

# Platelet Function Tests

## What, When, How

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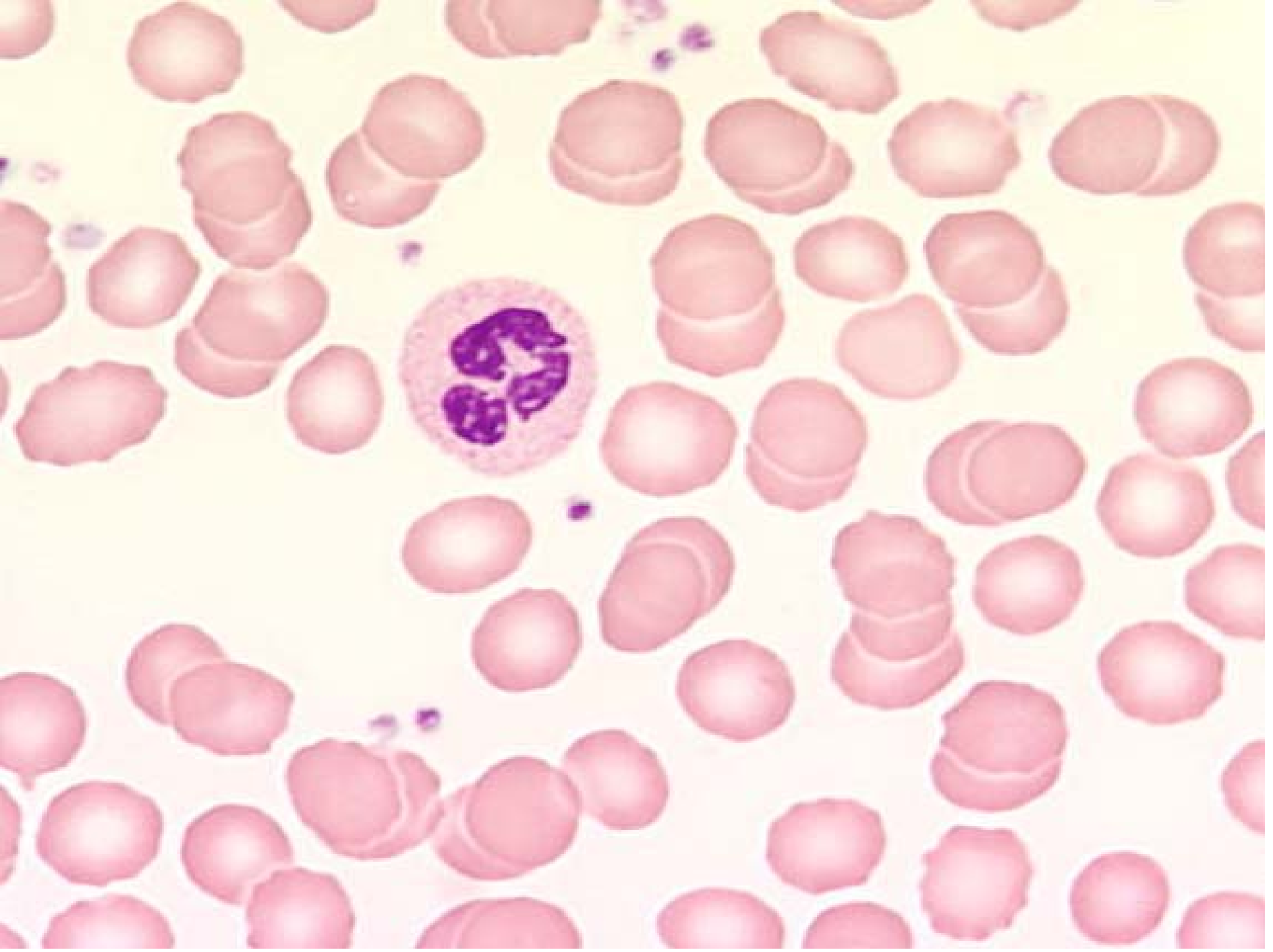
# Platelet Discovery

- Existence of the tiny cell called platelets was under dispute by light microscopy, which was resolved.
- German anatomist Max Schultze (1825-1874) who first offered a description of the platelet as "spherules".
- Giulio Bizzozero (1846-1901),  
**Named these «piastrine»,  
i.e. small plates (later platelets)  
and confirmed the role of  
platelets in coagulation**



# Platelet

- Remarkable mammalian adaptation.
- Required for human survival to prevent and arrest bleeding.
- Ironically, in the past century, this haemostatic activity became maladaptive.
- Develop age-dependent progressive atherosclerosis.
- Major contribution to ischaemic thrombotic vascular disease, the leading cause of death worldwide.



# Platelets

- **Produced in the bone marrow from mature megakaryocytes.**
- **Circulate in blood as disc-shaped anucleate particles.**
- **2–4  $\mu\text{m}$  in diameter with a mean platelet volume of 7 to 9 fl.**
- **150 – 450,000 / cmm**
- **The average lifespan of a platelet is about 5 to 9 days.**
- **Regulated by thrombopoietin, a hormone usually produced by the liver and kidney.**
- **Each megakaryocyte produces between 5,000 and 10,000 platelets.**
- **Contain many storage granules.**
- **A continuous membrane structure. Diverse cell surface receptors**
- **Signaling molecules that direct platelet adhesion, activation, and aggregation as well as coagulation**
- **Sequestered in the spleen & Liver**

# Platelets\_

## Cytoplasmic granules:

1.Alpha granules

2.Dense bodies

## Alpha granules

- Platelet factor 4 Heparinoid neutralisation
- Beta-thromboglobulin ? chemotaxis
- Thrombospondin? aggregation
- Platelet derived growth factor Mitogenesis, vessel repair
- vonWillebrand factor Adhesion ,aggregation
- Fibrinogen Aggregation ,coagulation
- Factor V Prothrombinase activity
- Fibronectin Fibroblast and platelet adhesion
- Plasminogen activator inhibitor 1 Inhibition of fibrinolysis

## **Dense Bodies:**

- ADP Aggregation ,vasoconstriction
- ATP Degrades to ADP
- 5 – HT Vasoconstriction , aggregation
- Calcium ?
- Pyrophosphate ?



## Plasma Membrane:

Number of specific receptors, often glycoproteins (GPs) through which platelets interact.

- Some important glycoproteins:
  - GP1a Collagen adhesion
  - GP1b Subendothelial microfibril adhesion:
  - GP II b-IIIa Fibrinogen binding, aggregation:
  - GP IV Thrombospondin binding
  - GP V Thrombin binding, aggregation
  - GP IX Platelet adhesion: part of GP – 1 b complex

# Platelets

- Platelets play a key role in both hemostasis and thrombosis.
- Accurate measurement of platelet function is critical for identifying patients with
  - platelet dysfunction
  - hyperfunction,
  - monitoring of modern antiplatelet therapy.
- majority of the 20th century the only means of assessing platelet function were a small number of fairly unreliable tests
  - manual platelet count,
  - inspection of the peripheral blood smear,
  - bleeding time.

# Platelets

- **A major problem**
- difficulty in simulating hemostasis in vitro.
- platelets are sensitive to manipulation,
- prone to artifactual in vitro activation.
- Early attempts to simulate hemostasis in vitro included methods in which platelets were counted before and after exposure to foreign surfaces (eg, glass columns) or thrombus formation was monitored within closed plastic tube loops.

# Platelets Disorders

- Platelet disorders are the most common cause of bleeding
- 1. Platelet number – Thrombocytopenia  
Thrombocytosis
- 2. Platelet Function defect

- Clinically suspected bleeding tendency.
- Following up an abnormal first line test.
- Acute haemostatic failure.

## Begin with-

- Clinical history (congenital or acquired ? Sec to)
- Family history (Hemophilia, vWF)
- Family tree
- Thorough clinical examination (Liver, spleen, LN )
- site ( Local or Haemostatic )
- Nature & frequency (spontaneous or post traumatic, duration, amount,transfusion etc.)
- Type ( petechia, purpura and mucosal bleeding – platelet disorder. hematoma, hemarthrosis-coagulation disorder)

# Defective Platelets Function

- A defect in function is suspected
  - if there is prolonged bleeding time
  - with or without skin or mucosal hemorrhage
  - in the presence of normal platelet count.
- 
- acquired platelet dysfunctions can occur at any age
  - range in severity from mild to life-threatening haemorrhages

# Disorders of Platelets Function

## Congenital

Rare

- Glanzman's disease
- Bernard Soulier's
- Storage granules defect

## Acquired

Common

- Drugs
- Uremia
- Myeloproliferative disorders
- Multiple myeloma



# Screening Coagulogram

- Bleeding time ( Ivy's method )
- Whole blood clotting time(Lee & White)
- Platelet count
- Platelet morphology
- Clot retraction
- Prothrombin Time ( P T )
- Activated partial thromboplastin time(APTT)
- Thrombin Time
- Clot solubility test

# Screening Coagulogram

- **Bleeding time** ..... ( upto 7 min )  
( Ivy's method )
- **Clotting time** ..... ( 5 to 11 min )  
( Lee & White)
- **Platelet count** ..... ( 150 – 450,000 /cmm )
- **Platelet morphology** ..... ( size & clumps )
- **Clot retraction** ..... ( good )
- **Prothrombin Time – T** ..... ( sec )  
( P T ) - C ..... ( sec )
- **Activated partial thromboplastin time - T ...** ( sec )  
( aPTT) C... ( sec )
- **Thrombin Time** ..... T... ( sec )  
C... ( sec )
- **Clot solubility test** ..... Insoluble

Impression -

# Screening Coagulogram

- Bleeding time ( Ivy's method )
- Whole blood clotting time(Lee & White)
- Platelet count
- Platelet morphology
- Clot retraction
- Prothrombin Time ( P T )
- Activated partial thromboplastin time(APTT)
- Thrombin Time
- Clot solubility test

- Bleeding time and clotting time extremely insensitive tests
  - Bleeding time remains normal until platelet count drops to 30,000/cmm.
  - Clotting time remains normal until factor VIII or IX is less than 1 %.

# Bleeding Time

- Template bleeding time
- Described by Duke in 1910 is the oldest test of platelet function
- Surprisingly it remains in wide use in the UK
- There is considerable variation in methodology between laboratories.

# Bleeding time

- The BT is highly dependent on –
- operator technique,
- is subjective
- is influenced by patient variables unrelated to haemostasis, such as age, gender, haematocrit, vascular pattern, skin thickness and skin temperature.
- The BT therefore has poor reproducibility, sensitivity and specificity, as well as being invasive; for these reasons it is not recommended.

# Laboratory Evaluation

- A reliably predictive “screening” test for platelet dysfunction does not exist.
- Neither the bleeding time (BT) nor the platelet function analyzer (PFA) is good for screening asymptomatic persons

# Bleeding

- Platelet count - 150 – 450,000 /cmm
- Thrombocytopenia - < 150,000 /cmm
  - Spontaneous bleeding < 20,000 /cmm
    - ( Giant platelet - ITP )
    - ( Small - marrow failure synd.)
  - will bleed on injury < 50,000 /cmm
  - If h/o bleeding > 40,000 /cmm
    - ( Suspect Platelet function defect )



# Platelet disorders

are common bleeding disorders

Congenital - Number  
Function

Acquired - Number  
Function

- The diagnostic evaluation of platelet disorders challenges both clinicians and clinical laboratories
- Testing for these conditions is complex,  
not well standardized  
time consuming.

<b>Platelet count ( x10<sup>9</sup>/L)</b>	<b>Risk of bleeding</b>	<b>Examples</b>
<b>&lt;75</b>	<b>Primary hemostasis impaired</b>	<b>After major surgery, trauma</b>
<b>&lt;50</b>	<b>Spontaneous bleeding</b>	<b>mostly seen in skin Petechiae, purpura</b>
<b>&lt;20</b>	<b>Noticeable hemorrhage</b>	<b>seen in skin and mucosa Epistaxis, gingival bleed</b>
<b>&lt;10</b>	<b>Possible life-threatening hemorrhage</b>	<b>mucosa and CNS Acute GI hemorrhage, intracranial hemorrhage</b>

# Laboratory tests

- **Laboratory tests for platelet disorders may include:**
- - **Assessing platelet number and size [MPV]**
  - **Assessing platelet morphology – blood film**
  - **Screening tests of platelet function**
    - Activated Clotting Time [ACT],**
    - Bleeding Time [BT] and**
    - PFA-100**
  - **Light Transmission Aggregometry**
  - **Assessment of platelet nucleotides**
  - **Flow cytometry e.g. to quantitate the presence or absence of platelet membrane glycoproteins**
  - **More specialised investigations which are primarily the province of research laboratories**

# LTA

- LTA is primarily regarded as the gold standard for platelet function testing. Invented in the 1960s,
- LTA measures the formation of platelet aggregates
- As a suspension of platelets in plasma (platelet-rich plasma [PRP]) is typically turbid, the formation of aggregates due to activation by an agonist added exogenously to the PRP results in increased light transmission through the platelet suspension.
- The extent of light transmission corresponds to the extent of platelet aggregation

- Venipuncture
- should only be collected from fasting and resting subjects
- refrained from smoking and caffeine ingestion on the day of testing
- Avoid non-steroidal anti-inflammatory drugs for 7 to 10 days
- Herbal remedies, garlic, alcohol and certain foods may also cause acquired platelet dysfunction

# Investigation of a suspected disorder of platelet function:

- Certain drugs and foods to be avoided for 7 days prior to test
- 12 hour fasting is advised
  - Bleeding time
  - Platelet count
  - Platelet size distribution
  - Examination of a stained blood film

# Case

- 4 year old child
- Petechial rash on the body
- Bleeding from mouth
- H/o fever 7 days ago
- No past history of significance
- No family history of bleeding tendency
- On examination – no organomegaly

# Screening Coagulogram

• Bleeding time .....	.....	> 10 min	( upto 7 min )
( Ivy's method )			
• Clotting time .....	.....	7 min 30 sec	( 5 to 11 min )
( Lee & White)			
• Platelet count .....	.....	10,000	( 150 – 450,000 /cmm )
• Platelet morphology	.....	Giant platelets	( size & clumps )
• Clot retraction	.....	Poor	( good )
• Prothrombin Time – T	.....	13	( sec )
( P T )	- C	.....	12
			( sec )
• Activated partial thromboplastin time - T ...		26	( sec )
( aPTT)	C...	24	( sec )
• Thrombin Time	..... T...	10	( sec )
	C...	10	( sec )
• Clot solubility test	.....	Normal	Insoluble

Impression - Thrombocytopenia. Other parameters within normal limits

Adv – Bone marrow examination

Bone marrow – Increased megakaryocytes, young forms

Impression : Peripheral platelet destruction ( ITP )





# Giant Platelets

- Larger than normal
- Increase in the mean platelet volume (MPV)
- Up to 10% of normal platelets are 'giant'
- When more than 20% of platelets are giant, other conditions must be considered

# Giant Platelet

## Causes

- Congenital
- Acquired

# Acquired

Acquired - more common.

- Immune thrombocytopenic Purpura
- Thrombocytopenia due to sepsis & infections
- Myeloproliferative disorders
- lymphoproliferative disorders
- Massive hemorrhage
- Prosthetic heart valve
- Splenectomy
- Vasculitis
- SLE
- TTP

# Inherited Giant Platelet Disorders

- **With structural defect**
  - Glycoprotein abnormalities**
    - Bernard-Soulier syndrome
    - Velocardiofacial syndrome
    - Association with mitral valve defect
    - Glycoprotein IV abnormalities
  - Calpain defect**
    - Montreal platelet syndrome
  - Alpha granules**
    - Gray platelet syndrome
- **With abnormal neutrophil inclusions**
  - May-Hegglin anomaly
  - Sebastian syndrome
  - With systemic manifestations**
    - Hereditary macrothrombocytopenia with hearing loss
    - Epstein syndrome
    - Fechtner syndrome
- **With no specific abnormalities**
  - Mediterranean macrothrombocytopenia
  - asymptomatic constitutional macrothrombocytopenia (ACMT) (Harris Platelet Synd.)

# Congenital

- Once considered rare.
- Now recognized with increasing frequency.
- Due to quantification of platelet number as a part of routine blood testing.
- Have variable bleeding symptoms.
- A bleeding tendency disproportionate to the platelet counts
- Often misdiagnosed as ITP

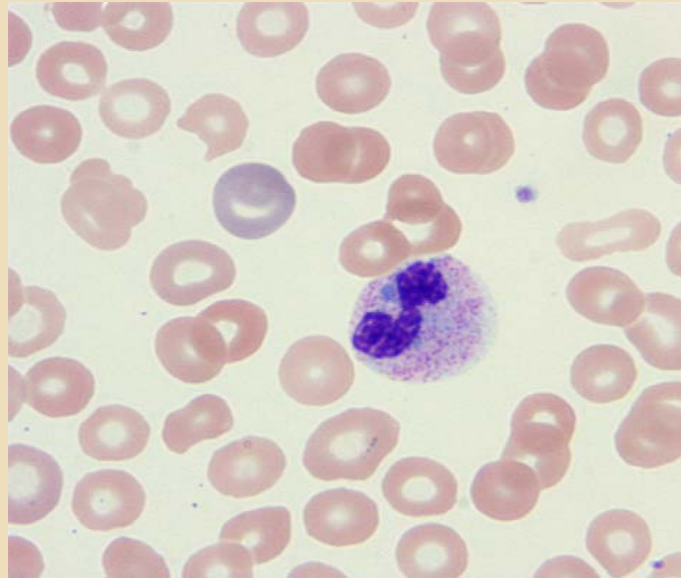
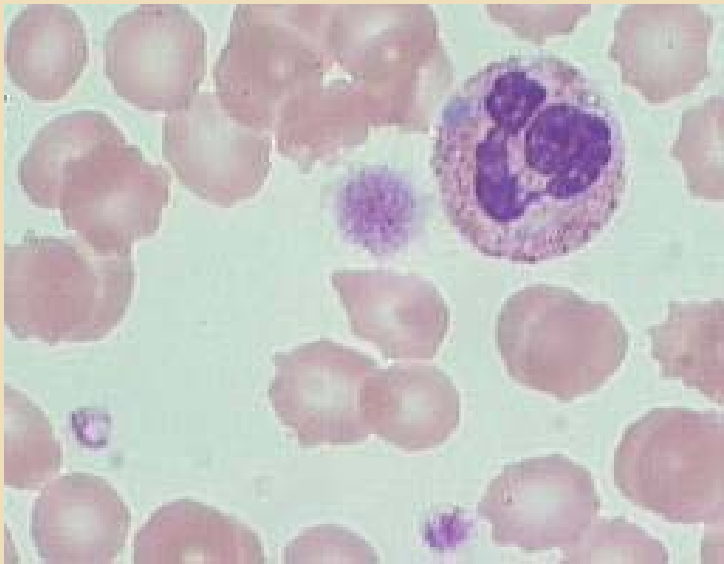
# Congenital

- Clinical presentation is widely heterogeneous
- Ranging from an asymptomatic condition to a severe life-threatening bleeding tendency.
- Recognized within the first few weeks of life, to mild conditions that may remain undetected even in adulthood.
- There are syndromic forms associated with physical stigmata

# **Macrothrombocytopenia with leukocyte inclusions/MYH9 disorders**

- Rare disorders
- Triad of giant platelets,
- Thrombocytopenia,
- Dohle body-like cytoplasmic inclusions in granulocytes





## Classification of neutrophil NMMHCA localization pattern in *MYH9* disorders.

### NMMHCA-positive granules

	Number	Shape	Size ( $\mu\text{m}$ )
Type I	1 or 2	oval spindle	0.5 2.0
Type II	3 20	circle oval	<1
Type III	20	< circle	<0.5

# Macrothrombocytopenia with leukocyte inclusions/MYH9 disorders

Phenotype	Macrothrombocytopenia	Leukocyte inclusions	Alport syndrome*
May-Hegglin anomaly	+	+	--
Sebastian syndrome	+	+	--
Fechtner syndrome	+	+	+
Epstein syndrome	+	--	+

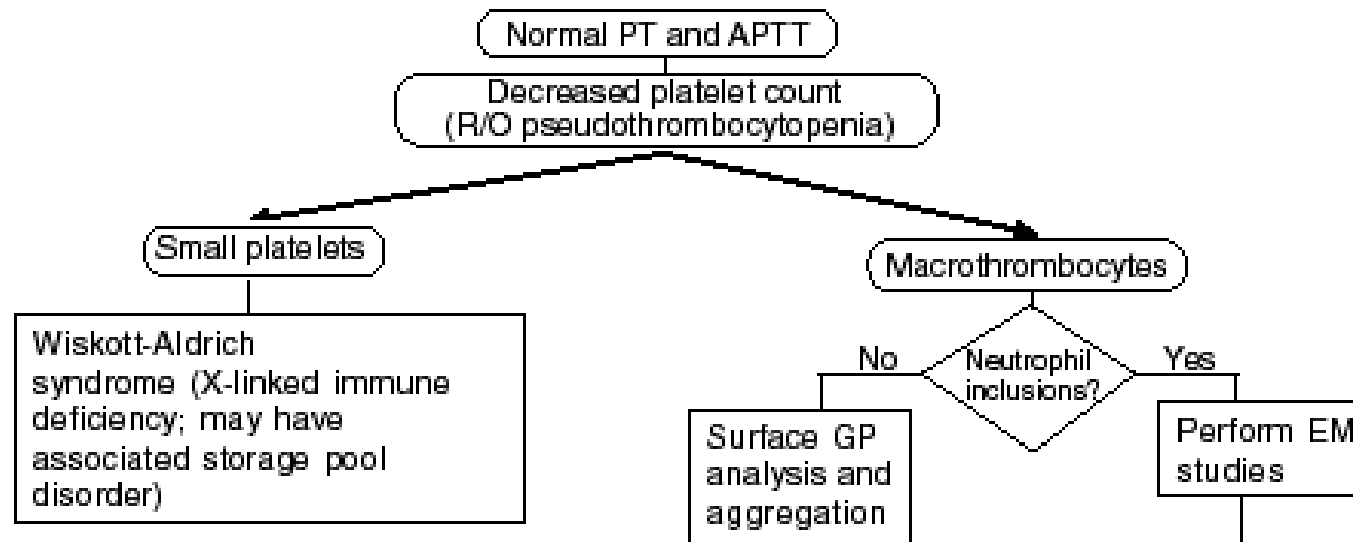
\* Nephritis, deafness and cataracts.

( Ref ; S. Kunishima, H. Saito Blood Reviews (2006) 20, 111–121 )

## **Macrothrombocytopenia with leukocyte inclusions/MYH9 disorders**

- Usually transmitted in an autosomal dominant manner.
- Approximately 20% of cases are considered to be sporadic.
- Although most patients with these disorders do not bleed, a few bleed and need only occasional treatment.

# Algorithm for evaluating a platelet disorder in patients with decreased platelet counts



Disorder	Abnormal GP	Abn aggregation	
Bernard-Soulier disease	GP Ib/IX/V	Ristocetin	♦ May-Hegglin anomaly (characteristic inclusions, nephritis) ♦ Fechtner syndrome (deafness, nephritis) ♦ Sebastian syndrome
Velocardiofacial syndrome	GP Ib $\beta$	NL	
GP IV abnormality	GP IV	Variable	
Mitral valve insufficiency	GP Ia, Ic, IIa	ADP, AA, Thr	
Gray platelet syndrome ( $\alpha$ -SPD)	P-selectin	Rist, Thr, Col	
Montreal platelet syndrome	Calpain def.	Thr	
Hereditary macrothrombocytopenia	NL	Epi, AA	
Epstein syndrome (nephritis)	Unknown	Col, ADP, Thr	
Mediterranean macrothrombocyt.	Unknown	Unknown	

# Disorders of Platelet Function

- Hereditary:
- Plasma membrane defects
  - Bernard-Soulier syndrome
  - Glanzmann's thrombasthenia
  - Platelet-type von Willebrand's disease
  - Defective response to collagen
  - Primary platelet coagulant defect

- Deficiency of storage organelles
- Dense body deficiency
  - Idiopathic (storage pool disease)
  - Hermansky – Pudlak syndrome
  - Wiskott- Aldrich syndrome
  - Chediak - Higashi syndrome
  - Thrombocytopenia with absent radii

- Alpha granule deficiency  
Grey platelet syndrome
- Deficiency of dense bodies and alpha granules
- Defects of thromboxane synthesis  
Cyclo – oxygenase deficiency  
Thromboxane synthetase deficiency



- Defects of response to thromboxane A<sub>2</sub> and ionophores

- Miscellaneous

Montreal platelet syndrome

Defects of response to adrenalin

Epstein's syndrome

# Acquired Disorders:

- Myeloproliferative disorders
  - Chronic myeloid leukaemia
  - Polycythemia vera
  - Myelofibrosis
  - Thrombocythaemia
- Acute leukaemias, preleukaemic states
- Renal disease ( Uremia )
- Dysproteinaemias

- Acquired storage pool deficiency
  - Disseminated intravascular coagulation
  - Auto immune diseases
  - Haemolytic – uraemic syndrome
  - Thrombotic thrombocytopenic purpura
  - Renal transplant rejection
  - Severe burns
  - Valvular heart disease
  - Cardiopulmonary bypass

- Chronic hypoglycaemia
  - Glycogen storage disease type I
  - Fructose 1,6- diphosphatase deficiency
  
- Bartter's syndrome

- Drugs

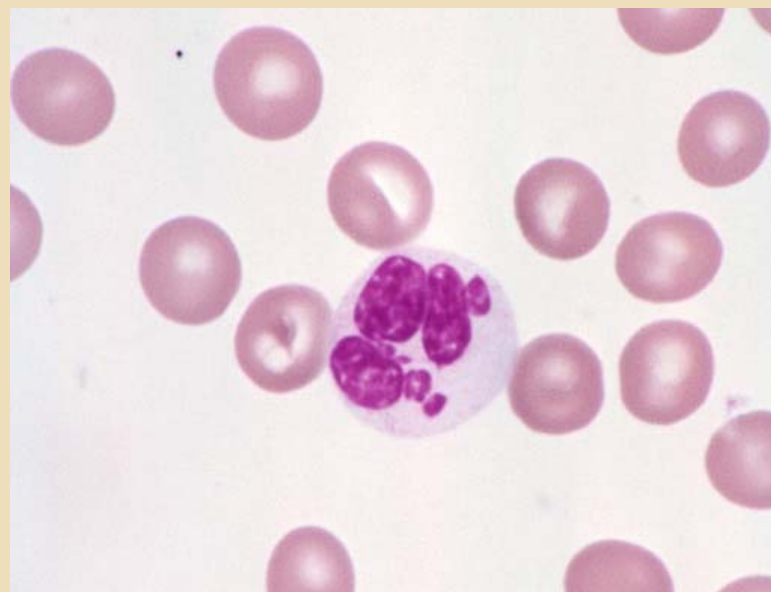
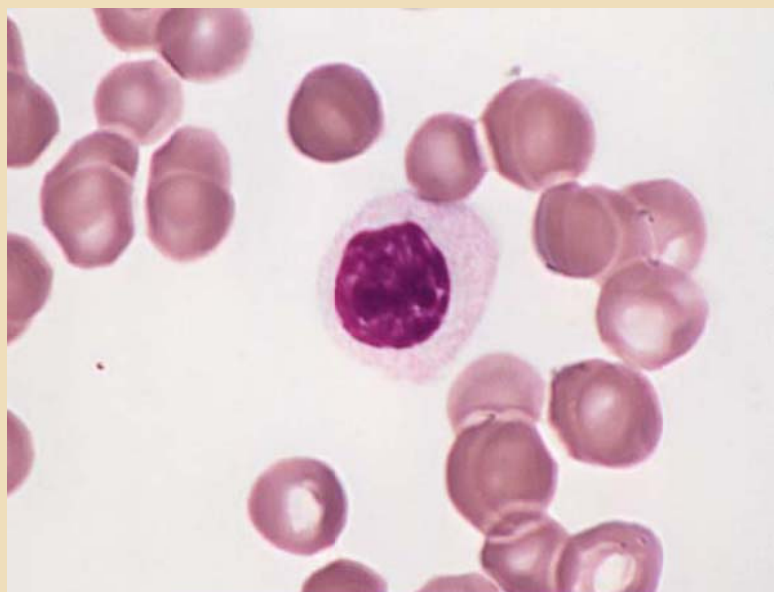
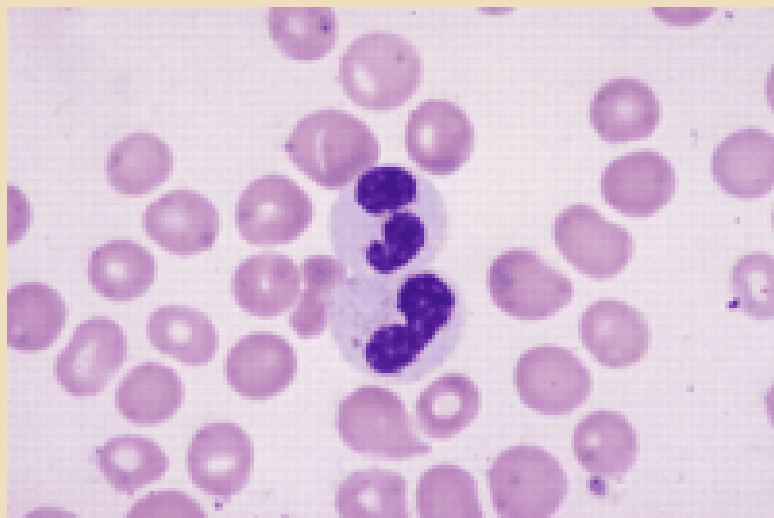
- Acetylesalicylic acid
- Indomethacin
- Sulphinpyrazone
- Phenylbutazone
- Dipyridamole
- Aminophylline
- Prostanoids
- Pennicillins
- Cephalosporins
- Heparin
- Dextrans
- Ethanol
- Radiographic contrast agents
- Clofibrate
- Phenothiazine
- Garlic

# Case

- 65 yr.old male
- Fever, wt. loss,
- Few blue patches patches on arm
- Bleeding gums , off & on
  
- Mild splenomegaly
  
- CBC – Hb – 10.5 gm %
  - TLC - 9,800 / cmm
  - DLC - Occ myelo , meta, nRBC
  - Platelet – 76,000/cmm, MPV – 10 fl, few giant platelet

Bone marrow - MDS

# Granulocytic series



# Case

- A 7yr old male child.
- Referred for complaint of red to blue coloured patches on lower limbs and arm since 4 months.
- These patches were spontaneous in onset, seen intermittently and were self resolving.



# Case

- There was no h/o of any previous episode of bleeding, history of umbilical cord bleeding was negative.
- Patient was not on aspirin and related drugs.
- SYSTEMIC EXAMINATION - NAD
- investigations-
  - Hb-13.6 gm%
  - TLC-19,000 /cmm
  - Plt Count-2,64,000
  - DLC-P27/L28/E41/M04

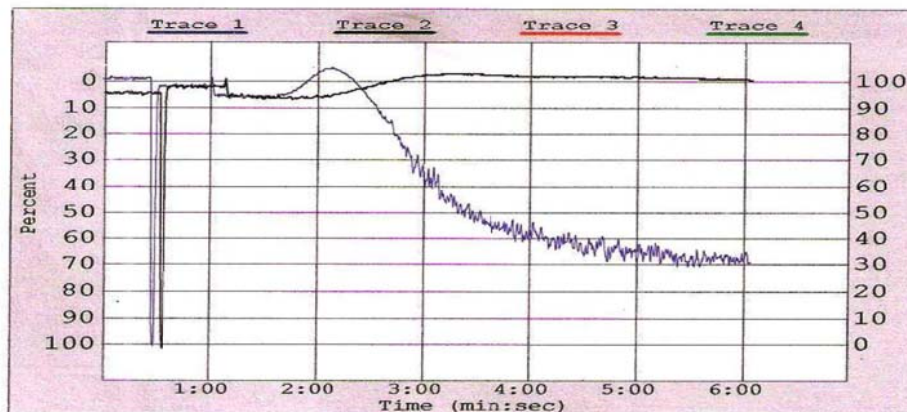
# Screening Coagulogram

• Bleeding time .....	<b>15</b>	( upto 7 min )
( Ivy's method )		
• Clotting time .....	<b>10</b>	( 5 to 11 min )
(Lee & White)		
• Platelet count .....	<b>2,64,000</b>	( 150 – 450,000 /cmm )
• Platelet morphology	<b>Normal</b>	( size & clumps )
• Clot retraction	<b>Good</b>	( good )
• Prothrombin Time – T	<b>15</b>	( sec )
( P T ) - C	<b>13</b>	( sec )
• Activated partial thromboplastin time - T ...	<b>31</b>	( sec )
(aPTT)	C... <b>30</b>	( sec )
• Thrombin Time	..... T... <b>10</b>	( sec )
	C... <b>10</b>	( sec )
• Clot solubility test	<b>Normal</b>	<b>Insoluble</b>

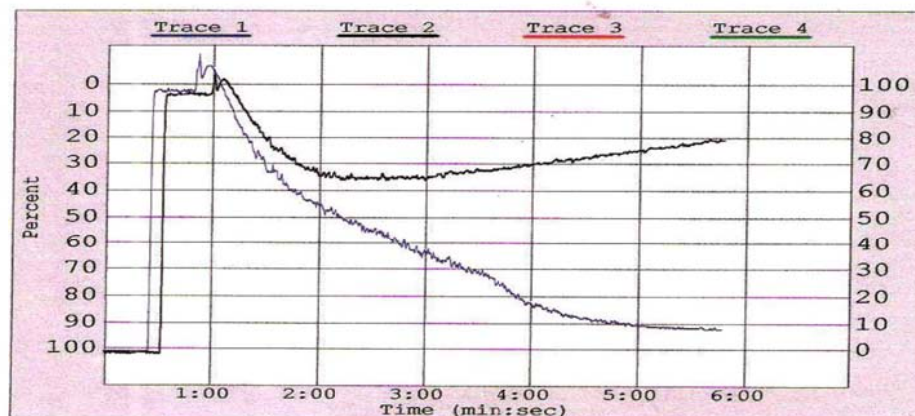
Impression - To rule out Platelet function defect

K.E.M.Hospital  
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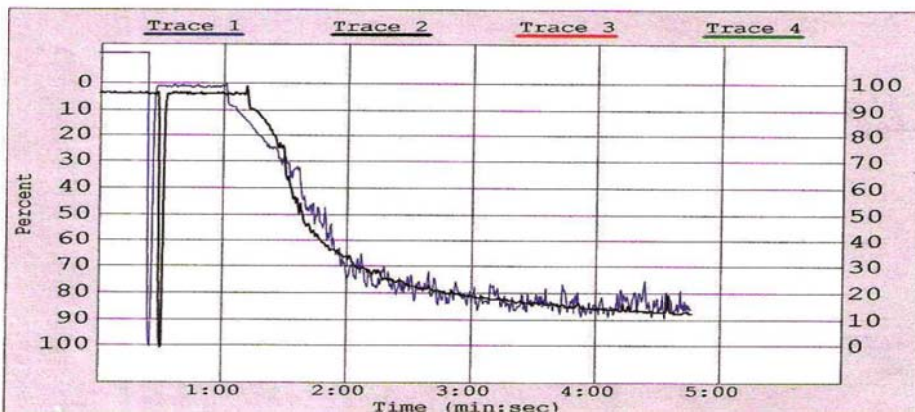
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Trace 1 Date: 11/19/2013 Time: 1:07:18 PM		
Name: control, ID : Lab :		
Blood Draw Time :		
Trace 2 Date: 11/19/2013 Time: 1:07:18 PM		
Name: SURAJ CHAUDHARY, ID : 10517, Lab : HAEMATOLOGY		
Blood Draw Time :		
Trace	1	2
Instrument	OPT	OPT
Reagent	Collagen	Collagen
Stirrer	2ug/mL	2ug/mL
Gain	1200	1200
Amplitude	75%	0%
Slope	80	3
Lag Time	0:27	1:18
Area Under	203.8	5.4
Comments		



Test ID : suraj chaudhary		
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Blood Draw Time :		
Trace 2 Date: 11/19/2013 Time: 12:57:40 PM		
Name: suraj, chaudhary/11years/M ID : 10517, Lab : haematology		
Blood Draw Time :		
Trace	1	2
Instrument	OPT	OPT
Reagent	ADP	ADP
Stirrer	10UM	10UM
Gain	1200	1200
Amplitude	96%	32%
Slope	84	62
Lag Time	0	0
Area Under	0	0
Comments		



Test ID : SURAJ CHAUDHARY		
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Name: CONTROL, ID : Lab :		
Blood Draw Time :		
Trace 2 Date: 11/19/2013 Time: 1:25:41 PM		
Name: SURAJ CHAUDHARY, 11/M ID : 10517, Lab : HAEMATOLOGY		
Blood Draw Time :		
Trace	1	2
Instrument	OPT	OPT
Reagent	Ristocetin	Ristocetin
Stirrer	1.0mg/mL	1.0mg/mL
Gain	1200	1200
Amplitude	89%	83%
Slope	113	132
Lag Time	0:24	1:12
Area Under	313.8	249.4
Comments		



- Platelet function tests revealed –

No aggregation with collagen.

Reduced aggregation with ADP.

Normal aggregation with ristocetin.

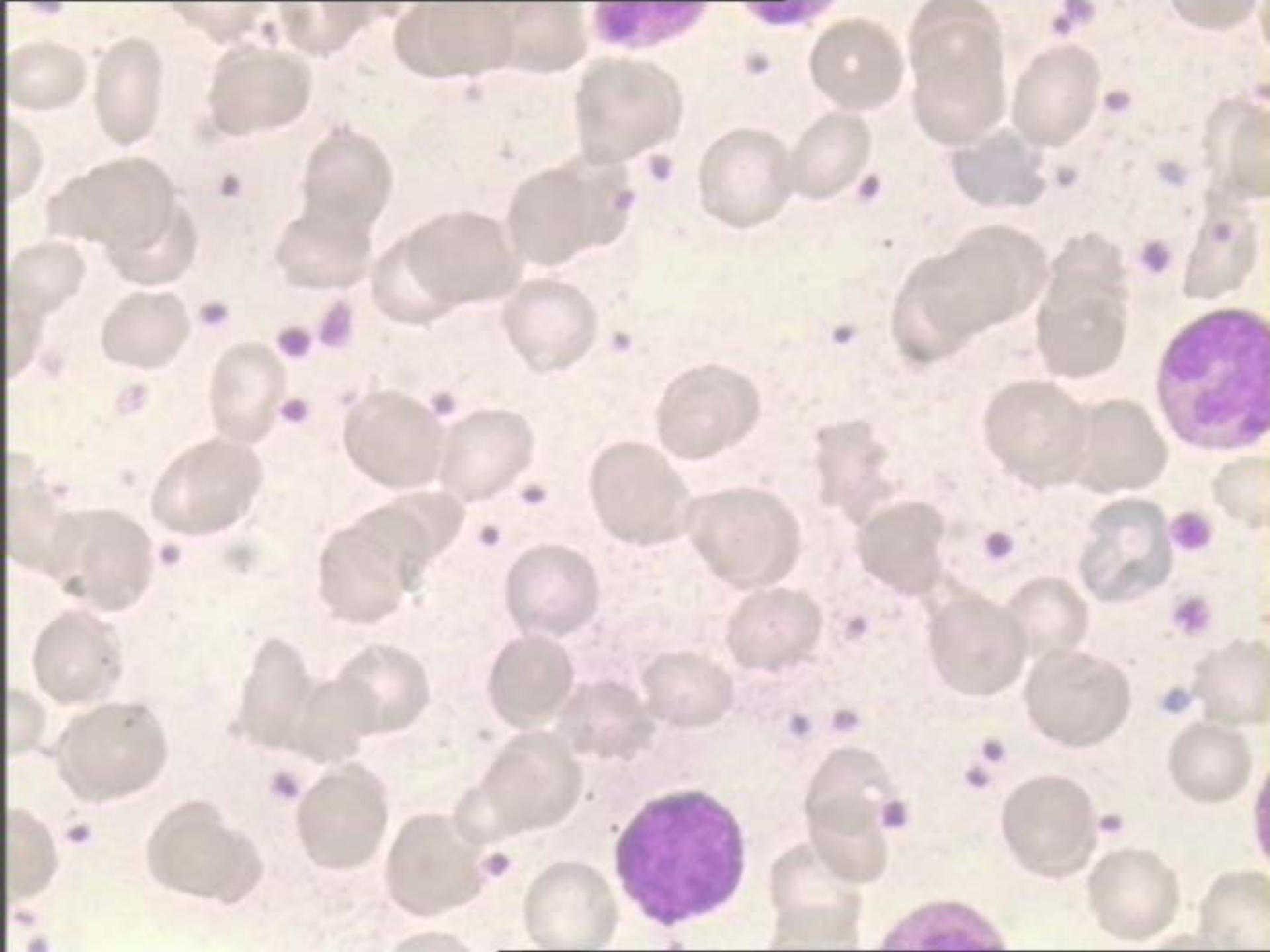
## Diagnosis

***Acquired Platelet Dysfunction with Eosinophilia.***

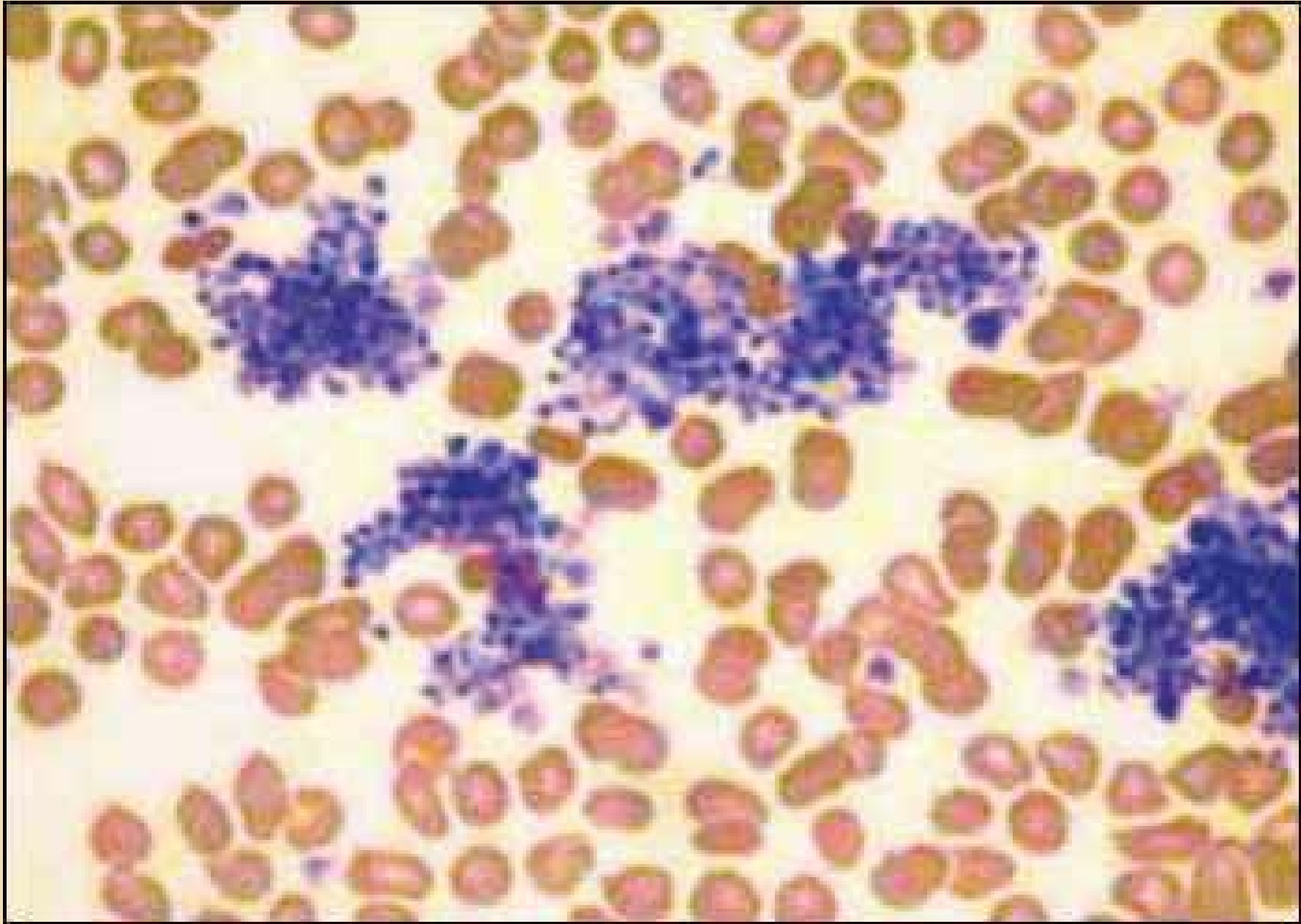
***( APDE )***

# Case

- 5 yr old girl
  - Bleeding from gums
  - Frequent nose bleed
  - No significant family history
- 
- Hb 9.0 gm %
  - Platelet 2,80,000 /cmm



# PBS & Bleeding



# Screening Coagulogram

• Bleeding time .....	<b>More than 20</b>	( upto 7 min )
( Ivy's method )		
• Clotting time .....	7.30	( 5 to 11 min )
( Lee & White)		
• Platelet count .....	2,80,000	( 150 – 450,000 /cmm )
• Platelet morphology	<b>no clumps</b>	( size & clumps)
• Clot retraction	<b>poor</b>	( good )
• Prothrombin Time – T	12	( sec )
( P T ) - C	12	( sec )
• Activated partial thromboplastin time - T ...32		( sec )
( aPTT)	C... 31	( sec )
• Thrombin Time	..... T... 11	( sec )
	C... 11	( sec )
• Clot solubility test	.....Normal	Insoluble

## Impression - Adv Platelet function test

( No aggregation with ADP, collagen - Normal agg. With Ristocetin

Highly suggestive of Glanzmann Thrombasthenia

Adv. Mol studies



# Global tests of platelet haemostatic function

- The most widely performed tests for screening platelet function disorders are currently
  - - Template BT
  - - Platelet Function Analyser (PFA-100 closure time )
  - - Platelet aggregometer
  - - Thromboelastography (TEG)
  - - Rotational Thromboelastometry (ROTEM)
- ROTEM and TEG provide global tests of haemostasis and platelet function and are mainly used within the surgical setting.

- The utility of most of these assay systems including TEG/ROTEM for the screening and diagnosis of platelet function defects has not yet been examined systematically and their use for this application is therefore not currently recommended.

# Thrombelastography= TEG

- Analyzes the entire hemostasis process
- Non-invasive instrument designed to analyze a whole blood sample to assess a patient's clinical hemostatic condition
- Primarily used during surgical procedures
- **Basic Principle**
  - Monitors hemostasis in its entirety
    - Clot initiation through clot lysis
    - Net effect of all hemostatic components interacting together
    - Activated blood maximizes thrombin generation and platelet activation in vitro
    - Demonstrates hemostatic potential of a blood sample at a given point

# The Utility of Platelet and Coagulation Testing of Antithrombotics

- An increasing need for the standardization of platelet function and coagulation testing for the assessment of antithrombotic therapies
- Identify ideal laboratory testing and monitoring procedures for acquired and inherited platelet function defects
- Evaluating patient status when treated with existing or emerging antithrombotics.

- Currently there are no instruments that reliably assess the risk of bleeding.
- The challenges that routinely faced are the complexity of physiology, the need for standardization of platelet testing methodology
- The necessity for appropriate interpretation of the test results.

Where?	How?	When?
<i>In vivo</i>	History & physical examination	The single best means for assessing platelet function <i>in vivo</i> at present
	Platelet count	Detecting hereditary and acquired disorders of platelet number. Following responses to therapy in patients with thrombocytopenia and thrombocytopenia.
	Bleeding time	Affected by qualitative and quantitative disorders of platelet function (hereditary and acquired). Can also be affected by major clotting disorders that affect thrombin generation and by disorders that affect vascular integrity.
<i>Ex vivo</i>	Light microscopy (peripheral blood smear) to assess platelet number, granularity and size.	Useful for assessing platelet number, granularity and size, and for detecting abnormalities of the other blood cell lineages. Disorders with rapid turnover. Hereditary and acquired defects of platelet formation. Detecting pseudothrombocytopenia and $\alpha$ -granule deficiency.
	Light microscopy (bone marrow aspirate and biopsy)	Evaluating megakaryocyte number and morphology, and for detecting abnormalities of the other blood cell lineages.
	Light transmission aggregometry (platelet rich plasma) and impedance aggregometry (whole blood)	Evaluating platelet responses to agonists (e.g. ADP, epinephrine, collagen, TRAP and arachidonate) and platelet activation in the absence of an agonist. Detecting hereditary and acquired defects of aggregate formation (e.g. Glanzmann's thrombasthenia).
	Dense granule secretion (lumi-aggregometers and $^{14}\text{C}$ -serotonin)	Detecting hereditary and acquired abnormalities of secretion that affect dense granules.
	ATP:ADP ratio	Storage pool disorders that affect dense granule storage of adenine nucleotides.
	Von Willebrand factor analysis (Ag, RCF, PTT and multimer analysis)	Diagnosing von Willebrand's disease
	Flow cytometry	Among other uses, can detect surface expression of common platelet proteins, evaluate $\alpha_{\text{IIb}}\beta_3$ (fibrinogen receptor) function, assess secretory mechanisms and assess the impact of P2Y <sub>12</sub> ADP receptor antagonists on VASP phosphorylation.
	PFA-100 (original and modified), VerifyNow, Multiplate, Impact, Plateletworks, VASP phosphorylation index	Monitoring the impact and, possibly, efficacy of antiplatelet agents
	ELISA-based assays for soluble platelet factor 4 (PF4), $\beta$ -thromboglobulin ( $\beta$ -TG) and CD40 ligand (CD40L)	Used for detecting states in which platelets are overly active <i>in vivo</i> and for monitoring the impact of antiplatelet agents and other disease interventions in clinical trials.

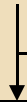
# Evaluating "new-onset" thrombocytopenia

<u>Review bleeding history</u>	<u>ITP</u>	<u>Congenital</u>
1. When was the onset of bleeding/bruising/petechiae?	Recent	Life-long
2. Have there been changes in general health? Any new medications?	Evaluate changes	No change
3. Has there been "excessive" bleeding after minor trauma during menses, surgeries, childbirth?	No	Yes
4. Are there any family members with increased bleeding or thrombocytopenia?	No	Yes
5. Have there been previous normal platelet values?	Yes	No
6. What has been the response to treatment, (steroids IVIG, anti-D, splenectomy)?	Increased platelets (approximately 80%)	Variable/small effect
7. Response to platelet transfusions /	Poor response/ short-lived	Good increment normal survival

macrothrombocytopenia



peripheral blood smear



acquired causes ( ITP, MDS etc )



granulocyte inclusion bodies

Dyserythropoiesis

Grey Platelets

Severe bleeding



+

--

GATA-1 sequencing

GPS

RIPA

NMMHCA IF

GATA-1 mutation

Absent

Increase

Abnormal

Normal

BSS

vWD 2B

MYH9 disorders

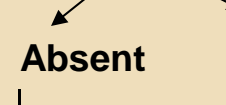
GPIb/IX FCM

No expression

-50% exp

BSS

BSS hetero





- Platelet count should be **confirmed** manually in a calculating chamber.
- Careful examination of a smear for morphological assessment of leukocytes and erythrocytes.
- If granulocyte inclusion bodies are obscure or absent, immunofluorescence analysis for neutrophil NMMHC -A ( nonmuscle myosin heavy chain-A ) localization is helpful to make a clear distinction.
- Flow cytometric analysis of platelet GP Ib/IX expression can differentiate BSS heterozygotes from patients with “true” isolated macrothrombocytopenia.
- Patients with congenital macrothrombocytopenia generally do not respond to standard ITP treatments,

# Approach

- Family history and blood cell morphology analysis for discriminating AITP from inherited thrombocytopenia in children with isolated chronic thrombocytopenia.
- Bone marrow examination and search for specific autoantibodies using the MAIPA test are of little help.
- An isotopic platelet life span study, when available, should be performed before considering splenectomy to exclude the diagnosis of inherited thrombocytopenia, especially when steroids and/or IgG IV administration failed to raise the platelet count.

# Approach

- **First acquired causes of macrothrombocytopenia, including ITP and myelodysplastic syndromes, should be ruled out.**
- **Complete history and physical examination should be carefully performed**
- **In syndromic forms, patients show complications of physical abnormalities such as facial, cardiac, renal disease, deafness, cataracts skeletal anomalies and/or mental retardation.**
- **If the patient previously had normal platelet counts, acquired rather than congenital conditions are more likely to be the underlying cause.**
- **In inherited macrothrombocytopenias, platelet counts are constantly decreased, ranging from as low as  $10 \times 10^9/l$  to near normal  $150 \times 10^9/l$ .**
- **On a peripheral blood smear, the majority of platelets are large, being similar to or larger than red blood cells or small lymphocytes**
- **In contrast, in patients with the much more common ITP, large platelets are present but the majority are of normal size**

# Approach

- It is important to make a proper diagnosis to avoid unnecessary treatment.
- Affected families should be educated about their diagnosis to avoid unnecessary medications and potentially dangerous treatments for presumed ITP
- When evaluating patients with refractory ITP or undifferentiated thrombocytopenia, congenital macrothrombocytopenias should be included in the differential diagnosis

**BLEEDING HISTORY**

No affected first degree relatives

Two sites

One site + transfusions

One site three times



**FIRST TIER TESTING**

-/+Bleeding time

-/+PFA closure time

Rule-out von Willebrand disease



**SECOND TIER TESTING**

Platelet Aggregometry

(esp. with ADP and arachidonic acid)



**THIRD TIER TESTING**

Platelet flow cytometry

Lumiaggregometry

Platelet electron microscopy

