SEEK AND YOU SHALL FIND:

NEWBORN SCREENING FOR HEMOGLOBINOPATHIES

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Pediatric Small Group Teaching
January 13, 2022

OBJECTIVES

- By the end of this presentation, you should be able to:
  - Explain the pathophysiologies of the hemoglobinopathies
  - Describe the diagnostic investigation of hemoglobinopathies
  - Discuss acute and chronic management strategies for patients with hemoglobinopathies
  - Evaluate the role of newborn screening for sickle cell disease and other hemoglobinopathies

CASE 1 - TE

- 14-month-old girl from Brandon
- Followed for panhypopituitarism – presented with jaundice and anemia
- Parents from Nigeria – Mom known AS, Dad uncertain
- Presents unwell with hand pain to the local ER
  - CBC shows anemia, sickle cells

CASE 2 – AA SIBLINGS

- 9 and 2-year-old brothers
- No known family history – Nigerian parents
  - Mom known AS and dad thought he was AS
- Older boy presents with leg pain
  - Investigations sent – confirm Hb SC
- Younger boy investigated when older boy seen in consult
  - Investigations confirm Hb SC

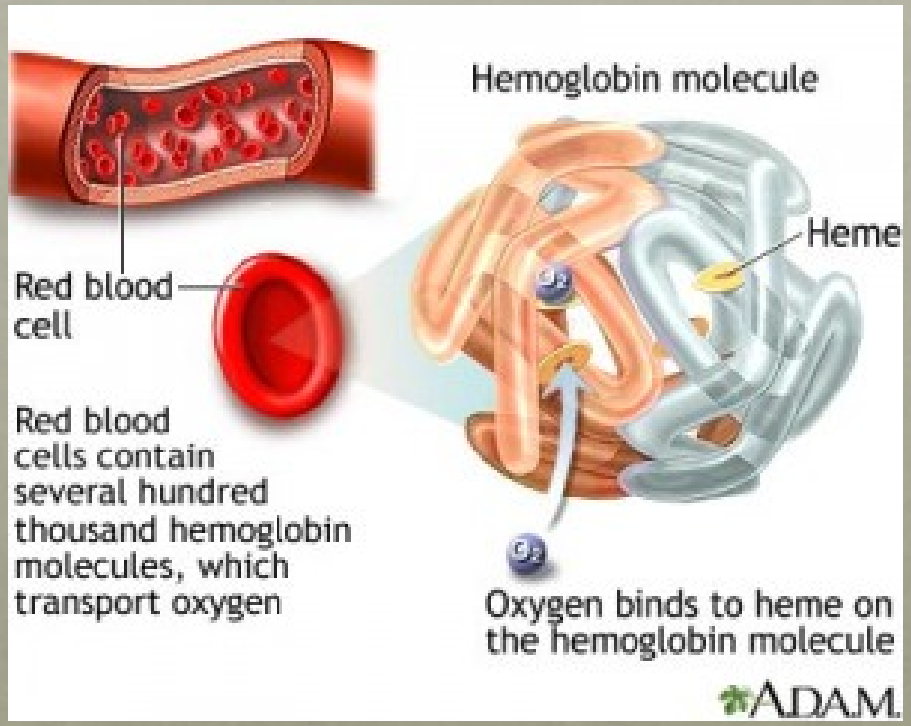
CASE 3 – OO

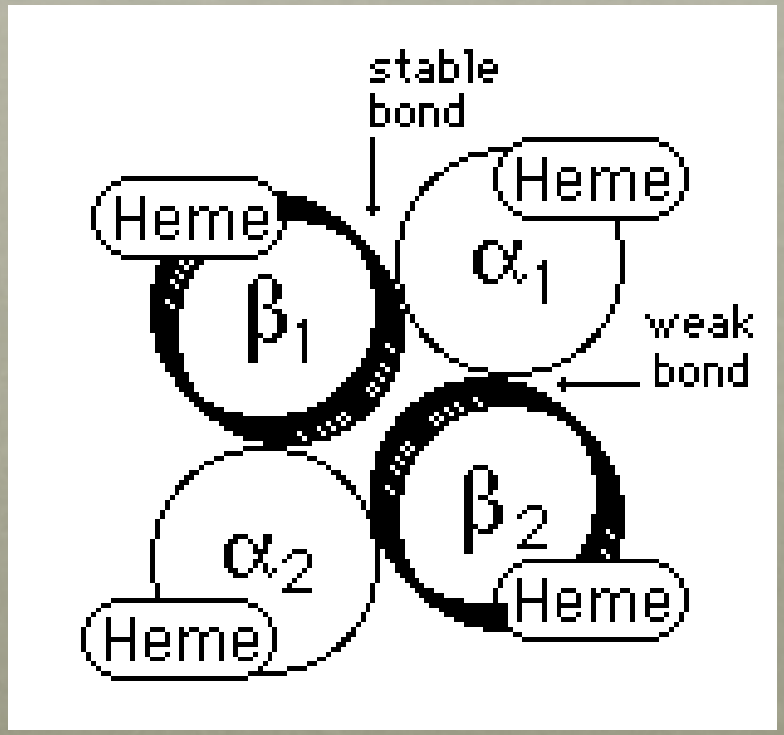
- 9-month-old girl
- Nigerian parents – Dad known AS, mom AA
- Presents with fever and irritability
  - Anemia and positive sickle screen
  - Investigations confirm Hb SS

CASE 4 - MS

- Refugee family from Myanmar – son admitted at 15 months of age with pallor and irritability
  - Significant anemia noted, ascribed to iron deficiency
- Review of family's immigration blood work shows both parents have slight microcytosis
- Physical exam reveals large head and marked hepatomegaly
- Investigations confirm Beta-thalassemia major

HEMOGLOBIN 101





KEY DISTINCTION

Hemoglobinopathy

- **Normal** production of an **abnormal** hemoglobin
  - Sickle cell syndromes
  - Unstable hemoglobins

Thalassemia

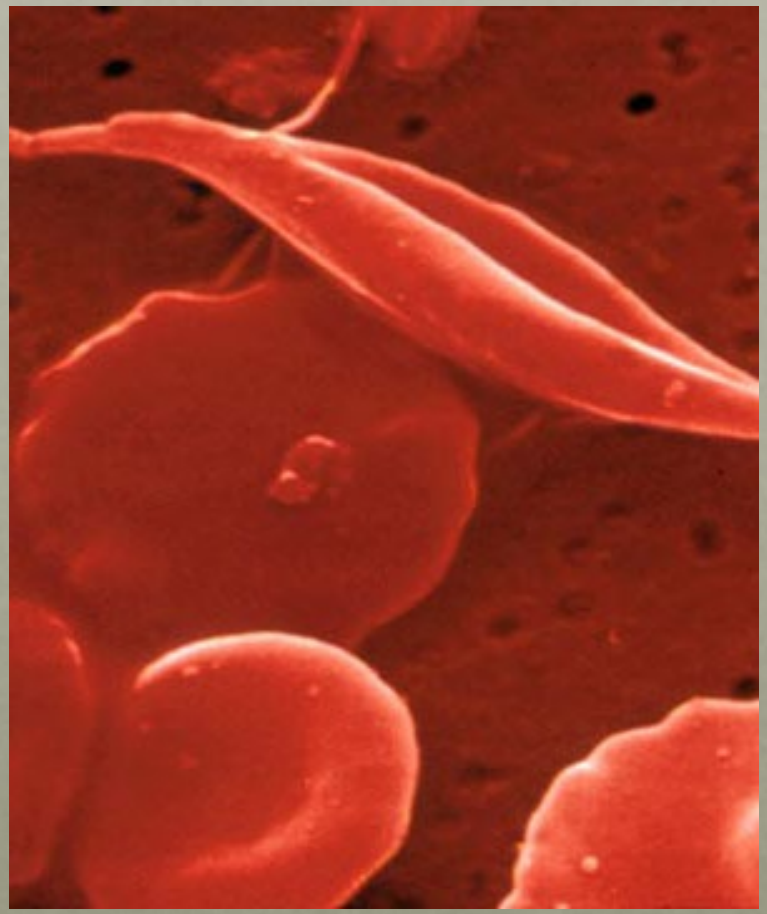
- **Decreased** production of a **normal** globin chain
  - Alpha
  - Beta
  - Gamma
  - Epsilon

PRIMARY HEMOGLOBINS

- A: Alpha – 2, Beta – 2
- F: Alpha – 2, Gamma – 2
- A2: Alpha – 2, Delta – 2
- S: Alpha – 2, Beta (sickle) – 2

SICKLE CELL ANEMIA

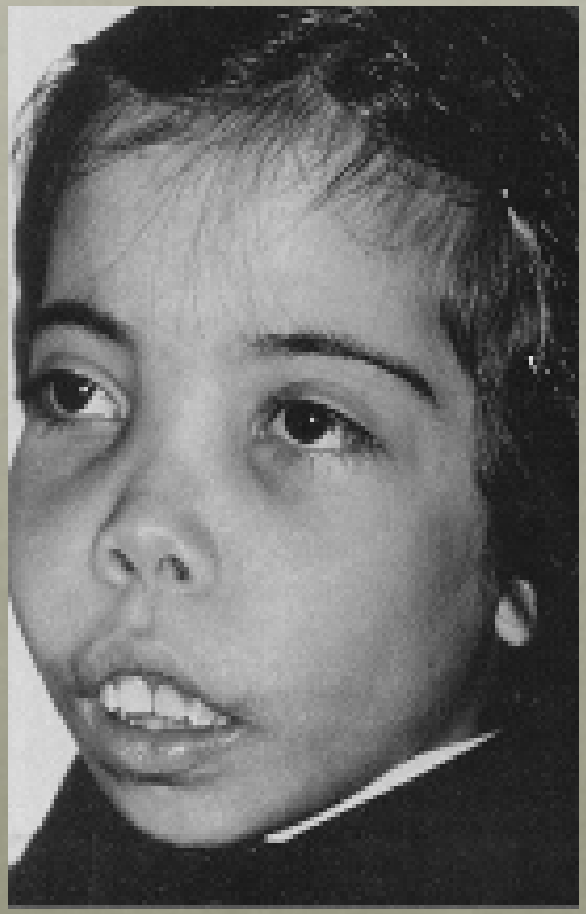
- Genetic mutation in the Beta-globin chain
  - Polymerizes in the deoxygenated state
- Abnormally shaped RBC
  - Not effective for oxygen transport
  - Occlude blood vessels
  - Shortened survival



THALASSEMIAS

- Symptoms due to anemia and increased red blood cell production

- Blood vessel blockages are *not* a problem

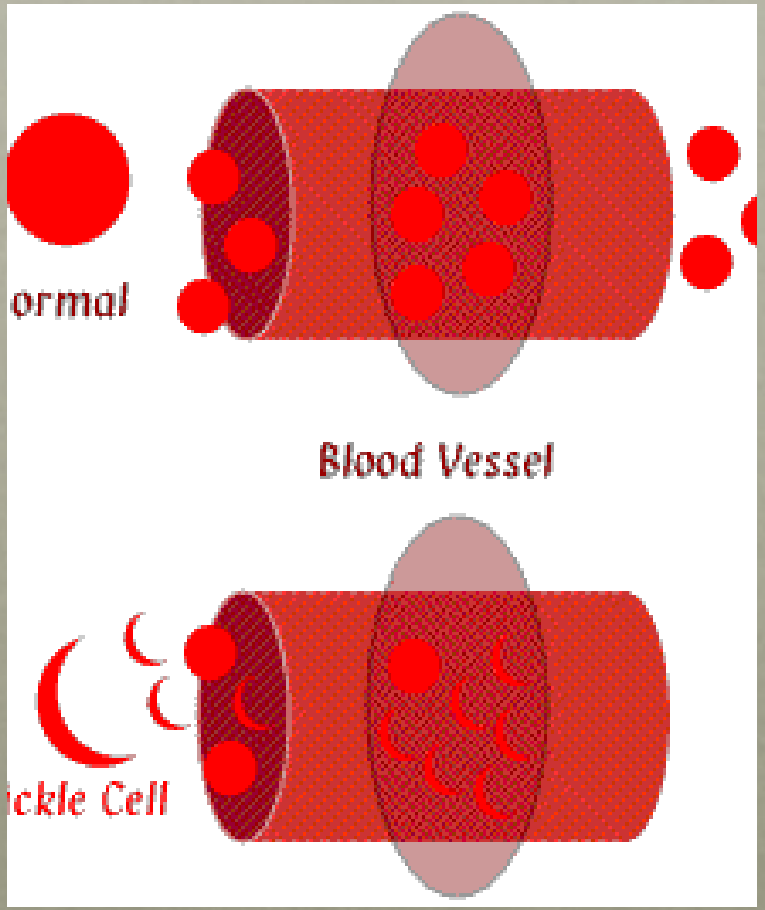


OUR NATIONAL DISEASE?

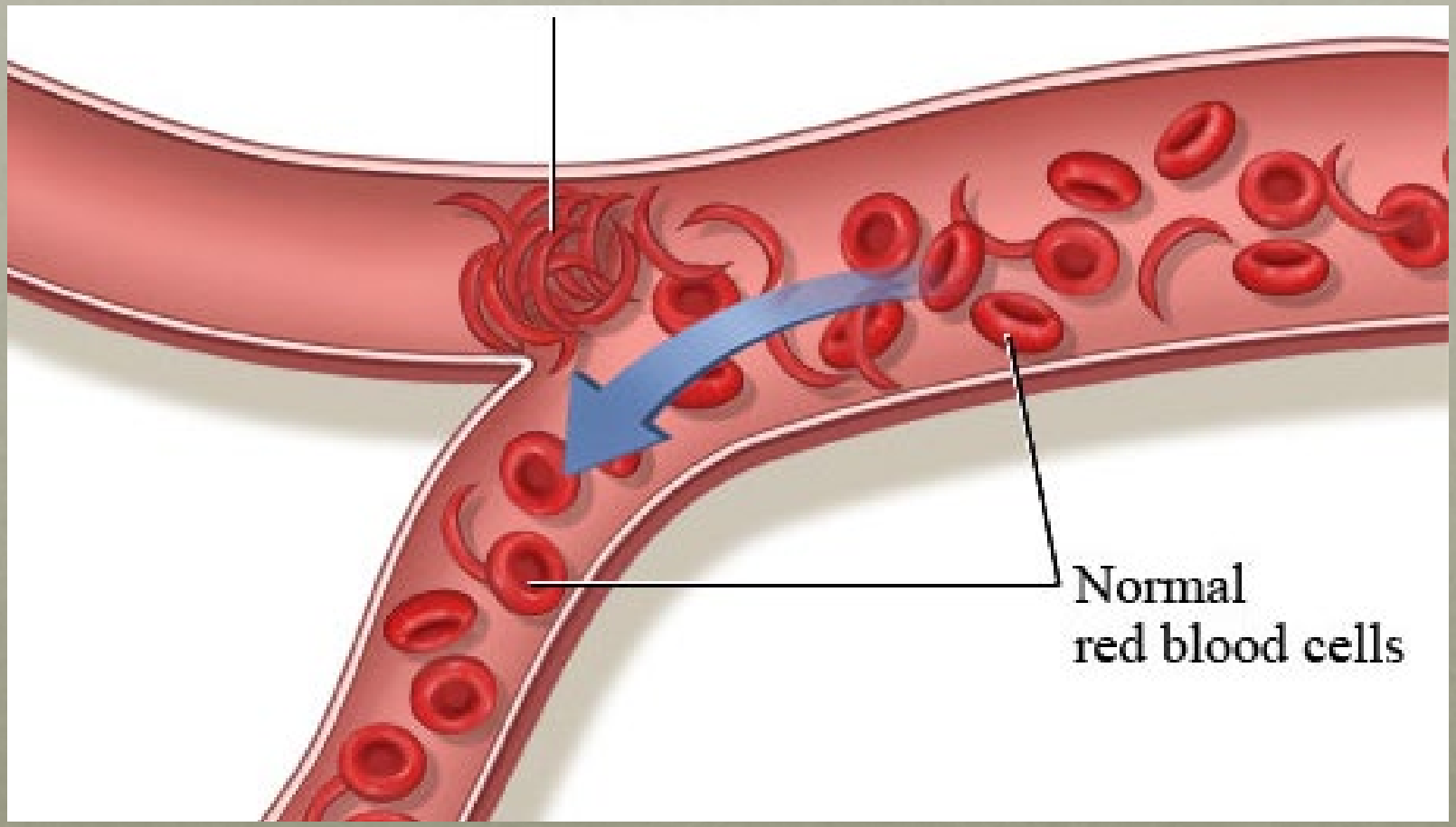
- Hemoglobinopathies represent the most common genetic disease worldwide
- Canada's ethnic diversity has resulted in an increasing frequency of SCD and thalassemia major, and an increased number of carriers
- Life expectancy of patients with SCD is reduced by ~25 years compared to normal population. Their QoL is also severely affected.

MANIFESTATIONS

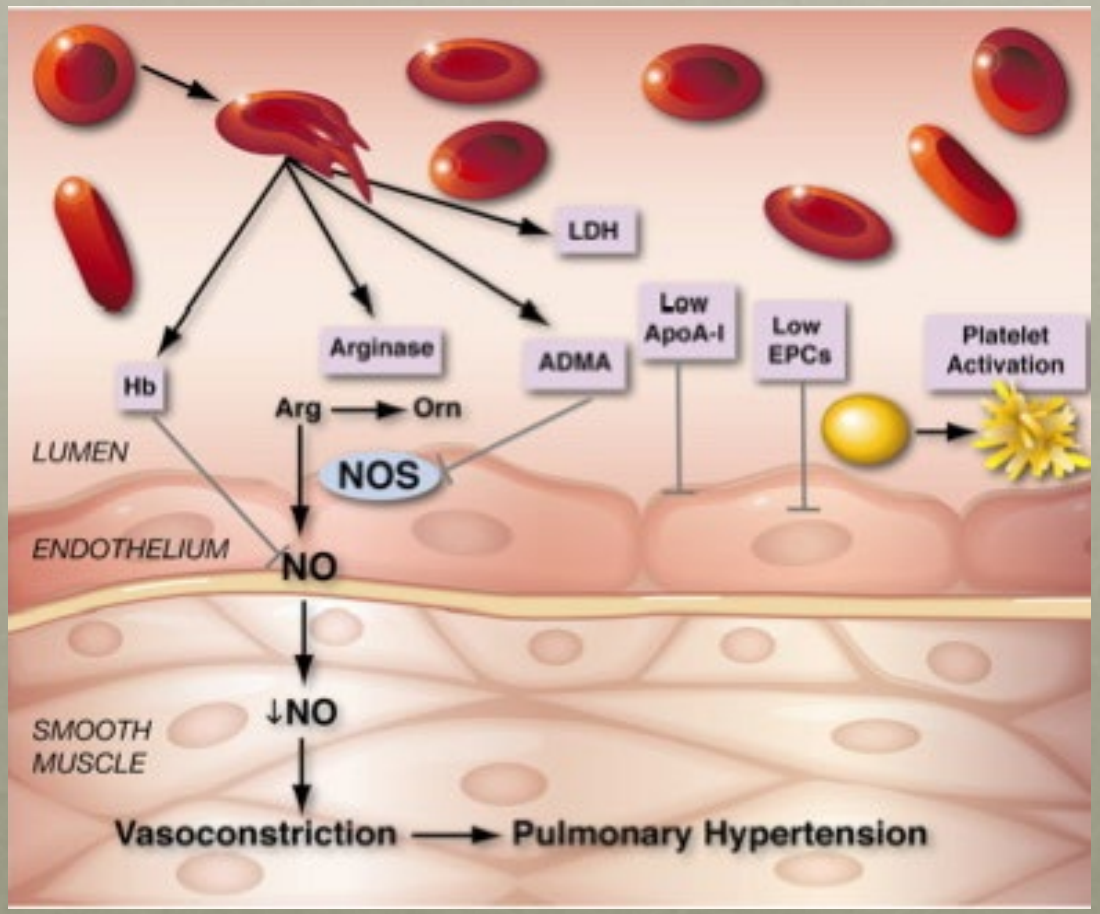
- Anemia
- Vaso-occlusive symptoms:
  - Pain
  - Acute Chest Syndrome
  - Dactylitis
  - Stroke
  - Splenic sequestration
  - Acute tubular necrosis
- Infectious risk



VASO-OCCLUSION

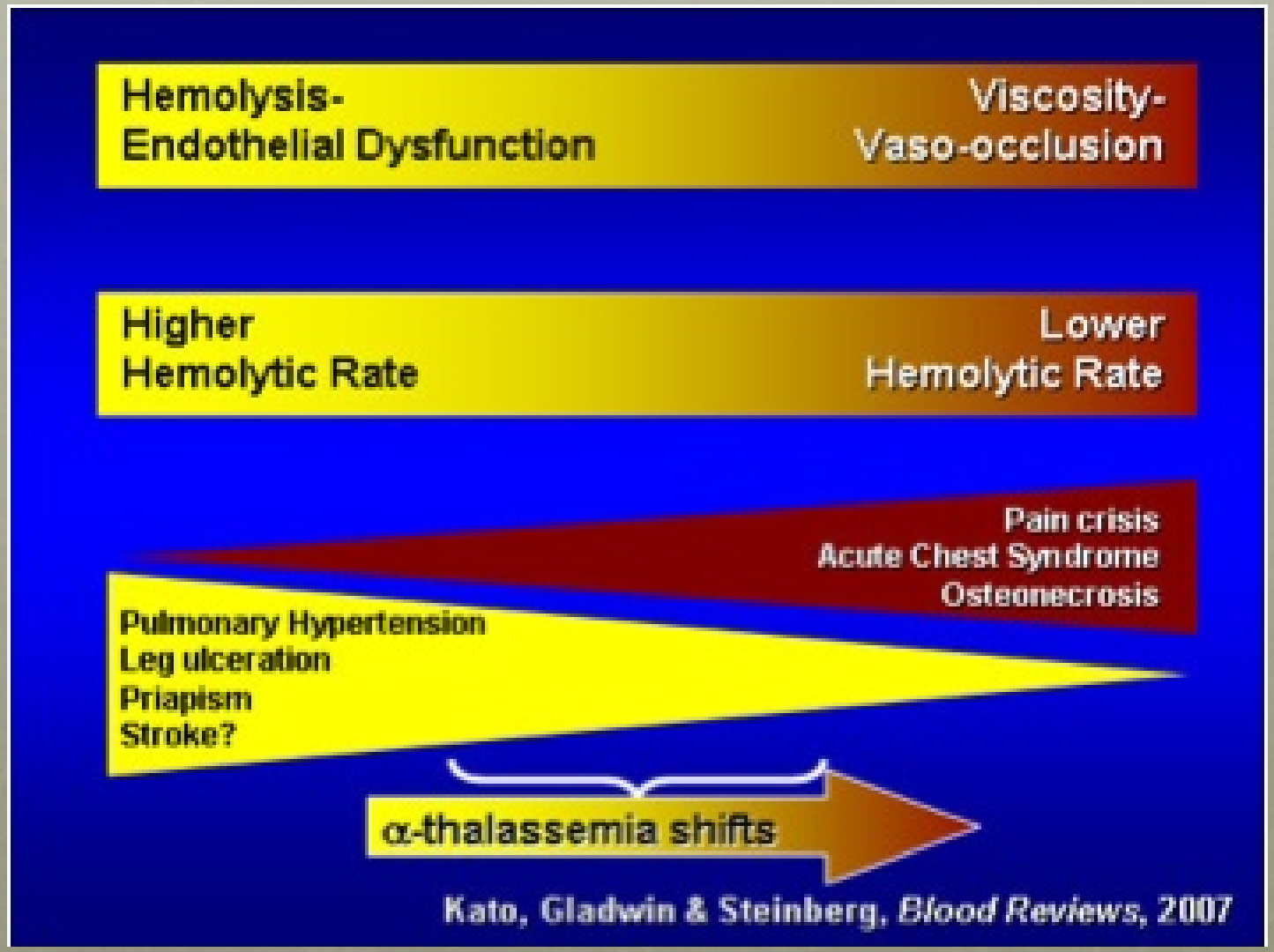


VASO-CONSTRICTION



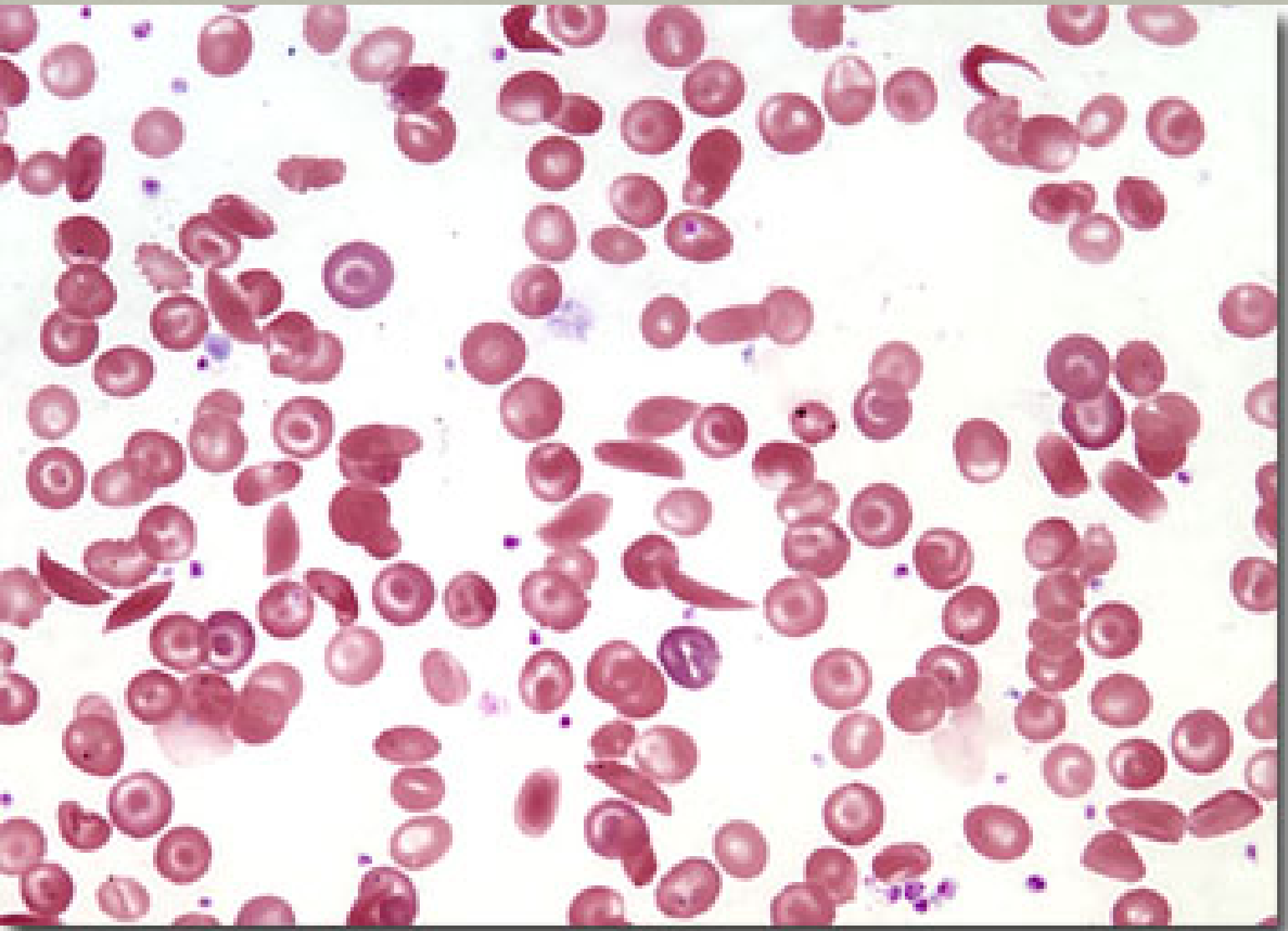
- Release of RBC contents leads to depletion of NO
  - Hemolysis contributes to vasoconstriction

PATHOPHYSIOLOGIC SPECTRUM



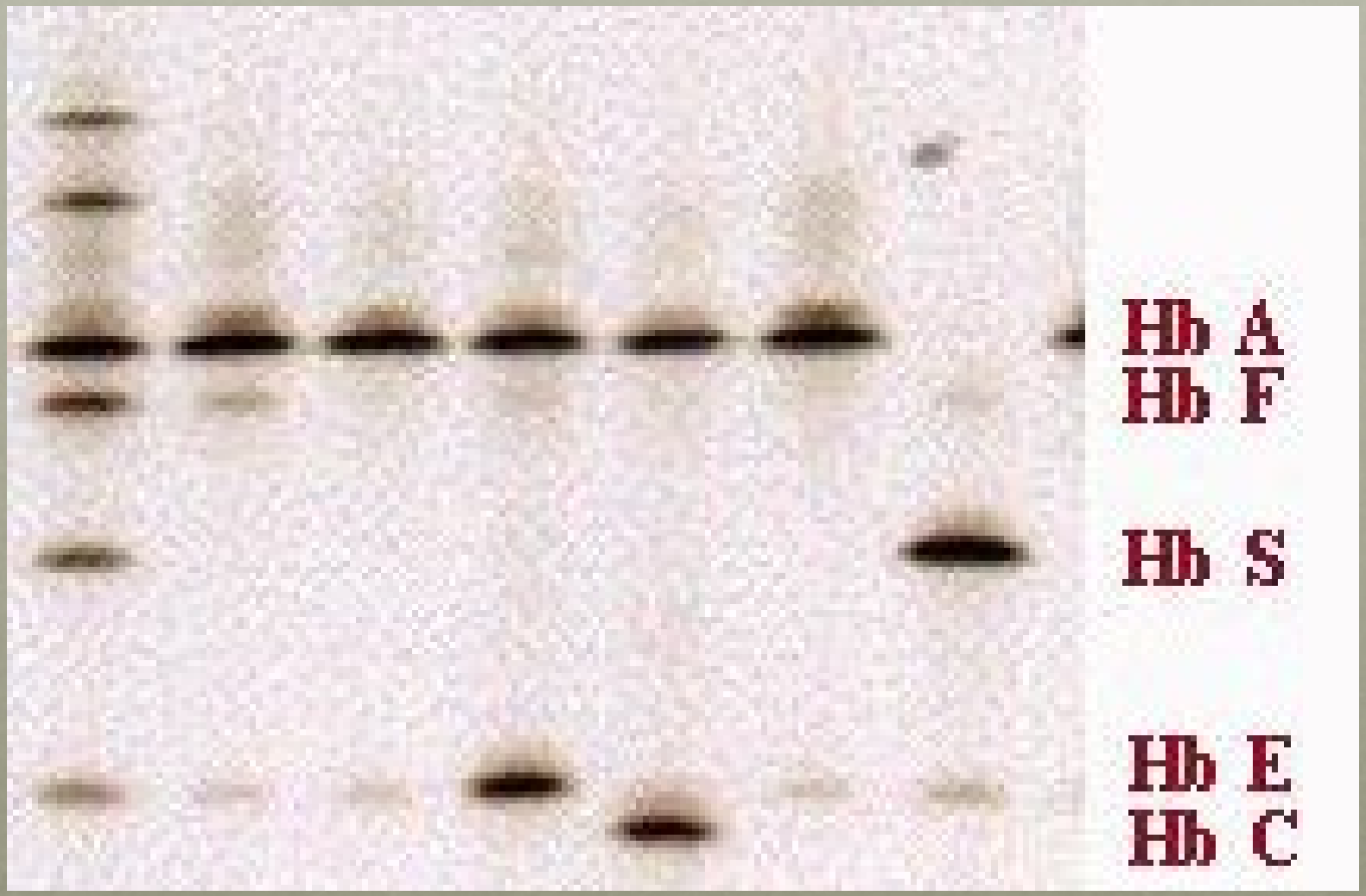
HEMOGLOBINOPATHY TESTING

- Screening tests:
  - Hb solubility test (Sickledex)
    - Sensitive but not specific
  - Complete blood count and smear

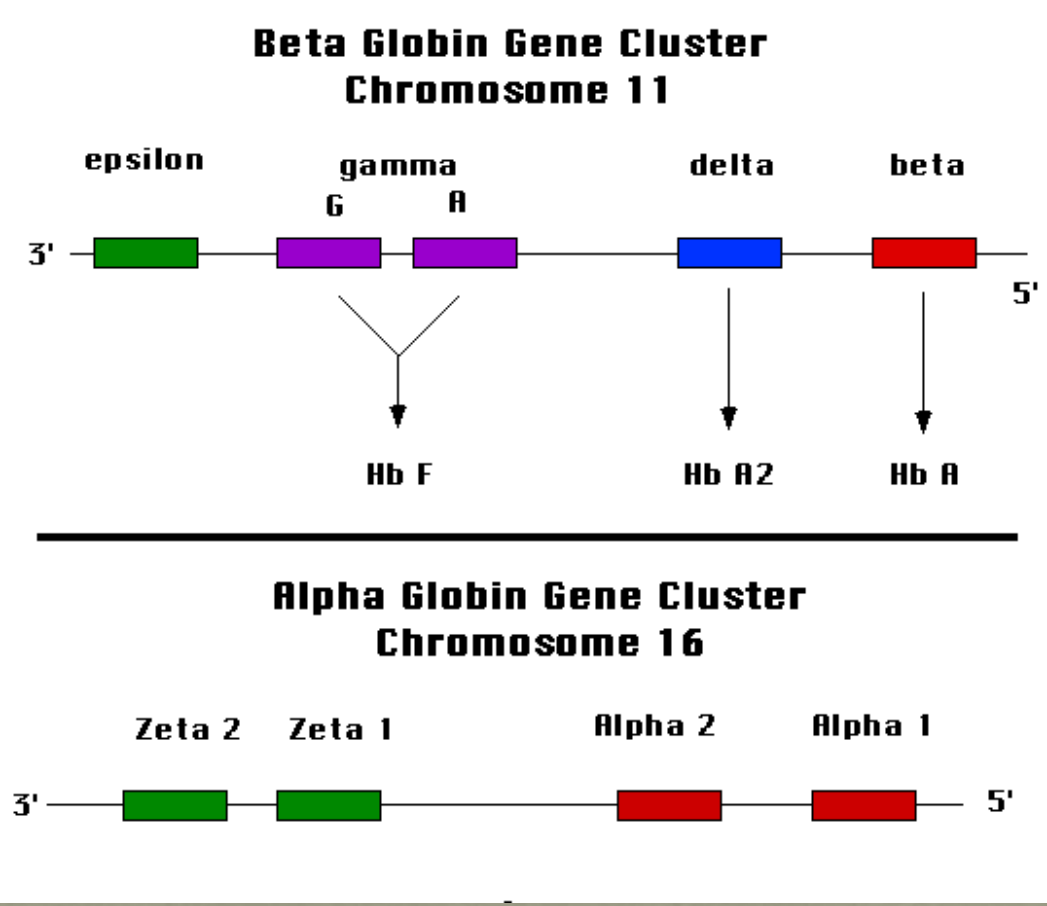


CONFIRMATORY TESTS

- Different modalities all based on separation of hemoglobins by molecular size and charge:
  - Isoelectric focusing
  - Electrophoresis
  - Capillary Zone Electrophoresis
  - High Purity Liquid Chromatography


Hb A
Hb F
Hb S
Hb E
Hb C

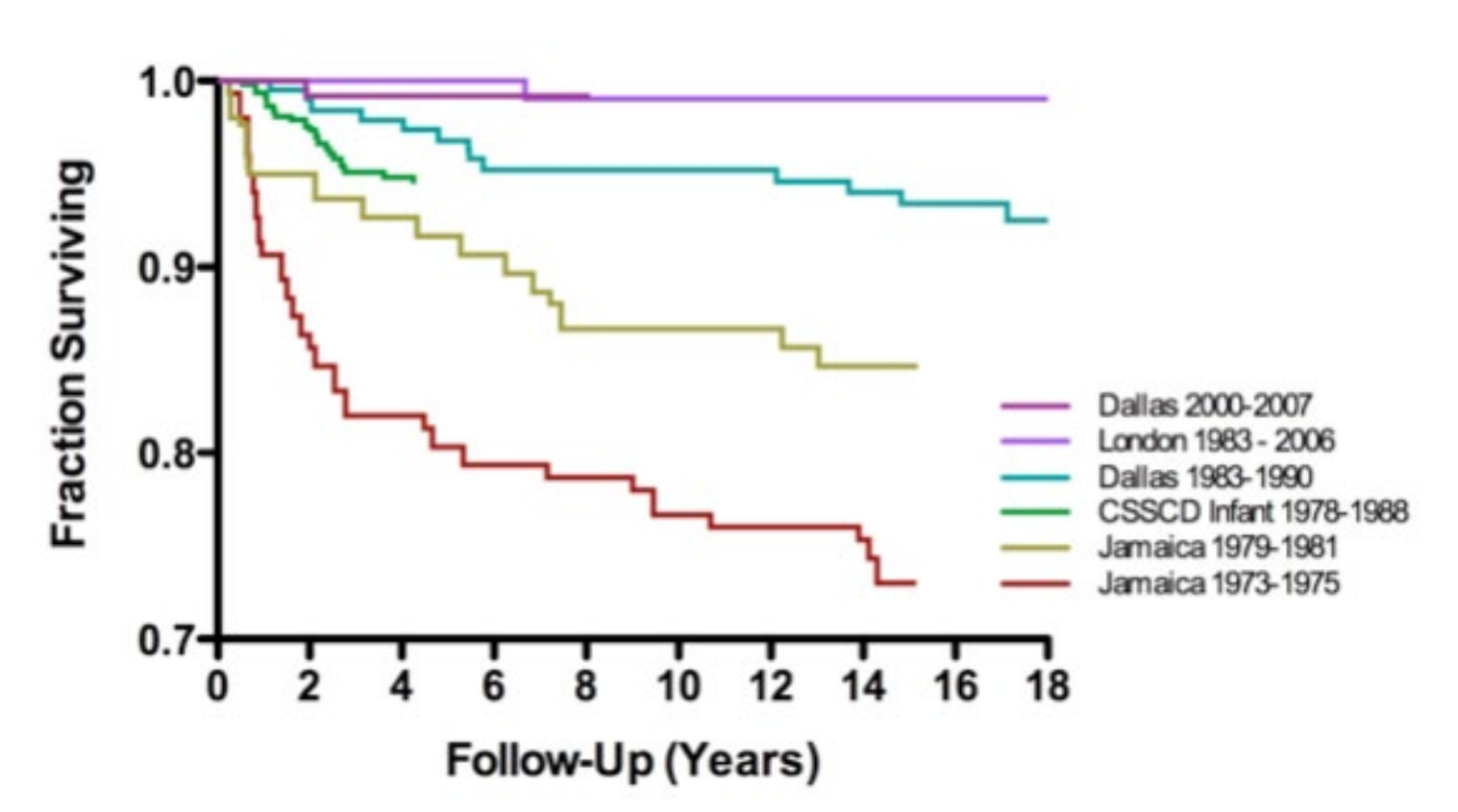
GENOTYPING



- Unequivocal results
- Useful in complex compound hemoglobinopathies or unusual FMHx
- Only available in Hamilton

WHO IS AT RISK?

- There are no good predictors of who will have high risk disease
  - Models may be highly cohort specific



ACUTE CHEST SYNDROME

- #1 cause of mortality, #2 cause of hospitalization in SCD
- Definition: New pulmonary infiltrate and at least three of: fever (> 38.5 deg), respiratory distress, chest pain, cough, wheeze
- ~50% of patients admitted for other complaints
  - Pain as a prodrome of the ACS
- Specific etiology identified in 38% of cases

Stroke in Sickle Cell Disease

- 11% risk of stroke by age 20; 24% by age 45
- Greatest risk from ages 2-5 – Approaches 1%/year
  - RR of stroke is 333 for children with SCD compared to healthy children
  - Mostly ischemic strokes from stenosis or occlusion of the large vessels
- 10-30% of patients with SCD have evidence of silent strokes on their MRI
  - 13% of infants with SCD had evidence of silent stroke

Verduzco and Nathan, Blood, 2009

MANAGEMENT

- Supportive care
  - Folic acid
  - Penicillin
  - Oxygen, hydration
  - Education and support
- Analgesia
  - Usually narcotics
- Transfusion
  - Simple
  - Chronic
  - Exchange
- Disease modifying agents
  - Hydroxyurea
  - Rivipansel
  - Hb F inducers
- Bone marrow transplantation

BLOOD TRANSFUSION

- Three options
  - Simple – Increase oxygen carrying capacity and decrease percentage of sickled cells
  - Chronic – Suppress hematopoeisis to maintain Hb S at less than 30%
  - Exchange – Acutely remove the sickled cells and replace with donor RBCs to achieve Hb S less than 30%
    - Automated or manual

RISKS OF TRANSFUSION

- Iron overload
  - Chelation therapy will be required
- Allo-immunization
  - Estimated 30% with simple transfusion
  - Value of extended RBC phenotyping?
- Infectious diseases
- Patient preferences/compliance

HYDROXYUREA

- Hydroxyurea approved in 1998 for adults with SCD
  - Induction of fetal Hb
  - Reduction of WBC, inflammatory mediators, cell-adhesion molecules than contribute to endothelial damage
  - Improved NO metabolism

- HUSOFT and HUG-KIDS studies showed that hydroxyurea was well tolerated and safe in infants and children, with some evidence of hematologic benefit

Strouse et al, Pediatr, 2008; Thompson et al, Pediatr Blood Cancer, 2010

- BABY-HUG – RCT in infants with SCD
  - Entry at 9-18 months
  - Primary endpoints of splenic and renal damage as surrogate markers of organ damage

- Unable to meet primary endpoints, but showed statistically significant improvement in hematologic parameters and decrease in acute crises

- HU should be considered for all young children with SCD

Wang et al, Lancet, 2011

BARRIERS TO HYDROXYUREA USE

- Concerns about efficacy
  - Uncertain of risks/benefits
- Concerns about impact on family
  - Taking pills, cost of medications, blood monitoring
- Concerns about potential side effects
  - Carcinogenicity

Oyeku et al, 2013

NOVEL AGENTS

- Rivipansel: 'Rationally designed' selectin inhibitor which decreased leukocyte adhesion to vascular endothelium at sites of inflammation
  - Potential role in decreasing pain in vaso-occlusive crisis
- Hb F Induction: Discovery of RBC-specific enhancer BCL 11A, which regulates the production of Hb F
  - Constitutionally weak in patients with Hereditary Persistence of Fetal Hemoglobin, inhibition might enhance Hb F

HEMATOPOEITIC STEM CELL TRANSPLANTATION

- The only potential cure for sickle cell disease
  - Has been more extensively investigated and used in thalassemia

- Several barriers to transplant in SCD:
  - Lack of information and limited understanding
  - Lack of HLA-suitable donors
  - Risk of transplant related toxicities

Thompson et al, 2013

GENE THERAPY

Table 1. Gene therapy clinical trials for TDT and SCD patients

Trial number	Phase	Sponsor	Site	Start date/ recruitment status	Number of patients	Vector and transgene (nuclease and DP name)	Cell source	Conditioning	DP administration	Last update (www.clinicaltrials.gov)	References
β-Thalassemia											
LG001	1/2	bluebird bio	France	September 2006/ completed	2*	HPV569 (β^{A-T87Q}-globin)	G-CSF mPBCs or BM	Myeloablative (busulfan)	IV	NA	26
NCT01639690	1	Memorial Sloan Kettering Cancer Center	United States	July 2012/active, not recruiting	4	TNS9.3.55 (β^{A}-globin)	G-CSF mPBCs	Nonmyeloablative (busulfan 8 mg/kg)	IV	6 June 2018	113
NCT02151526 (HGB205)	1/2	bluebird bio	France	July 2013/active, not recruiting	4	BB305 (β^{A-T87Q}-globin)	G-CSF + plerixafor mPBCs	Myeloablative (busulfan)	IV	31 January 2019	28
NCT01745120 (HGB204)	1/2	bluebird bio	United States, Australia, Thailand	August 2013/ completed	18	BB305 (β^{A-T87Q}-globin)	G-CSF + plerixafor mPBCs	Myeloablative (busulfan)	IV	8 May 2019	28
NCT02453477	1/2	IRCCS San Raffaele	Italy	May 2015/active, not recruiting	10	GLOBE (βA-globin)	G-CSF + plerixafor mPBCs	Myeloablative (thiotepa + threosulfan)	IO	4 May 2018	33
NCT02906202 (HGB207)	3	bluebird bio	United States, France, Germany, Greece, Italy, Thailand, United Kingdom,	July 2016/recruiting	23 (estimated)	BB305 (β^{A-T87Q}-globin)	G-CSF + plerixafor mPBCs	Myeloablative (busulfan)	IV	31 January 2019	31
NCT02906202 (HGB212)	3	bluebird bio	United States, France, Germany, Greece, Italy, Thailand, United Kingdom	June 2017/recruiting	15 (estimated)	BB305 (β^{A-T87Q}-globin)	G-CSF + plerixafor mPBC	Myeloablative (busulfan)	IV	31 January 2019	31
NCT03432364	1/2	Sangamo Therapeutics and Bioverativ Therapeutics	United States	February 2018/ recruiting	6	ZFN (ST-400)	mPBCs	Myeloablative (busulfan)	IV	4 February 2019	NA
NCT03655678	1/2	Vertex Pharmaceuticals and CRISPR Therapeutics	Germany, United Kingdom	September 2018/ recruiting	12 (estimated; may be expanded to 45)	CRISPR/Cas9 (CTX001)	$CD34^+$ human HSPCs (mobilization: NA)	Myeloablative (busulfan)	IV	3 May 2019	NA
SCD											
NCT02151526 (HGB205)	1/2	bluebird bio	France	July 2013/active, not recruiting	3	BB305 (β^{A-T87Q}-globin)	BM	Myeloablative (busulfan)	IV	31 January 2019	37
NCT02186418	1/2	Children's Hospital Medical Center, Cincinnati	United States, Jamaica	July 2014/recruiting	10	sGbG (γ-globin)	BM and plerixafor mPBCs	Reduced intensity conditioning (melphalan 140 mg/m^2 BSA)	IV	6 May 2019	44
NCT02247843	1	University of California Children's Hospital, Los Angeles	United States	July 2014/recruiting	6	βAS3-FB (β^{A-T87Q}-globin)	BM	Myeloablative (busulfan)	IV	29 March 2019	NA
NCT02140554 (HGB206)	1	bluebird bio	United States	August 2014/ recruiting	50 (estimated; 3 groups [A, B, C])	BB305 (β^{A-T87Q}-globin)	BM (A and B) plerixafor mPBCs (C)	Myeloablative (busulfan)	IV	20 May 2019	43
NCT03282656	1	David Williams, Boston Children's Hospital	United States	February 2018/ recruiting	7	BCH_BB-LCR shRNA(miR) shRNAmiR	Plerixafor mPBCs	Myeloablative (busulfan)	IV	24 May 2018	54
NCT03745287	1/2	Vertex Pharmaceuticals Incorporated and CRISPR Therapeutics	United States	November 2018/ recruiting	12 (estimated; may be expanded to 45)	CRISPR/Cas9 (CTX001)	NA	Myeloablative (busulfan)	IV	3 May 2019	NA

BM, bone marrow; BSA, body surface area; CRISPR, clustered regularly interspaced short palindromic repeat; DP, drug product; G-CSF, granulocyte-colony stimulating factor; IO, intraosseously; mPBC, mobilized peripheral blood cell; NA, not available; shRNA, short hairpin RNA; ZFN, zinc-finger nuclease.

*P1 failed to engraft and received the backup cells.

Magrin et al, Blood 2019

NEWBORN SCREENING

Gaston, M. H., et al. (1986). "Prophylaxis with oral penicillin in children with sickle cell anemia. A randomized trial." New England Journal of Medicine **314**(25): 1593-1599.

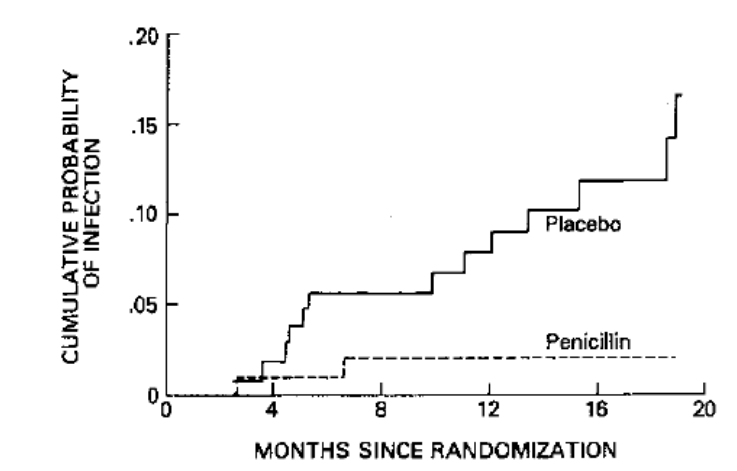


Table 2. Rate of *Streptococcus pneumoniae* Septicemia per 100 Person-Years, According to Treatment Group.

Age (Yr)	Rate of Infection*	
	Penicillin Group	Placebo Group
<1	0.0 (12.2)	20.1 (14.9)
1	2.2 (45.9)	9.1 (43.8)
2	0.0 (48.2)	10.8 (46.5)
3†	3.4 (29.8)	3.6 (27.6)
Total	1.5 (136.1)	9.8 (132.8)

- The trial was terminated 8 months early:
  - 84% reduction in the incidence of infection
  - 0 deaths from septicemia occurring in the Penicillin group, but 3 deaths from infection in the placebo group

- In 1987, NIH declared that new born screening when linked to timely diagnosis, parental education and comprehensive care reduces morbidity and mortality from SCD in infancy and childhood.

- Since then, universal newborn screening has become standard in all 50 states in US, UK and Brazil

ECONOMIC IMPACT

- Significant burden on healthcare resources, as well as reduced contribution to society by those affected
  - US study evaluated the cost of SCD care to be US$ 900-2500 per patient per month, and US$ 500,000 lifetime
- Newborn screening is essential:
  - Decreases infant mortality by allowing introduction of early antibiotic prophylaxis and other simple interventions
  - Early introduction of disease modifying therapies will reduce rate of acute and chronic complications

Am. J. Hematol. 84:323–327, 2009

NATIONAL EFFORTS

CanHaem

National physician group founded in 2013

- Consensus statement on Sickle Cell disease (2014, 2018)
- Consensus statement on Thalassemia (2009, 2018)

www.canhaem.org

SCDAC

National patient umbrella organization founded in 2012

- Universal screening for SCD and Thalassemia
- Accurate epidemiologic data
- Access to specialist and comprehensive care

www.sicklecelldisease.ca

LOCAL EFFORTS

CancerCare MB

- Active participants in the Sickle Transplant Advocacy and Research (STaR) Alliance
  - HSCT trial launching soon
  - Lead the country in BMT for pediatric sickle cell disease
- Active UGME and PGME research projects
- Awarded $105 000 for STaR initiative and related activities
  - Grant to launch a dedicated hemoglobinopathy program

Sickle Circle Manitoba

- Provincial patient advocacy group, affiliated with the SCDAC
- Annual fundraising walk
- Increasing patient outreach

www.sicklecirclemb.com

CADHAM LAB

- Hemoglobinopathy screening is next on the list for addition to the provincial newborn screening program
- Business case proposal with Manitoba Health for funding approval
- Culmination of many years of advocacy

"Ask and you shall receive"

OBJECTIVES

- By the end of this presentation, you should be able to:
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  - Describe the diagnostic investigation of hemoglobinopathies
  - Discuss acute and chronic management strategies for patients with hemoglobinopathies
  - Evaluate the role of newborn screening for sickle cell disease and other hemoglobinopathies

QUESTIONS?


So I'd like you to just explain your views on uranium waste, and maybe draw some parallels to Beowulf, and then explain how that relates to Roman culture with respect to the lessons we find in Shakespearean tragedies.