Platelet function testing

Blood platelets contribute to normal haemostasis in two main ways. They adhere to subendothelial microfibrils and collagen in the blood vessel wall, after which they change shape, undergo a specific release reaction, and aggregate with each other to form a primary haemostatic plug. As a consequence of these events, particularly during release and aggregation, procoagulant activities are generated, chiefly involving the platelet membrane phospholipids (platelet factor 3) so that blood coagulation is initiated at, and is largely localised to, the areas of platelet aggregation.

Quantitative or qualitative platelet disorders may result in a pronounced bleeding tendency, mainly due to failure of platelet plug formation, but also to a lesser extent to the suboptimal activation of blood coagulation. This Broadsheet sets out to describe the methods which can be used reliably to detect and classify such bleeding disorders.

Indications for platelet function tests

Defective platelet function or thrombocytopenia may present clinically with a variety of symptoms indicative of primary haemostatic failure, such as bruises or ecchymoses, epistaxis, gastrointestinal haemorrhage or menorrhagia. Platelet defects usually give rise to a fairly mild bleeding disorder, and patients may present with excessive bleeding only after surgery or dental extractions. Some, however, may have severe haemorrhagic symptoms early in life. In patients with mild symptoms it is usually impossible to be certain from the history alone whether the patient has a platelet or a coagulation defect. The initial investigation should include the following screening tests: blood film, platelet count, bleeding time, prothrombin time, activated partial thromboplastin time and assays for fibrinogen, factor VIII:C, von Willebrand factor antigen (vWF:Ag) and ristocetin cofactor activity. Only when a coagulation abnormality and von Willebrand’s disease have been excluded should platelet function be studied further. A suggested scheme for the investigation of platelet function is shown in the figure.

Because many commonly used drugs also affect platelet function, these should also be excluded, or, if this is impossible for clinical reasons, their influence should be taken into account when interpreting the results.

BLOOD FILM

Inspection of a well spread, well stained film is often most helpful and will allow an estimate of the platelet count and will show any gross morphological abnormalities in the platelets themselves. The presence of large platelets may be indicative of a congenital thrombopathy, such as Bernard-Soulier syndrome. The blood film also enables abnormalities in other cell lines to be observed, such as Döhle bodies in the granulocytes in the May-Hegglin anomaly. Perhaps the most important reason to inspect the blood film is to exclude another underlying haematological disorder—leukaemia, for example, in which platelet function may be impaired. It also allows other medical conditions, which may result in an acquired bleeding disorder, to be detected—liver, renal, or myeloproliferative diseases.

THE BLEEDING TIME

Of the many methods described for performing this useful in vivo test, the most sensitive, reproducible, and clinically valid is the template technique in which the bleeding time of one or more small cuts is determined under highly standardised conditions.

Technique

(a) Taking care to avoid superficial veins, scars, or bruises, a small area of skin on the lateral aspect of the volar surface of the forearm about two inches below the elbow joint, is cleansed with alcohol (and, if necessary, shaved), and allowed to air dry.

(b) A sphygmomanometer cuff is placed around the upper arm and kept inflated to a pressure of 40 mm Hg throughout the test, to standardise the intra-capillary pressure.

(c) A template device, preferably a semiautomatic, spring-loaded blade device such as the Simplate II (General Diagnostics), is placed firmly, but without excessive pressure, on the skin in the longitudinal
plane and the cut(s) made 5 mm long and 1 mm deep. After one second the device is removed and a stop-watch started.

(d) All blood emerging from the wounds is blotted off at 30 second intervals using Whatman No 1 filter paper. The wounds should not be touched so as to avoid dislodging any platelet aggregates forming within them.

(e) The time at which each wound stops bleeding is noted and individually reported.

(f) After completing the test the cuff is removed and dried blood around the wounds is cleaned away. The edges of the wound are drawn together and firmly held with sterile butterfly plasters to minimise scar formation.

**Interpretation**

The normal template bleeding time is up to 10 minutes, although in most healthy subjects it falls within the range two to eight minutes. A prolonged bleeding time in a person with a normal platelet count is most frequently due to either von Willebrand's disease or a functional platelet defect, although many other causes exist (table 1). A normal bleeding time does not exclude either of these two diagnostic possibilities.

*The bleeding time in thrombocytopenia:* When the whole blood platelet count is in the range 20–100 x 10⁹/l, the relation between it and the template bleeding time can be expressed by the Harker-Slichter equation, in which an “expected bleeding time” for a
Table 1  Causes of a prolonged bleeding time

<table>
<thead>
<tr>
<th>Category</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular defects</td>
<td>Ehlers-Danlos syndrome</td>
</tr>
<tr>
<td></td>
<td>Osteogenesis imperfecta</td>
</tr>
<tr>
<td>Coagulation factors</td>
<td>Afibrinogenaemia</td>
</tr>
<tr>
<td></td>
<td>Severe factor V deficiency</td>
</tr>
<tr>
<td>Von Willebrand's disease</td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td></td>
</tr>
<tr>
<td>Congenital thrombocytopeny</td>
<td>(table 3)</td>
</tr>
<tr>
<td>Acquired thrombocytopeny</td>
<td>Myeloproliferative diseases</td>
</tr>
<tr>
<td></td>
<td>Liver diseases</td>
</tr>
<tr>
<td></td>
<td>Uraemia</td>
</tr>
<tr>
<td></td>
<td>Some autoimmune diseases</td>
</tr>
<tr>
<td></td>
<td>Some drugs (table 2)</td>
</tr>
<tr>
<td></td>
<td>Dietary factors</td>
</tr>
</tbody>
</table>

Given platelet count can be calculated using the following:

Expected bleeding time = 30 - \( \frac{\text{Platelet count} \times (10^9/l)}{4} \) (in minutes)

This equation has been used as an alternative to platelet aggregometry where the latter is precluded in severe thrombocytopenia (see below). Because of the inherent variability of both the bleeding time and the blood platelet count, the results obtained using this equation should be interpreted with caution. If the actual bleeding time exceeds the expected bleeding time by more than about five minutes, however, a functional platelet defect is indicated.

Platelet aggregation

PRINCIPLE
Many methods for monitoring platelet aggregation have been described. In selecting a technique for routine use, the important considerations are its speed, simplicity, reproducibility and, above all, its ability to identify and classify those platelet defects which have clinical importance. The method recommended is based on the nephelometric principle originally developed by Born\(^1\) wherein the changes in the optical density of platelet-rich plasma was found to reflect the degree of platelet aggregation induced by one of a number of agonists.

PRECAUTIONS PRIOR TO STUDYING PLATELET AGGREGATION
Unless their effect on platelet aggregation is being specifically investigated, all drugs known to influence platelet function should be excluded for several days before testing, or for at least the time required for their elimination from the body. In particular, at least 10 days should have elapsed since the patient last ingested aspirin, as this compound permanently acetylates cyclooxygenase, one of the enzymes involved in thromboxane A\(_2\) formation in platelets. Most other drugs have a short term and less predictable effect, only influencing platelet function while they, or their metabolites, remain in the circulation. Some drugs which are known to affect platelet function are listed in table 2. This list is by no means comprehensive and, if any doubt exists about a particular compound, the company manufacturing it should be consulted.

Many normal dietary constituents such as alcohol, onions, garlic, peppers and ginger may also inhibit platelet function if ingested to excess and should be borne in mind when interpreting results of platelet aggregation studies. Because chylomicrons can interfere with the nephelometric evaluation of platelet function, studies should not be carried out shortly after a fatty meal. It is neither necessary nor desirable that the patient should be fasted overnight.

COLLECTION AND PROCESSING OF THE BLOOD SAMPLE
Platelets adhere to foreign surfaces, and it is important to minimise this process by using siliconised glass or plastic ware at all stages. Because of the vital role of calcium ions in platelet responses, the anticoagulant used must be standardised and for routine purposes; one volume of 0.106M tri-sodium citrate dihydrate to nine volumes of blood is recommended. Some workers prefer to add 1% dextrose to the anticoagulant, but this may cause confusion if it necessitates having a different collection tube from that used for routine blood coagulation tests. For critical studies, the ratio of citrate to blood should also be adjusted to take

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### Table 2  Drugs which can interfere with platelet function

<table>
<thead>
<tr>
<th>Category</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Anti-inflammatory drugs:</td>
<td>Aspirin</td>
</tr>
<tr>
<td></td>
<td>Non-steroidal anti-inflammatory drugs</td>
</tr>
<tr>
<td>2 Membrane-active drugs:</td>
<td>Local anaesthetics</td>
</tr>
<tr>
<td></td>
<td>Tri-cyclic antidepressants</td>
</tr>
<tr>
<td></td>
<td>( \beta ) blockers</td>
</tr>
<tr>
<td></td>
<td>Anti-histamines</td>
</tr>
<tr>
<td>3 Antibiotics:</td>
<td>Penicillin</td>
</tr>
<tr>
<td></td>
<td>Cephalosporins</td>
</tr>
<tr>
<td>4 Miscellaneous:</td>
<td>Heparin</td>
</tr>
<tr>
<td></td>
<td>Dextran</td>
</tr>
<tr>
<td></td>
<td>Dipyridamole</td>
</tr>
<tr>
<td></td>
<td>Aminophylline</td>
</tr>
<tr>
<td></td>
<td>Phenothiazines</td>
</tr>
<tr>
<td></td>
<td>Alcohol</td>
</tr>
</tbody>
</table>
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account of the haematocrit. This can be done by using the formula below, assuming a normal haematocrit of 40%.

Amount of citrate required for a 5 ml blood sample = \(0.5 \times \frac{(100 - \text{haematocrit})}{60}\)

Chilling activates platelets and so the citrated blood should be processed at 20–25°C. Because the function of the platelets deteriorates progressively in stored blood, it is advisable to start processing immediately after blood collection. Platelet rich plasma (PRP) is prepared by centrifugation for 10–15 minutes at 200 × g. The PRP is carefully removed, avoiding contamination with erythrocytes or the buffy coat, and stored at 20–37°C until tested. Although the precise temperature of storage is not critical, it does influence the results somewhat and so should be adhered to for all such samples.

Platelet aggregation depends on pH, and so the PRP should be maintained within the pH range 7.7–8.0. Satisfactory control of pH can be achieved by storing the PRP in full, tightly stoppered tubes and by completing the tests within two hours of blood collection. Buffers such as N-2-hydroxethylpiperazine-N'-2-ethanesulphonic acid (HEPES) can themselves influence platelet aggregation and are not recommended.

Immediately after centrifugation, platelets are refractory to aggregating agents and so the PRP should be allowed to “recover” for 30 minutes before commencing testing. After removal of the PRP, the remaining blood is centrifuged again for 20 minutes at 2000 × g to obtain platelet poor plasma, which is then used to blank the aggregometer and to dilute the PRP if necessary (see below).

PLATELET COUNT

A platelet count should be carried out on the PRP after which many workers adjust the count to some arbitrary “standardised” level. The rate of aggregation of the PRP within the cuvette is, to some extent, dependent on the platelet count. Over the range 200–400 × 10⁹/l such an effect is small but it is usual, particularly when studying hypoaggregable platelets, to standardise the count to about 300 × 10⁹/l with autologous platelet poor plasma (prepared as described above). Occasionally for research studies and particularly when testing patients with thrombocytopenia, it may be more appropriate to test the patient’s PRP without dilution instead of or in addition to testing at 300 × 10⁹/l. At PRP platelet counts below 200 × 10⁹/l there is a significant, progressive decrease of in vitro platelet aggregatory responses, due partly to biophysical factors such as the lower frequency of platelet-platelet collisions, and partly to the sensitivity limits of the aggregometer. It is recommended that

under these circumstances, the aggregation patterns of the patient are compared with those of healthy controls whose PRP has been adjusted to a similar count. Attempts to concentrate platelets by centrifugation followed by resuspension in a smaller plasma volume, virtually always induce a functional change in the platelets (usually refractoriness) and it is not recommended. Where the whole blood platelet count falls between 10 and 100 × 10⁹/l, the Harker-Slichter formula relating bleeding time to platelet count (see above) may give some indication of functional integrity.

PLATELET AGGREGOMETERS

A wide range of platelet aggregometers is available and the choice is largely a personal one. For a routine laboratory, a two channel aggregometer with pre-set temperature and stirring speed may be satisfactory; for research applications it may be desirable to purchase a machine on which these parameters can be varied.

Additional features are also available, such as the ability to measure adenosine triphosphate release or changes in ionised calcium concentrations simultaneously with platelet aggregation. Other machines which allow aggregation to be studied in whole blood rather than on PRP are also obtainable (see below). For diagnostic purposes, it is highly desirable that the aggregometer chosen should be able to distinguish clearly between the primary and secondary waves of platelet aggregation (see below).

Platelet responses are dependent on temperature and routinely, 37°C is selected for aggregation studies. Similarly, because stirring speed in the aggregometer influences the aggregation pattern, this should be set at 800–1000 rpm, which produces satisfactory results.

Another variable is the chart speed of the pen recorder. Although this is not so critical, a speed of 1 cm/minute is convenient because it allows a succinct record of the results to be copied and entered into the patient’s notes if required.

The final variable is the sample size accepted by the aggregometer. Most machines operate with a final volume of 0.5 ml in the cuvette, but with some (Payton aggregometers), a range of cuvettes are available allowing sample volumes of 0.1–1.0 ml of PRP to be used. It is important to remember that the aggregation patterns will vary somewhat with the sample volumes and so a normal range must be established for each size of cuvette used.

AGGREGATING AGENTS

The five aggregating agents listed below should be sufficient to discriminate between the various functional platelet defects. For research purposes, several other agonists can also be used including thrombin, serotonin, vasopressin, thromboxane A₂ (TXA₂)
anallogues, platelet activating factor (PAF) and the calcium ionophore A23187. Most of these are now commercially available.

1. **Adenosine 5-diphosphate (ADP):** A stock solution of 1 mmol/l of the di-sodium salt is prepared in 20% Owrén's barbitol buffer in isotonic saline, pH 7.35 (OBS), and stored in small aliquots at below −20°C, whence it is stable for at least three months. Once thawed, the solution should be used within three hours or discarded. Further dilutions as required are made in OBS.

The pattern of response to ADP depends on its final concentration. At 2 μmol/l, clearly defined primary and secondary waves of aggregation can usually be distinguished, the first representing the direct agonist-induced effect and the latter being due to the release of endogenous ADP and to a lesser extent of TXA₂, which itself induces platelet aggregation. Below 2 μmol/l, progressively fewer normal subjects show a secondary response and the primary wave usually reverses as the added ADP is enzymatically degraded. Above 3 μmol/l, the primary wave may be so intense that the distinction between it and the secondary phase is masked. ADP induces a change in the shape of platelets from a bi-convex disc to a spiky sphere. This change initially causes a slight increase in the optical density (OD) of the PRP which can usually only be distinguished clearly if primary aggregation is impaired.

2. **Adrenaline:** A stock solution of 1 mmol/l of the bitartrate salt is made in OBS. It should be stored and used exactly as prescribed for ADP. With adrenaline, the concentrations used and the aggregation patterns obtained are very similar to those seen with ADP. The primary wave, however, is not preceded by a shape change, does not reverse in the absence of a secondary wave, nor is it ever so intense that the secondary wave is masked.

3. **Collagen:** Several suitable collagen suspensions are commercially available, and for purposes of standardisation, are preferable to home-made preparations. The potency of different collagen preparations varies considerably and so it is advisable to keep to one supplier and to establish a normal range for the material used. A very potent, stable suspension of equine tendon collagen fibrils (1 mg/ml) is available from Hormon-Chemie, Munich, West Germany and is widely used. It is stored at 4°C and must be well mixed immediately before dilution in the buffer packaged with it. It should be used at a final concentration of 0.5−2.0 μg/ml and diluted suspensions are stable at 4°C for one week.

With collagen, no primary wave of aggregation occurs. The response is usually defined by the duration of the lag phase before aggregation begins and by the extent of the latter. As collagen induces release of endogenous platelet ADP, a slight increase in OD, due to the platelet shape change, usually precedes aggregation.

4. **Ristocetin:** Several sources of ristocetin (such as Lundbeck Ltd) are now available. The dry powder should be stored at 4°C until dissolved in OBS (5–15 mg/ml). Solutions should be stored frozen at below −20°C where they are stable for many months. At a final ristocetin concentration of 1.0–1.25 mg/ml in PRP, distinct primary and secondary waves of aggregation are usually discernible, but above this concentration the direct effect is often so intense that the two phases merge. The primary phase reflects the interaction between platelet membrane glycoprotein 1b and von Willebrand factor. Its intensity can be taken as a measure of the amount of von Willebrand factor in the plasma, while the second phase reflects the release of endogenous substances—for example, ADP from the platelets. It is becoming common practice also to test PRP with a lower ristocetin concentration (0.3–0.5 mg/ml) as this is reported to identify the type IIb variant of von Willebrand's disease. At this lower concentration, platelets from other vWD variants or normal subjects do not respond.

5. **Arachidonic acid:** Sodium arachidonate (99% purity) is dissolved in OBS to a concentration of 10 mmol/l. Small aliquots are placed in darkened glass phials which are then flushed with nitrogen to prevent oxidation, then tightly stoppered, and stored below −20°C. When added to PRP at final concentrations of 0.5–1.5 mmol/l, a large, monophasic wave of aggregation usually occurs, sometimes preceded by a short (<10 seconds) lag phase. Higher concentrations may cause some platelet lysis and should be avoided.

**Aggregation procedure**

The precise details for carrying out platelet aggregation studies will depend to a large extent on the machine used. Most will operate on a sample volume of 0.5 ml, in which case the protocol given below will give satisfactory results. It is important to use the correct size of cuvettes and stir bars as indicated in the manual supplied with the machine. It is preferable to use siliconised glass or plastic cuvettes and stir bars so as to minimise platelet activation by the foreign surfaces.

1. PRP (450 μl) is placed in a siliconised glass or clear plastic cuvette and a siliconised stir bar added. Any air bubbles present are dispersed by gentle tapping and the cuvette is placed in the aggregometer well.

2. With two-channel aggregometers it is convenient to use half of the width of the chart paper for each channel. With a 10 millivolt full scale deflection, the PRP settings used are 0.5 and 5.5 millivolts for channels one and two, respectively, and the corresponding blank values (which are set using platelet poor plasma) would be 4.5 and 9.5 millivolts.

For critical work it is advisable to compare the
patient and control responses to a given agonist using the same channel, as the sensitivity settings and hence the aggregation characteristics of each channel will be slightly different. For most purposes, however, this is unimportant.

3 Once the aggregometer is set up, the PRP cuvette is incubated in the well for about two minutes to warm to 37°C. Some machines have pre-warming ports which allows this stage to be bypassed.

4 Aggregating agent (50 μl) in an appropriate concentration is then delivered to the bottom of the cuvette using a semiautomatic pipette. This ensures adequate mixing and the transient interruption to the light path provides a useful marker of the starting point. Spring-loaded syringes, which inject the agonist under high pressure, are unsuitable as they introduce microbubbles which affect light transmission for some time, and may cause disruption of some platelets.

5 The change in light transmission of the PRP is monitored continuously for three to five minutes. This process is repeated with each agonist using appropriate concentrations. The final agonist concentration will, of course, be one tenth that of the strength added to the PRP.

**INTERPRETATION OF RESULTS**
When attempting to interpret platelet aggregation tracings it is generally more instructive to examine response to several concentrations of each agonist rather than to calculate a numerical value for the rate or extent of aggregation. Each agonist produces characteristic features (see above under aggregating agents) and the important point to determine is whether aggregation has occurred and, if so, can both primary and secondary waves be elicited? Using these criteria, several types of inherited platelet defect can be delineated (table 3). The most appropriate additional investigations to confirm the diagnosis may then be selected. The results with acquired platelet defects can sometimes be classified using similar criteria, but in many cases are more difficult to interpret.

If, for convenience of reporting results, a numerical value for aggregation is required, then some reproducible aspect of the curve can be measured, such as the percentage fall in OD between settings for PRP and platelet poor plasma three minutes after addition of a fixed dose of the agonist, and the patient’s result reported along with the normal range. Recommended final concentrations of different agonists for this purpose are ADP 2 μmol/l, adrenaline 2 μmol/l, collagen 1 μg/ml, ristocetin 1-25 mg/ml and arachidonic acid 1 mmol/l (table 3).

**Common sources of error**

No response on addition of aggregating agent Stir bar omitted or stuck in cuvette, stirrer motor not switched on.

Rapid large oscillations of pen before onset of aggregation Air bubbles or other foreign body in cuvette. A low platelet count will also cause this effect due to the high sensitivity setting required. The intense agglutination caused by ristocetin is also accompanied by large excursions of the pen.

Fall in optical density before addition of the agonist Spontaneous platelet aggregation which may be due to hyperaggregability or, more commonly, to a dirty cuvette or stir bar. A small fall in OD often occurs in lipaemic samples as some lipids dissolve on warming to 37°C.

Trace has normal biphasic shape but extent of fall in OD is reduced PRP is contaminated with red cells, white cells, or chylomicrons which contribute to the OD but not to aggregation. It may be difficult to eliminate microcytic red cells by differential centrifugation without concomitant loss of some larger platelets.

Fall in OD occurs on addition of agonist without oscillations of pen Agonist has caused platelet lysis so that the PRP becomes clear. This only usually occurs with high concentrations of arachidonic acid, TXA₂ analogues or A23187.

**Whole blood aggregometry**
As mentioned earlier, aggregometers which measure platelet responses in whole blood, using the principle of electrical impedance, are now available. To the extent that this permits aggregation to be monitored in the presence of red cells and leucocytes, these machines may be considered to be more physiological than those based on the nephelometric technique. Compared with the latter, however, the aggregation patterns in whole blood are rather less consistent and less readily distinguishable in defects of platelet release, such as

<table>
<thead>
<tr>
<th>Defect</th>
<th>ADP (2 μmol/l)</th>
<th>Adrenaline (2 μmol/l)</th>
<th>Collagen (1 μg/ml)</th>
<th>Arachidonate (1 mmol/l)</th>
<th>Ristocetin (1-25 mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bernard-Soulier syndrome</td>
<td>Biphasic</td>
<td>Biphasic</td>
<td>Normal</td>
<td>Normal</td>
<td>Absent</td>
</tr>
<tr>
<td>Thrombasthenia</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>First phase</td>
</tr>
<tr>
<td>Storage pool deficiency</td>
<td>First phase</td>
<td>First phase</td>
<td>Reduced</td>
<td>Normal or reduced</td>
<td>First phase</td>
</tr>
<tr>
<td>Defective release mechanism</td>
<td>First phase</td>
<td>First phase</td>
<td>Reduced</td>
<td>Reduced</td>
<td>First phase</td>
</tr>
<tr>
<td>Gray platelet syndrome</td>
<td>Usually normal</td>
<td>Usually normal</td>
<td>Usually normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>
storage pool disease. For this reason, measurement of platelet aggregability in PRP is preferred for routine purposes, whereas whole blood aggregometry remains mainly a research tool.

**FURTHER INVESTIGATION OF PLATELET FUNCTION**

The methods described above will assist in the detection of a functional platelet defect, although before a definitive diagnosis can be made, more specialised investigations may be needed. These include the determination of intraplatelet concentration of platelet adenine nucleotides (ATP and ADP) and intraplatelet or plasma concentration of platelet specific peptides such as β thromboglobulin and platelet factor 4, thromboxane A2, assays, calcium flux measurements and phospholipid turnover studies. Measurement of membrane glycoproteins is necessary for the unequivocal diagnosis of Bernard-Soulier syndrome and Glanzmann’s thrombasthenia. These specialised investigations are available at specialist or reference laboratories. The glass bead adhesion test, once quite popular for the detection of von Willebrand’s disease, platelet function disorders, and hypercoagulable states, has been superseded by platelet aggreometry and is no longer widely used. Similarly, the Prothrombin Consumption Index, formally used widely to detect both platelet and blood coagulating disorders, has fallen into disuse in most laboratories. It is, however, a sensitive marker of a defect in platelet procoagulant activity and is sometimes used for this purpose.

If an abnormal platelet aggregation pattern is observed, it is advisable to repeat the assessment on at least one further occasion to check for the consistency of the abnormality. A recent report suggested that a proportion of patients with storage pool deficiency showed a normal platelet aggregation pattern. If confirmed, this observation suggests that the assay of platelet adenine nucleotides should be carried out on all patients tested for platelet function. This may necessitate referral to a specialised centre where such tests are routinely carried out.

**Platelet sizing**

The growing literature on this subject suggests that under certain circumstances platelet sizing may be a valuable diagnostic discriminant of underlying pathological processes. Only time will tell if the initial enthusiasm is justified but, in any case, the blood or plasma concentration of various substances contained in or released by the platelets such as adenosine nucleotides, β thromboglobulin, and 5-hydroxytryptamine will depend to some extent on platelet volume as well as on platelet number and metabolic capacity. The potential to measure platelet volume accurately is a feature of many of the newer blood cell counters used in routine haematology laboratories. The principles involved and the detailed methodology used are beyond the scope of this Broadsheet and those interested should contact the manufacturers of these machines directly. Cell sizing is currently being assessed as a means of monitoring loss of single platelets induced by aggregating agents. Some interesting results are being produced, but at present, the value of this approach for diagnosis of platelet function defects is uncertain.

The technique of platelet sizing has a number of limitations:

(i) Platelet size is not constant in shed blood. When stored in vitro in anticoagulant, platelets swell progressively and this must either be prevented by fixation or the tests undertaken at a set time after blood collection.

(ii) Various physiological activities such as exercise and other adrenergic stress can affect both platelet numbers and platelet size, and this must be taken into account in evaluating results.

(iii) Racial variation in platelet count and volume have been described, the mean count being lower and the mean volume higher in Mediterranean races compared with Northern Europeans.

Results obtained of platelet volume in various pathological states must therefore be interpreted in the light of these physiological and technical influences.

**References**


**General reviews**


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