Blood platelet research in autism spectrum disorders: In search of biomarkers

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Abstract
Autism spectrum disorder (ASD) is a clinically heterogeneous neurodevelopmental disorder that is caused by gene-environment interactions. To improve its diagnosis and treatment, numerous efforts have been undertaken to identify reliable biomarkers for autism. None of them have delivered the holy grail that represents a reproducible, quantifiable, and sensitive biomarker. Though blood platelets are mainly known to prevent bleeding, they also play pivotal roles in cancer, inflammation, and neurological disorders. Platelets could serve as a peripheral biomarker or cellular model for autism as they share common biological and molecular characteristics with neurons. In particular, platelet-dense granules contain neurotransmitters such as serotonin and gamma-aminobutyric acid. Molecular players controlling granule formation and secretion are similarly regulated in platelets and neurons. The major platelet integrin receptor αIIbβ3 has recently been linked to ASD as a regulator of serotonin transport. Though many studies revealed associations between platelet markers and ASD, there is an important knowledge gap in linking these markers with autism and explaining the altered platelet phenotypes detected in autism patients. The present review enumerates studies of different biomarkers detected in ASD using platelets and highlights the future needs to bring this research to the next level and advance our understanding of this complex disorder.

KEYWORDS
autism spectrum disorders, blood platelets, gamma-aminobutyric acid, integrin αIIbβ3, melatonin, neurotransmitter agents, platelet dense granules, serotonin

Essentials
• Autism spectrum disorder (ASD) is a heterogeneous and complex neurodevelopmental disorder.
• Easily accessible, quantifiable, and reproducible biomarkers have not been found in ASD research.
• Blood platelets and neurons contain similar molecular interactors and neurotransmitters.
• Platelet-dense granule morphology, content, and function can be useful biomarkers for ASD.
1 | INTRODUCTION TO BIOMARKER RESEARCH RELATED TO AUTISM

Autism spectrum disorder (ASD) comprises a group of neurodevelopmental disorders that include autism, Asperger syndrome, childhood disintegrative disorder, and pervasive developmental disorder. ASD is characterized by difficulty in social interaction, communication, and repetitive behavior patterns. Normally, most of the symptoms start presenting during the first 2 years of life. The prevalence of ASD has been steadily growing, and according to recent studies, it is as high as 1 in 59 children under the age of 8 in the United States and around 1 in 100 in the European population. This increasing prevalence is most probably due to improved diagnostic methods. Because early intervention can help manage symptoms and improve the quality of life, identification of physiological biomarkers aiding in the early diagnosis of ASD is very relevant. Due to the high phenotypic heterogeneity and frequent comorbidities, ASD is often classified into 2 types: syndromic ASD, in which autism occurs as a comorbidity with known clinical syndromes; and idiopathic ASD, in which the patient exhibits only classical ASD features. Numerous studies have focused on the identification of biomarkers that would aid diagnosis and predict disease severity or evaluate treatment efficacy. A recent detailed overview of neuroimaging abnormalities and diverse metabolic and genetic biomarkers points out that though some possible candidates have been put forward, not a single highly predictive biomarker to diagnose and follow up ASD has yet been found. Many biomarker discovery studies try to focus on finding genetic variations linked to or causing the disorder, as it is well known that there is a high heritability in ASD, inferred from twin and family studies. These genetic studies suggest many possible contributing loci and different biological pathways underlying ASD, such as chromatin remodeling, synaptic plasticity, and neuronal connectivity.

Biomarker research in ASD is complicated by the wide variability in clinical presentation within core symptoms and variables such as language, cognition, and comorbid psychiatric or other symptoms. Due to the complex nature of the disease, any causative pathway or biomarker search would inevitably require a large sample size and more in-depth analysis of the genome. Therefore, ASD research, especially for idiopathic ASD cases, is recently more focused on using next-generation sequencing approaches. But with the discovery of more candidate gene variants, the genetic complexity of ASD has increased even more, and there is a scarcity of patient-derived material or relevant human cell models to study these genes and the associated defective molecular pathways. It is impossible to obtain neurons from the central nervous system (CNS) of living subjects for ex vivo research. Gene expression and imaging studies have been performed on postmortem brains, but there are many known limitations of this method, such as post-mortem interval, cause of death, storage procedures and biomatelial integrity. This has hindered research on candidate gene pathways, diagnostic biomarkers, and possible therapies for ASD. To overcome these challenges, it is still important to use alternative patient-derived cells (blood, fibroblasts, and stem cells) or material (cerebral spinal fluid, urine, feces, serum, and plasma) to gain more insights into pathophysiology and to discover biomarkers. This review focuses on studies that have used blood platelets as a patient-derived cell model to study ASD.

2 | PARALLELS BETWEEN PLATELETS AND NEURONS

Platelets are small anucleate cell fragments circulating in the blood, originally derived from megakaryocytes in the bone marrow. While the relevance of platelet activation for blood clot formation after vascular injury is well established, more exploratory research has shown that platelets contribute to the (patho)physiological processes important for cancer, inflammation, infection, and neurological diseases. The parallel between platelets and serotonergic neurons was drawn in the early 1970s. The link with neurological disorders still mostly relates to the similarities between the platelet release reaction of stored agonists following stimulation and the neurotransmitter release following an action potential in a neuron (Figure 1). Platelets and neurons have a strikingly similar calcium-dependent activation and secretion mechanism, secretory vesicles (containing neurotransmitters and activating molecules such as serotonin or 5-hydroxytryptamine [5-HT], dopamine, epinephrine, glutamate, gamma-aminobutyric acid [GABA], calcium, and ADP and ATP that are secreted upon activation), 5-HT transporters (serotonin transporter [SERT] and vesicular monoamine transporter 2 [VMAT2]), and cell surface receptors such as receptors for the listed neurotransmitters, integrin β3, ephrin, and many more. Surprisingly, certain neuron-specific markers are expressed in platelets such as reelin and amyloid precursor protein. Following endocytosis of different small molecules and proteins, multivesicular bodies are involved in the formation and sorting of granules in both neurons and platelets. Mutations disrupting these cargo sorting pathways have been known to cause disorders with bleeding and neurological defects.

Even though there are many studies reporting similarities between these cell types, existing literature knowledge cannot explain why it is so. In fact, they have different embryonic signatures, as neurons have an ectoderm origin, while platelets are formed by megakaryocytes that originate from the mesoderm. 5-HT mediates a wide range of neuropsychological processes. A very interesting recent review provides detailed insights into the 5-HT metabolism in and outside the CNS. Mammals have 2 major sites of 5-HT production, the brain and the gastrointestinal system, where 95% of total body 5-HT is found in the enterochromaffin cells of the gut, from where it can be released. After its release, 5-HT reuptake is coordinated by SERT into epithelial cells, where it is enzymatically degraded, or it enters the bloodstream, where it is transported into platelets and stored for future use. The blood-brain barrier is considered impermeable to 5-HT, though it is expected that peripheral 5-HT from the gastrointestinal system is released into the blood circulation, also flowing through the brain in the vicinity of neurons. However, there is no evidence that peripheral 5-HT that remains mostly stored in platelets plays any role in the
Platelets do not synthesize 5-HT, but they take it up from the circulating blood via SERT (or SLC6A4) to package into their dense granules via VMAT2. Platelets that are activated via diverse agonists and calcium signaling, release their dense granules including 5-HT, which is detected by 5-HT receptors for the amplification of platelet activation. The integrin receptor αIIbβ3 becomes activated after initial platelet activation to initiate platelet-platelet interactions via coupling with fibrinogen. Right panel shows neurons activated via calcium signaling and the arrival of the action potential, triggering release of synaptic vesicles containing neurotransmitters such as 5-HT, which bind to the 5-HT receptors of the postsynaptic neurons to complete neurotransmission. The released 5-HT is reabsorbed by the presynaptic neuron via SERT activity and transported back into synaptic vesicles through VMAT2.

Platelets Neurons

| Action potential | Presynaptic neuron |
|-----------------|-------------------|
|                 | 5-HT reuptake      |
| Ca2+            | Postsynaptic neuron |

Platelets Neurons

| Platelet activation | Platelet agonist | Ca2+ |
|---------------------|------------------|------|
| α-Granules          | β-Granules       |      |

Platelets Neurons

| Calcium channel |
|-----------------|

Platelets Neurons

| 5-HT uptake |
|-------------|

Platelets Neurons

| SERT       | VMAT2       |
|------------|-------------|

Platelets Neurons

| LDCV, large dense-core vesicle |
| SDCV, small dense-core vesicle |

Platelets Neurons

| 5-HT2A |

Platelets Neurons

3. **PLATELET-DENSE GRANULES AND AUTISM**

Platelets contain 2 types of specialized granules, alpha granules and dense granules, in addition to lysosomes (Figure 1). The more
abundant alpha granules (40-80 per platelet) are the largest (200-500 nm) and comprise several important proteins such as fibrinogen, P-selectin, von Willebrand factor, growth factors, and other small polypeptides. The smaller (~250 nm), scarcely populated (3-9 per platelet) dense granules contain signaling molecules (ATP, ADP, calcium) and neurotransmitters, of which some are critical for secondary platelet activation. These dense granules and their secretion pathway have been compared to the regulated secretion in neurons. In short, the neuronal secretion process uses mainly 2 types of secretory granules, the large dense-core vesicles (LDCVs) and the small synaptic vesicles (SSVs) (Figure 1). The LDCVs contain a variety of proteins, including slow-acting neuropeptides, growth factors, amines, and hormones and could be compared to alpha granules in platelets. However, the content of LDCVs in neurons and alpha granules in platelets is quite different, and no striking homology in their function or regulation has been found. A subgroup of the SSVs, the small dense-core vesicles, are comparable to dense granules in platelets because their cores appear similarly dense in electron microscopy images and they both contain, at least in part, the same small molecules and neurotransmitters. Therefore, it is not surprising that mainly platelet-dense granule contents or their formation or function seem to be perturbed in ASD cases, as discussed in the next section.

4 PLATELET-DENSE GRANULE MORPHOLOGY AND AUTISM

Alpha and dense granules are formed in megakaryocytes in the bone marrow prior to their transport into forming platelets. In platelets, granules can release their content following platelet activation. It is known that the BEACH (named after Beige and Chediak-Higashi) domain-containing proteins are important for platelet granule formation and secretion. For instance, genetic variants in NBEAL2 cause gray platelet syndrome, a bleeding disorder characterized by absence of alpha granules, while variants in LYST cause Chediak-Higashi syndrome, an immune disorder associated with a bleeding tendency characterized by a reduced number of dense granules. Together with the other BEACH domain–containing family members, these proteins are often described as playing a role in membrane dynamics and/or intracellular trafficking of endosome- or lysosome-related proteins and vesicles. However, exactly how these roles are executed by each of these family members remains largely obscure.

Interestingly, NBEA, the gene for the BEACH domain–containing protein neurobeachin (NBEA), was located on the autism-susceptible region of chromosome 13q, as identified by linkage studies. A de novo chromosomal translocation disrupting NBEA was later found in an ASD patient. Eight years later, Castermans et al showed defects in stimulated secretion of vesicles from mouse β-TC3 cells that were depleted of NBEA. In addition, defects in platelet-dense granule morphology were found in the ASD patient with the NBEA disruption. Electron microscopy showed platelets with smaller, more irregular, and differently localized dense granule cores when compared to control platelets. This study suggested the involvement of NBEA in ASD via its function in vesicle trafficking, morphology, and secretion. Heterozygous NBEA knockout mice suggested NBEA involvement in neurotransmitter release and synaptic functioning, and these mice also presented with defects in social behavior, conditioned fear response, spatial learning, and memory. Platelets from these NBEA knockout mice were smaller than controls and had abnormal dense-core halos. A proteomics study further revealed that these platelets had a reduction in actin-interacting elements, which could contribute to the cytoskeletal changes observed in the platelets. These studies highlight a promising avenue of using platelets to study ASD patients. Nonetheless, more studies on ASD subjects need to be performed to completely understand

FIGURE 2 PubMed search data for platelet studies related to autism. The number of studies in an interval of 10 years were plotted from 1970 to 2018. All platelet studies in autism were retrieved using the keywords “platelet OR platelets AND autism NOT PECAM NOT Platelet Factor 4” are shown in blue. All platelet studies related to the serotonin metabolism were found using the keywords “Platelet OR Platelets AND Autism AND serotonin” are shown in red. About 80% of all platelet studies have focused on the serotonin metabolism, while only in the most recent 8 years, studies appeared that focused on the many other platelet characteristics.
the role of NBEA in platelet-dense granule secretion and function. A recent study validated NBEA as a neurodevelopmental disorder gene, but patients presented with a broader phenotypic spectrum than ASD, including early generalized epilepsy. Platelet studies were not performed for this cohort.

5 | HYPERSEROTONEMIA IN AUTISM PLATELETS

In the search for a biomarker for ASD using platelets, the first studies appeared in the 1970s, when it was noted that platelet 5-HT levels were higher in patients with early-onset autism compared to controls. By refining our PubMed search for studies focusing on the 5-HT metabolism, it is clear that over the past 50 years, 80% of all the platelet-based research for ASD has focused on this topic (Figure 2). To inform the readers about this field, we selected research-based articles that each added a unique finding on top of measuring 5-HT levels in whole blood, plasma, or platelets, and this in cohorts ranging from 17 to 292 ASD cases. These studies are introduced in Table 1, together with their main conclusion. Most of these studies actually confirmed the initial discovery and hyperserotonemia was detected in about 17% to 40% of the ASD cases using whole blood or platelets. Studies that found normal or even lower 5-HT levels in ASD cases used small cohorts (<25 cases) and plasma samples, therefore excluding platelet-derived 5-HT. Hyperserotonemia was found to be correlated to age; ethnicity; sex; genetic variants in genes regulating 5-HT uptake (SLC6A4), synthesis (TPH1), or degradation (MAOA and MAOB); autoimmunity; gastrointestinal system, cognitive ability; and genetic variation in ITGB3, the gene coding for part of the important platelet activation receptor αIIbβ3 (Table 1). A recent meta-analysis by Gabriele et al. comparing 22 previously established studies involving a total of 739 autism subjects and 868 controls, revealed significantly elevated 5-HT levels in ASD cases compared to controls, and this for both whole blood (~23%) and platelet-rich plasma (~29%) data sets. According to Gabriele et al., the overall sensitivity of high platelet 5-HT in ASD is quite high (28% in whole blood and 22% in platelet-rich plasma when compared across various studies). In another study, a combination of 5-HT, N-acetyl serotonin (NAS) and melatonin together, resulted in a sensitivity of 80% and specificity of 85%, when distinguishing ASD cases from controls. There are different methods to quantify 5-HT. The most widely used are high-performance liquid chromatography electrochemical detection (HPLC-ECD) and ELISA. Both methods are sensitive, time consuming, and expensive and give comparable results, though ELISA can be slightly more sensitive but less specific compared to HPLC-ECD. Therefore, most studies seem to support the hypothesis that blood and/or platelet hyperserotonemia could serve as an ASD biomarker, though more larger-cohort studies are required.

Different studies have tried to explain the mechanism behind platelet hyperserotonemia detected in ASD. Recognizing the importance of SERT in both platelets and CNS, studies were performed to assess its potential role in ASD hyperserotonemia. It was found that ASD patients express higher levels of SERT on platelet membranes while the affinity of SERT for 5-HT remains the same, resulting in increased 5-HT internalization. Coding and noncoding variants in the SERT gene SLC6A4 have been identified, which are associated with elevated 5-HT levels in ASD. Interestingly, transgenric mice overexpressing the coding gain-of-function (GOF) variant Ala56 in SERT presented with behavioral impairment and hyperserotonemia. Therefore, enhanced SERT on platelet membranes can account for hyperserotonemia in at least some ASD cases, but other contributing factors have also been detected. Common variants in ITGB3, which encodes the β-chain of the platelet activation receptor integrin αIIbβ3 (glycoprotein GPIIbIIIa), have been linked to increased SERT activity and elevated blood 5-HT levels, as discussed in more detail in the next section. Another reason for whole blood hyperserotonemia could be the mildly increased platelet counts noted in some ASD studies, though this cannot explain the hyperserotonemia detected in most studies.

6 | PLATELET ACTIVATION RECEPTOR αIIbβ3 AND AUTISM

Platelet-platelet interaction during their activation is regulated via fibrinogen that binds to its membrane receptor integrin αIIbβ3. Following mild platelet activation, αIIbβ3 integrins undergo a conformational change to expose their binding site for fibrinogen (Figure 1). Numerous platelets are eventually linked by such fibrinogen bridges, resulting in a platelet plug. As mentioned above, the β-chain of the αIIbβ3 receptor is encoded by ITGB3 and variants for this gene have been associated with elevated blood 5-HT levels in ASD. The β3-subunit associates with αIIb to form the integrin αIIbβ3 in platelets, and with αv to form the integrin αvβ3 in neurons. In platelets, activated integrin αIIbβ3 interacts directly with the C-terminus of SERT and enhances the externalization and function of SERT. Similarly, in the midbrain and cortical neurons, integrin αvβ3 was also observed to be involved in the regulation of SERT. Preliminary data from Carter et al. showed that Itgb3 knockout mice were found to have altered social and repetitive behavior patterns, and they did not show interest toward social novelty. Such behavioral defect has not been validated in patients with Glanzmann thrombasthenia with inactivating variants in ITGB3, according to the literature. These patients have a recessive bleeding disorder with absent platelet aggregation responses to most agonists, but ASD phenotypes have never been reported for these patients. Severe psychomotor retardation was reported in only a single patient with Glanzmann thrombasthenia in combination with tuberous sclerosis, which is a multisystemic disorder, sometimes presenting with ASD. Interestingly, a recent study showed that a variant in the ITGB3 gene promoter is associated with enhanced promoter activity in megakaryocytes, higher β3-subunit expression in platelets, enhanced expression of SERT on the plasma membrane, and elevated 5-HT levels
### TABLE 1 Literature review on serotonin quantifications using blood or blood-derived substances from patients with autism

| Serotonin levels     | Number of ASD samples | Blood or blood-derived substances | Study outcome                                                                                                                                                                                                 | References          |
|---------------------|-----------------------|-----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|
| 1 Normal            | 6                     | Platelets                         | Endogenous 5-HT was slightly diminished but significant increase in platelet/mL plasma; 2-fold higher efflux of radioactive 5-HT from ASD patient platelets, which could indicate defective 5-HT turnover in the brain | Boullin et al 18    |
| 2 Hyperserotonemia  | 24                    | Whole blood                       | 5-HT level and platelet count are higher in ASD, while 5-HT level corrected for platelet count was similar between ASD and controls                                                                              | Ritvo et al 17      |
| 3 Hyperserotonemia  | 77                    | Whole blood                       | Higher 5-HT levels in ASD while normal in mentally retarded or cognitively impaired cases. Study points to the importance of matching for age and ethnicity                                                   | McBride et al 60    |
| 4 Hyposerotonemia   | 10                    | Plasma                            | Lower 5-HT levels in adults with ASD and inversely correlated with Overt Aggression Scale score                                                                                                             | Spivak et al 90     |
| 5 Hyposerotonemia   | 17                    | Plasma                            | Lower 5-HT levels in mothers of ASD cases supporting the hypothesis that maternal 5-HT would be a risk factor for ASD through effects on fetal brain development                                                   | Connors et al 91    |
| 6 Hyperserotonemia  | 53                    | Platelets                         | Higher 5-HT levels in 32% of ASD cases and a negative correlation with their speech development                                                                                                               | Hranilovic et al 92 |
| 7 Hyperserotonemia  | 109                   | Platelets                         | Higher 5-HT levels in ASD cases and this in association with common SLC6A4 and ITGB3 haplotypes, each separately but also via a significant interaction between those genetic markers                         | Coutinho et al 93   |
| 8 Hyperserotonemia  | 23                    | Platelets                         | Higher 5-HT levels in 17% of PDD cases without an elevation in intestinal permeability measured by sugar absorption                                                                                         | Kemperman et al 94  |
| 9 Hyperserotonemia  | 63                    | Platelets                         | Association between high 5-HT in ASD and common variants in genes regulating 5-HT synthesis (TPH1) and degradation (MAOA).                                                                                     | Hranilović et al 95 |
| 10 Hyperserotonemia | 50                    | Serum                             | Higher 5-HT and autoimmunity marker anti-myelin-basic protein (anti-MBP) levels in ASD but no correction between both markers                                                                               | Mostafa et al 96    |
| 11 Normal           | 23                    | Plasma                            | Normal 5-HT levels in plasma point out that hyperserotonemia in ASD platelets results from the platelet’s handling of 5-HT and not from their increased exposure to 5-HT                                               | Anderson et al 97   |
| 12 Hyperserotonemia | 279                   | Whole blood                       | Higher whole blood 5-HT levels in 40%, lower plasma melatonin in 51%, and higher platelet NAS in 47% of ASD cases. This study points to a disruption of the 5-HT/NAS/melatonin pathway in ASD                   | Pagan et al 90      |

(Continues)
in blood from ASD cases.\textsuperscript{63} This would mean that ASD is not associated with inactivating but rather GOF variants in \textit{ITGB3}, probably explaining the absence of obvious autism phenotypes in patients with Glanzmann thrombasthenia. However, a case report with a GOF variant in \textit{ITGB3} was reported with bleeding symptoms and mild thrombocytopenia, but a neurological phenotype was not reported.\textsuperscript{71} Platelets from this patient showed enhanced fibrinogen binding but also low $\alpha$IIb$\beta$3 expression levels, and this probably will not result in enhanced SERT expression on the platelet membrane, though it was not studied. Further studies are needed that focus on the role of this important platelet activation receptor in neuropathology.

### 7 | PLATELET FUNCTION, COUNT, AND SIZE STUDIES IN AUTISM

Using a light-transmission aggregometer, platelets are activated by adding an agonist such as ADP, collagen, thrombin, epinephrine or thromboxane A2 analog (U46619) that boosts platelet-platelet interactions and the release of their granules to achieve a full activation response\textsuperscript{20,72} (Figure 1). Table 2 presents the few studies that have performed platelet activation studies for ASD. In 1988, platelet aggregations were performed for children with infantile autism, and a consistent, though not significant, trend of lower platelet responses was detected after activation with collagen and ADP.\textsuperscript{73} About 20 years later, a study showed a borderline significant decrease in ADP- induced platelet aggregation for ASD cases with normal but not increased platelet 5-HT levels.\textsuperscript{74} Recently, detailed platelet function studies were performed for a larger cohort of 159 idiopathic ASD cases and their first-degree relatives.\textsuperscript{59} Though the maximal platelet aggregation response to epinephrine was not lower for ASD cases, its secondary wave response, which is dependent on dense granule secretion, was more frequently delayed or absent in ASD cases compared to controls. In addition, stimulated release of ATP from dense granules was reduced in ASD cases and their first-degree relatives compared to unrelated controls following activation with ADP and collagen.\textsuperscript{59} These findings suggest that at least for a subgroup of ASD cases, platelet granule secretion and subsequent activation is impaired, but it was not clear if this observation is linked to defects in 5-HT uptake or release. Again, larger cohort studies are required to validate platelet functional parameters. Other platelet parameters such as their size (mean platelet volume) and count are not widely

### TABLE 1 (Continued)

| Number of ASD samples | Blood or blood-derived substances | Study outcome | References |
|-----------------------|----------------------------------|---------------|------------|
| 20                    | Plasma                           | Higher 5-HT levels in ASD patients and their unaffected siblings, suggesting heritability of this trait | Bijl et al\textsuperscript{59} |
| 203                   | Platelets                        | Association between high 5-HT in male ASD cases and common variants in MAOB gene regulating 5-HT degradation | Chakraborti et al\textsuperscript{68} |
| 82                    | Whole blood                      | Correlation between high 5-HT in ASD and lower gastrointestinal symptoms | Marler et al\textsuperscript{79} |
| 292                   | Whole blood                      | The largest study performed to date showing higher 5-HT levels but only in prepubertal ASD patients (42%) and this more likely in males | Shuffrey et al\textsuperscript{100} |
| 213                   | Whole blood                      | Platelet NAS has higher heritability than hyperserotonemia and lowered melatonin in ASD | Benabou et al\textsuperscript{77} |
| 181                   | Whole blood                      | Negative correlation between maternal 5-HT levels and cognitive abilities in ASD | Montgomery et al\textsuperscript{101} |
| 176                   | Platelet rich plasma             | A promotor SNP in \textit{ITGB3} that results in enhanced promoter activity during megakaryocyte differentiation is associated with higher integrin \textit{\beta}3 protein expression and higher platelet 5-HT levels in ASD patients. \textit{ITGB3} is known to support SERT trafficking to the platelet membrane allowing enhanced 5-HT uptake in platelets | Gabriele et al\textsuperscript{63} |

Abbreviations: 5-HT, 5-hydroxytryptamine; ASD, autism spectrum disorder; NAS, N-acetyl serotonin; PDD, pervasive developmental disorder (part of ASD).
studied. Bijl et al\textsuperscript{59} reported only a mild increase in platelet count for ASD cases and their siblings.

8 | SEROTONIN DERIVATIVES N-ACETYL SEROTONIN AND MELATONIN IN AUTISM

The biosynthesis of melatonin occurs following 5-HT acetylation by the enzyme aryalkylamine N-acetyltransferase (AANAT) to NAS, which is then methylated by acetylsertotonin O-methyltransferase (ASMT) to melatonin\textsuperscript{75,76}. It has been shown in the literature that there is a reduction in the plasma level of melatonin in ASD cases\textsuperscript{50}. NAS is an important intermediate in the pathway of melatonin biosynthesis from 5-HT, and it has been found to be transported to platelet-dense granules, along with 5-HT\textsuperscript{75}. Recent studies found increased NAS levels in ASD platelets and that this observation was more heritable in ASD families than hyperserotonemia and decreased melatonin (Table 1)\textsuperscript{50,75,77}. Increased NAS, together with decreased melatonin, probably points to a defect in the melatonin synthesis pathway. Indeed, the activities of AANAT and ASMT, the 2 key enzymes contributing to melatonin synthesis, were reduced in postmortem samples of the pineal gland, gut, and platelets of ASD cases\textsuperscript{75}. Furthermore, it was noted that the heritability of reduction in ASMT activity was also higher than hyperserotonemia but similar to NAS\textsuperscript{77}. Melatonin has been well studied due to its use as a sleep inducer, but more recent studies have shown that NAS can regulate mood\textsuperscript{78,79}. It has also been shown to bind to the tropomyosin receptor kinase B receptor similar to the brain-derived neurotrophic factor, suggesting a role in neuronal growth and proliferation\textsuperscript{75,79}. This was confirmed in hippocampal neurons in sleep-deprived mice\textsuperscript{78,79}. Therefore, increased platelet NAS could echo its levels in the CNS, which in turn supports an abnormal growth acceleration in neural precursor cells from ASD cases as observed in a study by Schafer et al\textsuperscript{80}. Platelet NAS should be measured in larger ASD cohorts in addition to 5-HT to evaluate its potential as an ASD biomarker.

9 | OTHER NEUROTRANSMITTERS STORED IN PLATELETS AND STUDIED IN AUTISM

There is very limited evidence of other neurotransmitter changes in platelets of ASD patients. In one of the earliest studies focused on peripheral biomarkers for autism, Lake et al\textsuperscript{81} discovered an increased level of plasma norepinephrine, which they attributed to the low activity of the enzyme dopamine β-hydroxylase, which is responsible for the conversion of dopamine to norepinephrine. Ten years later, this observation was confirmed by another study that also detected a significant increase of epinephrine in plasma, together with a decrease in platelet epinephrine, norepinephrine, and dopamine in ASD subjects\textsuperscript{82}. Such data suggest a broader bioamine metabolism defect in ASD. Following these initial studies (performed in only 22 ASD cases), no substantial progress has been made regarding the status of these neurotransmitters in larger ASD cohorts.

Other platelet neurotransmitters studied in ASD are the major inhibitory neurotransmitter GABA and the major excitatory neurotransmitter glutamate. The imbalance in the excitation/inhibition pathways of these neurotransmitters has been reported in ASD\textsuperscript{83}. Platelets contain glutamate in their dense granules, which is released exclusively by activated platelets and this can enhance platelet activation\textsuperscript{84,85}. On the other hand, platelet aggregation is influenced by GABA, which is only 30% less abundant in platelets compared to cultured neurons\textsuperscript{86}. Though these neurotransmitters are actively studied in ASD cases, studies investigating their expression or function in autism platelets, are very limited. While early studies suggested decreased levels of both GABA and glutamate in ASD platelets,\textsuperscript{87}
subsequent studies have shown an elevation of plasma GABA and glutamate levels in ASD. 83,88,89

10 | FUTURE PERSPECTIVES USING PLATELETS FOR ASD RESEARCH

The exact pathophysiology of ASD has remained elusive over the years even though diagnostic and monitoring strategies have improved over time. This is in part due to ASD essentially being a spectrum that encompasses several disorders that seem to converge when it comes to the ASD core symptoms. Biomarkers, endophenotypes, and patient-derived peripheral cell models can prove very useful in understanding ASD, thereby providing better stratification along the spectrum. To this end, platelet studies have shed more light on the ASD, defined by platelet hyperserotonemia. It would be interesting to determine 5-HT and its related markers, NAS and melatonin, in very large cohort studies to study associations with diverse autism characteristics that are now all grouped under the term ASD. However, though hyperserotonemia in blood and platelets from ASD cases is a consistent observation that is over half a century old, the exact underlying mechanism deserves further molecular studies. Perhaps the answer lies in the basic biochemical properties shared by platelets and neurons, the reason behind which has never been explored in the literature. The small steps forward in this field are probably the result of the fact that blood and especially platelets are relatively foreign systems to most neuroscientists. Some initial studies have been performed to explain hyperserotonemia that have focused on genetic factors that modify SERT expression or activity to modify 5-HT uptake by platelets as described in this review. The main question remains as to where the extra 5-HT detected in ASD cases originates. As 95% of the total body 5-HT is produced by the enterochromaffin cells of the gut, alterations in this metabolism could result in altered 5-HT uptake in the blood and platelets. It would be interesting to study this in Itgb3 knockout mice. Future studies combining microbiome and platelet studies in ASD cases could also provide novel insights.

Furthermore, studies that focus on platelet functional tests can provide new tools for the diagnosis and management of ASD. However, more research is needed to fully understand the role of platelets in ASD and to develop effective therapeutic strategies. Platelets are also integral players in the immune system and play a role in inflammation and the immune response, which are important in the pathogenesis of ASD. Further studies are needed to investigate the role of platelets in immune-mediated processes in ASD.

RELATIONSHIP DISCLOSURE

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

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How to cite this article: Padmakumar M, Van Raes E, Van Geet C, Freson K. Blood platelet research in autism spectrum disorders: In search of biomarkers. Res Pract Thromb Haemost. 2019;3:566–577. https://doi.org/10.1002/rth2.12239