Measuring Thrombopoietin - 2012
A New Tool for Hematologists?

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James Homer Wright Established The Basic Elements Of Thrombopoiesis In 1906
Sir William Osler
(1849 – 1919)

Medicine at the bedside
Doctor Endre Keleman
(1921-2000)

Dr. Endre Kelemen Described Human Thrombopoietin In 1958
The Structure of Human Thrombopoietin

Amino-Terminal (EPO-like Domain) | Carboxy-Terminal (Glycosylated Domain)
A rabbit was made thrombocytopenic by the administration of busulfan on Day 0 and platelet counts and thrombopoietin levels measured thereafter.
SUMMARY

• A case of chronic thrombocytopenic purpura has been presented in which the pathogenesis appears to be due to congenital deficiency of a platelet-stimulating factor.

• The factor exists in normal plasma and is stable on storage under normal blood banking conditions and on freezing.

• The factor appears to act by stimulating megakaryocyte maturation and platelet production in an orderly and sequential manner.
The Harrington–Hollingsworth Experiment

Graph shows rapid development of thrombocytopenia, followed by a return to normal platelet levels, in healthy volunteers who received plasma from patients with idiopathic thrombocytopenic purpura.

William J. Harrington (1924–1992)
Case Study # 1

A 42 year - old woman with refractory immune thrombocytopenia (ITP) presents for a second opinion to a university hematologist after Undergoing an extensive treatment regimen including high dose dexamethasone, intravenous immunoglobulin and rituximab.

**Lab Results:**
- Platelet Count: 11,000 /µl
- Hemogram otherwise: Normal
- Mean Platelet Volume: 12.4 /fl (n. 7.5 – 11.5)
- Direct Glycoprotein Antibody (IIb/IIIa): Strongly Positive
- Blood Smear: No Schistocytes
- Immunoglobulins: Normal
- Serology for Epstein-Bar: Negative
- Hepatitis C: Negative
- Helicobacter Pylori: Negative
- Protein Electrophoresis: Negative
- Splenectomy offered: Patient declined

10/11/2012
Clinical Course:
The hematologist offers her a T.P.O. mimetic, Eltrombopag or Romiplostim which stimulates “Megakaryocytopenies”. A serum thrombopoietin level is drawn and comes back 621 pg/ml (n. < 99 pg/ml).

After 3 weeks of Romiplostim (1 mcg/kg), the platelet count remains low at 14,000 µl\(^3\) (n. 150 – 400 x10\(^3\)). The dosage of Romiplostim is increased to 10 mcg/kg without response at week # 12.

Final Decision:
A splenectomy is performed without incident and the platelet count is 151,000 / µl\(^3\) at month 9 after surgery.
What is the value of the elevated TPO level in this patient?

- The TPO level has no value in this case
- The patient has a thrombopoietin – producing tumor
- The assay for TPO is faulty due to poor Quality Control
- The patient has a marked elevation of erythropoietin (EPO) which is cross reacting with the TPO assay
## Thrombopoietin Levels in Blood Disorders

<table>
<thead>
<tr>
<th>Category (n)</th>
<th>Mean Age (yrs.)</th>
<th>Female (%)</th>
<th>Specific Diagnoses (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consumptive Thrombocytopenia (39)</td>
<td>51 (21-83)</td>
<td>24 (62%)</td>
<td>Primary or Secondary ITP (36) Thrombotic Thrombocytopenic Purpura (2) Antiphospholipid Antibody Syndrome (1)</td>
</tr>
<tr>
<td>Hypoproliferative Thrombocytopenia (49)</td>
<td>58 (31-87)</td>
<td>22 (45%)</td>
<td>Chemotherapy-Related (29) Primary or Secondary Bone Marrow Failure Syndromes (20)</td>
</tr>
<tr>
<td>Myeloproliferative Disorders (34)</td>
<td>65 (28-88)</td>
<td>20 (59%)</td>
<td>Essential Thrombocytosis (20) Polycythemia Vera (10) Myeloproliferative Disorder NOS (4)</td>
</tr>
</tbody>
</table>

Makar, R., Zhukov, O., Sahud, M. and Kuter, D. submitted for publication 2012
Case Study # 1

ANSWER…..

• Elevated TPO levels found in patients with ITP are less likely to respond to TPO mimetics drugs.
• Some ITP patients have elevated TPO levels suggesting that inadequate megakaryopoiesis is the predominant pathological feature

<table>
<thead>
<tr>
<th>TPO Level</th>
<th>Clinical Response</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \leq 95 \text{ pg/mL} )</td>
<td>( \leq 95 \text{ pg/mL} )</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 95 pg/mL</td>
<td>&gt; 95 pg/mL</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>49 (34 -66)</td>
<td>1001 (110 -1752)</td>
<td></td>
</tr>
</tbody>
</table>
Thrombopoietin: Why Should We Measure It?

- Patients with high TPO levels do not respond to TPO mimetic's
- Reimbursement for TPO mimetics may hinge on “normal” TPO levels prior to treatment

Methods of Measuring TPO:
- Home brew assay
- C-MPL responsive assay
- First market advantage
Case Study #2

RL, a 73-year-old man has a history of Thrombocytosis, with an initial platelet count of 1,730,000 with normal Hct and WBC. He is treated with Hydrea. The diagnosis is Essential Thrombocythemia (JAK2-neg). He remains on Hydrea at a dose of 500-1000 mg/day for over 3 months. His platelet count falls to 450,000/µl³.
Case Study # 2

- Four months into therapy his platelet count is now 150,000/µl. The Hydrea is discontinued, but over the next 3 months the platelet count continues to fall to 3,000 /µl.

- Patient receives platelet transfusions and bleeding is reduced.

- Diagnosis is uncertain. Treatment includes steroids, IVIG and Winrho.

- Referred to University Hospital for consideration of splenectomy or Thrombopoietin mimetic.
Lab Results

- Exam / diffuse ecchymoses, no splenomegaly, normal WBC count 3,900 and Hematocrit 41%
- Platelet count - 5,000 /µl³ and many large forms noted on smear.

Would a TPO level be of value in this patient?
- No, the patient requires a splenectomy as soon as possible
- No, the patient is surreptitiously taking Hydrea:
  - Obtain Plasma Hydrea level
- No, the patient is septic: draw 3 blood cultures
ANSWER:

- TPO level is 1,500 pg/ml (N. ≤ 75): Suggesting Bone marrow failure

Bone marrow is deferred and Romiplastin is not given

3 months later the platelet count has slowly returned to 115,000 /µl

DIAGNOSIS:

- Idiosyncratic reaction to Hydrea.....?
Case Study # 3

A 39 year-old woman presents for hematologic evaluation at SMC with elevated platelet count.

History
- An elevated platelet count was detected at age 19
- Transient ischemic attack 4 years ago (Platelet count 1.4 million μl³)
- Treatment for the last 18 months previously included:
  - Anegrelide, Interferon-α and Hydrea 500 mg
- Denies fever, sweating, weight loss, early satiety or vasomotor symptoms
- P.E. - No splenomegaly or bruising

Lab Results (at Stanford in March 2010):
- Platelet count - 1,015,000/ μl³ (without other abnormalities)
- WBC – 3,700: 42% neutrophils
- Hemoglobin – 11.6 gm/dl
- Peripheral blood smear - occasional large, hypogranular platelets.
- Reactive causes of thrombocytosis - excluded
- Examination - normal
- JAK2 V617F mutation analysis - negative
Case Study # 3

THIS CASE REPRESENTS A TYPICAL PRE-FIBROTIC STAGE OF ESSENTIAL THROMBOCYTHEMA

EXCEPT... IT ISN'T!!
Clinical Course:
A first year medical resident comes in to take a thorough history.

Doctor:
“Are there any blood disorders in your family?”

Patient:
“No, but my sister has high platelets also.”

Doctor:
“Do you have children?”

Patient:
“Yes, I have a boy age 9 and a girl age 11. They both have elevated platelet counts….so it must be catching!”

Doctor:
“Well, does your husband have elevated platelets?

Patient:
“Oh No! and no other family members have it, except my mother”
Case Study # 3

- Hereditary Thrombocythemia (HT) is suspected
- THPO or MPL mutations are investigated
- Serum TPO Levels are drawn in family members

![Graph showing TPO concentration in serum (pg/mL) for family members with and without mutations, and healthy controls.](image)

- *p=0.0378
- *p=0.0003

Jason Gotlib, et al
**Thrombopoietin Signaling**

**Glycoprotein:**
332 amino acids, 95 kDa Synthesized mainly in the liver

**Thrombopoietin**

![Thrombopoietin diagram with carbohydrate and COOH terminal domain labeled](image)

**Cytokine Receptor:**
Homologous to the oncogene in murine Myeloproliferative leukemia virus Present on megakaryocytes and platelets

**Receptor**

c-Mpl

![Receptor diagram with pathway involving Thrombopoietin receptor, STAT, JAK, MAPK, and increased platelet production](image)

Case # Study 3: THPO Germline Gene Mutation

Wild type

Exon 2 | Intron 2
T    G | G    T    G    A    G

Mutant

Exon 2 | Intron 2
T    G | G    T / C    G    A    G
Case Study # 3: Summary of THPO in HT

- To date, five HT families with three distinct \textit{THPO} mutations have been published, including Dutch, Japanese, and Polish pedigrees.

- No consistency in reports of thrombosis or clinical outcomes; our proband maintained on ASA

- In all cases, the mechanism of overproduction of platelets is related to alteration of the 5' UTR of the \textit{THPO} gene which results in enhanced translation of thrombopoietin (TPO) mRNA.
Case Study # 3: MPL Mutations in HT

<table>
<thead>
<tr>
<th></th>
<th>Essential thrombocythemia</th>
<th>~1-5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>WL:K (W515L/K)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary myelofibrosis</td>
<td></td>
<td>~5-10%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>MPL Ser505Asn (S505N)*</th>
<th>Japanese¹, Italian²,³</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT:K (P106L)</td>
<td></td>
<td>Arab⁴</td>
</tr>
</tbody>
</table>

*Rare frequency in PT-1 Cohort

# Gene Mutations

<table>
<thead>
<tr>
<th>Authors</th>
<th>Gene mutation</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wiestner</td>
<td>TPO, G&gt;C in intron3 position +1</td>
<td>Loss of uORF-mediated repression&lt;sup&gt;29&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kondo, Ghilardi</td>
<td>TPO, deletion of G in 5'-UTR</td>
<td>Loss of uORF-mediated repression&lt;sup&gt;32&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ghilardi</td>
<td>TPO, G&gt;T in 5'-UTR</td>
<td>Loss of uORF-mediated repression&lt;sup&gt;31&lt;/sup&gt;</td>
</tr>
<tr>
<td>Jorgensen</td>
<td>TPO, A&gt;G in intron3 position +5</td>
<td>Not studied</td>
</tr>
<tr>
<td>Ding</td>
<td>MPL, G&gt;A in exon 10 resulting in S505N in Mpl protein</td>
<td>Constitutively active Mpl protein</td>
</tr>
<tr>
<td>Moliterno</td>
<td>MPL-K39N</td>
<td>Co-dominant, mild thrombocytosis in homozygotes, function uncertain</td>
</tr>
<tr>
<td>El-Harith</td>
<td>MPL-P106L</td>
<td>Co-dominant, elevated Tpo serum levels</td>
</tr>
<tr>
<td>Kawamata</td>
<td>MPL-S204F</td>
<td>Found in uniparental disomy 1p, function uncertain</td>
</tr>
<tr>
<td>Williams</td>
<td>MPL-S204P</td>
<td>Function uncertain</td>
</tr>
<tr>
<td>Komatsu</td>
<td>MPL-S505N</td>
<td>Constitutive activation of Mpl protein, autosomal dominant thrombocytosis</td>
</tr>
<tr>
<td>Chaline</td>
<td>MPL-A506T</td>
<td>Function uncertain</td>
</tr>
<tr>
<td>Chaline</td>
<td>MPL-L510P</td>
<td>Function uncertain</td>
</tr>
<tr>
<td>Pikman</td>
<td>MPL-W515L</td>
<td>Constitutive activation of Mpl protein, sporadic ET or PMF</td>
</tr>
<tr>
<td>Pardanani</td>
<td>MPL-W515K</td>
<td>Constitutive activation of Mpl protein, sporadic ET or PMF</td>
</tr>
<tr>
<td>Vannucci</td>
<td>MPL-W515A</td>
<td>Constitutive activation of Mpl protein, sporadic ET or PMF</td>
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<tr>
<td>Chaline</td>
<td>MPL-A519Y</td>
<td>Function uncertain</td>
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<tr>
<td>Kawamata</td>
<td>MPL-Y591D</td>
<td>Found in uniparental disomy 1p, function uncertain</td>
</tr>
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</table>
Reactive Thrombosis

Hazard ratio = 5.3, 95% confidence interval 1.7–16; \( P = 0.004 \)
What Do These Case Studies Tell Us Today?

- **In case #1:**
  - We learned that serum thrombopoietin may give further insight into the nature of I.T.P. and perhaps save $1200 per month for a treatment that is unlikely to yield results.

- **In case #2**
  - We find out that serum thrombopoietin may allow one to predict imminent platelet recovery in a patient with a Hydroxyuea-induced hypoplastic marrow.

- **In case #3**
  - The features of Essential Thrombocytethemia suggesting a Myeloproliferative Disorder may in fact be hereditary in nature and that data is emerging that high platelets are risk factors for D.V.T. and P.E.
THANK YOU
Eltrombopag for the treatment of chronic idiopathic thrombocytopenic purpura.

Kuter DJ, Bussel JB, Lyons RM, et al.

Kuter DJ.
References

George JN.

Kaushansky K.