Hypercoagulable State in Hemoglobinopathies
Thromboembolic Events in a Large Cohort of Beta Thalassemia Patients

- Patients (N = 8,860)
  - 6,670 with TM
  - 2,190 with TI
- 146 (1.65%) thrombotic events
  - 61 (0.9%) with TM
  - 85 (3.9%) with TI

DVT = deep vein thrombosis; PVT = portal vein thrombosis; STP = superficial thrombophlebitis.

Cerebral lesions

- Focal bright lesions in the cerebral white matter.
- Most of the lesions in frontal lobes and bilateral.
- Maximal diameter up to 7 mm.
- All lesions negative in diffusion with no susceptibility artifacts.
Incidence of silent cerebral infarction (SCI) in 195 patients with β-TI obtained by MRI

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of patients</th>
<th>Age (years)</th>
<th>Prevalence of SCI (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manfre et al. 1999</td>
<td>16</td>
<td>Mean:29</td>
<td>37.5% (18.4-61.7)</td>
</tr>
<tr>
<td>Taher et al. 2010</td>
<td>30</td>
<td>Range: 9-48</td>
<td>60.0% (42.2-75.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean:32.1</td>
<td></td>
</tr>
<tr>
<td>Karimi et al. 2010</td>
<td>30</td>
<td>Mean: 24.3</td>
<td>26.7% (14.2-44.6)</td>
</tr>
<tr>
<td>Teli et al. 2012</td>
<td>24</td>
<td>Range: 18-34</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean:12</td>
<td></td>
</tr>
<tr>
<td>Karimi et al. 2012</td>
<td>95 **</td>
<td>Range: 23±8</td>
<td>15.8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean: 23</td>
<td></td>
</tr>
</tbody>
</table>

* 59 Splenectomized
** 46 Regularly transfused
3T MR Imaging of the Brain of a Multi-Transfused $\beta$-TM Patient

Coronal FLAIR thin section through parietal & occipital lobes and cerebellum demonstrates high intensity lesions as marked by arrows.

Axial FLAIR superior thin section demonstrates high intensity lesions in the frontal & parietal lobes as marked by arrows.
Pathophysiology of beta thalassemia

Normal synthesis of alpha chains

Deficiency of beta chains synthesis

Excess of alpha chains

Proteolysis

Oxidation precipitation

Insufficient of HbA synthesis

Oxidation of membrane proteins & lipids

Dyserythropoiesis

Hemolysis
Mechanism of denaturation of α or β hemoglobin changes and of sickle hemoglobin
Role of iron in oxidation

\[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^\bullet + \text{OH}^\bullet^- \]  
(Fenton reaction)

- Iron atom creates reactive oxygen species
Oxidative Stress in Hemolytic Anemias

Although oxidative stress is not the primary etiology of these diseases, it participates in causing damage to RBC (as well as neutrophils and platelets)

The Causes of Oxidative Stress

• Hemoglobin instability (hemoglobinopathies)
• Iron-overload (due to increased absorption and/or therapeutic blood transfusion
• Hemolysis
Oxidative status in RBC

Normal RBC

A

ROS

Counts

FL1-H

0 200 400 600 800 1000

Counts

FL1-H

0 200 400 600 800 1000

Thalassemic RBC

B

ROS

Counts

FL1-H

0 200 400 600 800 1000

GSH

C

Counts

FL2-H

10^0 10^1 10^2 10^3 10^4

GSH

D

Counts

FL2-H

10^0 10^1 10^2 10^3 10^4

Lipid Peroxidation

E

Counts

FL1-H

0 200 400 600 800 1000

Counts

FL1-H

0 200 400 600 800 1000

F

Lipid Peroxidation
<table>
<thead>
<tr>
<th>TYPE</th>
<th>NO of PATIENTS</th>
<th>µg Phosphorus /RBC 10^8</th>
<th>n moles MDA /RBC 10^8</th>
<th>n moles MDA /gm Hb</th>
<th>n moles MDA /µg phosph.</th>
<th>Vitamin E mg %</th>
<th>β Carotene O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>β THALASSEMIA MAJOR - ISRAEL with SPLENECTOMY</td>
<td>17</td>
<td>2.21</td>
<td>2.47</td>
<td>924</td>
<td>1.12</td>
<td>0.285</td>
<td>0.074</td>
</tr>
<tr>
<td>β THALASSEMIA MAJOR - ISRAEL without SPLENECTOMY</td>
<td>4</td>
<td>1.25</td>
<td>2.13</td>
<td>997</td>
<td>1.71</td>
<td>0.24</td>
<td>0.062</td>
</tr>
<tr>
<td>β THALASSEMIA MAJOR - USA without SPLENECTOMY</td>
<td>4</td>
<td>1.67</td>
<td>1.93</td>
<td>1,212</td>
<td>1.15</td>
<td>0.29</td>
<td>0.095</td>
</tr>
<tr>
<td>CONTROLS ISRAEL</td>
<td>2</td>
<td>1.29</td>
<td>1.35</td>
<td>513</td>
<td>1.00</td>
<td>0.66</td>
<td>0.08</td>
</tr>
<tr>
<td>CONTROLS USA</td>
<td>25</td>
<td>1.13</td>
<td>1.22</td>
<td>465</td>
<td>1.03</td>
<td>0.76</td>
<td>0.065</td>
</tr>
</tbody>
</table>
Prothrombin
Thrombin
THE PROCOAGULANT EFFECT OF THALASSEMIC ERYTHROCYTES (RBC)

PHOSPHOLIPIDS IN RBC MEMBRANE ARE ASYMMETRICALLY DISTRIBUTED WITH:
  - PHOSPHATIDYLCHOLINE (PC) IN OUTER LEAFLET
  - PHOSPHATIDYLSERINE (PS) IN INNER LEAFLET

EXPOSURE OF PS IN THE OUTER MEMBRANE LEAFLET OCCURS IN:
  ACTIVATED PLATELETS and SICKLE CELL RBC

PS REGULATES HEMOSTASIS BY SERVING AS A PROCOAGULANT PHOSPHOLIPID SURFACE IN THE PROTHROMBINASE COMPLEX
  (Zwaal, Molec Cell Bioch, 91:23, 1989)

OUR FINDING OF A PROCOAGULANT EFFECT OF THALASSEMIC RBC WHICH IS SIMILAR TO THAT OF SICKLE CELL RBC,
SUGGESTS A PS ASYMMETRY IN THE THALASSEMIC RBC MEMBRANES, WHICH MAY CONTRIBUTE TO THE ONGOING THROMBOTIC PROCESS
EFFECT OF THALASSEMIC ERYTHROCYTES ON THROMBIN GENERATION

AFIBRINOGENEMIC PLASMA (FACTORS X, V, II) + thrombin (0.02 U)

Xa, Va, II, Ca++, + washed erythrocytes (PL) (PROTHROMBINASE COMPLEX)

THROMBIN (IIa)

measured by amidolytic assay for thrombin
Red Blood Flow Properties:

- RBC self-aggregability
- RBC deformability
- RBC adherence to endothelium
Cell-Flow Properties Analyzer

Schematic diagram of the computerized image analyzer of RBC flow properties: aggregation, deformation, adherence.

S. Yedgar
RBC aggregability under flow

control  TM

Thalassemic RBC adhesion to EC is PS dependent

Ramot, BJH,
Blood Transfusion improves blood flow in vivo

Micrographs of retinal veins (angiograms) (1) and of RBC aggregates (2), corresponding diagrams of aggregate size distribution (3), from thalassemic patient, taken prior to (A) and after (B) transfusion of blood stored for 7 days.

AGGREGATE SIZE (No of RBC / Aggregate)

Borshtel'm, Transf Med Rev, 2010
The incidence of SCI in 28 transfusion dependent patients

15 males, 13 females - mean age 30 years

Exclusion criteria

- Diabetes mellitus
- History of thromboembolic event
- Aspirin or anticoagulation treatment

Pazgal I. et al. Thrombosis Research 2016
Results

Patients without SCI
N=11 (39.3%)

Patients with SCI
N=17 (60%)

FERRITIN (ng/ml) (P<0.031)

3910 (2600 – 12400)

6200 (5000-9600)
<table>
<thead>
<tr>
<th>Group Parameters</th>
<th>Patients with SCI N = 15</th>
<th>Patients without SCI N = 25</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years), Mean ±SD</strong></td>
<td>31.1 ±6.5</td>
<td>25 ±6.6</td>
<td>0.009*</td>
</tr>
<tr>
<td><strong>Sex (male/female ratio)</strong></td>
<td>6/9</td>
<td>5/20</td>
<td>0.273</td>
</tr>
<tr>
<td><strong>Hemoglobin (g/dL), mean ± SD</strong></td>
<td>9.35 ± 1.23</td>
<td>9.31 ±0.88</td>
<td>0.905</td>
</tr>
<tr>
<td><strong>Platelet/microliter, means ± SD</strong></td>
<td>423,571 ± 210,429</td>
<td>388,750 ± 152,073</td>
<td>0.559</td>
</tr>
<tr>
<td><strong>Ferritin (ng/mL), median, IQR</strong></td>
<td>2550, 2875</td>
<td>2400, 2330</td>
<td>0.868</td>
</tr>
<tr>
<td><strong>Blood transfusion (cc/kg/month), mean ± SD</strong></td>
<td>13.5 ± 5.9</td>
<td>11.1 ±4.4</td>
<td>0.150</td>
</tr>
<tr>
<td><strong>Splenectomized, (number %)</strong></td>
<td>12 (80)</td>
<td>9 (36)</td>
<td>0.01*</td>
</tr>
</tbody>
</table>

*Statistically significant
IQR interquartile range

CONCLUSIONS

1. The results of the 2 studies in 68 transfused patients with β-Thalassemia Major demonstrate an incidence of 67% (28 patients) and 37.5% (40 patients).

2. Significant correlations between the number of SCI were found with serum ferritin (28 patients) and with age, iron overload and splenectomy (40 patients).

3. These findings may suggest that the increased number of pathological circulating RBC's in β-TI may not be the only pathophysiological factor in the etiology of SCI.

4. Consequently, there are other factors that contribute to etiology of hypercoagulability in thalassemia.

5. *It should be noted that in sickle cell anemia, regular transfusions for 3 years decreased the number of SCI (M.R.DeBaun, NEJM:371;699; 2014)*
Unmasking of platelet procoagulant activity

2. exposure of phosphatidylserine
CD62p pos. platelets (%) vs. Annexin V binding (x10^3 molecules/RBC)
PLATELET LIFE SPAN (SURVIVAL) IN THALASSEMIA

HOURS

THAL. MAJOR

THAL. INTERMEDIA

THAL. MAJOR CONTROL THAL. INTERMEDIA CONTROL
ROS and GSH in platelets

\[ n = 46 \]

Mean 139
Mean 230
Mean 305
Mean 102

p < 0.0001, by student’s t-test.
Effect of thalassemic plasma on ROS generation by normal platelets

Normal platelets were incubated with autologous plasma or ABO compatible thalassemic plasma. ROS generation of the platelets was measured using the DCF assay.
Pathological findings on EEG related to protein C and platelets in NTDT patients

Statistically significant difference between pathological findings on EEG and low activity of protein C. EEG, electroencephalogram.

Statistically significant difference between the pathological findings on EEG and increased platelet (PLT) number. EEG, electroencephalogram.
Platelet Deposition Under Flow Conditions in Thalassemia

Fig. 1

- 0.2 ml WB
- Apply shear force (2 min.)
- Wash & stain (2 min.)
- IAS (2 min.)

Goldschmidt, Throb. Haemost. 2008
Platelet adhesion (SC) and average size (AS) in 22 patients and controls

Increased platelet deposition under flow conditions is induced by both thalassemic platelets and red blood cells. Thrombosis and Haemostasis, 2008.
A

No thrombosis

Thrombosis

P<0.005

P<0.01

Normal PRP+Thalassemic RBC from patients With and Without Thrombosis
Microparticles generation from RBC and platelets in HbE/β-thalassemia

- Decreased NR activity of HbE
- Decreased NO production from nitrite
- Decreased DDAH activity by oxidative stress
- Increased ADMA (endogenous NOS inhibitor)
- Degradation of L-Arg by arginase released from lysed RBC
- Increased hemolysis and microparticle generation from PS-exposed RBC
- Increased thrombin generation from PS-exposed RBC
- Microparticles from RBC and platelets
- Prothrombin
- Thrombin
- Increased ROS
- ROS-facilitated platelet activation
- Decreased NO availability
- Increased platelets activation
- Oxidative endothelial injury
- Endothelial dysfunction and diminished NO synthesis

Rhoda Elison Hirsch et al. Antioxidants and Redox Signaling, 2016
Increased TEE by circulating M.P. in β-Thal. Major (87 patients)

Significant factors

1. SPLENECTOMY
2. M.P. - RBC
3. M.P. - ENDOTHELIAL derived
4. HYPERBILIRUBINEMIA

Youssry I. et al Ann Hematol 2017 (96)
Circulating erythrocyte-derived microparticles are associated with coagulation activation in sickle cell disease

We conclude that the procoagulant state in sickle cell disease is partially explained by the factor XI-dependent procoagulant properties of circulating erythrocyte-derived microparticles.

Van Beers, Haematologica, 2009
Deep Venous Thrombosis and Pulmonary Embolism in Hospitalized Patients with Sickle Cell Disease

**CLINICAL SIGNIFICANCE**

- A higher than expected prevalence of apparent pulmonary embolism in patients with sickle cell disease suggests the occurrence of thrombosis in situ.

- Pulmonary embolism is not rare in patients with sickle cell disease.

- Imaging studies may be appropriate in sickle cell patients with respiratory symptoms to distinguish pulmonary embolism from thrombosis in situ.

Stein, AJM, 2006
Kalish Y. et al. Thrombosis Research 2014
Carotid Artery Occlusion Model
Carotid Artery – Time to Occlusion

Hypercoagulable state
Role of platelets

<table>
<thead>
<tr>
<th></th>
<th>w.t</th>
<th>w.t+ASA</th>
<th>Thal</th>
<th>Thal+ASA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>64.60</td>
<td>72.50</td>
<td>49.10</td>
<td>58.70</td>
</tr>
<tr>
<td><strong>Std. Deviation</strong></td>
<td>8.017</td>
<td>13.06</td>
<td>9.758</td>
<td>8.538</td>
</tr>
</tbody>
</table>
Opposite effects of TxA₂ and PGI₂ on hemostasis and thrombosis

Phospholipid- AA

Phospholipase

Cyclooxygenase

Arachidonic acid

Cyclooxygenase

Endoperoxides

PGI₂-synthase

Thrnoxane A₂ (blood platelets)

Prostacyclin (vessel wall)

Aggregation release & vasoconstriction

Hemostasis & thrombosis
EXCRETION OF URINARY PROSTAGLANDIN METABOLITES IN THALASSEMIA

- 2.3. din. TXB2
- 11 dehyd. TXB2
- 2.3. din. 6k-PGF1

[Bar chart showing ng/mole creatinine for different metabolites in Thal. Major, Thal. Intermedia, and Control groups.]
A = Normal Range  B = Adults  C = Children (2-12 years)
COAGULATION INHIBITORS IN BETA-THALASSEMIA MAJOR

AT III
Protein S (total)
Protein S (free)
C4b BP

Percent

A = Normal Range  B = Adults  C = Children (2-12 years)
The hypercoagulable state in thalassemia, Blood 2002
Platelets
- Increased platelet aggregation
- Increased expression of activation markers
- Presence of platelet morphologic abnormalities

Peripheral blood elements
- Expression of endothelial adhesion molecules and tissue factor on endothelial cells
- Formation of microparticles

Nitric oxide
- Hallmark of haemolysis
- ↓ Levels leading to vasoconstriction

RBCs
- Formation of reactive oxygen species
- Expression of negatively charged phospholipids
- Enhanced cohesiveness and aggregability

Hypercoagulability

Other factors
- Cardiac dysfunction
- Hepatic dysfunction
- Endocrine dysfunction

Thrombophilia
- No role for prothrombotic mutations
- Decreased levels of antithrombin III, protein C, and protein S
- Anti-phospholipid antibodies

Splenectomy
- High platelet counts and hyperactivity
- High levels of negatively charged RBCs
Summary

• **Hemoglobinopathies are associated with hypercoagulable state**

• **Iron over load and oxidative stress are major factors leading to:**
  - Increased RBCs and platelets’ activation and adhesion
  - Increased procoagulant effect of RBCs, platelets and MPs

• **Splenectomy is a major factor due to:**
  - Thrombocytosis
  - Elimination of a scavenger of pathological RBCs and MP

• **Clinical manifestations include VTE, AT, CVA, PAH and potentially others**

• **Increased number of SCI in SPX multitransfused TM and in NTDT patients**
Low dose Aspirin is indicated to prevent thromboembolic phenomena in SPX- regularly transfused TM an in NTDT patients.

The direct correlation between the degree of iron overload and the number of SCI suggests that effective iron chelation therapy is mandatory.
Effect of LD aspirin on TXB2 excretion

Eldor, Blood, 1991
This talk is dedicated to my colleague and dear friend, Amiram Eldor, who left us tragically a few years ago and who was one of the original contributors to this area.

**Acknowledgements**

- Eitan Fibach¹, Ada Goldfarb¹, Johnny Amer ¹, David Varon¹, Neta Goldschmidt ¹
- Ali Taher² · Khaled Mussalem²
- M. Domenica Cappellini³
- Rachid Merchant⁴
- Mehran Karimi⁵

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¹ Dept. of Hematology, Hadassah University Hospital, Ein-Kerem, Jerusalem, Israel
² Internal Medicine, American University of Beirut, Beirut, Lebanon
³ Centro Anemie Congenite, Policlinico Hospital IRCCS, Milano, Italy
⁴ Nanavati Hospital Mumbai, India
⁵ Shiraz University of Medical Sciences, Shiraz, Iran
Amiram’s contribution to the field

1. The hypercoagulable state in thalassemia.
   Eldor A, Rachmilewitz EA.

2. A chronic hypercoagulable state in patients with beta-thalassaemia major is already present in childhood.

3. In vivo platelet activation correlates with red cell anionic phospholipid exposure in patients with beta-thalassaemia major.

4. In vivo platelet activation in beta-thalassemia major reflected by increased platelet-thromboxane urinary metabolites.
   Eldor A, Lellouche F, Goldfarb A, Rachmilewitz EA, Maclouf J.

5. Enhanced aggregability of red blood cells of beta-thalassemia major patients.

6. Phosphatidylserine in the outer leaflet of red blood cells from beta-thalassemia patients may explain the chronic hypercoagulable state and thrombotic episodes.
   Borenstain-Ben Yashar V, Barenholz Y, Hy-Am E, Rachmilewitz EA, Eldor A.

7. A chronic hypercoagulable state and life-long platelet activation in beta-thalassemia major.

8. In vivo platelet activation in beta-thalassemia major reflected by increased platelet-thromboxane urinary metabolites.
   Eldor A, Lellouche F, Goldfarb A, Rachmilewitz EA, MacLoun J.


10. A chronic hypercoagulable state and life-long platelet activation in beta-thalassemia major.

11. Abnormal platelet functions in beta-thalassaemia.
    Eldor A.

    Eldor A.