; Plts 14K
- PT/PTT normal
- electrolytes, LFTs normal



Thrombocytopenia

DECREASED PRODUCTION

- Marrow Failure
- Drugs
- Myelophthysis
- Nutritional deficiency (megaloblastic anemia)

SEQUESTRATION

- Splenomegaly (rarely $<50,000$)

INCREASED DESTRUCTION

- ITP
- TTP
- DIC



Evaluation of Thrombocytopenia

History

- Intercurrent illnesses
- Medication history
- History of autoimmune disease, LPD

Physical

- Bleeding manifestations (purpura, petechiae)
- Splenomegaly
- Adenopathy

Laboratory

- Examination of the peripheral smear
- Platelet reticulocyte count
- Antiplatelet antibodies??
- Bone marrow examination??



ITP Therapy: Initial Treatment

Agents used for initial therapy of ITP:

Corticosteroids

- High dose methylprednisolone
- Prednisone
- Dexamethasone

IV Immunoglobulin

Anti-D



ITP Therapy: Initial Treatment

“Standard of care”

Prednisone 1mg/kg/day

Taper—schedules undefined

Response: 65-90%

Long term response: 5-30%

- Of note, study that reported 30% assessed at 6 months.
- Most studies with longer follow-up report <10% long term response to prednisone



ITP Therapy: Initial Treatment

Dexamethasone:

- Single course, 40mg/day X 4 (**NEJM 2003; 349: 831**)
 - Initial response: 85%
 - Relapse: 50%; 2nd course response: 100%
 - Sustained: 42%
 - Persistent response after D/C maintenance: 18%
- Multiple course, 40mg/day X 4 (**BLOOD 2007;109: 1401**)
 - Two protocols: 6 cycles q28 days/4 cycles q14 days
 - Initial response: 89%/86%
 - RFS at 15 mos: 90% /81%
 - Long term response:
 - median 26mos with 6 cycles: 68%
 - median 8 mos with 4 cycles: 74%
- Randomized trial Dex vs Prednisone (**BLOOD 2016;127: 296**)
 - Durable responses with less toxicity
 - Only a single course, so still need a trial of repeated courses of dexamethasone



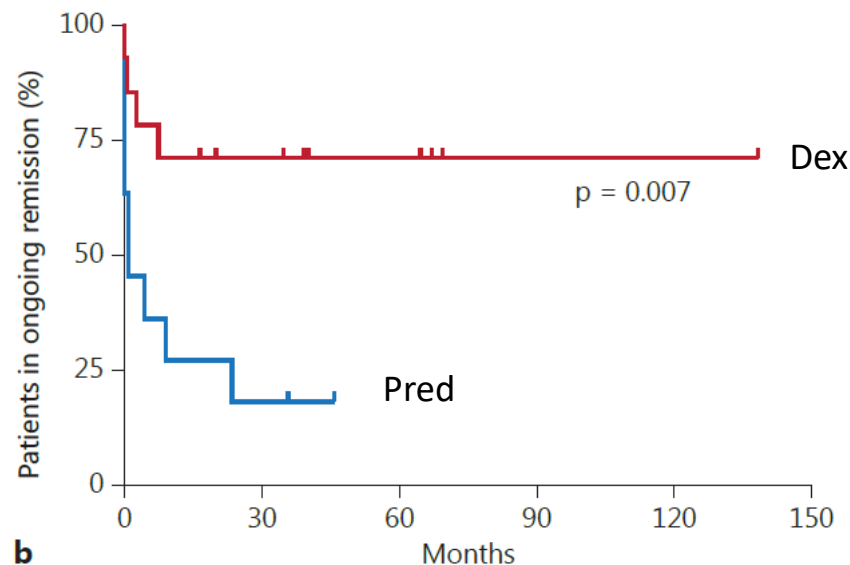
ITP Therapy: Initial Treatment

Randomized trial Dex vs Prednisone

Durable responses with less toxicity

- Only a single course, so still need a trial of repeated courses of dexamethasone

Small randomized single arm study (only 26 patients, stopped for low accrual)



All patients: 1 week pred

DEX: 40 qd X4 every 21 days X6

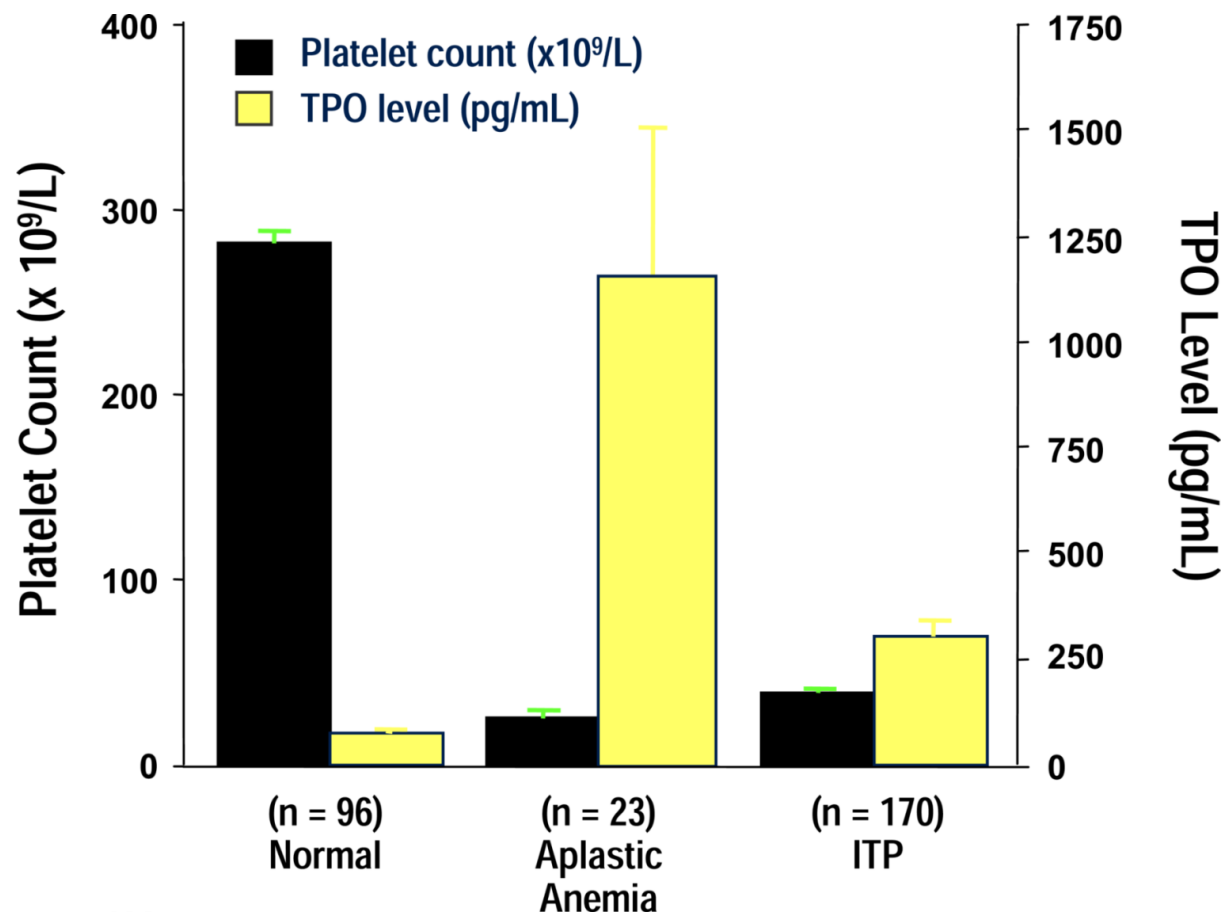
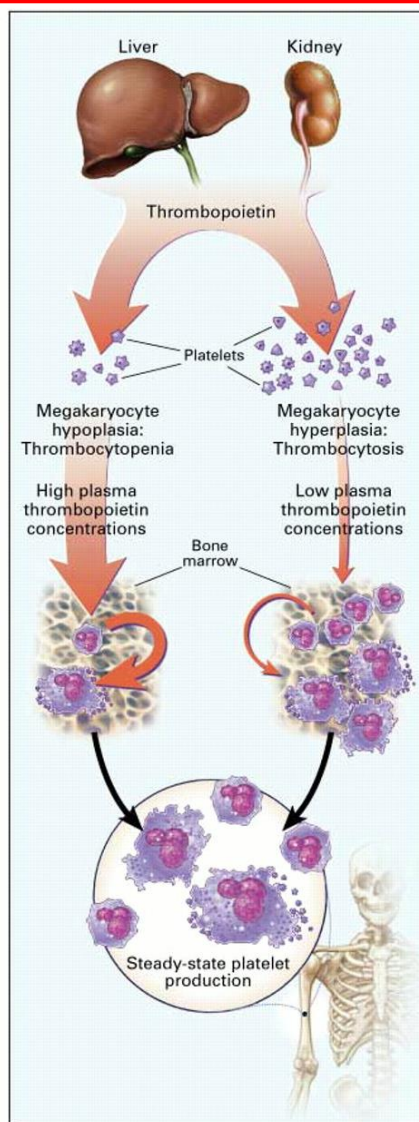


Therapy: Relapsed or Refractory ITP

Therapy	Response Rate	Time to Response	Toxicity	Duration of Response
Splenectomy	80%	1-24 days	Surgical complications, thrombosis	2/3 with no further Rx
Rituximab	60% response 40% complete	1-8 weeks	Allergic rxns; immune supp.	15-20% sustained Can be retreated
TPO Mimetics	>80% response (plts >50K)	2-3 weeks	Unknown long-term toxicity Increased reticulin Rapid fall of plts with D/C of drug	Up to 1.5-4 yrs with continuous drug



TPO Response to Thrombocytopenia



*Hematol Oncol Clin North Am.*23:1193, 2009.

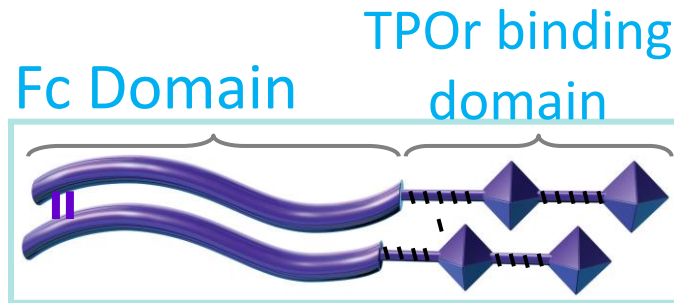
Thrombopoietin Mimetics

Romiplostim

Fc-peptide fusion
protein (peptibody)

Binds to and activates the
TPO receptor to increase
platelet counts

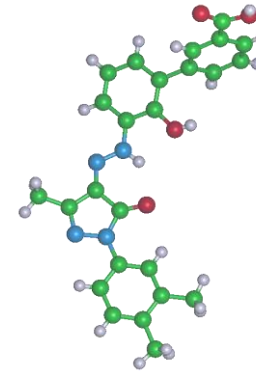
Approved for treatment of
chronic ITP in US, EU,
Canada and Australia



Eltrombopag

Small molecule, oral TPO
receptor agonist

Increases platelet production
by increasing
megakaryocyte growth



Both approved for the treatment of chronic ITP

Take Home Messages: Case 2

- The treatment of ITP continues to be somewhat controversial
- First-line therapy is corticosteroids—my recommendation is pulse dexamethasone
- Splenectomy remains excellent second-line therapy
- However, rituximab is effective in many patients, is probably the third most likely to give a long remission and can be repeated. Often preferred to splenectomy
- TPO mimetics work but have some known and many possible unknown downsides.



CASE 3

39 y/o female home maker, wife and mother of three with no significant past medical history

12/12/04: fevers, chills, rigors

- WBC 3.9, Hct 34.8, plt 148
- Fevers resolved after a 10 day course of doxycycline

1/05: recurrent fevers

- CBC and chemistries - normal

2/12/05: presented with fever and back pain

- ROS – fevers, chills, rigors, back pain, generalized fatigue



CASE 3

Transferred on vancomycin, ceftazadime, doxycycline

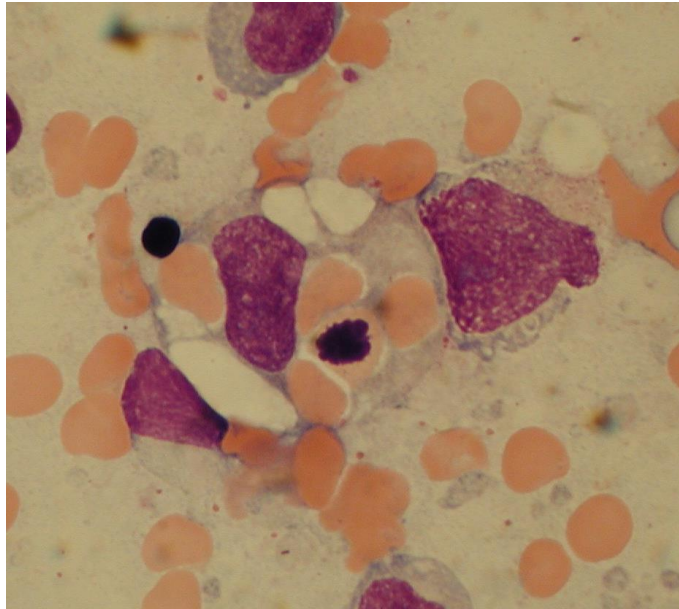
On arrival:

WBC 0.9; Hct 22.8 with reticulocyte count of 2.3%, platelets 73

Tbili 0.58, ALT 55, AST 128, AP 97, alb 3.2, LDH 1350

INR 1.47, PTT 30.5, D-dimer >1.0, FSP >40, fibrinogen 176

Iron 50, IBC 245, % iron saturation 20, ferritin 1460, CRP 5.3



Hemophagocytic Lymphohistiocytosis

- Related to impaired cytolytic activity of T cells and NK cells
- Uncontrolled T-cell activation with increased secretion of T_H1 cytokines, including $IFN\gamma$, IL-12, and IL-18, which activate macrophages
- Results in proliferation and activation of benign macrophages, and is associated with phagocytosis of hematopoietic elements throughout the RE system
- Familial HLH is associated with identified defects in cytotoxic T cells and NK cells
- Mechanism of acquired forms of HLH is unknown



HLH: Diagnostic Criteria

Molecular Diagnosis consistent with HLH
OR

Clinical and laboratory criteria (5/8)

- Fever
- Splenomegaly
- Cytopenia
- Hypertriglyceridemia and/or hypofibrinogenemia
 - Fasting triglycerides > 3mmol/l
 - Fibrinogen < 1.5 g/l
- Ferritin > 500
- sCD25 > 2400U/ml
- Decreased or absent NK-cell activity
- Hemophagocytosis in bone marrow, CSF, or nodes

These criteria are entirely based on PEDIATRIC patients



Hemophagocytic Lymphohistiocytosis

Genetic HLH

- Familial HLH
 - Known gene defects (perforin, munc 13-4, syntaxin 11)
 - Unknown gene defects
- Immune deficiency syndromes
 - Chediak-Higashi syndrome
 - Griscelli syndrome
 - X-linked lymphoproliferative syndrome

Acquired HLH

- Infection associated hemophagocytic syndrome
- Autoimmune disease (macrophage activation syndrome)
- Malignancy (T cell lymphoma)
- Drug hypersensitivity reaction (?)



Multifactorial Pathogenesis of HLH

Primary HLH:

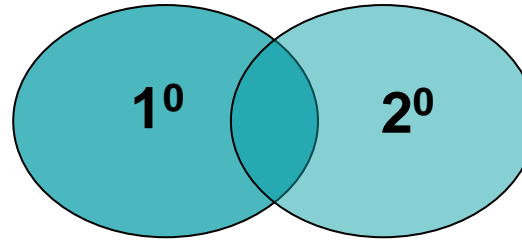
FHL, XLP, CHS

Infants

Spontaneous (?)

Recurrent (if untreated)

Fixed NK defect (?)



Secondary HLH:

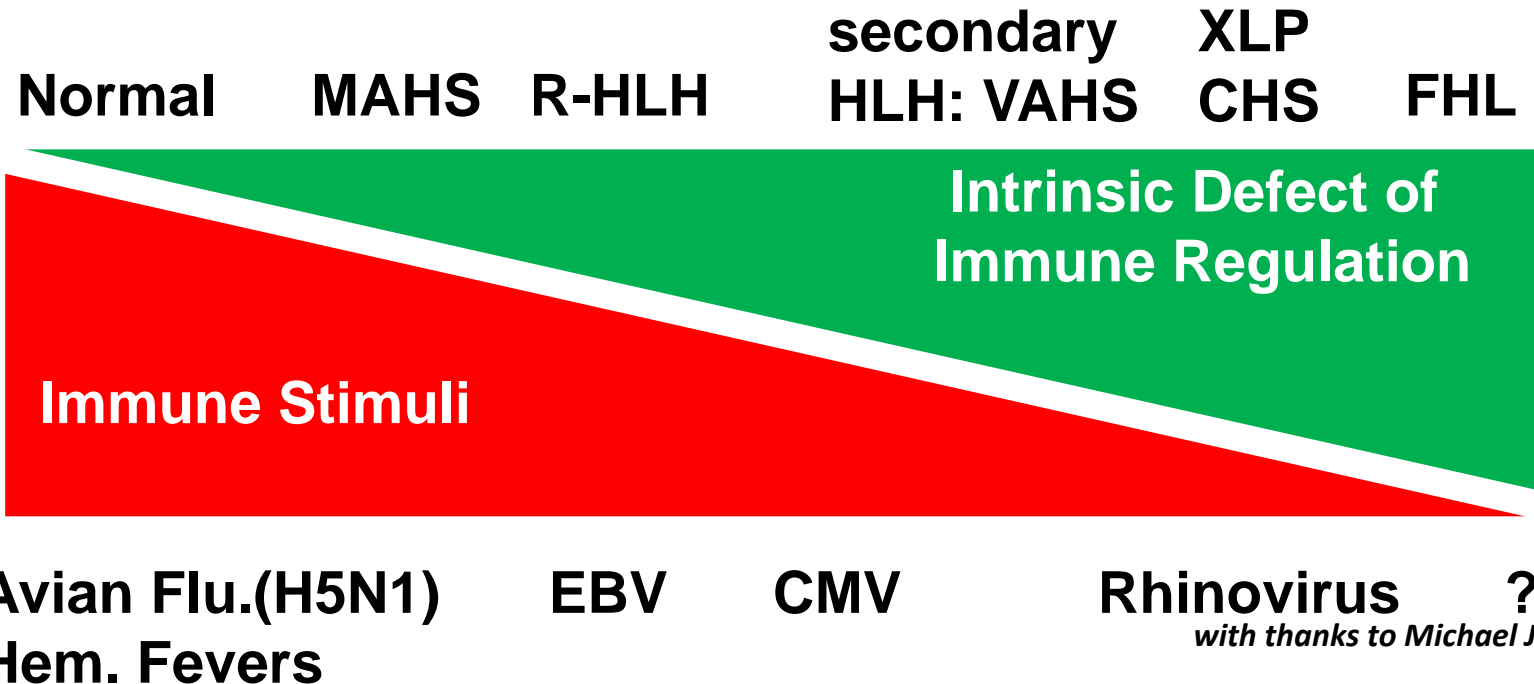
VAHS, MAHS, MAS

Older children

Clear triggers

Minimal recurrence risk (?)

No fixed NK defect (?)



Adult HLH: A newly recognized epidemic?

- In the last 15-20 years, there has been an explosion of reports and studies of adult HLH
- Apparent increase in HLH in adults is unexplained
- Newly-prevalent vs newly-recognized
- Either way, is an increasingly common diagnosis
- Despite this, the diagnosis continues to be driven by criteria developed in the pediatric population
- Diagnosis and treatment in adults is evolving



Surveys of adult HLH

103 adult HLH patients from single hospital in China:

- 48% hematologic malignancy
- 14% autoimmune disease
- 23% infectious disease
- **23% unknown**

Li et al, Medicine 2014; 93: 100

68 patients in Partners hospitals, Boston

- 49% malignancy
- 33% infectious
- 28% autoimmune disease
- **15% unknown**

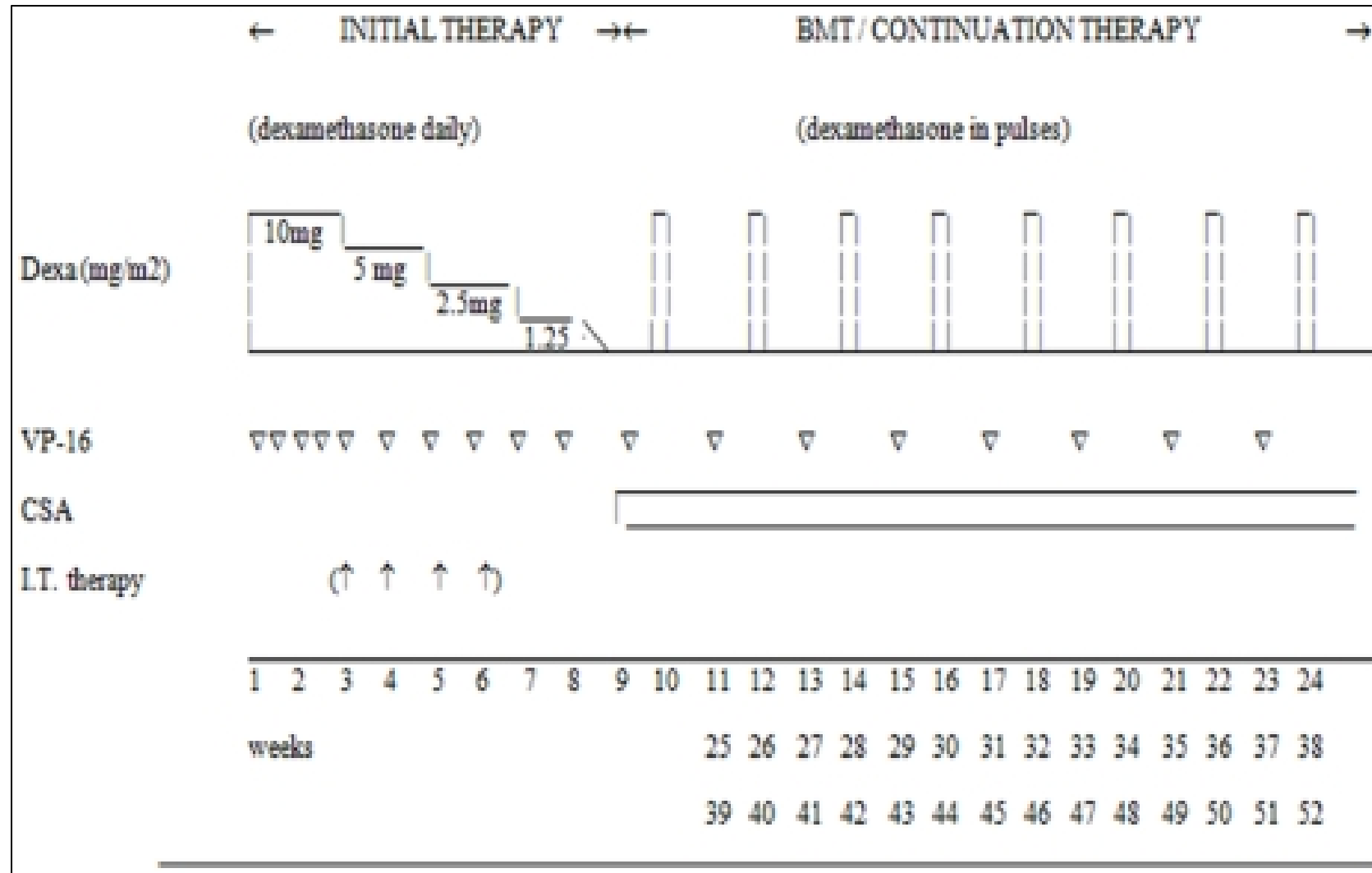
Schram et al, BrJHaematol 2015; in press

62 patients at Mayo Clinic

- 62% malignancy
- 34% infectious
- 8% autoimmune disease
- **6% unknown**



HLH-94 Protocol



Therapy for Adult HLH

Treatment in adults generally follow HLH-94

- Exception: MAS; usually respond to steroids + IST alone
- Some protocols modify the dose of etoposide in adults
- We generally use tacrolimus rather than CsA for less renal toxicity

Adults do less well than children

- May be delay in diagnosis and therapy
- May be more complex etiology
- Affected by much higher rate of associated malignancy

Salvage therapies: ATG, Alemtuzumab



Novel HLH Therapies: IFN γ inhibition

Emapalumab (NI-0501) = fully human, high affinity anti-IFN γ mAb that binds and neutralizes human IFN γ

- Open-label Phase 2 in US/Europe
- N=27 children with confirmed or suspected fHLH
 - Overall Response: 63% (7 CR, 8 PR, 2 improved)
 - All responding patients went on to transplant, so durability of response not assessable.
 - On basis of these data (which had not been published), emapalumab was approved in December 2018 by FDA for treatment of pediatric AND ADULT patients with fHLH. The EMA declined to approve it for either group.

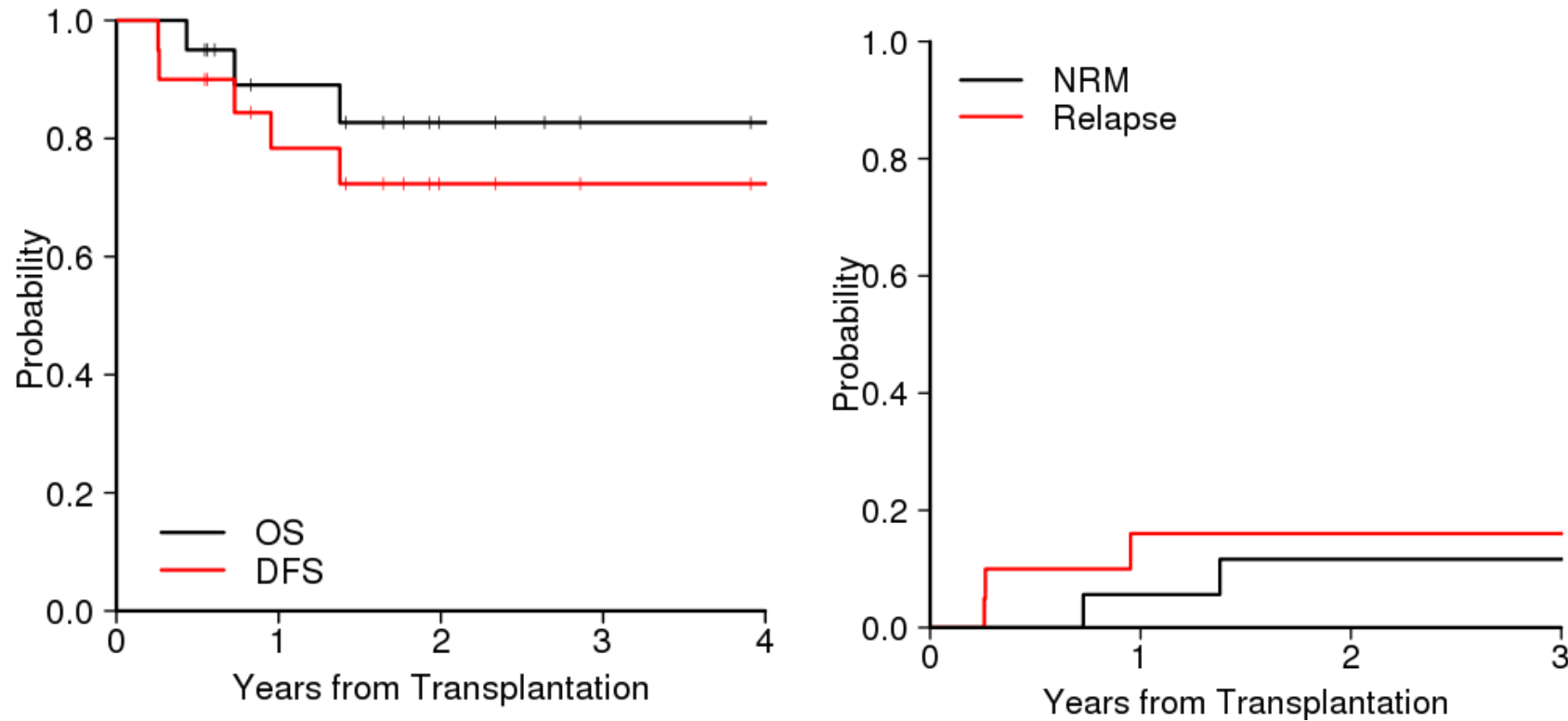


Novel HLH Therapies: JAK inhibitors

- Rationale: Uncontrolled activation T- cells and macrophages results in massive cytokine release which damages tissues
- $\text{INF}\gamma$, IL-2, IL-6 and IL-10 are released in these settings
 - Janus kinases (JAK) can transduce signals upon binding of various cytokines to their receptors
 - JAK inhibition is beneficial in other inflammatory conditions
 - Has been demonstrated to prevent and treat HLH in two mouse models of HLH
 - Currently in early clinical trials



Adult HLH: Allogeneic Transplant



21 patients: no relapse without lymphoma relapse



Take Home Points: Case 3

- Diagnosis of HLH in adults is still based on pediatric diagnostic criteria
- Etiology of HLH in adults is more skewed toward malignancy-associated disease
- Treatment of HLH in adults still parallels the approach to pediatric patients. There is increasing evidence that most patients should undergo HSCT
- **MOST IMPORTANT:** It is more common than you think; if you don't consider the diagnosis, you won't make it!



MOC Reflective Statement

Atypical HUS

- Remember that whereas TTP reflects deficiency of ADAMTS13, aHUS is caused by abnormal activation of the alternate complement pathway
- Half of patients with aHUS have mutations in the genes in the alternate complement pathway
- Therapy transformed by use of complement inhibitors (eculizumab, ravulizumab)

ITP

- Remember that first line therapy of ITP is corticosteroids
- Rituximab, splenectomy and TPO mimetics can be used as second line therapy

HLH

- Review the diagnostic criteria for HLH
- Remember that HLH in adults is associated with lymphoid malignancy in about 50% of patients



QUESTION 1

A 43-year-old man with no past medical history presents to the hospital with a two month history of fevers and night sweats. Repeated cultures by his PCP have been negative, and despite three admissions to the hospital, his illness has evaded diagnosis. He has lost 20 pounds, and he has been unable to work for 6 weeks.

Physical examination reveals a cachectic appearing young man with a palpable liver and spleen. He has no adenopathy. Laboratory studies reveal pancytopenia with an ANC of 726 and a platelet count of 15,000, elevated transaminases, and diffuse infiltrates on chest CT. He is begun on empiric antibiotics. Which of the following tests is LEAST likely to be helpful in making a diagnosis:

- A. Bone marrow aspirate and biopsy
- B. Liver biopsy
- C. Serum soluble CD25
- D. Serum ferritin



QUESTION 1

Which of the following tests is LEAST likely to be helpful in making a diagnosis:

- A. Bone marrow aspirate and biopsy
- B. Liver biopsy
- C. Serum soluble CD25
- D. Serum ferritin

ANSWER: B

This patient almost certainly has hemophagocytic lymphohistiocytosis (HLH), with pancytopenia, splenomegaly, and fever, with no obvious infectious source. Other diagnostic features supporting the diagnosis would be the finding of hemophagocytosis on bone marrow, an elevated serum ferritin, an elevated soluble CD25, elevated triglycerides, and low fibrinogen. A liver biopsy, while it might show hemophagocytosis, is likely to be dangerous in someone with this low a platelet count and is not considered a particularly helpful diagnostic procedure.



QUESTION 2

A 60-year-old woman goes to the doctor with a complaint of bruising and gum bleeding. CBC shows Hgb of 12.2g/dl, WBC of 8.3, and platelets of 6K. She is sent to the emergency department of admission. CBC a year ago was completely normal. Peripheral blood smear is unremarkable except for near absence of platelets and a few giant platelets.

The best next step is:

- A. Plasma exchange
- B. ANA, RF
- C. Corticosteroids
- D. Bone marrow examination
- E. Eculizumab



QUESTION 2

The best next step is:

- A. Plasma exchange
- B. Bone marrow examination
- C. Corticosteroids
- D. Eculizumab

ANSWER: C

This patient almost certainly has immune thrombocytopenia. Isolated thrombocytopenia with an otherwise normal CBC suggests ITP, especially with the presence of giant platelets. Therapy of choice is corticosteroids. ITP is a clinical diagnosis and bone marrow examination is rarely indicated in the absence of suspicion of another etiology. With a relatively normal hematocrit and absence of microangiopathic red cells on her smear, there is no reason to suspect TTP or aHUS, so plasma exchange or eculizumab therapy are not indicated.



REFERENCES

George JN, Al-Nouri ZL. Diagnostic and therapeutic challenges in the thrombotic thrombocytopenic purpura and hemolytic uremic syndromes. Hematology / the Education Program of the American Society of Hematology American Society of Hematology Education Program 2012;2012:604-9.

Legendre CM, Licht C, Muus P, et al. Terminal complement inhibitor eculizumab in atypical hemolytic-uremic syndrome. The New England journal of medicine 2013;368:2169-81.

Merrill SA, Brittingham ZD, Yuan X, Moliterno AR, Sperati CJ, Brodsky RA. Eculizumab cessation in atypical hemolytic uremic syndrome. Blood 2017; 130:368-72

Schram AM, Berliner N. How I treat hemophagocytic lymphohistiocytosis in the adult patient. Blood 2015;125:2908-14

Warkentin TE. Think of HIT. Hematology / the Education Program of the American Society of Hematology American Society of Hematology Education Program 2006:408-14.

Wei Y, Ji XB, Wang YW, et al. High-dose dexamethasone vs prednisone for treatment of adult immune thrombocytopenia: a prospective multicenter randomized trial. Blood 2016;127:296-302.

