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# Diagnostic Strategy for Congenital Thrombopathies

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**Abstract** **Review Article**

Congenital thrombopathies are a group of haemorrhagic disorders associated with platelet involvement causing bleeding of varying intensity. They are result of genetic abnormalities affecting the functionality and expression of proteins involved in the control of several stages of platelet activation including adhesion, secretion, aggregation and procoagulant activity. They are manifested in childhood and their diagnosis has recently benefited from advances in genetics and molecular biology; however, a number of constitutional thrombocytopenia remain unexplained and should benefit in the future from advances in next-generation sequencing techniques.
**Keywords:** congenital thrombopathy-haemorrhage-diagnosis.
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## INTRODUCTION

Congenital thrombopathies are a group of heterogeneous diseases that are revealed by a mucocutaneous haemorrhagic syndrome related to a functional or morphological abnormality associated or not with thrombocytopenia; these abnormalities limit the adhesion of platelets to the subendothelium, Or allows them to be activated or aggregated irreversibly. Their diagnosis involves an array of biological analyzes to chisel these different mechanisms. Despite the many tools available for diagnosis, more than 50% of thrombopathies remain untagged. We will try through this paper to show the contribution of each of these biological examinations.

### Constitutional Thrombopathies

Constitutional thrombopathies could be quantitative or qualitative abnormalities or both. Functional abnormalities are classified with respect to platelet dysfunction: adhesion, activation, secretion, aggregation, or procoagulant function.

More than 20 genes have been described as responsible for constitutional thrombocytopenia. The mode of transmission may be X-linked, autosomal dominant or autosomal recessive (homozygous or heterozygous composite). Forms with autosomal recessive inheritance are very often linked to a context of consanguinity (Table I).

The most common classifications of constitutional thrombocytopenia are those based on platelet size as measured by mean platelet volume (MPV) measurement by automated systems or platelet size measurement in May-Grünwald-Giemsa stained blood smear microscopy. (MGG) (Table II).

The distinction of platelet dysfunction remains very difficult because the steps of the haemostasis are intricate, that's why the classification based on the different platelet constituents is by far the most simplified: the membrane receptors, the receptors for the soluble agonists, cell signal transduction pathways and procoagulant phospholipids [1].

### Diagnostic strategy

Study of platelet morphology

#### *Platelet count and blood smear*

The blood count evokes bone marrow production insufficiency with evaluation of MPV. The presence of macrocytosis is related to a group of pathologies such as MYH9 syndrome, Bernard-Soulier diseases.

The blood smear can guide the diagnosis, for example: the presence of basophilic inclusions in neutrophils evokes an MYH9 syndrome, colorless platelets or very pale that evoke a syndrome of platelets, giant granules in neutrophils (PNN) in favor of Chediak-Higashi disease and the presence of platelets containing giant granules orients towards the Paris-Trousseau disease.

New automata allow a quantitative evaluation of young so-called crosslinked platelets thanks to the

detection of RNA residues, simple and fast realization, which reflects the thrombopoietic activity. It can be used as an initial orientation test in the etiological diagnosis of thrombocytopenia (Table II).

***Platelet immunofluorescence***

In a blood smear, platelet receptors are detected using monoclonal antibodies coupled to a fluorochrome. This technique is simple to perform and can detect deficits in certain receptors and structural abnormalities of platelets. An abnormality detected during this examination will guide specialized tests that will specify the type of thrombopathy [2].

***Examination under the electron microscope***

This examination allows the individualization of the whole of the ultrastructure platelet. The scanning electron microscope makes it possible to visualize the surface of the platelets and to study their change of shape, the emission of filopods. It also allows a study of the platelet ultrastructure through cuts and highlights the quantitative deficits in granules and the internal structures of the platelets.

Immunostaining with gold beads; The technique reserved for specialized laboratories makes it possible to visualize the localization of the targeted antigen by the labeled antibody. The double beam electron microscope thanks to a three D reconstruction of platelets and megakaryocytes allows a digital Acquisition of each section made [3].

**Anomalies of primary haemostasis**

***Bleeding time***

It is a simple test of achievement for the detection of thrombopathies. It is performed by an incision calibrated to the ear (Duke's test), or on the forearm and under 40mmHg pressure (Ivy-incision method). This test corresponds to the kinetics of formation of the platelet nail. Moreover, this invasive test is insensitive, not very specific and dependent operator.

***Platelet occlusion time***

The Platelet function analyzer is a small automaton that evaluates the platelet capabilities to occlude a micro-capillary covered with a collagen matrix in the presence of adenosine diphosphate (ADP) or adrenaline.

An increase in the occlusion time makes it possible to direct the diagnosis towards an abnormality of primary haemostasis in the absence of taking aspirin. This technique also has many limitations [4]:

- Limited sensitivity and specificity for the diagnosis of moderate abnormalities of primary haemostasis.
- False positive in cases of thrombocytopenia less than 80 000 / µl or hematocrit less than 30%.
- No discrimination between platelet diseases and von Willebrand diseases.
- Do not detect illness from empty pool.

***Study of platelet functions***

The standard technique for assessing platelet functions is platelet aggregation performed by photometric technique: a platelet suspension or washed platelets is stirred at 37 ° C, then the platelets are stimulated by a specific agent which will cause their agglutination or their aggregation. The curves obtained representing the kinetics of aggregation make it possible to appreciate the irreversible nature, its speed, its amplitude and also a change of shape of the packs.

Although it is considered as the Gold Standard, the aggregation technique, its implementation is limited outside the specialized laboratories [5]:

- Manual technique that requires significant expertise.
- Blood samples must be treated within 4 hours.
- Patients on an empty stomach.
- No international standardization.
- No external quality control assessment (Table III).

***Flow cytometry***

Attractive technique, thanks to the use of monoclonal antibodies, which makes it possible to quantify in a simple and fast manner the main platelet glycoproteins, to carry out certain functional tests and to evaluate the activability of certain platelet glycoproteins.

Quantification of platelet receptors is essential for the diagnosis of Glanzmann, Bernard and Soulier diseases and the detection of GPVI deficiency.

It is a sensitive, specific technique performed on small volumes of blood, regardless of the platelet count, and allows the study of platelets in their physiological state. It allows platelet glycoproteins to be quantified on the platelet surface at rest "basal state" and after activation by different agonists "activated state".

This technique also allows the realization of certain functional tests of platelet glycoproteins, including the binding of fibrinogen, the exposure of anionic phospholipids, the study of leuco-platelet aggregates.

The most commonly used monoclonal antibodies are anti-GPIIb-IIIa, anti-GPIbIX, anti-CD62P and anti-CD63. They make it possible to identify membrane and granular glycoprotein deficits, such as Glanzmann thrombasthenia (GPIIb-IIIa deficiency), Bernard Soulier syndrome (GPIb-IX deficiency), and platelet syndrome (CD62P deficiency after activation). Or a deficit in dense granules (CD63 deficiency after activation). It is also recommended to

confirm the first result on a second sample [6]. There are also ready-to-use kits for the exploration of these glycoproteins.

***Study of secretion***

Includes two types of examinations: direct granular content assay and study of the secretory process itself.

Most laboratories prefer to investigate secretory platelet capabilities rather than assaying the contents of beta thromboglobulin and PF-4 pellets after platelet stimulation using enzyme-linked immunosorbent assay (ELISA) [7] .

***Molecular biology***

Based on the search for genes coding for the proteins of interest, either by targeted sequencing in the event of a typical functional anomaly or by the new high-throughput sequencing techniques [8].

**Table-I: Classification of the main constitutional thrombocytopenia as a function of the genetic abnormality [1]**

| Syndrome | Gene mutated | Chromosomal location | Transmission |
|---|---|---|---|
| Wiskott-Alrich Syndrome | WAS | Xp11.23-p11.22 | X-linked |
| Thrombocytopenia related to XLT | WAS | Xp11.23-p11.22 | X-linked |
| Thrombocytopenia with absence of radius (TAR) | RBM8A | 1q21.1 | Recessive |
| Oculo-oto-radial syndrome or IVIC syndrome | | | Dominant |
| Amegakaryocytosis with radiocubital synostosis | SALL4 | 20q13 | Dominant |
| Congenital amegakaryocytosis | HOXA 11 | 7p15-14 | Recessive |
| Familial thrombocytopenia and predisposition AML | | | Dominant |
| Thrombocytopenia Paris-Trousseau | c-MPL | 1p34 | Dominant |
| DiGeorge Syndrome | RUNX1 | 21q22-12 | Dominant |
| MYH9 syndromes | | | Dominant |
| Thrombocytopenia ANKRD26 | FLI-1 | 11q23-q24 | Dominant |
| Thrombocytopenia ACTN1 | GP1b β | 22q11 | Dominant |
| X-linked thrombocytopenia with dyserythropoiesis (XLT) | MYH9 | 22q12-13 | |
| or thalassemic syndrome (XLTT) | ANKRD26 | 10P2 | X-linked |
| Bernard-Soulier syndrome | ACTN1 | | |
| | GATA-1 | Xp11.23 | Recessive |
| Gray platelet syndrome | GPIb α | 17p13 | |
| | GPIb β | 22q11 | Recessive |
| | GPIX | 3q21 | |
| | NBEAL2 | 3q21.1 | |

**Table-II: Classification of major constitutional thrombocytopenia as a function of platelet size and isolated or syndromic character [1]**

| Platelet size | Isolated thrombocytopenia | Syndromic thrombocytopenia |
|---|---|---|
| Small | X-linked thrombocytopenia | Wiskott-Aldrich |
| Normal | + Congenital amegakaryocytosis<br>+ Familial thrombocytopenia and predisposition to acute leukemias by AML1 gene mutation<br>+ Thrombopenia ANKRD26 | + Thrombocytopenia with no radius<br>+ IVIC Syndrome<br>+ Amagacaryocytosis with radioulnar synostosis<br>+ Stormorken Syndrome |
| Augmented | + Bernard Soulier Syndrome<br>+ Gray platelet syndrome<br>+ X-linked thrombocytopenia and GATA-1<br>+ Thrombocytopenia ACTN1<br>+ MYH9 syndrome | + Thrombocytopenia Paris-Trousseau<br>+ DiGeorge Syndrome<br>+ MYH9 syndrome<br>+ Sistostérolémie<br>+ Filaminopathies |

**Table-III: Correlation of Platelet Functional Abnormalities to Syndromic Constitutional Thrombopathies [1]**

| | |
|---|---|
| Abnormalities of membership (GPIb-IX-V) | Bernard-Soulier's disease<br>Pseudo-platelet Willebrand |
| Anomalies of primary aggregation (GPIIb-IIIa) | Thrombasthenia of Glanzmann |
| Abnormalities of soluble agonist receptors | TxA2 receivers<br>ADP receivers |
| Anomalies of cellular signaling | Cyclooxygenase deficiency<br>Thromboxane synthetase deficiency<br>Failure of calcium mobilization<br>Phosphatidylinositol synthesis failure<br>G protein system abnormalities |
| Anomalies of granular secretion | Empty delta pool<br>Empty pool alpha or gray platelet syndrome<br>Factor V Quebec |
| Anomalies of platelet procoagulant function | Scott's Syndrome<br>Stormorken Syndrome |

## CONCLUSION

Thrombopathies are most often responsible for a haemorrhagic syndrome, which makes it possible to evoke them and begin an etiological research. Their classification, based on the distinction of functional abnormalities of the platelet response, allowed to better understand the mechanism of these alterations and to identify the precise role of the various glycoproteins in platelet physiology. The establishment of a diagnostic strategy adapted to these various thrombopathies requires an increasingly accurate definition of platelet functional impairment, based largely on molecular biology, for each patient.

**Conflict of interest**

The authors declare that they have no conflict of interest in relation to this article.

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