Thrombogenesis in Sickle Cell Anemia

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HSS Day July 19th 2024

Learning Outcomes

Understanding the Pathophysiology

Diagnosis/Treatment

Sickle Cell Crisis

Complications - Thrombosis:

Hypercoagulable State in SCD

Markers of Thrombin Generation & Anticoagulant Proteins

Role of Platelets, Endothelial Cells, & Microparticles

Implications of TNF R1 and TNF Alpha

Management of Venous Thromboembolism (VTE) in SCD

Current and Potential Antithrombotic Therapies

Conclusions

Pathophysiology

Hemoglobin Abnormality:

- Caused by the production of abnormal hemoglobin such as Hemoglobin S (HbS)

Red Blood Cells (RBCs):

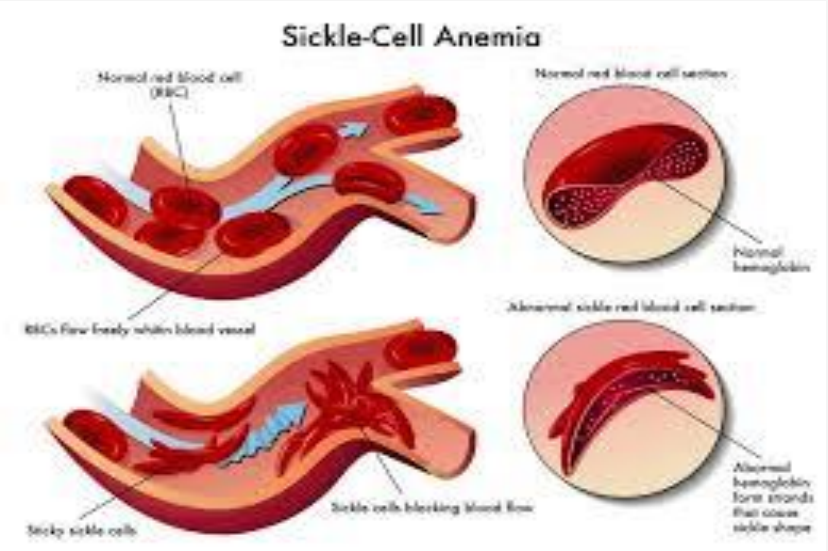
- Become rigid, sticky, and sickle-shaped
- Block blood flow

Cell Lifespan:

- Typical RBC life: 120 days
- Sickle cell life: 10-20 days

Condition:

- Anemic condition caused



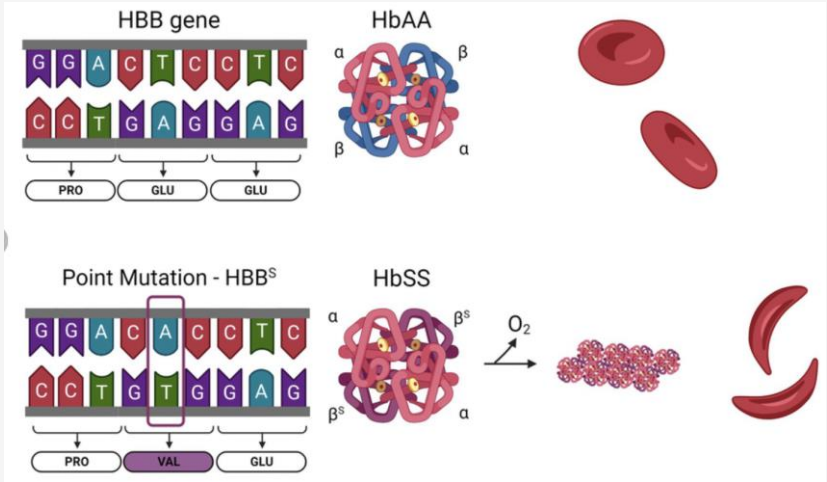
Pathophysiology Cont.

HBB Gene Mutation:

- Produces abnormal hemoglobin S (HbS)
- Single nucleotide substitution (adenine to thymine)
- Glutamic acid replaced by valine at the sixth position of the beta-globin chain

Effects:

- HbS polymerizes under low oxygen, less soluble
- Normal hemoglobin (HbA) remains soluble



Epidemiology

Most Prevalent in Individuals of:

- African
- Mediterranean
- Middle Eastern
- Indian Ancestry

Statistics:

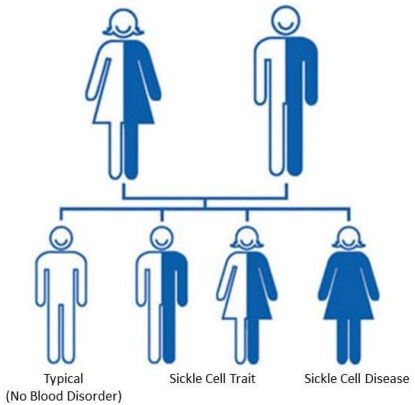
- 1 in 365 African-Americans have SCA
- 1 in 13 African-Americans carry the trait

Connection:

- Higher prevalence in regions with high rates of Malaria

Sickle Cell Trait & Hemoglobin Effects

- **Hemoglobin** is the iron-rich compound in red blood cells that allows cells to carry oxygen from the lungs to the rest of the body.
- **One copy** of the sickle cell gene
- **One normal** hemoglobin gene
- Abnormal hemoglobin s, gene beta (β) thalassemia, hemoglobin C, hemog hemoglobin E



Endothelium Interaction

Interaction with Endothelium:

- Sickled cells interact with the endothelium (inner lining of blood vessels), triggering inflammatory responses.

Adhesion and Binding:

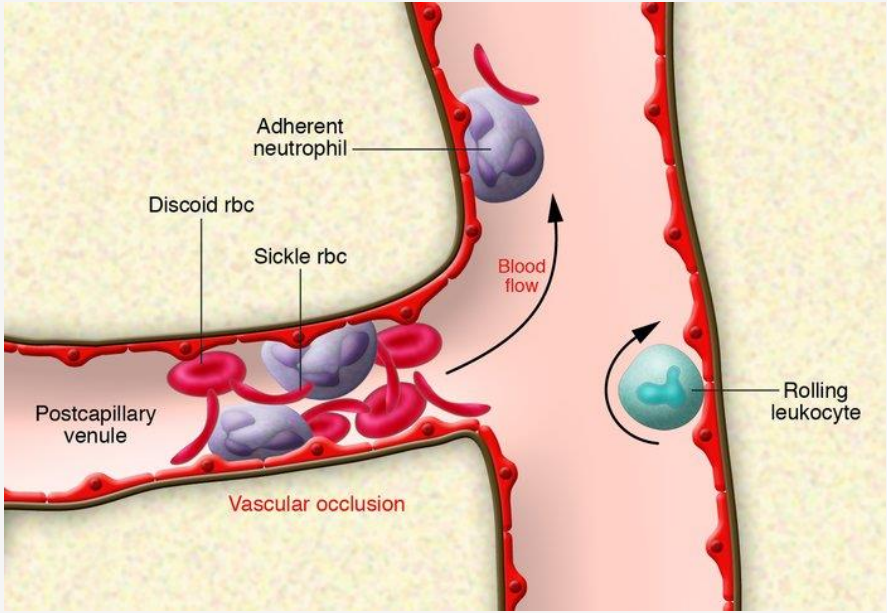
- Adhesion molecules on sickled cells and endothelial cells facilitate binding, worsening vascular occlusion.

Inflammatory Effects:

- Promotes endothelial damage
- Increases vascular permeability
- Activates coagulation pathways

Outcome:

- Contributes to the pro-thrombotic state observed in sickle cell anemia



Phospholipid Asymmetry

Phospholipid Asymmetry:

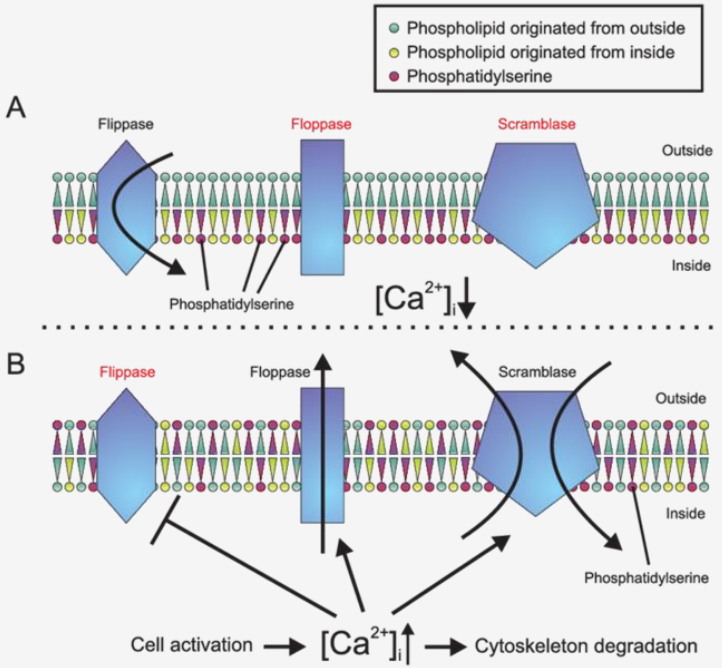
- Normally, phospholipids are arranged in a bilayer with amine-containing phospholipids in the inner layer.

Disruption:

- In sickle cell patients, this arrangement is interrupted, leading to membrane changes.

Effects:

- Decreased production of protein C and protein S



Symptoms

- **Fever**
  - High body temperature indicating infection or inflammation.
- **Blindness**
  - Partial or complete loss of vision.
- **Leg Ulcers**
  - Open sores on the legs due to poor circulation.
- **Organ Damage**
  - Impaired function of organs like the liver, kidneys, or heart.
- **Acute Chest Syndrome**
  - Severe chest pain, coughing, and difficulty breathing.
- **Stroke**
  - Sudden interruption of blood supply to the brain.
- **Avascular Necrosis**
  - Death of bone tissue due to lack of blood supply.
- **Anemia**
  - Reduced red blood cells causing fatigue and weakness.

Diagnosis

Blood and Genetic Tests:

- Hemoglobin S testing
- Hemoglobinopathy screening

Prenatal Testing:

- Prebirth sample of amniotic fluid

Newborn Screening:

- Included in newborn screening programs

Additional Tests:

- Complete blood count (CBC)

Treatment

Bone Marrow Transplants:

- Used in select cases to replace diseased bone marrow with healthy donor marrow.

Genetic Therapy:

- FDA-approved therapies targeting genetic causes of sickle cell disease.

Transfusions:

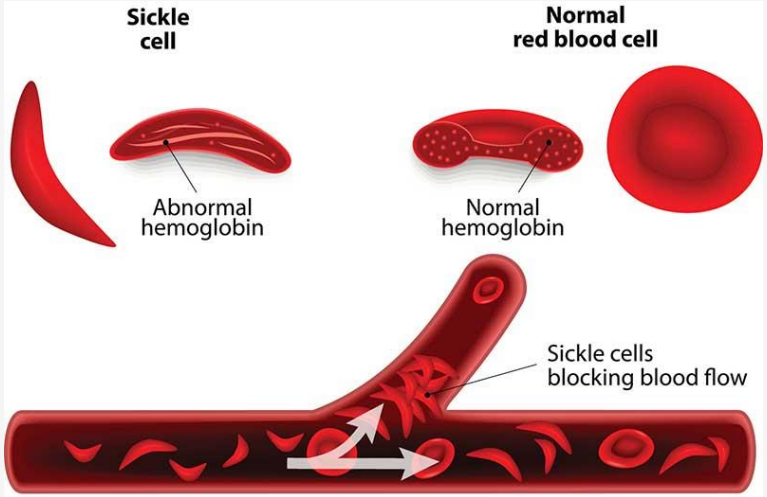
- Used to treat and alleviate symptoms by providing healthy red blood cells.

Medications:

- Hydroxyurea, Voxeltor, Crizanlizumab-ymca
- Over-the-counter medications for symptom management

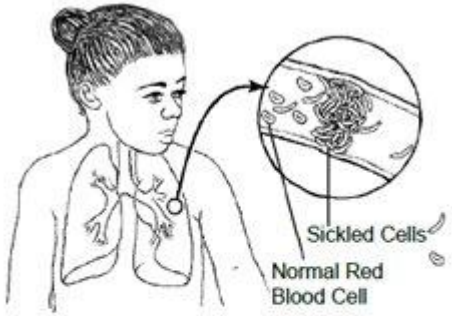
Sickle Cell Crisis

- Blood flow is **blocked** in an area because the affected and damaged cells have become stuck in the blood vessel
- **Immense pain** often occurring in the chest, arms, and legs
- **Blood flow interruption** can cause tissue and healthy cell death
- More severely in children
- Impaired organ functions
- A condition in which the bone marrow of someone with a condition such as sickle cell disease suddenly stops producing RBCs
- Complications: Vaso-occlusive crisis, myocarditis, splenetic sequestration, Acute Chest Syndrome



Sickle Cell Crisis - Acute Chest Syndrome

- A severe and potentially fatal complication of sickle cell disease
- Sickle red blood cells block blood vessels in the lungs, causing a pneumonia-like illness.

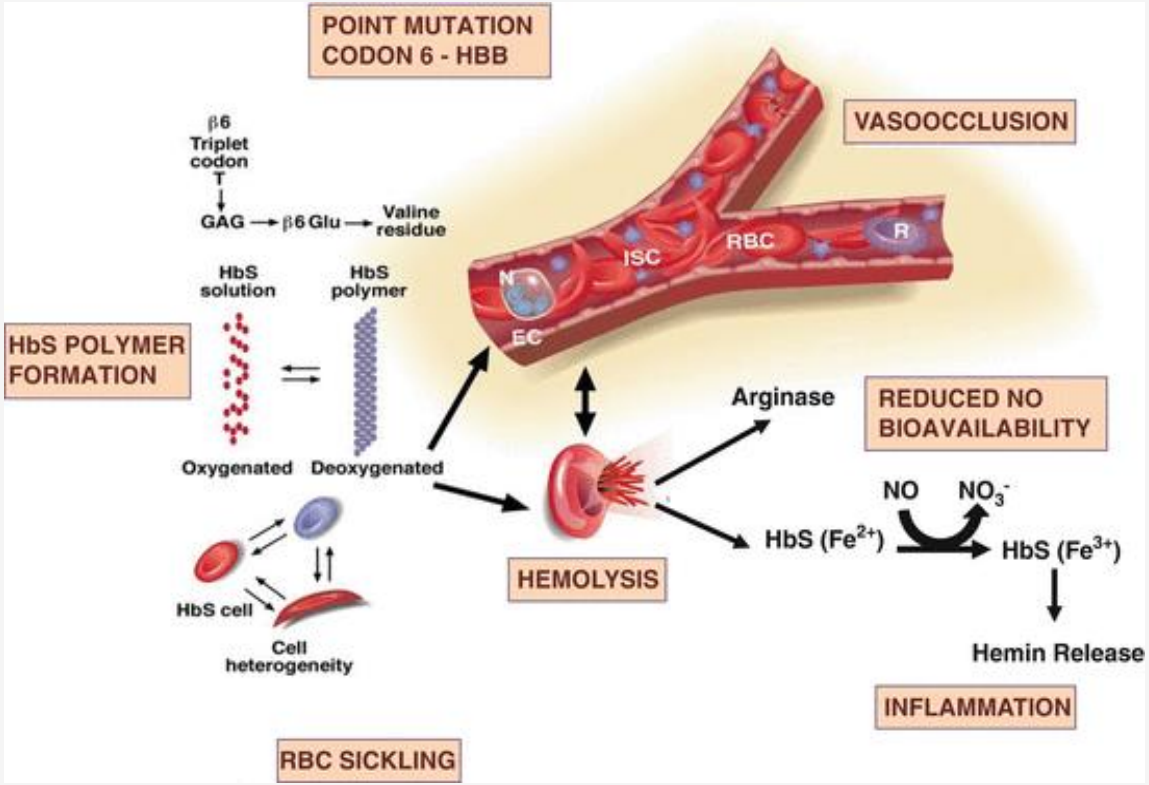


Picture 1 Sickled cells can clump together in the lungs, causing pain and trouble breathing.

Complications in Sickle Cell Disease

- **Sickle prothrombotic state** caused by sickle hemoglobin
- Responsible for other thrombotic events such as venous thromboembolism or strokes
- Risk factor increases with age
- Complications increase mortality rates
- New therapies have been developed to specifically target these complications

Prothrombotic State Observed in SCA



Sickle Cell Disease and acquired Hypercoagulable State

- Chronic hemolysis involvement
- Chronic inflammation and oxidative stress
- Elevated levels of pro-inflammatory cytokines
- Elevated levels of plasminogen inhibitor-1
- Increased thrombin generation and impaired fibrinolysis
- Enhanced platelet activation
- The release of procoagulant microparticles
- These all factors increase the risk of thrombotic complications in SCD patients

Hypercoagulability in SCD Patients Studies

Study produced by Naik et al:

- A 25% incidence of VTE by mean age 30
- Cumulative incidence rate of VTE is 11.3% by age 40
- Recurrence rate of VTE greater than 35% within 5 years.

Study produced by Austin et al:

- The presence of sickle hemoglobin is the main cause of a hypercoagulable state.
- The risk of VTE is increased approx. 2 fold among SCT patients.

Impaired Fibrinolysis in SCD Patients

- A result of chronic hemolysis and endothelial dysfunction which together create a pro-thrombotic environment
- Activated endothelial cells express higher levels of tissue factor, promoting thrombin generation
- Elevated thrombin levels not only facilitate clot formation but also impair fibrinolysis by controlling the fibrinolytic system

Alteration in Markers of Thrombin Generation & Anticoagulant Proteins

- Patients with SCD have increased plasma levels of markers of thrombin generation in the non-crisis steady state.
- Plasma levels of F1.2, TAT, D-dimers (inflammatory marker), PAP complexes, and fibrinopeptide A28 are elevated in patients with SCD
- The frequency of pain episodes in patients with SCD correlates with the extent of fibrinolytic activity (measured by d-dimer levels). This suggests that D-dimer levels may predict the frequency of pain crises.

Decreased Levels of Natural Anticoagulants

- Increased thrombin generation resulting from RBC prothrombinase activity.
- Increased binding of protein S by sickle RBC due to membrane PS exposure and inhibition of the binding of protein S to β2-glycoprotein 1 by antiphospholipid antibodies resulting in inactivation of protein S by circulating C4b-binding protein.
- Study by Tam DA - “Significantly decreased levels of proteins C and S were reported in patients with SCD who developed thrombotic strokes compared with neurologically normal children with SCD."

Platelets, Endothelial Cells, & Microparticles in SCD

- **Increased Circulating Endothelial Cells:** Higher numbers in steady state, even more during acute pain episodes.
- **Adhesion Marker Expression:** Cells express ICAM-1, VCAM-1, E-selectin, and P-selectin.
- **Tissue Factor (TF) Antigen:** Abnormally expressed in steady state; increased during acute pain episodes.
- **TF mRNA:** Circulating endothelial cells show TF mRNA, correlating with TF antigen and mRNA expression.

Platelets, Endothelial Cells, & Microparticles in SCD Cont.

- **Microparticles (MPs):** Small particles from cells after apoptosis; can come from RBCs, platelets, or endothelial cells. Higher numbers of MPs and TF-positive MPs in SCD patients, mainly from endothelial cells.
- **Coagulation Markers:** Plasma markers like D-dimer, TAT, and F1.2 correlate with total MP, TF-positive MP, monocyte-derived TF-positive MP, and RBC-derived MP.
- Suggests a role for MP in the hypercoagulable state observed in patients with SCD.

Study on Microparticles

- According to a study done by Shet, et al, attempting to prove that sickle blood might contain TF-positive MPs derived from these cells:
- **Total MPs (derived from erythrocytes (RBCs), platelets, monocytes (WBCs), and endothelial cells) elevated:**
  - **Crisis:** P = 0.0001
  - **Steady State:** P = 0.02
- **Erythrocyte-Derived MPs:**
  - **Crisis:** P = 0.0001
  - **Steady State:** P = 0.02
- **Monocyte-Derived MPs:**
  - **Crisis:** P = 0.0004
  - **Steady State:** P = 0.009

Sickle blood contains tissue factor-positive microparticles derived from endothelial cells and monocytes

Arun S Shet [1], Omer Aras, Kalpna Gupta, Mathew J Hass, Douglas J Rausch, Nabil Saba, Louann Koopmeiners, Nigel S Key, Robert P Hebbel

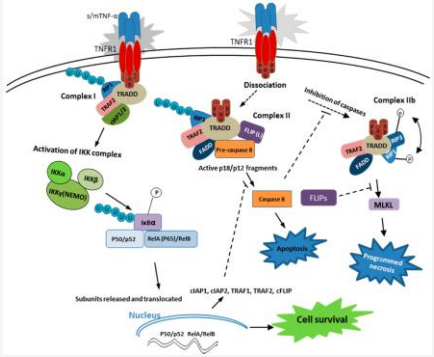
Study Cont.

- **Total TF-Positive MPs, derived from monocytes and endothelial cells, elevated:**
  - **Crisis vs. Steady State: P = .004**
  - **Crisis vs. Control Subjects: P < .0001**
- **Procoagulant Activity:**
  - Sickle MPs shortened plasma-clotting time compared to control MPs.
  - TF antibody partially inhibited this activity.
- **Coagulation Markers:** Elevated in SCD patients vs. control subjects.
  - **Total MPs:** P < .01
  - **TF-Positive MPs:** P < .01
- Support the concept that SCD is an inflammatory and procoagulant state with monocyte and endothelial activation and abnormal TF activity.

TNF R1 Study 1

Background Information

- **TNF-R1 (Tumor Necrosis Factor Receptor 1)**: A receptor for TNF-alpha, playing a critical role in mediating the effects of TNF-alpha, including inflammation, apoptosis, and immune response.
- **Role in Sickle Cell Anemia (SCA)**:
  - TNF-R1 binds TNF-alpha, triggering signaling pathways that lead to inflammation and cell death.
- **Significant Difference**: The p-value of 0.0014 indicates a significant difference in TNF-R1 levels between patients with mild and severe conditions.
- **Implications**:
  - Higher TNF-R1 levels in severe conditions suggest that TNF-R1 may be a marker of disease severity in SCA.
  - Elevated TNF-R1 could reflect increased TNF-alpha activity, contributing to more severe inflammation and complications.



Biomarker	Mild Median		Mild SD	Severe Median		Severe SD	P-Value
E-Selectin	81.60	±	37.46	79.73	±	77.29	8.9E-01
ICAM-1	320.40	±	122.33	354.55	±	150.80	2.7E-01
NO	15.76	±	11.15	11.37	±	9.43	4.1E-01
P-Selectin	39.67	±	40.79	39.32	±	18.83	3.2E-01
TNF-α	0.01	±	0.01	0.02	±	0.01	8.6E-01
TNF-R1	1.14	±	0.30	1.72	±	1.14	1.4E-03
VCAM-1	1157.25	±	486.54	2024.00	±	1366.35	1.8E-03

TNF Alpha Study 2

Tumor necrosis factor alpha in children with sickle cell disease in stable condition

S Kuvibidila [1], R Gardner, D Ode, L Yu, G Lane, R P Warrier

- NIH study done by S Kuvibidila et al determines TNF Alpha concentrations in children with SCD.
- **Method:** TNF was measured using enzyme immunoassay in 143 blood samples from 101 children.
- **Findings:**
  - TNF Alpha Levels were above normal:
    - 15% of controls, 34% of children with SCD, 52% of children with SCD and infection, 33% of children with SCD without infection
  - Higher TNF-alpha levels linked to Growth Deficits:
    - Weight Deficits: 46% (elevated TNF-alpha) vs. 31% (normal TNF-alpha)
    - Height Deficits: 50% (elevated TNF-alpha) vs. 28.6% (normal TNF-alpha)
- **Conclusion:** Most children with SCD in steady state have normal TNF-alpha levels; those with elevated TNF-alpha are more likely to have growth deficits.

Increased Platelet Aggregation

Increased Risk:

- SCD significantly increases the risk of prothrombotic events due to heightened platelet aggregation.

Mechanisms:

- Persistent inflammation and endothelial activation promote platelet adhesion and aggregation in SCD.

Thromboxane A2 Release:

- Activated platelets release thromboxane A2, intensifying aggregation and adherence to damaged endothelium.

Microthrombi Formation:

- Microthrombi form within blood vessels, particularly in the lungs, posing critical risks such as pulmonary embolism (PE).

Managing Thrombosis in Sickle Cell Disease

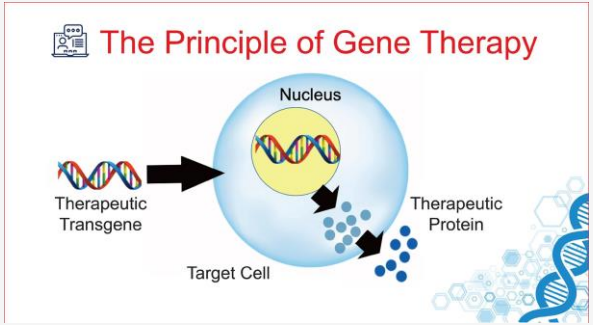
- **Diagnostic Procedures:**
  - Compression Ultrasound Dopplers for diagnosing DVT
  - Multidetector Computerized Tomographic Pulmonary Angiography scanning for PE
- **Treatment Phases:**
  - Active Treatment: Involves therapeutic dose of anticoagulation for three months to suppress the acute episode of thrombosis
  - Secondary Prevention: Includes a prophylactic dose of anticoagulation for an unspecified period of time aiming to prevent new VTE episodes from arising.
- **Indefinite Anticoagulation Therapy:**
  - This therapy is strongly recommended for patients with a low risk of bleeding and a risk of recurrent VTE >13% in the first year
  - Intermediate level recommendation for those with a risk of recurrent VTE of 8-13% in the first year

Managing Thrombosis in Sickle Cell Disease Cont.

- **VTE Recurrence Rate Avg:** High VTE recurrence rate ranging from approximately 25-40%
- SCD patients with less severe disease have VTE recurrence rates at an avg of 18% for 5 years
- **Anticoagulant Choice:**
  - Direct oral anticoagulants are preferred to other anticoagulants due to a better balance of efficacy and bleeding risk.
    - A meta-analysis revealed that DOACs were at least as effective as an alternative (warfarin) for patients with VTE and no active cancer, reducing the risk of major bleeding by 40%
    - In cancer-associated VTE, DOACs showed improved efficacy, but with a significantly increased risk of major bleeding (RR: 1.74; 95% CI: 1.05-2.88) and non-major bleeding (RR: 2.31; 95% CI: 0.85-6.28).

Gene Therapy for SCA

- Used to restore the normal function of proteins that are malfunctioning due to gene mutations
- Chronic transfusion therapy is commonly used in acute cases
- It is known to decrease the risk of VTE and strokes in SCA patients
- It also decreases the frequency of acute pain/chest episodes
- Hydroxyurea therapy is known to decrease plasma levels and protein C levels
- It also decreases Vaso occlusive crises frequency and severity



- Casgevy is approved for the treatment of SCA in patients with recurrent vaso-occlusive crises
- It utilizes CRISPR cas-9 technology to edit stem cells

Current Approved Risk Assessment Models for SCA

- Annual dilated retinal examination beginning at age 10
- Annual spot urine testing beginning at age 10
- Annual Transcranial Do studies starting at age 2 through 16
- Annual blood testing
- Mandatory newborn screenings (all 50 states)

Conclusions

Overview of Complications: SCA leads to chronic hemolytic anemia, vaso-occlusive crises, acute chest syndrome, stroke, and organ damage, driven by hemolysis, vascular obstruction, and chronic inflammation. Thrombosis is increasingly recognized as a significant complication in SCA, with blood clots forming within vessels and causing VTE, driven by hemolysis-induced oxidative stress, endothelial dysfunction, elevated TNF levels, and chronic inflammation.

Complex Pathophysiology: Thrombogenesis in SCA involves hemolysis releasing free hemoglobin and heme, scavenging nitric oxide (NO), promoting oxidative stress, and leading to endothelial activation. Chronic inflammation, elevated levels of TNF, circulating microparticles, and a hypercoagulable state further exacerbate thrombus formation. Markers such as D-dimer and fibrinogen indicate ongoing coagulation and inflammation.

Diagnosis and Treatment Summary: Diagnosing thrombotic complications involves Hemoglobin S and Hemoglobinopathy Testing as well as testing for markers like D-dimer, fibrinogen, and microparticles. Treatment includes hydroxyurea, transfusion therapy, genetic therapy, and anticoagulants, with emerging therapies targeting specific pathways in thrombogenesis under investigation.

Acknowledgements

- Thank you to our mentor Dr. Neha Thomas and director Dr. Jawed Fareed

- Thank you to The Global Thrombosis Forum and Loyola University for hosting this event and providing us with this opportunity to grow in our understanding of these serious conditions

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