# Sialylation and thrombocytopenia

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## Sialylation et thrombopénies



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**Abstract**

The sialic acid content on the surface of platelets decreases over the course of their life. This process is due to the mechanism of desialylation, involving sialidase enzymes. The absence of sialic acid exposes β-galactose residues, which are considered as senescent antigens. Genes regulating the synthesis and transfer of sialic acid, as well as mechanisms that exacerbate desialylation, are responsible for thrombocytopenia. In this review, we present basic and clinical data from the literature highlighting the important role of platelet sialylation. We also discuss the possibility of measuring platelet β-galactose exposure from a clinical perspective in order to identify patients with certain forms of thrombocytopenia who could benefit from an experimental treatment to suppress sialidase activity.

**Résumé**

Le contenu en acide sialique à la surface des plaquettes décroît au cours de la vie de la plaquette. Cette élimination correspond au mécanisme de désialylation qui implique des enzymes, les sialidases. Cette évolution se traduit par l'exposition de résidus de β-galactose, un sucre greffé sur les chaînes glycanes avant l'acide sialique. Ces résidus ainsi exposés suite à la désialylation, forment des antigènes de sénescence qui entraînent la clairance des plaquettes et sont des régulateurs de leur durée de vie dans la circulation. Certaines thrombopénies peuvent être causées par des altérations des gènes régulant la synthèse et le transfert de l'acide sialique, ou par des mécanismes physiopathologiques acquis qui exacerbent la désialylation. Dans cette revue, nous exposerons les données de la littérature aussi bien fondamentales que cliniques mettant en lumière le rôle important de la sialylation plaquettaire dans la régulation du compte plaquettaire. Nous aborderons également la possibilité de mesurer l'exposition du β-galactose à la surface des plaquettes et son intérêt en clinique afin d'identifier les patients thrombopéniques qui pourraient bénéficier d'un traitement – encore expérimental – inhibant les sialidases.

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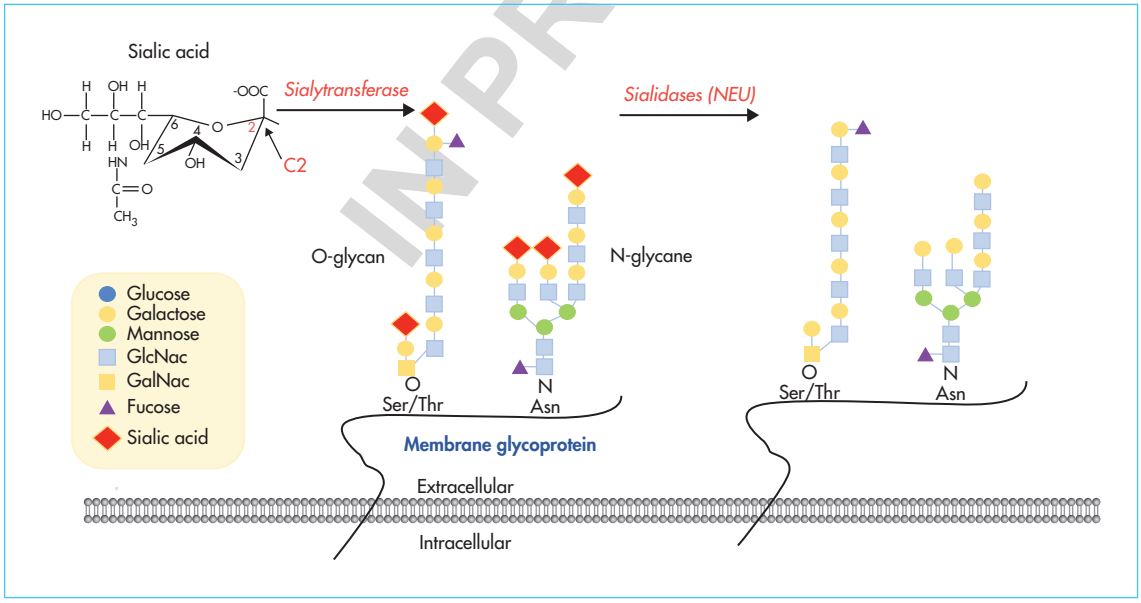
## Sialylation: the terminal stage of glycosylation

Glycosylation is a post-translational modification of proteins that takes place during their maturation involving the addition of carbohydrates to the protein chain. These carbohydrate chains become increasingly complex and divide into branches, which are referred to as "branched chains". Glycosylation takes place in the endoplasmic reticulum and termination of the glycan chains (branching, chain extension) takes place in the Golgi apparatus. Carbohydrate chains are linked to proteins by O-glycosidic (on serine or threonine) and N-glycosidic (on asparagine) bonds. The main carbohydrates added to the branched chains are N-acetylglucosamine (GlcNAc), xylose, mannose, fucose, galactose, glucose, N-acetylgalactosamine and sialic acid (N-acetylneuraminic acid, Neu5Ac), always at the end of the carbohydrate chain. Sialylation is therefore the final stage of glycosylation (*figure 1*). The term "sialic acid" was first used in 1952 to describe N-acetylneuraminic acid, a major product released by the hydrolysis of glycolipids in the brain or salivary mucins. Sialic acids are sugars with a nine-carbon backbone. The most common sialic acids in mammals are N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc) – the latter being absent in humans. Sialic acid (Neu5Ac) is synthesised in the cytoplasm of cells (in the case of platelets, in the mother cell: the megakaryocyte) through a series of biochemical reactions (*figure 1*) [1]. Biosynthesis of sialic acid begins with uridine diphosphate (UDP)-GlcNAc in the cytosol. The synthesis of sialic acid is catalysed by glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine (GNE; also called UDP-GlcNAc 2-



FIGURE 1




**Schematic representation of the sialylation and desialylation process on glycoproteins.** Sialic acid is positioned in the terminal position of N or O-glycans of a glycoprotein. It is linked by an osidic bond at position α2,3 or α2,6 to β-galactose (corresponding to the carbon position, C2, shown in red). The transfer to the glycosidic chain takes place via sialyltransferases, and elimination via sialidases (NEU), exposing the underlying sugar: β-galactose. GalNac: N-acetylgalactosamine; GlcNac: N-acetylglucosamine; Ser: serine; Thr: threonine; Asn: asparagine.

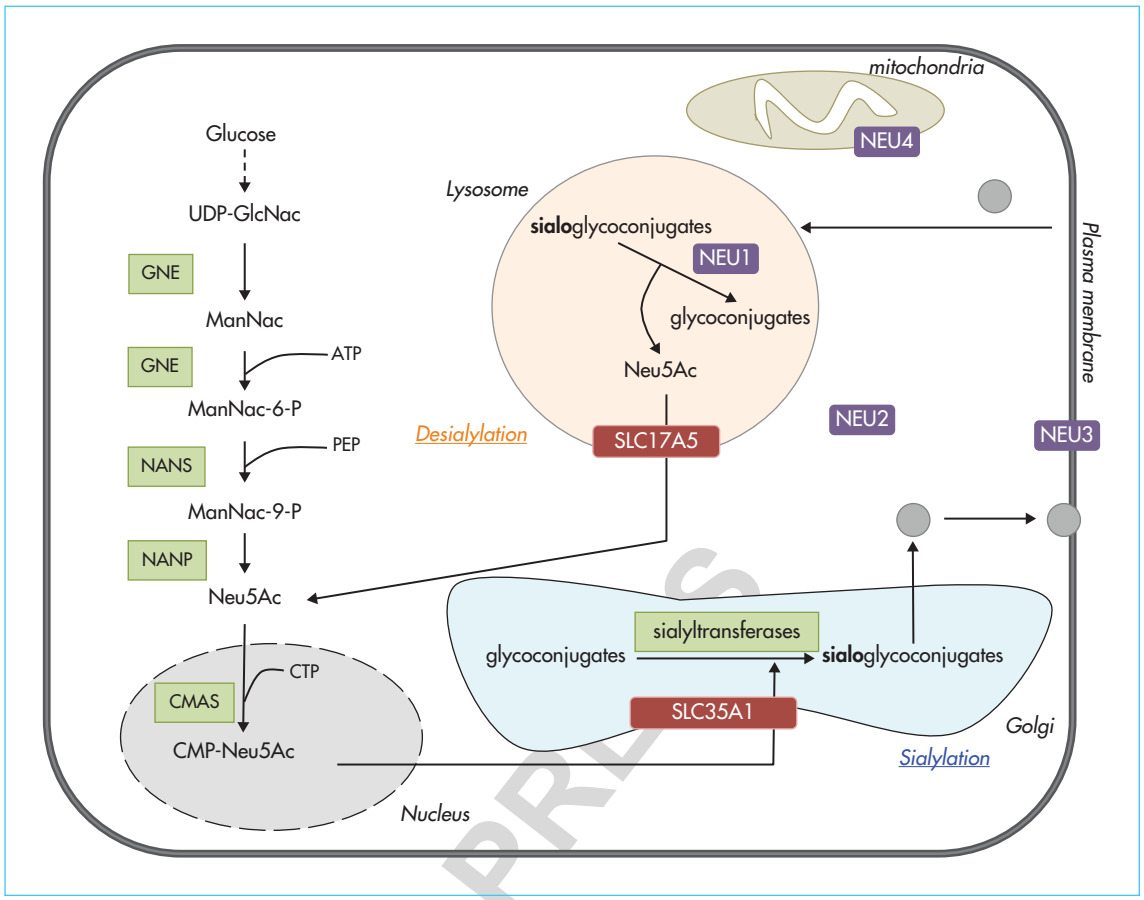
**Représentation schématique du processus de sialylation et de désialylation sur une glycoprotéine.** L'acide sialique est en position terminale des N ou O-glycanes d'une glycoprotéine. L'acide sialique est associé par une liaison osidique en position α2,3 ou α2,6 à un β-galactose (correspondant à la position des carbones, le C2 est représenté en rouge). Le transfert sur la chaÑne glycosidique se fait par des sialyltransférases. L'élimination se fait par des sialidases (NEU) exposant le sucre sous-jacent : le β-galactose. GalNac : N-acétylgalactosamine ; GlcNac : N-acétylglucosamine ; Ser : sérine ; Thr : thréonine ; Asn : asparagine.

epimerase/ManNAc-6-kinase), a bifunctional enzyme, which converts UDP-GlcNAc to ManNAc-6-P (N-acetylmannosamine 6-phosphate). ManNAc-6-P is then converted to Neu5Ac by Neu5Ac 9-phosphate synthase (NANS) in the presence of phosphoenolpyruvate (PEP) and Neu5Ac-9-phosphate phosphatase (NANP). Finally, Neu5Ac synthesised in the cytosol is transferred into the nucleus and activated by cytosine 5′-monophosphate N-acetylneuraminic acid synthetase (CMAS) to form cytosine 5′-monophosphate N-acetylneuraminic acid (CMP-Neu5Ac). This compound is transported in the Golgi apparatus by the transporter SLC35A1, where it is transferred to the glycoconjugates by various sialyltransferases (including ST3 β-galactoside α-2,3-sialyltransferase 4 [ST3GAL4], sT3GAL1 and ST6GAL1) by linking the C2 carbon of sialic acid with the C3 (ST3GAL4, ST3GAL1) and C6 (ST6GAL1) carbons of β-galactose, thus forming α2,3 and α2,6 bonds. Sialic acid is always positioned at a terminal position on β-galactose in glycoproteins. Finally, the sialoglycoconjugates that are formed are either transported to the plasma membrane or stored in storage and secretion vesicles/granules (*figure 2*). The sialic acid in the conjugates may also return to this cycle through the release of sialic acid from sialidases, called "neuraminidases" (NEUs). Currently, four NEUs are known to exist in humans: NEU1, 2, 3 and 4. Lysosomal sialidase (NEU1) initiates the degradation of sialoglycoconjugates. The released sialic acid is then transferred to the cytoplasm by a transporter, SCL17A5, to join a new biosynthesis cycle. Cytosolic sialidase (NEU2) and plasma membrane-associated sialidase (NEU3) exhibit maximal activity in the presence of gangliosides. Sialidase NEU4, which binds to outer mitochondrial membranes, has a broad substrate specificity, from glycoproteins to gangliosides and oligosaccharides. The elimination of sialic acid thus exposes the residues of β-galactose and GlcNAc bound to the glycosidic structure (*figure 1*).

## Sialylation as a determinant of platelet lifespan

Platelets are anucleated cells in mammals and play a major role in haemostasis. In humans, the reference value for the number of platelets in the blood is between 150 and 400 G/L. Below the minimum value, thrombocytopenia is considered. An insufficient number of platelets or the presence of non-functioning platelets may cause bleeding. Platelets have a lifespan of about eight to 10 days in humans, and the number of platelets in the blood is regulated by their production and elimination. This physiological elimination of platelets (outside the context of haemostatic activation) occurs via a mechanism of sialic acid loss, known as "desialylation". The net platelet count is therefore the result of platelet production and elimination. The importance of the role of sialic acid in platelet elimination was first suggested in the context of platelet transfusion. The life of transfused platelets is shortened at the time of transfusion to four to five days [2]. The initial clearance of a portion of the transfused platelets occurs within the first 24 hours, primarily in the spleen and liver, and determines the transfusion yield (or platelet recovery). The initial clearance of 40% of platelets is attributed to platelet storage lesions measured by changes in morphology, function and exposure of phosphatidylserine, but these lesions are independent of apoptosis [2]. In 1969, Murphy and Gardner demonstrated that transfused human platelets stored at 4°C are rapidly eliminated, with a lifespan of two to four days in the circulation [3]. Since this discovery, platelets are now stored at room temperature and are constantly agitated. Recent studies show that platelets stored in cold conditions can be partially activated after transfusion, which may result in a more favourable haemostatic outcome, especially in patients who are actively bleeding [4]. As a result, the Food and Drug Administration has re-approved the cold storage of platelets at between 1 and 6°C without agitation for up to three days for trauma patients. However, numerous studies have shown that cold-stored platelets sequentially lose their sialic acid and

FIGURE 2




**Metabolism of sialic acid in humans.** Sialic acid biosynthesis begins with UDP-GlcNAc (glucose derivative) in the cytosol. Sialic acid (Neu5Ac) is synthesised in the cytoplasm as a result of the action of various enzymes. The pathway leading to thrombocytopenia involves the enzyme GNE, the transporter SLC35A1 and sialyltransferases. The free form of Neu5Ac is transformed into CMP-NeuNAc in the nucleus, and then transported to the Golgi apparatus by a specific transporter, SLC35A1, where it is transferred onto glycoconjugates by various sialyltransferases. Sialylated glycoconjugates may be desialylated in the lysosome by sialidases (NEU). Released Neu5Ac is then transferred into the cytoplasm by a specific transporter, SCL17A5, to join a new biosynthetic cycle. The location of NEU in human cells is as follows: NEU1 = lysosome, NEU2 = cytoplasm, NEU3 = plasma membrane and NEU4 = mitochondrion. Only NEU1 and NEU2 are present in platelets. NEU1 may also be present in α granules and the plasma membrane and NEU2 in mitochondria and the plasma membrane.
ATP: adenosine triphosphate; CMAS: cytosine 5′ monophosphate N-acetylneuraminic synthetase; CMP-Neu5Ac: cytosine 5′-monophosphate-N-acetylneuraminic acid; PEP: phosphoenolpyruvate; GNE: glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine; ManNac: N-acetylmannosamine; ManNac-6-P: N-acetylmannosamine-6-phosphate; ManNac-9-P: N-acetylmannosamine-9-phosphate; NANP: Neu5Ac-9-phosphate phosphatase; NANS: Neu5Ac 9-phosphate synthase; Neu5Ac: N-acetylneuraminic acid (sialic acid); UDP-GlcNac: uridine diphosphate N-acetylglucosamine.

**Métabolisme de l'acide sialique chez l'Homme.** La biosynthèse de l'acide sialique commence avec l'UDP-GlcNAc (dérivé du glucose) dans le cytosol. L'acide sialique (Neu5Ac) est synthétisé dans le cytoplasme suite à l'action de différentes enzymes. L'enzyme GNE, le transporteur SLC35A1 et les sialyltransférases sont impliqués dans des thrombopénies. Neu5Ac est sous forme libre puis est transformé en CMP-NeuNAc dans le noyau, et ensuite transporté dans l'appareil de Golgi par un transporteur spécifique, SLC35A1, ou` il sera transféré sur les glycoconjugués par diverses sialyltransférases. Les glycoconjugués sialylés peuvent être désialylés dans le lysosome par des sialidases (NEU). Neu5Ac libéré est ensuite transféré dans le cytoplasme par un transporteur spécifique, SCL17A5, pour rejoindre un nouveau cycle de biosynthèse. La localisation des NEU dans les cellules humaines est la suivante : NEU1 = lysosome, NEU2 = cytoplasme, NEU3 = membrane plasmique et NEU4 = mitochondrie. Dans la plaquette, seules NEU1 et NEU2 sont décrites. Les localisations semblent différentes avec NEU1 dans les granules α et la membrane plasmique et NEU2 dans la mitochondrie et la membrane plasmique.
ATP : adénosine triphosphate. CMAS : cytosine 5′-monophosphate N-acétylneuraminique synthétase. CMP-Neu5Ac : cytosine 5′-monophosphate-acide N-acétylneuraminique. PEP : phosphoénolpyruvate. GNE : glucosamine (UDP-N-acétyl)-2-épimérase/N-acétylmannosamine. ManNac : N-acétylmannosamine. ManNac-6-P : N-acétylmannosamine-6-phosphate. ManNac-9-P : N-acétylmannosamine-9-phosphate. NANP : Neu5Ac-9-phosphate phosphatase. NANS : Neu5Ac 9-phosphate synthase. Neu5Ac : acide N-acétylneuraminique (acide sialique). UDP-GlcNac : uridine diphosphate N-acétylglucosamine.

galactose, exposing underlying residues of galactose and N-acetylglucosamine, respectively [5] (*figure 1*). Cooling the platelets also promotes aggregation of Von Willebrand factor (VWF) and glycoprotein Ibα (GPIbα) on their surface. Indeed, cooling combined with physiological shear forces causes GPIbα to unfold and VWF to bind to the chilled platelets. This binding leads to desialylation of the cold-stored platelets and accelerated clearance [5].

Platelet desialylation is achieved by NEUs, and only NEU1 and NEU2 have been described in platelets. NEU1 and NEU2 are located in the mitochondria and α-granules, respectively [6], but not in the lysosome, unlike other cell types. This desialylation occurs at the surface of the platelet plasma membrane, where NEU1/2 can be translocated [6-8]. The sialidase activity of NEU1 is regulated by its homodimerisation via transmembrane domain 2, however, this mechanism is not demonstrated in the platelet [9]. The sialic acid released by desialylation in the platelet, however, cannot be added back into the glycosylation cycle due to the absence of a nucleus in platelets; the nuclear step of transformation to CMP-sialic acid is necessary for its transfer to the Golgi apparatus. The mechanism of desialylation, therefore, results in the exposure of residues of β-galactose [10]. These exposed residues form senescent antigens that induce platelet clearance through their interaction with the Ashwell-Morell receptor (AMR) [11, 12] present on hepatocytes as well as CLEC4F, MGL and AMR receptors present on Kupffer cells that are liver-specific macrophages with phagocytic properties [12, 13]. Interestingly, the interaction between platelets and hepatocyte AMRs (particularly in hepatocyte villi) stimulates the production of thrombopoietin (TPO), which in turn stimulates thrombopoiesis. Furthermore, interaction with AMR leads to the expression of TPO mRNA via Janus kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3), creating a feedback mechanism to stimulate the production of platelets in the bone marrow [11,12]. The elimination of platelets by desialylation is therefore closely related to the generation of new platelets (*figure 3*).

AMR is an endocytic receptor, initially described in hepatocytes but also present in Kupffer cells [13]. This receptor is an oligomer of two subunits, asialoglycoprotein 1 (ASGR1) and ASGR2. There are many ligands for this receptor, including platelet and plasma glycoproteins, such as VWF, haptoglobin, amyloid protein and alkaline phosphatase. The key to recognition by the AMR, originally referred to as asialoglycoprotein hepatic receptor (asialo = absence of sialic acid), is the exposure of β-galactose and GalNAc (*figure 1*) following the cleavage of terminal sialic acid. Mice without functional AMR have increased platelet counts with a prolonged circulating half-life [11, 12]. This indicates that AMR plays a role in the elimination of circulating platelets.

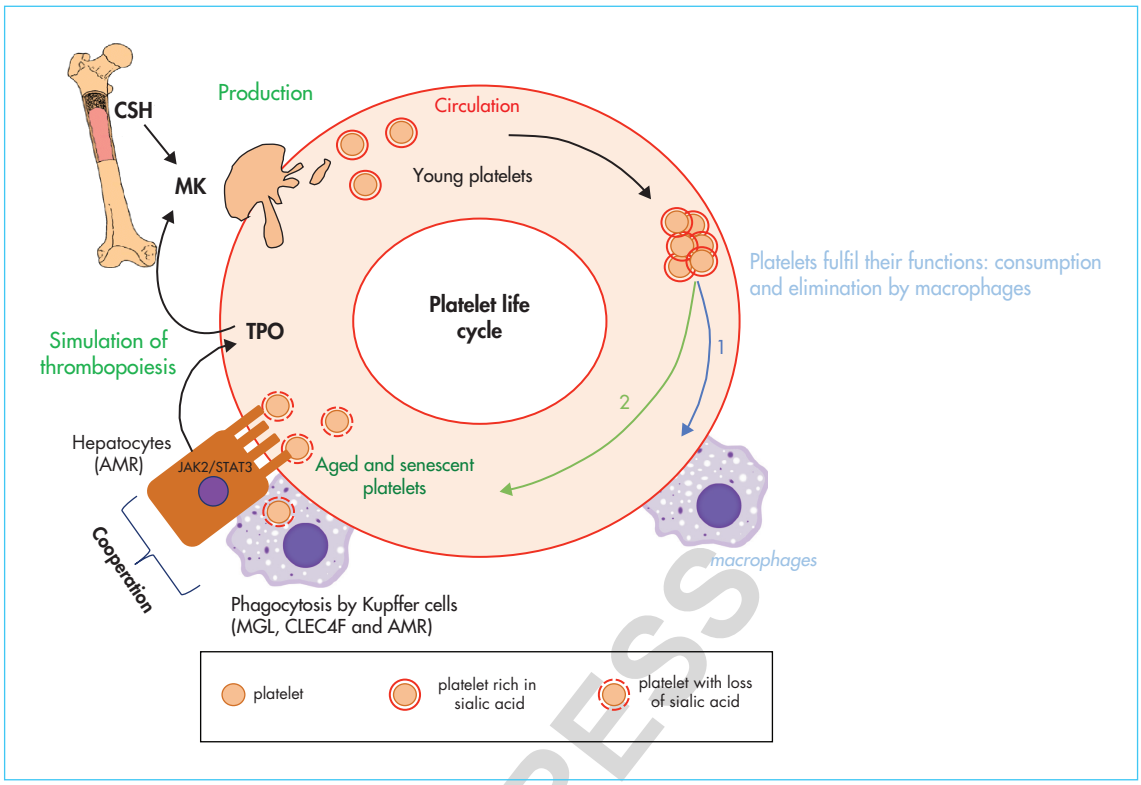
Some thrombocytopenias may be caused by a congenital deficiency in enzymes involved in biosynthetic cycles or sialic acid transporters, or caused by acquired pathophysiological mechanisms that exacerbate platelet desialylation, producing desialylated platelets. Both processes result in accelerated clearance and thrombocytopenia.

## Thrombocytopenia and lack of sialylation

Congenital deficiencies in enzymes in the sialic acid biosynthesis cycle can lead to thrombocytopenia, including mutations in the gene that encodes UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (*GNE*) (*figure 2*) [14-16]. Early studies show that *GNE* mutations are associated with progressive myopathy and thrombocytopenia [15, 17]. In more recent studies focusing on the role of platelet sialylation, patients showed no signs of myopathy [14, 15].

The sialic acid produced in the cells is transported to the Golgi apparatus by a specific transporter, SLC35A1. Transport takes place via an antiport mechanism, in

FIGURE 3




**Platelet life cycle.** Platelets are produced in the bone marrow as a result of haematopoietic stem cell (HSC) differentiation. Megakaryocytes (MK) release platelets into the circulation. These young platelets, rich in sialic acid, will: (1) either be consumed and then eliminated by macrophages; (2) or lose their sialic acid, exposing β-galactose, to become senescent platelets. The recognition of β-galactose by AMR receptors (expressed by hepatocytes) stimulates hepatic production of TPO, allowing the formation of new platelets, and the interaction between platelets and CLEC4F, MGL and AMR, allowing their phagocytosis by Kupffer cells; macrophages present in the liver. AMR: Ashwell-Morel receptor; CLEC4F: C-type lectin domain family 4 member F; MGL: galactose macrophage type C-lectin receptor.

**Cycle de vie de la plaquette.** Les plaquettes sont produites dans la moelle osseuse par différenciation des cellules souches hématopoïétiques (CSH). Les mégacaryocytes (MK) libèrent les plaquettes dans la circulation. Ces plaquettes jeunes sont très riches en acide sialique. (1) Soit elles seront consommées puis éliminées par des macrophages, (2) soit elles perdront leur acide sialique, exposant ainsi le β-galactose et deviendront des plaquettes sénescentes. La reconnaissance du β-galactose par les récepteurs AMR (exprimés par les hépatocytes) stimule la production hépatique de TPO, permettant la formation de nouvelles plaquettes, et l'interaction des plaquettes avec CLEC4F, MGL et l'AMR permet leur phagocytose par les cellules de Kupffer, des macrophages présents dans le foie. AMR : *Ashwell-Morel receptor* ; CLEC4F : *C-type lectin domain family 4 member F* ; MGL : *macrophage galactose type C-lectin receptor.*



other words, a CMP-sialic acid molecule enters, and a free CMP molecule exits via the transporter. Patients who lack this transporter are considered to have congenital disease of glycosylation (CDG), more specifically known as SLC35A1-CDG (CDGIIf in the old nomenclature). These patients may present with syndromes such as delayed psychomotor development, epilepsy, cerebral malformation, and muscle coordination and haematological disorders, including coagulation factor deficiencies and thrombocytopenia [18, 19]. The role of the transporter SLC35A1 has previously been alluded to in platelets, but evidence for its importance in platelet sialylation and its role in platelet survival were not investigated until 2018. A study on two siblings with an *Slc35a1* mutation [18], as well studies using a mouse model lacking this gene [20], demonstrated its role in regulating the quantity of platelets. Based on a study of platelets isolated from two patients harbouring a mutation in *Slc35a1* (p.Ser147Pro), our team showed that this mutation is associated with an increased elimination of platelets from the

circulation. Persistent thrombocytopenia was observed in both patients, with platelet counts of 95 ± 10 G/L in one and 60 ± 6 G/L in the other. Analysis of transferrin sialylation (a technique to identify a lack of N-glycosylation) demonstrated hyposialylation of this protein, suggesting a lack of maturation of N-glycosylation in the Golgi apparatus. Analysis of platelet sialylation, and specifically β-galactose exposure, was performed using samples from one of the two patients. An increase in the exposure of β-galactose was found, suggesting that the patient's platelets were hyposialysed. An alternative origin of thrombocytopenia was therefore explored. For this purpose, patient and control platelets were isolated, washed, and labelled with a fluorescent tracer and injected into immunodeficient mice in order to study their rate of elimination. After 90 minutes, approximately 50% of the control platelets were still present in the circulation while none of the patient's platelets were identified. This work shows that the platelets from these patients with dysfunctional SLC35A1 have an extremely short lifespan and clearly demonstrate hyposialylation. The mouse model also revealed fundamental anomalies of megakaryopoiesis. The number of megakaryocytes in the bone marrow of *Slc35a1-/-* mice was reduced and was associated with impaired maturation of these cells.

Sialyltransferases also play an important role in the lifespan of platelets. Although, to date no mutation or aberrant expression of ST3GAL4 sialyltransferase has been reported in humans, *St3gal4* knockout mice demonstrate deep thrombocytopenia [2]. This enzyme adds sialic acid to the α2,3 position of N-glycans. Crossing *St3gal4-/-*mice with AMR-deficient mice results in the recovery of platelet counts, implying that exposure of β-galactose in these mice leads to AMR clearance. Changes in O-glycosylation can also lead to severe thrombocytopenia. Several mouse models with O-glycan deficiency have been studied, including a model with suppressed ST3GAL1 sialyltransferase, which is responsible for the addition of sialic acid to α2,3 of O-glycans. *St3gal1-/-*mice have 50% fewer platelets than wild-type mice. However, the generation of ST3GAL1-disabled mice, specifically in the megakaryocyte and platelet lineage (flox/PF4-cre system), shows that the main cause of thrombocytopenia in these mice is due to impaired thrombopoiesis. More precisely, this desialylation leads to activation of immune cells which secrete interferon that inhibits thrombopoiesis [2]. This discovery, combined with that of the *St3gal1-/-*mouse model, opens up new avenues for studying the role of sialic acid in megakaryopoiesis, in addition to its role in the peripheral regulation of platelet number.



## Thrombocytopenia and desialylation

Some forms of thrombocytopenia may be caused by acquired mechanisms that exacerbate platelet desialylation, as has been observed in sepsis [21], dengue virus infection [22], Chagas disease [23], immune thrombocytopenic purpura (ITP) [24, 25], and in allogeneic haematopoietic stem cell transplant patients [26]. The antiviral drug, oseltamivir phosphate (Tamiflu®), acts as an inhibitor of influenza virus NEUs but also has inhibitory activity against human NEU. Its use in the context of thrombocytopenia, induced by exacerbation of desialylation inhibiting platelet NEUs, is therefore an interesting and inexpensive therapeutic possibility. Thus, our knowledge of the pathophysiology of ITP has recently evolved, from a model primarily involving the clearance of opsonised platelets by macrophages in the spleen via the FcRγ receptor, to a concept involving a FcRγ-independent mechanism that may be related to platelet elimination. In the case of ITP with anti-GPIbα antibodies, these antibodies bind to the platelet receptor, GPIbα, and increase platelet desialylation, resulting in accelerated platelet clearance and thrombocytopenia [8, 24]. These patients are less likely to respond to conventional treatments (corticosteroids, intravenous immunoglobulin, splenectomy). The

clearance of desialylated platelets is thought to be primarily via the liver and much less via the spleen, which may explain the lack of platelet recovery following splenectomy in some of these patients [8]. As a result, a few studies have shown an increase in platelet counts in patients who have received oseltamivir treatment [25, 27]. However, it appears that the combination of several therapies is more effective than oseltamivir monotherapy. Combining oseltamivir with other treatments has been shown to achieve a sustained platelet response in patients with anti-GPIbα ITP who do not respond to conventional therapies. Platelet counts for the four patients studied were 0, 3, 16 and 0 G/L before treatment, and 101, 134, 120 and 51 G/L after oseltamivir was combined with one or more other treatments (such as romiplostim, azathioprine, prednisone and dexamethasone) [27]. The synergy achieved by the addition of oseltamivir therefore appears to be a promising avenue for such forms of thrombocytopenia. Antibodies other than anti-GPIbα antibodies have recently been shown to induce desialylation. Indeed, anti-αIIbβ3 antibodies (GPIIb/IIIa) may also induce cleavage of sialic acid leading to exposure of β-galactose. For example, in a recent report of a patient with acquired Glanzmann thrombasthenia (ITP with anti-αIIbβ3) with Fc-γ IIa receptor-dependent platelet to trap their hosts' sialic acids, in particular, to escape the hosts' innate immune desialylation- (FcγRIIa), the use of anti-FcγRIIa antibodies was shown to inhibit this desialylation [28].

Pathogenic bacteria use sialidases response. Some pathogenic viruses also use sialidases. Several studies suggest that microorganism-induced thrombocytopenia is due to a process of platelet desialylation. However, the mechanisms leading to desialylation vary according to the infectious vector. In patients infected with dengue virus, increased binding of VWF to platelets is likely due to desialylation of platelets and VWF. The positive effect of oseltamivir on thrombocytopenia is presumably due to the inhibition of platelet-derived NEU1, as the dengue virus does not contain sialidase [22]. In Chagas disease, the parasite *Trypanosoma cruzi* releases trans-sialidase (which allows the transfer of sialic acid from the host surface to the parasite surface), which desialylates platelets causing thrombocytopenia [23]. In sepsis, which compromises the systemic host response to infection, thrombocytopenia is frequently observed. One study showed that the levels of platelet desialylation measured in 127 patients with thrombocytopenia were higher than those in 134 patients without thrombocytopenia. The addition of oseltamivir as treatment for thrombocytopenic patients with sepsis not only increased platelet counts but also reduced the amount of platelets transfused [21]. Finally, since VWF can induce platelet desialylation, the latter may be involved in thrombocytopenias in acquired Willebrand syndrome in which the VWF is activated – such as in circulatory support and in patients with aortic valve disease. Our group also studied the possible relationship between desialylation and thrombocytopenia in congenital Willebrand disease, particularly VWD type 2B, a haemorrhagic disease related to thrombocytopenia and thrombopathy [7]. Similar to immune thrombocytopenic purpura, mutated VWF spontaneously binds to platelet GPIbα and may, like anti-GPIbα antibodies, induce desialylation and contribute to thrombocytopenia. We explored the relationship between platelet desialylation and platelet count in 36 patients with VWD type 2B as well as in the Willebrand mouse model (2B mice) carrying the p.V1316M mutation, conferring severe Willebrand type 2B disease. Abnormally high levels of platelet desialylation were observed in patients with the p.V1316M mutation and platelets from 2B mice. In contrast, treatment of 2B mice with sialidase inhibitors was not associated with an increase in platelet count. We concluded that platelet desialylation plays a minor role in VWD type 2B and is not sufficient to be responsible for thrombocytopenia.

Treatments currently available for thrombocytopenic patients include platelet transfusion, immunosuppressive therapies, the use of intravenous immunoglo-

bulin, TPO agonists, and sometimes splenectomy, a surgical procedure. These expensive treatments are sometimes ineffective and not without side effects. Should oseltamivir prove to be effective in desialylation-related thrombocytopenia, the clinical and economic benefits would be significant given its low cost, low level of associated adverse events, and ease of use.

## Measurement of the exposure of β-galactose

Confirmation of the value of oseltamivir for the treatment of certain forms of thrombocytopenia requires large, randomised studies. However, a standardised test to quantify the desialylation/exposure of β-galactose on the surface of platelets is a prerequisite for identifying patients who may benefit from such treatment. There is no consensus on the interpretation of results on desialylation/exposure of β-galactose based on the currently available studies; the methods vary widely, and the interpretation does not take into account inter-individual variability as no reference values have been determined. In mice, reference ranges are determined in animals with the same genetic background, and a comparison of exposure of β-galactose on platelets under various conditions is often sufficiently informative. In humans, the determination of baseline values for exposure of β-platelet galactose in a healthy population is an essential prerequisite for identifying patients with thrombocytopenia who are likely to respond to sialidase inhibitors.

We have developed a standardised flow cytometry test to measure the exposure of β-galactose to platelet-rich plasma (PRP) prepared from blood collected in EDTA tubes; this provides the advantage of being able to use the same specimen used to diagnose or control thrombocytopenia in routine clinical practice. For this, we used a lectin called RCA-I (ricinus communis agglutinin-I) which specifically binds to β-galactose when exposed following elimination of terminal sialic acid. This lectin is coupled with a fluorochrome allowing it to be detected on the surface of the platelets and to measure fluorescence intensity. We determined the exposure of β-galactose in 120 healthy subjects with normal platelet counts, allowing us to determine a reference range in the healthy population. These values show that the exposure of β-galactose is independent of gender and blood group, thus allowing for an easy comparison of patient values, independently of these two parameters [30]. We propose that the development of this standardised protocol will allow large clinical studies to clarify the role of platelet desialylation in various forms of thrombocytopenia and the efficacy of NEU inhibitors such as oseltamivir.



## Open questions

A first question concerns the mechanism of control and activation of NEU platelets, both during platelet aging and pathologies in which desialylation is observed. Although studies show relocation of NEU to the platelet surface, agonist-induced platelet activation does not appear to result in this externalisation with the exception of VWF activation [6, 7]. This control is probably achieved via a "single" signalling pathway. Once on the surface, how are the NEUs activated? This is probably achieved by homodimerisation of transmembrane domains, as suggested by *in vitro* studies [9]. In other cell types, such as macrophages, NEU1 has been shown to interact with CD36 receptor and desialylate it [29]. In platelets, different receptors cooperate and form dimers (homo-/heterodimers), therefore we may envisage that protein complexes associated with NEUs may control the sialylation of glycoproteins.

What are the targets of sialidases? Many platelet glycoproteins contain sialic acid. GPIbα contains the highest levels, followed by the two integrin subunits αIIbβ3 and the glycoproteins GPV, GPVI, GPIbβ and GPIX, as part of the major human platelet receptors [12]. GPIbα and integrin αIIbβ3 can be desialylated, but

desialylation of these proteins alone does not appear to be sufficient to accelerate platelet clearance [6, 18]. Desialylation is likely a global phenomenon; therefore, the notion of a desialylation threshold could be useful to signal clearance from a quantitative, rather than a qualitative point of view [7].

In the coming years, the list of pathologies associated with thrombocytopenia linked to mechanisms of desialylation is likely to grow. These already include, for example, acquired pathologies associated with Willebrand disease, infections associated with bacterial and viral vectors, liver pathologies that could impact the functioning of the AMR receptor, but also pathologies linked to macrophage dysfunction. Also, the involvement of sialic acid in normal and pathological haematopoiesis should be reviewed, since one of the main functions of sialic acid is to (negatively) charge cells on the surface and to regulate cellular interactions. Finally, the measurement of platelet desialylation could be a marker of the quality of platelet concentrates before transfusion, thus optimising transfusion yield.

## References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# Questions à l'auteur

Q1 Merci de compléter la référence [20] en nous fournissant la tomaison et les folios.

Q2 Merci de compléter la référence [30] en nous fournissant le titre de l'article.