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BIOCHEMICAL, GENETIC DIAGNOSIS AND THERAPEUTIC PROGRAMS OF HEMOPHILIA IN CHILDREN

322.01 – PEDIATRICS AND NEONATOLOGY

Abstract of the PhD Thesis in Medical Sciences

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The thesis was elaborated within the Department of Pediatrics, Nicolae Testemițanu State University of Medicine and Pharmacy of the Republic of Moldova, founding consortium of the Doctoral School in Medical Sciences.

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INTRODUCTION

Hemophilia is a rare genetic disorder transmitted in a recessive manner and linked to the X chromosome, caused by a deficiency of coagulation factors VIII (hemophilia A) or IX (hemophilia B). Type A is the most common form, with an estimated incidence of 1:5,000–10,000 male newborns, while type B affects approximately 1:20,000 newborns [1]. The clinical and social impact of hemophilia is significant, leading to spontaneous or post-traumatic hemorrhagic episodes, progressive joint disability, and decreased quality of life. In the 20th century, the life expectancy of patients with severe forms rarely exceeded 20 years; however, the introduction of factor VIII–rich cryoprecipitate (1965) and subsequent prophylactic therapies radically changed the prognosis [2]. Globally, hemophilia is recognized as a major public health issue among rare diseases. Recent estimates indicate approximately 1.1 million patients, of whom fewer than 40% are diagnosed and over 70% do not receive appropriate treatment [3]. Inequities are particularly evident in low- and middle-income countries, where limited access to therapy increases the risk of disability and premature mortality. International organizations such as the World Federation of Hemophilia (WFH) and the World Health Organization (WHO) have launched global programs, including Treatment for All and the WFH Guidelines (2020 edition), which serve as key milestones for the standardization of care [4]. Therapeutic advances in the last decade (2015–2024) have brought unprecedented change. Extended half-life clotting factors, bispecific antibodies such as emicizumab, and the first gene therapies authorized for hemophilia B (Hemgenix, 2022) and hemophilia A (valoctocogene roxaparvovec, 2023) have been developed [5,6]. These innovations mark the beginning of a new era with the potential for long-lasting correction of the coagulation deficit. Nevertheless, persistent challenges such as inhibitor development (≈30% in severe forms), variable response, high costs, and long-term safety concerns still limit their widespread application [7,8]. Moreover, access to modern therapies remains unequal between high-income and resource-limited countries [9].

In the Republic of Moldova, hemophilia is included in the National Program for Rare Diseases, financed from public funds. Approximately 220 patients (≈52 children and 170 adults) are monitored, most of them in specialized centers in Chișinău [10]. In 2023, the authorities allocated ≈61.5 million Moldovan lei for the treatment of rare diseases, including hemophilia, reflecting the public commitment toward this patient group [11]. A major progress was achieved in 2019, when emicizumab was introduced for the treatment of children with hemophilia A with inhibitors [12]. However, the absence of a functional national registry, limited access to genetic testing, and insufficient multidisciplinary rehabilitation justify the need for applied research and personalized intervention models. In the regional context, Eastern European countries face similar challenges — underdiagnosis, limited access to modern therapies, and insufficient healthcare infrastructure. According to the European Hemophilia Consortium, in 2018 only 13% of the countries in the region had national registries and dedicated councils, while ≈37% of patients benefited from full prophylaxis, compared with ≈100% in Western Europe [13].

Based on these considerations, the **aim** of the present scientific study was established: To analyze the clinical manifestations, biochemical and genetic parameters in the diagnosis of hemophilia A and B in children, with the purpose of optimizing paraclinical diagnosis and fine-tuning an individualized therapeutic model adapted to the form and severity of the disease.

Objectives of the study

1. To analyze the clinical manifestations of the hypocoagulation syndrome in children with hemophilia;

2. To determine the quantitative and qualitative levels of coagulation factors in hemophilia A and B in children;
3. To analyze the relationship between clinical manifestations (phenotype) and genetic mutations (genotype) in pediatric hemophilia, in order to enhance the diagnostic utility of genetic testing;
4. To assess the presence of inhibitors to coagulation factors VIII and IX in patients with hemophilia and determine their titers in the context of therapeutic management;
5. To perform a comparative analysis of the efficacy and safety of substitution therapy with lyophilized/recombinant factors VIII/IX and cryoprecipitate in the treatment of hemophilia, in order to optimize therapeutic approaches for coagulation factor deficiency;
6. To develop a database on the online platform "Electronic Registry of Patients with Hemophilia in the Republic of Moldova."

Research methodology. The study was conducted within the Department of Pediatrics, *Nicolae Testemițanu State University of Medicine and Pharmacy of the Republic of Moldova*, in collaboration with the *Mother and Child Institute* (Department of Benign Hemopathies). The research included a sample of 90 children aged between 0 and 18 years, from both urban and rural areas. To achieve the proposed objectives, an observational, descriptive, cross-sectional, and selective study was designed. The research was carried out during 2018–2022 and included all 59 children diagnosed with hemophilia and monitored during this period in the clinic. To complement the analysis and obtain a detailed comparison of therapeutic strategies, an additional retrospective group of 31 children treated with cryoprecipitate during 2010–2013 was included. This retrospective phase enabled an extended evaluation of the effects of specific treatment over a longer duration, providing a comparative perspective on therapeutic methods. Participants underwent a comprehensive assessment including standard clinical evaluation and laboratory testing to determine coagulation indicators (Lee-White coagulation time, aPTT, PT, fibrinogen). Quantitative determinations of coagulation factors VIII and IX, inhibitor titers, and genetic mutation spectrum analyses were also performed. Subsequently, correlations were made between disease severity, clinical manifestations, genetic mutation types, and inhibitor titers. The obtained results were used to formulate conclusions and practical recommendations. Statistical analysis was performed using the SPSS (Statistical Package for the Social Sciences) software, version 20.

Scientific novelty of the research. For the first time in the Republic of Moldova, a comprehensive study was conducted on pediatric patients with hemophilia, focusing on the assessment of clinical and paraclinical features and identification of specific genetic mutations. The severity of hemophilia (assessed by clinical data and factor VIII/IX levels) was analyzed in association with the identified genetic mutations; inhibitor titration for coagulation factors VIII and IX was performed in children with hemophilia; and a database for National Registry for Monitoring Patients with Hemophilia was developed.

Theoretical significance. For the first time in the Republic of Moldova, the clinical and paraclinical features of pediatric hemophilia were comprehensively analyzed according to disease severity. Genetic mutations in hemophilia A and B were identified and classified by clinical form, and inhibitor titers to factors VIII and IX were determined for the first time in children. Correlations between genetic findings, inhibitor levels, and clinical severity were established, providing insight into phenotypic variability. The study proposes modern diagnostic and management approaches integrating clinical, genetic, and immunological data and led to the development of a National Pediatric Hemophilia Registry database, facilitating standardized data collection for this rare disease.

Applied value of the study. The study established the necessity of genetic testing for hemophilia A and B to facilitate early identification of severe forms. The results allow for personalized therapeutic guidance adapted to the genetic and immunologic profile of each patient. Determining inhibitor titers for coagulation factors VIII and IX provides essential support for preventing and managing treatment-related complications, including selection of alternative therapies such as bispecific monoclonal antibodies that bypass factor VIII and ensure effective control of bleeding in patients with inhibitors. The findings contribute to the optimization of therapeutic protocols and prophylactic strategies in pediatric hemophilia. The development of the database for National Registry for Monitoring Patients with Hemophilia serves as a practical tool for continuous surveillance, epidemiological assessment, and treatment planning. The study provides a valuable scientific foundation for the development of national public health policies regarding rare diseases in the Republic of Moldova.

Implementation of results. The research results have been integrated into the clinical practice of the Benign Hemopathies Department at the Mother and Child Institute and specialized clinics of the Oncology Institute of the Republic of Moldova, as well as into the educational process of the Nicolae Testemițanu State University of Medicine and Pharmacy, contributing to the theoretical and practical training of specialists in the field.

The thesis was discussed, approved, and recommended for public defense at the meeting of the Department of Pediatrics (minutes dated 12.05.2025), the Specialized Scientific Seminar 322. Pediatrics (minutes no. 2 of 08.10.2025), and the Scientific Council of the Consortium (minutes no. 1/1 of 17.11.2025), at the Nicolae Testemițanu State University of Medicine and Pharmacy of the Republic of Moldova. The positive opinion of the Research Ethics Committee of Nicolae Testemițanu SUMPh was issued under No. 6 on 10.11.2017.

Publications related to the thesis. The scientific findings have been presented in 25 publications (13 national, 12 international, 1 single-author), including: 2 articles in international scientific journals with impact factor (8.16), 3 articles in accredited national scientific journals (Category B+), 1 chapter in a monograph (sole author), 2 innovation patents, 2 implementation certificates, 3 conference abstracts, 4 oral presentations at international scientific conferences, 5 oral presentations at national conferences with international participation, and 4 poster presentations at international forums.

Keywords: Pediatric hemophilia, coagulation disorder, coagulation factors, phenotype-genotype, factor VIII inhibitors, substitution therapy, cryoprecipitate, recombinant factor, emicizumab, therapeutic models, national hemophilia registry.

Volume and structure of the thesis. The thesis follows the traditional format, comprising 146 pages, including the introduction, 6 chapters, discussions, general conclusions, recommendations, and a bibliography citing 139 sources. The iconography includes 37 tables and 15 figures (representing 22% of the core text volume).

Practical importance of the study. This study supports the optimization of individualized treatment and monitoring of children with hemophilia. Strengthening a national registry enables systematic data collection, evaluation of treatment effectiveness, and improved long-term management, contributing to research, epidemiological insight, and public health planning. Non-factor therapies offer an effective alternative for patients with inhibitors, significantly reducing bleeding episodes. Early detection and monitoring of inhibitors are essential for treatment adjustment and prevention of complications, ultimately improving quality of life.

1. MATERIALS AND RESEARCH METHODS

1.1. General Characteristics of the Research and Design of the Study Sample Volume

The project was conducted at the Department of Pediatrics, *Nicolae Testemițanu State University of Medicine and Pharmacy*, based at the *Mother and Child Institute (Department of Benign Hemopathies)*, and included 90 children (aged 2–17 years and 11 months) from both urban and rural areas.

Design of the study sample volume. An observational, descriptive, cross-sectional, and selective study was planned (2018–2022), including all children diagnosed with hemophilia and monitored in the clinic. For comparison of therapeutic strategies, a retrospective sublot of patients treated with cryoprecipitate (2010–2013) was added, allowing for the evaluation of treatment effects over 3 years and comparative analysis over time.

Study population. Between 2010 and 2022, 108 children with hemophilia were identified; 90 (83.3%) were included in the research through active participation (2018–2022) or retrospective analysis of records (2010–2013), ensuring representativeness and comparability between therapeutic methods.

Justification for retrospective inclusion. Given the rarity of hemophilia, the inclusion of previously treated patients expanded the analytical base and allowed evaluation of therapeutic model evolution across a relevant time frame.

Methodological justification. The low prevalence of the disease required near-complete inclusion of the eligible population within the department over the study period, minimizing selection bias and strengthening the external validity of the observational design.

Research Methodology. *Stage 1.* Selection of 90 children according to inclusion criteria: diagnosed hemophilia; age 0–18 years; Moldovan citizenship; ability to communicate and comply with study requirements; informed consent from the legal representative and assent from participants ≥14 years. Exclusion criteria: presence of other coagulopathies; lack or withdrawal of informed consent. Examinations included: clinical assessment of hemorrhagic syndrome; coagulation profile (aPTT, PT, fibrinogen, Lee–White test); anemia parameters (Hb, RBC, Ht); hepatic and cholestatic markers (ALT, AST, total and fractionated bilirubin); quantitative assays of FVIII and FIX. In 39 children, mutations of FVIII and FIX genes were analyzed; inhibitor titers were determined in the later phase following implementation of the National Protocol for Hemophilia with Inhibitors.

Stage 2. Grouping of patients according to the type of deficiency (Hemophilia A – FVIII; Hemophilia B – FIX) and sub-grouping by severity based on factor level: mild 5–50%, moderate 1–5%, severe: <1%.

Stage 3. Comparative analysis between subgroups according to: type and mode of inheritance of hemophilia; severity and clinical expression (e.g., hemarthroses); phenotype–genotype correlations.

Stage 4. Analysis of 32 cases followed for 3 years, divided by treatment type: 16 cryoprecipitate vs. 16 recombinant factor. The number of hospitalizations and clinical pattern of hemorrhagic syndrome were evaluated according to therapeutic model.

Stage 5. Formulation of conclusions and development of practical recommendations based on obtained results.

Ethical Aspects. All participants (and/or their legal representatives) were informed about the study procedures and requirements, and written informed consent (and assent for children ≥14 years) was obtained. No incentives were offered, and participation involved no costs. The study

adhered to international medical ethics standards established by the Declaration of Helsinki, ensuring confidentiality of participant data. The research protocol was approved by the Research Ethics Committee of the Nicolae Testemițanu State University of Medicine and Pharmacy (Minutes No. 06, dated 10.11.2017). The data obtained were communicated only to the respective participant; personal data were not used or disclosed for other purposes.

Children with hemophilia (n = 90)

↓

Evaluation of clinical findings and hypocoagulation syndrome

↓

Laboratory tests:

- Hemoleucogram: Hb, Er, Ht
- ALT, AST, Total bilirubin and fractions
- Thrombin time
- Secondary hemostasis: Prothrombin time, Fibrinogen, TTPa, Lee-White time

↓

Qualitative and quantitative analysis of plasma levels of factors VIII and IX

- Mild form: 5–50%
- Moderate form: 1–5%
- Severe form: <1%

↓

Molecular-genetic analysis of F8 and F9 gene mutations (n = 39)

↓

Investigation of anti-factor VIII/IX inhibitors (n = 41)

↓

Comparative study of replacement therapy regimens (n = 32):

- Treatment with cryoprecipitate (n = 16)
- Treatment with recombinant factor VIII/IX (n = 16)

↓

Correlation between genetic profile and clinical-hematological manifestations

↓

Development of the datebase for National Registry for Multidisciplinary Monitoring of Patients with Hemophilia in the Republic of Moldova

Figure 1. Study Design

1.2. Research Methods

The study applied historical, comparative, descriptive, clinical, and laboratory methods, each serving a specific role in the foundation and implementation of the research.

Historical method. Enabled the analysis of the evolution of hemophilia diagnosis and treatment in the Republic of Moldova through the study of literature, medical reports, and archival documents. This provided insight into the progress achieved—from traditional methods to genetic testing—and the impact of public health policies on access to modern therapies.

Comparative method. Facilitated identification of similarities and differences between local and international therapeutic approaches. By comparing clinical, biochemical, and genetic data, the efficacy of treatments applied in Moldova was assessed, and recommendations for optimization of care strategies were formulated.

*Descriptive method***.** Documented the clinical, biochemical, and genetic characteristics of pediatric patients with hemophilia, outlining the profile of hemorrhagic manifestations, biological parameters, and diagnostic and treatment peculiarities at the national level.

Clinical methods. Included detailed medical history and comprehensive physical examination with attention to external bleeding signs, cavitary hemorrhages, and recurrent hemarthroses. Major joints were assessed by inspection, palpation, and mobility testing. Differential diagnosis of hemorrhages was made according to localization and context (external, deep, intra-articular, visceral). In suspected cases of internal bleeding, imaging techniques (ultrasound, CT, MRI) were used. Joint monitoring was based on the Gilbert and HJHS scores, complemented by clinical observations regarding hemarthrosis recurrence and functional limitations. This integrated approach enabled precise clinical characterization and development of personalized therapeutic strategies.

Laboratory Methods. Biochemical, hematologic, and coagulation tests were performed in the Clinical Laboratory of the Mother and Child Institute using venous blood samples collected in the morning, fasting, from the cubital vein. Coagulation tests were performed in 3.2% citrate vacutainers (blood: anticoagulant ratio 9:1); samples were centrifuged within 4 hours, aliquoted after secondary centrifugation, and frozen. Exclusion criteria for samples: underfilled tubes, hemolysis/clotting, lack of freezing during transport. Biochemical tests were processed on a Cobas C311 analyzer; complete blood counts on a Yumizen H500 analyzer.

Quantitative determination of FVIII and FIX (coagulometry). *Principle:* Coagulation time measured in the presence of cephalin and an activator, mixed with "substrate" plasma containing all factors except the tested one. Activity is expressed as a percentage of normal.

FVIII: circulates bound to vWF; thrombin activation generates FVIIIa, cofactor in the tenase complex (FVIIIa–FIXa–Ca^{2+}) producing most FXa. Reference values: 50–150%. Note: In von Willebrand disease (especially type 2N), FVIII may be reduced with relatively preserved vWF:Ag/ristocetin ratio.

FIX: a vitamin K–dependent serine protease acting in the intrinsic tenase complex with FVIIIa; during propagation, it is the main generator of FXa. Reference FIX activity: <7 days – 35–60%; 8 days–1 year – 45–110%; 1–10 years – 60–100%; >10 years – 70–120%. Plasma stability: ≈4 h at room temperature, 3 weeks at −20°C, >1 year at −70°C.

Determination of anti-FVIII/FIX inhibitors. Context: IgG alloantibodies against FVIII (more frequent) or FIX appear as a reaction to substitution therapy, clinically manifesting through suboptimal therapeutic response and prolonged aPTT. Incidence varies (higher in severe hemophilia; increased risk in major F8/F9 mutations).

Steps: (1) **Screening** – aPTT mixing test (patient plasma + normal plasma): correction suggests absence of inhibitor; lack of correction → suspected presence. (2) **Quantification** – Bethesda assay; titer expressed in BU/mL (low responders <5 BU/mL; high responders >10 BU/mL). FIX inhibitors occur in ~3–5% and may be associated with anaphylactic events.

Preanalytical phase: fasting blood collection in 3.2% citrate; tourniquet <1 min; centrifugation 15 min at ~2500 g; plasma promptly frozen (stable ≈28 days at −20°C).

Basic hematological analysis. Complete blood count included RBC, Hb, Ht, and platelets. Cell counting was performed by **electrical impedance**, and hemoglobin concentration by **spectrophotometry**.

Evaluation of Secondary Hemostasis (Screening and Factor Assays)

Lee–White test (clotting time): whole blood without anticoagulant incubated at 37°C; reference interval 5–10 min (orientative utility).

Fibrinogen (Clauss method): coagulation time in excess thrombin inversely proportional to concentration; reflects fibrinogen function.

PT/INR (factor II/prothrombin complex): coagulometric method with thromboplastin; reference 80–130% activity.

aPTT: screening test of intrinsic pathway; reference <40 s; prolonged in hemophilia and in the presence of inhibitors.

Common preanalytical considerations (PT/aPTT/fibrinogen): morning collection, fasting; citrate 3.2%; centrifugation 10–15 min at ~3500 rpm; minimal venous stasis (tourniquet <1 min).

Liver and cholestatic biochemistry: ALT, AST, total and fractionated bilirubin determined photocolorimetrically to evaluate hepatic comorbidities and treatment-related effects.

Molecular–Genetic Analysis (F8/F9): Genomic DNA was isolated (proteinase K, phenol–chloroform). Detection of intron 22 and intron 1 inversions performed by long-fragment amplification and PCR. Coding and flanking regions of F8/F9 were amplified and analyzed by heteroduplex and automated sequencing (Genetic Analyzer 3100). *In silico* interpretation assessed mutation impact on splicing sites (SplicePort) and on FVIII structure/function (PolyPhen, CUPSAT). Intragenic polymorphisms were typed using RFLP and polyacrylamide gel electrophoresis.

Methods of Statistical Processing and Analysis

Statistical analysis was conducted using Microsoft Excel and IBM SPSS Statistics v.20. For comparison of means among three or more groups, Analysis of Variance (ANOVA) was used, decomposing total variability into between-group and within-group components to determine significance. When Levene's test indicated lack of homogeneity of variances, nonparametric tests, particularly Kruskal–Wallis, were applied (suitable for independent groups and non-normal distributions or small samples). For qualitative variables, the Chi-square test (χ^2) was used; when assumptions were not met, Monte Carlo simulation was applied to estimate the p-value. The threshold for statistical significance was set at $p < 0.05$; results with $p > 0.05$ were considered non-significant. Data were presented in tables, graphs, and figures — tables summarized data systematically, bar and box plots illustrated trends and inter-variable relationships, and images provided relevant clinical visuals, ensuring clear and accessible interpretation of the results.

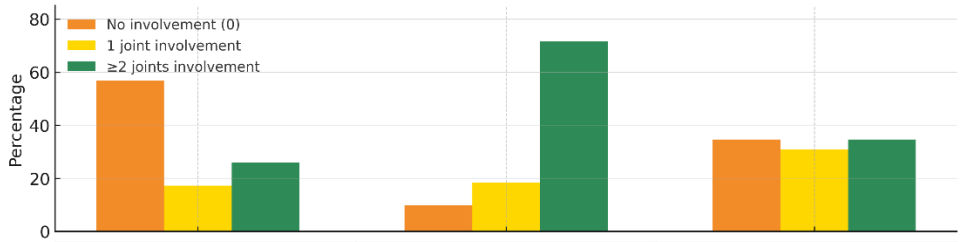
2. RESULTS

2.1. Results of the clinical spectrum analysis

Clinical evaluation of the joints was performed using standardized international scores (Gilbert and HJHS v2.1), which objectified the degree of impairment and allowed comparability between patients. The results showed that the number of joints involved ranged from 1 to 4, with a predominance of multiple joint involvement: 24.4% of children had simultaneous involvement of 4 joints (22 cases, 95% CI [15.57–33.32]), 23.3% had involvement of 2 joints (21 cases, 95% CI [14.60–32.07]), and 19.8% of 3 joints (16 cases).

Statistically significant differences were observed between hemophilia types. In hemophilia B, ankle joints were less frequently affected compared to hemophilia A ($\chi^2 = 7.04$, df = 2, p = 0.032), with a predominance of unilateral involvement (77.8%), in contrast to hemophilia A, where bilateral involvement was more frequent. Regardless of type, knees were the joints most frequently affected: 71.6% in hemophilia A and 77.8% in hemophilia B.

These findings confirm the role of recurrent hemarthroses in the pathogenesis of hemophilic arthropathy and highlight the importance of the Gilbert and HJHS scores in correlating disease severity with localization of joint involvement, providing benchmarks for prophylactic and therapeutic interventions (Figure 2).



	Elbow Joint	Knee Joint	Talo-crural Joint
No involvement (0)	56,8	9,9	34,6
1 joint involvement	17,3	18,5	30,9
≥2 joints involvement	25,9	71,6	34,6

*Statistically significant difference at p<0.05

Figure 2. Description of joint involvement in children with hemophilia in the Republic of Moldova

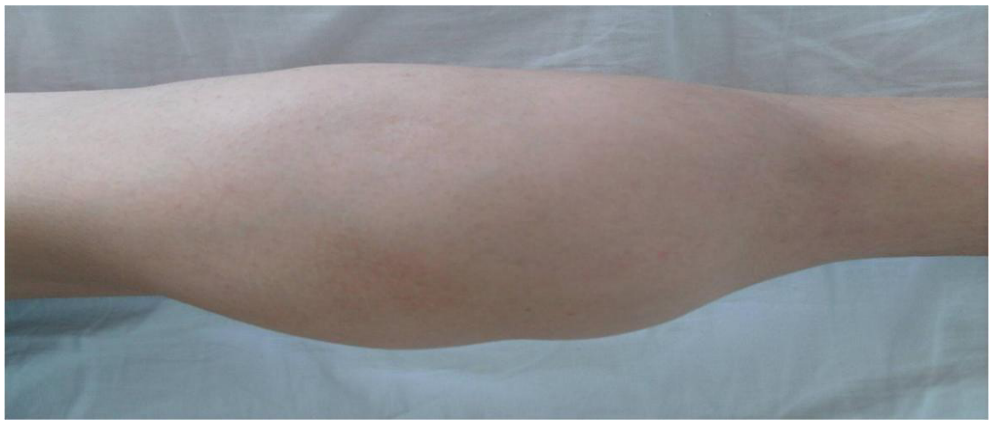


Figure 3. Clinical case. Stage IV knee hemarthrosis in a patient diagnosed with severe hemophilia

Note: The image shows significant swelling of the knee joint caused by intra-articular blood accumulation. The patient is monitored in the Hematology Clinic of the Mother and Child Institute.

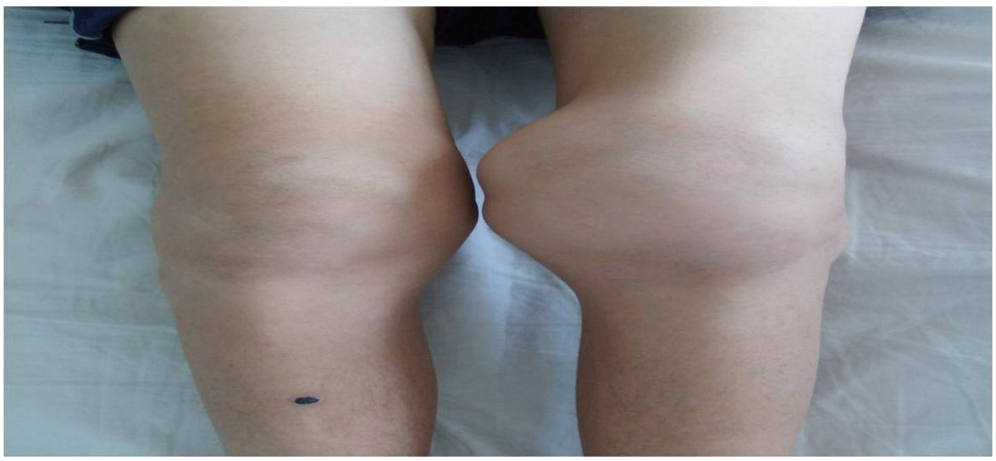


Figure 4. Clinical case. Stage V hemarthrosis of the left knee in a patient with severe hemophilia monitored in the Hematology Clinic of the Mother and Child Institute

Note: The image shows marked deformity of the knee joint, with pronounced swelling and visible asymmetry compared to the contralateral limb. These changes reflect severe chronic joint damage resulting from recurrent hemarthrosis episodes in patients with hemophilia.

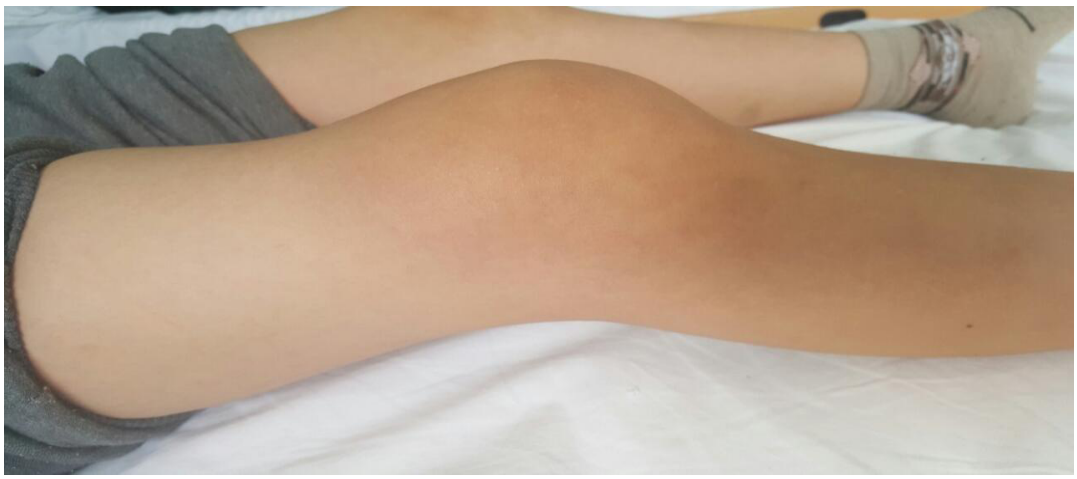


Figure 5. Clinical case in a patient with severe hemophilia presenting acute hemarthrosis of the right knee joint, with obvious swelling and alteration of the local contour

Note: This is a common manifestation in patients with severe hemophilia, particularly in the absence of adequate prophylactic treatment. The affected knee shows visible deformity associated with intra-articular blood accumulation, leading to pain, limited mobility, and inflammation. This case was encountered in a patient of the Hematology Clinic of the Mother and Child Institute, reflecting the need for specific emergency therapeutic interventions to prevent chronic joint complications.

These cases emphasize the increased risk of bleeding in children with severe hemophilia and the importance of early diagnosis and adequate therapeutic intervention.

Assessment of the type of genetic transmission is fundamental for family counseling and early diagnosis in hemophilia, an X-linked disease that predominantly affects males. In the studied group, in 39% of cases (35 children, 95% CI [28.82–48.96]) family history was unknown, and in 36.7% (33 children, 95% CI [26.71–46.62]) hemophilia was classified as sporadic, with no family history (35.8% hemophilia A and 44.4% hemophilia B). Only 24.4% of patients (22 children, 95% CI [15.57–33.32]) had known familial transmission, with similar distributions between hemophilia A (24.9%) and hemophilia B (22.2%). Statistical analysis did not reveal significant differences between types A and B regarding mode of transmission ($\chi^2 = 0.27$, df = 2, p = 0.911) (Table 1).

Table 1. Assessment of the type of disease transmission

		Type					
		A		B		Total	
		N	%	N	%	N	%
Type of transmission	Familial	20	24,9	2	22,2	22	24,4
	Sporadic	29	35,8	4	44,4	33	36,7
	Unknown	32	39,5	3	33,3	35	38,9
	Total	81	100	9	100	90	100

2.2. Results of the study of laboratory parameters

The aPTT was prolonged in all investigated children (mean 92.40 seconds, SD 28.52), with no significant differences between severity groups ($F = 0.153$, $p = 0.858$), confirming involvement of the intrinsic coagulation pathway characteristic of hemophilia. This parameter is a sensitive marker of factor VIII and IX deficiency, being essential both for correct diagnosis and for monitoring substitution therapy. In contrast, fibrinogen levels (mean 2.84 g/L, SD 0.27) and the prothrombin index (mean 90.06%, SD 4.43) were within normal limits, with no statistically significant differences according to disease severity ($p>0.05$), supporting the specificity of aPTT changes for hemophilia (Table 2).

Table 2. Descriptive analysis of coagulation parameters associated with secondary hemostasis

		Severity			
		Mild	Moderate	Severe	Total
PT Index %	N	13	33	44	90
	Mean	90,54	90,45	89,61	90,06
	SD	3,33	4,87	4,42	4,43
	95% CI	88,52 - 92,55	88,73 - 92,18	88,27 - 90,96	98,13 – 90,96
	Median	92,00	90,00	89,00	90,00
	25th Percentile	90,00	88,00	87,00	87,00
	75th Percentile	93,00	94,00	92,00	93,00
		F=0,904, p= 0.409 Kruskal-Wallis =1,47 , p= 0,480			
aPTT sec.	N	13	33	44	90
	Mean	96,00	92,79	91,05	92,40
	SD	26,04	28,91	29,43	28,52
	95% CI	80,27 - 111,73	82,54 - 103,04	82,10 - 99,99	86,43 – 98,37
	Median	110,00	91,00	91,50	98,00
	25th Percentile	104,00	68,00	63,00	63,00
	75th Percentile	112,00	110,00	109,00	110,00
		F=0,153, p=0,858 Kruskal-Wallis = 1,35 , p= 0,509			

Tabel 2. Descriptive analysis of coagulation parameters associated with secondary hemostasis (continued)

		Severity			
		Mild	**Moderate**	**Severe**	**Total**
Fibrinogen g/l	N	13	33	44	90
	Mean	2,92	2,80	2,85	2,84
	SD	0,35	0,30	0,23	0,27
	95% CI	2,71 - 3,14	2,70 - 2,91	2,78 - 2,92	2,78 – 2,90
	Median	3,10	2,80	2,90	2,85
	25th Percentile	2,70	2,70	2,70	2,70
	75th Percentile	3,20	2,90	2,90	2,90
		F=0,904, p= 0.409 Kruskal-Wallis =1,51 , p= 0,470			

The analysis of the distribution of patients with hemophilia by severity and Lee–White clotting time (Table 3) showed significantly prolonged clotting times (>1 hour) in 50% of patients with severe hemophilia (22 out of 44) and in 24.2% of those with moderate hemophilia (8 out of 33). No patient with mild hemophilia had a clotting time longer than one hour. Conversely, a clotting time ≤1 hour was recorded in all patients with mild hemophilia (100%), as well as in most patients with moderate (75.8%) and severe forms (50%). Statistical analysis revealed a significant difference ($p<0.05$) between severity groups regarding clotting time, suggesting a direct correlation between disease severity and the degree of prolongation. This finding reflects the functional deficiency of coagulation factors characteristic of hemophilia, with more pronounced impairment in severe forms where factor VIII or IX levels are below 1%. Thus, the Lee–White test may provide a useful indicator of global coagulation, but it needs to be correlated with specific investigations such as coagulation factor levels and global coagulation tests (aPTT, PT).

Table 3. Distribution of patients with hemophilia by severity and Lee–White clotting time

		Severity							
		Moderate		**Severe**		**Mild**		**Total**	
		N	%	N	%	N	%	N	%
Lee–White clotting time (5–10')	> 1 hour	8	24.2*	22	50.0*	0	0.0*	30	33.3
	<= 1 hour	25	75.8	22	50.0	13	100	60	66.7
	Total	33	100	44	100	13	100	90	100

*Statistically significant difference at $p<0.05$

The analysis of the relationship between Lee–White clotting time and coagulation parameters (PT, aPTT, fibrinogen) in 90 patients revealed relevant differences. Clotting time was >1 hour in 33.3% of cases and ≤1 hour in 66.7%. Prothrombin time (index) was comparable between groups (90.67 vs. 89.75%), with no significant differences ($F = 0.86$, $p = 0.358$). In contrast, aPTT was significantly higher in patients with prolonged clotting (103.43 vs. 86.88 seconds; $F = 7.21$, $p = 0.009$), confirming intrinsic pathway deficiency. Fibrinogen level was slightly lower in these patients (2.74 vs. 2.89 g/L; $F = 6.54$, $p = 0.012$), suggesting a moderate influence on overall coagulation. These data emphasize that aPTT is the main marker for

evaluating hemophilia, whereas PT and fibrinogen play a complementary role in characterizing the coagulation profile.

Table 4. Relationship between coagulation parameters and Lee–White clotting time Prothrombin Index (%)

		Lee-White Clotting Time		
		> 1 hour	</= 1 hour	Total
Prothrombin Time	N	30	60	90
	Mean	90.67	89.75	90.06
	SD	5.16	4.03	4.43
	95% CI	88.74 – 92,59	88,71 – 90,79	89,13 - 90,98
	Median	91.00	90.00	90.00
	25th Percentile	86.00	87.00	87.00
	75th Percentile	94.00	92.00	93.00
		F=0,86, p=0,358 Kruskal-Wallis = 0,95 , p= 0,330		
aPTT	N	30	60	90
	Mean	103.43*	86.88*	92.40
	SD	31.92	25.16	28.52
	95% CI	91,51 – 115,35	80,38 – 93,38	86,43 – 98,37
	Median	102.00*	90.00*	98.00
	25th Percentile	90.00	61.00	63.00
	75th Percentile	112.00	109.50	110.00
		F=7,21, p=0,009 Kruskal-Wallis = 4,15 , p= 0,042		
	N	30	60	90
	Mean	2.74*	2.89*	2.84
	SD	.23	.28	.27
	95% CI	2,65 – 2,83	2,82 – 2,96	2,78 – 2,90
	Median	2.80*	2.90*	2.85
	25th Percentile	2.55	2.70	2.70
	75th Percentile	2.90	3.10	2.90
		F=6,54, p=0,012 Kruskal-Wallis = 4,46 , p= 0,035		

*Statistically significant difference at p<0.05

Results of the quantitative assessment of coagulation factors VIII and IX

In the cohort of 90 children enrolled in the study, hemophilia A clearly predominated (90%, 81 cases) compared to hemophilia B (10%, 9 cases). Among patients with hemophilia A, the severe form was most frequent (51.9%, 42 cases), followed by the moderate form (35.8%, 29 cases) and the mild form (12.4%, 10 cases). In hemophilia B, the distribution differed, with predominance of the moderate form (44.4%, 4 cases), followed by the mild form (33.3%, 3 cases) and only 22.2% (2 cases) severe form. Overall, the severe form was present in 48.9% of patients (44 cases, 95% CI [38.56–59.22]), the moderate form in 36.7% (33 cases), and the mild form in 14.4% (13 cases, 95% CI [7.18–27.71]). Although severity distribution differed between hemophilia A and B, statistical analysis did not reveal significant differences ($\chi^2 = 4.09$, df = 2, p = 0.129). These results

confirm the predominance of severe forms, with major clinical implications for the need for continuous prophylaxis and multidisciplinary monitoring.

Table 5. Quantitative assessment of coagulation factors VIII and IX

		Tip					
		A		B		Total	
		N	%	N	%	N	%
Severity	Mild	10	12,4	3	33,3	13	14,4
	Moderate	29	35,8	4	44,4	33	36,7
	Severe	42	51,9	2	22,2	44	48,9
	Total	81	100	9	100	90	100

Results of the study of molecular–genetic changes

Of the 90 children included, blood sampling for identification of the genetic mutation type associated with hemophilia was performed in only 43.3% of cases (39 children). The most frequent mutation was missense, found in 17.8% (95% CI [9.88–25.68]) of cases (16 children). This mutation was observed in 18.5% of hemophilia A cases (15 children) and 11.1% of hemophilia B cases (1 child). The intron 22 inversion (Inv Intr 22) was recorded in 14.8% (95% CI [6.31–20.36]) of cases (12 children), being observed exclusively in hemophilia A. Another mutation type encountered was frameshift, in 7.8% (95% CI [2.24–13.31]) of cases (7 children), of which 7.4% were associated with hemophilia A (6 children) and 11.1% with hemophilia B (1 child). Nonsense mutation was the rarest, identified in 3.7% (95% CI [0–7.04]) of cases (3 children), all associated with hemophilia A (Table 6).

Table 6. Assessment of genetic mutation type

		Tip					
		A		B		Total	
		N	%	N	%	N	%
Mutation	Frameshift	6	7,4	1	11,1	7	7,8
	Intron 22 inversion	12	14,8*	0	0,0*	12	13,3
	Missens	15	18,5	1	11,1	16	17,8
	Nonsense	3	3,7	0	0,0	3	3,3
	Negative	1	1,2	0	0,0	1	1,1
	Not subjected to control	44	54,3	7	77,8	51	56,7
	Total	81	100	9	100	90	100

*Statistically significant difference at $p<0.05$

Table 6 shows the distribution of genetic mutations in hemophilia according to Lee–White clotting time, highlighting the relationship between mutation type and coagulation severity. Severe mutations, such as large deletions and intron 22 inversions, were predominantly associated with clotting times >1 hour, indicating profound hemostatic impairment. Nonsense, splicing, and frameshift mutations also clustered in this group, reflecting major defects in factor VIII or IX synthesis. In contrast, missense mutations and small deletions, typically linked to moderate or mild disease, were more common among patients with clotting time ≤1 hour.

These findings demonstrate that mutation type directly influences Lee–White prolongation and disease severity, underscoring the importance of molecular diagnosis for prognosis, patient stratification, and individualized management.

Table 6. Distribution of genetic mutations according to Lee–White clotting time values in patients with hemophilia

Mutation Type	> 1 hour (%)	≤ 1 hour (%)
Frameshift	3.0	10.0
Intron 22 Inversion	23.33	8.33
Missense	13.33	20.00
Negative	0.0	1.67
Nonsense	6.67	1.67
Not Tested	53.3	58.33

Results of the study of inhibitor titers

Table 7 presents inhibitor status in hemophilia A and B with corresponding proportions and 95% confidence intervals. Overall, 49 of 90 patients (54.4%) were not tested, predominantly in hemophilia A (60.5%), whereas all patients with hemophilia B were tested. Detectable inhibitors were found only in hemophilia A (4 patients, 4.9%; 95% CI 0.19–8.70%), with none in hemophilia B. Absence of inhibitors was recorded in 28 patients with type A (34.6%) and all patients with type B (100%), totaling 37 of 90 patients (41.1%).The high proportion of untested cases in hemophilia A suggests limited access to inhibitor assays and has implications for diagnostic and management strategies (Table 7).

Table 7. Assessment of inhibitor titer

		Type					
		A		B		Total	
		N	%	N	%	N	%
Inhibitors	Not performed	49	60,5*	0	0,0*	49	54,4
	Present	4	4,9	0	0,0	4	4,4
	Absent	28	34,6*	9	100*	37	41,1
	Total	81	100	9	100	90	100

*Statistically significant difference at $p<0.05$

2.3. Study of the interrelation between clinical and paraclinical parameters depending on disease severity

Interrelation between disease severity and mode of transmission

Familial transmission was most frequently associated with moderate hemophilia (45.5%; 15 cases), significantly higher than in severe forms (15.9%; 7 cases) and absent in mild forms. In severe (54.5%) and mild cases (76.9%), the transmission type was most often unknown, compared with only 3.0% in moderate forms ($\chi^2 = 32.216$, $p < 0.001$). Sporadic transmission also predominated in moderate hemophilia (51.5%), with lower frequencies in severe (29.5%) and mild forms (23.0%). These findings highlight the heterogeneous relationship between inheritance patterns and disease severity in hemophilia (Table 8).

Table 8. Interrelation between transmission type and disease severity

		Severity							
		Mild		Moderate		Severe		Total	
		N	%	N	%	N	%	N	%
Mode of inheritance	Familial	0	0,0*	15	45,5*	7	15,9*	22	24,4
	Sporadic	3	23,0	17	51,5	13	29,5	33	36,7
	Unknown	10	76,9*	1	3,0*	24	54,5*	35	38,9
	Total	13	100	33	100	44	100	90	100

* Statistically significant difference at p<0.05

Interrelation between disease severity and incidence of hemarthroses. We analyzed the association between hemarthrosis (the most common lesion in hemophilia) and disease severity and found: severe hemophilia was associated with involvement of multiple joints, most commonly ≥4 joints, while single-joint involvement was rare (4.5%). The elbow was affected more frequently in severe forms (54.5%), predominantly bilaterally, compared with moderate (39.4%) and mild forms (23.0%) ($\chi^2 = 11.81$, $p = 0.019$). The knee was the most commonly involved joint overall, with high rates of bilateral involvement in severe (72.7%) and moderate forms (84.9%), significantly exceeding mild forms (38.5%) ($\chi^2 = 10.07$, $p = 0.039$). Ankle involvement was also more frequent in severe (72.7%) and moderate cases (66.7%), while largely absent in mild hemophilia (92.3%) ($\chi^2 = 7.0$, $p = 0.030$).

Overall, disease severity correlates with the number of affected joints and bilateral involvement, supporting the need for severity-adapted management (Table 9).

Table 9. Interrelation between hemarthroses and disease severity
Number of affected joints

		Severity							
		Mild		Moderate		Severe		Total	
		N	%	N	%	N	%	N	%
Number of affected joints	0	2	15,4	1	3,0	0	0,0	3	3,3
	1	5	38,5	0	0,0	2	4,6	7	7,8
	2	6	46,2	7	21,2	8	18,2	21	23,3
	3	0	0,0	8	24,2	8	18,2	16	17,8
	4	0	0,0	10	30,3	12	27,3	22	24,4
	5	0	0,0	3	9,0	8	18,2	11	12,2
	6	0	0,0	4	12,1	6	13,6	10	11,1
	Total	13	100	33	100	44	100	90	100
Elbow	0	10	76,9	20	60,6	20	45,5	50	55,6
	1	3	23,1	1	3,0*	10	22,7*	14	15,6
	2	0	0,0	12	36,4	14	31,8	26	28,9
	Total	13	100	33	100	44	100	90	100
Knee	0	3	23,1	2	6,1	5	11,4	10	11,1
	1	5	38,5	3	9,1	7	15,9	15	16,7
	2	5	38,5*	28	84,9*	32	72,7	65	72,2
	Total	13	100,	33	100	44	100	90	100
Ankle	0	12	92,3*	11	33,3*	12	27,3*	35	38,9
	1	1	7,7	11	33,3	13	29,6	25	27,8
	2	0	0,0	11	33,3	19	43,2	30	33,3
	Total	13	100	33	100	44	100	90	100

*Statistically significant difference at p<0.05

Interrelation between disease severity and genetic mutation type. We analyzed the types of recorded genetic mutations by disease severity, describing the genotype–severity association: In mild forms of hemophilia, the most frequent mutation was missense (7.7%; 1 child). Inv Intr-22 occurred in 13.3% (12 children), but only in moderate (15.2%, 5 children) and severe forms (15.9%, 7 children). Frameshift mutations were found in 7.8% (7 children), with 12.1% (4 children) in moderate forms and 6.8% (3 children) in severe forms. Nonsense mutation was the rarest – 3.3% (3 children), with 4.6% (2 children) in severe and 3.0% (1 child) in moderate forms.

A statistically significant difference at $p<0.05$ was found between the frequency of missense mutations in moderate forms (36.4%, 12 children) and in severe forms (6.8%, 3 children) ($\chi^2 = 24.015$, df = 10, p = 0.008).

These findings highlight the diversity and complexity of genetic mutations involved in hemophilia and their relevance in determining disease severity (Table 10).

Table 10. Results of phenotype–genotype interrelation

		Severity							
		Mild		Moderate		Severe		Total	
		N	%	N	%	N	%	N	%
Mutation	Frameshift	0	0,0	4	12,1	3	6,8	7	7,8
	Intron 22 inv	0	0,0	5	15,2	7	15,9	12	13,3
	Missense	1	7,7	12	36,4*	3	6,8*	16	17,8
	Nonsense	0	0,0	1	3,0	0	0,0	1	1,1
	Negative	0	0,0	1	3,0	2	4,6	3	3,3
	Not subjected to	12	92,3*	10	30,3*	29	65,9*	51	56,7
	Total	13	100	33	100	44	100	90	100

*Statistically significant difference at $p<0.05$

Interrelation between disease severity and inhibitor titer. The analysis of the interrelation severity–inhibitor titer (Table 11) presents the distribution of patients according to severity and inhibitor determination results in two evaluation stages.

Out of 90 patients, 49 (54.4%) were not tested for inhibitors. The highest proportion of untested patients was in the moderate group (84.9%), followed by severe (43.2%) and mild (15.4%). This distribution may reflect more frequent testing in severe cases, where inhibitor presence is more likely and significantly affects treatment.

In the first testing stage, inhibitors were detected only in patients with severe hemophilia (16.0%); in mild and moderate forms, no inhibitors were identified. Most tested patients (90.2%) had no inhibitors, indicating relatively low prevalence in this cohort. The second testing stage showed identical results, confirming inhibitors only in severe forms (16.0%). This concordance suggests stable findings over time and emphasizes the predisposition of severe forms to inhibitor development.

Results indicate a clear correlation between severity and inhibitor risk, with inhibitors present exclusively in severe hemophilia. The high percentage of untested patients, especially in moderate forms, highlights the need for stricter screening strategies. The statistically significant difference at $p<0.05$ confirms the clinical relevance of these results.

Table 11. Interrelation between disease severity and inhibitor titer

	Severity							
	Mild		Moderate		Severe		Total	
	N	%	N	%	N	%	N	%
Not performed	2	15,4	28	84,9	19	43,2	49	54,4
Determination of inhibitor titer in stage I								
Present	0	0,0*	0	0,0*	4	16,0*	4	9,8
Absent	11	100	5	100	21	84,0	37	90,2
Total	13	100	5	100	25	100	41	100
Determination of inhibitor titer in stage II								
Present	0	0,0*	0	0,0*	4	16,0*	4	9,8
Absent	11	100	5	100	21	84,0	37	90,2
Total	11	100	5	100	25	100	41	100

*Statistically significant difference at $p<0.05$

2.4. Analysis of the distribution of genetic mutations and clinical severity according to the status of anti–factor VIII inhibitors

The distribution of mutation types and severity of hemophilia A was analyzed in 90 patients according to the presence of specific anti–factor VIII inhibitors, divided into three categories: patients not tested for inhibitors (n = 49), patients with inhibitors present (n = 4), and patients without inhibitors (n = 37) (Table 12).

Table 12. Distribution of genetic mutations and clinical severity according to anti–factor VIII inhibitor status

		Inhibitors against factor VIII							
		Not performed		**Present**		**Absent**		**Total**	
		N	%	N	%	N	%	N	%
Type of mutation	Frameshift	6	12.2	0	0.0	1	2.7	7	7.8
	Intron 22 inv	12	24.5	0	0.0	0	0.0	12	13.3
	Missense	15	30.6	0	0.0	1	2.7	16	17.8
	Nonsense	1	2.0	0	0.0	0	0.0	1	1.1
	Negative	3	6.1	0	0.0	0	0.0	3	3.3
	Not subject to	12	24.5	4	100.0	35	94.6	51	56.7
	Total	49	100.0	4	100.0	37	100.0	90	100.0
Severity	Mild	2	4.1	0	0.0	11	29.7	13	14.4
	Moderate	28	57.1	0	0.0	5	13.5	33	36.7
	Severe	19	38.8	4	100.0	21	56.8	44	48.9
	Total	49	100.0	4	100.0	37	100.0	90	100.0

The most frequent mutation in the total group was missense (16 patients, 17.8%), followed by intron 22 inversion (13.3%) and frameshift mutations (7.8%). Notably, 56.7% (51 patients) were not genetically tested, limiting genotype–phenotype correlation.

In patients not tested for inhibitors (n = 49), missense mutations (30.6%), intron 22 inversion (24.5%), and lack of genetic testing (24.5%) had similar proportions, suggesting genetic heterogeneity in the absence of inhibitor evaluation. In the group with inhibitors (n = 4), all patients (100%) lacked genetic evaluation, raising concerns about limited access to genetic testing for

complex cases. Among patients without inhibitors (n = 37), most had unknown genetic status (94.6%), with missense and frameshift mutations each present in only 2.7%.

Regarding clinical severity, severe hemophilia A predominated (n = 44; 48.9%), followed by moderate (n = 33; 36.7%) and mild forms (n = 13; 14.4%). In the non-tested inhibitor group, moderate severity was most frequent (57.1%), while severe forms represented 38.8%; mild forms were rare (4.1%). In the inhibitor-positive group, all cases (100%) were severe, supporting the literature that inhibitor development is strongly associated with severe disease. In the inhibitor-negative group, 56.8% were severe and 29.7% mild, potentially reflecting effective prophylaxis or favorable genetic background.

The distribution of mutation types and clinical severity suggests an association between severe hemophilia A and inhibitor presence, with a high proportion of cases lacking both genetic and inhibitor testing. This underscores the need to improve access to molecular and immunologic diagnostics in hemophilia management, especially in severe forms.

2.5. Results of the comparative study according to type of treatment administered

General characteristics of the groups. From the initial group of 90 confirmed hemophilia patients, 32 were rigorously selected and divided into two groups: 16 treated with cryoprecipitate and 16 with recombinant FVIII. Medical records were reviewed from the first admission (diagnosis) and clinical evolution analyzed over three years. The number of hospitalizations per year and clinical manifestations of hemorrhagic syndrome were meticulously documented, allowing comprehensive assessment of treatment efficacy and impact on disease progression and patients' health (Table 13).

Table 13. General characteristics of the groups

		Replacement therapy					
		Cryoprecipitate		FVIII		Total	
		N	%	N	%	N	%
Type	HA	15	93,8	15	93,8	30	93,8
	HB	1	6,2	1	6,2	2	6,2
	Total	16	100,00	16	100,00	32	100,00

Hemophilia, a genetic disorder with a significant impact on quality of life, is characterized by the early onset of symptoms, which necessitates prompt and efficient diagnosis. In our study, we observed that the children included were identified with this condition within the first two years of life. We found that 68.8% of cases (22 patients) received diagnostic confirmation during the first year of life, while 31.2% of cases (10 patients) were diagnosed in the second year of life. These data clearly illustrate the early impact of hemophilia and emphasize the need for a practical approach to the detection and management of this condition, providing essential insights for improving patients' quality of life [Table 14].

Table 14. Age at primary diagnosis

		Replacement therapy					
		Cryoprecipitate		FVIII		Total	
		N	%	N	%	N	%
Age at primary diagnosis	1-12 months	11	68,8	11	68,8	22	68,8
	12-48 months	5	31,2	5	31,2	10	31,2
	Total	16	100	16	100	32	100

In the first year of follow-up, most patients required at least one hospitalization for substitution therapy: 56.25% of those treated with cryoprecipitate (9 children) and 31.25% treated with recombinant FVIII (5 children). Repeated hospitalizations (2–4 per year) were reported in both groups (18.75–25%). Notably, in the FVIII group some patients had up to 7 hospitalizations (12.5%, 2 children), indicating more complex therapeutic management in these cases (Table 15).

Table 15. Number of hospitalizations in the first year after diagnosis

		Replacement therapy					
		Cryoprecipitate		FVIII		Total	
		N	%	N	%	N	%
Number of admissions during the first year of follow-up	1	9	56,25	5	31,25	14	43,75
	2	3	18,75	4	25,0	7	21,89
	3	3	18,75	2	12,5	5	15,6
	4	1	6,25	3	18,75	4	12,5
	7	0	0,0	2	12,5	2	6,25
	Total	16	100	16	100	32	100

In the second year of follow-up, only 6.25% (1 child) required no hospitalization, while 25% (4 children) had at least one admission for substitution therapy, regardless of treatment. Recurrent hospitalizations ranged from 2 to 10 episodes per year, with comparable frequencies between cryoprecipitate and FVIII. Extreme cases were seen in both groups, including up to 11 hospitalizations per year in a child treated with cryoprecipitate. These data reflect complex and variable therapeutic needs, underlining the importance of individualized management (Table 16).

Table 16. Number of hospitalizations in the second year after diagnosis

		Replacement therapy					
		Cryoprecipitate		Factor VIII		Total	
		Nr	%	Nr	%	Nr	%
Number of admissions during the second year of follow-up	0	1	6,25	0	0,0	1	3,1
	1	4	25,0	4	25,0	8	25,0
	2	1	6,25	2	12,5	3	9,4
	3	3	18,75	4	25,0	7	21,9
	4	4	25,0	3	18,75	7	21,9
	5	0	0,0	1	6,25	1	3,1
	7	1	6,25	0	0,0	1	3,1
	9	0	0,0	1	6,25	1	3,1
	10	1	6,25	1	6,25	2	6,3
	11	1	6,25	0	0,0	1	3,1
	Total	16	100	16	100	32	100

In the third year of follow-up, 6.25% (1 child) required no hospitalization, and 21.9% (7 children) had at least one admission, with similar frequencies between FVIII (25.0%) and cryoprecipitate (18.75%). Rehospitalizations ranged from 2 to 10 per year, with different distributions between groups; extreme cases of up to 11 hospitalizations were again seen in patients treated with cryoprecipitate (6.25%, 1 child). These results highlight persistent complex therapeutic needs and the necessity of tailored clinical management (Table 17).

Table 17. Number of hospitalizations in the third year after diagnosis

		Replacement therapy					
		Cryoprecipitate		F VIII		Total	
		N	%	N	%	N	%
Number of admissions during the third year of follow-up	0	1	6,25	2	12,5	3	9,4
	1	3	18,75	4	25,0	7	21,9
	2	2	12,5	4	25,0	6	18,75
	3	3	18,75	1	6,25	4	12,5
	4	2	12,5	3	18,75	5	15,6
	5	2	12,5	0	0,0	2	6,25
	6	1	6,25	0	0,0	1	3,1
	7	1	6,25	0	0,0	1	3,1
	8	0	0,0	1	6,25	1	3,1
	10	0	0,0	1	6,25	1	3,1
	11	1	6,25	0	0,0	1	3,1
	Total	16	100	16	100	32	100

2.6. Comparative results of cryoprecipitate versus recombinant factor treatment in controlling hemorrhagic syndrome relapses

The analysis of the data presented in the table shows the distribution of substitution treatment with cryoprecipitate and Factor VIII (FVIII) for the management of hematomas, which represented the admission diagnosis. The data are expressed as the number of admissions and the corresponding percentages for different degrees of hematoma severity throughout the treatment. The figures also suggest that, despite balanced therapeutic preferences, there are variations in treatment efficacy depending on hematoma severity. The results indicate that, for higher-grade hematomas (grades 2 and 3), FVIII treatment was predominant, possibly reflecting greater efficacy of this therapy in severe cases. This aspect is crucial, as it suggests that Factor VIII may be more effective in the management of severe hematomas, while cryoprecipitate may be sufficient for milder cases. These findings are fundamental for improving treatment protocols and personalizing therapy according to patients' individual needs, with the ultimate goal of optimizing clinical outcomes and reducing treatment-related risks [Table 18].

Table 18. Comparative characteristics of hospitalizations with diagnosis of hematoma by treatment type

		Replacement therapy					
		Cryoprecipitate		F VIII		Total	
		Nr	%	Nr.	%	Nr.	%
Hematoma	0	3	18,75	0	0,00	3	9,4
	1	4	25,0	0	0,0	4	12,5
	2	1	6,25	5	31,25	6	18,75
	3	2	12,5	5	31,25	7	21,9
	4	1	6,25	1	6,25	2	6,25
	5	1	6,25	1	6,25	2	6,25
	6	1	6,25	1	6,25	2	6,25
	7	1	6,25	0	0,0	1	3,1
	8	0	0,0	1	6,25	1	3,1

	10	1	6,25	0	0,0	1	3,1
	12	1	6,25	0	0,0	1	3,1
	15	0	0,0	1	6,25	1	3,1
	18	0	0,0	1	6,25	1	3,1
	Total	16	100	16	100	32	100

The comparative analysis of hemarthrosis treatment showed that 28.1% of cases (9 children) had involvement of 3 joints, managed with both FVIII (25%, 4 cases) and cryoprecipitate (31.25%, 5 cases). In the cryoprecipitate group, very severe forms with up to 24 affected joints were recorded (6.25%, 1 child), whereas in the FVIII group the maximum number of affected joints was 5 (18.75%, 3 cases). These findings suggest superior efficacy of FVIII in limiting hemarthrosis severity compared to cryoprecipitate (Table 19).

Table 19. Comparative characteristics of hospitalizations with diagnosis of hemarthrosis by treatment type

		Replacement therapy					
		Cryoprecipitate		**F VIII**		**Total**	
		N	%	N	%	N	%
Hemarthrosis	0	3	18,75	1	6,25	4	12,5
	1	2	12,5	2	12,5	4	12,5
	2	1	6,25	6	37,5	7	21,9
	3	5	31,25	4	25,0	9	28,1
	4	1	6,25	0	0,0	1	3,1
	5	0	0,0	3	18,75	3	9,4
	6	1	6,25	0	0,0	1	3,1
	8	1	6,25	0	0,0	1	3,1
	11	1	6,25	0	0,0	1	3,1
	24	1	6,25	0	0,0	1	3,1
	Total	16	100	16	100	32	100

Data regarding the distribution of substitution treatment with cryoprecipitate and Factor VIII (FVIII) for the management of massive hemorrhages were analyzed. Two massive hemorrhages were recorded in one patient managed with cryoprecipitate. This observation is important, as it suggests that the efficacy of cryoprecipitate treatment in preventing or controlling massive hemorrhages may be lower than that of Factor VIII therapy. Overall, the data indicate that, in cases of massive hemorrhage, treatment with Factor VIII may be more effective than cryoprecipitate. However, it is essential to consider each case individually and to carefully evaluate the benefits and risks according to the patient's specific needs and clinical conditions [Table 20].

Table 20. Comparative characteristics of the number of admissions with the diagnosis of massive hemorrhages of different localization depending on the type of treatment administered (cryoprecipitate or recombinant factor)

		Replacement therapy					
		Cryoprecipitate		**F VIII**		**Total**	
		Nr	%	Nr	%	Nr	%
Massive hemorrhages	0	15	93,8	16	100	31	96,9
	2	1	6,2	0	0,0	1	3,1
	Total	16	100	16	100	32	100

The analysis of minor hemorrhages showed superior efficacy of FVIII compared to cryoprecipitate. In the FVIII group, 43.75% of patients (7 children) had no minor hemorrhages, compared with only 12.5% (2 children) in the cryoprecipitate group. The proportion of patients with more than 3 minor hemorrhages was higher in the cryoprecipitate group (25%, 4 children) than in the FVIII group (12.5%, 2 children). These results confirm better control of minor hemorrhages with FVIII (Table 21).

Table 21. Comparative characteristics of the number of admissions with the diagnosis of minor hemorrhages of different localization depending on the type of treatment administered (cryoprecipitate or recombinant factor) type

		Replacement therapy					
		Cryoprecipitate		**F VIII**		**Total**	
		Nr	%	Nr	%	Nr	%
Minor hemorrhages (bleeding episodes)	0	2	12,5	7	43,75	9	28,1
	1	3	18,75	2	12,5	5	15,6
	2	4	25,0	5	31,25	9	28,13
	3	3	18,75	0	0,0	3	9,4
	4	4	25,0	1	6,25	5	15,6
	6	0	0,0	1	6,25	1	3,1
	Total	16	100	16	100	32	100

The comparative analysis of planned hospitalizations by treatment type (Table 22) highlights a significant difference between cryoprecipitate and recombinant FVIII. Patients treated with cryoprecipitate had no planned admissions (100% emergency-only episodes), reflecting the impossibility of using prophylaxis. In contrast, FVIII treatment allowed prophylactic organization: 68.75% had one or more planned admissions, and 31.25% were managed on an outpatient basis. These results confirm the superiority of FVIII prophylaxis in reducing recurrent bleeding and hemophilic arthropathy compared with episodic cryoprecipitate. The comparative analysis of the number of planned admissions according to the type of substitution treatment (Table 22) highlights a significant difference between the use of cryoprecipitate and recombinant Factor VIII (FVIII) in the organization of planned treatment.

The comparative analysis of the number of planned admissions revealed significant differences between treatment with cryoprecipitate and treatment with recombinant Factor VIII ($p < 0.05$). Patients treated with cryoprecipitate had no planned admissions (100% of episodes were emergency-based), reflecting the impossibility of prophylactic use. In contrast, treatment with recombinant Factor VIII allowed for prophylactic organization, with 68.75% of patients having one or more planned admissions and 31.25% being managed on an outpatient basis.

These results confirm the superiority of FVIII prophylaxis in reducing the risk of recurrent bleeding episodes and hemophilic arthropathy, compared with cryoprecipitate, which is used exclusively on an episodic basis.

Table 22. Comparative characteristics of the number of planned admissions depending on the type of treatment administered (cryoprecipitate or recombinant factor)

		Replacement therapy					
		Cryoprecipitate		F VIII*		Total	
		Nr	%	Nr	%	Nr	%
Planned	0	16	100	5	31,25	21	65,6
	1	0	0,0	3	18,75	3	9,4
	2	0	0,0	3	18,75	3	9,4
	3	0	0,0	1	6,25	1	3,1
	6	0	0,0	3	18,75	3	9,4
	8	0	0,0	1	6,25	1	3,1
	Total	16	100	16	100	32	100

*Statistically significant difference at $p<0.05$

2.7. Development and implementation of the datebase for electronic registry of patients with hemophilia in the Republic of Moldova

As a result of this research, an electronic datebase for national registry for patients with hemophilia was created and implemented – a dedicated tool for integrated monitoring and management of this rare disease. Its main purpose is to centralize data on hemophilia type, severity, hospitalization history, and treatment regimens, ensuring continuous surveillance and a unified information flow between pediatric and adult centers.

The registry complies with legal requirements for medical data confidentiality and offers strictly controlled access only to authorized personnel. Its interactive structure allows continuous data entry and updating, generation of clinical reports and statistics, and longitudinal patient analysis. The system has two components: one for pediatric patients, managed at the Mother and Child Institute, and one for adult patients, managed at the Oncology Institute, thus ensuring continuity of care from diagnosis to adulthood.

The database allows filtering and analysis by multiple criteria (age, hemophilia type and severity, inhibitor titer, therapeutic regimen), enabling clinicians to access both current and historical data. The intuitive interface facilitates use without advanced technical training, and automatically generated reports support treatment evaluation and resource planning.

Developed since 2018, datebase for the registry is a strategic resource for national research and a tool for alignment with international standards in rare disease management. Through data digitalization, the Republic of Moldova strengthens its capacity to participate in multicenter studies and increases its scientific visibility. The registry also supports public health policies by providing relevant data for resource allocation, expansion of access to innovative therapies, and integration of personalized medicine.

In conclusion, implementation of the database for electronic registry of patients with hemophilia represents a decisive step in modernizing medical infrastructure, offering a solid foundation for standardizing care, optimizing prognosis, and improving quality of life. Expanding its functionalities and periodic data updates will consolidate a national model of best practice in the management of hemophilia and other rare diseases.

SYNTHESIS OF CHAPTERS

The research provides a comprehensive assessment of pediatric hemophilia in the Republic of Moldova, integrating clinical, laboratory and genetic data, with direct implications for severity stratification and treatment personalization [14–16]. Hemophilia, an X-linked monogenic disorder (FVIII/FIX deficiency), manifests through spontaneous or post-traumatic bleeding, with hemarthrosis as the dominant complication and major driver of functional impairment [16].

Clinical manifestations and joint involvement. Most patients present with 2–4 affected joints, the knee being the most frequent site (≈72% in A, 78% in B), followed by the ankle and elbow. The ankle is less frequently affected in hemophilia B, with a predominance of unilateral involvement ($\chi^2 = 7.04$; $p = 0.032$) [17–19]. These patterns justify targeted monitoring (ultrasound/MRI) and early prophylaxis according to the type of hemophilia [8].

Genetic transmission and counseling. Case distribution is heterogeneous: familial, sporadic (≈37%) and with unknown family history (≈39%). The frequency of de novo mutations is consistent with the literature (≈30–35%) and is higher in hemophilia A, explained by the larger size of the F8 gene [16,20]. No significant differences were observed between hemophilia A and B ($p = 0.911$), underlining the importance of genetic testing and carrier identification [21,22].

Hematologic and biochemical profile. Hemoglobin and red blood cell counts decrease with increasing disease severity (Hb: $F = 10.21$; $p < 0.001$; erythrocytes: $F = 5.13$; $p = 0.008$), reflecting chronic blood loss and a risk of iron deficiency anemia [23]. Hepatic parameters may vary (higher total bilirubin in mild forms), and the thymol turbidity test is more elevated in severe forms ($F = 5.27$; $p = 0.007$), suggesting the need for liver monitoring in patients exposed to repeated therapies [23].

Coagulation and global tests. aPTT is prolonged in all patients (≈92 s), while PT, TT and bleeding time remain within normal limits, confirming intrinsic pathway involvement [24,25]. Lee–White clotting time > 1 hour was observed in 50% of patients with severe forms and 24% of those with moderate forms ($p < 0.05$), correlating with elevated aPTT; fibrinogen generally remained within normal limits [26].

Genotype–phenotype associations. Genetic testing was performed in 39 patients (43%), identifying missense mutations (17.8%), intron 22 inversion (14.8%, exclusively in hemophilia A), frameshift (7.8%) and nonsense (3.7%) variants. Intron 22 inversion is associated with severe forms and an increased risk of inhibitor development [25,27,28]. Overall, missense mutations predominate in mild/moderate forms, whereas frameshift and nonsense variants are more frequent in moderate–severe disease [29,30].

Inhibitors. Inhibitors were detected exclusively in severe hemophilia A (≈16% of tested patients), confirming the need for regular screening (Bethesda assay), risk stratification (mutation type, cumulative exposure days) and the use of bypassing agents or emicizumab [30,31].

Therapy: evolution and comparisons. The therapeutic sequence has evolved from plasma/cryoprecipitate to lyophilized and recombinant concentrates, extended half-life (EHL) factors and non-factor therapies (emicizumab), while gene therapies define the current stage [31,32]. In the comparative cohort ($n = 32$, severe forms), rFVIII reduced hospitalizations and hemarthroses and enabled planned prophylaxis, whereas cryoprecipitate was used predominantly in emergencies, with greater variability of hemostatic control [33].

Epidemiology and underdiagnosis. Globally, prevalence is ≈10.6/100,000 inhabitants (≈830,000 cases, ≈280,000 severe), but only ~426,000 patients had been identified according to the 2022 WFH report, highlighting underdiagnosis in resource-limited countries [20]. Europe

identifies over 80% of cases, yet gaps persist in Eastern Europe [17]. The introduction of prophylaxis (2019) and emicizumab (2024) improves disease control [30,32].

An electronic datebase for Registry of patients with hemophilia has been created and implemented, aligned with international confidentiality standards, with pediatric/adult modules and analytical reports for planning and research; it represents a strategic tool for standardization, comparability and health policy design [31].

The study consolidates the links between severity, hematologic profile, mutation type and inhibitor risk, justifying genetic testing, inhibitor screening and personalized prophylaxis. Integration of these data into the National Registry and expansion of access to modern, including non-factor, therapies are essential for reducing morbidity and harmonizing care with international best practices [34].

GENERAL CONCLUSIONS

1. In hemophilia A and B, the clinical picture was predominantly characterized by hematoma-type hemorrhages, which constituted the initial manifestation of the hemorrhagic syndrome, while joint involvement subsequently became the dominant clinical component, identified in over 70% of patients with severe forms. Knee hemarthrosis was the most frequent localization for both types of hemophilia, followed by the elbow and ankle joints.

2. Quantitative assessment of coagulation factors demonstrated a direct relationship between low levels of factors VIII and IX and the severity of hemophilia. In hemophilia A, the mean FVIII level was 0.7 IU/dL in the severe form and 3.5 IU/dL in the moderate form, whereas in hemophilia B the mean FIX level was 0.9 IU/dL and 4.1 IU/dL, respectively, confirming the value of these measurements in monitoring the effectiveness of replacement therapy.

3. Genetic analysis revealed a predominance of missense and frameshift mutations, associated with moderate and severe forms of the disease. Intron 22 inversion was identified exclusively in moderate and severe forms of hemophilia A (15.2% and 15.9%), confirming its major pathogenetic role and the relevance of genetic testing for etiological diagnosis and prediction of inhibitor risk. The most frequent mutations were missense (17.8%) and intron 22 inversion (14.8%).

4. Inhibitor testing revealed an occurrence rate of 16.0%, observed exclusively in patients with severe hemophilia. This finding highlights the importance of periodic inhibitor monitoring in this patient category, in order to adjust treatment and prevent therapeutic failure due to hyperergic response to replacement therapy.

5. Comparative analysis of treatment strategies demonstrated superior efficacy of recombinant and lyophilized factor VIII preparations in controlling severe hemorrhages and preventing hemophilic arthropathy, compared with cryoprecipitate, which proved effective mainly in mild forms of the disease. These results support the need to orient therapy toward concentrated, standardized, and virologically safe products, while emphasizing the importance of avoiding transfusion of substrates with infectious potential associated with plasma-derived preparations ($p<0.05$).

6. The development and implementation of the Web-based National Electronic Registry of Patients with Hemophilia in the Republic of Moldova enabled the centralization and standardization of clinical and paraclinical data, facilitating continuous patient monitoring, individualized treatment, and evaluation of therapeutic effectiveness, while ensuring data confidentiality and security requirements, and contributing to the optimization of medical resource utilization as well as supporting research activities and scientific cooperation.

PRACTICAL RECOMMENDATIONS

1. Mandatory implementation of genetic testing for all patients diagnosed with hemophilia and for potential carriers of mutant genes is essential for early diagnosis, identification of asymptomatic carriers, and application of primary prophylaxis measures in families at increased risk.

2. Individualization of treatment according to the patient's genotype should become a central component of hemophilia management, as disease severity and therapeutic response are closely correlated with the type of identified genetic mutation.

3. Rigorous clinical and paraclinical monitoring is necessary for patients carrying the Intron 22 inversion mutation, given its high frequency in severe forms of hemophilia A and its association with an increased risk of inhibitor development. Dynamic adjustment of treatment according to clinical evolution contributes to the prevention of complications and optimization of prognosis.

4. The development and implementation of a comprehensive monitoring algorithm for patients at risk of inhibitor development, based on the integration of genetic, clinical, and paraclinical markers, would enable a proactive therapeutic approach and the early introduction of modern biological non-factor replacement therapies, thereby reducing the risk of long-term complications and improving patients' quality of life.

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LIST OF PUBLICATIONS AND PARTICIPATIONs IN SCIENTIFIC FORUMS

of Ms Dorina Agachi, PhD graduate, Department of Pediatrics,
Nicolae Testemițanu State University of Medicine and Pharmacy
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published on the topic of the Doctoral Thesis in Medical Sciences

Biochemical and genetic diagnosis and therapeutic programs of hemophilia in children

specialty 322.01 – Pediatrics and neonatology

Scientific papers

• **Contributions in monographs:**

• Agachi D. *Hemophilia: A Medical-Social Problem*, pp. 307–323. In: Mazur-Nicorici Lucia, Diaconu Camelia Cristina, *Compendiu de Boli Rare*. Chișinău: Tipografia Impressum; 2020, 506 p. ISBN 978-9975-3426-6-7.

• **Articles in international scientific journals:**

✓ *articles in ISI, SCOPUS journals and other international databases*

1. Agachi D., Țurea V., Esanu G. *The comparative study between treatment with Cryoprecipitate and recombinant Factor VIII in Hemophilia in children.* World Journal of Biology Pharmacy and Health Sciences. 2024; 20(01): 483–493. eISSN: 2582-5542, doi: 10.30574/wjbphs.2024.20.1.0793.

2. Agachi D., Țurea V., Esanu G. *Vaccination in Hemophilic Children: Challenges, Innovations, and Future Directions.* World Journal of Biology Pharmacy and Health Sciences. 2024; 20(02): 162–170. eISSN: 2582-5542, doi: 10.30574/wjbphs.2024.20.2.0863.

• **Articles in accredited national scientific journals:**

✓ *articles in category B journals*

3. Agachi D., Turea V., Mihalachi-Anghel M. *Hemophilia in a female patient – case report.* Buletin de Perinatologie. 2019; 2(83): 108–111. ISSN 1810-5289.

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• **Articles in scientific conference proceedings:**

✓ *Abstracts/theses in national and international scientific conference proceedings*

6. Agachi D., Sirocov N., Țurea V., Ușurelu N., Esanu G., Mihalachi-Anghel M. *The genetic profile of hemophilia in children in the Republic of Moldova.* In: Abstract Volume of the

National Conference of Pediatrics: *Guidelines and Protocols in Pediatrics*. Bucharest; 2020, p. 55. ISSN 1454-0398 / e-ISSN 2069-6175 / ISSN-L 1454-0398 CNCSIS/CNCS Code 767.

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• Patents, certificates, registrations, materials presented at invention exhibitions:

9. Agachi D., Turea V., Eșanu G., Malai O. *Record of hospitalized hemophilia patients in public medical-sanitary institutions in the Republic of Moldova. Database on the platform of the "Electronic Registry of Patients with Hemophilia in the Republic of Moldova".* Innovator Certificate No. 6065, 30.05.2023.

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11. Turea V., Eșanu G., Popovici E., Balanuța A., Agachi D. *National Clinical Protocol-108 "Hemophilia in children".* Chișinău, 2022. 38 pp.

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✓ National

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• Participations with posters at scientific forums

✓ National with international participation

21. Agachi D., Turea V., Mihalachi-Anghel M. *Hemophilia in a female patient.* National Conference with International Participation, Chișinău–Sibiu Biennial, 3rd Edition, "Interdisciplinarity in Pediatric Infectious Diseases". Chișinău, 16–18 May 2019.

✓ International

22. Agachi D., Turea V., Mihalachi-Anghel M., Eșanu G. *Physiotherapy models for children with hemophilia.* National Conference "Zilele Pediatriei Ieșene N.N. Trifan", 33rd Edition. Iași, Romania, 13–15 May 2021.

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24. Agachi D., Turea V., Eșanu G. *Inhibitory hemophilia. Contemporary treatment with Emicizumab. Particularities for pediatric practice.* National Conference of Pediatrics "Guidelines and Protocols in Pediatrics". Bucharest, Romania, 3–7 April 2024.

ANNOTATION

Dorina Agachi, "Biochemical, genetic diagnosis and therapeutic programs of hemophilia in children", PhD thesis in medical sciences, Chișinău, 2026.

Structure of the thesis. The thesis comprises 146 pages and consists of an introduction, 6 chapters, discussions, general conclusions, recommendations, and a bibliography citing 139 sources. The iconographic material includes 37 tables, 15 figures, and 4 annexes. The scientific results obtained were reflected in 25 publications.

Keywords: hemophilia, coagulation, factor VIII, factor IX, bleeding, hemarthrosis, inhibitors, molecular-genetic mutation, aPTT, recombinant and lyophilized replacement factor.

Field of study: pediatrics and neonatology – 322.01.

Aim of the study. To analyze the clinical manifestations, biochemical and genetic parameters in the diagnosis of hemophilia A and B in children, with the goal of optimizing paraclinical diagnosis and developing an individualized therapeutic model adapted to the type and severity of the disease.

Objectives of the study. Analysis of clinical manifestations of hypocoagulation syndrome in children with hemophilia; determination of quantitative and qualitative levels of coagulation factors in hemophilia A and B; analysis of the relationship between clinical manifestations (phenotype) and genetic changes (genotype) in pediatric hemophilia to strengthen the utility of genetic diagnosis; evaluation of the presence of coagulation factor VIII and IX inhibitors and determination of their titers in the context of therapeutic management; comparative analysis of the efficacy and safety of replacement therapy with lyophilized/recombinant factors VIII/IX and cryoprecipitate for optimizing treatment approaches; development of a database on the web platform "Electronic Registry of Patients with Hemophilia in the Republic of Moldova."

Scientific novelty. For the first time in the Republic of Moldova, a comprehensive study of pediatric patients with hemophilia was carried out, focusing on clinico-paraclinical features and the identification of specific genetic mutations. The severity of hemophilia (based on clinical data and factor VIII/IX levels) was analyzed in association with identified mutations; titration of factor VIII and IX inhibitors in children was performed; and the National Registry for Monitoring Patients with Hemophilia was developed.

Theoretical significance. For the first time nationally, clinico-paraclinical features of hemophilia in children were analyzed, highlighting manifestations by severity and classifying genetic mutations associated with hemophilia A and B. The titers of coagulation inhibitors (factors VIII and IX) were determined for the first time, and their correlation with genetic and clinical data provided a theoretical basis for understanding phenotypic variability. Modern directions for integrated diagnosis and therapeutic management were proposed, and the datebase for National Monitoring Registry was developed to systematize and standardize data on this rare disease.

Practical significance. The study demonstrates the importance of genetic testing in hemophilia A and B for the early identification of severe forms and for guiding personalized therapy. Determination of inhibitor titers against factors VIII and IX supports prevention and management of substitution therapy complications, including the use of emicizumab in patients with inhibitors. The results contribute to the optimization of therapeutic and prophylactic protocols, while the creation of the datebase for National Monitoring Registry provides a practical tool for patient surveillance, planning, and for supporting public health policies regarding rare diseases in the Republic of Moldova.

Implementation of results. The findings have been integrated into the practice of the Department of Benign Hemopathies at IMSP Mother and Child Institute and specialized clinics of IMSP Oncology Institute in the Republic of Moldova, as well as into the educational process at the "Nicolae Testemițanu" State University of Medicine and Pharmacy, contributing to the theoretical and practical training of specialists in this field.