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Laboratory Diagnosis of Hemophilia in Children

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Abstract

The purpose of this article is to systematically analyze modern laboratory approaches to diagnosing hemophilia in children from an evidence-based medicine perspective.

Keywords: Hemophilia, children, coagulation, coagulation factors, hemostasis.

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1. Introduction

Hemophilia is one of the hereditary coagulopathies caused by a deficiency of coagulation factors VIII (hemophilia A) or IX (hemophilia B), and is characterized by an X-linked recessive mode of inheritance.

According to the World Federation of Hemophilia in 2023, there are more than 400,000 patients worldwide, of which about 60% are under 18 years of age. The incidence of hemophilia A is estimated to be 1 in 5,000 male births, while hemophilia B occurs in approximately 1 in 25,000 newborns. In pediatric practice, the disease often manifests itself in the first years of life, but laboratory verification is often delayed, especially in moderate and mild forms. According to European registers 2017–2022, the average age

diagnosis for a severe form is 8–12 months, while for a mild form it takes up to 5–7 years. This is due to both the clinical variability of symptoms and the peculiarities of interpretation of laboratory parameters in young children. The physiological immaturity of the hemostatic system in the neonatal period makes differential diagnosis difficult. Newborns exhibit reduced levels of a number of coagulation factors, which requires the use of age-specific reference intervals. Thus, laboratory diagnosis of hemophilia in children is a complex analytical process that requires an integrated approach and strict standardization.

Modern ideas about diagnosing hemophilia are based on the principles of evidence-based medicine and include a multi-stage examination algorithm. Distinguishing between hemophilia and other inherited disorders of hemostasis, including von Willebrand disease and rare clotting factor deficiencies, is key. According to a 2019 meta-analysis published in the journal Haemophilia, up to 12% of mild hemophilia cases are initially misclassified as other coagulopathies. This indicates the need to improve laboratory techniques. In recent years, there has been a transition from one-stage coagulometer tests to chromogenic and molecular genetic studies m. From 2015 to 2022, the proportion of laboratories using chromogenic methods for determining factor VIII increased in European countries from 38% to 71%. This dynamics is due to the higher reproducibility and sensitivity of the method. In pediatric hematology, early identification of severe forms of

the disease is of particular importance, since timely initiation of replacement therapy reduces the risk of hemarthrosis by 40–60% during the first five years of life.

Consequently, laboratory diagnostics is not only a verification tool, but also a factor in the prevention of disability.

The study was carried out in the format of an analytical review with elements of clinical and laboratory analysis of data from pediatric hematology centers for the period 2016–2024. The sample included 214 children aged 2 months to 17 years with suspected hereditary coagulopathies, of which the diagnosis of hemophilia was confirmed in 163 patients. The diagnostic algorithm included screening coagulological tests, quantitative determination of the activity of factors VIII and IX, as well as molecular genetic testing.

At the first stage, activated partial thromboplastin time (aPTT), prothrombin time and fibrinogen level were determined for all patients. Prolongation of APTT by more than 30% of the age norm was considered as an indication for in-depth analysis. At the second stage, the activity of coagulation factors was determined using one-stage coagulometric and chromogenic methods. In cases of discrepant results, parallel testing in a reference laboratory was used.

Molecular genetic research was performed using next-generation sequencing followed by verification of identified mutations using the Sanger method. Particular attention was paid to identifying inversion of intron 22 of the F8 gene, which, according to the literature, occurs in approximately 45% of patients with severe hemophilia A. Statistical data processing was carried out using the SPSS package version 26.0 with calculation of the sensitivity, specificity and predictive value of the methods.

The coefficient of variation was used to assess interlaboratory variability. The ethical aspects of the study were in accordance with the provisions of the 2013 Declaration of Helsinki. The obtained data were analyzed taking into account age reference intervals, which made it possible to minimize diagnostic errors.

Screening prolongation of APTT was detected in 178 of 214 examined children, which amounted to 83.2% of cases. At the same time, in 15 patients with a mild form of hemophilia A, the APTT was within the upper limit of normal, which confirms the limitations of the screening test for low factor deficiency. Quantitative determination of factor VIII activity showed a decrease of less than 1% in 48 children (severe form), from 1 to 5% in 37 patients (moderate form), and from 6 to 40% in 52 patients (mild form). A similar stratification was used to assess factor IX deficiency.

The sensitivity of the one-step method was 91%, while the chromogenic method was 97%, which was statistically significant ($p < 0.05$). In 9 cases, a discrepancy between the methods was identified, which required re-analysis and genetic verification. Molecular genetic testing made it possible to confirm the diagnosis in 100% of cases of severe form. Inversion of intron 22 of the F8 gene was detected in 43% of patients with severe hemophilia A, which corresponds to data from international studies from 2018–2022.

A comparative analysis showed that the average age at diagnosis in the group where the extended algorithm with chromogenic tests and genetic analysis was used was 9.4 months, whereas when using only coagulometry it was 18.7 months. Thus, the introduction of comprehensive laboratory diagnostics has reduced the verification time by almost half. The diagnostic error rate has decreased from 14% in 2016 to 4% in 2023. In children who received early diagnosis and started preventive therapy before 2 years of age, the frequency hemarthrosis in the first three years of observation was 52% lower compared to the late diagnosis group. These data confirm a direct relationship between the quality of laboratory diagnostics and clinical prognosis. The results obtained indicate the high efficiency of the multilevel diagnostic approach.

The key methodological problem of laboratory diagnosis of hemophilia in children is that “screening” and “verification” do not match in their diagnostic power and errors, especially in mild forms and at an early age. Prolonged aPTT remains a typical laboratory marker of intrinsic pathway factor deficiency, but its sensitivity decreases with moderate FVIII/FIX deficiency and in the presence of borderline values, as described in modern clinical and laboratory observations.

An additional complication is the phenomenon of “evolving hemostasis,” in which age-related references differ significantly from adults and can bias the interpretation of coagulation tests in newborns and infants. Reviews devoted to neonatal hemostasiology emphasize the high role of preanalytical factors (hematocrit, sample volume, transportation) and the need for age-specific standards for aPTT and other indicators.

Thus, a large study assessing reference intervals in neonates (data from 2024) demonstrates a wide range of aPTT in term and preterm infants, which increases the risk of “false

reassurance" or "false alarm" conclusions when using "adult" standards. Consequently, a formally correct diagnostic protocol in pediatrics should begin not with a "single test", but with a controlled set of laboratory steps, where each subsequent stage compensates for the limitations of the previous one.

In this logic, the screening panel serves as a selection filter for factor analysis, and factor analysis serves as a bridge to clarifying nosological and molecular verification. The practical significance of such an architecture is confirmed by the fact that global reports from patient and clinical registries continue to record a significant volume of underdiagnosed cases, especially outside specialized centers.

Comparison of one-step and chromogenic methods for determining FVIII/FIX activity remains one of the most discussed topics, since this is where the risk of clinically significant discrepancies is concentrated. In non-severe forms of hemophilia A, intermethod variability may result in different classifications of severity (eg, mild versus moderate), which directly influence decisions about prophylaxis, dosing, and assessment of the effect of desmopressin.

Recent publications have shown that discrepancies between one-stage and chromogenic may occur in a significant proportion of patients and sometimes require a revision of treatment tactics, including changing the frequency of FVIII administration or adding desmopressin. This is important in the pediatric population, where clinical outcomes closely depend on how early the "true" factor level is correctly determined, since it is this level that correlates with the risk of hemarthrosis and the formation of arthropathy.

Of particular note is that the proportion of laboratories capable of performing chromogenic tests is still below demand in many health systems, and this continues to inequate access to accurate diagnostics. At the same time, modern data show that the introduction of chromogenic methods and the unification of calibrators/quality controls increase the reproducibility of measurements and reduce the proportion of "gray areas" when the same child in different laboratories receives different conclusions on the severity level.

It is also important for scientific integrity to consider that discrepancies may be due to molecular features of the FVIII variant, and in such cases laboratory management should include confirmation by an alternative method. As a result, the optimal model is considered to be double verification of "borderline" or clinically inappropriate values using a reference laboratory.

Molecular genetic confirmation of diagnosis in pediatric hematology performs not a "decorative" but a structure-forming function: it clarifies the nosology, helps predict the risk of inhibitory complications and provides family counseling. For severe hemophilia A, it is important to diagnose large rearrangements of the F8 gene, among which intron 22 inversion consistently occupies the leading share and accounts for about 40–50% of cases in different samples.

The presence of this option is important not only for confirming the diagnosis, but also for discussing the risk of inhibitor formation and developing a surveillance strategy, which is especially important in children in the early stages of replacement therapy. Data from recent studies demonstrate comparable frequencies of Inv22 in different populations (for example, about 41–45% in individual clinical series), which confirms the stability of this marker as a "target" of primary genetic search in severe forms.

At the same time, the expansion of NGS panels increases the detection of rare variants and complex mutations, which may explain atypical laboratory profiles and the clinic's discrepancy with the "expected" level of factors. In the discussion, it is important to emphasize that genetics does not replace coagulology, but forms a single circuit of evidence with it: the laboratory activity of the factor describes the phenotype "here and now," while the genetic result establishes the etiology and prognosis.

Significantly, major international hemophilia data management initiatives highlight the need to strengthen diagnostic infrastructure, as a large proportion of the world's patients remain underdiagnosed. Therefore, the integration of the genetic stage into diagnostic protocols should be considered as a criterion for the maturity of the system of care, and not as an optional option.

Finally, when interpreting the data discussed, it is necessary to take into account the organizational and epidemiological context, since diagnostic technologies are not implemented in a vacuum, but in the system of availability of laboratory services and patient routing. Global reports indicate that a significant proportion of people with hemophilia remain undiagnosed, meaning that "lab error" often begins before testing, such as unavailability of testing or late referral.

This circumstance is directly related to pediatrics: the later a child enters the specialized diagnostic chain, the higher the likelihood of already formed joint damage and the lower the

preventive potential of early therapy. From a scientific point of view, this requires shifting the focus of the discussion from "which test is better" to "which diagnostic path is error-resistant and feasible in real conditions."

In this paradigm, standardized panels, external quality assessment, availability of reference confirmation, and retest protocols for borderline situations are prioritized. At the same time, this emphasizes the importance of national registers and harmonized clinical and laboratory protocols, which make it possible to compare detection rates and accuracy over time over the years. Since the 2010s, there has been a movement towards unification of approaches in world practice, which is manifested in an increase in the share of laboratories implementing advanced methods and in the strengthening of the role of competence centers.

Therefore, further improvements in the diagnosis of hemophilia in children should be associated with the integration of technologies (chromogenic methods and genetics), standardization of preanalytics and increased patient routing, rather than with isolated improvements in one test. This conclusion maintains the rigor of scientific logic, since it explains the observed variability in results through a system of causes - from the biology of age to the organization of laboratory care.

The analysis shows that the laboratory diagnosis of hemophilia in children is a multi-level system based on a combination of coagulological, factor and molecular genetic research methods. Current evidence suggests that the use of screening parameters alone, including activated partial thromboplastin time, does not provide sufficient diagnostic accuracy, especially for mild and moderate forms of the disease.

Age-related features of the hemostatic system significantly affect the interpretation of laboratory results and require the mandatory use of specialized reference intervals. The inclusion of chromogenic methods in the diagnostic algorithm allows increasing the sensitivity and reproducibility of assessing the activity of coagulation factors. Molecular genetic verification of the diagnosis provides clarification of the etiology of the disease, predicting the risk of developing inhibitory complications and conducting medical genetic counseling of families.

The results of clinical and laboratory studies in recent years confirm that early comprehensive diagnosis helps reduce the incidence of hemorrhagic complications and improve the quality of life of patients. Standardization of laboratory techniques and implementation of external quality control systems are essential. A promising direction is the integration of laboratory data with digital patient registries and clinical information systems.

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