Platelet Function Disorders (PFD)

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Platelet Function in Haemostasis

- Essential to primary haemostasis (platelet adhesion and “plug” formation
- Platelet activation by trace amounts of thrombin (collagen, vWF) leads to catalytic –ve charged surface for assembly coagulation
- Delivery of molecules for effective thrombus formation and would healing and repair
Overview - PFD

Inherited
- Adhesion Defects (BSS, Plt-type VWD, others)
- Agonist Receptor (Collagen receptor α2B1 GPIV; ADP - P2Y12; TXA2 receptor deficiency)
- Signalling (various)
- Secretion (SPD; Dense granule deficiency, alpha granule deficiency, others)
- Aggregation (GT, Congenital afibrinogenaemia)
- Membrane defects (Scott’s Syndrome)

Acquired
- Anti-platelet medications
- Uraemia
- Primary BM disease (MPN, MDS, Leukaemia)
- Dysproteinaemia
- Acquired VWD
- Acquired Storage Pool Disease
- ITP (anti-platelet Abs)
- Liver Disease

The Patient….
- Muco-cutaneous bleeding
- Usually mild bleeding/bruising but variable in severity
- Generally provoked bleeding (occasionally spontaneous)
- Maybe a family history of bleeding
The Patient’s Dr.:

- Nuisance bleeding/bruising
- Contribution to Iron deficiency
- Concerns of bleeding and worse outcomes with surgery as patient is a “bleeder”
- “What exactly is the problem with your platelets?”
- “Do you need a platelet transfusion?”

The Haematologist:

- Confirm abnormal bleeding phenotype (patient assessment, ? Bleeding Score)
- Acquired PFD – medications, renal failure, bone marrow disease
- Specific physical signs (petechiae, purpura, splenomegaly, eczema, deafness, cataracts, albinism, developmental abnormalities)
- Platelet count and blood film
- Screening assessments (SBT & PFA-100 …not very useful)
- Platelet function testing – LTA, Mepacrine staining dense granules by flow and quantitation of release, EM whole mount assessment
- Investigation particular defects as required
  - Platelet membrane glycoprotein expression
  - MYH9 immuno-histochemistry
  - Ultrastructural examination by TEM
  - Molecular genetics (GT, BSS, others)
Platelet Aggregation (LTA)
Method of Born GV

Patient LP - BSS
Platelet Granules

- **Alpha granules**
  - Largest, most abundant (~80/plt), heterogeneous contents growth factors coagulation proteins adhesion molecules cytokines angiogenic factors

- **Dense granules**
  - Less abundant (~7/plt), molecules for cell activation (Nucleotides, ions, serotonin)

- **Lysosomes (endosomes)**
  - Primary & secondary lysosomes, involved endosomal-lysosomal degradative pathway (? clathrin-independent)

Cellular Events in Platelet Secretion
Dense Granule SPD

- Decreased/absent dense granules with reduced serotonin and ADP/ATP stores
- Reduced thrombus formation and mild-moderate bleeding phenotype believed to parallel the degree of dense granule deficiency
- Associated with disorders pigmentation and lysosomal storage (HPS and CHS genes, other signalling genes)
- Diagnosis in patients with muco-cutaneous bleeding by aggregation findings (reduced 2 phase aggregation to collagen, ADP, Epinephrine) and reduced mepacrine labelling by flow.
- Diagnosis often missed by aggregation
- Gold standard test is EM of unstained whole mount platelets

Whole Mount Methods (1)

- Platelet preparation and fixation (need patient)
  - Citrate or ACD
  - Make PRP
  - Drop on grid for 3-5s
  - Drain and dry excess with filter paper
  - Drop 0.1% glutaraldehyde in White’s saline for 3-5s
  - Rinse H2O, drain excess with filter paper, air-dry and into EM unstained
Whole Mount Methods (2)

Whole Mount Methods (3)

JEOL-1400 TEM
40-120keV
Whole Mounts

1000x

6000x

Dense Granule EM RR

<table>
<thead>
<tr>
<th></th>
<th>Sum</th>
<th>N</th>
<th>Sum/100 plats</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>% 0 score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>480 (±113)</td>
<td>5 (± 0.87)</td>
<td>33</td>
<td>7.6%</td>
<td></td>
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<tr>
<td>Abnormal</td>
<td>&lt;250</td>
<td>&lt;2</td>
<td>&gt;10%</td>
<td></td>
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</tbody>
</table>

Platelet Dense Granule Count
### Patients

<table>
<thead>
<tr>
<th></th>
<th>Sum</th>
<th>N</th>
<th>Sum/100 plats</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>% 0 score</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChGabb</td>
<td>453</td>
<td>205</td>
<td>&lt;250</td>
<td>221</td>
<td>1</td>
<td>22</td>
<td>&lt;10%</td>
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<tr>
<td>DSt</td>
<td>87</td>
<td>100</td>
<td>&lt;2</td>
<td>87</td>
<td>0</td>
<td>0</td>
<td>55%</td>
</tr>
<tr>
<td>KSt</td>
<td>41</td>
<td>90</td>
<td>&lt;2</td>
<td>46</td>
<td>0</td>
<td>8</td>
<td>74%</td>
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<tr>
<td>JL</td>
<td>141</td>
<td>100</td>
<td>&lt;2</td>
<td>141</td>
<td>1</td>
<td>11</td>
<td>37%</td>
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#### Platelet Dense Granule Count

<table>
<thead>
<tr>
<th>Platelet Dense Granule Count</th>
<th>No. Dense Granules/Platelet</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChGabb</td>
<td>0.0</td>
</tr>
<tr>
<td>DSt</td>
<td>0.0</td>
</tr>
<tr>
<td>JL</td>
<td>0.0</td>
</tr>
</tbody>
</table>

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#### Platelet Dense Granule Count

- **ChGabb**
- **DSt**
- **Control**
- **JL**
Platelet Function Disorders

- Often iatrogenic
- Inherited PFD
  - Common
  - Mild bleeding phenotype
  - Laboratory investigation is complex
  - Characterisation of defect is valuable as the specific diagnosis facilitates sound management advice.