Mistery in the Mediterranean

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The mistery…..

- Mrs AC, aged 43, admitted to emergency unit for respiratory failure with severe pulmonary hypertension and cardiac failure
- She died within one month of hospitalization

Clues……

- She was diagnosed at the age of 8 yrs as carrier of beta-thalassemia
- **Hematological data at diagnosis**

Hb: 10.2g/dl

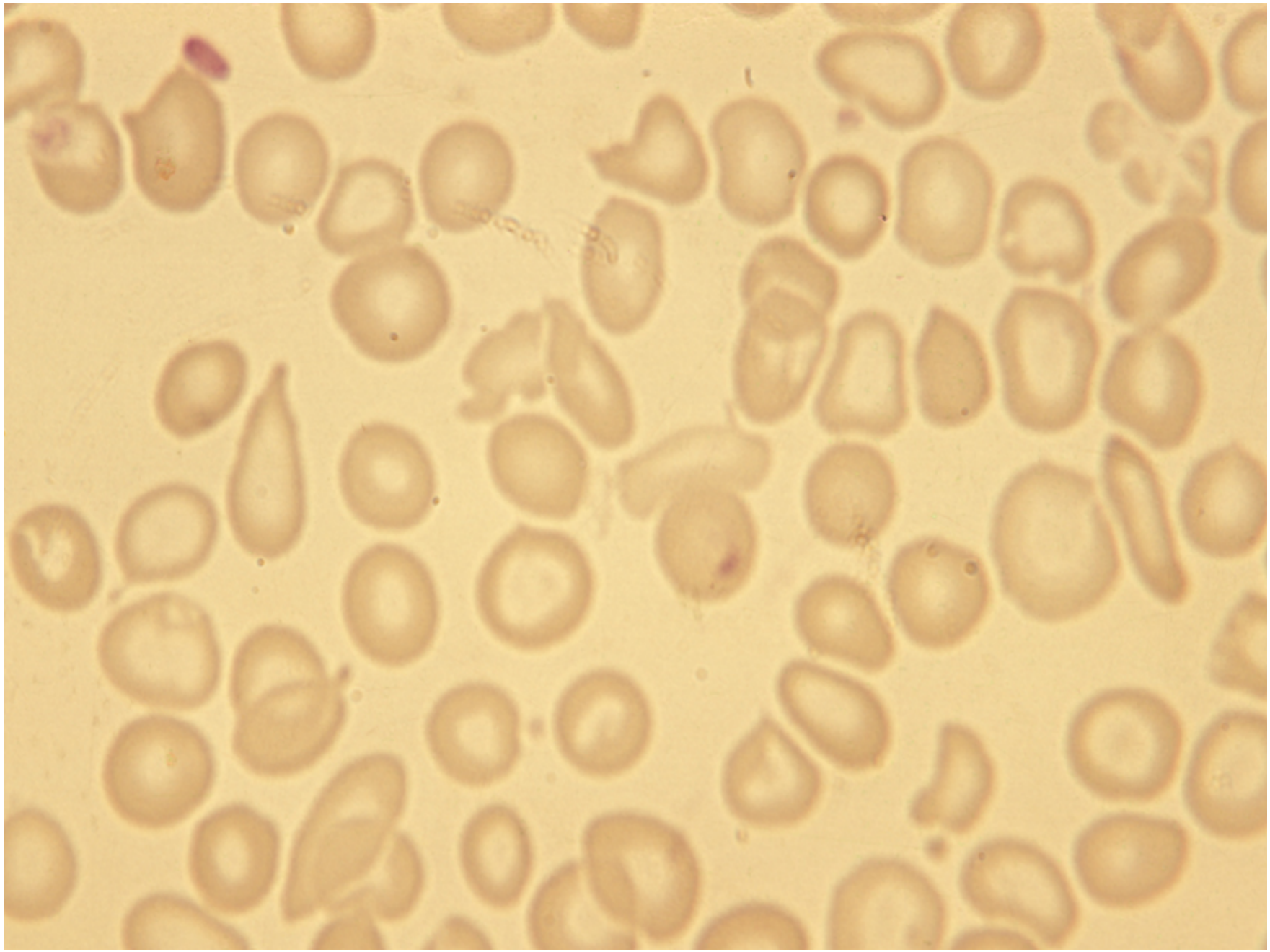
RBC: $5.1x10^{12}$/l; MCV 78fl

WBC: $4.5x10^{9}$/l

Platelets: $150x10^{9}$/l

HbA 93.6%; HbA2: 4.2%; HbF: 2.2%

Ferritin: 200ng/ml



Question 1

- Was the diagnosis of beta-thalassemia carrier correct?

1. Yes
2. No

Clues……

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Hematological indices in b° thalassemia mutations

Mutation	Hb (g/dL)	MCV (fL)	MCH (pg/cell)	Hb A2 (%)
cod. 39	11.8±1.6	63±3	20.4±2.3	5.5±1.1
IVS I-110	12.3±1.4	64±4	26.7±2.7	5.3±0.4
IVS I-1	12.8±1.5	67±5	23.9±1.8	4.5±0.7
IVS II-1	12.4±1.3	66±6	21.8±2.6	4.9±0.8
cod. 6	11.3±1.1	69±7	23.5±2.6	5.2±0.3

Hematological indices in b^+ thalassemia mutations

Mutation	Hb (g/dL)	MCV (fL)	MCH (pg/cell)	HbA2 (%)
-87	13.0±1.7	76±4	25.4±2.3	6.2±1.1
-101	14.0±1.5	81±8	26.7±2.7	3.3±0.4
IVS I-6	12.5±1.5	71±5	22.9±1.8	3.9±0.7
IVS II-745	11.0±1.3	65±6	20.8±2.6	4.9±0.8
IVS II-844	14.3±1.1	83±7	29.6±2.6	3.3±0.3

More….

- She married at 24yrs
- She underwent splenectomy and cholecystectomy at the age of 25 yrs because of :

✓ palpable spleen – 12 cm
✓ cholelitiasis
✓ worsening of anemia: Hb 9.3g/dl
✓ serum iron 185ug/dl; transferrin saturation: 90%;
✓ ferritin 350ng/ml

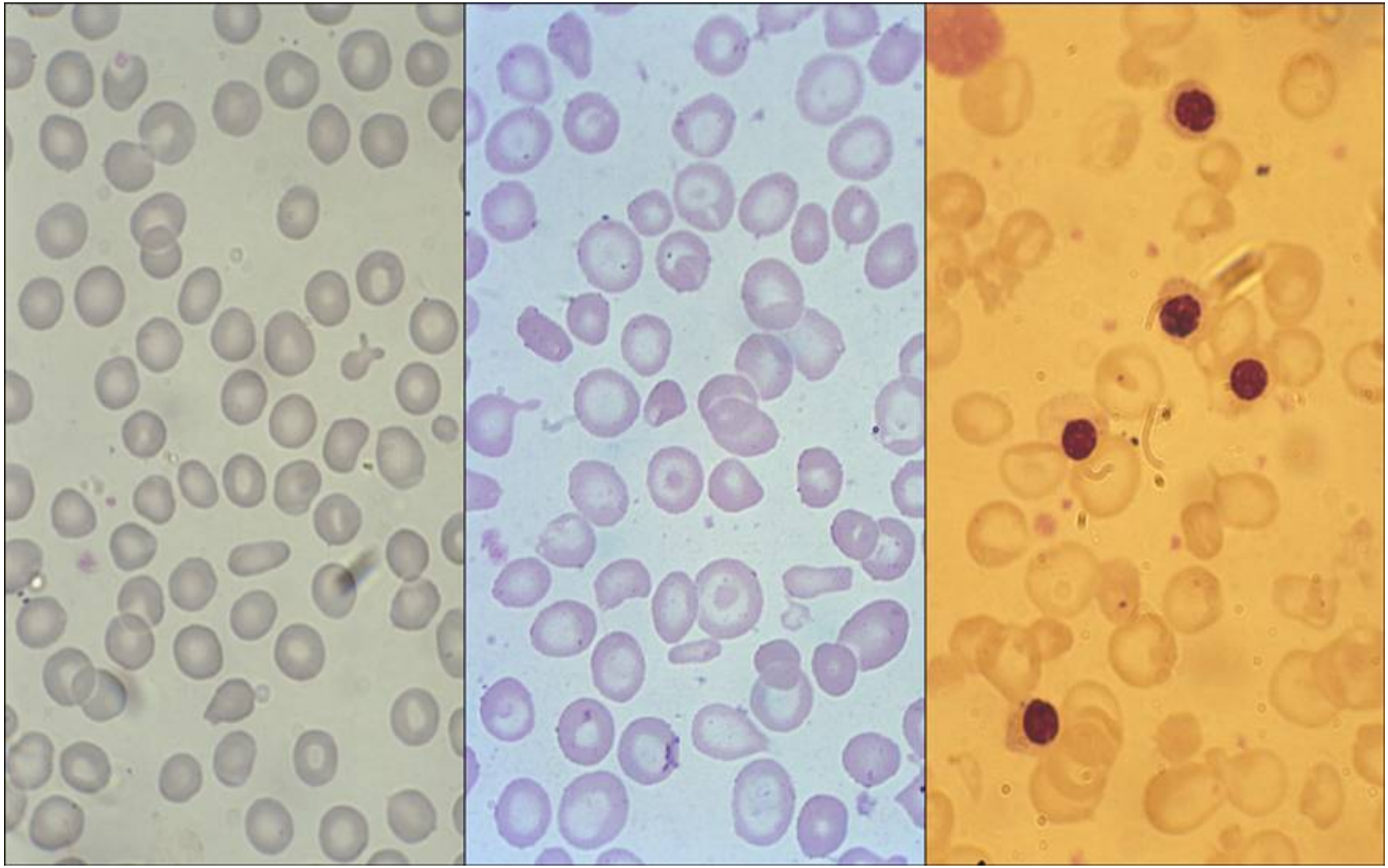
Question 2

- Is splenomegaly common in carriers of beta thalassemia?

1. Yes
2. No

Post-splenectomy…..

- ✓ Hb: 10.0g/dl
- ✓ Platelets: $700x10^9/l$
- ✓ Circulating Erythroblasts: $70x10^9/l$
- ✓ AST: 55U/l; Alk Phos: 150U/l
- ✓ Bil. Tot: 3.2mg/dl, Ind. 2.8mg/dl
- ✓ Blood film: marked anysopoichilocytosis, erythroblasts, aggregated platelets



Beta-thalassemia carrier Proband pre-splenectomy Proband post-splenectomy

- She was advise to have periodic checks on hematological status, iron levels, hepatic and cardiac function
- She was feeling well thus she did not undergo regular follow-up
- She was on Folic Acid
- She completed pregnancy at the age of 31yrs after three miscarriages

The misterious event…

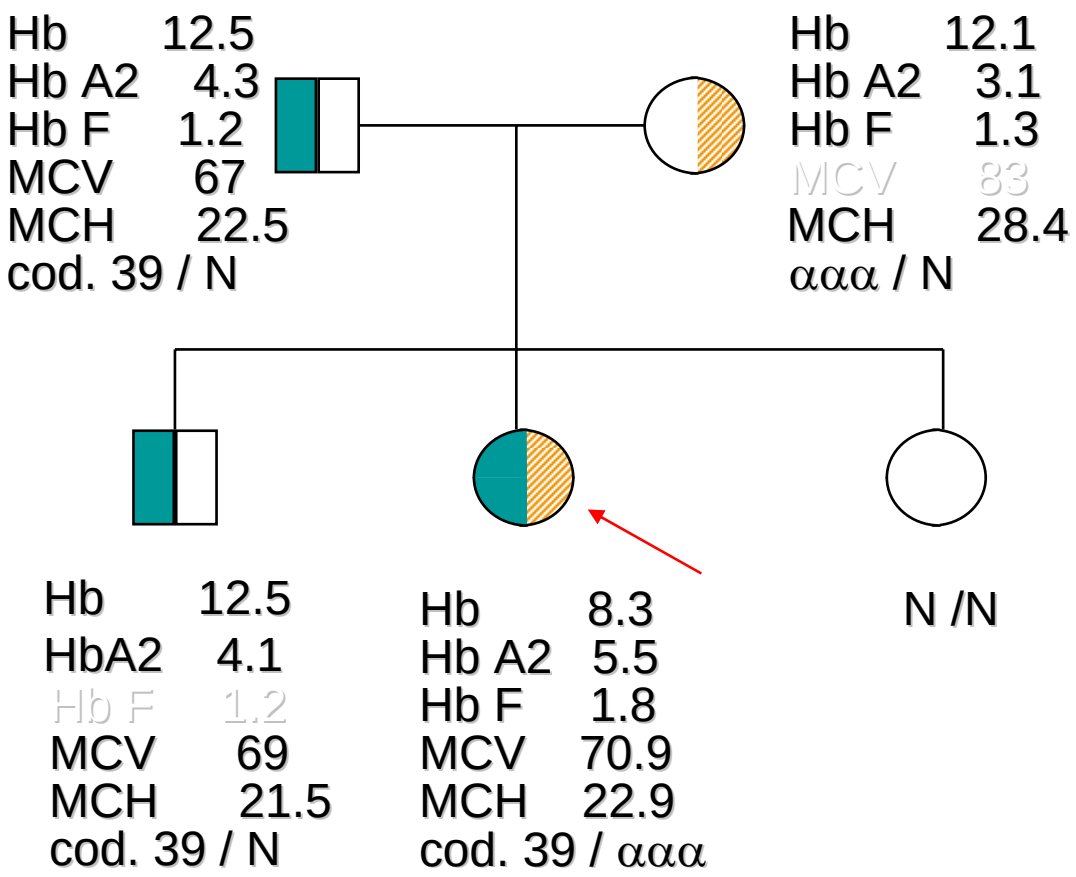
- 2 months before admission she started to complain dyspnoea on effort that gradually became worse
- ECG, Echocardiogram, respiratory function tests at that time were normal
- Hb: 8.5g/dl
- Platelets: $800x10^9/l$
- Erythroblasts: $120x10^9/l$
- Tot.Bil.: 6.5mg/dl; Ind. 5.3mg/dl
- Ferritin: 350ng/ml

Question 3

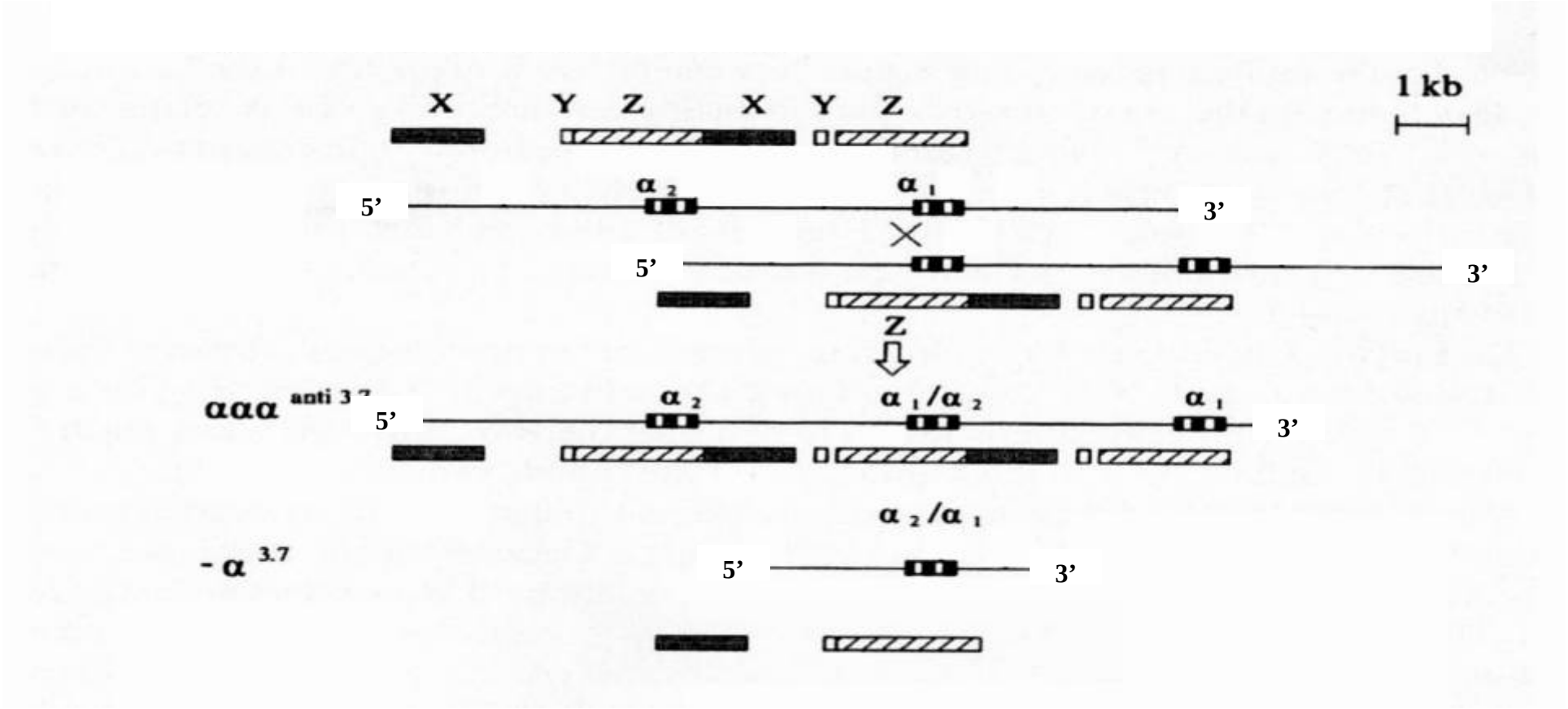
- Which other hemathological evaluations do you suggest?

1. Membrane skeleton proteins
2. Glucose-6-phosphate deydrogenase activity
3. Gilbert syndrome
4. Molecular analysis of globin genes

C. A.


Hb 12.5
Hb A2 4.3
Hb F 1.2
MCV 67
MCH 22.5
cod. 39 / N
Hb 12.1
Hb A2 3.1
Hb F 1.3
MCV 83
MCH 28.4
ααα / N
Hb 12.5
HbA2 4.1
Hb F 1.2
MCV 69
MCH 21.5
cod. 39 / N
Hb 8.3
Hb A2 5.5
Hb F 1.8
MCV 70.9
MCH 22.9
cod. 39 / ααα
N /N

Triplication alpha genes

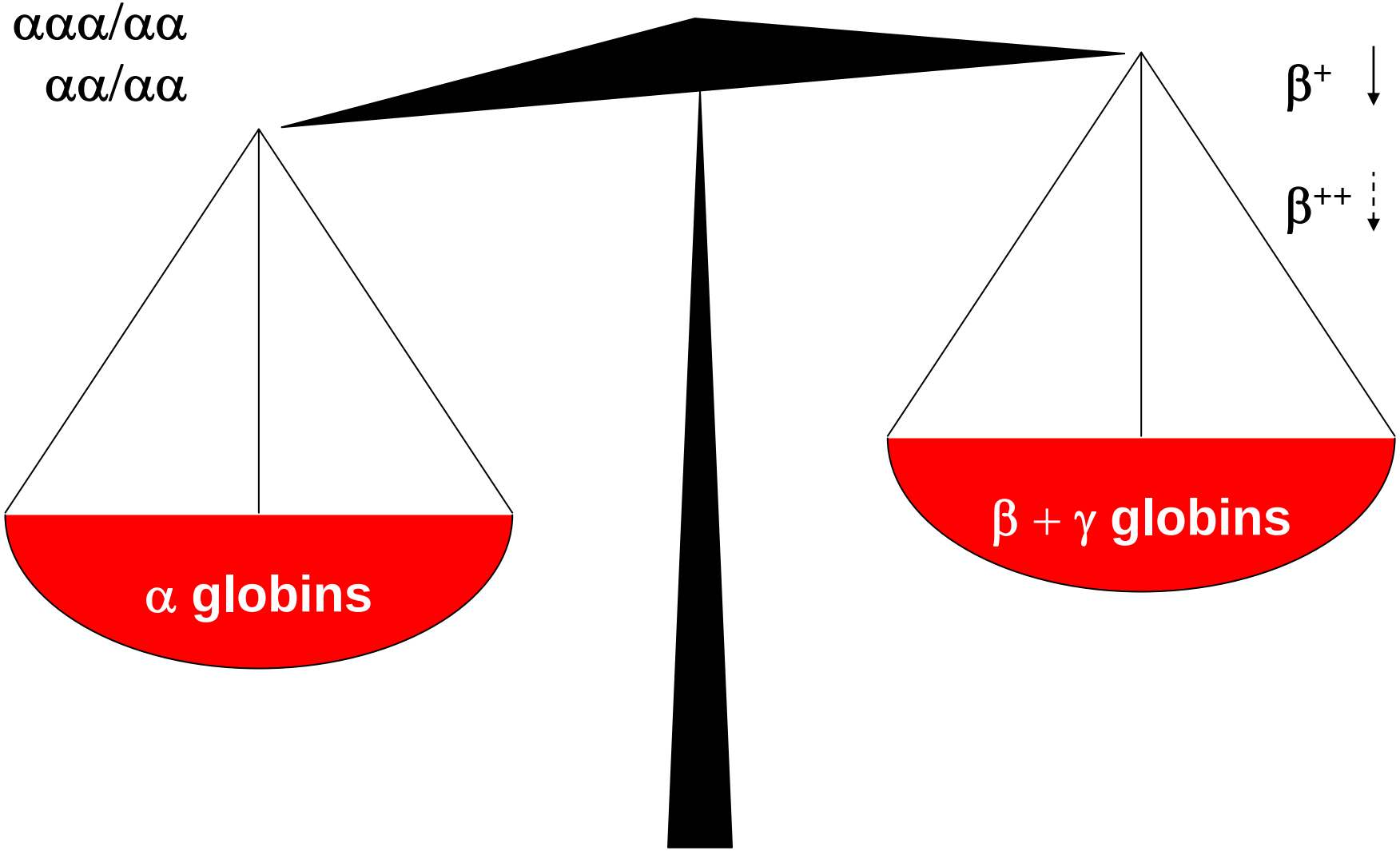


Cohinerited with beta globin gene defect cause “Thalassemia Intermedia”

β Thalassemia Intermedia

αααα/αα
ααα/αα
αα/αα

β^0 ↓
β^+ ↓
β^{++} ⇣



The tragedy…

At admission:

- Dispnoea for minor effort
- ECG: right axis deviation
- EGA in air: pO2 74mmHg
- Echocardiogram: medium tricuspid deficit, PAP 91mmHg
- Pulmonary scintigraphy: multiple perfusion defects due to multiple microthrombi

The tragedy…

At admission:

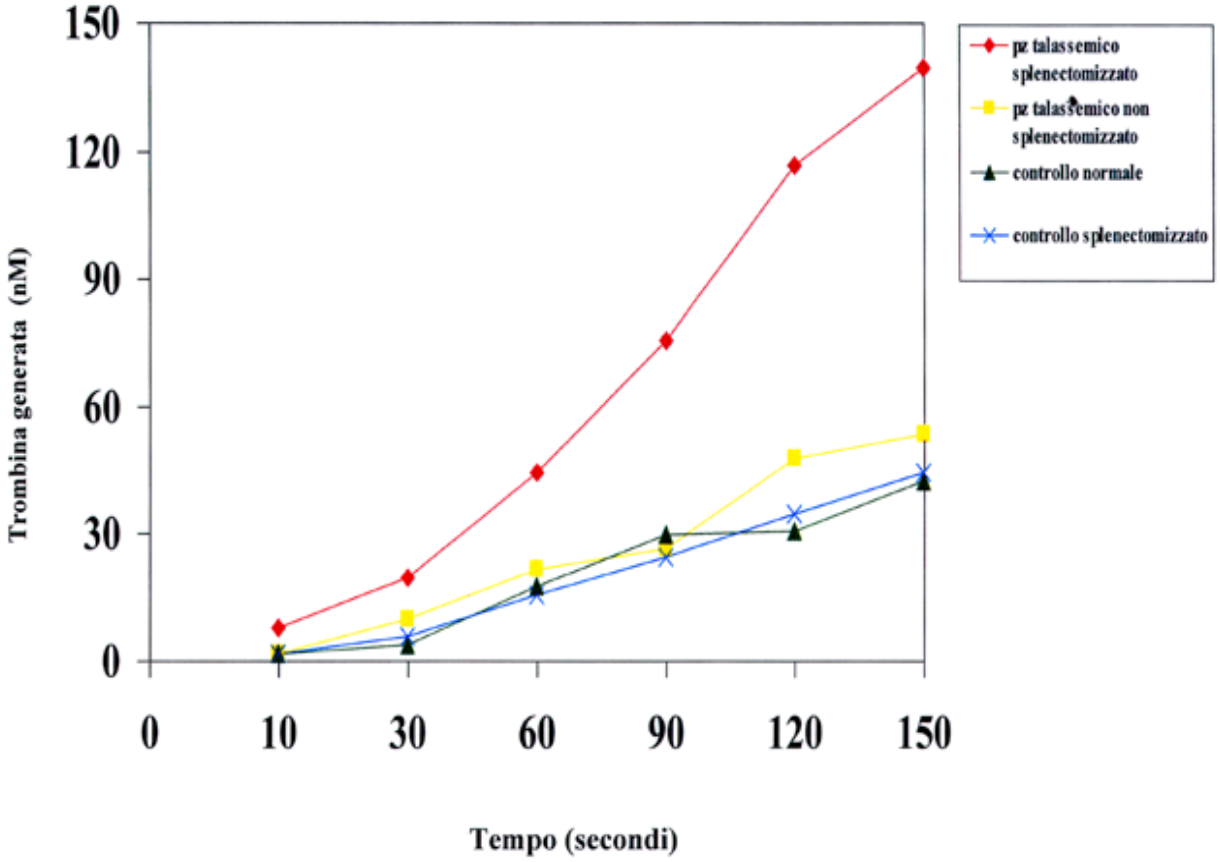
- biochemical and scanning tests excluded underlying disease which might result in pulmonary microthrombi
- Genetic defects of coagulation were excluded
- Free intracellular globins (emichromes); intra-cell free iron; intraerythrocytic oxidative reduction agents suggested RBC membrane lipid-peroxidation

Question 4

- Are thalassemia intermedia patients at risk for thrombosis?

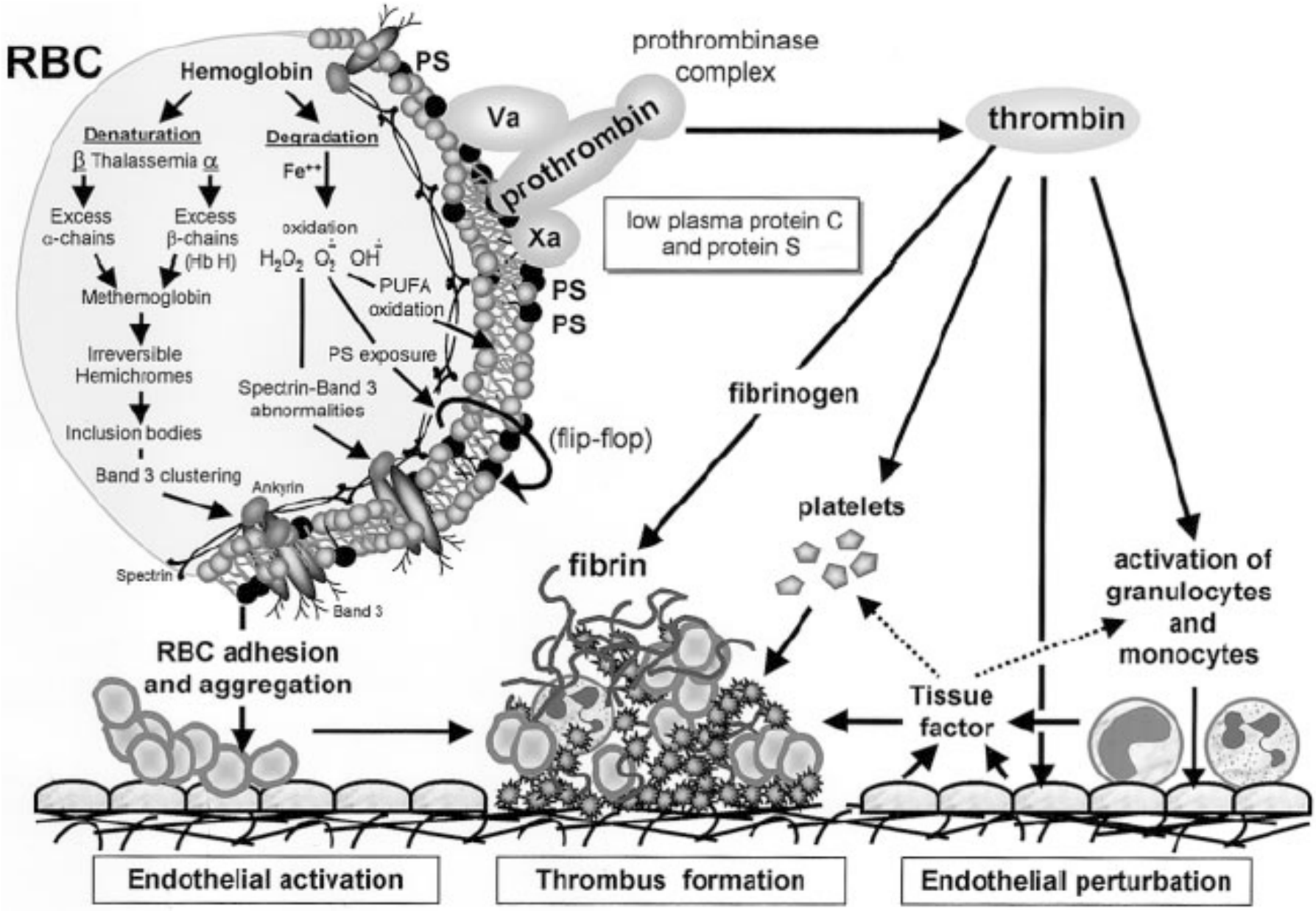
1. No
2. Yes
3. Not known

Abnormal Thalassemic RBC and Hypercoagulable state



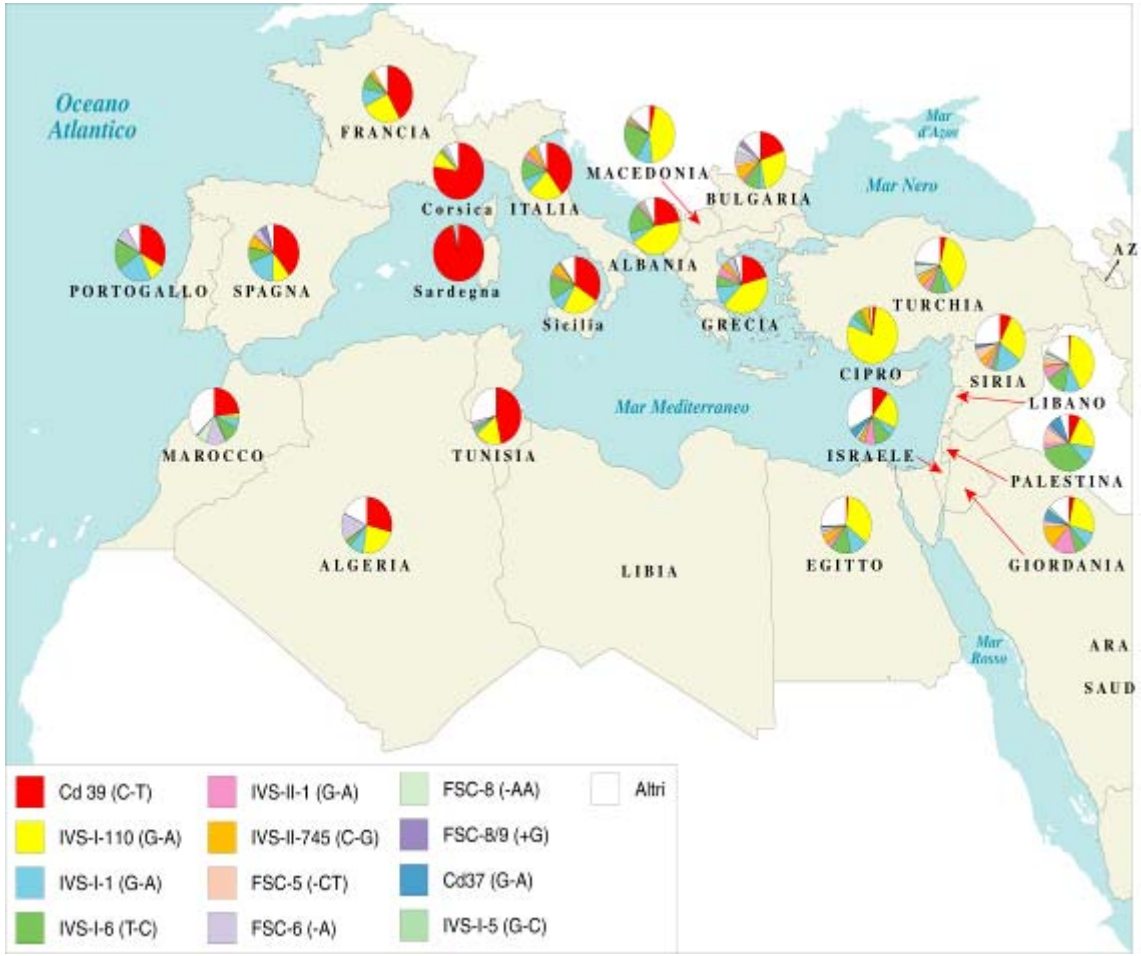
Representative examples of time course of thrombin generation in the presence of erythroid thalassemic cells as source of phospholipids.

Capelllini M et al. Br J Hematol, 2000.



Eldor & Rachmilewitz, Blood 2002

The Mediterranean anemia



Message to take home…

- Thalassemia carriers with Hb levels <11g/dl without associated iron deficiency deserve to be genotyped
- Triplicated alpha genes cohinerited with beta° mutations can be responsible of thrombotic complications
- Splenectomy is not advisable

1. Question

Is Pulmonary Hypertension in Thalassemia Intermedia a problem?

PAH Definition

mPAP >25 mmHg at rest or >30 mmHg during exercise

- Tricuspid regurgitant Doppler Jet velocity (TRV): > 2.5 m/sec; moderate to severe > 3m/sec (PAP at least 41 mmHg)

PAH in Hemoglobinophaties

- **Thalassemia Major:** **10%**
  (Derchi et al Am Heart J 1999)

- **Thalassemia Intermedia:** **40- 47 %**
  (Aesoppos A et al Chest 1995;
  Chest 2005)

- **SCD:** **60%**
  (Gladwin M et al NEJM 2004)

Pulmonary Hypertension In Thalassemia Intermedia

131 TM patients who received regular chelation transfusions and were highly compliant with treatment (mean age, 28 +/- 6 years [+/- SD]), and 74 age-matched, TI patients who did not receive chelation transfusions.

- **Considerable pulmonary hypertension (systolic tricuspid gradient > 35 mm Hg) was only present in TI (23.0%).**

Aessoposs A., et al. Ann.NY Acad.Sci 2005

THALASSEMIA INTERMEDIA AND THROMBOSIS

The estimated total number of patients treated for thalassemia in the participating centers was 8860

- 6670 had TM
- 2190 had TI

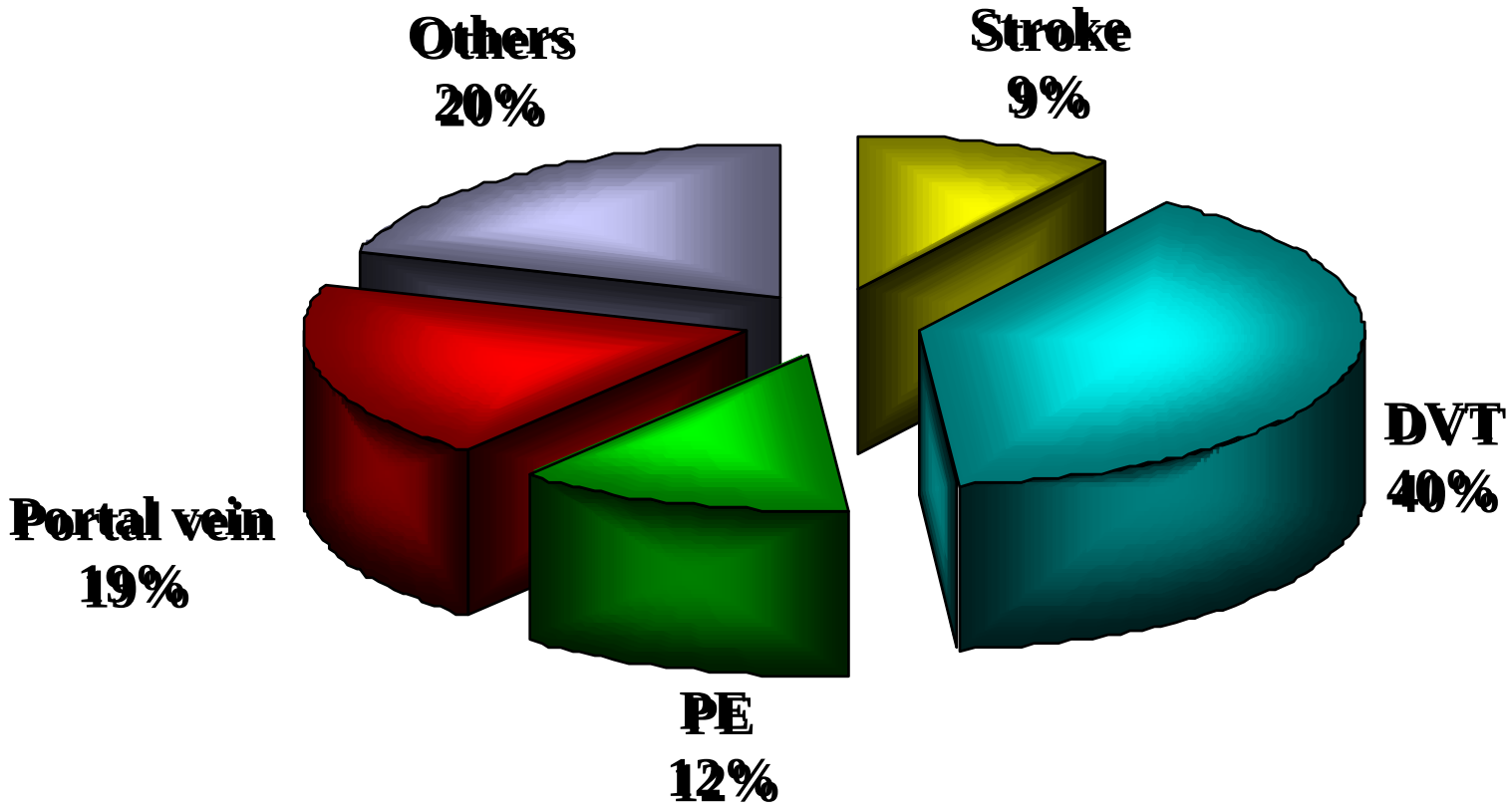
The total number of patients who had a thrombotic event was 155 (1.75%)

- 61 (0.9%) had TM
- 85 (4%) had TI

45% patients were males, and 55% were females

Taher A. et al. 2005

THALASSEMIA INTERMEDIA AND THROMBOSIS



A. Taher et al 2005

THALASSEMIA INTERMEDIA AND PAH

15/85 (17.6%) TI patients had pulmonary hypertension that was associated in half of them with pulmonary embolus and/or deep vein thrombosis. All these 15 patients were splenectomized and 10 had an average pre-transfusion hemoglobin less than 9.

Taher A. et al. 2005

Pulmonary Hypertension in Haemoglobinopatic patients without LVD

Tricuspid Regurgitant Jet Velocity	Thal.Maj. 485 pts	Thal Int 458 pts	SCD 178 pts	% pts
TRV < 2.5 m/sec	436 (89.9%)	395 (86.2%)	166 (93.2%)	89%
2.5<TRV<3.5 m/sec (mild)	46 (9.4%)	57 (12.5%)	11 (6.2%)	10.%
TRV>3.5 m/sec (severe)	3 (0.6%)	6 (1.3%)	1 (0.9%)	

Derchi G. et al, Blood 106;11,756a

2. Question

What is the patophysiology of PAH in Thalassemia Intermedia?

Risk Factors in PAH in Thalassemia Intermedia

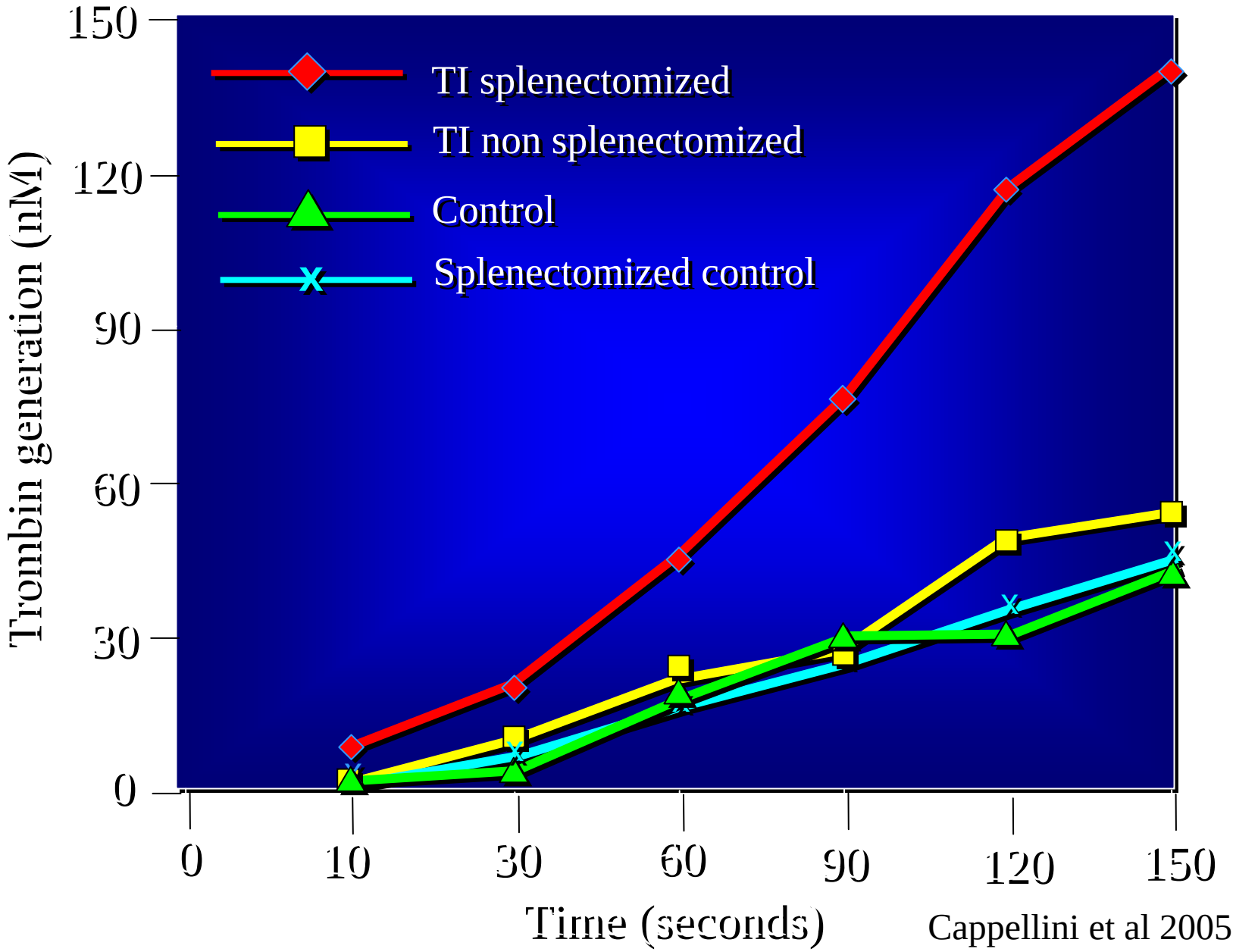
- **Splenectomy/Hypercoagulability**
- **Chronic Hemolysis**
- **Chronic Hypoxemia**
- **Iron Overload**
- **Liver disease**

Splenectomy
Hypercoagulability

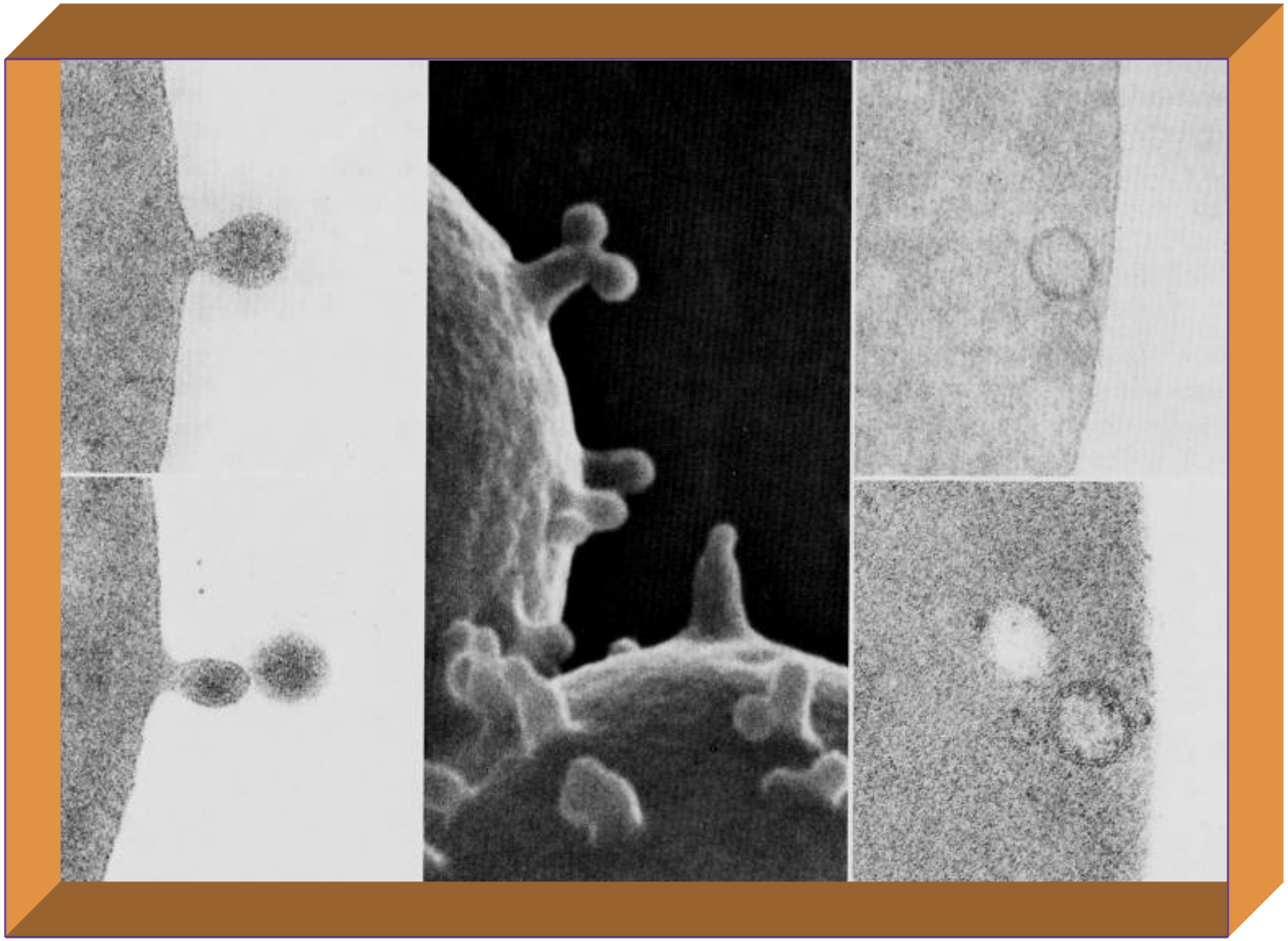
The loss of splenic function causes:

increase of the circulation of platelet derived mediators

- increase of the senescent and abnormal erytrocytes in the circulation that have procoagulant activity
- increase the plasma hemoglobin



Cappellini et al 2005



Chronic Hemolysis
Evidences (?)

In SCD hemolysis contributes to impaired nitric oxide bioavailability and to endothelial disfunction (Morris et al. Ann.N.Y.Acad Sci.2005)

- Hemolysis releases red-cell arginase that convertes arginine to ornithine and urea
- High arginase activity reduces the availability of arginine to NO-synthase resulting in a NO deficiency

Hemolysis-associated Pulmonary Hypertension in TI

Plasma amino acid levels and arginase activity were studied in 14 Thalassemia patients:

- 8 TM
- 4 HbE/β thal
- 2 HbH

Morris et al. Ann.N.Y.Acad.Sci, 2005

Hemolysis-associated Pulmonary Hypertension in TI

Plasma arginine concentration trends lower (median 50uM)

- Ornithine levels are increased
- Ratio arginine to ornithine is low
- Plasma arginase activity is increased

Morris et al. Ann.N.Y.Acad.Sci, 2005

Chronic Hypoxemia

Pulmonary restrictive pattern was observed in 14% of Thalassemia Intermedia patients

- reduced total lung capacity(TLC)
  < 70% corrected for age and size

Piatti G. et al 2006

Risk Factors in PAH in Thalassemia Intermedia

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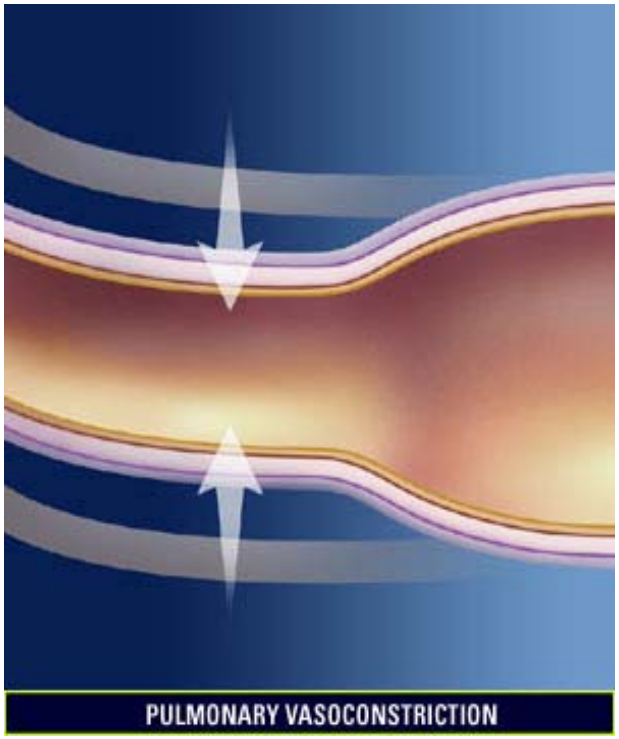
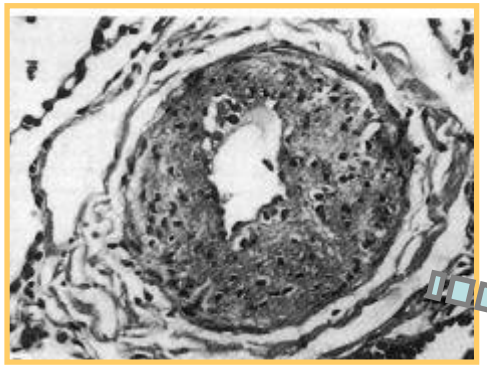
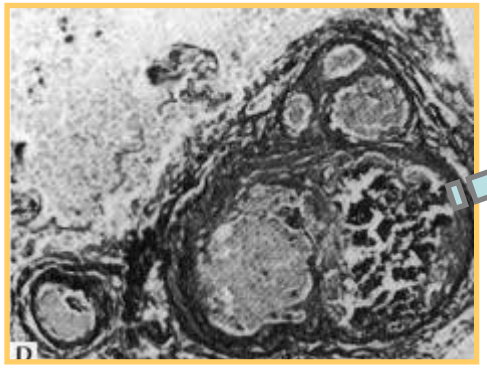
Patophysiology

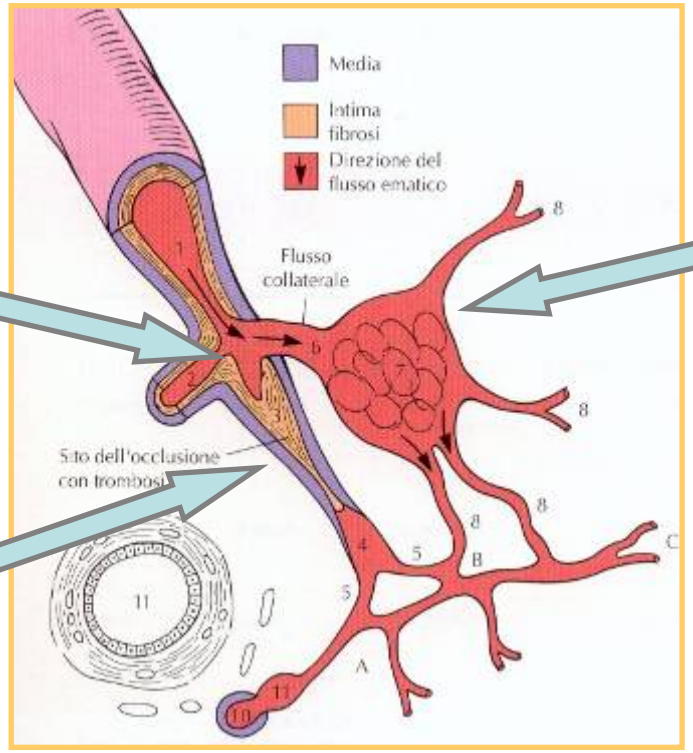


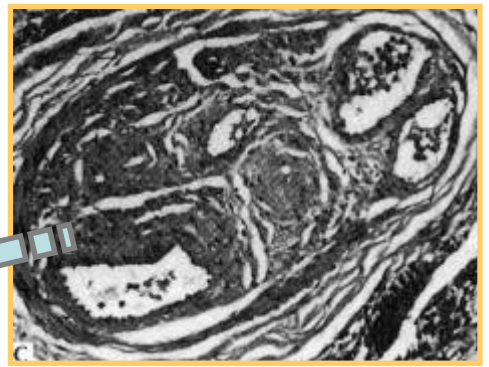
PAH: histological feature

Increased intimal and medial thickness









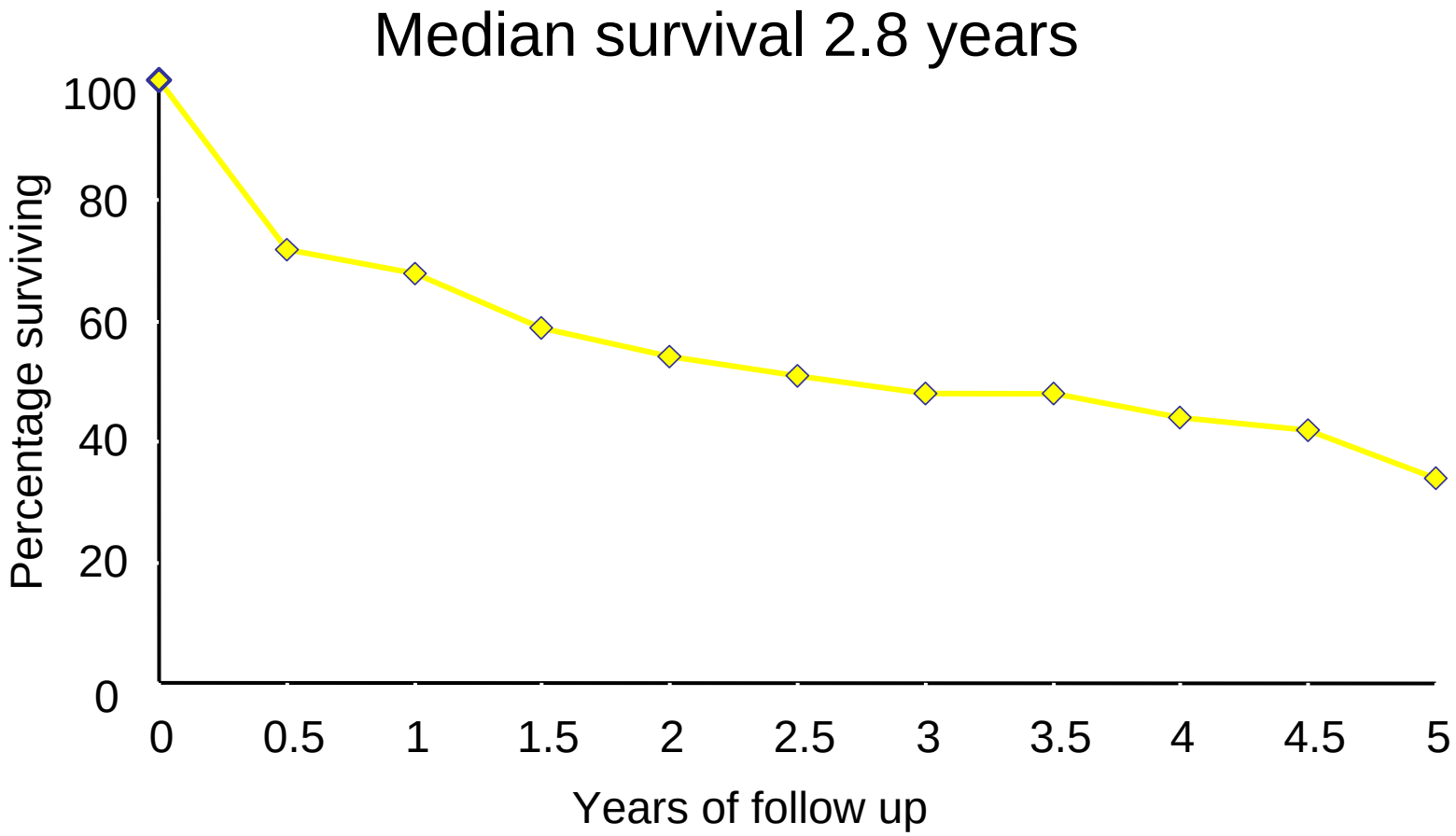
Plexiform lesion

Intimal fibrosis and Pulmonary arteriolar occlusion

3. Question

How to early diagnose PAH in Thalassemia Intermedia?

Prognosis of PAH is poor



D'Alonzo et al. Ann Internal Med 1991;115:343

Diagnosis in PAH

AWARENESS

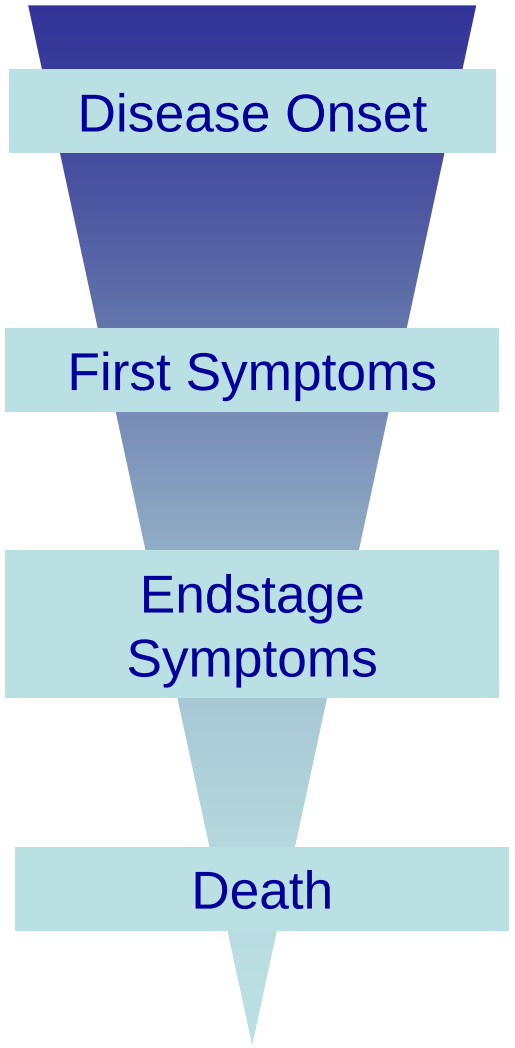
Key to early diagnosis

ECHO
- RV enlargement
- Decreased LV cavity size
- Abnormal septal configuration consistent with RV overload

DOPPLER
- Marked dependence on atrial systole for ventricular filling

RIGHT HEART CATHETERIZATION
- Mandatory to confirm & characterize disease

Signs and symptoms of PAH



No early symptoms of PAH

Annual screening in high risk populations mandatory

Progressive dyspnea on exertion, fatigue, palpitations, chest pain, syncope, coughing

Symptoms and signs of right heart failure

Edema, ascites

Median survival is 2.8 years

. Am.Coll. Cardiol. 2004

4. Question

- **How to treat PAH in Thalassemia Intermedia?**

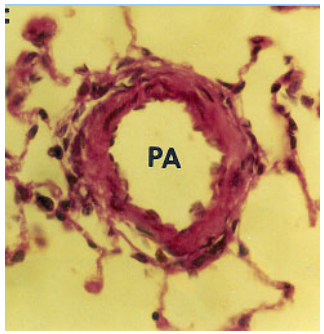
Prevention of PAH in Thalassemia Intermedia

1. Avoid splenectomy when possible
2. Control the hypercoagulable status
3. Reduce hemolysis
   - Transfusions
   - Hydroxyurea
   - anti-oxidants
   - Nitric Oxide

Specific Therapeutic approaches

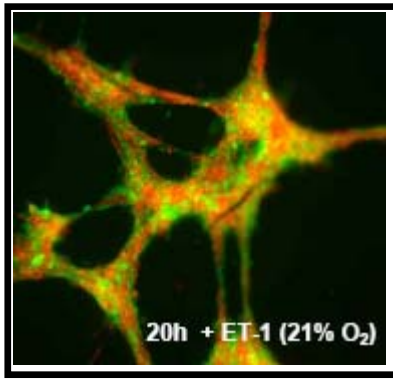
- Calcium channel blockers
- Prostacyclin analogues
- Endothelin-1 receptor antagonists
- Tromboxane inhibitors

Bosentan:endothelin-receptor antagonist



Smooth muscle cells

Vasoconstriction, hypertrophy, proliferation [1-2]



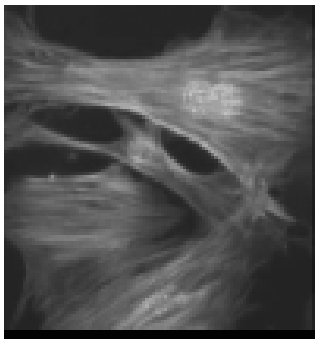
Endothelial cells

Proliferation, dysfunction [3-6]

Endothelin

Inflammation

NF-kB and cytokine release,increased vascular permeability [10-13]

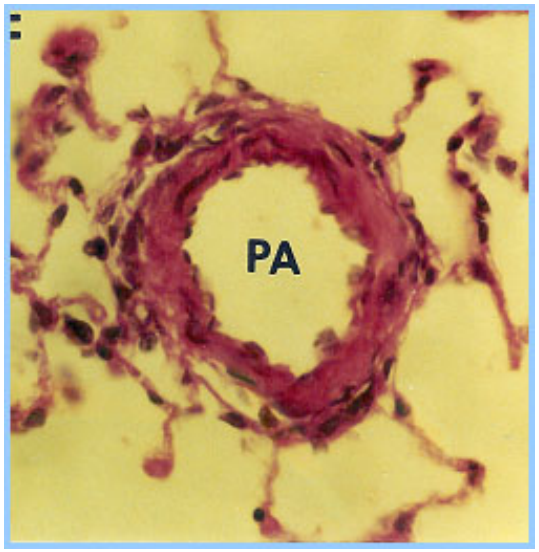


Fibroblasts

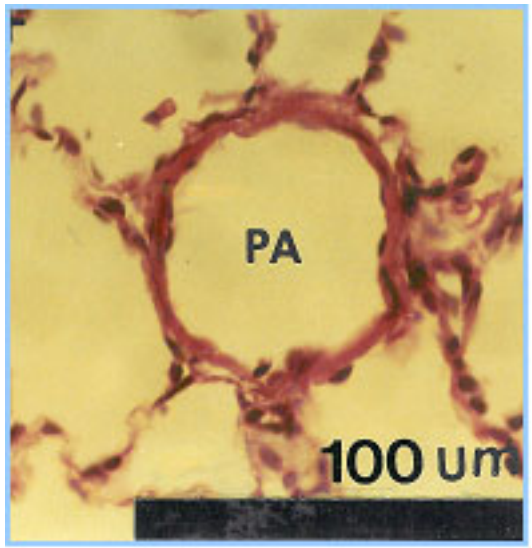
Fibrosis [7-9]

[1] Clozel M et al. J Pharmacol Exp Ther 1989 **;** [2] Yang Z et al. Circulation 1999; [3] Kuhlmann et al. Acta Physiol Scand 2005; [4] Davie N. Univ. Colorado Health Sciences Center. With permission; [5] Girgis et al. Am J Respir Crit Care Med 2005 ; [6] Amiri et al. Circulation 2004; [7]Cambrey et al. Am J Respir Cell Mol Biol 1994; [8] Shi-Wen et al. J Invest Dermatol 2001; [9] Shi-Wen et al. Mol Biol Cell 2004; [10] Yang et al. Circulation 2004; [11]Wilson et al. Biochem Piophys Res Comm 2001; [12]Helset et al. Am J Physiol 1996; [13]Salani et al. Am J Pathol 2000.

Bosentan prevents PAH in Mice



10% O_2 6 weeks
+ 1 week placebo



10% O_2 6 weeks
+ 4 week bosentan 100 mg/kg/d

Adapted from: Chen S-J, et al. *J Appl Physiol.* 1995;79:2122.

Sildenafil in PAH

Selective and potent inhibitor of cGMP-spoecific phosphodiesterase-5

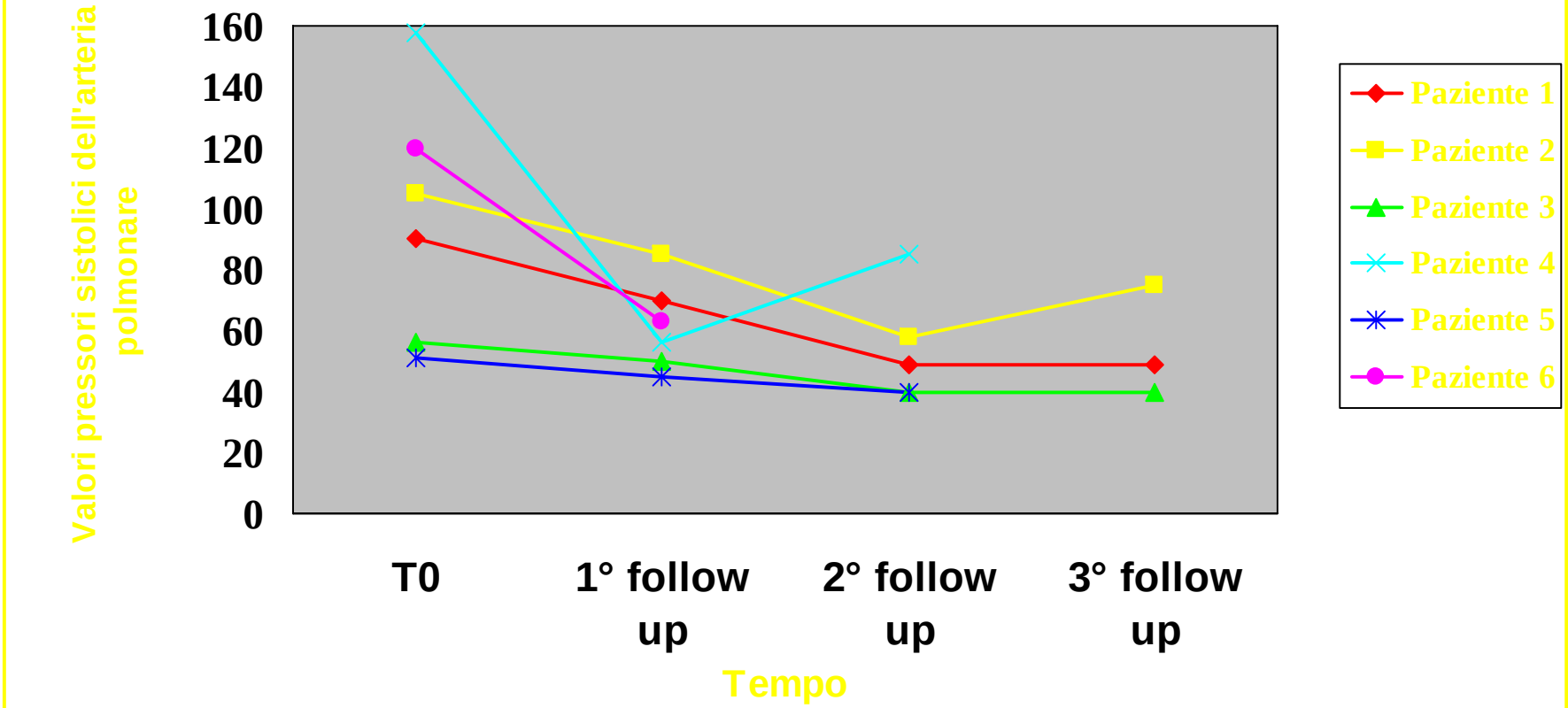
- Promotes smooth muscle relaxation in lung vasculature
- It has been used successfully in primary and secondary PAH

Long-term treatment with sildenafil in a thalassemic patient with pulmonary hypertension.

Littera R, Derchi G et al.

Blood. 2002 Aug 15;100(4):1516-7

PAP during Sildenafil treatment



Derchi et al. Haematologica 2005

Conclusions

✓ Thalassemia Intermedia patients are at risk for PAH

✓ Preventing approaches have to be implemented

✓PAH has to be diagnosed as early as possible

✓ Adequate treatments still need to be explored