

# A review of inherited platelet disorders with guidelines for their management on behalf of the UKHCDO

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## Summary

The inherited platelet disorders are an uncommon cause of symptomatic bleeding. They may be difficult to diagnose (and are likely to be under-diagnosed) and pose problems in management. This review discusses the inherited platelet disorders summarising the current state of the art with respect to investigation and diagnosis and suggests how to manage bleeding manifestations with particular attention to surgical interventions and the management of pregnancy.

**Keywords:** inherited platelet disorders, platelet glycoproteins, thrombocytopenia, platelet receptors, platelet bleeding.

For most, if not all, of the inherited platelet disorders, there is little adequate evidence (a lack of randomised controlled trials) in the literature upon which to base recommendations for management. There are some large case series for a number of the severe, more clearly defined disorders, such as Glanzmann thrombasthenia, but for most disorders the published evidence about management consists of case reports or very small series. The advice given herein is therefore based on personal practice of several haemophilia centre directors and is 'expert opinion'. Investigation and treatment are discussed in broad terms for 'mild' and 'severe' disorders (defined clinically) as there is little that is specific to a single disorder. Where relevant, additional

details (including the management of neonates in some of the conditions) are included in the disorder-specific sections. There have been some very interesting advances in recent years in the understanding and investigation of platelet molecular biology, which are in a process of evolution and, in due course, are anticipated to influence diagnosis.

## Platelet physiology

Human platelets are small anucleate cells derived from bone marrow megakaryocytes. Platelets are discoid and measure approximately  $2.0\text{--}4.0 \times 0.5 \mu\text{m}$  with a mean volume of 7–11 fl. The normal platelet count is  $150\text{--}450 \times 10^9/\text{l}$ . Platelets are formed from their parent cells, the bone marrow megakaryocytes (MK), which are characteristically very large polyploid cells, reaching up to 50  $\mu\text{m}$  in diameter. During maturation MKs undergo endomitosis (nuclear replication without cell division) to form cells with DNA ploidy values ranging from 4 to 128 n. Megakaryopoiesis is regulated by the cytokine thrombopoietin (TPO) which is constantly synthesised by the liver. TPO binds a specific receptor (c-mpl) on both circulating platelets and bone marrow MKs, signals and is then internalised and degraded. The total mass of cells therefore determines the free level of TPO that ultimately regulates megakaryopoiesis and platelet production (Kaushansky, 2005; Pang *et al*, 2005; Patel *et al*, 2005). Platelets have a mean life span in the circulation of 10 d. The shape and small size of the platelet enables them to flow centrifugally in blood vessels, allowing them to interact optimally with damaged endothelium. Upon vessel wall damage, platelets undergo a highly regulated set of functional responses including

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adhesion, spreading, granular release reactions, activation of phospholipase A<sub>2</sub>, aggregation, exposure of a procoagulant surface, microparticle formation and clot retraction. These platelet responses enable the rapid formation of a haemostatic plug that occludes the site of blood vessel damage and limits blood loss.

### *Platelet adhesion*

Subendothelial components [e.g. collagen, von Willebrand factor (VWF), fibronectin and laminin] are exposed upon vessel wall damage. Platelets have surface receptors for all these proteins to enable rapid adhesion to the damaged area. VWF facilitates the initial adhesion via binding to the glycoprotein (GP)Ib/IX/V complex, especially under high shear conditions. These interactions enable the platelets to slow down sufficiently so that further binding interactions can take place with other receptor–ligand pairs resulting in static adhesion. In particular, the initial interaction between collagen and GPVI induces a conformational change (activation) in the platelet integrins GPIIb/IIIa ( $\alpha$ -IIb $\beta$ -3) and GPIa/IIa ( $\alpha$ -2 $\beta$ -1). VWF and collagen form strong bonds with GPIIb/IIIa and GPIa/IIa, respectively, anchoring the platelets in place.

Decreased or absent expression of the GPIb/IX/V receptor due to mutations in the *GPIBA*, *GPIBB* or *GP9* genes gives rise to Bernard–Soulier syndrome. Decreased or absent expression of GPIIb/IIIa or qualitative defects of the receptor due to mutations in either the *ITGA2B* or the *ITGB3* genes gives rise to the disorder Glanzmann thrombasthenia.

### *Platelet aggregation and secretion*

As platelets are recruited to the area of blood vessel damage they become activated by a range of agonist substances including adenosine diphosphate (ADP), thrombin and thromboxanes, which interact with seven transmembrane receptors. Receptor stimulation results in G protein interactions, which enable activation of enzymes involved in cellular metabolic pathways, in particular, phosphatidylinositol 3-kinase and phospholipase C. Metabolic pathway activation results in the elevation of cytoplasmic calcium and phosphorylation of substrate proteins, which bring about changes in the cytoskeleton, enabling platelet shape change and spreading, release of  $\alpha$ - and dense-granular contents, stimulation of phospholipase A<sub>2</sub> and liberation of thromboxane A<sub>2</sub> (TXA<sub>2</sub>), induction of a procoagulant surface and activation of GPIIb/IIIa receptors.

A rare, diverse group of disorders of ‘platelet signal transduction’ have been described including defects in the agonist receptors for, TXA<sub>2</sub>, ADP and collagen, the membrane G proteins and in the prostaglandin pathway enzymes cyclooxygenase and TXA<sub>2</sub> synthetase. Disorders of the ‘platelet storage granules’ are also well described and include dense-granule deficiency,  $\alpha$ -granule deficiency, and combined dense and  $\alpha$ -granule deficiency.

A suggested classification for the most clearly defined heritable platelet disorders together with their estimated frequencies are shown in Table I.

## **Approach to the diagnosis of a platelet function disorder**

Patients are investigated for a possible inherited defect of platelet number or function in various circumstances. There may be a history of bleeding or easy bruising, a family history of a platelet function defect or thrombocytopenia. Patients will usually be undergoing investigation for a bleeding disorder, so investigation for a platelet function disorder will be undertaken as part of this process. Patients may have multiple haemostatic defects, for example, von Willebrand disease (VWD) or factor XI deficiency combined with a platelet function defect (Bolton-Maggs *et al*, 1995) or defects of both platelet number and function that combine to produce a clinical bleeding phenotype.

A schema for analysis of patients with suspected platelet disorders is shown in Fig 1 with an algorithm which is discussed below.

There are numerous ‘acquired’ causes of thrombocytopenia and platelet dysfunction (George & Shattil, 1991; Rao, 2002). These will not be covered in this review, which will only deal with inherited disorders.

### *The patient history*

History taking is a key part of the assessment of a possible bleeding disorder and is the best screening method for possible platelet function defects. Bleeding histories are subjective, variable and evolve throughout a person’s lifetime. Children may not have had sufficient haemostatic challenges to develop a positive clinical history.

There is overlap between symptoms suffered by people with mild platelet function disorders and the normal population. The bleeding history in severe platelet function disorders will be clearly abnormal.

Patients should be assessed for disorders that may cause acquired platelet dysfunction or vasculitis. A drug history should be taken, including medicines bought ‘over the counter’ and herbal remedies, as non-steroidal anti-inflammatory drugs (NSAIDs) and other drugs are the commonest cause of platelet dysfunction (Hassan & Kroll, 2005).

Inherited disorders of platelet function, and in particular platelet number, may be associated with other clinical features that represent a defined syndrome. The specific associations with other medical manifestations are covered in the relevant disease-specific sections.

Bleeding manifestations typical of platelet function defects are:

- 1 unexplained or extensive bruising;
- 2 epistaxis – particularly lasting more than 30 min, causing anaemia or admission to hospital;

Table I. A suggested classification of the heritable platelet disorders.

	OMIM number*	Site of gene defect†	Estimated no. cases	
			UK	Worldwide
<i>Disorders of platelet number</i>				
<i>MYH9 disorders</i>				
May–Hegglin anomaly	155100	<i>MYH9</i>	<100	<1000
Sebastian syndrome	605249			
Fechtner syndrome	153640			
Epstein syndrome	153650			
Congenital amegakaryocytic thrombocytopenia	604498	<i>MPL</i>	<10	<100
Amegakaryocytic thrombocytopenia with radioulnar synostosis	605432	<i>HOXA11</i>	<10	<100
Thrombocytopenia absent radius syndrome	274000	Unknown	<10	<100
X-linked thrombocytopenia with dyserythropoiesis	300367	<i>GATA1</i>	<10	<100
<i>Severe disorders of platelet function</i>				
Wiskott–Aldrich syndrome	302000	<i>WAS</i>	<100	<1000
Glanzmann thrombasthenia	273800	<i>ITGA2B, ITGB3</i>	<100	<1000
Bernard–Soulier syndrome	231200	<i>GP1BA, GPIBB, GP9</i>	<100	<1000
<i>Disorders of receptors and signal transduction</i>				
Platelet cyclo-oxygenase deficiency	605735	Unknown	<10	<100
Thromboxane synthase deficiency	274180	Unknown	<10	<100
Thromboxane A2 receptor defect	188070	<i>TBXA2R</i>	<10	<100
ADP receptor defect (P2Y <sub>12</sub> )	600515	<i>P2RY12</i>	<10	<100
<i>Disorders of the platelet granules</i>				
Idiopathic dense-granule disorder ( $\delta$ -storage pool disease)	185050	Unknown	<100	<1000
Hermansky–Pudlak syndrome	203300	<i>HPS1, AP3B1, HPS3, HPS4, HPS5, HPS6, DTNBP1, HPS8</i>	<100	>1000
Chediak–Higashi syndrome	214500	<i>LYST</i>	<100	<1000
Grey platelet syndrome	139090	Unknown	<10	<100
Paris–Trousseau/Jacobsen syndrome	188025 and 147791	<i>11q23 deletion (FLI1)</i>	<10	<100
Idiopathic $\alpha$ - and dense-granule storage pool disease	185050	Unknown	<100	<1000
<i>Disorders of phospholipid exposure</i>				
Scott syndrome	262890	<i>ABCA1</i>	<10	<10

This classification includes the major heritable disorders and some rare disorders that represent distinct clinico-pathological entities. The classification excludes disorders that are poorly characterised or that have been described in very small numbers of subjects. The estimated numbers of affected individuals represent the consensus view of the authors and are derived from the published world literature describing each disorder.

\*Online Mendelian Inheritance in Man accession number: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>.

†Human Genome Organisation nomenclature: <http://www.gene.ucl.ac.uk/nomenclature/>.

- 3 menorrhagia, particularly if this has been present since the menarche;
- 4 oral cavity bleeding;
- 5 bleeding during childbirth;
- 6 bleeding following invasive procedures;
- 7 bleeding following dental extraction.

A history of bleeding is more likely to be significant if it is longstanding, the more symptoms that are present, the more severe these symptoms are and the more frequently they occur. The requirement for blood transfusion due to bleeding, medical intervention to stop the bleeding or the development of anaemia adds weight to the bleeding history. For example, epistaxis that has required packing or repeated cauterisation and menorrhagia resulting in anaemia

(particularly if the menorrhagia has been present since the menarche) are more significant symptoms. A bleeding tendency is less likely if a person has undergone invasive procedures without bleeding.

#### *History suggestive of a severe platelet function disorder*

Severe platelet function disorders are likely to present from early childhood onwards and may cause problems at any age, including the neonatal period. Intracranial haemorrhage and subdural haemorrhage may occur following delivery and the antenatal care of affected women coupled with the planned delivery of at-risk babies are essential components of care in these disorders.

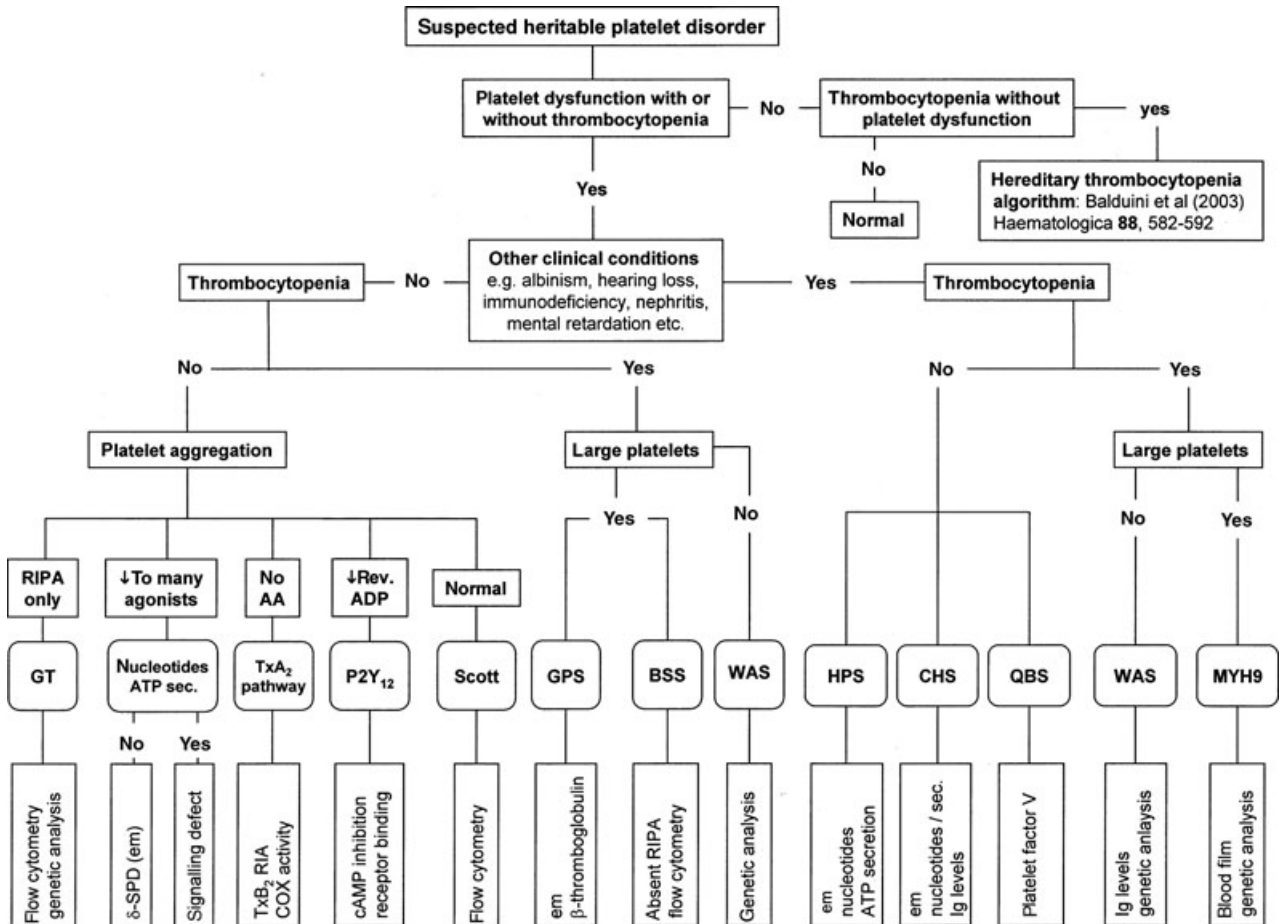


Fig 1. Scheme of analysis of patients with suspected hereditary platelet-based bleeding disorders: for patients with a clinical history suggestive of a heritable platelet disorder, laboratory analysis is required to confirm a defect in platelet number or function and to enable refinement of diagnosis to the level of individual disorders or groups of disorders. In many cases, this may be achieved by performing an automated full blood count, blood film, platelet aggregometry and quantification of platelet nucleotides. It is recognised that in most clinical settings, these investigations are usually performed simultaneously at the same clinic visit. This algorithm is intended as a guide to the systematic interpretation of these laboratory assays rather than a suggested sequence of investigations. Where appropriate, specialist investigations that are necessary for diagnosis are included for specific disorders. Many of these assays are available only through laboratories with a research interest in platelet function. These include flow cytometry to measure expression of the integrin subunits GPIIb or IIIa (GT), GPIIb- $\alpha$  (BSS) and annexin V binding (Scott syndrome) and genetic analysis to confirm a mutation in the relevant gene, e.g. GPIIbIIIa (GT), WAS (WAS and XLA) and MYH9. Genetic analysis has not been included as a definitive test in all cases as several disorders can be caused by mutations in more than one gene, e.g. HPS and BSS. The algorithm encompasses the most frequent or best characterised heritable platelet disorders but excludes disorders that are described in very small numbers of kindreds or that require esoteric tests to be fully characterised. Down arrow, reduced aggregation; AA, arachidonic acid; BSS, Bernard-Soulier syndrome; CHS, Chediak-Higashi syndrome; COX, cyclo-oxygenase;  $\delta$ -SPD, dense-granule disorder; em, electron microscopy; GPS, grey platelet syndrome; GT, Glanzmann thrombasthenia; HPS, Hermansky-Pudlak syndrome; MYH9, MYH-9-related disorder; P2Y<sub>12</sub>, deficiency of P2Y<sub>12</sub> ADP receptor; QBS, Quebec platelet syndrome; Rev., reversible aggregation; RIA, radioimmunoassay; RIPA, ristocetin-induced platelet aggregation; Sec., secretion; TXA<sub>2</sub>, thromboxane A<sub>2</sub>; WAS, Wiskott-Aldrich syndrome.

Excessive bleeding from the umbilical stump or after circumcision may be presenting features, as may the development of bruising after handling. Guthrie tests and vaccination pose haemostatic challenges for babies and excessive bleeding may be noted when teething. As the child's mobility increases, easy or extensive bruising after mild trauma may be noted.

Prolonged epistaxis is a common problem throughout childhood for children with severe platelet function disorders and may in itself be life threatening. Menorrhagia and bleeding during childbirth are common and may be very severe. Bleeding following invasive procedures is almost inevitable.

In contrast to patients with haemophilia, inherited platelet disorders do not usually present with haemarthroses or muscle bleeds and bleeding occurs at the time of trauma rather than after a delayed onset.

#### *History suggestive of a mild platelet function disorder*

Mild platelet disorders are likely to become evident later in life, and only after some haemostatic challenge, such as surgery or dental extractions. Easy bruising is a very non-specific symptom, and in many of these patients it may be

difficult to decide if bleeding manifestations are abnormal or not.

### *Family history*

A family history compatible with the dominant forms of platelet disorders should be sought. Consanguineous partnerships increase the likelihood of a recessive platelet disorder. Investigation of family members may be helpful in establishing a diagnosis of an inherited platelet disorder.

### *Examination*

Examination assesses the type of bleeding, if any, at the time of the consultation but often the main purpose is to exclude an underlying disease or diagnose a recognised syndrome. Scars from previous trauma or surgery should be examined in case the defect in primary haemostasis is due to a collagen disorder and skin hyperelasticity should be sought. Urine should be tested for blood and protein.

### *Investigations for a suspected inherited platelet disorder*

All patients under investigation for a possible platelet disorder should be investigated for other or additional potential causes of bleeding. In practice, this will occur in parallel to investigation of platelet function.

### *Full blood count and film*

The platelet count should be measured. A peripheral blood film should be examined to confirm the platelet count and assess platelet size and morphology and any white cell or red cell changes. Changes seen in specific disorders will be described in the appropriate section. If clumping is seen, a platelet count in a citrated sample should be analysed. Giant platelets may not be identified automatically and in syndromes with large platelets an inappropriately low platelet count may be reported on an impedance analyser. Optical platelet counters give more accurate counts in samples with macrothrombocytopenia. A reference method for platelet number using flow cytometry for platelet counting is available (Harrison *et al*, 2001).

There are many potential causes of thrombocytopenia but previous platelet counts and family studies may be helpful in distinguishing between congenital and acquired causes.

### *Coagulation screen*

All patients should have a prothrombin time, activated partial thromboplastin time, Clauss fibrinogen and thrombin time performed. Laboratories should determine their own age-related reference range and be aware of the variable sensitivity of the tests to reduced levels of clotting factors (Bolton-Maggs *et al*, 2004).

All patients with symptoms suggestive of a platelet function disorder should be investigated for VWD (Laffan *et al*, 2004). This disorder is more common than platelet function disorders, presents with a similar bleeding phenotype and can interact with platelet function disorders to affect their phenotype.

### *Bleeding time*

The use of the bleeding time has reduced over the last decade. Its clinical utility is limited because the test is poorly reproducible, invasive, insensitive and time consuming (Rodgers & Levin, 1990; Lind, 1991). The bleeding time is frequently normal or only minimally prolonged in mild platelet function disorders (Lind, 2002), but will be prolonged in the more severe forms.

The bleeding time does not correlate with the bleeding tendency within individual patients and it is widely considered that an accurate bleeding history is a more valuable screening test (Burns & Lawrence, 1989; Peterson *et al*, 1998).

Patients with collagen disorders usually have a normal bleeding time (De Paepe & Malfait, 2004) and a prolonged bleeding time should prompt further investigation of platelet function.

The bleeding time is not suitable as a screening test but should be considered in specific circumstances in the investigation of patients with bleeding disorders if other tests have not demonstrated a defect.

### *Platelet function analyser-100*

Platelet function analyser-100 (PFA-100) measurements (Jilma, 2001; Francis, 2002) will be significantly abnormal in Glanzmann thrombasthenia and Bernard-Soulier syndrome with closure times typically >300 s on both ADP/collagen and adrenaline/collagen cartridges. Therefore, it can be used to screen patients to exclude these diagnoses.

PFA-100 may be sensitive to platelet storage pool disorders, primary secretion defects, Hermansky-Pudlak syndrome (HPS) and Quebec syndrome. However, its utility as a screening test as part of an investigation for these disorders is limited, because false-negative results occur in patients with all these disorders (Favaloro, 2001; Harrison *et al*, 2002a; Liesner *et al*, 2004; Quiroga *et al*, 2004). Patients with a history suggestive of a platelet function defect will need further investigation whether the PFA-100 is normal or abnormal.

PFA-100 can be affected by platelet count, haematocrit, diet and aspirin, and is very dependent on VWF levels (Favaloro, 2001; Jilma, 2001). A prolonged PFA-100 closure time must therefore be further investigated by measurement of VWF levels. Abnormal tests should be repeated to exclude a transient-acquired defect.

### *Platelet aggregation*

Platelet aggregation in platelet-rich plasma may give important information about platelet function, especially as it provides

information on the time course of activation. Typical agonists for platelet aggregation are ADP, adrenaline, collagen, arachidonic acid, ristocetin, U46619 (thromboxane receptor agonist) or thrombin receptor activating peptide (TRAP). It is recommended that full dose–response curves to each agonist are obtained and compared relative to a reference range obtained from several healthy volunteers (British Committee for Standards in Haematology, BCSH, 1988; Moffat *et al*, 2005; Zhou & Schmaier, 2005). If thrombin is used, an inhibitor of fibrin polymerisation, such as GPRP (glycine–proline–arginine–proline) peptide or washed platelets, must be used. Platelet counts below  $120 \times 10^9/l$  can severely influence responses to some agonists. There is no need to normalise the platelet count of the test plasma relative to the control above this value, especially as there is evidence that dilution of a plasma sample will change the responses to various agents. In thrombocytopenic samples, where the platelet count is below  $120 \times 10^9/l$ , the best options are to adjust a control sample to the same count as the patient or to perform studies on washed platelets where the platelet number can be normalised. In both cases, it is necessary to generate standard concentration–response relationships from a pool of control donors. However, neither case is ideal in that dilution of the control sample with platelet-poor plasma is reported to impair agonist activation and concentration of samples using washed platelets is likely to generate a very small sample volume for analysis (as a result of the thrombocytopenia). Consideration should therefore be given to more specialist tests that are suited to analysis of samples with a low platelet yield, such as analysis of P-selectin expression or activation of integrin GPIIb/IIIa by flow cytometry.

Aggregation responses associated with specific diagnoses will be covered in appropriate sections.

#### *Platelet nucleotides content and release*

The measurement of platelet adenosine triphosphate (ATP)/ADP content and release is useful in the diagnosis of storage pool and release defects (Summerfield *et al*, 1981). Normal platelet aggregation does not exclude the diagnosis of storage pool disease, especially depending upon the agonists and the range of concentrations that have been used. In one study, 25% of patients with storage pool disease had normal platelet aggregation (Nieuwenhuis *et al*, 1987), and in another, 17 of 46 (35%) patients with normal platelet aggregation had storage pool disease (Israels *et al*, 1990). At present it is recommended that, unless the laboratory has demonstrated that their platelet aggregation assay is sensitive to defects in platelet nucleotide amount or release, patients suspected of having a bleeding disorder caused by platelet dysfunction should have both platelet aggregation and platelet nucleotides/ATP release performed.

Normal ranges in children: ideally, age-related local normal ranges should be established for total and released levels of ATP and ADP and their ratios but for ethical reasons this is seldom possible. Recent studies have shown that platelet

aggregation, nucleotide content and nucleotide release in children over 12 months of age do not differ from adult values, whereas collagen-induced platelet nucleotide release has been shown to be reduced in neonates compared with children older than 1 year; agonist-induced secretion of platelet granule contents has been shown to be reduced in both term and premature babies due to immature signal transduction pathways (Rajasekhar *et al*, 1994, 1997).

#### *Platelet flow cytometry*

Flow cytometry may be used to measure platelet activation as well as surface GPs,  $\alpha$ -granule release, phospholipid expression and microvesicle production. Flow cytometry assays have also been reported to measure dense-granule content and release (Gordon *et al*, 1995; Wall *et al*, 1995). The most common applications, however, are the assessment of GPIb/IX/V and GPIIb/IIIa in the diagnosis of Bernard–Soulier syndrome and Glanzmann thrombasthenia. Heterozygous states of these disorders can also be identified. The measurement of GPIb-IX-V in the heterozygous state needs to be corrected for platelet size.

The main benefits of flow cytometry include the small quantities of blood that are required, making this technique particularly useful in young children and in thrombocytopenic individuals, and unequivocal demonstration of defects in levels of specific GPs.

#### *Electron microscopy*

Transmission electron microscopy (EM) of thin sections of fixed/embedded platelets is available in only a few specialised laboratories but may be of use in assessing platelet granule defects and changes in platelet ultrastructure, for example, in assessing patients with MYH-9 defects. Whole mount EM is particularly useful for quantifying dense-granule numbers, as they are very easily identified by EM of unstained preparations (White, 1969). Dense bodies can be counted in many normal platelets to determine a normal range. In HPS the dense granules are completely absent.

#### *Molecular analysis*

Some families with severe platelet function disorders may benefit from identification of their molecular defect(s) to allow antenatal diagnosis to be offered. Molecular analysis of platelet GPs is available in a small number of specialist laboratories.

#### *Other methods*

A wide variety of tests are available in specialist laboratories that may provide further information on the disorder, including analysis of receptor expression, protein phosphorylation and formation of second messengers. In addition, several laboratories are at an early stage of developing

genomic- and proteomic-based approaches for the analysis of individuals with platelet disorders, although the practical significance of these tests is presently unclear.

## Specific platelet disorders

For investigation refer to the algorithm on approach to diagnosis in Fig 1. The clinical management of the platelet disorders is discussed later and divided broadly into severe and mild. Where specific details apply to individual disorders these are included in the disease-specific section.

## Inherited thrombocytopenias

In the following, sections 1–6 refer to disorders with thrombocytopenia (excluding Bernard–Soulier syndrome).

### Introduction

The inherited thrombocytopenias are a heterogeneous group of uncommon conditions that result in early onset thrombocytopenia, which may occur either as an isolated finding or in association with other abnormalities. Although some cases remain unclassifiable, recent advances in molecular genetics have improved our understanding of the nature of many of these conditions. Various different classifications have been proposed based on variables that include the mode of inheritance, platelet size, genetic mutations and co-existing abnormalities (Drachman, 2004). A recent publication has proposed a simplified scheme for the investigation of these disorders based on platelet size and the presence of co-existing clinical abnormalities (Balduini *et al*, 2002). It is important to note that, although many of these conditions are very rare, they are likely to have been misdiagnosed in the past (often as immune thrombocytopenia), which may have led to the use of inappropriate treatment.

### 1. MYH-9-related thrombocytopenia syndromes

**Definition.** MYH-9-related disorder is an autosomal dominant macrothrombocytopenia syndrome that includes entities previously classified as May–Hegglin anomaly, Sebastian, Fechtner and Epstein syndromes and some non-syndromic heritable thrombocytopenias. This disorder is defined by the presence of deleterious mutations within *MYH9*, the gene that encodes non-muscle myosin II-A heavy chain (NMMHC-IIA) (Seri *et al*, 2000, 2003; Pecci *et al*, 2002).

**Molecular genetics.** The NMMHC-IIA is part of the non-muscle myosin IIA hexamer that is a component of the contractile cytoskeleton in MK, platelets and other tissues. *MYH9* mutations associated with MYH-9-related disorder are predicted to disrupt stability of the non-muscle myosin IIA hexamer or to disrupt its interaction with other regulatory proteins (Seri *et al*, 2000). Thrombocytopenia results from

defective megakaryopoiesis. Additional defects in platelet function may arise from defective shape change or expression of GPIb/IX/V (Balduini *et al*, 2002; Di Pumpo *et al*, 2002). There is poor correlation between *MYH9* genotype and clinical phenotype and there is variability in phenotype amongst individuals with the same mutations (Seri *et al*, 2003).

**Clinical presentation.** The clinical features of MYH-9-related disorder include, with decreasing frequency, bleeding, sensorineural hearing loss, glomerulonephritis and cataract (Seri *et al*, 2003). Affected individuals usually show a mild bleeding phenotype although this may be more severe than expected from the platelet count because of abnormal platelet function. Life-threatening bleeding is recognised although uncommon. Other phenotypic manifestations are highly variable and may present in infancy or in adult life.

MYH-9-related disorder should be considered in individuals previously classified as ‘non-syndromic macrothrombocytopenia’, in which autosomal dominant or sporadic macrothrombocytopenia exists in isolation. In all patients with suspected MYH-9-related disorder, evidence of glomerulonephritis, sensorineural deafness and cataract should be sought with specialist investigation.

**Minimal diagnostic criteria.** The association of macrothrombocytopenia and one or more of the recognised clinical associations of MYH-9-related disorder is highly suggestive of this diagnosis. Platelet counts are typically in the range of  $20\text{--}130 \times 10^9/l$  and most patients show an elevated mean platelet volume and a conspicuous population of very large platelets (Balduini *et al*, 2003; Seri *et al*, 2003; Lova *et al*, 2004). However, the platelet phenotype is variable and the platelet count and mean platelet volume may be underestimated by automated counters. Döhle-like bodies within neutrophils on May–Grünwald–Giemsa stained peripheral blood are highly suggestive of MYH-9-related disorder. However, the proportion of neutrophils containing inclusions and the inclusion morphology are variable, with some individuals showing absent inclusions using standard microscopy. Abnormal staining of neutrophil inclusions with anti-NMMHC-IIA may offer greater sensitivity (Pecci *et al*, 2002; Seri *et al*, 2003). Definitive diagnosis requires the demonstration of a causative mutation within *MYH9*.

**Management.** As bleeding phenotype is highly variable in MYH-9-related disorders, treatment of individual patients should be guided, where possible, by personal and family haemostatic history. In general, the bleeding disorder should be managed as for other mild platelet disorders (see below).

### 2. Congenital amegakaryocytic thrombocytopenia

**Definition.** Congenital amegakaryocytic thrombocytopenia (CAMT) is a rare disorder characterised by a severe

thrombocytopenia associated with the almost complete absence of MK in the bone marrow.

*Molecular genetics.* The molecular cause of CAMT has now been identified in a number of cases or families and is due to mutations in the *MPL* gene, which results in altered expression or function of the TPO receptor *c-mpl* (van den Oudenrijn *et al*, 2000; Ballmaier *et al*, 2001). CAMT is a recessive disorder and both parents have normal platelet numbers and function.

Studies in mice have demonstrated a wider importance of TPO and *c-mpl* in haemopoiesis. This is in keeping with observations in humans that some individuals with CAMT develop pancytopenia and reduced numbers of all haemopoietic progenitors (Kimura *et al*, 1998).

*Clinical presentation.* Congenital amegakaryocytic thrombocytopenia usually present in the neonatal period or soon after with bleeding symptoms due to severe thrombocytopenia. At diagnosis thrombocytopenia is usually an isolated finding but the condition is associated with progressive haemopoietic failure and the development of severe aplastic anaemia. Haemopoietic failure usually develops over a period of 5–10 years, although it can be more rapid (Ballmaier *et al*, 2003).

*Minimal diagnostic criteria.* The presence of severe congenital thrombocytopenia associated with markedly reduced or absent MK in the bone marrow is highly suggestive of a diagnosis of CAMT. The differential diagnosis of CAMT includes Fanconi's anaemia and other congenital bone marrow failure syndromes and definitive confirmation of the diagnosis currently requires molecular analysis of the *MPL* gene.

*Management.* Platelet transfusion is the most important aspect of the initial management of CAMT (see also generic section). Given the severity of the thrombocytopenia and the eventual progression of this condition to severe aplasia, CAMT is best managed by haemopoietic stem cell transplantation (HSCT) (see below).

### 3. Amegakaryocytic thrombocytopenia with radioulnar synostosis

*Definition.* Amegakaryocytic thrombocytopenia with radioulnar synostosis is a recently described syndrome where radioulnar synostosis occurs in association with an amegakaryocytic thrombocytopenia.

*Molecular genetics.* This condition has been described only in a small number of families to date and appears to be dominantly inherited (Thompson & Nguyen, 2000; Thompson *et al*, 2001). Molecular analysis has demonstrated the presence of mutations in the *HOXA11* gene. It is not yet clear how failure of expression of *HOXA11* causes abnormal megakaryopoiesis and other abnormalities of haemopoietic development.

*Clinical presentation.* Similar to CAMT, this condition usually presents in the first few days of life with bleeding symptoms secondary to severe thrombocytopenia. Platelet morphology is normal and MK are virtually absent from the marrow. Infants with the condition have a proximal radioulnar synostosis and may have other skeletal abnormalities. Sensorineural deafness has also been reported. Again, like CAMT, the condition is associated with the development of pancytopenia as a consequence of haemopoietic failure.

*Minimal diagnostic criteria.* Amegakaryocytic thrombocytopenia in association with characteristic skeletal malformation. Molecular analysis may demonstrate the presence of mutations in the *HOXA11* gene.

*Management.* Initial management is with platelet support but a number of affected cases have subsequently undergone HSCT (see below).

### 4. Thrombocytopenia and absent radii

*Definition.* Thrombocytopenia with absent radii (TAR) is a rare but highly distinctive form of severe congenital thrombocytopenia that characteristically improves through childhood. Patients with TAR may show an additional defect in platelet function (Day & Holmsen, 1972).

*Molecular genetics.* Thrombocytopenia with absent radii is associated with defective response of MK to TPO and the functional defect may lie in the signalling pathways downstream of the TPO receptor *c-mpl* (Ballmaier *et al*, 1997). Although in most cases TAR shows autosomal recessive inheritance, apparently dominantly inherited cases have also been reported. The molecular basis of TAR is unknown.

*Clinical presentation.* Thrombocytopenia with absent radii is associated with the combination of severe congenital thrombocytopenia and bilateral absent radii but is often with associated clinical features, such as gastrointestinal problems (47%), particularly cow's milk intolerance, skeletal defects of the lower limbs (47%), renal (23%) and cardiac anomalies (15%), and facial capillary haemangioma (Hedberg & Lipton, 1988; Greenhalgh *et al*, 2002). The thrombocytopenia is associated with reduced numbers of MK in the bone marrow and defective MK maturation.

Although the thrombocytopenia is initially severe, the platelet count gradually improves. There is no history of progressive haemopoietic failure in this condition, although there are occasional reports of the development of acute myeloid leukaemia in later life (Go & Johnston, 2003).

*Minimal diagnostic criteria.* The diagnosis is based on the typical clinical and bone marrow abnormalities.



*Management.* Platelet transfusions are usually required initially but this is not usually required beyond infancy as the platelet count often improves. Bleeding risk may therefore be more severe than expected from the platelet count. Some clinicians suggest that a platelet function test is performed in TAR patients, including those with normal platelet number, to accurately assess bleeding risk before major surgery.

### 5. X-linked thrombocytopenia with dyserythropoiesis (GATA1 mutations)

*Definition.* This is an X-linked disorder in which thrombocytopenia is associated with red cell abnormalities due to mutations in the *GATA1* gene, which encodes the erythroid and MK transcription factor GATA1.

*Molecular basis.* GATA1 and its co-factor FOG-1 are crucial for normal MK development (Shivdasani, 2001). GATA1 is also important for erythroid development and *GATA1* knockout mice die *in utero* from a severe anaemia. Mutations in the *GATA1* gene can give rise to a macrothrombocytopenia associated with variable red cell abnormalities depending upon the site of the mutation.

*Clinical features.* A small number of kindreds have now been described where a peripheral blood macrothrombocytopenia is associated with mild to moderate dyserythropoiesis (Nichols *et al*, 2000; Freson *et al*, 2001; Mehaffey *et al*, 2001; Yu *et al*, 2002). The condition is X-linked and, clinically, patients often have significant bleeding problems, which may be reported from birth onwards. The thrombocytopenia is usually severe and the platelet size is increased. Small defects in platelet function have also been recorded, although platelet aggregation to most agents is normal (when corrected for platelet counts) (Hughan *et al*, 2005). The anaemia is of variable severity and there may be evidence of haemolysis. The marrow is hypercellular with evidence of dysplasia in both the erythroid and myeloid series. The condition has not been associated with leukaemic transformation or progressive bone marrow failure. Female carriers may also have a mildly increased reticulocyte count and evidence of imbalanced globin chain production (Thompson *et al*, 1977).

*Minimal diagnostic criteria.* Thrombocytopenia in association with red cell abnormalities is suggestive of the diagnosis, which can be confirmed by finding a mutation in the *GATA1* gene.

### 6. Wiskott–Aldrich syndrome

*Definition.* Wiskott–Aldrich syndrome (WAS) is a rare X-linked recessive disease classically characterised by microthrombocytopenia, eczema and immunodeficiency (Wiskott, 1937; Aldrich *et al*, 1954), with an incidence of about 4 per million live male births.

The clinical phenotype is variable, with milder forms of the syndrome previously described as X-linked thrombocytopenia (XLT).

*Molecular genetics.* Wiskott–Aldrich syndrome is a monogenic disorder, arising from defects in the *WAS* gene, located at Xp11.22. The *WAS* gene encodes a 502 amino acid protein termed WASP that is expressed in all haemopoietic lineages but shows reduced or absent expression in WAS (Derry *et al*, 1994). It has been shown that isolated XLT is also caused by mutations in the *WAS* gene and that this is a variant of the same disease (Derry *et al*, 1995; Kwan *et al*, 1995; Zhu *et al*, 1995; Ochs, 1998). Defects of signal transduction and in the maintenance of the cytoskeleton result from the aberrant WASP, giving rise to the diverse clinical and laboratory manifestations of the syndrome (Remold-O'Donnell *et al*, 1996; Ochs, 1998; Higgs & Pollard, 2000).

*Clinical presentation.* The infant often presents with bruising and purpura in the neonatal period and there is an increased risk of intracranial haemorrhage. Bleeding from the gastrointestinal tract may occur, and prolonged bleeding after circumcision may be the presenting feature.

Eczema develops during the first year of life and may be of varying severity, often being widespread and debilitating in children with 'classic' WAS and mild or absent in those with the 'XLT' phenotype.

Infections occur early, often in the first 6 months of life. Bacterial infections occur most frequently, particularly otitis media and respiratory tract infections, with severe viral infections and opportunistic infections occurring less frequently (Sullivan *et al*, 1994). In the 'XLT' phenotype, eczema and infection do not occur.

Autoimmune disorders occur frequently with increasing age in WAS, haemolytic anaemia and vasculitis being the most frequent (Sullivan *et al*, 1994). The other life-threatening complication of WAS is of malignancy, usually though not exclusively lymphoreticular in origin. In the series reported by Sullivan *et al* (1994), malignancy occurred in 13% of individuals, the average age at diagnosis being 9.5 years.

*Minimal diagnostic criteria.* The diagnostic triad of thrombocytopenia, eczema and immunodeficiency are typical, but the full set was absent in most individuals at the first evaluation and the average age at diagnosis in 122 individuals was 21 months, ranging from birth to 25 years (Sullivan *et al*, 1994).

Thrombocytopenia with small platelets is present at birth and may vary between 5 and  $50 \times 10^9/l$ . The average diameter of WAS platelets is reported to be  $1.82 \pm 0.12 \mu\text{m}$  compared with the normal platelet diameter of  $2.3 \pm 0.12 \mu\text{m}$  (Kenney, 1990). Bone marrow examination shows normal numbers and morphology of MK; but there may be ineffective thrombopoiesis (Ochs *et al*, 1980) with reduced platelet survival (Murphy *et al*, 1972). Various defects of platelet function have been reported in WAS (Baldini, 1972), although the

degree of thrombocytopenia will always affect the interpretation of such results.

Immune defects are not present at birth and usually develop in later childhood, when they are variable and progressive, affecting both T and B lymphocytes. T lymphocytes are depleted (Ochs *et al*, 1980) and show abnormal function. Immunoglobulin (Ig)G levels are usually normal but serum IgM levels are reduced, resulting in low titres of iso-haemagglutinins (Murphy *et al*, 1972). Antibody formation to polysaccharide antigens is absent or minimal. Antibody responses to other protein antigens may be normal or reduced. Genotypic confirmation of the diagnosis through analysis of the WAS gene is undertaken by a small number of genetic laboratories in the UK. Identification of non-random inactivation of the X chromosome in haemopoietic cells may be helpful in the diagnosis of female carriers when gene analysis is not possible.

*Management.* The management of WAS should address bleeding manifestations, recurrent infections, eczema, autoimmune disorders and the risk of malignancy; these patients benefit from multidisciplinary management, which should include an immunologist. In 1968, the life expectancy for infants with WAS was reported as 3.5 years (Cooper *et al*, 1968): developments in antimicrobial therapy, the introduction of intravenous immunoglobulin, the ready availability of blood product support and the more frequent use of splenectomy have increased survival to the second and third decade (Mullen *et al*, 1993). However, the only curative treatment for WAS remains bone marrow transplantation.

Bleeding – see under severe platelet disorders in next section. Whenever possible, human leucocyte antigen (HLA)-selected platelets should be used in order to avoid sensitisation and, because of the immune deficiency, should always be irradiated and cytomegalovirus (CMV) negative.

Splenectomy may result in an increase both in platelet number and size; the risk of postsplenectomy sepsis is increased so that the risk–benefit analysis of this operation must be evaluated for individual patients. A median survival of 25 years has been reported in a series of 39 patients following splenectomy coupled with the daily use of antibiotics (Mullen *et al*, 1993; Litzman *et al*, 1996). It is recommended that prophylactic antibiotics should be lifelong for individuals who have undergone splenectomy. Splenectomy does not influence the development of malignancy or autoimmune disorders. It may be expected to be successful in the milder phenotype of this syndrome (XLT) where immunodeficiency is not a feature.

*Immune deficiency.* The immunodeficiency in WAS is severe and progressive: all infections must be thoroughly investigated and appropriate antimicrobial therapy commenced early. Prophylactic antibiotics and antifungals may be indicated and intravenous immunoglobulin should be given at least monthly when recurrent bacterial infections occur. Otherwise, the use of Ig has been successfully restricted to periods of infection.

*Eczema.* Eczema can be severe and debilitating and may require aggressive local therapy as well as systemic steroids. Food allergies may be an exacerbating factor and should be sought routinely.

*Haemopoietic stem cell transplantation* See later.

*Pregnancy* If the mother is a carrier for WAS or the carrier status is unknown but the fetus is male, delivery should be managed as for a potentially severely affected child (see sections below). Where the gene defect is known in a particular family, antenatal diagnosis can be offered with the option of termination of pregnancy if the fetus is found to be affected.

## Glanzmann thrombasthenia (normal platelet count)

### Definition

Glanzmann thrombasthenia, is a rare autosomal recessive disorder characterised by a deficiency or functional defect of platelet GPIIb/IIIa (Nurden & Caen, 1975; Phillips & Agin, 1977; Ginsberg *et al*, 1986), which mediates aggregation of activated platelets by binding the adhesive proteins, fibrinogen, VWF and fibronectin. The incidence is higher in communities where consanguineous partnerships are frequent (Khanduri *et al*, 1981; Awidi, 1984; Seligsohn & Rososhansky, 1984; Ahmed *et al*, 1988).

### Molecular genetics

The genes that encode GPIIb and GPIIIa, termed *ITGA2B* and *ITGB3*, respectively, are located within a single 260-kb segment in the q21–23 band on chromosome 17. More than 100 mutations in either gene have been reported. A diversity of mutational defects are described, including point mutations leading to single amino acids substitutions, insertions or deletions, nonsense mutations and splicing abnormalities (Sosnoski *et al*, 1988; Ramasamy, 2004).

### Clinical presentation

The clinical features are summarised in a large cohort of 177 patients (George *et al*, 1990). Most present before the age of 5 years, often during the neonatal period with purpura or petechiae or in early childhood with excessive bruising. Major bleeding complications during the neonatal period, such as intracranial bleeding are rare (Awidi, 1992).

Purpura, epistaxis, gingival bleeding and menorrhagia are the commonest clinical features (George *et al*, 1990). Epistaxis is the commonest cause of serious bleeding and is more severe in childhood. Severe epistaxis is less frequent in adults. Gingival bleeding is common especially if dental hygiene is poor. Iron deficiency anaemia is common in children.

Menorrhagia is often a serious problem in women and severe bleeding can occur at the menarche. Occasionally, patients may present with severe menorrhagia with no history of bleeding prior to menarche (Markovitch *et al*, 1998).

Gastrointestinal haemorrhage was reported in 22 of the 177 cases (George *et al*, 1990). This may be sometimes associated with angiodysplasia (Nardone *et al*, 1999; Poon *et al*, 2004). Haematuria occurred in 10 of the 177 patients. Haemarthrosis is rare (Cronberg & Nilsson, 1968; Reichert *et al*, 1975; Kloforn & Lightsey, 1979; George *et al*, 1990), and occurred in five of 177 cases (George *et al*, 1990). Intracranial haemorrhage is unusual; it was reported that three of the 177 cases, two of these were following head trauma.

Bleeding after trauma or surgical procedures can be severe. Ten of 12 untreated infants bled following circumcision (Seligsohn & Rososhansky, 1984). Dental extractions are usually associated with excessive bleeding and troublesome bleeding may also occur with exfoliation of deciduous teeth.

Pregnancy and delivery represent a severe haemorrhagic risk (see below). With the exception of menorrhagia and the threat posed by pregnancy, severity of bleeding diminishes with age.

#### *Minimal diagnostic criteria*

Glanzmann thrombasthenia is characterised by a normal platelet count and morphology, a prolonged bleeding time, absent or diminished clot retraction and defective platelet aggregation. If available, testing with PFA-100 shows prolonged closure times (Buyukasik *et al*, 2002; Harrison *et al*, 2002a). The coagulation screen is normal. Thrombasthenic platelets do not aggregate in response to agonists, such as ADP, collagen, thrombin and adrenaline, but do agglutinate in the presence of ristocetin. Affected platelets adhere normally to damaged subendothelium but fail to spread normally and do not form platelet aggregates (Weiss *et al*, 1986). Definitive diagnosis is made by flow cytometry using antibodies to GPIIb (CD41) and GPIIIa (CD61). In the neonate, flow cytometry is easily performed on small volumes of blood, but aggregation is necessary to identify those with normal quantities of receptors with functional defects (Montgomery *et al*, 1983; Jennings *et al*, 1986).

#### *Management*

See the following section for bleeding in severe disorders. Bone marrow transplant should be considered in patients with recurrent severe bleeding. It has been used successfully in a small number of patients (McCull & Gibson, 1997). As affected individuals are most often found in consanguineous families, the chance of a compatible sibling donor is increased.

#### *Neonates*

Although presentation of Glanzmann thrombasthenia in the neonatal period is common, symptoms are not usually severe and are usually confined to purpura and petechiae (George

*et al*, 1990). Occasionally more severe bleeding, such as haematemesis, may occur. Intracranial haemorrhage in the neonatal period is rare.

Neonatal alloimmune thrombocytopenia may occur and is discussed later in the section on management of pregnancy.

### **Bernard–Soulier syndrome (thrombocytopenia with large platelets)**

#### *Definition*

Bernard–Soulier syndrome is a congenital bleeding disorder characterised by thrombocytopenia and large platelets and a prolonged bleeding time (Bernard & Soulier, 1948). Platelets fail to aggregate with ristocetin. The incidence in the USA is thought to be about one in a million population (Lopez *et al*, 1998). However, inherited as an autosomal recessive condition, it is commoner in populations with high incidence of consanguineous partnerships (Macheta *et al*, 1997).

#### *Molecular genetics*

The underlying defect is the absence or decreased expression of the GPIb/IX/V complex on the surface of the platelets. This complex is the receptor for VWF, so that defects result in deficient binding of VWF to the platelet membrane resulting in defective platelet adhesion (Nurden & Caen, 1975). GPIb is the major sialylated GP on the platelet surface, so the absence of or reduction in this protein can lead to thrombocytopenia because platelets with absent or reduced sialic acid have reduced survival (Grottum & Solum, 1969). The complex also facilitates thrombin activation of platelets (Lopez *et al*, 1998). The enlarged platelets may relate to abnormal membrane development observed in abnormalities of GPIb- $\alpha$  (Poujol *et al*, 2002). A potentially important observation is that one copy of *GPIBB*, the gene that encodes GPIb- $\beta$ , is lost in people with chromosome 22q11 deletions (di George and Velocardiofacial syndromes). Some of these patients develop clinically significant thrombocytopenia and giant platelets, but their platelet function is normal (Van Geet *et al*, 1998). The 22q11 deletion syndromes may be associated with a severe Bernard–Soulier syndrome phenotype if the single remaining *GPIBB* gene contains an independently inherited mutation (Budarf *et al*, 1995). Children with 22q11 deletions may require major cardiac surgery in infancy and therefore may have a significant bleeding risk (Lascone *et al*, 2001; Nakagawa *et al*, 2001).

The four components of the complex are encoded by genes on different chromosomes – *GPIBA* on chromosome 17, *GPIBB* on chromosome 22, *GP9* and *GP5* on chromosome 3. Mutations causing Bernard–Soulier syndrome have been described for all these except *GPV* (consistent with the finding that *GP5* knockout mice are normal (Poujol *et al*, 2000)). A mutation database is available at <http://www.bernardsoulier.org>. Currently (accessed 18 January 2006) this contains

22 references to mutations in the *GPIBA* gene, 14 for the *GPIBB* gene and 10 for the *GP9* gene. There is also a mutation in the *GPIBB* gene (Ala156Val 'Bolzano') (Savoia *et al*, 2001) which causes hereditary macrothrombocytopenia and symptoms in heterozygotes (see below).

### *Clinical presentation*

In its most severe form, Bernard–Soulier syndrome is a serious disorder that can be difficult to manage. The inheritance of Bernard–Soulier syndrome is normally recessive (i.e. no symptoms in heterozygotes) when homozygosity is often due to consanguinity. Males and females are equally affected. Heterozygotes usually have normal investigations and no or mild bleeding problems (Lopez *et al*, 1998). However, dominant heterozygous Bernard–Soulier syndrome has been reported (Miller *et al*, 1992) and may be more common than previously thought. An analysis of 12 consecutive patients (Italian) thought to have 'hereditary macrothrombocytopenia' revealed 10 to have the same mutation in the *GPIBB* gene (Ala156Val 'Bolzano') (Savoia *et al*, 2001) that was associated with a reduction in GPIIb- $\beta$  expression similar to Bernard–Soulier syndrome heterozygotes. However, 22 of the 46 affected subjects/family members had mild to moderate bleeding symptoms (epistaxis, gingival bleeding, menorrhagia, easy bruising or excessive bleeding after dental surgery) but no bleeding after major surgery or childbirth (Savoia *et al*, 2001). A careful review of the literature by this group identified 38 heterozygous relatives of Bernard–Soulier syndrome patients, some with bleeding symptoms similar to the patients in their own study. This study suggests that heterozygotes for Bernard–Soulier mutations may be symptomatic. Patients with familial macrothrombocytopenia should be considered as possible candidates for this class of mutation.

In classical Bernard–Soulier syndrome, typical symptoms are those of frequent nosebleeds, bleeding from gums, and easy bruising that is more severe than expected from the degree of thrombocytopenia. Bleeding often starts in childhood. Life-threatening bleeds are associated with major trauma or surgery. Dental extraction may be troublesome. Women often suffer with menorrhagia and severe bleeding may occur after childbirth. While severe cases are usually diagnosed in childhood, the diagnosis may be missed or confused with immune thrombocytopenia. Patients may be first diagnosed in pregnancy (Prabu & Parapia, 2006).

### *Minimal diagnostic criteria*

In this severe disorder, the bleeding time is nearly always prolonged and closure time on the PFA-100 is usually >300 s on both cartridges. The platelet count is very variable, ranging from  $<30 \times 10^9/l$  to the normal range (Lopez *et al*, 1998) and may be underestimated by automated counters. A more accurate measurement is obtained by manual counts and by using CD61 (GPIIIa) antibodies (flow cytometric method).

Platelet size is increased. Examination of a blood film is mandatory. Bernard–Soulier syndrome should be distinguished from other causes of thrombocytopenia and giant platelet syndromes. Children may be misdiagnosed with immune thrombocytopenia (Cuthbert *et al*, 1988) or, in infancy, as alloimmune thrombocytopenia, leading to inappropriate therapy (Pinto da Costa *et al*, 2003). Bone marrow examination is unnecessary. Platelet aggregation studies should be carried out at experienced centres. Platelets fail to aggregate with ristocetin. This is not corrected by the addition of normal plasma, as seen in VWD (Shapiro, 2000). Flow cytometry offers a rapid method of confirmation and is recommended using analysis of GPIIb- $\alpha$  surface density. EM is unnecessary for diagnosis. Ultrastructural studies show that the surface-connected system, the tubular system and the micro-tubular system are much increased and prominent.

### *Management*

The bleeding tendency is variable and needs to be assessed individually. In addition to the investigations mentioned above patients should have full blood count, liver function tests (adults), blood group and iron status measured. Children and adolescents, and adult women are often anaemic and iron deficient.

Surgery and pregnancy are covered in a separate section below. Normal infants of mothers with Bernard–Soulier syndrome who have antiplatelet antibodies are at risk of alloimmune thrombocytopenia.

### *Neonates*

Infants with thrombocytopenia may present a diagnostic dilemma (Pinto da Costa *et al*, 2003). Consanguinity of the parents may be an important indicator of a possible autosomal recessive condition. In the affected infant with Bernard–Soulier syndrome, normally no treatment is needed unless there is bleeding or in rare cases where the platelet count is low, for example,  $<30 \times 10^9/l$ , and there is danger of spontaneous haemorrhage.

## **Disorders of platelet receptors and signal transduction pathways (normal platelet count)**

### *Definition*

The heritable disorders of platelet receptors and signal transduction pathways are a heterogeneous, ill-defined group of defects of platelet activation. They are associated with widespread inhibition of platelet activation to one or more agonists, including defects in aggregation, granule secretion and cytoskeletal regulation, and arise from abnormal function of platelet membrane receptors or their associated signalling pathways. Disorders previously classified as 'primary secretion defects' may be a subset of this group.

### *Molecular genetics*

Disorders within this group may arise through molecular defects in a wide range of receptors or their signalling proteins. As a consequence, identification of the causative molecular defects is often difficult and has been successful in only a small number of isolated cases (Rao *et al*, 2004).

### *Clinical presentation*

Affected individuals show abnormal primary haemostasis usually manifesting as a mild bleeding tendency due to the presence of compensatory receptors or signalling pathways. Patients with defects in signal transduction pathways are likely to have additional clinical manifestations because of the contribution of the signalling pathways to the regulation of other cell types. For example, pseudohypoparathyroidism type 1b, which is associated with a mild bleeding disorder, is caused by a defect in the heterotrimeric G protein subunit  $G_{\alpha}$  which regulates adenylyl cyclase (Freson *et al*, 2002, 2004).

### *Minimal diagnostic criteria*

These disorders are nearly always associated with normal platelet count and morphology. Platelets usually show a decrease in primary aggregation to one or more agonists, which is often accompanied by an absence of secondary aggregation in response to some, or all, standard laboratory agonists. This group of disorders may be distinguished from storage pool defects by the demonstration of normal platelet nucleotide content and nucleotide release.

Most disorders within this group are heterogeneous in laboratory phenotype and require specialist investigation for definitive diagnosis. This could include, for example, measurement of surface receptor expression or activation of signal transduction pathways. However, some disorders have been described with sufficient frequency to allow 'provisional' diagnosis in non-specialist laboratories by platelet aggregation using standard laboratory agonists.

### *Defects in TXA<sub>2</sub> formation or TXA<sub>2</sub> receptor function*

Defective TXA<sub>2</sub> receptor function is associated with impaired platelet aggregation to arachidonic acid and a variable response to other agonists, sometimes comprising a reduced primary wave and absent secondary wave. The response to ristocetin is preserved (Fuse *et al*, 1993, 1996; Lages *et al*, 1981; Samama *et al*, 1981; Wu *et al*, 1981). Defects in platelet cyclo-oxygenase (prostaglandin H<sub>2</sub> synthase-1) (Malmsten *et al*, 1975; Lagarde *et al*, 1978; Horellou *et al*, 1983) or thromboxane synthase (Mestel *et al*, 1980; Defreyn *et al*, 1981; Rao *et al*, 1985) are associated with a similar laboratory phenotype using standard laboratory agonists but may be distinguished from TXA<sub>2</sub> receptor defects by preservation of platelet aggregation in

response to prostaglandin-H<sub>2</sub> or the synthetic thromboxane U46619.

### *ADP receptor defects*

Platelets express two G protein-coupled receptors for ADP, P2Y<sub>1</sub> and P2Y<sub>12</sub>. The P2Y<sub>12</sub> receptor is the target for clopidogrel and plays a major feedback role in supporting platelet activation by a wide range of agonists, following release of ADP. Five patients with defects in the P2Y<sub>12</sub> receptor have been described. The patients have absent or impaired platelet aggregation with ADP, and variable impairment in aggregation to other agonists (Cattaneo *et al*, 1992, 2000, Nurden *et al*, 1995). This disorder shows autosomal recessive inheritance but heterozygous individuals may have a reduced, rather than absent, secondary aggregation response to ADP (Cattaneo *et al*, 1992, 2003). The importance of the P2Y<sub>12</sub> receptor in supporting platelet activation *in vivo* indicates that more patients with defects in this receptor may be identified. Patients with defects in the P2Y<sub>1</sub> ADP receptor have not been reported.

Platelets express a ligand-gated ion-channel receptor, P2X<sub>1</sub> which is activated by ATP. Patients with an inherited defect in this receptor have not been described.

### *Collagen receptor defects*

A small number of patients (<10) have been identified with defects in platelet activation by collagen, which have been attributed to defects in GPVI or GPIa/IIa (Moroi *et al*, 1989; Ryo *et al*, 1992; Arai *et al*, 1995). In these cases, however, it is unclear whether the defect is due to a secondary factor, such as, for example, immune thrombocytopenia or the presence of a myeloproliferative disorder.

### *Adrenoceptor defects*

Selective absence of an aggregation response to adrenaline has been reported as a heritable trait in association with easy bruising and reduced expression of platelet  $\alpha_2$ -adrenoceptors (Tamponi *et al*, 1987; Rao *et al*, 1988). However, the aggregation response to adrenaline is variable in normal individuals and impaired aggregation with adrenaline may be a common asymptomatic population variant (Weiss & Lages, 1988). The relationship between  $\alpha_2$ -adrenoceptor defects and bleeding requires further clarification.

### *Management*

Very little is known about management since the disorders of platelet receptors and signal transduction pathways are heterogeneous and historically have been poorly characterised. In general, this group of disorders should be managed as for other mild platelet disorders (see below).

## Abnormalities of the platelet granules (normal platelet counts)

### Definition

Defective platelet function can result from deficiencies in the number of granules, granule content or failure of normal secretory mechanisms upon stimulation. Many conditions are associated with a defect in either dense- or  $\alpha$ -granules, although both can be affected in a small number of cases. In many cases, defects in secretion from platelet granules are part of a more complex condition affecting other body systems.

### Dense-granule disorders (normal platelet count)

- 1 HPS;
- 2 Chediak–Higashi syndrome (CHS);
- 3 idiopathic dense-granule deficiency.

Dense granules are a lysosome-related organelle that are localised to platelets and MK. They form part of a family of highly specialised, cell-type-specific organelles that includes melanosomes and lytic granules of cytotoxic T lymphocytes and natural killer cells. Dense-granule disorders are generally part of a more complex congenital disorder that can be attributed to defects in several of the other related lysosome-related organelles. Overall, the platelet defect in dense-granule disorders causes a bleeding diathesis of mild–moderate severity with easy bruising, epistaxis and menorrhagia, but the possibility of significant surgical- or trauma-induced bleeding.

### Hermansky–Pudlak syndrome

Hermansky–Pudlak syndrome is a genetically, clinically and biologically diverse disorder. It was first described by two Czechoslovakian pathologists (Hermansky & Pudlak, 1959) and consists of a collection of genetically distinct defects that have common clinical and laboratory findings (Huizing *et al*, 2001). The group of autosomal recessive disorders affects related subcellular organelles, including platelet dense granules and melanosomes, with each disorder being defined by the nature of the mutated gene. Oculocutaneous albinism and platelet dense-granule defects are common to all forms of HPS with additional manifestations that are characteristic of certain forms of HPS, including pulmonary fibrosis, granulomatous colitis, neutropenia and mild immunodeficiency. For further clinical information on HPS, the reader is referred to the recent reviews by Gunay-Aygun *et al* (2004) and Spritz *et al* (2003).

Although extremely rare worldwide, it is the commonest genetic disorder in Puerto Rico affecting one in 800 and giving rise to more than 500 known cases (Witkop *et al*, 1990). There are also clusters of affected kindreds in the Swiss Valais, southern Holland and Japan and a recently reported series of UK residents of Turkish extraction (Harrison *et al*, 2002b).

*Molecular genetics.* To date, HPS has been associated with mutations in eight human genes: *HPS1*, *AP3B1/HPS2*, *HPS3*, *HPS4*, *HPS5*, *HPS6*, *HPS-7/dysbindin* and *HPS8* (Gwynn *et al*, 2000; Li *et al*, 2003; Starcevic & Dell’Angelica, 2004; Di Pietro & Dell’Angelica, 2005; Morgan *et al*, 2006). All the proteins encoded by the HPS genes are involved in intracellular vesicular trafficking, controlling processes such as protein sorting, vesicle docking and fusion. Additional manifestations of HPS are characteristic of mutations in specific HPS genes. Examples include pulmonary fibrosis associated with *HPS1* and *HPS4* mutations and neutropenia and immunodeficiency associated with *HPS2* mutations. The proteins encoded by the *HPS1* and *HPS4* genes are believed to play a co-ordinated role in trafficking of proteins to newly synthesised organelles (Chiang *et al*, 2003).

Most human HPS patients are known to have mutations of either *HPS1* or *HPS4*. In the HPS patients from Puerto Rico, a specific genetic abnormality in *HPS1* is almost ubiquitous; that is a 16-bp frameshift duplication affecting codons 491–496 in exon 15 of *HPS1* at chromosome segment 10q23 (Oh *et al*, 1996). A number of other mutations in *HPS1* have been described in HPS patients from Europe and Japan (Oh *et al*, 1998).

It is noteworthy that between eight and 10 genes have been identified in mouse models of HPS, which as yet have not been implicated in a dense-granule disorder in humans. The precise number of genes that fall into this group is determined by the way that HPS is defined (Di Pietro & Dell’Angelica, 2005). For example, the mocha mouse displays neurological defects that have not been reported in HPS patients, even though it encodes for a protein that forms a complex that includes the gene encoded by *HPS-2*.

### Chediak–Higashi syndrome

Chediak–Higashi syndrome is an autosomal recessive dense-granule disorder which, in common with HPS, is associated with deficient pigmentation of oculocutaneous albinism (Introne *et al*, 1999; Huizing *et al*, 2001). In addition, infections and a lymphoproliferative accelerated phase affect the majority of patients with death usually occurring in the first decade. The classical finding in this condition is very large peroxidase-positive cytoplasmic granules in haemopoietic (neutrophils) and non-haemopoietic cells. The dense-granule disorder results in similar laboratory features and bleeding diathesis to HPS (Buchanan & Handin, 1976) but some CHS patients have reduced or irregular dense bodies rather than total absence.

*Molecular genetics.* Chediak–Higashi syndrome is caused by a series of frameshift and nonsense mutations in the *CHS* gene which result truncation of the protein lysosomal trafficking regulator (LYST). LYST is believed to play a role in organelle trafficking, but its precise function is not known.

### Idiopathic dense-granule disorders

Idiopathic dense-granule disorder or  $\delta$ -storage pool disease is used to describe a relatively large cohort of patients with mild bleeding problems that have been attributed to a defect in dense-granules. The genetic basis of the underlying defect is unclear. The large variability in the extent of aggregation and the absence of other characteristic features means that this may be a relatively miscellaneous group of disorders. Unless a clear demonstration of a defect in dense-granule content/release has been demonstrated, it is prudent to consider other causes of the disorder, such as a defect in ADP receptor signalling or a defect in an intracellular signalling pathway.

### Diagnosis of dense-granule disorders

A defect in platelet dense-granule content/release is common to HPS, CHS and the idiopathic dense-granule disorders. Analysis of  $\alpha$ -granule function, however, must be performed to distinguish this group of disorders from combined  $\alpha$ - and dense-granule disorders.

Hermansky–Pudlak syndrome and CHS have in common oculocutaneous albinism, but can be distinguished by the large peroxidase-positive cytoplasmic granules in neutrophils in CHS. In addition, CHS is usually lethal within the first decade.

Platelet dense-granule disorders may result in defects in platelet aggregation that range from an abnormal response to all agonists to more subtle changes, which may only be seen with low concentrations of agonists. Characteristic features are: (i) the absence of second wave aggregation to adrenaline (however, note that this is seen in a proportion of control donors); (ii) a delayed and reduced response to collagen; (iii) impaired aggregation to low concentrations of agonists, such as arachidonic acid and TRAP; and (iv) high concentrations of ADP elicit full, irreversible aggregation.

A marked reduction in both the content and ratio of ADP to ATP or absence of release of ATP (measured with a lumi-aggregometer) is diagnostic of a platelet dense-granule disorder. Reduced numbers or absence of dense granules can be confirmed by EM.

### Management

The bleeding symptoms of dense-granule disorders should be managed as for other mild platelet disorders (see below). In the case of CHS, the only curative therapy is bone marrow transplantation.

### $\alpha$ -Granule disorders (some may have thrombocytopenia)

- Grey platelet syndrome (GPS; also known as gray platelet syndrome);
- Paris–Trousseau or Jacobsen syndrome;
- Quebec platelet syndrome;
- arthrogryposis-renal dysfunction-cholestasis (ARC).

### Grey platelet syndrome (may have thrombocytopenia)

This is an extremely rare condition with <100 cases reported worldwide. It is the only known granular disorder that is associated with an absence of  $\alpha$ -granules when analysed by EM (Raccuglia, 1971; Hayward, 1997). Due to the absence of  $\alpha$ -granules, the platelets appear agranular and grey in peripheral blood smears. In addition, the platelets are large and misshapen. The contents of  $\alpha$ -granules are absent, including proteins which are synthesised within the MK, such as platelet factor 4,  $\beta$ -thromboglobulin and platelet-derived growth factor, and those that are taken up from the blood, including platelet factor V and fibrinogen. The  $\alpha$ -granule marker P-selectin, however, is retained and is redistributed to the surface upon activation. Drouin *et al* (2001) identified three GPS patients who also had grey neutrophils, demonstrating that this disorder is not necessarily restricted to platelets. The defect in GPS is believed to be due to defective targeting and packaging of endogenously synthesised proteins in the platelet  $\alpha$ -granules, although the responsible gene(s) or biochemical defects have not been identified.

Both autosomal recessive and autosomal dominance patterns of inheritance have been described raising the possibility that GPS can be caused by defects in more than one gene. Mild to moderate myelofibrosis has been described in some but not all GPS patients (Jantunen *et al*, 1994). The myelofibrosis has been attributed to the spillage of fibroblast growth factors in the bone marrow, although other explanations have also been proposed (Falik-Zaccai *et al*, 2001).

*Minimal diagnostic criteria.* The appearance of ‘grey’, misshapen and slightly large platelets on a blood film is characteristic. In addition, the platelet count is often reduced and can be as low as  $50 \times 10^9/l$ . The levels of platelet  $\alpha$ -granule contents are reduced or absent, including  $\beta$ -thromboglobulin, platelet factor 4, fibrinogen, VWF, factor V and platelet-derived growth factor. Platelet nucleotides fall within the normal range. EM of platelets demonstrates reduced numbers or absence of  $\alpha$ -granules. Platelet aggregation to a range of agonists has been shown to be normal in some GPS patients (e.g. Falik-Zaccai *et al*, 2001), although a number of cases are associated with defects in aggregation to one or more agonists, e.g. loss of response to collagen (Nurden *et al*, 2004).

### Paris–Trousseau or Jacobsen syndrome (thrombocytopenia)

Paris–Trousseau or Jacobsen syndrome is an autosomal dominant-inherited thrombocytopenia that is characterised by the presence of giant  $\alpha$ -granules in a low percentage of platelets and the presence of two morphologically distinct populations of MK in the bone marrow, some of which present with signs of abnormal maturation. In addition, there are several other congenital abnormalities, including mental retardation, cardiac abnormalities and cranio-facial abnormalities. The bleeding diathesis is relatively mild.

Paris–Trousseau or Jacobsen syndrome patients have deletions on the long arm of chromosome 11q that include the *FLII* gene, which encodes a transcription factor that is essential for megakaryocytopoiesis. The hemizygous deletion of *FLII* generates a subpopulation of progenitor cells that are unable to undergo normal differentiation, thereby forming a large population of small, immature MK that undergo massive lysis (Raslova *et al*, 2004). The proportion of platelets with giant  $\alpha$ -granules cannot release their contents normally upon stimulation (Breton-Gorius *et al*, 1995). It is an extremely rare disorder with only 10 cases reported in children (Favier *et al*, 2003).

#### *Quebec platelet syndrome (or factor V Quebec)*

Quebec platelet syndrome is an autosomal dominant, extremely rare disorder which was originally characterised by the low level of factor V within platelet  $\alpha$ -granules but normal plasma levels of the coagulation factor (Hayward *et al*, 1997). There are currently about 40 members of a single family characterised in Canada. There are no characteristic morphological or platelet aggregometry features, so cases may be undiagnosed. The defect in factor V is now known to be caused by overexpression of urokinase plasminogen activator leading to activation of plasmin. The protease cleaves multimerin which would otherwise stabilise factor V (Kahr *et al*, 2001; Sheth *et al*, 2003). There is also degradation of many other  $\alpha$ -granules proteins, including P-selectin, although platelet  $\alpha$ -granule structure is conserved (Hayward, 1997). Quebec platelet syndrome is associated with defective procoagulant activity due to the resulting failure in assembly of the prothrombinase complex (Hayward, 1997; Hayward *et al*, 1997).

Urokinase plasminogen is released in unusually large amounts upon activation and this may explain why the bleeding responds to fibrinolytic inhibitors rather than to platelet transfusions.

Another related defect, which is also extremely rare, is platelet factor V New York where the intracellular factor V appears to be decreased but is not proteolysed as in factor V Quebec (Weiss *et al*, 2001).

#### *Arthrogryposis-renal dysfunction-cholestasis*

A mutation in the gene *VPS33B*, which encodes for a protein that is implicated in protein trafficking has recently been identified as the cause of ARC syndrome (Lo *et al*, 2005). ARC is an autosomal recessive multisystem disorder associated with platelet dysfunction caused by a deficiency in platelet  $\alpha$ -granules (Gissen *et al*, 2004). This disorder is readily identified based on the presence of other distinguishing features and affected individuals usually die within their first year.

#### *Management of $\alpha$ -granule disorders*

The rare nature of these disorders means that there is little evidence on which to base treatment of the bleeding problem.

In general, they should be managed as for other mild bleeding disorders (see below) with the exception of Quebec platelet syndrome which has been reported to be unresponsive to platelet transfusions (Hayward, 1997; Hayward *et al*, 1997). Recent studies support the use of fibrinolytic inhibitors to control bleeding in Quebec platelet syndrome (Hayward *et al*, 1996, 1997).

#### *Alpha- and dense-granule disorders*

*Idiopathic  $\alpha$ - and dense-granule disorders.* There are very few reports of patients with deficiencies in both  $\alpha$ - and dense-granules and little information on which to base treatment. Measurement of  $\alpha$ -granule contents and secretion is essential to distinguish this group of disorders from those of platelet dense granules. Based on present information, the clinical phenotype and laboratory abnormalities are believed to be similar to dense-granule deficiency (Weiss *et al*, 1993), but it is likely that this will represent a much more heterogeneous group of disorders in view of the number of proteins implicated in vesicle trafficking, many of which will be associated with widespread organ dysfunction because of their role in other cell types.

A number of mice models have been identified with defects attributed to defects in platelet dense- and  $\alpha$ -granules. The defect in dense granules alters the coat colour, as is the case for defects in genes that cause HPS in mice, but the distinguishing feature is the accompanying defect in  $\alpha$ -granule content. The genes responsible for these defects encode for proteins involved in membrane trafficking. Examples include Rab geranylgeranyl transferase, which encodes for a protein that regulates geranylation of the small G protein, rab27a (Swank *et al*, 1993; Detter *et al*, 2000), and the pallidin gene, which encodes for a protein that is associated with syntaxin 13, a SNARE (soluble N-ethylmaleimide-sensitive fusion factor attachment receptor) protein involved in vesicle trafficking (Huang *et al*, 1999).

#### *Management of $\alpha$ - and dense-granule disorders*

The rare nature of these disorders means that there is little evidence on which to base treatment of the bleeding problem. In general, they should be managed as for other mild bleeding disorders (see below).

### **Scott syndrome and related disorders**

#### *Definition*

Scott syndrome was originally described in 1979 in a patient (from whom the name was derived) presenting with a relatively severe bleeding syndrome (Weiss *et al*, 1979). Later studies on this and other families demonstrated that the defect is inherited in an autosomal recessive pattern (Dachary-Prigent *et al*, 1997; Weiss & Lages, 1997; Munnix *et al*, 2003).



However, the defect is extremely rare with only three cases described so far in the literature. Scott syndrome is characterised by a significantly reduced ability to promote both tenase and prothrombinase activity on the platelet surface. This is caused not only by reduced exposure of negatively charged phospholipids but also reduced generation of microvesicles within activated platelets (Bever *et al*, 1992; Kojima *et al*, 1994).

A similar clinical condition, termed Stormorken syndrome, has also been described in one family that presented with platelets with so-called 'inverse Scott syndrome' (Stormorken *et al*, 1995). The platelets in this very rare condition demonstrate full procoagulant activity and exposure of phosphatidylserine (PS) in the absence of activation along with higher levels of circulating microvesicles. Platelet function tests reveal abnormal clot retraction and reduced aggregation responses and ATP secretion in response to collagen. The platelets also have a tendency to spontaneously aggregate. Another related syndrome with deficient microvesiculation but normal procoagulant activity has been recently described in four patients from three unrelated families (Castaman *et al*, 1997).

#### *Molecular genetics*

Recently, a novel missense mutation has been found in the gene that encodes the lipid transporter ABCA1, which resulted in altered protein trafficking and reduced PS translocation in a patient with Scott syndrome (Albrecht *et al*, 2005). The molecular basis for Stormorken syndrome is not yet known.

#### *Clinical presentation*

Patients with Scott syndrome bleed following invasive procedures such as dental extraction. Postpartum bleeding has been reported and can be very severe.

Patients with Stormorken syndrome (platelet number low normal) exhibit a mild bleeding disorder associated with musculodystrophy and asplenia (Stormorken *et al*, 1985, 1995; Stormorken, 2002). Although one might expect patients with Stormorken syndrome to present with a thrombotic condition the patients paradoxically all have a moderate bleeding tendency.

#### *Minimal diagnostic criteria*

Scott syndrome and Stormorken syndrome can be easily diagnosed using flow cytometry. Annexin V labelled to fluorescein isothiocyanate now provides a calcium-dependent high-affinity probe to assess whether platelets express PS upon activation (Bettache *et al*, 1998; Munnix *et al*, 2003). The generation of microvesicles can also be simultaneously monitored on the flow cytometer (Sims *et al*, 1989). Platelets are activated with calcium ionophore (A23187) or a combination of thrombin and collagen. Normal platelets will bind only annexin-V after activation. In contrast, Scott syndrome platelets will fail

to significantly expose PS and therefore bind less annexin V upon activation whereas Stormorken syndrome platelets will significantly bind annexin V in the absence of stimulation.

The prothrombin consumption index is also a useful screening test for Scott syndrome that can be performed in any laboratory (Parry *et al*, 1980).

#### *Management*

Given the rarity of these disorders there is very little literature on their treatment and management. The mainstay of treatment for Scott syndrome is platelet transfusion, which corrects the primary defect. Tranexamic acid is useful for mucosal bleeding. Although there are no published data it might be anticipated that recombinant FVIIa would be less efficacious in Scott syndrome than other diseases but could be considered if first-line therapy is unsuccessful.

### **The management of inherited platelet disorders**

Inherited platelet disorders are rare, with some described in single or a handful of families (e.g. Scott, Stormorken and Quebec syndromes). These disorders require specialist management. All people with such disorders should be registered with a haemophilia centre with appropriate facilities for investigation, treatment and 24-h open access. Affected individuals should also be issued with a card describing their condition, and it is advisable to give the patient and his/her primary care doctor some written information on the condition as many of these disorders are uncommon and will be unfamiliar to many medical personnel. Children who present with easy bruising are therefore often initially thought to suffer non-accidental injury rather than hereditary bleeding disorders.

Advice should be given where necessary about life-style issues (e.g. individuals with severe disorders should avoid contact sports) and patients with severe platelet disorders should avoid medication which interferes with platelet function, i.e. salicylates and other NSAIDs. These agents may be used with caution in patients with milder disorders where other medical conditions, such as cardiovascular disease indicate. The use of such drugs must be balanced against the risks. People with inherited platelet disorders should be immunised against hepatitis A and B (Makris *et al*, 2003), have baseline liver function tests performed at diagnosis, and these should be routinely monitored especially in those who receive blood products. Children commonly suffer from iron deficiency and this is likely to be particularly prominent where there is an additional severe platelet disorder.

Pregnancy should be managed in close collaboration with a haemophilia centre, with a written plan of management for the mother, and also a plan for investigation and management of the neonate, in collaboration with neonatologists, if appropriate. Further guidance is given below. For some disorders

molecular genetic analysis is available, and antenatal diagnosis may be possible for some of the severe disorders.

### Specific treatment options

The following agents may be considered and are discussed in relation to specific indications later:

- 1 Antifibrinolytic agents, e.g. tranexamic acid, either orally at a dose of 15–25 mg/kg t.d.s. (or i.v. at a dose of 10 mg/kg t.d.s. in the case of more serious bleeding). This is useful for control of menorrhagia and other mild bleeding manifestations from mucous membranes, such as epistaxis. It may also be useful as a mouthwash (10 ml of a 5% w/v solution q.d.s. which, if swallowed, is equivalent to a dose of 500 mg q.d.s.) for local mouth bleeding (e.g. bleeding from inflamed tonsils).
- 2 Desmopressin (DDAVP): useful reviews are by Mannucci (1997) and Wun *et al* (1995). Patients with storage pool disorders usually (but not always) respond. It is also not clear if laboratory correction (e.g. of the bleeding time) will correlate with clinical efficacy. The effects of DDAVP may involve an increase in the levels of circulating VWF. A recent review examined the cellular mechanism of action (Kaufmann & Vischer, 2003) and noted that effects on platelet function remain undefined. DDAVP may cause flushing and hypotension. It should not be used in individuals with evidence of atherosclerosis. It causes fluid retention, and patients should be advised to restrict fluid intake in the subsequent 24 h. Intravenous fluids should be given with caution because of the risk of water retention, which can result in hyponatraemia and fits. For this reason, it is not generally given to children under the age of 2 years in whom the risk of hyponatraemia may be greater. If given for more than a single dose it is advisable to monitor daily weights and plasma electrolytes.

Desmopressin may be the agent of choice for mild bleeding problems where tranexamic acid alone is ineffective. There is no convincing evidence to support the practice of doing a DDAVP correction of the bleeding time. The effect must be assessed by the clinical response. DDAVP can be given as:

- (i) Intravenous infusion over 30 min – the usual dose regimen is 0.3 µg/kg of a 4 µg/ml solution diluted to 30–50 ml in 0.9% saline, and infused over 30 min, but there is some evidence that a smaller dose of 0.2 µg/kg is also effective when used with tranexamic acid 10 mg/kg (Schulman *et al*, 1987).
- (ii) Subcutaneous injection at a dose of 0.3 µg/kg (may be useful in the control of menorrhagia where tranexamic acid fails). A more concentrated product is available containing 15 µg/ml.
- (iii) Intranasal spray – available in a concentrated form (150 µg per dose) to be administered at 300 µg for an adult and 150 µg for a child (over 30 kg in weight)

equivalent to 0.2 µg/kg. This may be useful for the control of menorrhagia or for a spate of nosebleeds although some individuals find it troublesome to administer.

- 3 Platelet transfusions – are appropriate in severe disorders and when other agents have failed. However, these are blood products and carry risks of transfusion-transmitted infections and allergic reactions. In the UK there is a possibility of transmission of the prion agent associated with variant Creutzfeldt–Jakob disease. The risk is unknown but is thought to be higher with cellular products (Seghatchian, 2003). Experimental transmission by blood transfusion has been demonstrated in sheep (Hunter *et al*, 2002), and two cases of probable prion infection by blood transfusion are now reported in the UK (Ironsides & Head, 2004; Llewelyn *et al*, 2004). It is likely that multiple donor exposures increase the risk, so that platelet transfusions should only be given if essential. Children born since the beginning of 1996 have not been exposed to prions through the food chain, and it is preferable to avoid donor exposures wherever possible. Because of these considerations, platelet transfusions should not be given without clear indications. Patients with platelet disorders may be subject to repeated episodes of transfusion, putting them at risk of developing alloantibodies either to HLA antigens or missing GPs in Bernard–Soulier syndrome and Glanzmann thrombasthenia (see below). For these reasons it has been custom and practice to HLA-type these patients at diagnosis and where possible, to transfuse HLA-selected platelets (unless the delay in obtaining these would compromise the clinical situation). Current BCSH guidelines do not support this practice (BCSH, 2003a). Evidence suggests that the incidence of HLA alloimmunisation is reduced with prestorage leucocyte depletion (Novotny, 1999), but such evidence as there is has been gathered from immunosuppressed recipients and may not hold true for immunocompetent people with inherited disorders who may need repeated transfusions. Because the consequences of sensitisation may be so deleterious in these patients (failure to obtain haemostasis), our recommendation is that HLA-selected platelets are preferable. A systematic gathering of information about platelet sensitisation in patients with platelet disorders is warranted. Platelets are available as:
  - (i) Random donor platelets – usually available as pheresis units, one adult therapeutic dose (ATD) is equivalent to four to six single donor units. In children the dose is 10–15 ml/kg. This is often sufficient given before surgical procedures and a further ATD afterwards, depending upon the nature of the intervention or bleed. Patients with severe disorders may need higher doses or two to four ATDs.
  - (ii) HLA-selected platelets (as pheresis units) – product of choice for all patients with inherited platelet disorders.
- 4 Recombinant factor VIIa (rFVIIa): this is an alternative therapeutic agent whose use is being evaluated. An international registry collects data on use in platelet

disorders (Poon & d'Oiron, 2000) and participation is encouraged. Experience in platelet disorders is limited apart from in Glanzmann thrombasthenia (see below) (Poon *et al*, 2004). Recombinant FVIIa is licensed for use in Glanzmann thrombasthenia where refractoriness to platelet transfusions has been demonstrated. It is not licensed for any other platelet disorder, although there is some published data on use in Bernard–Soulier syndrome (see below).

### **Management of spontaneous bleeding in patients with severe disorders (especially Glanzmann thrombasthenia, Bernard–Soulier syndrome, and the more severe forms of Wiskott–Aldrich syndrome)**

Bruising is very common in all the severe disorders and does not require any treatment. For patients who have moderate or severe bleeding, it is important to have a low threshold for admission to hospital. Young children with epistaxis may lose much more blood than is clinically apparent. If bleeding is mild or moderate, treatment with local measures and anti-fibrinolytic drugs may suffice, especially if the bleeding is mucocutaneous. Local measures may include compression, use of gelatin sponge or gauze dipped in tranexamic acid or topical thrombin applied to superficial wounds (Bellucci & Caen, 2002). Epistaxis may be controlled by gel foam soaked in topical thrombin or by nasal packing (Bellucci & Caen, 2002). Packing can irritate nasal mucosa resulting in local inflammation and rebleeding when the packing is removed. Oral tranexamic acid (combined with tranexamic acid mouthwash) may stop gingival bleeding.

When local measures and antifibrinolytics fail to control bleeding, the choice of treatment lies between platelet transfusion, rFVIIa or a combination. Platelet transfusions are usually effective in controlling haemorrhage but have several disadvantages, particularly in Glanzmann thrombasthenia and Bernard–Soulier syndrome where there is a risk of alloimmunisation by HLA antigens or the specific missing GPs resulting in refractoriness to transfusions.

The use of rFVIIa in Glanzmann thrombasthenia was described in the treatment of severe epistaxis in a 2-year-old boy (Tengborn & Petruson, 1996). There are other reports of successful treatment with rFVIIa, but the total number of patients treated is small (Chuansumrit *et al*, 1999; Poon *et al*, 1999; Poon, 2001; Caglar *et al*, 2003; Kaleelrahman *et al*, 2004). Recombinant FVIIa is licensed for use in Glanzmann patients who are refractory to platelet transfusions. A commonly used dose has been 110 µg/kg. Recombinant FVIIa has a short half-life and the dose may need to be repeated at 90-min intervals. Higher doses of up to 270 µg/kg have been used when the standard dose was not effective (Chuansumrit *et al*, 1999). The number of doses required to treat bleeding episodes has also varied. The dose given and the number of doses

should be assessed individually for each patient; this agent is not always successful. Recombinant FVIIa may be less effective in more severe bleeding or if there is a delay from onset to treatment (Almeida *et al*, 2003). Recombinant FVIIa is generally safe, but thrombotic complications have been recorded (Aledort, 2004). Recombinant FVIIa could be used in preference to platelet transfusion for non-life-threatening bleeding where the site can easily be assessed, such as epistaxis or oral bleeding, when local measures and oral tranexamic acid are insufficient to stop the bleeding. Intracranial bleeding is rare in Glanzmann thrombasthenia, and any life-threatening bleeding should be treated with platelet transfusions without necessarily waiting for HLA-selected platelets where this would cause delay.

Menorrhagia can be a major problem in women with Glanzmann thrombasthenia. Oral tranexamic acid should be tried, and hormone therapy may be helpful. Bleeding may be severe enough to require blood transfusions.

### **Management of dental procedures and surgery in patients with inherited platelet disorders**

Dental extractions and surgical procedures in patients with inherited platelet disorders should always be carried out in close collaboration with a haemophilia centre that has expertise in the management of these conditions. Prior to dental extractions or elective surgery patients who have previously received platelet transfusions should be screened for HLA and platelet antibodies.

#### *Patients with 'severe' platelet disorders, e.g. Glanzmann thrombasthenia and Bernard–Soulier syndrome*

*Dental extractions.* Effective local haemostatic measures including suturing and the use of topical haemostatic agents are essential. Oral tranexamic acid should be commenced the day before the extractions and continued for 5–7 d. The patients should be kept under close clinical supervision.

It is desirable to avoid platelet transfusion if possible for two reasons; first, to reduce alloimmunisation, and secondly in the UK, to avoid unnecessary risk of exposure to prions. Recombinant FVIIa may be used to cover dental extractions in patients with Glanzmann thrombasthenia and Bernard–Soulier syndrome. The International Registry for the use of rFVIIa in patients with hereditary platelet disorders reported successful use of rFVIIa to cover dental extractions in all nine reported patients with Glanzmann thrombasthenia (Poon *et al*, 2004). The dose and total number of treatments is not always stated. Two patients (Almeida *et al*, 2003) were given a total of three doses of rFVIIa (100 µg/kg per dose), the first 30–45 min prior to extraction and the subsequent doses 90 min apart. There is a single case of dental extraction in Bernard–Soulier syndrome (Almeida *et al*, 2003) using rFVIIa with four doses of rFVIIa (100 µg/kg) given 90 min apart.

We suggest a trial of rFVIIa at a dose of at least 90 µg/kg, given immediately prior to the procedure and two to three further doses given at 90-min to 2-hourly intervals. If bleeding recurs, further doses of rFVIIa should be given. If bleeding is not controlled at the time of the extraction or further rFVIIa is not effective in controlling rebleeding the patient should receive an adult dose (or appropriate paediatric dose) of HLA-selected platelets.

*Minor surgery.* The International Registry for the use of rFVIIa in patients with hereditary platelet disorders has reported successful use of rFVIIa to cover 11 of 12 minor surgical procedures in patients with Glanzmann thrombasthenia (Poon *et al*, 2004). We recommend that patients with Glanzmann thrombasthenia and Bernard–Soulier syndrome undergoing minor surgical procedures should be given a trial of rFVIIa treatment. A suggested regime would be a dose of 90 µg/kg given immediately prior to the procedure and repeated at 2-hourly intervals for 12 h, then at increasing intervals of 3–4 h, depending upon the clinical situation, until the patient is considered to be no longer at risk of rebleeding. It should be noted that the initial dose may need to be higher in children in whom rFVIIa also has a shorter half-life (Villar *et al*, 2004). If bleeding is not controlled at the time of surgery (i.e. no response to two or three doses of rFVIIa) or rebleeding does not respond to further doses of rFVIIa, the dose could be escalated or an infusion of one adult dose of HLA-selected platelets should be administered and repeated as necessary. Oral tranexamic acid should also be prescribed during the postoperative period.

*Major surgery.* The international registry reports the successful use of rFVIIa in six of nine major surgical procedures in patients with Glanzmann thrombasthenia. However, due to uncertainties concerning the efficacy of rFVIIa, it is recommended that HLA-selected platelets are used for elective major surgery in patients with Glanzmann thrombasthenia and Bernard–Soulier syndrome. An adult dose of platelets should be given immediately prior to the procedure and further doses given depending on clinical need. For emergency procedures random donor platelets may have to be given rather than delay while HLA-selected platelets are located. Oral tranexamic acid should also be prescribed during the postoperative period.

*Patients with ‘mild’ platelet disorders – including most of the other inherited disorders (inherited thrombocytopenias are described separately, below)*

Spontaneous bleeding is not likely to occur.

*Dental extractions and minor surgery.* Desmopressin has been reported to be haemostatically effective for dental extractions and minor surgery in patients with milder platelet defects, including storage pool disease (Kohler *et al*, 1985; Kentro

*et al*, 1987; DiMichele & Hathaway, 1990; Rao *et al*, 1995; Kosch *et al*, 1999; Piot *et al*, 2002). This should be the agent of choice. A single dose given immediately prior to the extraction should be sufficient. If rebleeding occurs after more than 12 h a further dose may be given. If DDAVP is contraindicated or is not haemostatically effective at the time of the extraction or for the treatment of rebleeding, patients should receive an appropriate dose of HLA-selected platelets or possibly rFVIIa.

*Major surgery.* Due to the unpredictability of the efficacy of repeated doses of DDAVP, it is recommended that patients with milder platelet defects undergoing major surgical procedures should receive an adult (or appropriate paediatric) dose of HLA-selected platelets prior to operation and further doses given depending on clinical need. As in Glanzmann thrombasthenia and Bernard–Soulier syndrome, random platelets may have to be given to cover emergency procedures to avoid delay.

Effective local haemostatic measures should be applied. Oral tranexamic acid may also be appropriate, depending on the circumstances. The patients should be kept under close clinical supervision.

#### *Management of surgery in patients with hereditary thrombocytopenia*

The platelet count in this group is variable and patients may also have platelet dysfunction of varying severity. Treatment must be individualised and based on knowledge of the past bleeding history, platelet number and function, and known response to previous platelet infusions and haemostatic agents. The risk of bleeding due to thrombocytopenia is likely to be a continuum and the target platelet count will depend on the risk of bleeding related to the specific surgical procedure and the severity of the consequences of haemostasis failure.

#### *Dental extraction and surgery*

In patients with no clinical or laboratory evidence of platelet dysfunction, management should be based on the platelet count. In the context of idiopathic thrombocytopenic purpura (ITP), BCSH guidelines recommend that the platelet count should be raised above  $30 \times 10^9/l$  for dental extraction and inferior dental block and to a level of at least  $50 \times 10^9/l$  for minor operative procedures (BCSH, 2003b). The platelet transfusion guidelines recommend a platelet count of at least  $50 \times 10^9/l$  for procedures such as liver biopsy, laparotomy and central line insertion (BCSH, 2003a).

The ITP guidelines (BCSH, 2003b) recommend a platelet count of at least  $80 \times 10^9/l$  for major surgery whilst the platelet transfusion guidelines (BCSH, 2003a) recommend a platelet count of at least  $50 \times 10^9/l$  for major surgery and  $100 \times 10^9/l$  for procedures on the eye and brain.

Similar target platelet counts are recommended for patients with inherited thrombocytopenia. Tranexamic acid may also be useful, especially for dental procedures, and DDAVP and further platelet infusion should be tried for patients who bleed despite apparently adequate platelet counts.

In patients who also have platelet dysfunction, management depends on the previous history of bleeding and known response to haemostatic agents. Platelet counts should be raised to at least the target levels recommended above for patients without platelet dysfunction but higher target platelet counts are likely to be required for some patients. Depending on the individual's past history and previous known responses, DDAVP may also be required. Patients whose baseline platelet count is above the target level may respond to DDAVP alone. If bleeding occurs despite these measures, further platelets should be transfused and if this fails, rFVIIa should be considered.

### Management of pregnancy in women with inherited disorders of platelet function and number

Pregnancy in women who have an inherited platelet disorder or may be carrying a child with an inherited platelet disorder should be managed with close collaboration between haemophilia centre staff and the obstetric unit. A written birth plan for obstetric and haematological management should be available. Appropriate blood products and laboratory monitoring should be immediately available. Close clinical observation by staff experienced in the management of bleeding disorders is required.

Regional anaesthesia in patients with platelet dysfunction is contraindicated because haemostasis cannot be guaranteed and a plan for pain relief in labour should be made with the patient antenatally. If there is co-morbidity that makes the risk of general anaesthesia high, a management plan should be made in advance between the haematologist and anaesthetist in case a Caesarean section becomes necessary.

Thromboprophylaxis should be considered on an individual basis, particularly in patients who have received haemostatic treatment.

Uterine contraction is an important part of perinatal haemostasis, especially in women with inherited platelet disorders, and should be planned in advance between the obstetrician and haematologist. Standard therapy is to use intramuscular syntometrin during the second stage of labour. If intramuscular injections are contraindicated an intravenous infusion of syntocinon should be used to ensure uterine contraction. Close attention should be paid to possible surgical causes of bleeding. Haemostatic management depends on the severity of the bleeding disorder and is discussed below.

If the fetus is at risk of having inherited a platelet function disorder, ventouse extraction and high forceps are contraindicated and fetal scalp monitoring and blood sampling should be avoided. A written management plan for diagnosis and

treatment at birth should be available and a neonatologist should be involved with management.

### Women with 'severe' platelet disorders, particularly Glanzmann thrombasthenia and Bernard–Soulier syndrome

Women with severe platelet disorders are at significant risk of haemorrhage at the time of delivery, which may be life threatening and necessitate hysterectomy. Women should be informed of this risk before becoming pregnant. Antepartum haemorrhage is less common; vaginal bleeding severe enough to require transfusion occurred once in 21 pregnancies of women with Glanzmann thrombasthenia. Postpartum haemorrhage may occur 1–2 weeks after delivery (George *et al*, 1990). In Bernard–Soulier syndrome there are few reports in the literature ( $n = 11$ ; Prabu & Parapia, 2006). Bleeding has been reported in the antenatal period but is more common at and after delivery, including as late as 6–8 weeks postpartum.

Women should be monitored antenatally for the development of anti-HLA and GPIIb/IIIa or GPIb iso-antibodies. The normal fetus may be at risk of alloimmune thrombocytopenia as a result of transplacental haemorrhage followed by sensitisation. In mothers who have already developed antibodies, an anamnestic response can occur (Vivier *et al*, 1989). Antiplatelet antibodies may cross the placenta and cause neonatal thrombocytopenia and intrauterine fetal haemorrhage. In neonates at risk of thrombocytopenia, instrumental delivery should, ideally, be avoided although this must be balanced against the risk of a Caesarean section in the mother. Plasma exchange during pregnancy has been used to reduce the antibody titre (Vivier *et al*, 1989).

*Vaginal delivery.* Maternal haemorrhage is likely at the time of vaginal delivery if no haemostatic treatment is given. One option is to use rFVIIa combined with tranexamic acid. This avoids the use of platelets and the associated risk of formation of iso-antibodies that may increase the chance of neonatal alloimmune thrombocytopenia and bleeding in a future pregnancy. There are no data related to dosing schedules but 90 µg/kg (early pregnancy weight) given as close to delivery as possible, followed by further infusions 2-hourly for three to four doses or until haemostasis is clinically secure, is a reasonable approach. If bleeding occurs despite this treatment HLA-selected platelets should be infused.

The other option is to give HLA-selected platelets prior to delivery combined with tranexamic acid. There is no evidence to guide the amount of platelets but one or two adult doses given prior to delivery and further doses given dependent on observed bleeding could be tried. Reports describe platelet transfusion for 6 d postdelivery (George *et al*, 1990). If HLA antibodies or GPIIb/IIIa antibodies are present, rFVIIa can be used in conjunction with HLA-selected platelets.

Severe late postpartum haemorrhage has been described 1–2 weeks after delivery. Apparently uncomplicated deliveries in

women without signs of bleeding complications need to continue to be closely observed and tranexamic acid continued for at least 2 weeks, and to have ready access to the haemophilia/obstetric service.

A useful algorithm for the management during surgery and delivery is available (Bell & Savidge, 2003).

*Caesarean section.* Bleeding at the time of Caesarean section is inevitable without haemostatic cover and should be treated as for any major surgical procedure. HLA-selected platelets and tranexamic acid are recommended as first-line treatment with one to two adult units given immediately before the procedure and further doses postoperatively, dependent on the clinical evidence of bleeding. The use of surgical drains and regular testing of haemoglobin in the first 12–24 h will help identify bleeding early. Bleeding may be concealed if it is into the uterine wall.

*Neonates.* The neonate is not at risk of inheriting the full platelet function disorder unless the father is a carrier, although it should be noted that a limited number of symptoms are seen in individuals who are heterozygous for Bernard–Soulier syndrome. This may be important to consider because of the high rate of consanguinity in some communities. Screening of the father's platelets by flow cytometry (to quantify the relevant GP) will identify carrier fathers and so help to identify neonates who are potentially at risk. If the neonate is at risk of a severe platelet function disorder, ventouse and instrumental delivery and fetal scalp monitoring are contraindicated.

Neonates of mothers with Glanzmann thrombasthenia or Bernard–Soulier syndrome complicated by antiplatelet antibodies are at particular risk of alloimmune thrombocytopenia caused by transplacental passage of the antibodies to the fetus, which can result in fatal intrauterine intracranial haemorrhage. In such neonates, platelet transfusion should be ABO and RhD identical, CMV screened and free of clinically significant irregular blood group antibodies. If possible HLA-selected platelets should be used (Gibson *et al*, 2004).

#### *Women with 'mild' inherited platelet function disorders*

The variable severity of this group of disorders means that some are likely to respond to DDAVP, whilst other women will require platelets. Haemostatic management of these patients will depend on previous knowledge of their bleeding phenotype and experience of their response to treatment modalities.

*Use of epidural anaesthesia.* In women with inherited thrombocytopenia and no platelet dysfunction BCSH guidelines can be followed. The ITP guidelines recommend that epidural and spinal anaesthesia should be avoided if the platelet count is  $<80 \times 10^9/l$  (BCSH, 2003b). The platelet transfusion guidelines (BCSH, 2003a), however, state that, for lumbar puncture and epidural anaesthesia, the platelet count should be raised to at least  $50 \times 10^9/l$ .

In women with normal coagulation and platelet count the risk of spinal haematoma is higher after an epidural than a spinal anaesthetic (Tyagi & Bhattacharya, 2002). The risk of spinal haematoma in relation to the platelet count is likely to be a continuum and a cutoff is necessarily arbitrary. In the absence of good quality data, each patient must be assessed individually and the risk of spinal haematoma weighed against the risk of general anaesthesia and potential benefit of pain relief in the individual.

In patients with inherited thrombocytopenia the platelet count should be raised to at least  $80 \times 10^9/l$  when an epidural is both placed and removed. Although the risk of spinal haematoma is less, the platelet count should also ideally be raised to at least  $80 \times 10^9/l$  for a spinal anaesthetic.

*Vaginal delivery.* Depending on the known severity and previous responses to haemostatic agents, vaginal delivery could be managed in a number of ways.

Observation with no haemostatic treatment or tranexamic acid alone may be suitable for some patients. If bleeding occurs, DDAVP or platelets can be given depending on previously observed responses.

Some patients will have a clinical history that suggests bleeding is likely following delivery. In this case, DDAVP or platelets should be infused at the time of delivery depending on previously observed responses.

If DDAVP fails to achieve an adequate haemostatic response, platelets should be used initially at one to two adult doses. If bleeding continues after platelet transfusion and a surgical cause of bleeding is excluded then rFVIIa should be tried at a dose of 90 mg/kg repeated every 2 h until haemostasis is secured.

*Caesarean section.* This should be managed as for any other major surgical procedure and in most cases HLA-selected platelets should be given prior to Caesarean section in association with tranexamic acid. Further platelets should be given depending on haemostatic response.

Some patients may be adequately managed with DDAVP, and platelet transfusions are used only if bleeding occurs.

If bleeding continues after platelet transfusion and a surgical cause of bleeding is excluded then rFVIIa should be tried at a dose of 90  $\mu$ g/kg, repeated every 2 h until haemostasis is secured.

*Management of the neonate.* Some platelet function disorders have a dominant mode of inheritance. In these cases the neonate will be at risk of bleeding at the time of delivery and an appropriate plan should be made.

#### *Inherited thrombocytopenia.*

This group of patients varies in severity and may also have platelet dysfunction. Treatment is based on knowledge of the past bleeding history, platelet number and function.

If there is no clinical evidence of platelet dysfunction then management should be based on platelet number.

**Vaginal delivery.** In the context of ITP, BCSH guidelines recommend that the platelet count should be raised above  $50 \times 10^9/l$  for a vaginal delivery (BCSH, 2003a). In women with inherited thrombocytopenia a platelet count of  $50 \times 10^9/l$  is likely to be sufficient for haemostasis during vaginal delivery. However, in women with a history of mild bleeding, close observation and the use of tranexamic acid may be sufficient to cover vaginal delivery at platelet counts between 30 and  $50 \times 10^9/l$ , thus avoiding the use of blood products (Lichtin, 1996).

**Caesarean section.** The risk of bleeding related to platelet count is likely to be a continuum. The platelet count should be raised at least to a level of  $50 \times 10^9/l$  to cover Caesarean section and the decision to raise the count to  $80 \times 10^9/l$  should be made on a case-by-case basis, dependent on the patient's previous history and the planned mode of anaesthesia.

#### *Patients with inherited thrombocytopenia and associated platelet dysfunction*

**Vaginal delivery.** The platelet count should be raised to at least  $50 \times 10^9/l$  and tranexamic acid was used. Depending on the individual's past history and previous known responses, DDAVP and platelets may also be needed at the time of delivery.

**Caesarean section.** The platelet count should be raised to  $80 \times 10^9/l$  and tranexamic acid was used. Depending on the individual's past history and previous known responses, DDAVP and platelets may also be needed at the time of delivery.

## Haemopoietic stem cell transplantation

There are a number of platelet disorders which ultimately may be most appropriately managed by HSCT. This may reflect

either the severity of the clinical bleeding problems or the natural history of the underlying condition where there is a tendency for progression to marrow aplasia or for malignant transformation. As with allogeneic bone transplantation for other benign conditions, these transplant procedures are increasingly performed using reduced-intensity conditioning (RIC) regimens, which are associated with reduced toxicity and may expand the potential use of such procedures in this area (French *et al*, 2004).

In both CAMT and amegakaryocytic thrombocytopenia with radioulnar synostosis, severe aplasia is likely to develop after a variable period of time. A number of successful transplant procedures have been reported in CAMT and this now includes matched unrelated and RIC transplant procedures (Lackner *et al*, 2000; Yesilipek *et al*, 2000; Al-Ahmari *et al*, 2004; Muraoka *et al*, 2005; Steele *et al*, 2005). Successful HSCT has also been reported in amegakaryocytic thrombocytopenia with radioulnar synostosis (Thompson *et al*, 2001).

Both Glanzmann thrombasthenia and Bernard-Soulier syndrome may be associated with potentially life-threatening bleeding problems. Where severe bleeding is a recurrent problem, HSCT may be an appropriate form of therapy and a small number of cases have now been reported (McColl & Gibson, 1997). As affected individuals are most often found in consanguineous families, the chance of a compatible sibling donor is increased and again, RIC transplantation may be feasible (Flood *et al*, 2005).

Transplantation can be curative for all components of WAS and has the potential to cure upwards of 90% of patients depending on the nature of the transplant undertaken. A total of 170 transplants for WAS have been reviewed. All children with WAS should be considered for HSCT at diagnosis. Transplantation is currently recommended for those with an HLA-identical sibling donor and matched-unrelated donor transplant is also recommended for those with an excellent HLA match, using T-cell depletion and add-back if appropriate (Filipovich *et al*, 2001). Haploidentical transplantation is

**Table II.** Topics for further research and audit.

Area	Suggested research/audit
Diagnosis	Production of guidelines to standardise laboratory methods Publication of a network of laboratories able to perform more detailed tests Contribution to mutation databases for specific conditions e.g. Bernard-Soulier syndrome at <a href="http://www.bernardsoulier.org">http://www.bernardsoulier.org</a> Improvements in molecular and genetic diagnostic methods
Clinical presentation	Contribution to established registries to share knowledge about the rare disorders
Treatment	Gathering of data about human leucocyte antigen sensitisation in all patients treated with platelet transfusions Database for registration of treatment with rVIIa in Glanzmann thrombasthenia (funded by Novonordisk) at <a href="http://www.glanzmann-reg.org">http://www.glanzmann-reg.org</a> Improved definition of dose of platelets required for effective haemostasis in different disorders
International collaboration	These disorders are rare and we encourage international collaboration through the International Society for Thrombosis and Haemostasis, which could establish an international congenital platelet disorders clinical group

less successful and should be considered for severe clinical phenotypes who have not responded to splenectomy or who have recurrent serious infections. A strong correlation between type of gene mutation, presence or absence of WASP and the clinical phenotype of affected individuals has recently been described (Jin *et al*, 2004): if such studies can be verified then this information may be useful in predicting long-term prognosis and enabling informed decisions to be made about selection of patients for bone marrow transplantation or splenectomy.

## Conclusion

The heritable platelet disorders are a heterogeneous group of disorders, but for clinical purposes can usefully be divided into severe or mild. Treatment options for bleeding can therefore be generalised. Stem cell transplantation is an option for the most serious disorders. The state of the art is advancing rapidly with the discovery of new molecular interactions that, in future, will lead to better understanding of platelet physiology. Most of our recommendations are not able to be based on the highest level of evidence, but nevertheless can act as a basis from which to audit future practice. Table II gives information about topics for further research and audit.

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