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PRIMARY HEMOSTASIS DISORDERS IN HEMATOMESENCHYMAL DYSPLASIA SYNDROME. LITERATURE REVIEW

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Abstract

Relevance: Hematomesenchymal dysplasia (HMD), as a background pathology in recurrent clotting disorders, is reported with a frequency of 54.9% and manifests as combined and concomitant forms in 45.1% of patients, undifferentiated forms in 22.1%, and differentiated forms in 9.8% as Marfan, Ehlers-Danlos, Whrolick-Lobstein, Franceschetti syndrome, etc. Hemorrhagic disorders are one of the obligate syndromes of HMD, characterized by an early onset and recurrent course, and the nosological structure is quite heterogeneous, due to genetic defects in various parts of the hemostatic system. Angiopathies were detected in 12.3% of patients, thrombocytopathies - 25.5%, Willebrand syndrome (disease) - 11.5%, hemophilia - 4.3%, their combinations - 45.1%, 1.3% had latent (asymptomatic) defects [24].

Aim: To review the literature on disorders of the primary hemostasis in hematomesenchymal dysplasia.

Search strategy: Sources were searched in the following databases: UpToDate, BMJ, PubMed, Scopus, Wiley, Medline, The Cochrane Library, Springer Link, Web of Science. The depth of the search was 18 years: from 2004 to 2022. Thirty-one articles were included in the literature review, which were available in full text and underwent a critical appraisal process.

Algorithm for selecting literary resources → Study of clinical guidelines, monographs reporting the concept of undifferentiated connective tissue dysplasia, mesenchymal dysplasia syndrome, hematomesenchymal dysplasia, joint hypermobility syndrome → Review of articles from journals, academic journals, dissertations → Systematization of the material → Literature analysis and article writing.

Results and conclusions: The problem of hemostasis disorders in HMD is understudied and requires more attention to cover this narrow field of hematology as variants of the clinical picture is various and the most life-threatening complications are both profuse bleeding and thrombophilic manifestations [24].

Keywords: *hematomesenchymal dysplasia, children, primary hemostasis, systemic mesenchymal dysplasia, thrombocytopathy.*

Резюме

НАРУШЕНИЯ ПЕРВИЧНОГО ЗВЕНА ГЕМОСТАЗА ПРИ СИНДРОМЕ ГЕМАТОМЕЗЕНХИМАЛЬНОЙ ДИСПЛАЗИИ. ОБЗОР ЛИТЕРАТУРЫ

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Актуальность: Гематомезенхимальная дисплазия (ГМД), как фоновая патология при рецидивирующих нарушениях свертывания крови, регистрируется с частотой 54,9% и проявляется как комбинированными и сочетанными вариантами у 45,1% пациентов, недифференцированными формами – у 22,1%, так и дифференцированными - у 9,8% в виде синдромов Марфана, Элерса-Данлоса, Вролика-Лобштейна, Франческетти и др. Геморрагические расстройства являются одним из облигатных синдромов ГМД, характеризуются ранним дебютом и рецидивирующим течением и по нозологической структуре весьма неоднородны, что обусловлено генетическими дефектами в различных звеньях системы гемостаза. Ангиопатии обнаруживаются у 12,3% пациентов, тромбоцитопатии – 25,5%, синдром (болезнь) Виллебранда – 11,5%, гемофилия – 4,3%, их комбинации – 45,1%, у 1,3% - скрытые (бессимптомные) дефекты [24].

Цель: обзор литературных ресурсов по нарушениям тромбоцитарного звена системы гемостаза при гематомезенхимальной дисплазии.

Стратегия поиска: Поиск источников проводился в базах: UpToDate, BMJ, PubMed, Scopus, Wiley, Medline, The Cochrane Library, SpringerLink, Web of Science. Глубина поиска составила 18 лет: с 2004 по 2022 годы. В обзор литературы были включены 31 статья, которые были доступны в виде полного текста и прошли критический процесс оценки.

Алгоритм отбора литературных ресурсов → Изучение руководств, монографий, отражающих концепцию недифференцированной дисплазии соединительной ткани, синдрома мезенхимальной дисплазии, гематомезенхимальной дисплазии, синдрома гипермобильности суставов → Изучение статей из журналов, научных сборников, диссертаций → Систематизация материала → Анализ литературы и написание статьи.

Результаты и выводы: Проблема гемостазиологических расстройств при ГМД недостаточно изучена и требует большего внимания с целью освещения данной узконаправленной области гематологии, так как варианты клинической картины разнообразны и наиболее жизнеугрожающими осложнениями являются как профузные кровотечения, так и тромбофиллические проявления [24].

Ключевые слова: *гематомезенхимальная дисплазия, дети, первичное звено гемостаза, системная мезенхимальная дисплазия, тромбоцитопатия.*

Түйіндеме

ГЕМАТОМЕЗЕНХИМАЛЫҚ ДИСПЛАЗИЯ СИНДРОМЫНДАҒЫ БАСТАПҚЫ ГЕМОСТАЗ ЖҮЙЕСІНІҢ БҰЗЫЛУЫ. ӘДЕБИЕТТІК ШОЛУ.

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Кіріспе: Гематомезенхималық дисплазия (ГМД) қайталанатын қан ұюының бұзылуларындағы фондық патология ретінде 54,9% жиілікпен тіркеледі және пациенттердің 45,1%-ында біріктірілген және ілеспе формалар, 22,1%-да бөлінбеген формалар және 9,8% - да сараланған формалар түрінде Марфан, Элерс-Данлос, Вролик-Лобштейн, Франческетти және т.б. геморрагиялық бұзылулар ГМД облигациялық синдромдарының бірі болып табылады. басталу және қайталанатын курс, ал нозологиялық құрылым гетерогенді, бұл гемостаз жүйесінің әртүрлі бөліктеріндегі генетикалық ақауларға байланысты. Ангиопатиялар науқастардың 12,3% - да, тромбоцитопатиялар - 25,5% - да, Виллебранд синдромы (ауру) - 11,5% - да, гемофилия - 4,3% - да, олардың комбинациясы - 45,1% - да, 1,3% - да жасырын (асимптоматикалық) ақаулар бар [24].

Мақсаты: гематомезенхимальды дисплазиядағы гемостаз жүйесінің бұзылуы туралы әдебиеттік шолу.

Іздеу стратегиясы: дереккөздер келесі мәліметтер базасынан табылды: Up To Date, BMJ, Pub Med, Scopus, Wiley, Medline, Cochrane кітапханасы, Springer Link, Web of Science. Іздеу тереңдігі 18 жыл болды: 2004 жылдан 2022 жылға дейін. Әдебиеттерге шолу толық мәтінде қол жетімді және сыни бағалаудан өткен отыз бір мақаланы қамтыды.

Әдеби ресурстарды таңдау алгоритмі → Дәнекер тінінің бөлінбеген дисплазиясы, мезенхималық дисплазия синдромы, гематомезенхималық дисплазия, буындардың гипермобилділік синдромы туралы түсініктерді, монографияларды зерттеу → Журналдардан, ғылыми жинақтардан, диссертациялардан мақалаларды зерттеу → Материалды жүйелеу → Әдебиеттерді талдау және мақала жазу.

Нәтижелер мен қорытындылар: ГМД гемостазының бұзылуы мәселесі жеткілікті түрде зерттелмеген және гематологияның осы тар аймағын қамтуға көп көңіл бөлуді қажет етеді, өйткені клиникалық көріністің нұсқалары әртүрлі және өмірге қауіпті асқынулар-бұл ауыр қан кету және тромбофильді көріністер [24].

Түйінді сөздер: *гематомезенхималық дисплазия, балалар, гемостаздың бастапқы буыны, жүйелік мезенхималық дисплазия, тромбоцитопатия.*

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Introduction

The importance of studying diseases of the hemostasis system in children is due to the wide variety of clinical symptoms, combination with pathologies of other organs and systems, difficulty in diagnosis, and even the possibility of disability. Hemostasis is a complex multi-stage (cascade) process of two links, a primary, vascular-platelet, and a final, plasma-coagulation process. The pathological conditions of each vary widely, with congenital and acquired ones differentiated according to genesis, hemorrhagic and thrombotic variants determined according to the nature of the clinical manifestations. A balance between hemorrhagic syndrome and thrombosis is necessary to maintain physiological levels of the hemostasis system. Despite the classified variants of clotting disorders, there is a wide range of unexplored pathological syndromes which require more detailed investigation. The pathogenetic mechanism of thrombohemorrhagic manifestations is associated with abnormal development of the mesenchymal layer of the vascular endothelium, manifested by the hematomesenchymal dysplasia (HMD) syndrome. HMD is a variant of undifferentiated connective tissue dysplasia (UCTD) [5]. UCTD refers to cases in which the complex phenotypic features do not match any differentiated diseases. The concept of HMD was created by *Barkagan Z.S.,* followed by further studies on hemorrhagic and thrombotic conditions in connective tissue dysplasia carried out by a group of research students and followers of the professor's school. However, the nature of bleeding manifestations in children with hemorrhagic mesenchymal dysplasia did not sufficiently analyze in the available literature [*Andreeva N.N., 2005*].

An urgent problem of connective tissue dysplasia is hemostasis disorders. The hemorrhagic syndrome is one of the manifestations of mesenchymal abnormalities [*Barkagan Z.S.,1988*]. The changes affect both the coagulation and platelet-vascular stages of hemostasis. The severity of HMD can range from mild to severe, even life-threatening conditions (intracranial hemorrhage, uterine, gastrointestinal hemorrhage). A variety of hemorrhagic manifestations - recurrent epistaxis, mild bruising, and excessive bleeding of the gums - are often seen in various forms of dysplasia [*Barkagan Z.S. et al., 1994*].

The worldwide prevalence of HMD is unknown; separate studies suggest that the incidence varies according to the severity of the clinical manifestations, with mild to moderate variants occurring in 60-80% of cases and severe variants occurring less frequently in the range of 10-20% [2].

Aim: to review the bibliographic resources on disorders of the primary hemostasis in hematomesenchymal dysplasia.

Search strategy: The list of sources in the following databases: UpToDate, BMJ, Pub Med, Scopus, Wiley, Medline, The Cochrane Library, Springer Link, Web of Science. The depth of the search is eighteen years: from 2004 to 2022. The literature review included thirty-one papers available in full text and reviewed through a critical appraisal process.

Algorithm for selecting literary resources → Study of clinical guidelines, monographs reporting the concept of undifferentiated connective tissue dysplasia, mesenchymal dysplasia syndrome, hematomesenchymal dysplasia, joint hypermobility syndrome → Review of articles from journals, academic journals, dissertations → Systematization of the material → Literature analysis and article writing.

This literature review was carried out as part of the PhD Dissertation on "Complex assessment of the hemostasis system and genetic screening in children with hematomesenchymal dysplasia". The study theme was approved by the ethical committee.

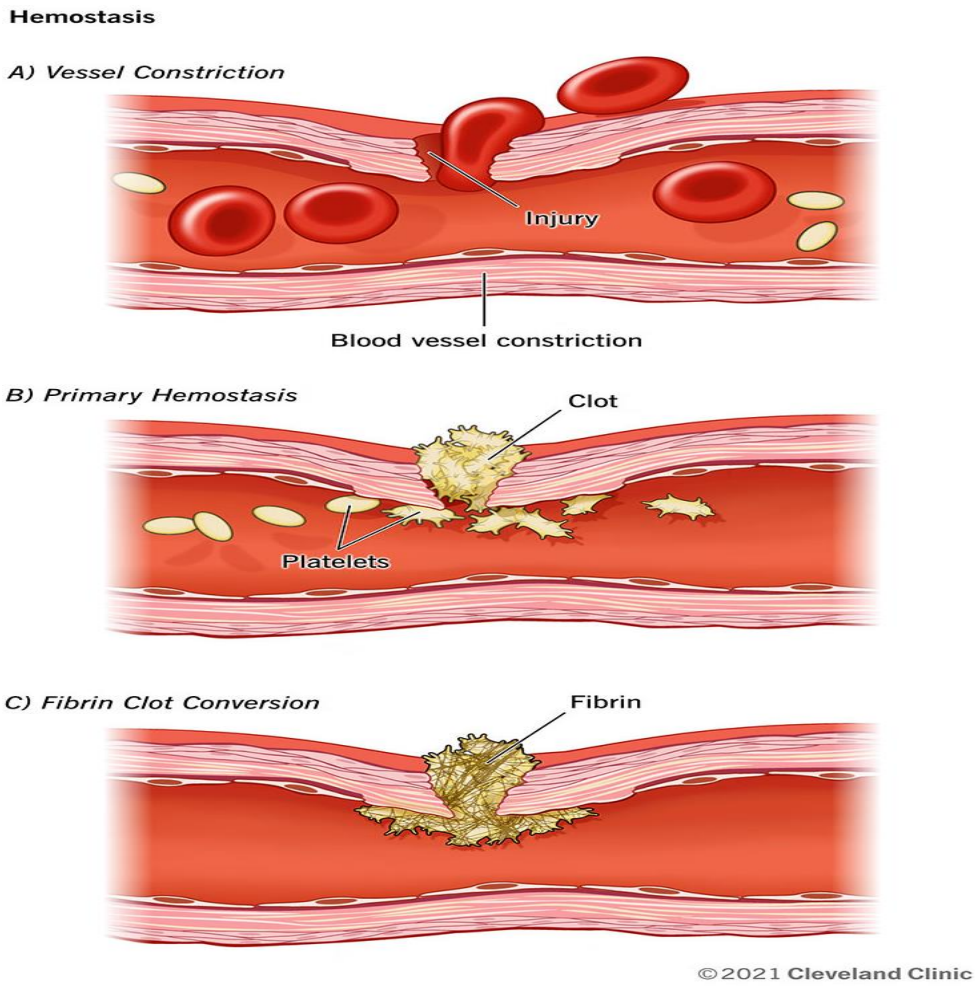
The results of the search and discussion section.

I. Primary hemostasis disorders in thrombocytopathies associated with GMD syndrome.

The primary site of hemostasis is the vascular and platelet stage of the clotting system, in which platelets play a significant role. When a blood vessel, artery, or vein, is damaged, platelets immediately begin the bleeding arrest phase by forming clumping clot plates at the site of damage to the vascular wall. This process consists of three consecutive steps: adhesion, secretion, and aggregation. During the adhesion phase, platelets attach themselves to the site of the vascular wall defect and spread across the vessel surface to stop bleeding. The secretion phase follows, marked by platelet activation, as platelets are inactive without vessel wall disruption. In turn, activated platelets release the contents of their granules. The final phase of primary hemostasis is aggregation, during which platelets stick together under the influence of biologically active amines, resulting in the formation of plugs. The outer part of the platelets contains receptors, while the inner part includes granules, which play a crucial role in the clotting process [19].

There are conditions in the development in which platelet function does not perform as it should, and its function is impaired. Due to impaired platelet plug formation, bleeding time might have prolonged, and patients with impaired platelet function are prone to form a hemorrhagic syndrome, which manifests as hematoma and spontaneous, prolonged bleeding. The underlying cause of platelet dysfunction can be related to a problem with the platelets themselves or with a single receptor or granule. Platelet dysfunction can be congenital or acquired [31].

Clinically, the hemorrhagic syndrome in thrombocytopathies is manifested by a microcirculatory type of bleeding, which can occur either alone or in combination with other hemorrhagic diatheses. Thus, congenital thrombocytopathy can be associated with hemophilia. According to *Suvorova N.M.,* the study revealed that 1/5 of children with hemophilia had manifestations of the microcirculatory type of bleeding, namely nasal bleeding, petechiae, ecchymoses, and gastrointestinal bleeding, the frequency of which was higher in the group of children with manifestations of connective tissue dysplasia syndrome. In addition, impaired platelet aggregation function was confirmed in 1/2 of the children with hemophilia. Among them, 80% had symptoms of connective tissue dysplasia, and 7.5% were detected in combination with an unexpressed reduction of the Willebrand factor. Platelet quality defects were detected during the assessment of serotonin concentration and release, manifested by impaired serotonin storage pool and serotonin release response, and the incidence of these defects was also higher in children with connective tissue dysplasia. Further, neonates with hemophilia, combined with hypocoagulation symptoms, were found to have both impaired platelet aggregation function and serotonin release from platelets [9, 25].



Picture 1. Hemostasis is how body plugs and repairs a wound
[https://my.clevelandclinic.org/health/symptoms/21999-hemostasis].

To verify the diagnosis of thrombocytopathy, the traditional method of diagnosis, light transmission aggregometry, and a more modern test of platelet functional activity based on flow cytometry before and after platelet activation is used. The clinical presentation of bleeding symptoms is assessed using the ISTHBAT scale. It is not always possible to correlate clinical signs of hemorrhage with laboratory findings, as there are cases in the literature where a severe hemorrhagic syndrome may have laboratory coagulogram indicators within normal limits, but asymptomatic patients show significant changes in hemostatic tests at the time of the prophylactic examination. According to a group of researchers in a retrospective evaluation of 50 children with thrombocytopathy, in which thrombocytopenia was an exclusion criterion, a comparative analysis of aggregometry and PFA with the ISTHBAT bleeding scale revealed that aggregometry had little susceptibility to platelet granule deficiency. In addition, abnormal results from the PFA test and without the defect were not differentiated when the ISTHBAT bleeding scale was applied [10].

In a further study, the subject was the detection of primary clotting pathology in children with bleeding manifestations in the absence of concomitant coagulopathy and thrombocytopenia and the evaluation of the correlation between the degree of clinical manifestations and platelet dysfunction. Of 32 children aged 1 to 17 years with various hemorrhages, the PFA study revealed that two children showed no defects, four children had a deficiency of integrin IIb3, and twenty-six patients had a variety of quantitative and functional platelet granule defects, among which, dense granule deficiency was detected in twelve patients. Combined defects were found in fourteen children. A direct correlation was found between the clinical bleeding index and the degree of integrin activation and dense granule release ($p<0.05$), besides a statistically significant correlation between the clinical bleeding index and isolated disorders of integrins or dense granules, was not found. It follows that the degree of change in the phenotypic features of platelets may correlate with the severity of manifestations of the hemorrhagic syndrome, which makes the use of the test of platelet functional activity promising in the diagnosis of the etiology of the hemorrhagic syndrome among the child population [6].

Inherited defects of platelet function are a heterogeneous cluster of rare clotting disorders, with symptoms ranging from mild to life-threatening. Accurate diagnosis of platelet function defects remains a challenge, even for specialists. Despite advances in understanding the etiology of these defects, in the vast majority of patients with inherited platelet disorders, the underlying mechanisms remain unknown. The treatment of platelet dysfunction will depend on the specific type of disorder as well as the severity of the bleeding. Platelet transfusion remains the

main therapy for severe bleeding. Alternatively, rFVIIa (90-120 μg/kg bolus infusion) can be given. In contrast to receptor defects, platelet function defects can be treated with desmopressin (0.3 μg/kg body weight intravenously or in case of mild bleeding with Octostim (Ferring) spray) and/or cyclocapron (tranexamic acid 20 mg orally) [21,13].

Moderate types in general, are very difficult to diagnose correctly. Current methods for assessing platelet function are hardly standardized and require proper laboratory equipment and staff competence. Analyses need to be carried out immediately after venipuncture, which is only possible in specialized central laboratories. Consequently, the prevalence of hereditary thrombocytopathies in the population remains underestimated.

The use of flow cytometry is currently one of the most promising methods for the diagnosis of inherited platelet abnormalities. This method detects several platelet functions simultaneously, including in patients with low platelet counts. In addition, flow cytometry requires a very small amount of blood, which is a priority for pediatric patients. One of the advanced diagnostic trends in congenital thrombocytopathies is molecular genetic diagnosis. The growth of molecular genetic diagnostics has been driven by advances in next-generation sequencing (NGS) technology, a method that allows a large number of samples to be examined in a single time frame, hence being financially and time-saving for the study.

It cannot be excluded that, in the next few years, molecular genetic diagnostic methods will replace first-line functional tests in the identification of thrombocytopathies. The interpretation of the sequencing results is challenging and it is sometimes difficult to establish a link between the phenotypic manifestations of hemorrhagic bleeding and the mutation. In addition, moderate hemorrhagic events are in most cases a combined condition that is the result of complex inheritance of several defects that do not individually induce hemorrhagic events. Consequently, the stepwise application of functional tests according to international guidelines is now basic in the diagnostics of platelet abnormalities of hereditary origin [11].

The morphological and functional parameters of the platelets of newborns and their mothers in norm and thrombohemorrhagic complications of pregnancy were analyzed. The state of platelets was assessed by automatic hematologic analysis, computer cytomorphometry, and aggregation capacity. Pathological changes in the platelet hemostasis of mothers suffering from thrombopathy have been found to affect the morphofunctional cell activity of their children, which is expressed in an increase in the quantitative, optical, geometrical, and functional properties of platelets [1].

The diagnosis of thrombocytopathies has its difficulties and peculiarities. The clinical manifestations of thrombocytopathies can be nasal bleeding, menorrhagia, and another bleeding. The absence of a poor family history does not exclude the presence of a spontaneous mutation leading to the development of the disease in the patient. In the presence of any history of bleeding of the microcirculatory type (easily formed ecchymoses, nasal and gingival bleeding), a detailed history and comprehensive examination of both plasma and platelet hemostasis must be performed. Genetic analysis is of course important to identify the specific mutation and, if possible, trace it back into the family tree. Patients with hereditary thrombocytopathy should be seen in specialized hematology centers [8].

The diagnostic search for hereditary thrombocytopathies must exclude thrombocytopenia, and plasma hemostasis disorders, followed by specific tests characterizing platelet aggregation properties and functional activity. For the differential diagnosis of Hermansky-Pudlak syndrome and Chediak-Higashi syndrome, morphological examination of peripheral blood cells and genetic analysis are essential [3, 19].

The light transmission aggregometry (LTA) method has been recognized as the "gold standard" for assessing platelet functional activity. The method is based on the photometer estimation of the light transmission capacity (% aggregation) of citrate-rich platelet plasma when an aggregation agonist (ADP, epinephrine, collagen, arachidonic acid, thromboxane, ristocetin) is added to it. Platelet aggregation induced by ristocetin, which activates Willebrand factor binding to the glycoprotein complex GPIb-IX-V, is also measured using LTA. In this test, data on the intake of medications and homeopathic drugs that may affect platelet aggregation must be considered [20].

Aggregometric features of several thrombocytopathies are known to be present. The absence of aggregation with all agonists except ristocetin indicates Glanzmann thrombasthenia. This diagnosis can be confirmed by flow cytofluorimetry, a quantitative assessment of the platelet membrane receptor IIb/IIIa. A significantly reduced response to all concentrations of ADP indicates a defect in the ADP receptor P2Y12. Decreased second wave aggregation by ADP and epinephrine and decreased aggregation with collagen may indicate a storage pool deficiency. To confirm dense granule deficiency, the functional status of dense granules should be examined by flow cytofluorimetry and electron microscopy. Thus, the recognition and differential diagnosis of thrombocytopathies should be based on a comprehensive study of hemostasis, examination of platelet morphology by light and electron microscopy, assessment of functional activity by flow cytofluorimetry, and genetic analysis to identify mutations correlating with different types of thrombocytopathies [4, 18].

A study by Russian researchers found that the acquired form of thrombocytopathies prevails over hereditary thrombocytopathies in children. It was observed more frequently in children from 11 to 14 years of age. In 55.5% of cases, the cause of acquired thrombocytopathy was the intake of drugs, NSAIDs, antibiotics, and antihistamines. The hemorrhagic syndrome manifested typical recurrent epistaxis and ecchymosis in 55% and 25%, respectively, with a normal platelet count, against a background of increased mean platelet volume, hypochromic anemia, prolonged Ivy bleeding time, addition coagulogram values were within reference values, in contrast, aggregation with ADP and adrenaline was impaired.

The initial diagnosis of platelet dysfunction in the pediatric population should include a set of hematological tests: analysis of mean platelet volume, Ivy bleeding time, coagulogram, measures of degree, time, and rate of platelet aggregation with ADP agonists, adrenaline, ristomycin. To

definitively verify the diagnosis of platelet dysfunction, abnormal aggregation must be recorded three times at 45-day intervals, with a precise protocol for the hemostasiogram tests [12,7].

Adolescents with menorrhagia or other mucocutaneous bleeding symptoms suggestive of clotting disorders should be screened for platelet dysfunction in conjunction with other clotting disorders, including Willebrand disease and factor deficiency. A complete blood count, including platelet count and platelet size, should be performed to determine thrombocytopenia. In addition, a blood smear test should be performed as part of the assessment. Previous platelet counts can help to distinguish acquired thrombocytopenia from congenital thrombocytopenia. Before testing platelet function, a thorough history of medication should be obtained and, if medically possible, medications that depress platelets should be discontinued 10-14 days before testing. Neither a bleeding time nor a platelet function assay -100 has sensitivity or specificity for screening or diagnosis of platelet dysfunction. Platelet light transmittance aggregatometry in platelet-rich plasma is considered the benchmark for diagnosing mild abnormalities of platelet dysfunction. Typical agonists include collagen, ADP, arachidonic acid, adrenaline, and ristocetin. Nonthrombocytopenic freshly prepared samples are required for platelet aggregometry. Platelet adenosine triphosphate release, valuable for diagnosing storage pool and secretion disorders, can be performed with Lumiaggregometry. Flow cytometry helps diagnose Bernard-Soulier syndrome and Glanzmann thrombasthenia [27, 29].

Table 1.

Primary hemostasis diagnostic tests.

Primary hemostasis diagnostic tests		
	Screening tests	Description
1	Complete blood count: platelet count and platelet size, mean platelet volume	helps to distinguish acquired thrombocytopenia from congenital thrombocytopenia, exclude thrombocytopenia
2	Ivy bleeding time	time more than 10 minutes are concerning for coagulopathy, method is more accurate but has an increased scarring risk. Normal bleeding time less than 8 minutes
3	Duke bleeding time	times greater than 5 minutes indicates to coagulopathy, method is less accurate and carries a higher hematoma rate. Normal bleeding time less than 3 minutes
4	Coagulogram	blood clotting factors assays (Factor I, Factor II, Factor VIII, IX, for exclusion secondary hemostasis disorders
5	Platelet light transmittance aggregatometry	detection of time and rate of platelet aggregation with ADP agonists, adrenaline, collagen, arachidonic acid, tromboxane, ristomycin.
6	Lumiaggregometry	method for detection of platelet function disorders, platelet adenosine triphosphate release, valuable for diagnosing platelet storage pool and secretion disorders
7	Flow cytometry	helps diagnose Bernard-Soulier syndrome and Glanzmann thrombasthenia
8	POCT (Point-of-care testing of platelet count)	provides real-time data for rapid decision method, similar results with the reference method and good correlation between capillary and venous blood samples, provides point-of-care assessment of normal and thrombocytopenic platelet counts from fingerprick blood with high precision and limited interferences

A comparative review of platelet function assessment tests has shown that work continues to transform the various platelet function assessment tests into diagnostic tools for assessing clotting disorders and monitoring antiplatelet therapy. The available POCT platelet function assessment system (point-of-care testing or testing at the patient's bedside) makes in laboratories and intensive care units, allowing their use in various clinical settings, such as inherited clotting disorders, intensive cardiovascular care, traumatic coagulopathy, liver transplantation and obstetric care for bleeding prognosis. Similarly, the use of these POCT tests could be extended not only at the patient's bedside in critical areas outside the specialist laboratory, but also in centralized and remote laboratories. Newly available POCTs can be considered useful supplements to existing well-established functional platelet tests, but further prospective studies are needed to determine the use of these tests. Future improvements in the study of the platelet genome and proteome may include knowledge of platelet function testing with notable implications for the diagnosis and management of patients with hemorrhagic or thrombotic defects [28, 14].

In the same way, according to a study by *Connie H. Miller,* gender, race, diet, and testing system affect platelet function test results in healthy individuals, and these differences should be considered when interpreting results in patients referred for bleeding evaluation. Adrenaline and ristocetin are particularly problematic; abnormal results observed with only one of these agonists may reflect population changes and should be interpreted with caution. Exclusion of flavonoid-rich foods from the diet 24 h before testing may reduce false-positive results. Systems with the lowest intra-individual variability and fewest false positives might be the best choice for testing patients; increased specificity often leads to decreased sensitivity. A similar comparison of methods using patients with known defects in platelet function would be required to determine whether methods demonstrating the least variability retain sufficient

sensitivity to detect mild platelet defects and whether measurements that reduce specificity significantly improve diagnostic efficacy. Because of the high frequency of abnormal results observed in normal individuals, it is necessary to confirm all abnormal results by demonstrating the reproducibility of the defects in another sample, as well as examining specific receptors, granules, or DNA, to ensure an accurate diagnosis and avoid mislabelling patients as having platelet dysfunction [23, 30].

Despite recent advances in this field, there is no uniform approach to the assessment of a child with symptoms of mild bruising and/or bleeding. A detailed personal and family history of bleeding symptoms should be obtained. Future research will show whether tools such as the pediatric bleeding questionnaire can be used as screening tools to identify patients who require additional laboratory tests when initial screening is normal. The physical examination cannot be forgotten as an important part of the assessment, and assessment of joint mobility should be included in every initial visit, especially in pediatric patients. Platelet dysfunction is one of, if not the most common inherited clotting disorders. However, testing for such disorders is time-consuming and requires a stepwise approach. Differences in methodology and interpretation of platelet aggregation testing adversely affect our ability to differentiate the literature and make meaningful clinical decisions. Hematologists need to understand the indications and limitations of platelet function testing methods used by their institutions. Although recently published guidelines will hopefully improve the standardization of techniques such as platelet aggregometry, population studies are still needed to better understand the expected laboratory results of common facilities, such as platelet storage and release defects [26, 16,17].

The diagnosis of hereditary blood clotting disorders (HBCs) remains a challenge, especially for inherited platelet disorders, due to the heterogeneity of the clinical and laboratory phenotype, the limited specificity of platelet function tests, and a large number of potential causative genes. Disclosure of the underlying molecular defect provides an effective diagnosis of HBCs, facilitating prognosis and clinical care, which is particularly important in implicating severe clinical syndromes and may be associated with an increased risk of malignancy. Until recently, Sanger sequencing of candidate genes was the only method of molecular diagnosis, but this approach is time-consuming and expensive and requires phenotype-based identification of any obvious candidate gene(s). High-tech sequencing (HTS) now enables simultaneous and rapid investigation of several genes at an affordable cost. This HTS technology, which includes targeted sequencing of predefined genes, whole-genome sequencing, or complete genome sequencing, is revolutionizing the genetic diagnostics of human diseases. With its widespread adoption in research and clinical practice, HTS is rapidly improving the molecular characterization of HBCs. However, despite this powerful approach, many patients still do not receive a diagnosis. As HBCs are complex and rare diseases, the development of better laboratory assays, improved bioinformatics systems, and the formation of interdisciplinary teams are recommended to improve our understanding of HBCs [22, 15].

Conclusion. Based on the above literature review, the difficulty in accurately verifying hereditary thrombocytopathies is that platelet defects can often be complex, combining granule and platelet receptor defects. Thrombocytopathies are an integral component of GMD, often accompanying coagulopathies such as hemophilia, and Willebrand's disease. In addition, the phenotype of the disease may not correlate with the genotype, hence the diagnosis of platelet dysfunction requires a multidisciplinary examination of platelet function, performing several methods, standard and genetic, as well as correlation analysis according to the ISTHBAT bleeding scale. Such a comprehensive approach to the diagnosis of thrombocytopathies based on GMD facilitates a much easier understanding of the etiopathogenesis of platelet defects and will help to interpret the results more effectively, allowing a more accurate algorithm for the management of primary hemostasis pathology to be defined.

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