bjh guideline

Clinical and laboratory diagnosis of heritable platelet disorders in adults and children: a British Society for Haematology Guideline

Keith Gomez,[1] Julia Anderson,[2] Peter Baker,[3] Tina Biss,[4] Ian Jennings,[5] Gillian Lowe,[6] Sean Platton[7] and on behalf of the British Society for Haematology Guidelines

[1]Haemophilia Centre and Thrombosis Unit, Royal Free London NHS Foundation Trust, London, [2]Haemophilia Thrombosis and Immunology Centre, Royal Infirmary, NHS Lothian, Edinburgh, [3]Haemophilia and Thrombosis Centre, Oxford University Hospitals NHS Foundation Trust, Oxford, [4]Haemophilia Comprehensive Care Centre, Royal Victoria Infirmary, Newcastle-upon-Tyne Hospitals NHS Foundation Trust, Newcastle-upon-Tyne, [5]UK NEQAS for Blood Coagulation, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, [6]Haemophilia Comprehensive Care Centre, Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham, and [7]Haemophilia Centre, The Royal London Hospital, Barts Health NHS Trust, London, UK

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Methodology

This guideline was compiled according to the British Society for Haematology (BSH) process at https://b-s-h.org.uk/. The Grading of Recommendations Assessment, Development and Evaluation (GRADE) nomenclature was used to evaluate levels of evidence and to assess the strength of recommendations. The GRADE criteria can be found at http://www.gradeworkinggroup.org. A literature search was carried out using the terms given in Appendix I.

Review of the manuscript

Review of the manuscript was performed by the BSH Haemostasis and Thrombosis Task Force, the BSH Guidelines Committee and the sounding board of BSH. It was also on the members section of the BSH website for comment. It has also been reviewed by the Platelet Society which does not necessarily approve or endorse the contents.

This guideline replaces the previous British Committee for Standards in Haematology guideline published in 2011 on laboratory diagnosis of heritable disorders of platelet function.[1] The remit has been expanded to include clinical diagnosis and heritable thrombocytopenia under the overarching term heritable platelet disorder (HPD). Acquired disorders such as immune thrombocytopenia and drug-induced platelet dysfunction are not covered.

Since the previous guideline there have been significant advances in the clinical assessment of HPD. For example, bleeding assessment tools (BATs) have been developed to standardise clinical evaluation and provide a quantitative analysis of bleeding symptoms.[2] A major laboratory advance has been in the use of high-throughput sequencing (HTS) which is now available in routine clinical practice in the UK. As a result there has been a large increase in distinct HPD with a better understanding of their underlying molecular basis and clinical effects.[3] Previously there were five categories of HPD captured in the National Haemophilia Database (NHD) of which three were distinct disorders: Glanzmann thrombasthenia, Bernard–Soulier syndrome and platelet-type pseudo-von Willebrand disease. These have now been replaced by the single HPD overarching category with several sub-categories covering nearly 50 distinct disorders (http://www.ukhcdo.org/nhd/). While these are individually rare disorders, collectively HPD comprise 10% of registered cases in the NHD, the fourth most common group of disorders after von Willebrand disease, haemophilia A and factor XI deficiency.[4]

The International Society on Thrombosis and Haemostasis (ISTH) has produced guidance on the order in which laboratory assays may be used to investigate platelet function disorders[5] and consenting of patients for genomic testing.[6] There are also North American guidelines for laboratories on light transmission aggregometry.[7] However, there remains a need for guidance for clinicians and scientists involved in the diagnosis of these disorders that is up to date and specific to UK practice.

Correspondence: BSH Guidelines Administrator, British Society for Haematology, 100 White Lion Street, London N1 9PF, UK.
E-mail: bshguidelines@b-s-h.org.uk

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Clinical evaluation

Taking a bleeding history and the use of a BAT

Individuals with a HPD frequently present with a history of haemorrhagic symptoms. These may include easy bruising, prolonged bleeding from minor wounds, epistaxis, gum bleeding, bleeding from the gastrointestinal or urinary tract, menorrhagia, bleeding after childbirth and bleeding after dental extraction or surgery. Haemarthroses, intramuscular bleeding or intracranial haemorrhage are less frequently seen compared with haemophilia. A careful bleeding history is necessary to determine the frequency and severity of the bleeding symptom(s) as well as the interventions that have been necessary to control or prevent bleeding. Any history of surgery, dental extractions or childbirth without significant bleeding should also be noted.

Identification of a significant bleeding history in children can be difficult. Symptoms such as easy bruising and epistaxis are relatively common in children who do not have a bleeding disorder, and haemostatic challenges such as invasive procedures or menarche are less likely to have occurred. Detailed questioning about specific neonatal bleeding symptoms, such as cephalohaematoma, umbilical cord bleeding or bleeding after immunisations or heel prick sampling, may be informative in younger children.[8]

The use of a BAT to quantify haemorrhagic symptoms and signs has been studied in relation to the diagnosis of mild bleeding disorders. These tools allocate a score for each haemorrhagic symptom. A higher score indicates a greater severity of bleeding and/or that a more significant intervention has been required in order to manage the bleeding. A negative score is obtained for a history of haemostatic challenge(s) without bleeding in some scoring systems. The ISTH-BAT has been shown to improve pretest probability in the investigation of type 1 von Willebrand disease and can be administered by a healthcare professional or patient.[9,10] Although the ISTH-BAT can differentiate between individuals with or without an established diagnosis of platelet function disorder,[11] its ability to predict laboratory abnormalities in newly presenting subjects with a history of excessive bleeding has not been consistently shown.[2,12]

The timing of onset of haemorrhagic features is important. Severe platelet disorders are likely to present at a younger age, sometimes with severe neonatal bleeding. A later presentation with haemorrhagic symptoms is more typical of a less severe disorder.

Other aspects of clinical history taking

It is important to exclude causes of acquired platelet dysfunction and thrombocytopenia. Non-steroidal anti-inflammatory drugs have a well-documented effect on platelet function while other classes of drugs, such as antidepressants, seem to have a lesser effect.[13] Diseases of other systems such as renal failure and myeloid neoplasms can be associated with platelet dysfunction with clinically significant bleeding.[14] Dietary components such as caffeine, garlic and turmeric may influence platelet function if taken regularly in high doses such as found in food supplements.[15]

The presence of a family history of haemorrhagic symptoms and signs, particularly when linked to knowledge of abnormal platelet number or function in symptomatic family members, may help to identify the mode of inheritance of the condition. Consanguinity increases the likelihood of an autosomal recessive disorder. Obtaining historical blood counts and assessing trends is essential when an inherited thrombocytopenia is being considered.

Examination

Clinical examination may reveal evidence of current or recent bleeding. However, more relevant is the role of examination in identifying other potential causes of bleeding, such as hypermobility (which can be formally assessed using the Beighton score), or features that are indicative of a recognised syndrome.[16]

Associated features in syndromes which involve reduced platelet number or abnormal platelet function

There are many syndromes or conditions that result in reduced platelet number and/or function.[17] Some of these have phenotypic features that are obvious, for example skeletal malformation in Thrombocytopenia with Absent Radii syndrome or oculocutaneous albinism in Hermansky–Pudlak syndrome, and may be noticed before a bleeding phenotype manifests. In others, the platelet disorder is identified first and screening for associated abnormalities may be necessary once the diagnosis has been made, such as sensorineural hearing loss, presenile cataract, liver enzyme abnormalities and glomerulonephritis in association with the *MYH9*-related macrothrombocytopenias.[18,19] Recognition of the potential for serious complications, such as premature cardiovascular disease in hereditary sitosterolaemia, can enable preventative action to be taken.[20] Appendix II lists some heritable conditions that may be associated with a platelet disorder and their phenotypic features. More complete listings of HPD with detailed descriptions of phenotype can be found in online resources such as omim.org and orpha.net.

Recommendations

- **The use of a BAT is suggested as a means of taking and documenting a standardised bleeding history but is not sensitive enough to determine the need for laboratory testing (2C)**

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Collection and transfer of samples

The reliability, accuracy and reproducibility of results are improved by the standardisation of pre-analytical variables, particularly for light transmission aggregometry (LTA). Although a tourniquet may be used initially, it should be released after venepuncture and blood collected using a minimum 21-mm-gauge needle. The first few millilitres of blood drawn should be discarded to reduce the possibility of platelet activation. Haemolysis leads to activation or desensitisation of platelets and therefore haemolysed samples should be rejected. Samples should be drawn into 105–109 mmol/l buffered trisodium citrate anticoagulant to maintain a stable pH with care taken to ensure the tubes are correctly filled. As maintaining the correct concentration of citrate is critical, underfilled or overfilled samples should be rejected. The minimum volume of blood required depends on which assays are required and is detailed in the appropriate sections below. Ensuring a good quality sample of adequate volume for LTA in babies can be challenging, and testing should be deferred until at least 12 months of age if clinically appropriate.

Although previous guidelines have recommended refraining from smoking and caffeine prior to sampling there is no good evidence that this has an effect on LTA results using standard agonists.[21] If it is safe to do so, medications that affect platelet function should be stopped 7–10 days before testing. As many patients will be unable to discontinue their medications, a record of medications should be available when interpreting the results. Although there is some evidence that samples can be taken at another site and transported to a specialist laboratory for testing, this has not been reliably reproducible in clinical practice.[23] Therefore, samples should be collected at the same site as testing occurs to avoid any deterioration in functional responses due to mechanical activation during transportation or sample ageing. Studies using pneumatic tube transport systems have not shown any significant effects on tests of platelet function.[24,25] Pre-analytical errors such as clotted or inadequately mixed samples, platelet clumping in ethylenediaminetetraacetic acid (EDTA) and the presence of cryoglobulins or red cell heat damage during transport need to be excluded.[26] Lipaemia impacts the baseline turbidity of the sample, and traces will be similar to those obtained in samples with low platelet counts. These samples may be tested but the final report should indicate that the sample was lipaemic.

Recommendations

- **Haemolysed or incorrectly filled samples should be rejected (1B)**
- **Medications that may affect platelet function should be discontinued 7–10 days before testing if appropriate (1B)**
- **It is preferable for samples to be taken at the same site as the testing laboratory to prevent transport artefacts (2C)**

Assessment of platelet count, size and morphology

Platelet count

Routine platelet counting is now performed on automated analysers that provide high throughput with greater precision and reproducibility than original manual microscopic methods. The majority of routine counting is based upon electrical impedance. Other options are optical light scatter and fluorescence or flow cytometry based immunological labelling. Macrothrombocytopenia, and to a lesser extent, microthrombocytopenia, is seen in many HPDs, resulting in abnormalities in size distribution histograms or scatter plots produced by impedance methods. This requires further evaluation using optical/fluorescence-based systems or microscopy.

The International Council for Standardization in Haematology's recommended reference method for counting platelets is the use of CD41 and CD61 monoclonal antibody-labelled whole blood analysed on a flow cytometer.[27] The count is derived from the ratio of fluorescent-labelled platelets to red cells in the same preparation (produced from a reference impedance technique). The use of immuno-labelled platelet counting with a flow cytometer provides a very accurate count particularly in thrombocytopenic patients ($<20 \times 10^9$/l). However, this requires larger volumes of blood and is more time-consuming and costly. Methods have been described on analysers using only CD61, although this cannot be done accurately in Glanzmann thrombasthenia where the target αIIbβ3 is reduced or absent, or in the presence of therapeutic antagonists to the target such as abciximab. Manual microscopic platelet counting was previously used as a reference method but the coefficient of variation is wider than with the automated method.[28-30]

Impedance counting is recognised as accurate when platelet counts are normal ($>150 \times 10^9$/l) and there are no significant abnormalities of red cell or platelet size or shape, as these impair the separation of adjoining populations by size alone. This becomes prone to artefact if other cells or cellular debris cross into the platelet populations such as red and white cells, bacteria or lipids.[31] Similarly, abnormally sized platelets may be underestimated by impedance counting. Optical platelet counting uses laser light scatter detection at low and high angles to differentiate size (volume) and refractive index (granularity) respectively. Optical methods are therefore recommended for resolving abnormal count and size distribution when granular content can be interrogated to add to the identification profiles. This is particularly important in differentiating inherited thrombocytopenia associated with changes in platelet size and granularity.[32] The use of multiple detectors at different angles optimises the discrimination of platelets by size and granularity from red-cell debris. Macrothrombocytopenias are better identified using optical detection systems rather than impedance counters.[33]

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Platelet volume

Mean platelet volume (MPV) is a readily available derived parameter which can be reported alongside platelet count, being inversely proportional in normal populations. Many inherited and acquired factors such as age, gender, ethnicity and lifestyle have been reported as influencing both platelet count and MPV.[34] This is confounded by the lack of standardisation of pre-analytical collection procedures such as resting time, temperature and anticoagulant impacting on platelet size.[35,36] Modern full blood count analysers also contribute to the variation with differences of up to 25% in MPV reported between manufacturers, probably due to differences in detection methods (impedance *versus* optical) and their different responses to macrothrombocytes and red cell fragments.[37-39] Laboratories should undertake local validation of instrument-specific MPV ranges being wary of cross platform comparisons. As abnormal platelet size is often a distinguishing feature of HPD, MPV has become an important diagnostic parameter.

Fluorescent labelling involving staining of immature red cell and platelet RNA allows further differentiation when used alongside scattered light. The immature platelet fraction or reticulated platelet count has proven to be a useful marker of thrombopoietic activity. It can differentiate between increased and decreased platelet production and may be significantly raised in macrothrombocytopenias, sometimes disproportionately compared to granule content.[40] As the test requires additional reagents with associated costs it is often used as an add-on test triggered by abnormal impedance flagging.

Microscopy

Pitfalls remain in all the counting techniques in patients with abnormal platelet size, shape or content. Blood film microscopy should be performed to confirm platelet numbers and identify morphological abnormalities. Conventional light microscopy can detect some basic morphological abnormalities, such as the loss of α-granules in the grey-platelet syndrome, but is of limited diagnostic value because it does not provide the resolution required for viewing platelet ultrastructure. More specialist techniques such as immunofluorescence or electron microscopy are required for more accurately demonstrating morphological abnormalities of platelet organelles and the cytoskeleton. Although electron microscopy was first used to assess platelet ultrastructure in the 1940s, it is still not readily accessible in clinical practice. Immunofluorescence with panels of antibodies targeting key platelet glycoprotein receptors, α- and δ-granules and cytoskeletal proteins has been shown to be useful in the differentiation of HPD.[41] Similarly superresolution light microscopy can overcome the resolution limits imposed by conventional light microscopy and has high sensitivity for structural platelet disorders.[42] This technique provides quantitative, high-throughput, automated morphometric analysis of thousands of platelets from a single patient. As these techniques can be performed on peripheral blood smears sent by ordinary post at room temperature, they may well become a key component of the diagnostic pathway for HPD in the future.

Recommendations

- **Optical or fluorescence platelet counts should be run on abnormal plots or samples with unexplained impedance-derived low platelet counts (1B)**
- **Blood film morphology should be examined in new cases of unexplained thrombocytopenia to document morphological abnormalities of platelets and other blood cells (1B)**
- **MPV should be reported routinely alongside the platelet count (1B)**

Light transmission aggregometry

Light transmission aggregometry was first described in the 1960s and measures the transmission of light through platelet-rich plasma (PRP), which increases as platelets aggregate or agglutinate after addition of an agonist.[43,44] Despite the advent of automated techniques that have the potential to reduce intra-assay variability, preparation of PRP and analysis is time-consuming, and interpretation of results and correlation with clinical phenotype still needs to be undertaken by those with experience.[45,46]

Sample preparation for LTA

There are a few guidelines from other organisations that deal with sample preparation but relatively little reliable data on the effects of methodological variation.[7,47,48] The methods described in this section reflect protocols developed in UK haemophilia centres through practical experience. Citrated blood samples obtained as described above are centrifuged to prepare PRP and platelet-poor plasma (PPP). Samples should be left to rest at room temperature for 15 min after collection. To prepare PRP, whole-blood samples should be centrifuged at either 170 ***g*** for 15 min or 200 ***g*** for 10 min in a swing-out rotor without refrigeration or application of the brake.[49,50] Autologous PPP is prepared by centrifugation (after removal of PRP or using whole samples) at a minimum of 1500 ***g*** for at least 15 min at room temperature. At the end of the centrifugation steps a plastic pipette should be used to separate the PRP or PPP, which should be carefully removed without disturbing the buffy coat layer or red cells. The PRP or PPP should then be transferred into separate polypropylene tubes, capped and stored upright at room temperature for at least 30 min prior to testing to avoid false refractoriness of platelets to epinephrine.[51] All analysis

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should be performed within four hours of sample collection to reduce the chances of storage artefacts affecting platelet function.

A platelet count should be performed on the PRP. There is no effect on LTA if the platelet count in whole blood is between 100 and 600 $\times$ 10^9/l, and so adjustment of platelet count for samples with PRP platelet count below 600 $\times$ 10^9/l is not recommended as it may impair responsiveness of platelets to agonists.[52-54] In samples with PRP platelet counts above 600 $\times$ 10^9/l, abnormalities of platelet aggregation may be more frequent when using PPP-adjusted PRP than using native PRP,[55] and adjustment with physiological saline may be more appropriate.[54,56] A control sample diluted with physiological saline to the same PRP platelet count should be analysed alongside the patient sample.

Results of LTA after analysis of PRP with a platelet count below 100 $\times$ 10^9/l could be inaccurate, therefore caution should be exercised in the interpretation of results from such samples. In conditions where thrombocytopenia and abnormal LTA are characteristic features (e.g. Bernard–Soulier syndrome, type 2B and platelet-type von Willebrand disease), tracings should be compared with a diluted control sample with a similar platelet count. Samples from patients with macrothrombocytopenia (for example in Bernard–Soulier syndrome or *MYH9*-associated thrombocytopenia) may have PRP prepared by sedimentation at room temperature for 30 min.[57]

Agonists for LTA

A panel for LTA should include agonists at doses that are able to discriminate between normal and abnormal platelet function. The agonists that should be included in clinical panels for LTA are adenine diphosphate (ADP), epinephrine, collagen (type I, tendon), arachidonic acid and ristocetin. The concentrations used should be sufficient to cause maximum aggregation of more than 50% and no complete disaggregation in 95% of normal controls, and appropriate concentrations are shown in Table I below; laboratories may use lower concentrations of agonists as long as these criteria are met. Many laboratories use analysers with eight channels, and Table I includes a panel of eight agonists that are sufficient to demonstrate normal platelet aggregation in routine clinical practice.

When a more detailed investigation is required, additional agonists can be used to further characterise a defect found in the standard panel. Higher concentrations of agonists can be used if there is an abnormal response in the initial panel, but this should not be so high as to risk over-activating abnormal platelets and generating potentially misleading normal responses. For example: collagen at 2·00 or 2·50 μg/ml if inadequate response to 1·25 μg/ml collagen; 10 μM epinephrine if inadequate response to 5 μM. PRP should be observed for spontaneous aggregation for 1 min before addition of agonists but this is not normally possible in automated or semi-automated aggregometers, so the addition of physiological saline as a mock agonist can be used.[46] The decision to test with additional agonists often requires timely expert review, which may not necessarily be available at the time of analysis. A pragmatic approach would be to perform a full panel on all samples or in follow-up testing. This would require a total of 2·0-3·2 ml of PRP depending on the analyser used, which should be obtainable from 12 to 20 ml of citrated whole blood, depending on patient haematocrit and analyser used.[46,58]

When sample volume is restricted, for example in young children, a cut-down panel of six agonists (Table I) requiring 0·9–1·4 ml of PRP (depending on the analyser used), which should be obtainable from 5·1–9·0 ml citrated whole blood (depending on patient haematocrit), will be sufficient to exclude Glanzmann thrombasthenia, Bernard–Soulier syndrome and type 2B von Willebrand disease.

Table I. Recommended final concentrations of agonists for initial, full and minimal aggregometry panels.

Agonist	Suggested initial panel (for use with eight-channel analyser)	Full panel	Minimum panel (when sample volume is limited and aim is to exclude a severe disorder)
Adenine diphosphate (ADP)	5 and 10 μM	5 and 10 μM	10 μM
Arachidonic acid (AA)	1 mM	1 mM	1 mM
Collagen (type I, tendon)	1·00 or 1·25 μg/ml	1·00 or 1·25 μg/ml and 2·00 or 2·50 μg/ml	2·00 or 2·50 μg/ml
Epinephrine	5 and 10 μM	5 and 10 μM	10 μM
Ristocetin	0·5 and 1·25 g/l	0·5 and 1·25 g/l	0·5 g/l and 1·25 g/l
U46619 (thromboxane A2 receptor agonist)	–	1 μM	–
Thrombin receptor agonist peptide (TRAP) (PAR-1)	–	5 and 10 μM	–
Normal saline	–	0·9% w/v	–
Approximate volume of whole blood required*	7–12 ml	12–20 ml	5–9 ml

*Dependent on technology used for aggregation assays.

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If there is no response with 1·25 g/l ristocetin then mixing equal volumes of patient PRP with control PPP will correct this if it is due to von Willebrand disease but not if it is due to defects in the glycoprotein (Gp) Ib-V-IX receptor.[59] If platelets fail to show spontaneous aggregation (with normal saline as the agonist) but agglutinate with 0·5 g/l ristocetin then type 2B or pseudo-(platelet-type) von Willebrand disease (VWD) should be suspected. Washed platelets from the patient re-suspended in normal PPP will agglutinate with the addition of 0·5 g/l ristocetin in pseudo-VWD but not in true type 2B VWD. Conversely, washed platelets from a normal control re-suspended in PPP from a patient with type 2B VWD will agglutinate with 0·5 g/l ristocetin in type 2B VWD but not in pseudo-VWD.[60] Platelet-type VWD can also be diagnosed by flow cytometry.[61]

There are some situations when additional agonists not listed in the table may also be considered, for example, γ-thrombin, PAR-4 (AYPGKF), collagen-related peptide, convulxin, calcium ionophore A23187 or phorbol 12-myristate-13-acetate. These are mostly used in research studies, and in clinical practice further investigation using the other assays described in this guideline will be more appropriate.

Performing aggregometry

Platelet aggregometers should be calibrated with autologous PPP (considered to allow 100% light transmission) and PRP (0% light transmission) at 37°C. The agonist is mixed with PRP in a ratio of 1:9 to initiate aggregation and the final concentration of agonist within PRP is recorded, taking into account the 10-fold dilution factor. Some automated techniques use a ratio of 1:7 without apparent effect on the results obtained.[45,46,62] A stir speed of 1000 rpm is recommended unless otherwise specified by the manufacturer. It is important that samples are preincubated for at least 5 min at 37°C prior to agonist administration to obtain stable baseline traces. When using non-automated techniques care must be taken to add agonists directly to the PRP, rather than the side of the tube, with the avoidance of air bubbles. The aggregation tracing should be observed for 10 min, unless laboratories can locally verify that most control samples will aggregate before 5 min for a particular agonist, in which case observations can cease after 5 min for that agonist. Any unexpected observations should be repeated on a fresh sample taken on another day.

Once the assays are completed the traces should be visually interpreted. Numerical data can be generated for parameters including maximum and final aggregation as a percentage of full light transmission with PPP, lag time and primary aggregation slope. Subjective observations can be made on shape change, disaggregation (and whether fully reversible or not), and the presence or absence of secondary aggregation. Where numerical data are reported by the laboratory, this should ideally include local, normal cut-off values, which should be calculated using non-parametric statistics on 40 or more normal subjects.[63] This may not be possible due to the inherent variability of the test and the large number of subjects required. Therefore, it is acceptable to subjectively evaluate the shape of the aggregation curve for each agonist concentration, considering the following parameters: lag phase, maximal amplitude, primary aggregation slope and disaggregation.[64] It is important that clinical interpretation is performed by experienced staff in a multidisciplinary team meeting.

Recommendations

- **We suggest that the full panel of agonists for clinical use consists of adenine diphosphate (ADP), epinephrine, collagen (type1, tendon), arachidonic acid, ristocetin, U46619 (thromboxane-A2 receptor agonist), thrombin receptor agonist peptide (PAR1) and normal saline at the final concentrations shown in Table I. The concentrations used should be sufficient to cause maximum aggregation of more than 50% and no complete disaggregation in 95% of normal controls (except for low-dose ristocetin and normal saline) (2C)**
- **Reports should include quantitative measures of aggregation with a subjective review of the shape of aggregation curves (1B)**
- **Where numerical data are reported by the laboratory, this should include local, normal cut-off values, which should be calculated using non-parametric statistics on 40 or more normal subjects (1B)**
- **Tracings and results should be interpreted in a multidisciplinary team meeting by individuals experienced in the techniques used (1C)**
- **Any abnormal unexpected results for individual agonists should be repeated on a fresh sample taken on a separate day, to avoid making a confirmed diagnosis on a single result (1B)**
- **Results obtained on samples with PRP platelet counts $<100 \times 10^9$/l may be tested to exclude Glanzmann thrombasthenia, Bernard–Soulier syndrome, type 2B or platelet-type VWD, but results should be reported with caution (2C)**

Flow cytometry and additional phenotype tests

Flow cytometry is essential for the characterisation of platelet receptor disorders, allowing quantification of receptor density and confirming genotype. Although requiring relatively expensive flow cytometry equipment and technical expertise, the addition of simplified commercial Conformitè Europëenne (CE)-marked reagents makes its use relatively straightforward. Antibodies against GpIIb (CD41) and GpIIIa (CD61) for the diagnosis of Glanzmann thrombasthenia and GpIbα (CD42b), and GpIX (CD42a) for the diagnosis of

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Bernard–Soulier syndrome are readily available and are the most frequently tested. Abnormalities of collagen binding due to defective GpIa/IIa (CD31/CD49b) and GpVI can be detected in conjunction with abnormal LTA response to collagen. These have been rarely described with mild to moderate bleeding phenotypes and mild macrothrombocytopenia.[65]

Extended antibody panels can be performed as second-line investigations to differentiate rarer activation disorders demonstrating impaired and enhanced annexin V binding (Scott and Stormorken syndrome respectively) in conjunction with genetic investigation. Guidelines and reviews have already been published setting out the stepwise approach to diagnosis in conjunction with appropriate clinical history and/or consideration of other abnormal platelet function tests.[66,67]

It is essential to minimise platelet activation during the preparation of samples for flow cytometry. Citrate or EDTA anticoagulated whole blood can be used with the need for only a relatively small sample volume, with a low platelet count (usually obtained through dilution) being required making it attractive in paediatric cases.[68] The dilution associated with this does, however, mean that platelet–platelet interactions cannot be investigated and physiological shear stress is lost.[69] PRP (prepared as described in the LTA section) may give a cleaner preparation to help accurate gating of the population but must be weighed up against the risk of activation or selective removal of larger platelets.[50] Incubation and mixing should be performed with gentle agitation and matched isotype antibody controls should be run in parallel. An EDTA sample should also be drawn for platelet count and morphology at the same time. Calibrated beads now allow quantification of flow results in terms of either mean fluorescence or copy number per platelet. Local normal ranges should be derived for adult and paediatric samples allowing for the fact that neonates may have significantly lower receptor densities.[70]

Platelet activation can also be measured in a flow cytometer using antibodies to markers of activation such as P-selectin (CD62P) and uptake/secretion of mepacrine over a fixed time period.[71] Practically these assays are poorly controlled and standardised due to problems associated with sample handling and lability. They are not suitable for routine clinical practice but may be of use in individual cases.

Measurement of platelet nucleotides

LTA may not be sensitive to all δ-granule storage pool defects. Total platelet adenine nucleotides and their release have long been measured as a more specific test for these abnormalities.[72] However, only half of respondents to a 2016 UK NEQAS survey that tested platelet function offered nucleotide analysis.[73] The proportion was similar in an ISTH worldwide survey.[5] In the UK NEQAS survey in 2016, the 12 laboratories that reported a nucleotide measuring technique all used in-house constructed luciferin/luciferase-based luminometry. This measures adenosine triphosphate (ATP) concentration from a platelet lysate with the benefit that samples can be frozen and transported.[74] Conversion of the stored ATP via the addition of pyruvate kinase to ADP allows for the generation of an ATP:ADP ratio. This is important in the identification and quantification of the metabolic and storage pools located in the platelet, with the latter accounting for approximately 60% of the total content. Storage pool defects affecting δ-granules are associated with a decrease in ADP concentration leading to increased ATP:ADP ratios.[75]

In addition to measurement of nucleotide amount, ATP release assesses the secretion of nucleotides during degranulation. This can indicate defects of the release mechanism that may be missed by assays that only quantify nucleotides. Real-time incorporation of ATP release into platelet aggregometry (lumi-aggregometry) enables assessment of nucleotide release simultaneously with aggregation.[72,76] However, commercially available lumi-aggregometers are only semi-automated and do not differentiate between defects of nucleotide content and defects in secretion, but may be useful as a preliminary nucleotide screen.

Recommendations

- **Local age-specific reference ranges for receptor numbers in flow cytometry should be established (1B)**
- **Consider flow cytometry for the diagnosis of Glanzmann thrombasthenia and Bernard–Soulier syndrome (2B)**
- **Consider platelet nucleotide quantification and secretion assays in the presence of normal light transmission aggregometry (2C)**

Rapid/point-of-care platelet function testing

There are many tests available for the rapid assessment of drug-induced platelet inhibition, but these are outside the scope of this guideline. Those that may be useful in the diagnosis of HPD include aggregometry-based methods and tests involving a measure of platelet adhesion.

Platelet function analyser

The PFA-100 and PFA-200 devices use citrated whole blood aspirated at high shear rates (5000–6000/s) through disposable cartridges containing an aperture coated with either collagen and epinephrine, or collagen and ADP, or P2Y12.[77,78] These agonists trigger platelet adhesion, activation and aggregation, leading to rapid occlusion of the aperture and cessation of blood flow, reported as the closure time. This requires 0·8 ml citrated whole blood for each cartridge, making it attractive for paediatric samples. Normal test results can exclude severe platelet defects, but not milder disorders.[79,80] Abnormal results

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need to be repeated, and are not specific for a particular disorder. The platelet function analyser (PFA) is subject to a number of variables including platelet count, haematocrit, and elevated von Willebrand factor or fibrinogen.

Whole-blood aggregometry

Impedance aggregometry, as used in the Multiplate® instrument, is a method of aggregometry allowing use of whole blood. Following adhesion of platelets to two metal wire electrodes, impedance increases as platelet aggregates form on the electrodes, following treatment of the sample with agonists. Use of whole blood is possibly more physiological as the sample contains all the blood components normally present when platelets aggregate and assesses platelet adhesion to a solid phase. However, biphasic and reversible ADP responses cannot be determined, and weak or absent epinephrine responses have been described in normal controls.[81-83] The method has been shown to be of value in screening for severe HPDs but has a low sensitivity for milder defects.[80] This may be because the agonist concentrations required are much higher in whole blood compared with PRP. Other aggregometry-based rapid point-of-care tests of platelet function, including VerifyNow® (Platelet Works) have been shown to be useful in monitoring antiplatelet therapy but have not been shown to have utility in the diagnosis of HPDs.[84]

Other rapid, point-of-care methods

These include the Impact-R® cone and plate(let) analyser and the global thrombosis test, both based on shear forces stimulating platelet adhesion, and viscoelastic methods such as the ROTEM® and TEG® devices. There are currently insufficient data to indicate any utility in the diagnosis of HPDs. In selected cases with abnormal point-of-care results at baseline, these tests may have some utility in the monitoring of the response to treatments such as desmopressin.

Recommendations

- **Point-of-care tests should not be used to screen for HPDs as they lack sensitivity for milder disorders (1C)**

Quality assurance and tests of platelet function

Internal quality control (IQC) and external quality assessment (EQA) are important components of quality assurance in all tests of haemostasis. EQA is available for platelet counting and morphology, but not for MPV. Laboratories also face difficulties in performing EQA or IQC with platelet function assays (such as LTA, nucleotide or glycoprotein assays) which are not easily included in EQA surveys.[85] This is because there is a relatively narrow window of time after blood samples are taken before platelet function is compromised and in contrast to other haemostasis parameters, it is not possible to stabilise this by separating and freezing samples. Lyophilisation is also not currently an option. The requirement for fresh samples also restricts availability of abnormal IQC.

Splitting a sample from a donor between two sites is an option in cases where the sites are geographically close enough for samples to be tested within the required timescale. This can provide an additional form of quality control, but is not possible for some laboratories and so should not be regarded as essential. This approach requires careful monitoring of sample storage conditions prior to testing. Laboratories taking this approach would need to define what 'acceptable' agreement between the sites is and what action is to be taken if this agreement is not achieved. Moreover, similar reagents and aggregometers should be employed to allow comparability, and split sample testing should not be a mandatory component of platelet aggregometry quality control for accreditation.[86]

Approaches have been developed to provide a form of 'abnormal' EQA through the provision of a platelet inhibitor solution to which locally obtained donor blood is added followed by testing,[85] and also to provide post-analytical interpretative challenges in the form of aggregometry traces and electron micrographs.[87] While these approaches may help increase confidence in the quality of results reported by the laboratory, the subjectivity inherent in the assessments impedes standardisation and uniform acceptance.

Recommendations

- **Laboratories should run a fresh sample from a donor expected to give a normal aggregation pattern for each new lot of reagents and whenever an abnormal aggregation pattern is seen in a patient. Each set of investigations should include at least one normal aggregation trace for each agonist as part of IQC (1C)**

Use of genetic or genomic testing

Since the previous version of this guideline there have been major advances in genetic testing methodology. Genetic testing refers to the study of genes and the passage of traits and disorders between generations, whereas genomic testing involves studying all of a person's genes. High-throughput sequencing techniques (HTS, also referred to as next-generation sequencing or NGS) were developed during the human genome project and provide access to much greater amounts of DNA sequence than was possible with polymerase chain reaction (PCR)-based sequencing. Crucially this allows for multiple genes to be analysed in a single assay. HTS has allowed the identification of numerous new genes in which variants cause platelet dysfunction and/or thrombocytopenia.[88-91] These advances mean that it is now possible to easily undertake genetic testing for all heritable disorders of platelet function or number for which the causative gene has been

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identified. This can be useful even in the absence of a family history as will be the case in many recessive disorders. Our understanding of the molecular basis of heritable thrombocytopenias is generally more complete than for non-syndromic platelet function disorders. Consequently the yield, in terms of pathogenic variants identified, is higher in the former. Testing should include all the potential causative genes for HPD as set out in the list which is annually updated by the ISTH.[92]

In many cases the platelet abnormality is only one component of a multisystem syndrome and recognising the potential for clinical manifestations in other organs and systems is a key part of patient management.[3,17] Some of these manifestations are already well known in clinical practice such as the propensity for pulmonary fibrosis and granulomatous colitis in Hermansky–Pudlak syndrome.[93] These clinical features are associated with variants in *HPS1, HPS4* and *AP3B1* but not with variants in the other genes known to cause Hermansky–Pudlak syndrome. Knowledge of the causative variant indicates which patients require monitoring for these manifestations and prevents unnecessary testing in other patients. Other important examples of additional clinical features of genes causing heritable thrombocytopenia include an increased risk of leukaemia with *RUNX1, ETV6, GATA1* and *ANKRD26* mutations,[94] renal impairment, deafness and cataracts associated with *MYH9*[18] and premature atherosclerosis associated with *ABCG5* and ABCG8.[20] Lists of the clinical features associated with specific genes are available in online databases such as www.omim.org and www.genecards.org.

The use of HTS has led to new insights even for those disorders with a well characterised genetic basis. Bernard–Soulier syndrome has been known for many years to be caused by autosomal recessive variants in the *GP9, GP1BA* or *GP1BB* genes but more recently autosomal dominant forms of this condition due to variants in the *GP1BA* and *GP1BB* genes have been described.[95,96]

High-throughput sequencing has greatly improved the efficiency and accessibility of testing for single nucleotide variants. Other types of genetic abnormality such as copy number variation or gross rearrangements are less easy to detect by these methods or may not be detected at all. Clinicians need to be aware of the limitations of different HTS platforms when requesting analysis and the sensitivity for different types of genetic variation. For some diseases this is critical. For example Quebec platelet disorder is caused purely by copy number variation (a duplication in the *PLAU* gene) rather than single nucleotide variants.[97] In genes that are poorly characterised there may be insufficient knowledge to allow classification of a variant as pathogenic or benign. Compared with variants in coagulation factor genes there is a relatively high proportion of variants of uncertain significance. As the analysis technique will often cover multiple genes there is a possibility of incidental findings. Guidance on strategies for dealing with these issues and discussing them in the consent process has recently been published.[6,98,99]

Recommendations

- **Genetic analysis should be offered to all patients suspected of having a HPD (1B)**
- **If a HTS panel is used, it should include all the genes listed by the ISTH as causative of HPDs (1B)**
- **The consent process for genetic testing should discuss the limitations and drawbacks of the methodology used, including the possibility of variants of uncertain significance and incidental findings, and implications for patients and family members (1B)**

Interpretation of results

The decision to diagnose or exclude a HPD requires careful evaluation of all the evidence outlined in the sections above. Discussion in a multidisciplinary setting is recommended prior to confirming or excluding a diagnosis because it is often a multimodal process. This facilitates correlation of reproducible abnormal laboratory assays with expert clinical evaluation of the bleeding history. There are situations where uncertainty will remain, for example an isolated abnormal response to a single weak agonist (ADP or epinephrine) or borderline platelet nucleotide levels. There may be inconsistencies between the presumed effects of genetic variants and the results obtained from functional laboratory assays. Appendix III describes data interpretation in some example cases to highlight these issues and in particular the multiple lines of evidence that may be required to arrive at a diagnosis.

In some pedigrees it may be necessary to evaluate several potentially affected and unaffected members before coming to a conclusion. When investigating relatives of individuals with a mild HPD, those that have not had haemostatic challenges may have little in the way of bleeding symptoms. It may be better to defer testing of children until the clinical phenotype can be assessed with greater confidence or in the event that they require an invasive procedure. It is essential to understand implications for family members when interpreting an individual's results and to counsel the patient accordingly so expectations are appropriately managed. Co-segregation studies may not be feasible due to pedigree size or unavailability of members, in which case comparison with unrelated cases in national and international databases may assist in interpretation. These databases are currently in development and registration of cases with detailed phenotypic data is essential for our further understanding of HPD.

In the absence of established EQA schemes, abnormal results should be discussed within local multidisciplinary teams involving experienced laboratory scientists and

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clinicians, and if uncertainty persists further discussion with external colleagues experienced in platelet function testing may be considered. Recruitment to research studies looking at bleeding disorders (especially investigation of unclassified bleeding disorders) may be offered when there is low confidence regarding whether an HPD is present or not.

Suggested criteria for diagnosis of a HPD

- The index case in a pedigree should have a clinical history of unexpected or excessive bleeding as evaluated (and quantified) by a clinician with expertise in the diagnosis of bleeding disorders
- Reproducible abnormality in one or more of the laboratory assays discussed above
- Other haemostatic defects (e.g. VWD) excluded or bleeding felt to be disproportionate to any co-existing diagnoses

Recommendations

- **Diagnoses should be confirmed in a haemophilia centre with expertise in diagnosing platelet disorders (1B)**
- **All confirmed cases should be registered in the NHD and issued with a bleeding disorders card (1B)**

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Conflicts of interest

The BSH paid the expenses incurred during the writing of this guidance. All authors have made a full declaration of interests to the BSH and Task Force Chairs which may be viewed on request. None of the authors have any relevant conflicts of interest to declare.

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Appendix I. Literature review details

Searches were performed using the online search engine Medline (PubMed). Search terms were: ('Platelet function disorder OR heritable thrombocytopenia') AND; 'diagnosis' AND; 'transmission aggregation'; 'molecular genetic testing'; 'platelet function testing'; 'platelet function analyser'; 'secretion tests'; 'flow cytometry'; 'light transmission aggregometry'; 'lumi-aggregometry'; 'electron microscopy'; 'light transmission aggregation'; 'nucleotide'; 'multiplate'; 'verify now'. Filters were applied to include only publications written in English, studies carried out in humans, clinical trials, clinical studies, comparative studies, evaluation studies, guidelines, meta-analysis, multicentre studies, observational studies, practice guidelines, reviews, validation studies, and which were published between 1 January 2008 and 10 July 2020, inclusive.

Review process

Members of the writing group will inform the writing group Chair if any new evidence becomes available that would alter the strength of the recommendations made in this document or render it obsolete. The document will be reviewed regularly by the relevant Task Force and the literature search will be re-run every three years to search systematically for any new evidence that may have been missed. The document will be archived and removed from the BSH current guidelines website if it becomes obsolete. If new recommendations are made an addendum will be published on the BSH guidelines website (https://b-s-h.org.uk/guidelines/).

Audit tool

See https://b-s-h.org.uk/guidelines/for template

Appendix II. Non-haemostatic phenotypic features of some heritable platelet disorders

Syndromes associated with thrombocytopenia	Phenotypic features
Amegakaryocytic thrombocytopenia with radioulnar synostosis	Abnormal bony or soft tissue connection between the radius and ulna
DIAPH1-associated thrombocytopenia	Sensorineural deafness
MYH9-associated thrombocytopenia	Sensorineural deafness
	Glomerulonephritis
	Presenile cataract
	Neutrophil inclusions (Döhle-like) bodies
RUNX1, *ETV6* and *ANKRD26*-associated thrombocytopenia	Propensity to myeloid malignancy
Sitosterolaemia	Elevated plant sterol levels with premature cardiovascular disease
	Stomatocytosis
	Splenomegaly
SRC-associated thrombocytopenia	Facial abnormalities
	Myelofibrosis
Thrombocytopenia with absent radius (TAR) syndrome	Bilateral absent radii
	Cow's milk intolerance
	Lower limb skeletal defects
	Cardiac anomalies
	Renal tract anomalies
	Cranio-facial abnormalities
	Facial capillary haemangioma
Wiskott–Aldrich syndrome	Eczema
	Immunodeficiency
	Vasculitis
	Lymphoid malignancy
	Autoimmune disorders
Syndromes associated with abnormal platelet function	Phenotypic features
Chédiak–Higashi syndrome	Oculocutaneous albinism
	Immunodeficiency
	Haemophagocytic lymphohistiocytosis

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Appendix II. (*Continued*)

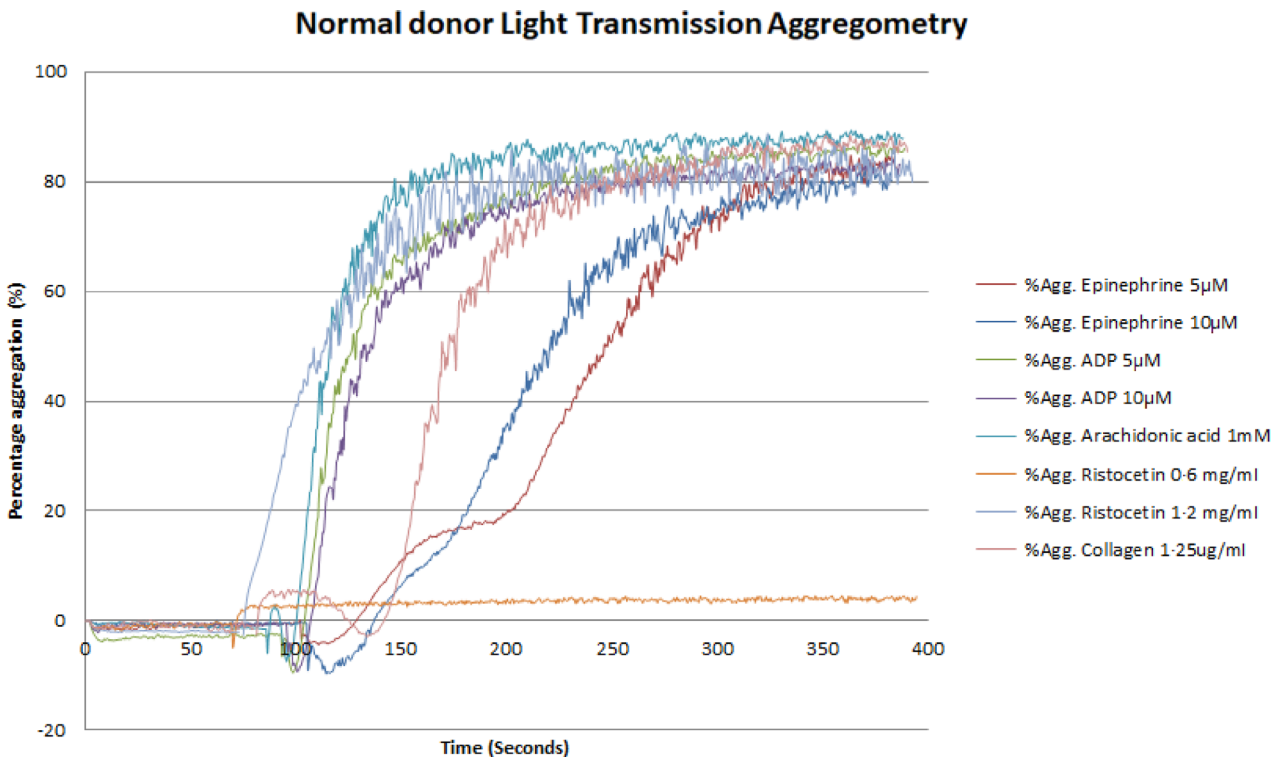
Syndromes associated with thrombocytopenia	Phenotypic features
Grey-platelet syndrome	Myelofibrosis with pancytopenia and splenomegaly
	Autoimmune disease
Hermansky–Pudlak syndrome	Oculocutaneous albinism
	Pulmonary fibrosis
	Granulomatous colitis
	Immunodeficiency
Paris–Trousseau/Jacobsen syndrome	Developmental delay
	Cardiac anomalies
Stormorken syndrome	Cranio-facial abnormalities
	Myopathy
	Miosis
	Asplenia

Appendix III. Case studies highlighting issues that might arise during diagnosis of heritable platelet disorders

Case 1. Normal donor results

Normal control with no bleeding history, not on any medication.

Laboratory results



Platelet aggregometry report: Bi-phasic epinephrine response, absent ristocetin 0·6 mg/ml as expected. Longer lag phase with collagen but within normal limits.

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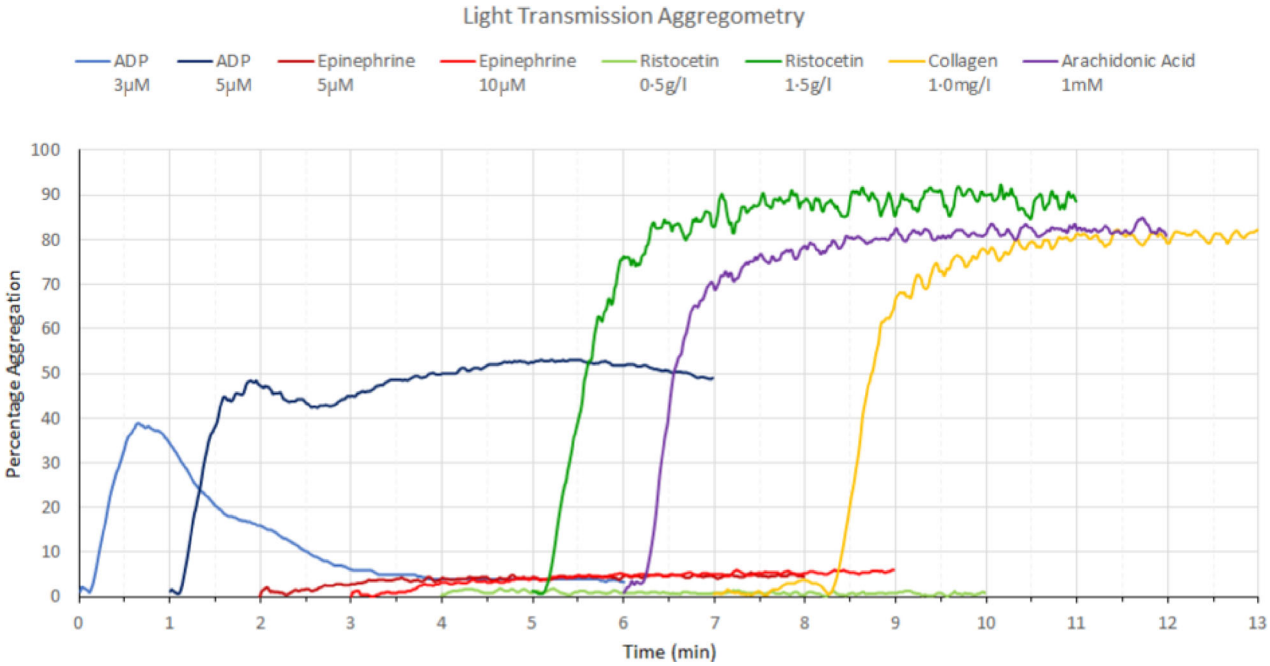
Case 2. Mild abnormalities with some agonists in light transmission aggregometry

Twenty-year-old female, transfusion required for menorrhagia and after delivery. Cauterisation for epistaxis. BAT score = 10.

Mother, older sister and grandmother all required transfusion after delivery and hysterectomy before the age of 40 years.



Laboratory results

Platelet aggregometry report: ADP 3 µM first wave followed by disaggregation, ADP 5 µM first wave only. Epinephrine 5 and 10 µM no response. Normal response with other agonists. Normal platelet nucleotides and ATP release.

Mother and sister have similar results. Whole genome analysis carried out on trio in BRIDGE-BPD study: no potentially pathogenic variants identified.

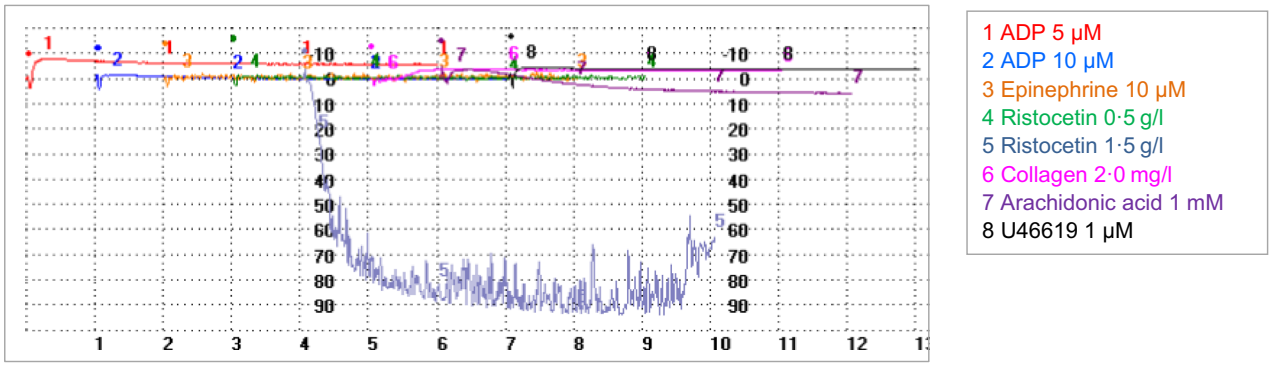
Interpretation

Heritable platelet disorder with molecular basis uncharacterised. Referred to as a 'Weak Agonist Response Defect' in some laboratories.

Case 3. Inconsistent aggregometry and flow cytometry results

Twelve-year-old boy with epistaxis requiring cauterisation on three occasions. Bled heavily after tooth extraction requiring re-suturing. Parents consanguineous. BAT score 8. A female cousin (also a product of a consanguineous marriage) had menorrhagia and died from post-partum haemorrhage.

Laboratory results



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Platelet aggregometry report: no response to any agonist except ristocetin 1·5 g/l. Consistent with Glanzmann thrombasthenia.

Platelet glycoprotein analysis:

CD41 GpIIb/IIIa7 MFC (control 8)

CD42b GpIb9 MFC (control 5)

CD61 GpIIIa5 MFC (control 7)

Comment: Glycoprotein expression is slightly reduced but >70% of the control result and not consistent with Glanzmann thrombasthenia.

Genomic panel analysis: Homozygous for c.2248C>T in *ITGB3* causing p. Arg750Ter in GpIIIa.

Interpretation

The inconsistency in the platelet aggregometry and flow cytometry results is explained by the genomic findings. Although homozygosity for a premature stop codon would normally result in a null allele, in this case the variant truncates the cytoplasmic domain leaving the extracellular and transmembrane regions intact. This preserves the epitopes recognised by the antibodies in the flow assay but interferes with signalling and cytoskeletal structure explaining the aggregometry results. This would be expected to produce a moderate, rather than severe, bleeding phenotype which is what was observed in this pedigree.

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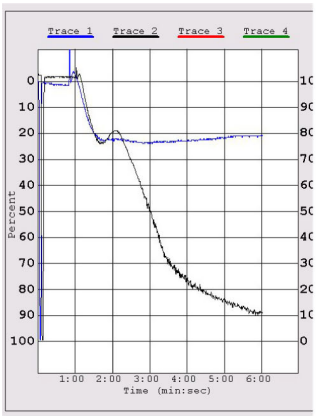
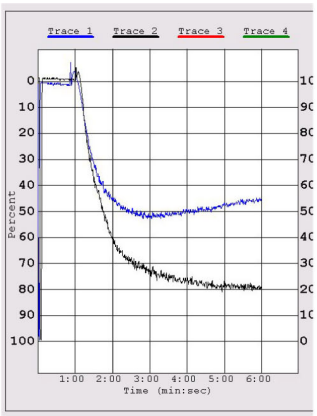
Case 4. Aggregometry indicating a specific molecular defect

Sixty-four-year-old female with previous hysterectomy due to menorrhagia. Numerous instances of post-surgical bleeding throughout lifetime. ISTH BAT score 13.

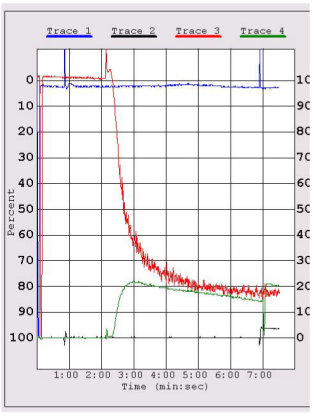
Laboratory results



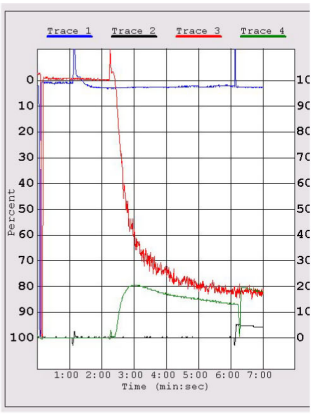
ADP 3 µM
Black – control aggregation
Blue – patient aggregation



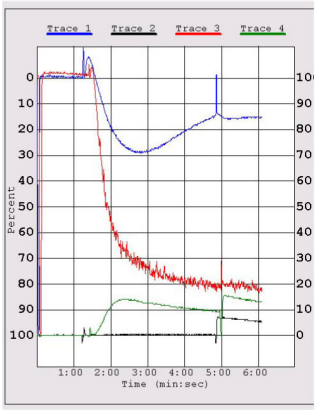
ADP 10 µM
Black – control aggregation
Blue – patient aggregation



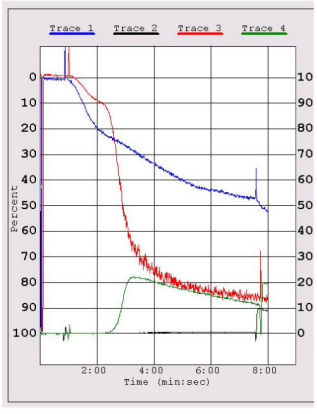
Arachidonic acid 1 mM
Red – control aggregation
Green – control secretion
Blue – patient aggregation
Black – patient secretion



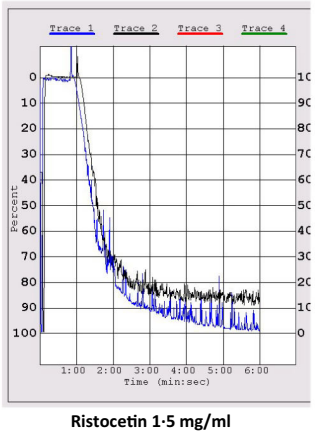
Arachidonic acid 1·5 mM
Red – control aggregation
Green – control secretion
Blue – patient aggregation
Black – patient secretion



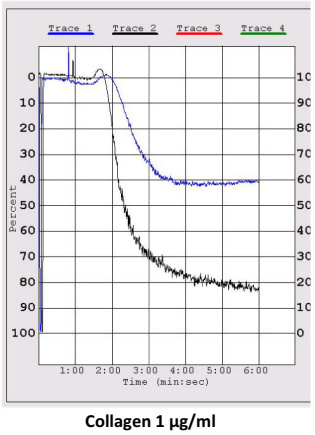
U46619 3 µM
Red – control aggregation
Green – control secretion
Blue – patient aggregation
Black – patient secretion



Epinephrine 10 µM
Red – control aggregation
Green – control secretion
Blue – patient aggregation
Black – patient secretion



Ristocetin 1·5 mg/ml
Black – control agglutination
Blue – patient agglutination



Collagen 1 µg/ml
Black – control aggregation
Blue – patient aggregation

Aggregometry report: ADP and collagen show reduced aggregation. Epinephrine and U46619 show reduced aggregation and ATP secretion. Arachidonic acid shows absent aggregation and secretion.

Genetic analysis: Heterozygous c.125A>G in *TBXA2* resulting in p. Asn42Ser. This is a novel heterozygous thromboxane receptor variant. Asn42 is conserved across all class A G-protein coupled receptors, suggesting a vital role for receptor structure and function. Further studies revealed that the variant reduces surface expression of the receptor (Nisar *et al.*, Thromb Haemost 2014; 111: 923–932).

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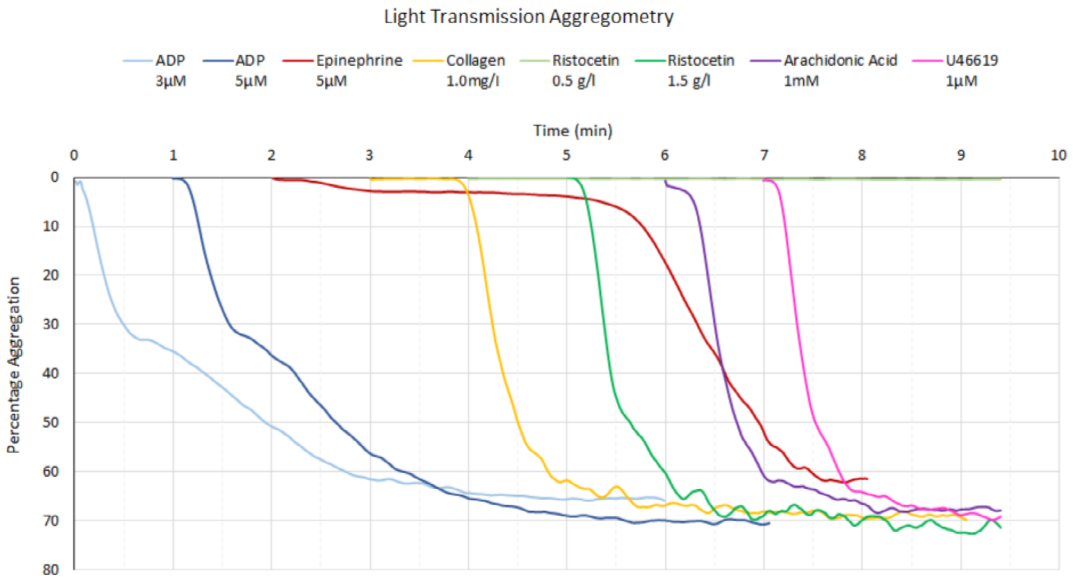
Interpretation

Heritable platelet disorder due to a thromboxane A2 receptor defect.

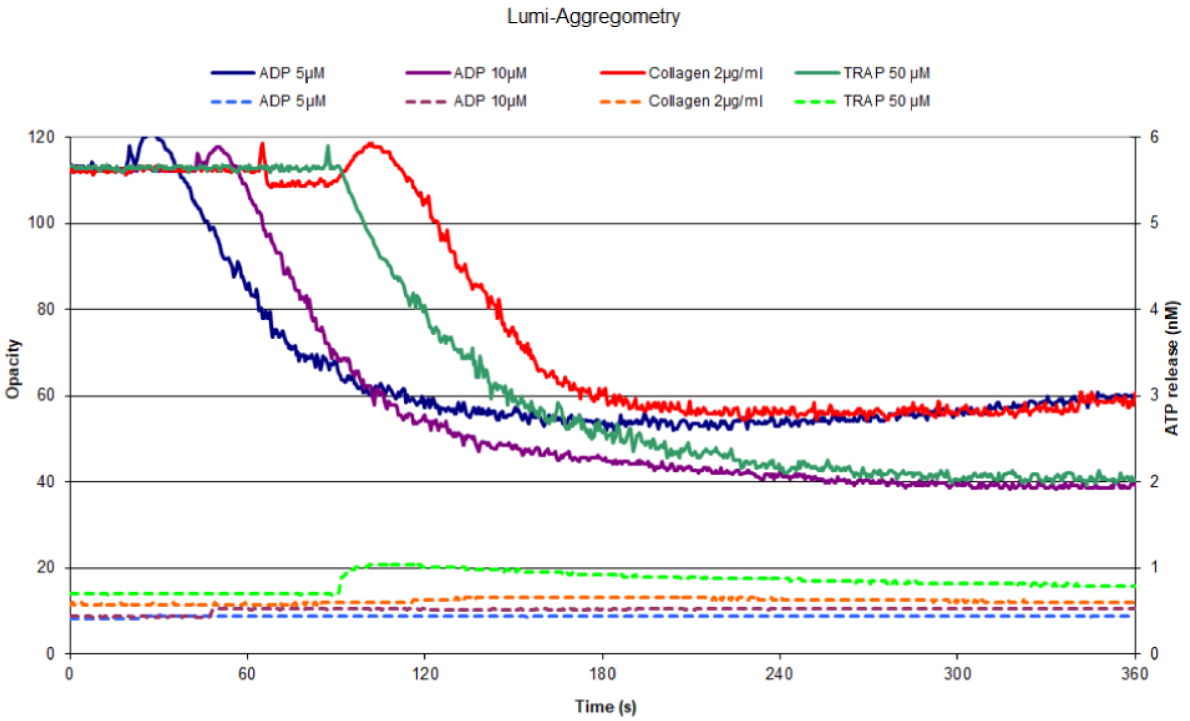
Case 5. Storage pool disorder not detectable by aggregometry

Fifty-year-old female with long history of menorrhagia, easy bruising and excessive bleeding after surgery. No non-haemostatic clinical features. BAT score = 6.

Laboratory results



Light transmission aggregometry report: normal responses with all agonists suggesting no abnormality in platelet function



Lumi-aggregometry report: Normal aggregation responses but absent ATP release

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Lumi-aggregometry report: Normal aggregation responses but absent ATP release.
Platelet ATP0·55 amol/platelet [0·6–1·39]
Platelet ADP0·12 amol/platelet (L)[0·22–0·59]
Platelet ATP: ADP ratio4·45 (H) [0·86–2·26]

Interpretation

Although the aggregometry responses are normal in both light transmission aggregometry and lumi-aggregometry, there is absent ATP release in the latter. This is corroborated by significantly reduced platelet ADP and an increased ATP : ADP ratio indicating a dense granule defect that is not detectable using standard aggregometry.

Diagnosis: Non-syndromic storage pool disorder.

Case 6. Identification of a syndrome underlying a heritable platelet disorder

Seventy-seven-year-old Caucasian woman with lifelong bleeding tendency, reduced visual acuity and nystagmus. Intractable rectal bleeding for which she was being considered for surgery. Bleeding requiring surgical intervention after dental surgery.

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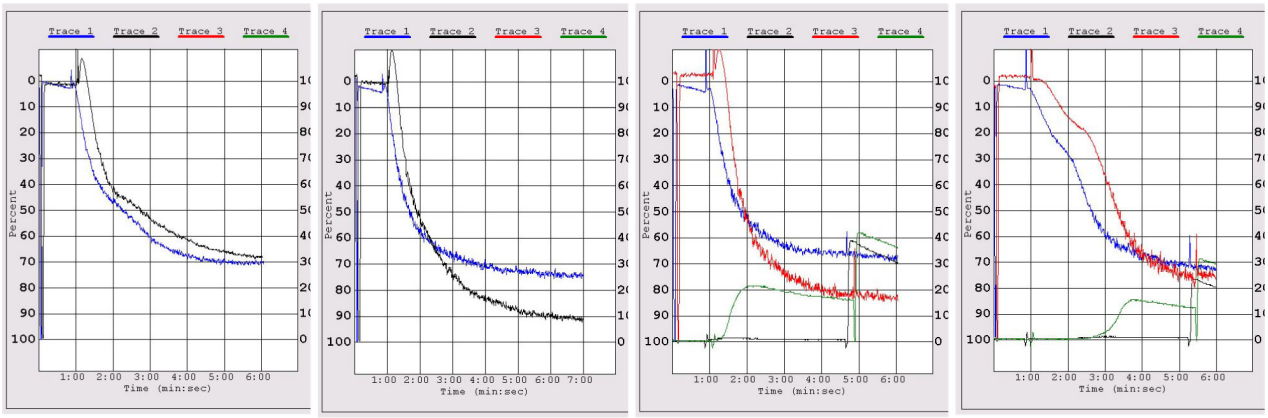
Laboratory results

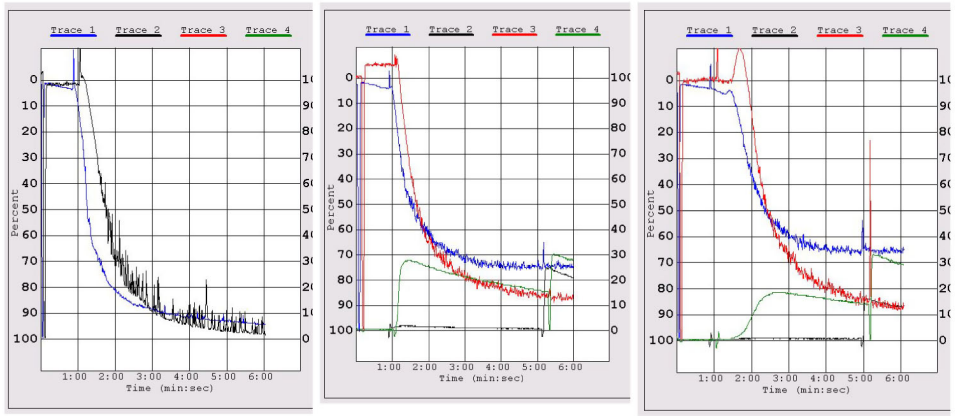


ADP 3 µM
Black – control aggregation
Blue – patient aggregation

ADP 10 µM
Black – control aggregation
Blue – patient aggregation

Arachidonic acid 1·0 mM
Red – control aggregation
Green – control secretion
Blue – patient aggregation
Black – patient secretion

Epinephrine 10 µM
Red – control aggregation
Green – control secretion
Blue – patient aggregation
Black – patient secretion



Ristocetin 1·5 mg/ml
Black – control aggregation
Blue – patient aggregation

PAR-1 peptide 100 µM
Red – control aggregation
Green – control secretion
Blue – patient aggregation
Black – patient secretion

Collagen 3 µg/ml
Red – control aggregation
Green – control secretion
Blue – patient aggregation
Black – patient secretion

Aggregometry report: All agonists show normal aggregation but, where measured, absent secretion.

Genetic analysis: Homozygous c.177G>A in *DTNBP1* resulting in p. Trp59Ter.

Interpretation

Lumi-aggregometry consistent with a dense granule deficiency. As with the previous case, aggregometry traces are similar to those in the control. Non-haemostatic clinical features and confirmatory genetics led to a final diagnosis of Hermansky–Pudlak syndrome type 7.

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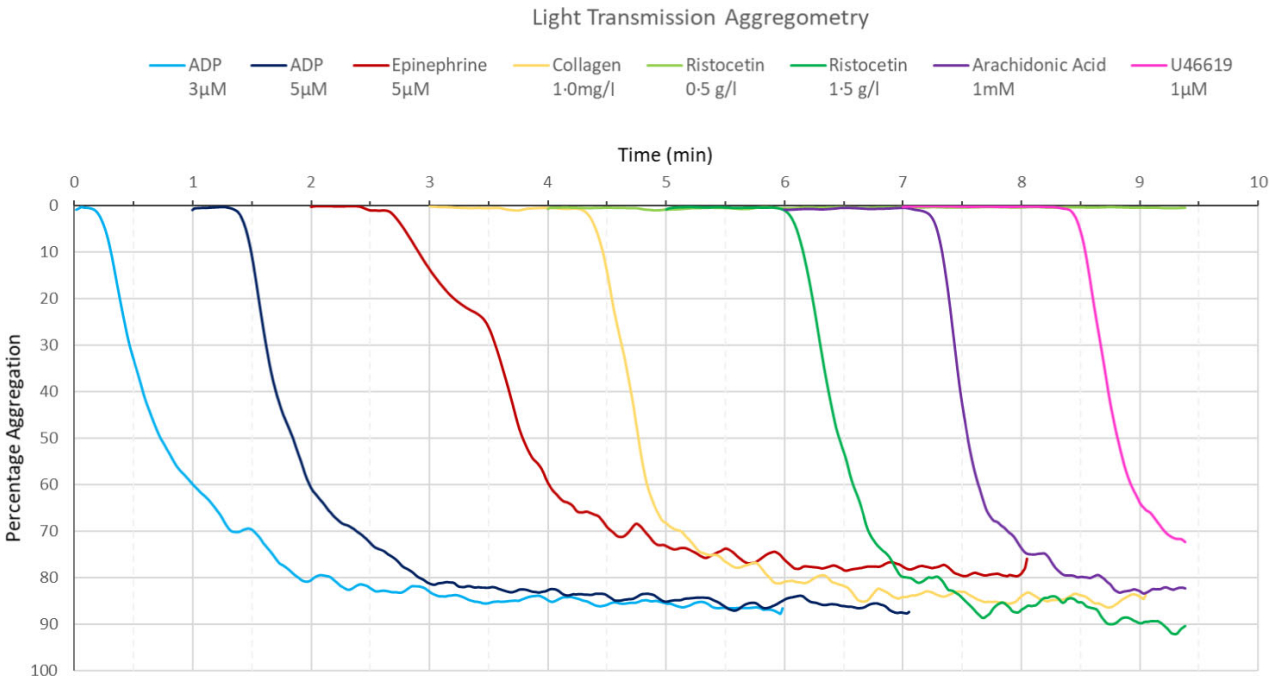
Case 7. Unexpected findings after genomic testing

Thirty-two-year-old male referred with a history of petechial rash, prolonged bleeding after surgery and thrombocytopenia. His father had a diagnosis of von Willebrand disease but further details were not available as the family was estranged.

Laboratory results

VWF:Ag49/dl [45–175]
VWF:RCo20 iu/dl [45–170]
VWF:CB22 iu/dl [47–170]
VWF multimers: Reduced high-molecular weight multimers.
Platelet count 60 × 10^9/l.



Light transmission aggregometry report (PRP platelet count 100×10^9/l): Normal responses to all agonists including ristocetin 0·5 g/l (absent response).

Genomic analysis was carried out on the R90 Bleeding and Platelet disorders panel:

- Heterozygous c.817C>T in *VWF* resulting in p. Arg273Trp.
  Pathogenic. Previously reported in association with type 2A VWD.
- Heterozygous c.958C>T in *RUNX1* resulting in p. Arg320Ter.
  Pathogenic. Previously reported in a case with myelodysplastic syndrome developing aged 50.

Interpretation

The presumptive diagnosis on initial review had been type 2B VWD as this would explain all the clinical features. Subsequently the lack of response to low-dose ristocetin excluded this diagnosis. Genomic analysis indicated that the deficiency in VWF was due to a type 2A defect and that the thrombocytopenia was separately caused by a *RUNX1* variant. Explaining these results to the patient was initially difficult because the consent process had only covered VWD and the patient had not been prepared for an alternative cause for the thrombocytopenia. This raises the importance of discussing the possibility of unexpected outcomes from genomic analysis. Note that the *RUNX1* variant is not an incidental finding in this case as it explains part of the phenotype (thrombocytopenia) under investigation.

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Case 8. Heritable platelet disorder initially treated as immune thrombocytopenic purpura (ITP)

Seventy-year-old female. Chronic thrombocytopenia, first diagnosed after stillbirth 28 years previously with no response to intravenous immunoglobulin or steroids. No history of major bleed, either spontaneous or following trauma/surgery. Bone marrow done many years ago without any bleeding (report not available). Cataract surgery four years ago covered with platelet transfusion, no bleeding. BAT score 0 (bruises are only symptom, all <5 cm). Daughter has thrombocytopenia. Parents and three brothers all died but none had bleeding problems.

Laboratory results

Platelet count 29 $\times$ 10^9/l.
PRP platelet count 21 $\times$ 10^9/l.

Light-transmission aggregometry:

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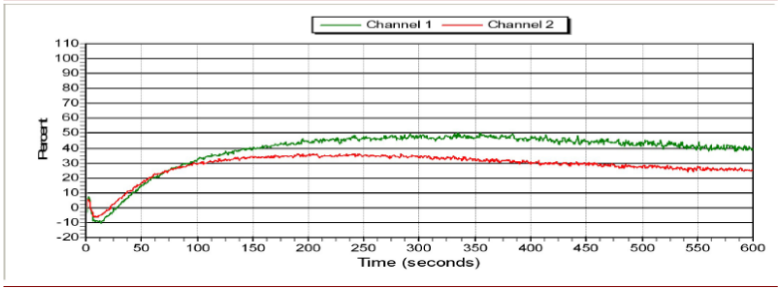
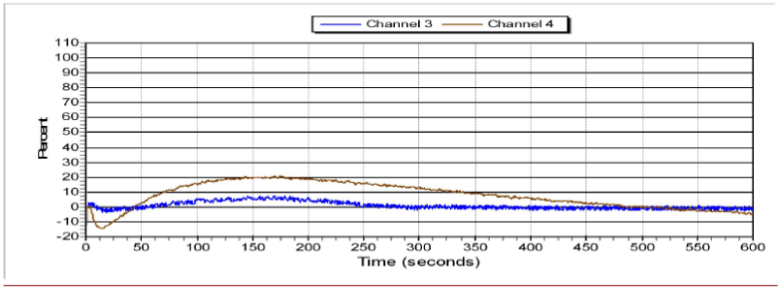
Chnl	Reag	Lot No.	Conc.	Units	PPP	PRP	Max %	TMax
1	Adenosine Diphosphate		5	uM	0·075	0·158	50·1	602
2	Adenosine Diphosphate		2·5	uM	0·065	0·152	36·2	601

Chnl	Slope	Slope2	Lag	Date and Time
1	43·1		13·3	21/08/2019 15:54:42
2	40·1		7·7	21/08/2019 15:54:50



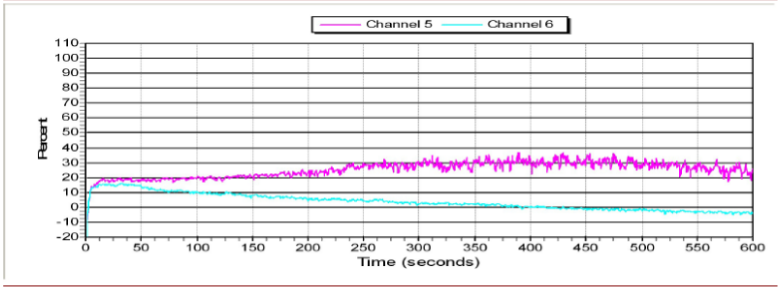
Chnl	Reag	Lot No.	Conc.	Units	PPP	PRP	Max %	TMax
3	Arachidonic Acid		1	mM	0·057	0·159	7·6	600
4	U46619		1	µM	0·070	0·149	20·8	601

Chnl	Slope	Slope2	Lag	Date and Time
3	8·9		19·0	21/08/2019 15:55:01
4	31·5		13·8	21/08/2019 15:55:09



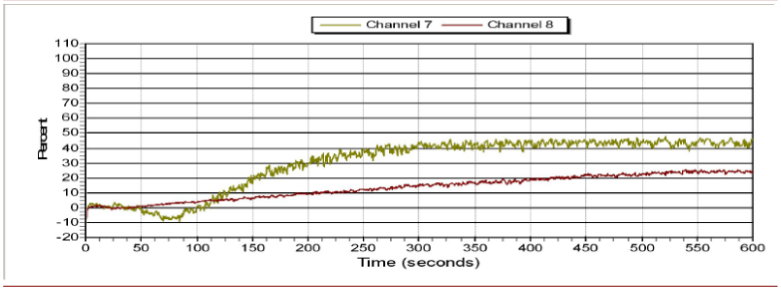
Chnl	Reag	Lot No.	Conc.	Units	PPP	PRP	Max %	TMax
5	Ristocetin		1·5	mg/mL	0·068	0·148	36·8	600
6	Ristocetin		·5	mg/mL	0·072	0·147	16·2	600

Chnl	Slope	Slope2	Lag	Date and Time
5	25·3		0·0	21/08/2019 15:55:16
6	8·9		582·1	21/08/2019 15:55:24



Chnl	Reag	Lot No.	Conc.	Units	PPP	PRP	Max %	TMax
7	Collagen		1·25	ug/mL	0·078	0·154	48·0	601
8	Epinephrine		10	uM	0·071	0·171	25·5	601

Chnl	Slope	Slope2	Lag	Date and Time
7	32·1		84·7	21/08/2019 15:55:32
8	7·9		23·5	21/08/2019 15:55:40



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Aggregometry report: Note low PRP platelet count of 21 × 10^9/l. Reduced responses to all agonists including ristocetin.

Genomic testing using ThromboGenomics Bleeding and Platelet Disorders panel:

Heterozygous c.97T>C in *GP1BA* resulting in p. Cys33Arg. Classified as pathogenic and previously reported in association with autosomal dominant Bernard–Soulier syndrome.

No potentially pathogenic variants identified in any other genes on the panel.

Interpretation

The lack of response to immune treatments and the identification of another family member with thrombocytopenia raised the possibility of a heritable thrombocytopenia. The aggregometry was not particularly helpful because, although there was a reduced response to ristocetin, there were similar reductions with other agonists and this could be explained by the thrombocytopenia. The genomics result indicated that the thrombocytopenia was due to the very rare autosomal dominant form of Bernard–Soulier syndrome.

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Case 9. Severe bleeding in infancy

Two-year-old female. Delivered by caesarean section, no initial neonatal issues. Heel prick was done with no excess bleeding. No problems with cord separation. Bleeding from vaccination sites which lasted 4–5 h. Intermittent epistaxis on and off, usually brought on by crying, that stops after 5–6 min. Admitted to hospital with gum bleeding that only settled after treatment with tranexamic acid and platelet transfusion. Parents consanguineous. Five-year-old brother had no bleeding history.

Laboratory results

Platelet count 240 $\times$ 10^9/l.
Normal morphology.

Light-transmission aggregometry:

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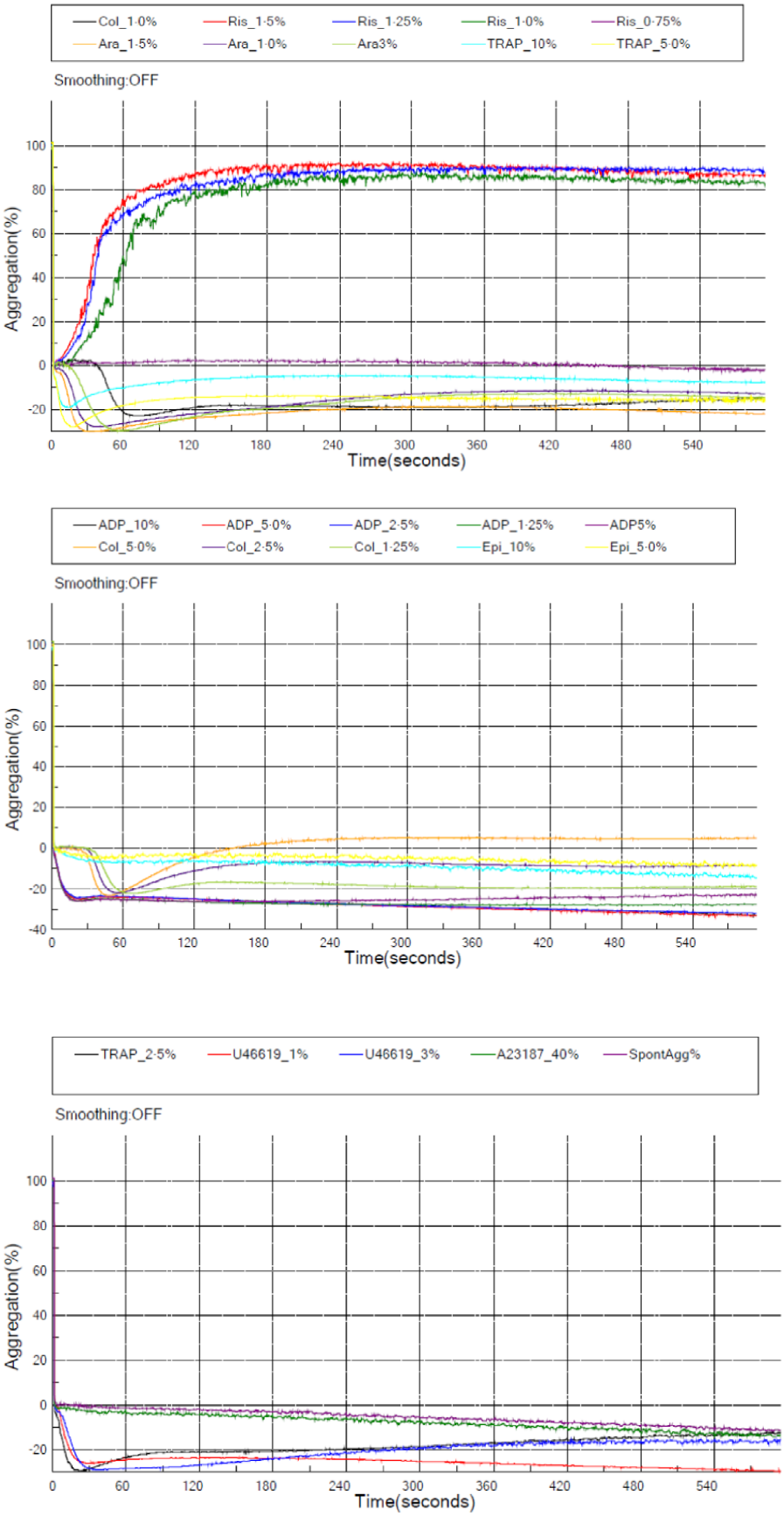

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No response to any agonists except ristocetin to which there is a normal response. Consistent with Glanzmann thrombasthenia.

Platelet glycoprotein analysis:
CD41 GpIIb/IIIa0 MFC (control 20)
CD42b GpIb19 MFC (control 13)
CD61 GpIIIa1MFC (control 14)
Comment: GpIIb/IIIa receptors are almost completely absent consistent with Glanzmann thrombasthenia.

Genomic testing using ThromboGenomics Bleeding and Platelet Disorders panel:
Homozygous c.856G>C in *ITGB3* resulting in p. Gly286Arg. Classified as Likely Pathogenic
No potentially pathogenic variants identified in any other genes on the panel.

Interpretation

A straightforward case of severe Glanzmann thrombasthenia

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