

## Diagnosis and Management of Inherited Platelet Disorders

a report by

**Paquita Nurden and Alan Nurden**

*French National Reference Centre for Inherited Platelet Diseases*

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### Inherited Platelet Disorders

Inherited platelet disorders constitute a group of rare diseases giving rise to bleeding syndromes of varying severity. The defects concern the number or the function of platelets or, in some cases, both.<sup>1–3</sup> Platelets are essential for haemostasis as they adhere to the site of the vascular lesion and, due to their capacity to aggregate, form a mass able to stop bleeding. This process, particularly important in small vessels, is finely controlled to avoid obstruction of the circulating blood.<sup>4</sup> Excessive platelet activity is the cause of coronary artery syndromes where the obstruction of coronary arteries by platelet-rich thrombi formed in the vicinity of ruptured atherosclerotic plaques can lead to myocardial infarction, the principal cause of death in the Western world. Identification of the molecular defects giving rise to bleeding syndromes has been instrumental in defining the mechanisms underlying haemostasis.

The classic disorders of platelet adhesion and platelet aggregation are Bernard–Soulier syndrome (BSS) (with giant platelets) and Glanzmann thrombasthenia (GT).<sup>1,3</sup> In BSS, mutations in the genes encoding glycoprotein (GP)Ib $\alpha$ , GPIIb $\beta$  or GPIX give rise to a block in GPIIb-IX-V maturation and a molecular deficiency of this essential adhesion receptor for von Willebrand factor (VWF).<sup>5</sup> Rare mutations affecting a disulfide loop in GPIIb $\alpha$  lead to upregulated binding of VWF and platelet-type von Willebrand disease (vWD). In GT, a wide panoply of mutations in the genes encoding the  $\alpha$ IIb $\beta$ 3 integrin lead to a block in its biosynthesis and surface expression. Rare defects give rise to an expressed but non-functional integrin.<sup>6</sup> Other inherited defects affect receptors of primary agonists, including the G-protein (Gi)-coupled ADP receptor P2Y<sub>12</sub>.<sup>7</sup>

Another large series of disorders affects intracellular constituents of platelets. As an example, in the Hermansky–Pudlak and Chediak–Higashi syndromes, defects in pathways resulting in dense granule (and melanosome) biogenesis provoke a deficient platelet storage pool of nucleotides and serotonin.<sup>8</sup> In gray platelet syndrome (GPS), a defective  $\alpha$ -granule maturation and an inability to store growth factors and cytokines promote myelofibrosis.<sup>9</sup>

Platelets are produced in the bone marrow by budding in great numbers from megakaryocytes (MKs).<sup>10</sup> Familial thrombocytopenias (FTs) result from an abnormal platelet production and are often associated with the formation of giant platelets.<sup>3,11</sup> Macrothrombocytopenia is seen for a group of disorders assembled under the name of myosin heavy chain 9 (MYH9)-related disease, as each results from an abnormality of the non-muscle myosin heavy chain IIA (NMMHC-IIA) encoded by the MYH9 gene.<sup>12</sup> Defects in transcription factors account for many FTs and are often accompanied by abnormalities in other blood cells, or even development. For example, mutations in the GATA1 gene can be responsible for thrombocytopenia, a low GPIIb content, defects in alpha granules and also a  $\beta$ -thalassaemia-like phenotype involving red cells.<sup>13</sup> Mutations of CBFA2 (RUNX1/AML1) are responsible for a dominant familial platelet disorder with predisposition to acute myelogenous leukaemia (AML).<sup>14</sup> Nevertheless, even though a large number of platelet disorders have been identified, many platelet defects remain to be classified.

### Diagnosis

#### *Clinical Pattern at Presentation*

Mucosal-type bleeding such as spontaneous cutaneous bruising, nose bleeds, menorrhagia and gastrointestinal (GI) bleeding are symptoms of platelet disorders.<sup>1,15,16</sup> For a severe platelet disease such as GT or BSS, bleeding is seen at or shortly after birth. In the more moderate syndromes, bleeding is occasional and often trauma-related. It is essential to interrogate the patient to ascertain his/her bleeding history. The physician should establish whether there is a family history of bleeding and/or related illnesses. If possible, a drug-related or immunological cause must be ruled out. A coagulation factor screen should be performed.

Occasionally, diagnosis is made in the absence of bleeding, for example during platelet-function testing prior to surgery or when there is a familial notion of thrombocytopenia. The common practice of epidural anaesthesia for delivery requires a control of the platelet count during pregnancy and frequently becomes the first occasion to reveal a thrombocytopenia. Diagnosis during pregnancy can be difficult because of physiological changes, e.g. increased plasma VWF



Paquita Nurden is Co-ordinator of the French National Reference Centre for Inherited Platelet Diseases and a Haematologist at the Centre Hospitalier Universitaire de Bordeaux (CHU). She is a member of the American Society of Haematology (ASH) and the French Society of Haematology (SFH), among others. Dr Nurden has written over 100 original articles in national and international journals and gained her degree in medicine from the University of Bordeaux.



Alan Nurden is a Scientific Advisor at the French National Reference Centre for Inherited Platelet Diseases and Research Director of the French National Centre for Scientific Research (CNRS). He is a member of the American Society of Haematology (ASH) and the International Society of Thrombosis and Haemostasis (ISTH), among others. Dr Nurden has written over 200 original articles and over 50 book chapters.

E: alan.nurden@cnrshtl.u-bordeaux2.fr

levels. These can complicate the interpretation of results.<sup>17</sup> Many patients with congenital thrombocytopenia are identified only after they have been considered as idiopathic thrombocytopenic purpura (ITP) and have received unsuccessful medications including corticotherapy, intravenous immunoglobulin G (IVIgG) or even splenectomy.<sup>18</sup> FT with or without giant platelets can be associated with deafness, cataracts, kidney disease, eczema, infections, skeletal abnormalities or cardiac disease.

### Biological Tests

The first task is to perform a complete blood count and a differential smear review. Automated haematology analysers recognise platelets by their size, so the presence of enlarged platelets may lead to an underestimation of platelet count. Examining a slide stained with May–Grunwald–Giemsa (MGG) will provide information about platelet size, the presence of agglutinates and the platelet granule content. This first simple screening can help to classify the patient within the FT group.<sup>19</sup>

Further screening for a platelet defect is possible with one of the new mechanical devices that substitute for the bleeding time test.<sup>20</sup> Here, the Platelet Function Analyzer (PFA-100, Dade Behring, Newark, US) is the most widely used and is very sensitive for the more severe platelet defects (especially those involving VWF), although its usefulness for patients with mild bleeding syndromes is controversial.<sup>21</sup> Mostly, platelet function testing requires a specialised laboratory. Platelet aggregation is a crucial test and involves measuring the light transmittance through citrated platelet-rich plasma (PRP) agitated with a soluble agonist.<sup>22,23</sup> Among the stimuli to be tested are ADP, collagen, arachidonic acid (AA), epinephrine and ristocetin at both high and weak doses. Thrombin-receptor-activating peptide (TRAP) is a useful alternative to thrombin in citrated PRP. Quality control is essential and each laboratory must establish a response range for each agonist in control subjects.

When there is a lack of response to collagen, convulxin, a specific agonist of GPVI, can be used to examine this receptor.<sup>24</sup> When the platelet does not respond to AA, specific agonists of the thromboxane A<sub>2</sub> receptor such as U46628 are tested. An abnormal aggregation with some but not all agonists implies a defect within the signalling pathways responsible for the transmission of signals from the agonist receptors. The release of secretory pools of nucleotides from dense granules can be simultaneously measured with a Chronolog Lumi-aggregometer (Chrono-Log Corporation, Havertown, US).<sup>23</sup> Clot retraction is another useful test, while optical evaluation of platelet adhesion to specific substrates is starting to be used.<sup>25</sup>

Platelet receptors can be quantified by flow cytometry using panels of antibodies directed against specific epitopes of the receptors.<sup>26</sup> For the  $\alpha$ IIb $\beta$ 3 complex, monoclonal antibodies recognising activation-dependent determinants enable an evaluation of its functional capacity.<sup>27</sup> Measures of the surface expression of P-selectin or CD63 provide information about platelet secretion. P-selectin is associated with the membranes of  $\alpha$ -granules of unstimulated platelets and translocates to the plasma membrane of activated platelets.<sup>28</sup> Procoagulant activity can be measured by assessing annexin-V binding to phosphatidylserine.<sup>29</sup> More

sophisticated analyses include the study of platelet ultrastructure by electron microscopy<sup>2,24</sup> and the evaluation of protein phosphorylations by Western blotting.<sup>30</sup> Study of megakaryocytopoiesis in culture starting with peripheral blood CD34+ cells can yield information about defects in the formation and liberation of platelets from MKs.<sup>31</sup> Assaying plasma levels of thrombopoietin (TPO) is important in FT and can reveal an abnormality of the c-Mpl receptor.<sup>32</sup>

In some diseases, diagnosis is relatively easy. Thus, an absence of aggregation with all physiological agonists, a normal platelet count and a normal response of platelets to ristocetin suggest GT.<sup>1,6</sup> Diagnosis is confirmed by flow cytometric analysis of platelet  $\alpha$ IIb $\beta$ 3 receptors, a particularly useful method for newborns, in whom the quantity of blood available is limited.<sup>28</sup> Thrombocytopenia with giant platelets and a specific absence of the response to ristocetin corresponds to the profile of BSS, a finding confirmed by the absence or abnormal functioning of the GPIb-IX-V complex.<sup>5</sup>

Macrothrombocytopenia and a normal response with ristocetin should be accompanied by MGG staining to look for Döhle bodies in leukocytes (MYH9-related disease) and also to verify the presence of  $\alpha$ -granules (absent in gray platelet syndrome).<sup>19</sup> A simple immunofluorescence test can point to MYH9-related disease.<sup>33</sup> Thrombocytopenia associated with small platelets suggests Wiskott-Aldrich syndrome (WAS) or the X-linked thrombocytopenia (XLT) form if the major clinical signs of WAS, such as infections and eczema, are absent.<sup>34</sup>

In FT it is important to verify the response of platelets to small doses of ristocetin. A positive ristocetin-induced platelet aggregation (RIPA) with an upregulated interaction between GPIb and VWF is suggestive of type 2B VWD or platelet-type VWD.<sup>17,31</sup> Molecular biology is the final step in establishing diagnosis for patients with rare platelet diseases.<sup>35,36</sup> It constitutes the only method of identifying abnormalities of transcription factors and confirming the basis of new diseases.

## Management of a Platelet-based Bleeding Syndrome

### Spontaneous Bleeding Syndrome

A number of factors must be taken into account when managing patients with a severe bleeding diathesis.<sup>1,15</sup> Close attention must be paid to children with scattered petechiae and superficial bruising. If bleeding occurs, the child must immediately go to hospital. Local procedures to reduce blood loss need to be discussed with experts at specialist centres. If blood loss is significant, transfusion of red cell concentrates is advised. Platelet transfusions are required for extensive blood loss. It is important, if possible, to transfuse platelets that have been prepared recently. A problem of platelet transfusion for patients lacking receptors is the development of isoantibodies directed against the missing protein.<sup>1,37</sup> The presence of inhibitors may compromise the efficacy of new transfusions. Nowadays, it is common to use recombinant FVIIa rather than platelets, especially when inhibitors are present.<sup>38,39</sup>

The occurrence of GI angiodysplasia in elderly patients with GT is a problem. The clinical course often involves multiple episodes of GI bleeding and a resistance to classic treatments (plasma argon

coagulation, platelet and packed red cell transfusions or even recombinant FVIIa). The association of octeotride with or without an oestrogen–progesterone combination should be considered in the management of recurrent GI bleeding due to angiodysplasia and has been successfully used in GT.<sup>39,40</sup> It should be proposed early for patients with repeated GI bleeding.<sup>40,41</sup> The most difficult situation to deal with is intracranial haemorrhage with the risk of lesions.

## Preventative Treatment Prior to Surgery

A challenge is to predict the haemorrhagic risk for patients with moderate bleeding symptoms during surgery or childbirth. It is important to know about the recent and past history of nose or gum bleeding, easy bruising, the presence of menorrhagia or excessive bleeding during previous surgery or childbirth. Not only is the intensity of the bleeding episodes important, but also their frequency; haemoglobin and ferritin levels can reflect severity. The notion of a bleeding score is useful. This should take into account a patient's age, as bleeding frequency often decreases with age.

For patients with repeated and severe bleeding, haemorrhagic risk is important and platelets are systematically transfused. The quantity of normal (transfused) platelets required to avoid bleeding has been assessed as 50,000/μl and this level should be maintained

for three or four days. For GT or BSS patients in whom isoantibodies are suspected, the survival of transfused platelets can be assessed by flow cytometry. For patients with a moderate or weak bleeding tendency, and depending on the type of surgery, local procedures can be used. One option is to perfuse tranexamic acid during the immediate period after surgery.<sup>15</sup>

Desmopressin, commonly used for patients with vWD, is also recommended for patients with moderate thrombocytopenia or a mild platelet functional defect; while increasing VWF levels at the injured site, it may also have a direct effect on platelets.<sup>42</sup> Some disorders, such as WAS, Chediak–Higashi syndromes, Griscelli syndromes or AML, require specialist treatment and haematopoietic stem cell transplantation at an early age.<sup>15</sup>

## Conclusions

Growing evidence suggests that inherited diseases of platelets are more frequent than previously thought. Efforts are being made to distinguish FT from ITP to avoid diagnosis after patients have received medications for controlling an immune-mediated process. Recent progress in diagnosis will not only be valuable for the patients, but will also continue to identify targets, such as new treatments for patients with ischaemic disease, for controlling platelet functions clinically. ■

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