Clinical Diagnosis of ITP

Nichola Cooper
September 2016
Disclosures for

In compliance with COI policy, ISTH requires the following disclosures to the session audience:

<table>
<thead>
<tr>
<th>Research Support/P.I.</th>
<th>Pfizer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Employee</td>
<td>No relevant conflicts of interest to declare</td>
</tr>
<tr>
<td>Consultant</td>
<td>Novartis, Amgen</td>
</tr>
<tr>
<td>Major Stockholder</td>
<td>No relevant conflicts of interest to declare</td>
</tr>
<tr>
<td>Speakers Bureau</td>
<td>No relevant conflicts of interest to declare</td>
</tr>
<tr>
<td>Honoraria</td>
<td>Amgen, Novartis</td>
</tr>
<tr>
<td>Scientific Advisory Board</td>
<td>Amgen, Novartis</td>
</tr>
</tbody>
</table>

Presentation includes discussion of the following off-label use of a drug or medical device:

<N/A>
Immune Thrombocytopenia (ITP)

- Isolated thrombocytopenia (platelets <100)

- Exclusion of other causes:
  - Inherited platelet disorders
  - Increased destruction: (other autoimmunity, viruses)
  - Decreased production: (viruses, infiltration, marrow failure)

- 1 in 40,000 F>M, approximately 1-2% familial

- Definitions:
  - Newly diagnosed (0-3 months)
  - Persistent (3-12 months)
  - Chronic (>12 months)
  - Refractory
ITP terminology: 3 ITP phases

Possibility of spontaneous remission:

- Newly diagnosed ITP: 0–3 months
- Persistent ITP: 3–12 months
- Chronic ITP: >12 months

Challenges in managing ITP

**Diagnosis**
- Bone marrow
- APAs

**Pathogenesis**
- T cell
- B cell
- Megakaryocyte

**Treatment**
- **Who:**
  - Platelet count, symptoms, psychology
- **When:**
  - Newly diagnosed, persistent, chronic
- **What:**
  - Steroids, IVIg, rituximab, TPO-RAs, MMF, other...

APA, against phospholipid antigens; IVIg, intravenous immunoglobulin; TPO-RA, thrombopoietin receptor agonist; MMF, mycophenolate mofetil
Overview

- There is no diagnostic test for ITP, it is a diagnosis of exclusion
- The pathology of ITP is unclear and may have multiple causes
- This is relevant when considering treatment, which should be tailored to individual patient details
Pathogenesis (known and presumed)

- Genetic susceptibility vs environment
- Serum derived effects: antiplatelet antibodies
- Unregulated T helper cells and activated cytotoxic T cells
- Inhibition of megakaryopoiesis (?)
Genetic susceptibility vs infection

SLE 5%
APS 2%
CVID 1%
CLL 2%
Evan's 2%
ALPS, post-tx 1%
HIV 1%
Hep C 2%
H. pylori 1%
Post vaccine 1%
Misc. systemic infection 2%

Cines DB et al. Blood 2009;113:6511-6521
What is the evidence for immune pathology in ITP? B-cell disease

Antiplatelet antibodies: 1951

Inhibition of megakaryocytes by plasma from ITP patients: 2004

Platelet count after infusion with patient plasma

What is the evidence for immune pathology in ITP? T-cell-mediated

T-cell proliferation in response to own platelets and Th1-mediated disease: 1990s\(^1\)

Cytotoxic T-cell attack against platelets: 2003\(^2\)

\[\text{\(^3\)H-thymidine incorporation (x10\(^{-3}\))} \]

\[
\begin{array}{c|c|c|c}
\text{Platelets (x10\(^9\)/L)} & 0 & 50 & 100 \\
\hline
\text{Control} & 4 & 8 & 12 \\
\text{Active ITP} & 16 & 20 & 24 \\
\text{ITP in remission} & 8 & 12 & 16 \\
\end{array}
\]

\[
\begin{array}{c|c|c|c}
\text{Platelet lysis} & 0 & 10 & 20 \\
\hline
\text{Control} & 5 & 10 & 15 \\
\text{Active ITP} & 20 & 30 & 40 \\
\text{ITP in remission} & 10 & 20 & 30 \\
\text{Control} & 5 & 10 & 15 \\
\text{Active ITP} & 20 & 30 & 40 \\
\text{ITP in remission} & 10 & 20 & 30 \\
\end{array}
\]


CD, cluster of differentiation; IFN-\(\gamma\), interferon-\(\gamma\); IL, interleukin; TGF-\(\beta\), transforming growth factor-\(\beta\); Th, helper T cell
Immunological profile in ITP

- Activated T cells, Th1, Th17 profile $^{1-4}$
- Pro-inflammatory cytokines $^{1-6}$
- Impaired Treg function $^7$

Complex ITP pathophysiology underlies platelet destruction

- Impaired megakaryocyte maturation
  - Reduced platelet production
- Platelet autoantibody production
- Tc-cell-mediated platelet destruction
- Platelet phagocytosis
- Epitope spreading
- Platelets

Adapted from Stasi R et al. Thromb Haemost 2008;99:4–13

MHC, major histocompatibility complex; Tc, cytotoxic T cell; TCR, T-cell receptor
Many factors are involved in making platelets

TPO

MK development:
GATA1, RUNX1, FLI1, ANKRD26, NBEAL2, GFI1N

Pro-platelet development:
Cytoskeleton: Rock, myosin (inhibition Inc)

Collagen, VWF, fibrinogen (GpIb-IX-V, aIIbB3)

Migration: SD1a

Bone

Stem cell niche

Vascular niche

Blood vessel

Sheer stress

Adapted from Pecci A & Balduini CL. Br J Haematol 2014;165:179–192

MK, megakaryocyte; TPO, thrombopoietin
Old platelets are recognized by the Ashwell–Morell receptor (AMR) in hepatocytes causing thrombopoietin production
Thrombopoietin levels are lower than expected in ITP

- No correlation between platelet count and TPO levels in patients with ITP\(^1\)
- No significant difference in TPO levels between patients with ITP and controls\(^2\)

Causes of Thrombocytopenia

- Platelet production and regulation is not fully understood

- Thrombocytopenia can be the end result of many events
  - Gene defects in megakaryocyte development
  - Bone marrow infiltration
  - Infection
  - Increased destruction (APAs and T cells)
  - ? Abnormalities of thrombopoietin regulation
Clinical diagnosis of ITP: medical history

• Presenting Complaint
  – Extent of bleeding (*inherited platelet disorders*)

• Past Medical History
  – Infections (*immunodeficiency*)
  – Previous bleeding (*tonsils, teeth*)

• Review Of Systems
  – Joints, rashes, hair loss, mouth ulcers, night sweats, weight loss (*lupus, lymphoproliferative disorders*)

• Family History
  – Bleeding disorders, thrombocytopenia, autoimmune diseases
Examination

- Extent of bleeding, area of bleeding
  - Bleeding scores

- Lymphadenopathy *(not consistent with ITP)*

- Splenomegaly *(not consistent with ITP)*

- Hepatomegaly *(not consistent with ITP)*
Blood film examination

pseudothrombocythaemia

Erythrocytes
- Fragmentation: TTP/HUS/DIC/HELLP
- Macrocytes: megaloblastosis, haemolysis
- Spherocytes: Evans

Platelets
- Large: hereditary thrombocytopenias
- Small: Wiscott-Aldrich syndrome
- Large and small: ITP

Leukocytes
- Toxic granules
- PMN Inclusions
- Atypical lymphocytes (infection, ITP)
- Blasts: leukaemia
Other causes of thrombocytopenia based on platelet size

Drachman J G Blood 2004;103:390-398
<table>
<thead>
<tr>
<th>Small platelets MPV &lt;7fL</th>
<th>Normal platelets MPV 7-11 fL</th>
<th>Large/Giant platelets, MPV &gt;11 fL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wiskott-Aldrich syndrome</td>
<td>Familial platelet disorder/AML</td>
<td>MHY9-related diseases</td>
</tr>
<tr>
<td></td>
<td></td>
<td>May-Hegglin anomaly</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sebastian syndrome</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fechtner syndrome</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Epstein syndrome</td>
</tr>
<tr>
<td>X-linked thrombocytopenia</td>
<td>Chromosome 10/THC2</td>
<td>Bernard-Soulier syndrome</td>
</tr>
<tr>
<td></td>
<td>Congenital Amegakaryocytic</td>
<td>Paris-Trousseau</td>
</tr>
<tr>
<td></td>
<td>thrombocytopenia</td>
<td>thrombocytopenia/Jacobsen</td>
</tr>
<tr>
<td></td>
<td>Thrombocytopenia and absent</td>
<td>Velocardiofacial/DiGeorge</td>
</tr>
<tr>
<td></td>
<td>radii</td>
<td>syndrome</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GATA1 mutation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Grey platelet syndrome</td>
</tr>
</tbody>
</table>
Platelet size for distinguishing between inherited thrombocytopenias and immune thrombocytopenia: a multicentric, real life study
Investigations in persistent ITP

- Immunodeficiency:
  - Immunoglobulins
  - Lymphocyte subsets
- Platelet disorders:
  - vWAg (children)
- Autoimmunity:
  - DRVVT
  - ACL
  - Autoimmune profile
  - TFTs
- Infections:
  - Hep B, Hep C, HIV, CMV
  - H pylori
Controversial Investigations in persistent ITP

**Bone marrow examination:**
Guidelines recommend only in those with *abnormal features*, those over 60 and pre-splenectomy

**Anti-platelet antibodies:** not recommended (not sensitive or specific enough to direct treatment)
Bone marrow in ITP

- Useful to confirm no other features causing thrombocytopenia
- Useful to describe megakaryocyte features
- Useful for a baseline test pre long term treatment (3 of our cohort have developed other bone marrow conditions – OS, MF, MDS)
- May be useful in the future to direct treatment
We analysed the interaction of **CD4 cells** and **CD8 cells** with megakaryocytes in bone marrow
ITP patients have higher MGK density in the bone marrow but no difference in size.
ITP patients have similar T-cell numbers to disease controls

Thomas Mayo
But more CD4/MK interactions and clustering (more than 1 CD4 per MK)
Increased CD4/MK interactions in patients with chronic disease

CD4 IPs % (CD4+MGK) by disease phase

No. of IPs % (CD4+MGK)

p=0.0154

ND/Persistent phase

Chronic phase

Thomas Mayo
No difference in CD8/MK interactions or CD8/MK clustering
Peripheral blood inflammatory CD8 is significantly higher in ITP patients

**P value <0.01**
Expression of proinflammatory markers is not increased in CD4 cells of ITP patients

CD8 TNF α

CD8 IL2

* P value <0.05
Regulatory T cells are significantly reduced in the periphery in ITP patients

* P value <0.05

Anwar Sayed
Bone marrow changes

- May reflect disease activity
- Correlation with clinical findings and peripheral blood abnormalities is in progress
Antiplatelet antibodies are difficult to measure.
Successful treatment with oseltamivir phosphate (tamiflu) in a patient with chronic immune thrombocytopenia positive for anti-GP Ib/IX autoantibody.
Potential use for APAs

- Identification of specific APAs may allow:
  - Identification of types of ITP
  - May be able to predict bleeding and
  - May predict treatment responses

- New methods are needed
Thrombopoietin levels may distinguish between consumptive or hypoproliferative causes of thrombocytopenia

Maker et al Haematology 2013: Thrombopoietin levels in patients with disorders of platelet production: Diagnostic potential and utility in predicting response to TPO Receptor agonists
Reduced absolute immature platelet fraction (IPF), but increased % IPF in ITP

IPF may allow a better understanding of treatment effects

Relevance to ITP patients

- Variable findings in the pathology
  - Only 60% have identifiable antibodies
  - T cell and bone marrow changes variable

- Variable responses to many treatments with no biomarkers
  - 60% respond to splenectomy
  - 50% respond to rituximab
  - 30-50% respond to immunosuppression

- Wide variability in responses shows a diverse disease
Treatment recommendations based on platelet count

- **Platelet count**
  - 0
  - 10x10⁹/L
  - 30x10⁹/L
  - 150x10⁹/L

- **Treat adults**
  - Treatment depends on individual patient factors: Age, other comorbidity, success of treatment
  - Only treat if bleeding, requiring surgery or requiring anticoagulation or antiplatelet agents

- **Only treat children with symptoms**

Images courtesy of N Cooper
Risk of fatal/severe bleeding in ITP

- Presence of other conditions such as hypertension or cerebrovascular disease

- Increased rates of fatal haemorrhage rates with age
  - 0.004 per patient-year <40 yrs
  - 0.012 per patient-year 40 to 60 yrs
  - 0.130 per patient-year >60 yrs

- Increased risk of VTE

Management of immune thrombocytopenia (ITP)

Thrombocytopenia
Diagnosis: infection, drugs, inherited, acquired

Newly diagnosed ITP (0-3 months)
Steroids or IVIG

Persistent ITP (3 to 12 months)
MMF or thrombopoietin receptor agonists or Rituximab

Chronic ITP (>12 months)
Continuous TPO-RAs
Repeated rituximab (+/- dexamthasone)
Continuous MMF
Other: (danazol, dapsone, hydroxychloroquine)
Splenectomy
What factors are involved in treatment decision making?

Consider other factors:
- Rituximab: Increased response in women within first 2 years of diagnosis
- MMF: Less effective in patients with virus-associated ITP
- TPO-RAs: Potential risk of thrombosis
Refractory ITP (approx 5%)

- Consider the diagnoses, is it ITP?
- Role at this stage for bone marrow examination
- Role for antiplatelet antibody testing
- ? Role for NGS
- There is a real need for better understanding of ITP and better diagnostic markers
Summary

- ITP is a diagnosis of exclusion of other causes of thrombocytopenia
- Better understanding of the underlying pathology is needed
- Anti-platelet antibodies may be helpful
- TPO levels, bone marrow biopsies and NGS have potential
Plan

- Establish better antiplatelet antibody testing
- Establish a B and T cell immunophenotyping panel to differentiate patients into subtypes
- Analyse more bone marrow samples
- Correlate TPO levels with outcome
- Prospective studies to establish biomarkers
- Better phenotyping will help to guide genomic studies