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ORIGINAL PAPER

CONTEMPORARY ASPECTS OF HEMOPHILIA IN CHILDREN

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Abstract

Introduction. Hemophilia is a major public health concern among rare diseases, prompting the active involvement of international organizations. Less than half of patients have a confirmed diagnosis, highlighting a significant degree of underdiagnosis, especially in resource-limited countries.
The aim of the research was to study the clinical manifestations and genetic profile of hemophilia in children, for improving the accuracy of diagnosis and developing a personalized therapeutic model appropriate to the form and severity.
Materials and methods. An observational, descriptive, cross-sectional study with specific participant selection included a group of 90 children aged between 0 and 18 years. The coding and flanking regions of F8/F9 were amplified and analyzed by heteroduplex and automatic sequencing (Genetic Analyzer 3100).
Results. In terms of clinical severity, severe hemophilia A was the most common (48.9%), followed by moderate (36.7%) and mild (14.4%). In severe forms, six joints were affected in 13.6% of cases. The most common mutation identified in the total patient group was the Missense mutation, followed by Inversion of intron 22 and Frameshift. In the group with no inhibitors, most patients had unknown genetic status (94.6%), and Missense and Frameshift mutations were

Résumé

Aspects contemporains de l'hémophilie chez l'enfant

Introduction. L'hémophilie constitue un enjeu majeur de santé publique parmi les maladies rares, ce qui explique l'implication active des organisations internationales. Moins de la moitié des patients disposent d'un diagnostic confirmé, révélant un sous-diagnostic important, en particulier dans les pays à ressources limitées.
L'objectif de cette étude était d'examiner les manifestations cliniques et le profil génétique de l'hémophilie chez l'enfant afin d'améliorer la précision du diagnostic et de développer un modèle thérapeutique personnalisé adapté à la forme et à la sévérité de la maladie.
Matériels et méthodes. Une étude observationnelle, descriptive et transversale, avec sélection spécifique des participants, a inclus 90 enfants âgés de 0 à 18 ans. Les régions codantes et flanquantes des gènes F8 et F9 ont été amplifiées et analysées par hétéroduplex et par séquençage automatique (Genetic Analyzer 3100).
Résultats. L'hémophilie A sévère était la plus fréquente (48,9%), suivie des formes modérée (36,7%) et légère (14,4%). Dans les formes sévères, six articulations étaient atteintes dans 13,6% des cas. La mutation la plus fréquente était la mutation faux-sens, suivie

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present in a small proportion (2.7% each). The database created allows physicians access to both current and historical data.
Conclusions. The findings support the improvement of pediatric hemophilia approach. Continuous monitoring and adequate management planning will reduce the patient's disability risks and increase their life quality.

Keywords: hemophilia, registry, children, inhibitors, severity.

List of abbreviations:
ANOVA – Analysis of Variance
APTT – activated partial thromboplastin time
CI – confidence interval
CRM – cross-reactive material
PHMI – Public Health Medical Institution
PT – prothombin time
SD – standard deviation

de l'inversion de l'intron 22 et du décalage du cadre de lecture. Chez les patients sans inhibiteurs, la majorité présentait un statut génétique inconnu (94,6%). La base de données créée permet l'accès aux données actuelles et historiques.
Conclusions. Ces résultats soutiennent l'amélioration de la prise en charge pédiatrique de l'hémophilie, réduisant le risque de handicap et améliorant la qualité de vie.

Mots-clés: hémophilie, registre, enfants, inhibiteurs, sévérité.

Introduction

Hemophilia A and B are rare congenital coagulation disorders, with comparable frequency in different populations worldwide. Hemophilia A, which represents the predominant form (about 85% of cases), occurs in approximately 1 in 5,000 male newborns, while hemophilia B is less frequent, occurring in approximately 1 in 25,000 male newborns[1,2]. The same proportion is observed globally, with about 85% of cases being type A and 15% type B[1]. Because of X-linked inheritance, the disease almost exclusively affects males, although rare cases of symptomatic females may occur, usually when they are carriers with severe factor deficiency (less than 5% of patients)[3].

In the Republic of Moldova, hemophilia is classified as a rare disease and has been monitored through a functional National Registry since 2023. The distribution of hemophilia types reflects international trends, with approximately 85% of cases being hemophilia A[1]. Compared to 2013, when 227 affected individuals were reported, a slight decrease in the number of patients has been observed, influenced by population decline, migration, and mortality caused by disease complications[4]. The current prevalence, estimated at 63–79 patients per million inhabitants, is lower than the European and global average (106 per million)[4,5]. Analysis by age group shows that approximately one quarter of registered patients are children, reflecting both the congenital nature of hemophilia and historical limitations in long-term survival without adequate treatment.

In recent years, access to genetic testing and inhibitor evaluation has expanded due to international collaboration and funding dedicated to rare diseases. However, the absence of a strong national network of specialized centers and the lack of uniformly trained personnel across all regions remain important challenges in correct detection and monitoring of all cases[6,7]. To improve epidemiological surveillance, authorities have developed additional functionalities of the National Registry, allowing digital recording of clinical and therapeutic data, which facilitates analysis of disease evolution, resource management, and planning of innovative treatments[6].

The F8 gene, located on Xq28, is approximately 186 kb long and consists of 26 exons[8], while the F9 gene, located on Xq27, is about 33 kb long and includes 8 exons[9]. The size and complexity of these genes, especially F8, explain the large diversity of mutations identified to date. In severe hemophilia A, 30–45% of patients present intron 22 inversion (Inv22), and 2–5% intron 1 inversion[9,10], both altering mRNA processing and preventing factor VIII synthesis, resulting in a severe phenotype[10]. The remaining patients, including those with moderate or mild forms, usually present point mutations, such as missense, nonsense, small deletions, insertions, or splice-site mutations[9].

In hemophilia B, point mutations in the F9 gene predominate, especially missense and nonsense mutations, while large deletions or inversions are very rare[9]. In hemophilia A, null mutations lead to complete absence of FVIII protein, whereas in hemophilia B many severe mutations are missense, allowing

synthesis of an antigenically present but nonfunctional protein[11]. Approximately 30% of mutations occur de novo, without family history[12]. Identification of the exact genetic mutation through molecular testing is essential for confirming diagnosis, evaluating relatives, and providing genetic counseling.

Research has shown that the type of mutation in FVIII and FIX genes influences factor activity levels and bleeding severity. Major genetic defects generally cause a more severe clinical phenotype. However, it has not been systematically evaluated whether patients with null mutations in hemophilia A and B have similar bleeding patterns. The different frequency of null mutations also explains the higher proportion of cross-reactive material (CRM)-positive patients in hemophilia B. Null mutations prevent synthesis of any detectable FVIII or FIX[13]. Approximately 5% of hemophilia A patients are CRM-positive and may have FVIII levels up to 30% of normal. CRM in hemophilia A is often associated with missense mutations in the A2 domain of factor VIII. In hemophilia B, about one third of patients are CRM-positive and can produce varying amounts of FIX, which may explain why hemophilia B generally has a milder bleeding phenotype than hemophilia A, although clinical evidence is still limited. This mutation distribution may also explain why inhibitors, usually associated with severe mutations, occur much less frequently in hemophilia B[14].

Some missense mutations associated with mild hemophilia A may favor inhibitor development, a phenomenon not reported in mild hemophilia B. Certain null mutations of the FIX gene may be partially compensated by ribosomal readthrough, allowing synthesis of minimally functional protein, which can influence clinical evolution.

A particular form is hemophilia B Leyden, a rare condition characterized by bleeding manifestations at birth that spontaneously remit during puberty, when FIX levels reach normal values. This phenomenon is correlated with increased growth hormone levels after puberty[6,15].

Overall, although the Republic of Moldova has made significant progress in diagnosis and care of pediatric hemophilia patients, epidemiological data still indicate a difference between the number of registered patients and the theoretically estimated incidence, highlighting the need to strengthen early detection, treatment, and reporting mechanisms.

The objective of the study was to evaluate clinical manifestations, biochemical markers, and the genetic profile in hemophilia A and B in children, to improve paraclinical diagnosis and to develop a personalized therapeutic model adapted to the type and severity of the disease.

Materials and methods

The study was conducted in the Department of Pediatrics of the State University of Medicine and Pharmacy "Nicolae Testemitanu", Chisinau, Republic of Moldova, in consortium with the Public Health Medical Institution (PHMI) Institute of Mother and Child, Hematology Department. It included a sample of 90 children aged 0–18 years, who were under the care of a hematologist and diagnosed with hemophilia. An observational, descriptive, cross-sectional, and selective study was initiated. The study was carried out during the period 2018–2022 and included all children diagnosed with hemophilia and monitored in the clinic during this time. To complete the analysis and obtain a detailed comparison of the therapeutic strategies used, an additional retrospective group of 31 children treated with cryoprecipitate during 2010–2013 was included. This retrospective stage allowed evaluation of the effects of specific treatment over a longer period, providing a comparative overview of the therapeutic methods used. The participants underwent a comprehensive examination, which included standard clinical assessment and laboratory investigations to determine coagulation parameters (Lee–White clotting time, APTT, PT, fibrinogen). Quantitative measurements of coagulation factors VIII and IX, inhibitor titer, and analyses to identify the spectrum of genetic mutations were also performed. Correlations were then established between disease severity, clinical manifestations, type of genetic mutation, and inhibitor titer. The results obtained were used to formulate conclusions and develop practical recommendations. Statistical data processing was performed using tests appropriate to the study design, including ANOVA, Kruskal–Wallis, chi-square, Monte Carlo tests, etc.

Results

The pathogenesis of hemophilia is directly responsible for the tendency toward recurrent bleeding, with repeated hemarthroses that over time lead to the development of hemophilic arthropathy. The difference between bleeding and hemorrhage lies in severity and the volume of blood lost; however, the repeated impact on the joints is decisive in their functional deterioration.

The data obtained in the study showed that the number of affected joints ranged from 1 to 4, with multiple joint involvement being more frequent. Thus, 24.4% of the examined children (22 cases, 95% CI [15.57–33.32]) had simultaneous involvement of 4 joints, 23.3% (21 cases, 95% CI [14.60–32.07]) of 2 joints, and 19.8% (16 cases, 95% CI [0–0.00])

Table 1. Assessment of the type of disease transmission

		Type					
		A		*B*		*Total*	
		N	%	*N*	%	*N*	%
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

of 3 joints. These results were quantified using the mentioned scores, with each joint evaluated individually according to specific parameters (pain, mobility, edema, deformity, muscle atrophy, etc.).

Statistically significant differences were identified between the clinical forms of the disease. In hemophilia B, the ankles were affected significantly less frequently compared to hemophilia A (χ^2 = 7.04, ds = 2, p = 0.032), suggesting a phenotypic particularity of this form. In hemophilia B, unilateral ankle involvement was predominant (77.8%), whereas in hemophilia A, bilateral involvement was more frequent (only 34.6% of cases had unilateral involvement).

On the other hand, regardless of the type of hemophilia, the knee joints were the most frequently affected: 71.6% of patients with hemophilia A and 77.8% of those with hemophilia B presented clinical signs and scores compatible with recurrent hemarthrosis at this level. These results reflect the high incidence of involvement of weight-bearing joints in the context of repeated bleeding, with potential progression to deforming arthropathy if not properly managed.

The study found that in almost 39% of cases (95% CI [28.82–48.96]; 35 children), the family history was unavailable or unknown. This suggests that, in these cases, information regarding disease transmission within the family was either incomplete or unavailable. In a similar proportion of cases, namely 36.7% (95% CI [26.71–46.62]; 33 children), hemophilia was classified as sporadic, meaning that no known family history of the disease existed. Within this category, 35.8% (29 children) had hemophilia A and 44.4% (4 children) had hemophilia B.

In a smaller number of cases, specifically 24.4% (95% CI [15.57–33.32]; 22 children), the familial mode of disease transmission was known. This indicates that a family history of the disease was identified, and the specific pattern of inheritance was recognized. In these cases, the proportions of hemophilia A and hemophilia B were relatively similar, with 24.9% (20 children) for hemophilia A and 22.2% (2 children) for hemophilia B.

Statistical analysis did not reveal significant differences between hemophilia A and B regarding the mode of disease transmission. These findings suggest that the pattern of inheritance does not differ significantly between the two types of hemophilia (χ^2 = 0.27, ds = 2, p = 0.911) and that this characteristic may be similar in both forms of the disease (Table 1).

A detailed evaluation of key biological parameters provides indirect information about the patient's overall condition, the degree of systemic involvement, and potential comorbidities or consequences of recurrent bleeding. The statistical results obtained highlight significant differences between the clinical forms of hemophilia—mild, moderate, and severe—with respect to several hematological and biochemical markers, confirming the hypothesis that disease severity is reflected not only clinically but also at the paraclinical level. The mean hemoglobin level was significantly higher in the mild form of the disease (122.77 g/L, SD: 14.24) compared to the moderate (116.24 g/L, SD: 18.11) and severe forms (110.73 g/L, SD: 13.53), with a statistically significant difference (F=10.21, p <0.001). This finding likely reflects a better functional status of the bone marrow and a lower overall blood loss in mild forms. Lower Hb levels in moderate and severe forms may indicate chronic recurrent bleeding, gradual depletion of iron stores, and the development of hypochromic, microcytic, or mixed anemia. The mean red blood cell counts also differed between groups. The severe form showed markedly lower values (3.53×10^6/µL, SD: 0.54) compared to the mild form (3.97×10^6/µL, SD: 0.41) (F=5.13, p=0.008). This parameter confirms the tendency toward anemia in severe forms, potentially explained by persistent and uncontrolled bleeding frequently observed in these patients. A surprising aspect of the biochemical analysis was the higher total bilirubin values in the mild form of the disease (17.63 µmol/L, SD: 5.56) compared to the moderate (10.30 µmol/L, SD: 2.59) and severe forms (9.76 µmol/L, SD: 2.98), with a statistically significant difference (F=29.44, p < 0.001). This may be explained by increased erythrocyte turnover in the context of effective compensation, with active hepatic conversion.

Table 2 reflects the distribution of clinical severity of hemophilia according to disease type—hemophilia A (factor VIII deficiency) and hemophilia B (factor IX deficiency)—in a sample of 90 children included in our study. Of the total participants, 81 children (90%)

Table 2. Quantitative assessment of coagulation factors VIII and IX

		Type					
		A		*B*		*Total*	
		N	*%*	*N*	*%*	*N*	*%*
Severity grade	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

Table 3. Assessment of genetic mutation types

		Type					
		A		*B*		*Total*	
		N	*%*	*N*	*%*	*N*	*%*
Mutation type	Frameshift	6	7.4	1	11.1	7	7.8
	Inv Intr 22	12	14.8	0	0.0	12	13.3
	Missense	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 tested	44	54.3	7	77.8	51	56.7
	Total	81	100	9	100	90	100

were diagnosed with hemophilia A, and the remaining 9 children (10%) with hemophilia B.

Detailed subgroup analysis shows a marked prevalence of severe forms in Hemophilia A, where 51.9% of patients (42 cases) had severe factor VIII deficiency (<1%). The moderate form (FVIII activity 1–5%) was identified in 35.8% of Hemophilia A patients (29 cases), while the mild form (activity >5%) was much less frequent, occurring in only 12.4% (10 cases). This profile aligns with international literature, where Hemophilia A tends to present more frequently in severe forms, with direct implications for clinical management and the need for early implementation of prophylactic treatment.

In contrast, the distribution of severity in hemophilia B (factor IX deficiency) showed a different pattern. In this group, the moderate form was the most common (44.4%, 4 children), followed by the mild form (33.3%, 3 children). The severe form was identified in only 22.2% of cases (2 children), suggesting a trend toward a milder phenotypic expression in hemophilia B compared to hemophilia A, an observation supported by other international clinical studies.

Across the entire cohort, regardless of hemophilia type, the severe form predominated (48.9%, 44 cases, 95% CI [38.56–59.22]), followed by the moderate form (36.7%, 33 cases) and the mild form (14.4%, 13 cases, 95% CI [7.18–27.71]). These data support the notion that nearly half of the children in the study require sustained therapeutic management, including continuous prophylaxis and multidisciplinary monitoring to prevent hemorrhagic complications. Although the percentage differences between Hemophilia A and B in terms of disease severity are notable, statistical analysis ($\chi^2 = 4.09$, ds = 2, p = 0.129) did not identify a statistically significant difference between the two hemophilia types regarding severity. Nevertheless, the results provide an important perspective on clinical distribution and can guide risk stratification and adaptation of therapeutic strategies.

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

The presence of inhibitors in patients with hemophilia was analyzed according to the two types: hemophilia A and hemophilia B. The proportions are indicated for each category, along with the 95% confidence interval (CI) for each group. In hemophilia A, inhibitor testing was not performed in 60.5% of cases

Table 4. Assessment of inhibitor titers

		Type					
		A		B		Total	
		N	%	N	%	N	%
Inhibitors	Not tested	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

Table 5. Phenotype–genotype correlation results

		Severity grade							
		Mild		Moderate		Severe		Total	
		N	%	N	%	N	%	N	%
Mutation	Frameshift	0	0.0	4	12.1	3	6.8	7	7.8
	Inv Intr 22	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
	Negative	0	0.0	1	3.0	0	0.0	1	1.1
	Nonsense	0	0.0	1	3.0	2	4.6	3	3.3
	Not tested	12	92.3*	10	30.3*	29	65.9*	51	56.7
	Total	13	100	33	100	44	100	90	100

(95% CI [44.16–64.73]; 49 patients), indicating moderate uncertainty. In hemophilia B, all patients underwent inhibitor titration. Overall, 49 out of 90 patients did not undergo inhibitor testing, representing 54.4% of the entire study population. In hemophilia A, 4 patients (4.9%) had detectable inhibitors, with a 95% CI ranging from 0.19% to 8.70%, indicating a low incidence of this phenomenon. No cases of inhibitors were observed in hemophilia B (0%). Overall, 4 patients (4.9%) out of the 90 included in the study had detectable inhibitors. In hemophilia A, 28 patients (34.6%) had no inhibitors, but this percentage is significantly lower than the group that was not tested. The 95% CI for this proportion ranges from 30.95% to 51.28%, indicating moderate frequency. In hemophilia B, all patients (9 patients, 100%) had no inhibitors, which is characteristic for this type of hemophilia. Overall, 37 patients (41.1%) out of the 90 included in the study had no inhibitors, of which 28 were hemophilia A and 9 were hemophilia B. Hemophilia A showed a significant prevalence of untested cases, suggesting possible limited access to this analysis for most patients. These observations provide important information regarding diagnostic approaches and patient management, as well as the prevalence of inhibitors in different types of hemophilia (Table 4).

The types of genetic mutations were analyzed in relation to disease severity, thereby describing the association between genotype and the degree of disease severity.

In mild forms of hemophilia, the most frequent mutation was Missense, observed in 7.7% of cases (16 children). The Inv Intr-22 mutation was recorded in 13.3% of cases (12 children) and was found only in moderate (15.2%, 5 children) and severe forms (15.9%, 7 children). Another type of mutation identified was Frameshift, present in 7.8% of cases (7 children), with a frequency of 12.1% (4 children) in moderate forms and 6.8% (3 children) in severe forms. The least frequent mutation was Nonsense, observed in 3.3% of cases (3 children), with 4.6% (2 children) in the severe form and 3.0% (1 child) in the moderate form.

A statistically significant difference was identified at $p < 0.05$ between the frequency of the Missense mutation in moderate forms, which occurred in 36.4% of cases (12 children), and in severe forms, which occurred in 6.8% of cases (3 children) ($\chi^2 = 24.015$, ds = 10, $p = 0.008$).

These findings highlight the diversity and complexity of genetic mutations involved in the etiology of hemophilia and their relevance in determining disease severity (Table 5).

Data regarding the distribution of replacement therapy with cryoprecipitate and factor VIII (F VIII) for the management of massive hemorrhages were analyzed. Two massive hemorrhages were recorded in one patient treated with cryoprecipitate. This observation is important, suggesting that the efficacy of cryoprecipitate in preventing or controlling massive hemorrhages may be lower than that of factor VIII therapy. Overall, the data suggest that in cases of massive hemorrhage, treatment with factor VIII may be more effective than cryoprecipitate. However, it is important to consider each individual case and to evaluate the benefits and risks according to patient-specific needs and conditions (Table 6).

Following the completion of all research results, the "Electronic Registry for Hemophilia Patients in

Table 6. Comparative characteristics of hospitalizations for massive hemorrhages at different sites according to the type of treatment administered

		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 Republic of Moldova" was created and implemented. This is an information system dedicated to monitoring and managing data on hemophilia patients at the national level. The registry represents an essential component of public health infrastructure, with a direct impact on the quality of care provided to hemophilia patients.

The primary objective of the registry is to consolidate and centralize information on hemophilia patients, including the type of hemophilia (A or B), severity grade, hospitalization history, and recommended treatment regimens. It contributes to continuous patient monitoring by ensuring a coherent flow of information that allows data transfer to specialized departments, such as the Hematology Department of the PHMI Mother and Child Institute, and subsequently to the Oncology Institute for patients over 18 years of age.

The registry was developed to comply with confidentiality requirements and personal data protection, applying national legislation on data protection and medical information confidentiality. Its interactive structure allows for continuous collection and storage of patient data, ensuring that information is accessible only to authorized personnel.

This research makes a valuable contribution by providing a comprehensive evaluation of the clinical and paraclinical characteristics of hemophilia in children in the Republic of Moldova. The results have important implications for justifying the need to identify genetic mutations and inhibitor titers in the assessment of children with hemophilia. These parameters, together with clinical manifestations and coagulation factor levels, are essential for an accurate assessment of disease severity and for developing personalized therapeutic strategies. Thus, the present research strengthens the theoretical and practical foundation for hemophilia management in children in the Republic of Moldova. Hemophilia is a hereditary monogenic disorder characterized by a pronounced tendency for spontaneous or post-traumatic bleeding, caused by a severe deficiency of coagulation factor VIII (in hemophilia A) or factor IX (in hemophilia B). The severity of clinical manifestations varies according to the level of coagulation factor activity, and hemarthrosis represents one of the most frequent and debilitating complications of the disease, significantly impacting patients' quality of life.

Discussion

The present study highlights the clinical, genetic, and hemostatic characteristics of pediatric hemophilia in the Republic of Moldova, emphasizing the importance of integrated clinical, paraclinical, and molecular assessment for individualized therapeutic management. From an epidemiological perspective, hemophilia prevalence in the Republic of Moldova remains below the European average, suggesting persistent underdiagnosis, particularly in mild forms and rural regions[14]. Hemophilia A represented the predominant form, while severe and moderate phenotypes accounted for most cases. Musculoskeletal manifestations, predominantly involving the knees and ankles, constituted the most frequent complications, with disease severity influenced by coagulation factor deficiency and therapeutic history, described in similar studies[1,3]. Genetic analysis confirmed the X-linked recessive inheritance pattern, with a substantial proportion of sporadic cases suggestive of de novo mutations. Molecular investigations demonstrated the predominance of missense mutations, while intron 22 inversion was associated with severe hemophilia A and an increased risk of anti-FVIII inhibitor development, supporting a strong genotype–phenotype correlation. Inhibitors were identified exclusively in patients with hemophilia A, underscoring the need for standardized monitoring algorithms and genetic risk stratification in the researched populations[8,10]. Hemostatic evaluation revealed significantly prolonged activated partial thromboplastin time (aPTT) in all patients, confirming intrinsic pathway impairment, whereas other coagulation parameters remained within normal ranges. Severe forms were associated with anemia secondary to recurrent hemorrhagic episodes and biochemical alterations suggestive of hepatic involvement, highlighting the necessity for continuous hematological and hepatic monitoring. Therapeutic outcomes demonstrated the superiority of recombinant factor VIII (rFVIII) over cryoprecipitate in controlling severe bleeding episodes, preventing hemarthroses, and limiting the progression of hemophilic arthropathy. Prophylactic rFVIII therapy significantly reduced recurrent hemorrhages and long-term disability risk, contributing

to improved quality of life in pediatric patients. The introduction of modern prophylactic strategies, Emicizumab therapy, and the national electronic database has represented a major advancement in disease monitoring and therapeutic management.

Conclusions

Genetic analysis highlighted the predominance of Missense and Frameshift mutations, which were associated with moderate and severe forms of the disease. The intron 22 inversion was identified exclusively in moderate and severe forms of Hemophilia A (15.2% and 15.9%), confirming its major pathogenic role and the relevance of genetic testing for establishing an etiologic diagnosis, predicting inhibitor risk, and providing family counseling. Frameshift and Nonsense mutations were also more frequently observed in moderate and severe forms.

Quantitative assessment of coagulation factors demonstrated a direct correlation between low levels of factors VIII and IX and the severity grade of hemophilia.

Inhibitor testing revealed an occurrence rate of 16.0%, observed exclusively in patients with severe hemophilia. This finding underscores the importance of periodic monitoring of inhibitors in this patient category to adjust treatment and prevent therapeutic failures due to immune resistance.

Comparative analysis of treatment strategies demonstrated superior efficacy of recombinant and lyophilized factor VIII preparations in controlling severe hemorrhages and preventing hemophilic arthropathy, compared to cryoprecipitate, which was mainly effective in mild forms.

The development and implementation of the National Registry represent a major advancement in centralizing and standardizing clinical and paraclinical information, facilitating continuous monitoring, personalized treatment, and evaluation of therapeutic efficacy.

Author Contributions:

V.I., D.A., I.S., G.E., V.T. were responsible for clinical diagnosis, paraclinical investigations, treatment decisions and follow up of the patients. V.I., I.S. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Compliance with Ethics Requirements:

"The authors declare no conflict of interest regarding this article"

"The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study"

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