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

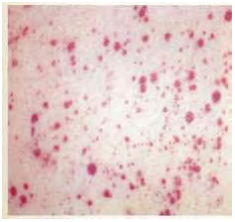
- Adhesion Defects (BSS, Plt-type VWD, others)
- Agonist Receptor (Collagen receptor a2B1 GPIV; ADP - P2Y12; TXA2 receptor deficiency)
- Signalling (various)
- Secretion (SPD; Dense granule deficiency, alpha granule deficiency, others)
- Aggregation (GT, Congenital afibrinogenaemia)
- Membrane defects (Scott's Syndrome)

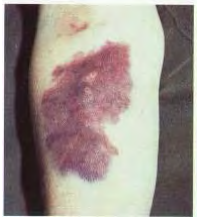
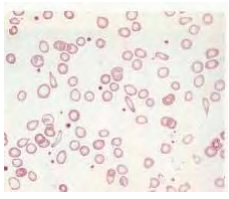
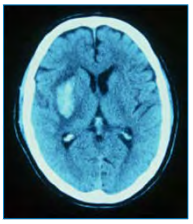
Acquired

- 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….

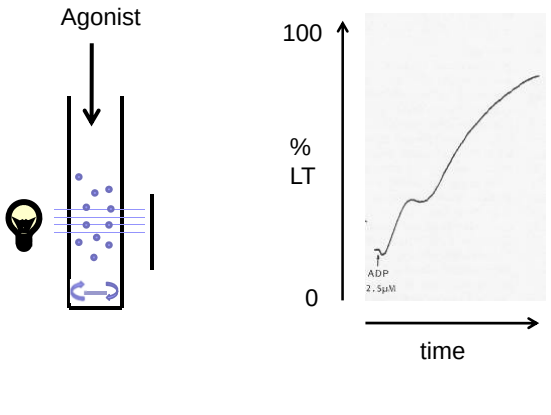
- Nuisance bleeding/bruising
- Contribution to Iron deficiency
- Concerns of bleeding and worse outcomes with surgery as patient is a "bleeder"
- "What exactly is the problem with your platelets?"
- "Do you need a platelet transfusion?"

The Haematologist….

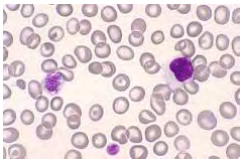
- Confirm abnormal bleeding phenotype (patient assessment, ? Bleeding Score)
- Acquired PFD – medications, renal failure, bone marrow disease
- Specific physical signs (petechiae, purpura, splenomegaly, eczema, deafness, cataracts, albinism, developmental abnormalities)
- Platelet count and blood film
- 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)

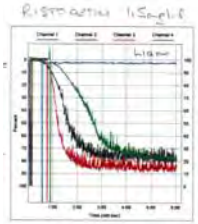
Platelet Aggregation (LTA)

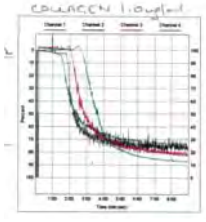
Method of Born GV

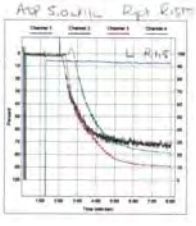


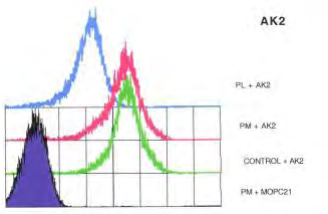
Patient LP - BSS

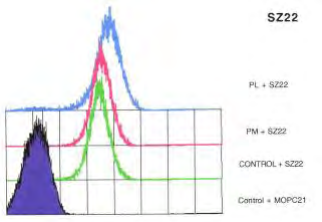












Platelet Granules

- 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

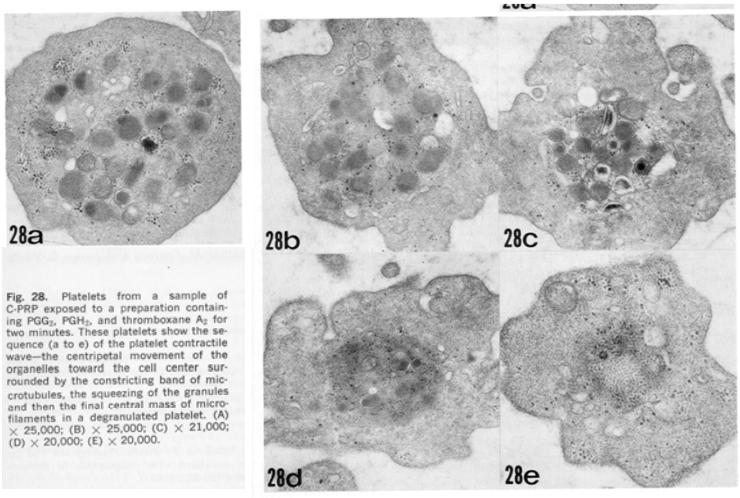


Fig. 28. Platelets from a sample of C-PRP exposed to a preparation containing PGG_2, PGH_2, and thromboxane A_2 for two minutes. These platelets show the sequence (a to e) of the platelet contractile wave—the centripetal movement of the organelles toward the cell center surrounded by the constricting band of microtubules, the squeezing of the granules and then the final central mass of microfilaments in a degranulated platelet. (A) × 25,000; (B) × 25,000; (C) × 21,000; (D) × 20,000; (E) × 20,000.

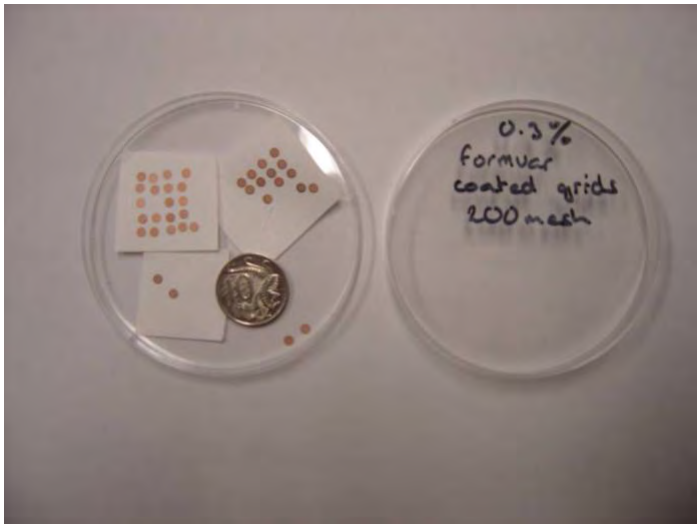
Dense Granule SPD

- 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

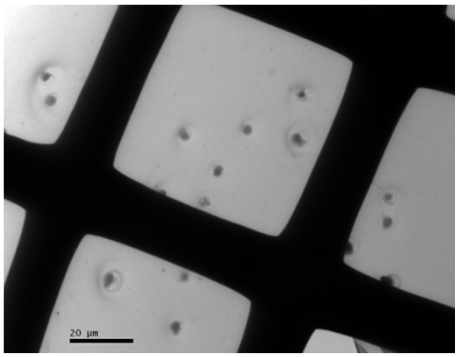
Whole Mount Methods (2)

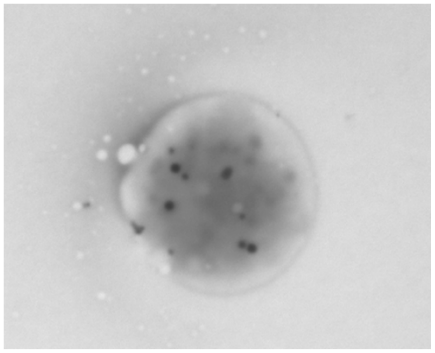


Whole Mount Methods (3)

JEOL-1400 TEM
40-120keV

Whole Mounts



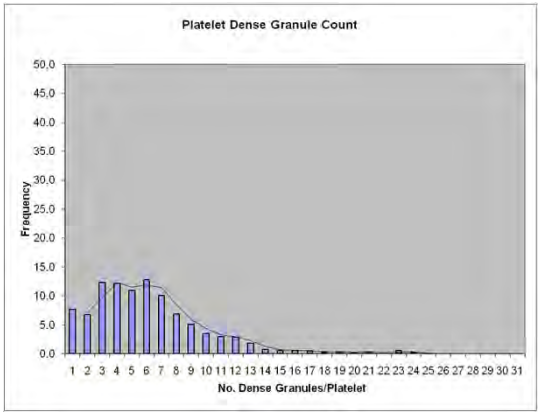


1000x

6000x

Dense Granule EM RR

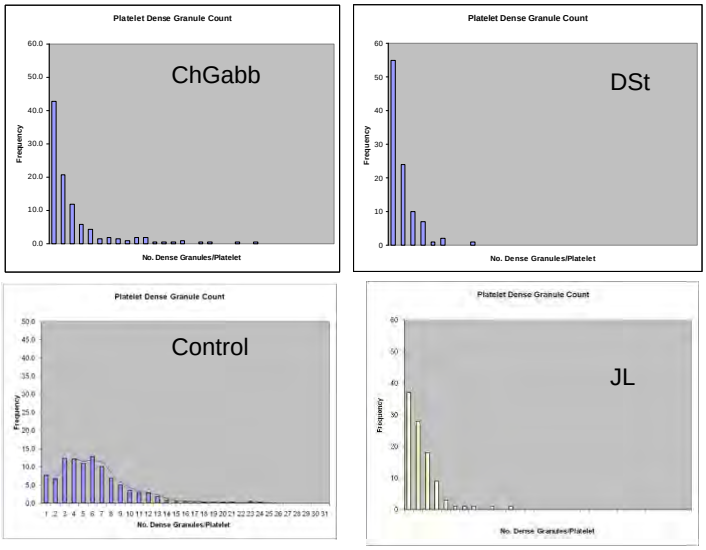
	Sum	N	Sum/100 plats	Median	Min	Max	% 0 score
Controls			480 (± 113)	5 (± 0.87)	0	33	7.6%
Abnormal			<250	<2			>10%



Patients

	Sum	N	Sum/100 plats	Median	Min	Max	% 0 score
			<250	<2			<10%
ChGabb	453	205	221	1	0	22	43%
DSt	87	100	87	0	0	8	55%
KSt	41	90	46	0	0	8	74%
JL	141	100	141	1	0	11	37%

Patients



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.