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Review Article

Biomarkers in Hemophilia: Diagnostic, Prognostic, and Treatment-Selection Utility: A Narrative Review of the Literature

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ABSTRACT

Background: Hemophilia A and B are inherited bleeding disorders in which outcomes are shaped by factor levels, inhibitor development, inter-individual pharmacokinetics, and progressive joint disease. With the emergence of extended half-life therapies, non-factor therapies, and gene therapy, there is an increasing need for biomarkers to support diagnosis, risk stratification, treatment selection, and monitoring.
Methods: We conducted a narrative review of English-language studies indexed in PubMed, Scopus, Web of Science, and Google Scholar (January 2000 to October 2025). We prioritized primary studies, trials, registries, and assay-validation reports on diagnostic, prognostic, predictive, or monitoring biomarkers in hemophilia A/B; guidelines were used for context.
Results: Established biomarkers include FVIII/FIX activity assays, inhibitor testing (Bethesda/Nijmegen-modified), F8/F9 genotyping, and imaging-based joint assessment. Emerging candidates include global hemostasis assays (thrombin generation, viscoelastic testing), PK/PD metrics for prophylaxis individualization, and exploratory inflammatory/angiogenic and tissue-turnover markers linked to arthropathy.
Conclusions: Evidence for biomarkers in hemophilia is heterogeneous, and many non-traditional markers remain exploratory; standardized assays and prospective multicenter validation with clinically meaningful endpoints are needed before routine adoption. Limitation: This narrative synthesis was conducted in English and did not include a formal risk-of-bias assessment.

1. Introduction

Hemophilia is a hereditary disease characterized by impaired blood clotting due to a deficiency of clotting factors, especially factor VIII (hemophilia A) or factor IX (hemophilia B). Hemophilia A is more common than hemophilia B, representing approximately 80 – 85% of all cases. Both types are inherited in an X-linked recessive pattern, primarily affecting males. While the majority of females are asymptomatic carriers, rare symptomatic cases have emerged due to skewed X-chromosome inactivation or homozygous mutations [1–4].

Clinical manifestations of hemophilia vary from case to case according to disease severity. Patients with severe hemophilia suffer from spontaneous bleeding episodes, especially in the muscles and soft tissues. The most common manifestation is joint bleeding. Milder forms of hemophilia appear in the form of prolonged bleeding after trauma or surgery. Severe joint involvement in hemophilia significantly impairs quality of life for affected individuals. Chronic joint bleeding leads to synovitis, cartilage degeneration, and progressive arthropathy, which significantly affects individuals' well-being and overall activities of daily living [1–4].

Hemophilia A results from mutations in the F8 gene, which is specific to hemophilia A, and hemophilia B results from mutations in the F9 gene, which is specific to hemophilia B; both are located on the X chromosome. These mutations result in the absence of clotting factors or defects in their function. Developments in molecular genetics have enabled accurate identification of these mutations, thereby facilitating the detection of disease carriers [1, 4].

Biomarkers are defined as measurable characteristics that indicate normal biological processes, pathogenic processes, or responses to therapeutic interventions. Biomarkers can be classified into diagnostic, prognostic, predictive, and monitoring categories according to their intended clinical application. Accordingly, biomarkers have become central to evaluating hemophilia severity, guiding therapy, and predicting outcomes. We can measure the quality of the disease,

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its progression, and its response to treatment using some vital indicators, which are:

- Protein-based biomarkers: Factor VIII/IX activity levels, thrombin generation tests.
- Genetic biomarkers: F8 and F9 gene mutations.
- Imaging modalities: MRI and ultrasound for joint health assessment [2, 5].

Several reviews have previously addressed biomarkers in hemophilia; however, most have focused on specific biomarker domains rather than providing a comprehensive overview. Some reviews emphasize imaging-based assessment of joint disease, while others primarily address genetic mutations or individual laboratory markers.

The goal of this narrative review is to compile the most recent data regarding hemophilia biomarkers and their clinical significance. It specifically focuses on protein-based biomarkers, such as Factor VIII/IX activity and thrombin-generation profiles; genetic biomarkers (F8 and F9 mutations); and imaging biomarkers that assess joint damage and disease progression. This review aims to clarify the role of these biomarkers in clinical practice and to identify areas requiring further hematology research by classifying them and linking them to diagnostic and therapeutic decision-making.

2. Methods

Search strategy and sources: PubMed, Scopus, Web of Science, and Google Scholar databases were searched for English-language records published from January 1, 2000, through October 31, 2025. The core terms combined hemophilia ("hemophilia", "hemophilia A", "hemophilia B") with biomarker concepts (e.g., biomarker, genetic marker, inhibitor, thrombin generation, viscoelastic testing, pharmacokinetics, microRNA, inflammatory markers, imaging) and clinical intent (diagnosis, prognosis, prediction, monitoring).

2.1. Study selection

Two reviewers independently screened titles/abstracts and then full texts for relevance to biomarker use in hemophilia A/B. Disagreements were resolved by discussion and, when needed, adjudication by a third reviewer. Reference lists of included primary studies and key reviews/guidelines were checked to identify additional eligible primary studies.

2.2. Eligibility and data handling

We included primary human studies (original research articles, clinical trials, systematic reviews, registry analyses, and assay-validation studies) that evaluated diagnostic, prognostic, predictive, or monitoring biomarkers in hemophilia A or B. Guidelines were used for background only and were not treated as primary evidence. We excluded case reports, editorials, animal-only studies, and reports without biomarker-related outcomes. For each included primary study, we extracted biomarker type, specimen matrix, assay/platform (when reported), diagnostic or prognostic performance, and study design and cohort context. The synthesis employed a narrative (qualitative) approach, emphasizing key trends, biomarker validation efforts, and clinical implications.

2.3. Quality assessment

Given the narrative design and heterogeneity of included study designs and outcomes, we did not perform a formal risk-of-bias assessment using standardized tools. Instead, studies were descriptively appraised based on study design, sample size, outcome definitions, and clarity of biomarker measurement and reporting.

2.4. A new step in treating hemophilia

2.4.1. Advances and Challenges in Hemophilia Management

Hemophilia management has long faced barriers, including the high cost of treatment and unequal patient access. These challenges were highlighted in the latest hemophilia management guidelines issued by the International Society on Thrombosis and Haemostasis (ISTH) in June 2024. Currently, five classes of treatment and prophylaxis options have been identified, ranging from well-established therapies to emerging interventions. These include standard half-life (SHL) clotting factor concentrates, extended half-life (EHL) clotting factor concentrates, bispecific monoclonal antibodies, rebalancing agents, and gene therapy products [6].

Prophylactic treatment is widely recommended to prevent bleeding episodes in the management of hemophilia. Guidelines from ISTH and the World Federation of Hemophilia (WFH) continuously refine recommendations based on emerging evidence and advocate specific treatment strategies tailored to different patient conditions. These efforts reflect the dynamic nature of hemophilia research aimed at optimizing management approaches [7].

Factor replacement therapy remains a cornerstone of hemophilia management, whether for bleeding prevention or episodic treatment. SHL factor replacement therapies are increasingly being replaced by EHL products, which reduce the frequency of administration, thereby improving patient adherence and reducing emotional distress, ultimately enhancing quality of life (QoL). Despite their widespread use, factor replacement therapies fail to completely prevent joint bleeding, the most common bleeding site in hemophilia, prompting the exploration of alternative treatment strategies. Notably, in 2024, the FDA approved new non-factor therapies, including monoclonal antibodies such as marstacimab (Hympavzi) and gene therapy agents like fidanacogene elaparvovec (Beqvez) [6, 7].

Several novel therapies are currently available or in clinical development. These include efmoroctocog alfa, a factor VIII (FVIII) protein engineered with three half-life-extension technologies to overcome the half-life limitations imposed by von Willebrand factor; next-generation, high-potency bispecific monoclonal antibodies; enhanced factor molecules; gene therapies; and rebalancing agents. Among these, rebalancing therapies are particularly promising, as they restore hemostatic balance by inhibiting natural anticoagulants. These therapies are especially beneficial for patients with inhibitors or those who do not respond adequately to traditional clotting factor replacement. For instance, the rebalancing agent fitusiran has demonstrated superiority over on-demand clotting factor concentrates in reducing annual bleeding rates in both hemophilia A and B. Further details on current and future therapeutic approaches are discussed elsewhere [6].

Personalized medicine and stratified treatment approaches have recently been introduced in hemophilia management to better tailor therapies to individual patient needs. A single prophylactic regimen – such as one based solely on weight or age – is unlikely to be optimal for all patients. Instead, personalized prophylaxis is largely guided by a patient's individual pharmacokinetic (PK) profile, particularly their FVIII levels [6, 7].

Recent efforts to optimize prophylaxis through PK-guided approaches have shown promise in improving patient outcomes. Compared with conventional prophylaxis, PK-guided personalized prophylaxis using fourth-generation recombinant human FVIII has been associated with reduced annualized bleeding rates, extended dosing intervals, and lower overall doses. Beyond FVIII-based personalization, ongoing research aims to elucidate the early molecular mechanisms involved in inhibitor development upon initial

exposure to clotting factor concentrates. A better understanding of these processes could enable the prediction of inhibitory antibody formation, further refine the stratification of patients with hemophilia, and guide treatment decisions [6, 7].

2.4.2. Laboratory and Genetic Tests for Hemophilia Diagnosis

Hemophilia requires a stepwise diagnostic approach that begins with clinical suspicion and screening with basic coagulation tests. Routine tests, such as a complete blood count (CBC), prothrombin time (PT), and activated partial thromboplastin time (aPTT). We can identify the deficiency of the intrinsic pathway by prolonged aPTT with normal PT, prompting factor assays for factor VIII (FVIII) and factor IX (FIX). Its severity is classified in relation to the residual factor activity: severe (<1%), moderate (1-5%), and mild (>5-40%). When bleeding doesn't stop despite replacement therapy, an inhibitor assay, typically the Nijmegen-modified Bethesda assay, is ordered to quantify neutralizing antibodies. As the inhibitor testing requires experienced laboratories and standardized conditions, access remains limited in low-resource settings [6].

Genetic analysis is the most reliable confirmatory tool for identifying carriers, prenatal diagnosis, and classifying patients for gene therapy. Recent sequencing methods (e.g., targeted F8/F9 gene panels and next-generation sequencing) can identify point mutations, deletions, or inversions in more than 98% of cases. Prenatal or preimplantation genetic testing is allowed to be used on fetal DNA from chorionic villus sampling, amniocentesis, or free fetal DNA in maternal plasma. Still cost-effective in families showing mutations, linkage, or restriction fragment length polymorphism (RFLP) analysis. There are reliable tools for carrier detection and reproductive planning, as recent studies have validated several polymorphic markers (e.g., FXS1108, DXS9897, F8int22, DXS9901). Genetic Tests also help in personalized treatment, including eligibility assessment for gene therapy or prophylactic protocols [6].

2.4.3. Imaging Studies for Joint Evaluation

Damage to the joints is considered one of the most serious long-term complications in hemophilia, so imaging becomes an essential biomarker to monitor the disease. Recurrent hemarthroses stimulate synovial hypertrophy, cartilage degradation, and eventual hemophilic arthropathy. We use imaging modalities as radiologic biomarkers to detect early structural and functional joint changes before irreversible damage occurs [8].

The most widely used imaging modality for patients with hemophilia is Musculoskeletal ultrasound (MSK-US). It provides a rapid, non-invasive evaluation of joint effusion, synovial hypertrophy, and cartilage thickness. We can conduct qualitative monitoring and intercenter reproducibility using scoring systems such as the Hemophilia Early Arthropathy Detection with Ultrasound (HEAD-US) and the Joint Tissue Activity and Damage Examination (JADE) scores [8].

The gold standard for comprehensive assessment of joint structures is Magnetic resonance imaging (MRI). To determine disease severity, the World Federation of Hemophilia (WFH) recommends MRI scoring systems, such as the Denver or IPSG scores. Updates to imaging recommendations, including quantitative MRI and ultrasound elastography, have expanded the role of these modalities as biomarkers of disease progression. Quantitative MRI metrics, such as T2 relaxation times and dGEMRIC indices, correlate with biochemical cartilage degradation and can serve as surrogate endpoints in prophylactic trials. The use of imaging biomarkers with longitudinal follow-up enables clinicians to identify factor replacement or non-factor prophylaxis strategies, thereby reducing joint morbidity and improving quality of life [8].

2.5. Significance of biomarkers in different studies

Significance of biomarkers in different studies Biomarkers are broadly defined by the United States Food and Drug Administration (FDA) as molecular, histologic, radiographic, or physiologic characteristics that can be assessed for diagnostic, monitoring, prognostic, predictive, pharmacodynamic, and safety purposes. Not only can diagnostic markers be used to identify a disease, but some can also identify a particular subtype, thereby guiding future management. Other monitoring biomarkers can be used to follow up on disease progression or treatment response. Prognostic biomarkers can identify high-risk individuals, determine resource allocation, and guide clinical decision-making. Certain biomarkers can stratify patients to predict the risk of certain outcomes, proving to be particularly useful in clinical trials [6, 9, 10].

In real life, biomarkers are not just numbers from lab reports. They provide important information about how hemophilia manifests in each patient and how they might respond to a particular treatment. Based on the BEST framework, biomarkers can be divided into four main groups: diagnostic, prognostic, predictive, and monitoring. This system helps make the scientific data more practical for daily clinical work [9, 10].

Diagnostic markers, such as FVIII or FIX activity, thrombin generation, and inhibitor testing, are used to define the type and severity of hemophilia. In addition, genetic analysis for F8, F9, or F11 mutations can confirm the disease and detect carrier status. Prognostic biomarkers include baseline factor levels, high-risk genotypes (like intron 22 inversion), and inflammatory molecules such as CRP or VEGF. These can help predict the frequency of bleeding or the extent of joint involvement. Predictive markers are useful in determining which therapy will be most effective. For instance, a patient's baseline thrombin generation may influence their response to emicizumab or gene therapy. Finally, monitoring biomarkers, including D-dimer and viscoelastic tests (TEG, ROTEM), is used to assess hemostatic stability and to follow the effects of therapy over time. Thinking in this structured way helps physicians integrate laboratory results, genetic data, and imaging findings into a single picture. This approach improves individual care and supports early recognition of complications [7, 9, 10].

Motion analysis, including gait analysis and surface electromyography (sEMG), is being studied as an indicator of early joint changes and muscle affection, which are often missed by radiography and clinical examination. The non-invasive nature of this tool makes it especially suitable for pediatric patients. Magnetic resonance imaging (MRI) is also very sensitive to early joint changes, such as synovial hypertrophy. In addition, it can detect the presence of hemosiderin, indicating joint bleeding. However, it is not ideal in the pediatric population, as they often require sedation to tolerate the procedure. This makes ultrasound a better option for children with hemophilia despite its relatively lower sensitivity. Ultrasound can assess effusions and synovial changes, but cannot detect hemosiderin deposition. Although X-rays are sometimes used, they can only detect late joint changes [10].

The role of biochemical markers in hemophilic arthropathy is also being explored. For instance, a study by Xu et al (2020; n=144 severe hemophilia A patients and 90 healthy controls; cross-sectional study, China) found levels of leukocytes, C-reactive protein (CRP), macrophage migration inhibitory factor (MIF), and vascular endothelial growth factor (VEGF) to be elevated in patients with hemophilia, and even more so in those with active bleeding. Though

this could indicate their potential use as biomarkers, the study is limited by a relatively small sample size and inadequate long-term follow-up (55). A case-control study by Knowles et al (2023; n=59 hemophilia A/B and 54 healthy controls; cross-sectional with longitudinal sub-analysis, Germany) found serum levels of interleukin-6 (IL-6), CRP, LPS-binding protein (LBP), and soluble IL-6 receptor α (sIL-6Rα) to be elevated in hemophilia patients with acute bleeding or recent bleeding within the last month, suggesting a possible diagnostic role [7].

C2M, COMP, and CTX-II are biomarkers of cartilage degradation that are elevated in patients with hemophilia. Compared with controls, CTX-1, a marker of bone resorption, was elevated, and PINP, a marker of bone formation, was increased in patients with hemophilia. In early joint bleeding, serum levels of D-dimer, fibrin degradation products (FDPs), and plasminogen were significantly decreased. On the other hand, plasma levels of epidermal growth factor (EGF), colony-stimulating factor 2 (CSF2), interleukin 4/13 (IL4/13), fibroblast growth factor 2 (FGF2), and MIP-1α were significantly lower during acute bleeding [7, 11].

One study, López-Jiménez et al. (2019; n=50 pediatric patients with hemophilia; cross-sectional genetic association study, Mexico), examined the association between genetic biomarkers and the severity of hemophilic arthropathy in pediatric patients. After analyzing data from 50 patients, those expressing the MTHFR 677TT genotype were found to have more affected joints. On the other hand, those expressing the MTHFR 1298AC genotype had more severe effusions, as assessed by MRI. Subchondral cysts were significantly more common among participants with the TNFα-308GA genotype (58). However, further studies are needed to identify the mechanisms underlying the relation between genetic polymorphisms and the clinical and radiographic manifestations in pediatric patients with hemophilic arthropathy [9, 10].

Interestingly, a cross-sectional study by Susanah et al. (2021; n=216 patients with severe hemophilia A; cross-sectional observational study, Indonesia) found a significant correlation between high serum tumor necrosis factor α (TNF-α) levels and high factor VIII inhibitor titers, suggesting a potential role in treatment selection. However, further studies are needed to further explore this correlation [9, 10].

Beyond treatment selection, biomarkers are being explored to monitor therapeutic efficacy. For instance, rurioctocog alfa pegol is a PEGylated recombinant factor VIII used to prevent bleeding in patients with hemophilia A. Manon-Jensen et al (2023; n=98; secondary biomarker analysis of a phase 3 randomized trial (PROPEL), multinational) evaluated the use of collagen turnover markers to assess the efficacy of rurioctocog alfa pegol. PRO-C4 is a biomarker that reflects type 4 collagen formation, while C4M represents type 4 collagen degradation. Both together can assess basement membrane remodeling. PRO-C5 reflects type 5 collagen formation, and C3M reflects type 3 collagen degradation; they assess interstitial matrix remodeling. PRO-C2 reflects type 2 collagen formation, and C2M reflects type 2 collagen degradation. Thus, they can assess cartilage remodeling [9, 10].

Serum levels of these biomarkers were measured at baseline and at 3-month intervals until 1 year post-treatment. PRO-C4, C4M, PRO-C5, and C3M levels decreased after treatment with rurioctocog alfa pegol [12].

On the other hand, PRO-C2 had increased at 6 months. However, this increase was not observed in C2M levels. In addition to their potential use in monitoring treatment response, these biomarkers have allowed us to better understand the mechanisms through which the medication works. Thus, they can be valuable in research settings and clinical trials. Though promising, the small number of participants in this study limits the generalizability of findings [12].

2.6. Biomarker validation in cases of hemophilia

For biomarkers to be truly useful, they must be validated and standardized. Many are promising, but only a few are well tested for routine practice. According to FDA BEST and ISTH guidance, validation should include analytical accuracy, clinical significance, and reproducibility. Analytical validation focuses on the consistency and precision of measurements. For example, FVIII and FIX assays should yield identical results regardless of the reagents or instruments used. Thrombin generation and viscoelastic tests also require careful calibration to minimize inter-laboratory variability. Clinical validation means linking biomarker levels to real outcomes like bleeding frequency or joint health. However, most studies remain small, and differences in methods complicate comparisons. Reproducibility across laboratories remains a major challenge. Global efforts led by WFH and WHO reference centers aim to unify testing methods, use shared reference materials, and define acceptable ranges of variation. Standardization of how and when samples are collected will help produce comparable results between studies. When this is achieved, biomarkers will become reliable tools not only for research trials but also for personalizing therapy in everyday hemophilia care [1, 2, 4–6].

Historically, the life expectancy for individuals with hemophilia was less than 11 years. Still, the introduction of clotting factor concentrates has significantly improved the quality of life and life expectancy, now extending to 60-70 years. In the Asia-Pacific region, prophylactic treatment for young children with hemophilia has been adopted based on successful randomized controlled trials in the US. However, many healthcare systems still struggle with advanced hemophilic arthropathy (HA) due to various challenges, including inhibitor development. To better understand HA, researchers are using advanced imaging, molecular biology, and genetic assessment [4, 5, 7].

Biomarkers can aid in diagnosing challenging cases, predicting disease progression, and developing targeted therapies. Biochemical markers in serum, urine, or synovial fluid can reflect joint tissue changes, enabling early detection and potentially preventing joint damage. Further research on biomarkers is essential to improve decision-making and management of HA [4].

Validating biomarkers involves evaluating their efficacy in documenting disease progression and activity, thereby enabling their widespread use in therapeutic interventions. While imaging techniques such as MRI are preferred for detecting arthropathy in patients with hemophilia, biomarker analysis has also shown promise. Studies have identified biomarkers with high sensitivity and specificity for diagnosing acute joint bleeding, such as CRP and VEGF. Combinations of biomarkers, such as cartilage degradation markers, have been used to monitor joint damage and assess treatment efficacy [1, 4, 7].

However, the heterogeneity of biomarkers and inter-individual differences pose challenges in decision-making. Further research is needed to identify novel biomarkers specific to hemophilic arthropathy, leveraging omics approaches like proteomics. Personalized treatment strategies, including clotting factor replacement and physiotherapy, may benefit from biomarker-driven monitoring of joint health. Potential biomarkers, such as cartilage iron and inflammatory factors, hold promise for early diagnosis and primary prophylaxis [13].

Research has identified several biomarker combinations that can accurately distinguish between patients with joint disease and healthy individuals. For instance, serum levels of bone turnover markers, such as sRANKL and OPG, are lower in patients with hemophilia, and these levels correlate with joint outcomes. Conversely, elevated sclerostin levels, a regulator of bone formation, have been associated with joint damage [13].

The proinflammatory cytokine TNF-α is elevated in patients with hemophilic arthropathy and correlates with joint deterioration. However, its lack of specificity limits its use as a biomarker. Other potential biomarkers, such as iron accumulation in cartilage and vascularization factors like VEGF, have been associated with joint damage and disease progression. Additionally, low bone mineral density and vitamin D deficiency have been associated with poor joint health and reduced quality of life in patients with hemophilia. Despite these findings, biomarker research in hemophilia faces challenges, including heterogeneous patient populations, small sample sizes, and the lack of a reference standard for assessing arthropathy [4, 14].

Several limitations constrain biomarker research in hemophilic arthropathy (HA). One major challenge is the lack of a standardized reference for assessing HA severity. Physical function scores and imaging techniques have limitations in reflecting joint damage, and discrepancies between clinical function and imaging findings are common. Radiographic assessments, such as X-rays, are useful for visualizing bone structures but may not capture changes in cartilage and synovial tissue. Alternative imaging modalities, such as ultrasonography and MRI, can provide more representative assessments, but they also have limitations [4, 7, 8].

Moreover, biomarkers can be influenced by various factors, including co-infections like HIV or HCV, which can affect inflammatory marker levels. Bone markers may not accurately reflect bone status due to age-dependent variations and differences between children and adults. Tissue specificity and assay variability can also contribute to heterogeneous assessments and contradictory results. To overcome these challenges, future studies should prioritize homogeneous study designs, minimize inter-individual variability, and consider combined biomarker indices to improve correlations [4, 15].

Biomarkers can be categorized by utility, and numerous biomarkers have been reported in the literature, including those related to cartilage, synovium, bone, and inflammation. Examples include miRNAs, soluble vascular cell adhesion molecule-1 (sVCAM-1), and markers of collagen turnover, bone formation, and resorption [1, 4, 7, 10].

MicroRNAs (miRNAs) play a crucial role in regulating gene expression and have been identified as potential biomarkers for hemophilic arthropathy. Research has shown that specific miRNAs, such as miR-208a-3p and miR-524-3p, are associated with severe hemophilic arthropathy and may serve as early diagnostic or prognostic tools. These miRNAs target genes involved in bone and cartilage degradation, including PTEN, which regulates the PI3K/AKT pathway and endothelial growth [16].

Studies have also found changes in miRNA expression patterns in response to joint bleeding and arthropathy development. While some miRNAs, such as miRNA-15b, have been linked to hemophilia in animal models, others, such as miRNA-155 and miRNA-9, have yielded inconsistent results in human studies. Further research is needed to validate the potential pathogenic role of miRNAs in hemophilic arthropathy and explore their utility as biomarkers [16].

Research on microRNAs (miRNAs) as biomarkers for hemophilia arthropathy (HA) is limited. One major concern is the uncertainty about the tissue source of miRNAs and whether plasma levels accurately reflect changes in osteochondral tissues. Without analyzing osteochondral tissue, it's challenging to draw conclusive correlations between tissue activity and circulating miRNA levels. Studies have focused on patients with advanced arthropathies, but future research should investigate miRNA signatures indicative of early arthropathy. Overlapping miRNA levels between controls and patients can complicate the determination of thresholds for early diagnosis. To overcome these challenges, homogeneous study designs, minimized inter-individual variability, and combined biomarker indices are essential [4].

Although microRNA (miRNA) has been proposed as a potential biomarker for rheumatoid arthritis and hemorrhagic arthritis, as demonstrated in this study (Pasta et al.), the evidence remains limited due to several factors that affect the quality of results, such as small sample sizes and inconsistencies in analytical methods. There are several uncertainties regarding the accuracy of pathological tissue analysis. Consequently, miRNA-based biomarkers should currently be considered research tools rather than clinically validated indicators. Therefore, more clinical trials are needed to clarify the accuracy of the results [4]. Similarly, genetic polymorphisms beyond causative F8 and F9 mutations have been associated with disease severity and joint outcomes; however, these findings lack direct therapeutic implications and therefore have a very limited role in clinical decision-making. Further research and verification are needed before their routine and fundamental use in clinical practice can be established to ensure optimal patient outcomes [4].

2.7. Prognostic biomarkers in Hemophilia

Prognostic biomarkers help us understand how hemophilia evolves, who is likely to bleed more and who might develop complications such as arthropathy or inhibitor formation later on. One of the most important indicators remains the baseline factor level. Patients with FVIII or FIX activity $< 1\%$ usually have spontaneous bleeding and more rapid joint damage, whereas those with moderate levels tend to have milder symptoms and fewer spontaneous episodes [1, 4, 5].

Genetic changes also carry strong prognostic value. For example, large deletions, nonsense mutations, and intron 22 inversions in the F8 gene have consistently been associated with a substantially higher risk of inhibitor development than smaller or missense mutations. There are also immune-related genes, such as IL10, TNFA, and CTLA4, that may modify this risk, although results across populations remain somewhat inconsistent. In recent years, inflammatory markers have become another useful group of prognostic tools. Higher levels of C-reactive protein (CRP), vascular endothelial growth factor (VEGF), and matrix metalloproteinases (MMP-1, MMP-9) have been associated with persistent joint inflammation and early cartilage degradation. Studies that follow patients over time have shown that when these markers remain elevated, joint damage and functional decline typically progress more rapidly – even in patients receiving regular prophylaxis. A newer approach is to analyze multiple markers simultaneously, combining genetic, coagulation, and inflammatory data to develop predictive models for bleeding severity and inhibitor risk. Still, these tools need validation in larger, more diverse groups [6, 7, 13, 14].

Unfortunately, there is no specific prognostic biomarker to predict the risk of inhibitor development during the treatment of patients with hemophilia. However, many studies investigate potential biomarkers, but they are not yet fully validated. The study by Işık et al. (2021; n=3,248 hemophilia A cases with missense F8 variants; retrospective

database study) reported that elevated levels of G-CSF and IL-6, reduced levels of IL-10, higher core-fucosylation and galactosylation, and lower sialylation were observed in patients with inhibitors [4, 14, 17].

Large registry studies and systematic reviews have consistently shown that specific F8 mutation types, particularly large deletions, nonsense mutations, and missense variants affecting the A2, A3, and C2 domains, are associated with an increased risk of inhibitor development in hemophilia A [13].

Exploratory transcriptomic analyses reported in conference abstracts have suggested up-regulation of B-cell – related pathways in patients with inhibitors; however, these findings remain preliminary and require validation in peer-reviewed prospective studies [16].

Numerous prognostic biomarkers have been studied for their ability to predict hemophilic arthropathy; however, MRI remains the gold standard for evaluation. López-Jiménez et al.'s study revealed that certain genetic variants, such as MTHFR 677TT and MTHFR 1298AC, were associated with more affected joints and increased joint effusion. In contrast, the TNFα-308GA genotype was associated with subchondral cysts [8, 18].

Additionally, increased levels of inflammatory biomarkers such as TNF-α and cartilage and bone remodeling biomarkers, including CTX-II, COMP, CS-846, and collagen turnover markers such as C4M, PRO-C4, C3M, PRO-C5, and PRO-C2, were associated with joint bleeding and joint tissue bleeding. Future studies should aim to standardize cutoff values and laboratory techniques to improve the reliability of these biomarkers for clinical decision-making [1, 12, 19, 20].

2.8. Diagnostic biomarkers in Hemophilia

Diagnostic biomarkers are still the foundation for identifying hemophilia type and severity. Routine tests include prothrombin time (PT), activated partial thromboplastin time (aPTT), and specific factor assays. A pattern of prolonged aPTT with normal PT almost always suggests FVIII or FIX deficiency. One-stage or chromogenic assays confirm the diagnosis and help distinguish hemophilia A from B. It's also essential that reagents be standardized and assays calibrated using WHO reference plasma to avoid interlaboratory variability [1, 4, 5].

Beyond these basic tests, newer diagnostic markers are making the process more detailed. Global coagulation tests, such as thrombin generation (TGT) and viscoelastic methods (ROTEM, TEG), provide a broader view of the hemostatic system. They are especially helpful when monitoring patients receiving non-factor therapies, such as emicizumab, for which routine FVIII assays are unreliable [21].

Genetic biomarkers now play a central role as well. Sequencing the F8 and F9 genes not only confirms the diagnosis but also supports genetic counseling, prenatal diagnosis, and gene therapy planning. Next-generation sequencing (NGS) panels can test multiple coagulation genes at once, saving time and increasing diagnostic yield [4, 6, 7, 21].

Other experimental markers, such as circulating microRNAs (miR-15b, miR-30e, miR-150) and inflammatory mediators (IL-6, TNF-α), are being studied to detect subclinical inflammation or early joint changes. While early results are promising, they still need to be validated in large, standardized studies before being used in clinical practice [21].

In short, combining traditional factor assays with molecular and genetic testing provides a clearer diagnostic picture, enabling physicians to tailor management and move toward precision medicine in hemophilia [1, 4, 5, 7, 21].

2.9. Monitoring treatment efficacy and safety

Monitoring biomarkers is critical for tracking therapeutic response and identifying potential problems such as inhibitor formation or insufficient hemostatic protection. Routine monitoring includes FVIII or FIX activity, aPTT, and inhibitor titers measured by the Bethesda or Nijmegen-modified Bethesda assay. Checking trough levels helps ensure patients, especially those on extended half-life or gene therapy products, are adequately protected from bleeding [1, 4, 5, 7, 12, 21].

However, standard factor assays don't always reflect the true clinical picture, particularly for patients on non-factor therapies such as emicizumab, fitusiran, or concizumab. In such cases, global assays such as thrombin generation (TGT), ROTEM, and TEG are more informative. Thrombin generation parameters – such as lag time, peak thrombin, and endogenous thrombin potential – have been shown to correlate with bleeding tendency and to guide dose adjustment or the timing of prophylaxis [21].

Newer molecular and inflammatory markers (for example, D-dimer, VEGF, MMPs, and CRP) can reveal ongoing inflammation or vascular stress. If these markers remain elevated even when factor levels appear adequate, this may indicate silent joint bleeding or early arthropathy, prompting imaging or review of treatment. For patients receiving gene therapy, markers such as vector DNA copy number, transgene expression, and immune response signals (ALT levels, anti-AAV antibodies) are essential for assessing the durability and safety of the therapy. Long-term monitoring helps detect any early loss of vector activity or immune-mediated reduction in gene expression. Ultimately, integrating laboratory data, molecular markers, and imaging into a single monitoring plan enables physicians to personalize prophylaxis, detect complications early, and improve long-term outcomes for people living with hemophilia [14, 21].

Hemophilia treatment has transitioned from standard factor replacement therapy to extended half-life (EHL) concentrates, non-factor therapies, and gene therapy. While these classes of therapies and biomarkers have pioneered the monitoring of biomarkers uses for (i) dose tailoring and safety monitoring and (ii) surrogate endpoints in clinical trials and longitudinal studies [12].

For EHL factor products and PK-guided prophylaxis, factor activity (FVIII/FIX), PK metrics (e.g., trough and time below target), and global hemostatic function assays (thrombin generation; viscoelastic assays) are often used as pharmacodynamic outcome monitoring biomarkers to bleed outcomes [12].

Lastly, because the clinical burden is predominantly attributable to hemophilic arthropathy, imaging and tissue-turnover biomarkers are used more frequently as outcome measures alongside the annualized bleeding rate. Musculoskeletal ultrasounds (e.g., HEAD-US) and MRIs are paired with synovitis, osteochondral damage, and hemosiderin scores. They are often used in conjunction with inflammatory and cartilage/bone turnover markers to assess and document disease activity and progression at the joint level. Consequently, the following subsections are organized by biomarkers, biological family, and intended use within the BEST framework (diagnostic/prognostic/monitoring/predictive), with an emphasis on the level of validation and the pragmatic issues that limit the routine use of biomarkers [1, 8, 12, 21].

Table 1: Summary of biomarkers for hemophilia: diagnostic and follow-up utility

Biomarker	Specificity/Sensitivity	Category	Use	Limitations
Factor VIII, IX	Specificity 100% / Sensitivity 99%	Diagnosis	Diagnosis of hemophilia and classification of disease severity	Does not predict inhibitor development or subclinical joint damage
miRNA-150	NR	Follow-up	Exploratory molecular biomarker: altered expression associated with synovial inflammation and disease progression	Sampling requires synovial fluid
IL-4, IL-13	NR	Follow-up	Exploratory immunologic markers: associations reported with inhibitor development in selected cohorts	Limited and heterogeneous evidence; requires serial measurement; not validated for routine use
aPTT	Sensitivity 90–95%	Diagnosis	Initial coagulation screening	Requires confirmatory tests
Genetic markers (Factor VIII/IX)	Specificity 98–100% / Sensitivity 95%	Diagnosis	Detect mutation	Expensive; poor correlation with severity
CRP	Specificity 67.87% / Sensitivity 88.43%	Follow-up	Non-specific marker of acute inflammation; elevated during active bleeding or synovitis	Not specific
VEGF	Specificity 68.3% / Sensitivity 82.8%	Follow-up	Associated with synovial hypertrophy and vascular remodeling in hemophilic joints	Affected by infection and trauma
CTX-II	NR	Follow-up	Marker of cartilage degradation; elevated in hemophilic arthropathy and associated with joint damage	Not specific; elevated in osteoarthritis
D-dimer	NR	Follow-up	Reflects activation of fibrinolysis during acute bleeding episodes	Not specific; affected by age, inflammation, and surgery
miRNA-15b	NR	Follow-up	Exploratory molecular biomarker; altered expression reported during synovitis	Sampling requires synovial fluid
Inhibitor assays (Bethesda test)	Specificity 95% / Sensitivity 98%	Follow-up	Quantify antibodies against Factor VIII, IX	False negative results in low-titer inhibitor levels

NR, Not reported; CRP, C-reactive protein; VEGF, vascular endothelial growth factor; CTX-II, C-terminal telopeptide of type II collagen; aPTT, activated partial thromboplastin time.

The majority of the above studies are small, single-center, and often cross-sectional. Inflammatory markers are more consistently raised in participants with severe hemophilic arthropathy than in well-controlled patients on effective prophylaxis. However, the studies differ substantially in age (pediatric vs. adult cohorts), definitions of joint disease (clinical scores vs. ultrasound/MRI), timing of sampling relative to bleeding episodes, and the assays used. The reported effects are often similar (with greater inflammation associated with increased joint damage), yet they lack effect sizes and clinical relevance. Additionally, neutral and negative results are more common in smaller cohorts, in studies with low-grade inflammation, and in studies with diverse target outcomes. In summary, inflammation-related biomarkers remain of interest but lack the necessary standardization to enable routine joint health assessments without cross-sectional imaging and detailed clinical information [1, 8, 12].

2.10. Analytical Assay Methodology and Standardization

The lack of consistency in assay methodologies is a key limitation in hemophilia biomarker studies, reducing comparability across studies. The same biomarker may yield different results, and, depending on the analytical platforms used, many reports do not specify assay details [1, 4, 22].

Inflammatory and Angiogenic biomarkers (e.g., CRP, VEGF, cytokines) are commonly measured using ELISA or multiple assays; both are affected by interassay and interlaboratory variability. On the other hand, factor VIII and IX activity assays are supported and standardized by World Health Organization reference standards. However, their reliability is decreased in patients taking non-factor therapies. Cartilage turnover and molecular biomarkers, particularly circulating microRNAs, are still poorly standardized and lack validated reference ranges. Only factor activity assays are well harmonized; therefore, most other biomarker classes require further methodological standardization to confirm reproducibility and clinical utility [5, 12, 14, 21, 22].

2.11. Analytical Considerations and Assay Platforms

Biomarker validation in hemophilia often disregards the validation of different analytical assays. Avoiding technology selection that entails considerable variability is advisable to mitigate issues in data interpretation. The Enzyme-Linked Immunosorbent Assay (ELISA) is still the 'go-to' approach for assessing quantifiable biomarkers because of cost and sensitivity. The single-analyte detection inherent to ELISA approaches, along with the higher sample volumes required, are constraints of the approach. There are also multiplex bead-based arrays (e.g., Luminex) and plate electrochemiluminescence sensors (e.g., Meso Scale Discovery), which are more widely used because they enable simultaneous assessment of multiple markers of tissue turnover and cytokines from small sample volumes. The more common multiplex approach, particularly with respect to cost, still does not provide a defined marker for some ELISA assays and, in fact, yields a broader range for some less abundant markers. There is also the option of more

sophisticated research mass spectrometry (LC-MS/MS), which is even less common due to the high cost and specialized expertise required. The primary challenge when comparing findings across studies is inter-assay variability. A substantial portion of this issue is attributable to the lack of a consistent standard for antibody selection [22].

Each commercial kit will ultimately use antibodies that bind to different epitopes on the same model protein. Therefore, when using Kit A, one study may show a biomarker level as 'high,' while another study using Kit B may show the same biomarker level as 'normal.' This is purely a function of the different analytical measurement systems used, not of any biological differences. This phenomenon is even more pronounced when assessing bone and cartilage turnover biomarkers (COMP, CTX-II, and C2M), as they are not standardized, unlike coagulation factors [22].

Although assays for FVIII, FIX, and CRP are standardized against World Health Organization (WHO) International Standards, ensuring globally comparable results, most biomarkers for the musculoskeletal system are still governed by "research-use-only" (RUO) manufacturing standards. These RUO assays use arbitrary internal calibrators that vary from lot to lot. Data unification is impossible until international reference materials are created for these specific markers of joint tissue. Consequently, researchers are left with no choice but to provide specific details about the assay manufacturer, catalog number, and whether lot-to-lot control samples are available. The most pressing need for future validation is the creation of Certified Reference Materials (CRMs) to close the gap between discovery and clinical use [5, 12, 22].

2.12. Pre-analytical variables

Some previous analytical considerations have a major role in influencing the biomarkers of hemophilia, as the choice of specific biological matrices, such as using plasma or synovial fluid, has a clear and significant role in the concentration of chemical markers. The latest studies (Van Bergen et al.) have shown differences between plasma and serum C-reactive protein (CRP) levels, underscoring the need to select the appropriate matrix, as an incorrect choice can lead to inconsistent results [22, 23].

Furthermore, circulating microRNA (miRNA) levels may vary between serum and plasma. This necessitates expanding the number of clear protocols for collecting standardized data to ensure highly reliable data. Although synovial fluid plays a significant and clear role in the joint environment, many recent studies have focused primarily on urinary and hematological markers because they are noninvasive [23–25]. Recent studies (Pasta et al.) have highlighted the importance of timing in sample collection for clinical events, as it is a crucial variable that significantly influences the results. Several joint tissue indices exhibit transient elevations following joint hemorrhage. Specifically, urinary carboxylate peptide-1 (uCTX-II) and serum chondroitin sulfate 846 (CS846) levels show a substantial and noticeable increase lasting from two to seven days after joint bleeding. Similarly, inflammatory markers like macrophage migration inhibitory factor (MIF) and CRP show marked intra-individual changes when measured during an acute bleed compared to baseline levels one month later [23, 24]. Methodological differences in sample handling and storage conditions significantly affect biomarker stability. As this study (Leuci et al.) demonstrated, plasma samples for microRNA (miRNA) analysis must be processed immediately after collection to ensure measurement accuracy. The use of different laboratory assays, varying reagent batches, and materials from multiple suppliers contributes to the observed lack of standardization across the literature. Ideally, biomarkers should demonstrate high stability during both the collection phase and long-term storage to be clinically feasible [22, 23].

Several individual patient factors influence the results, including the timing of sample collection, which clearly affects the interpretation of vital signs data. It is also known that common co-infections in hemophilia patients, such as hepatitis C virus or HIV, have a significant impact on inflammatory marker levels. However, several studies do not account for these variables, which significantly affect the interpretation of the results [23, 24].

Moreover, bone turnover markers are highly age-dependent, showing significant variations between pediatric and adult populations, which complicates the establishment of universal reference ranges. Recent studies (van Bergen et al.) (2025; in press; narrative review of hemophilia biomarker methodology) have shown that levels of physical activity and the presence of systemic inflammation in conditions such as rheumatoid arthritis play a significant role in the metabolism of indicators traditionally used to assess joint damage. This necessitates verifying all results and considering all factors that influence their interpretation. These diverse pre-analytical factors collectively contribute to substantial inter-study heterogeneity and limited reproducibility in hemophilic arthropathy research [23, 24].

Several obstacles still significantly hinder the success of clinical applications of biochemical markers. Among the most important are the lack of a global reference standard for assessing joint diseases, the small sample size, which contributes significantly to trial failures, and the variation in laboratory techniques between different laboratories. All these obstacles must be overcome to achieve the best clinical outcomes for patients. Establishment of more homogeneous study designs and longitudinal measurements within the same patient cohort is required to overcome these challenges [23].

2.13. Temporal and kinetic considerations in biomarker assessment

From a clinical perspective, understanding the fundamentals of kinetic and temporal dynamics has numerous crucial applications to biomarkers of rare diseases. The complexity of hemophilia patients' situations necessitates a broad and appropriate interpretation, compounded by missing and underexplored data on clinical events and therapeutic interventions [23, 24, 26].

2.14. Acute-phase vs chronic biomarker patterns

Classifying the biomarkers mentioned in this review will clarify their responses and place them within a systematic behavioral timeline. (CRP, IL-6) They are protein-based inflammatory markers and act as acute-phase reactants. Their function rises after hemarthrosis within the early hours and peaks at 1-2 days post-bleed. Reaching 7-14 days following the resolution of bleeding can be considered as the point of returning to baseline. We can infer that they can detect acute episodes and treatment responses [23–26].

On the other hand, cartilage turnover markers (CT-II, COMP, PRO-C4), joint damage markers such as VEGF, and other microRNA species act as chronic-phase reactants through the slow pathway over weeks to months. Cumulative tissue damage and continuous pathological remodeling are widely covered in the chronic pathway, with less suitability for individual bleeding events. Cartilage turnover markers require sustained use over the next 6-12 months to prevent bleeding and ensure measurable improvement. A possible delay in detectable response may overlap with a lack of experience, leading to an inaccurate suggestion of treatment failure. Prolonged observation can provide us with the real benefits of prophylactic action [23–26].

2.15. Expected time to normalization after bleeding cessation

(D-dimer and thrombin generation) reach the normalization point within 3-7 days in the effective hemostasis period. On the contrary, Factor VIII/IX levels respond immediately to replacement therapy within hours. All those variable changes alarm us to focus on measuring the time of every event with clear necessity [8, 10, 23–26].

2.16. Time to detectable change after treatment initiation

We can highlight the variation in therapeutic response times by providing examples of the aforementioned classes under the initiative's prophylaxis. Factor VIII/IX levels reach the normalization point immediately post-infusion, paving the way for acute pharmacokinetic follow-up. The inflammatory biomarker class requires 2-4 weeks of continuous prophylaxis before reaching the point of decline, after which it reaches maximal suppression at 2-3 months. We can't depend on cartilage degradation markers because of the minimal early change during the first 3-6 months. Improved values can be measured only after more than 6 months of effective therapy. Emicizumab, a novel therapy, can alter thrombin generation within weeks, whereas changes in inflammatory and joint damage markers persist for longer, requiring follow-up beyond 1 year [8–10, 23–28].

2.17. Optimal sampling frequency for monitoring

Few studies have specified the timing of biomarker sampling relative to clinical events, limiting interpretation. Optimal strategies likely include: daily to every-other-day monitoring of inflammatory markers during acute bleeding; monthly to quarterly assessments during prophylaxis optimization; and 6-12 month intervals for long-term prognostic markers. Standardization of sampling protocols – including time since last bleed, time since factor infusion, and time of day – is essential for reproducibility [8, 10, 23, 24, 26–28].

Although we have gathered all relevant data on hemophilia biomarkers, there remains a knowledge gap regarding sampling frequency and timing. We still need standards for sampling protocols to record bleeding episodes, time since last bleeding, time since factor infusion and treatment administration, and diurnal insights relevant to assessing reproducibility. Optimal checkpoints can be matched with a factor class, such as: 2-3 days follow-up during acute bleeding using inflammatory markers; 1-4 months follow-up during prophylaxis using factor VIII/IX; and 6-12 months follow-up intervals for prognostic assessment over a long time by using cartilage markers and microRNA forms [23, 26, 28].

2.18. Are single measurements or serial measurements more informative?

The included studies shared a common methodological limitation: reliance on single-checkpoint biomarker measurements, without accounting for dynamic variation. Serial measurement provides us with more and superior privileges, such as: controlling for high biological variability and temporary events (trauma grade, recent activity, illness presence), trajectory analysis allowance instead of absolute numbers and values in decreasing or increasing forms to guarantee more prognostic data, setting better individualized treatment response data, and perfect capability of detecting subclinical disease activity before progression and clinical case deterioration. It is difficult to establish a correlation between single-measurement risk misclassification and accurate findings at a large scale [23, 28].

We recommend, in the near future, focusing on longitudinal study designs that use predefined protocols with clear specifications for hemophilia biomarkers. Prepared protocols with specific serial sampling at well-known intervals can establish evidence-based medicine. Our best options are: bleeding, timing, factor handling, physical activity, and time of day. There are necessary steps for reproducing and translating clinical findings; serial measurements are the preferred method for longitudinal studies [23].

2.19. Publication bias

Interpretations within the same category of our review must be critically evaluated, particularly when they concern publication bias in the scientific committee. Most reviewers and researchers prefer hearing good news about a disease such as hemophilia, so we observe a tendency to publish only studies with promising positive statistical findings rather than studies with negative or null outcomes. The diversity of reasons has shaped the current situation, including rare epidemiology, with studies reporting fewer than 50 patients, the absence of a pre-registered protocol with specific hypotheses, leading to more selective and statistical reporting of biomarker efficacy, and overreliance on exploratory biomarkers. (miR-15b, miR-208a-3p, miR-524-3p), As novel microRNAs, added to (IL-4, IL-13), as specific inflammatory mediators, and cartilage markers, have more attention to follow. The disproportionate emphasis on initial studies with positive reports has led to unfair validation, with neglected studies receiving less scrutiny and potentially yielding poorer results.

2.20. Biomarker-Specific Concerns

As noted earlier, the class of biomarkers can have significant consequences, leading to the standardization of use among clinicians across multiple medical centers. Factor VIII/IX and F8/F9 genetic variants have been widely validated and used for many years without being affected. However, Novel markers require fairer interpretation through involvement in large, prospective, multicenter validation studies. Another suitable solution for all relevant limitations is to receive all submitted studies in the future, subject to prior registration. Documenting the prognostic value with equal numbers of positive and negative outcomes is also highly recommended, followed by a follow-up on clinical effects, while reducing the number of current limitations [8, 10, 12, 23].

2.21. Comparison with previous literature

Previous reviews have largely focused on hemophilic arthropathy or imaging-based assessment of joint disease. In contrast, the present review integrates biochemical, molecular, genetic, and imaging biomarkers within a unified framework, highlighting both potential clinical applications and persistent gaps in validation and standardization. While our findings are generally consistent with prior reviews, this synthesis emphasizes the limited readiness of most non-traditional biomarkers for routine clinical use [1, 4, 8, 12, 23, 25].

2.22. Summary of the main findings

This narrative review summarizes current evidence on biomarkers in hemophilia across protein-based, genetic, molecular, inflammatory, and imaging domains. Established biomarkers such as factor VIII/IX activity levels, inhibitor assays, and imaging modalities remain central to diagnosis and disease monitoring. In contrast, emerging biomarkers, including inflammatory markers, cartilage turnover products, and molecular signatures, are supported primarily by small and heterogeneous studies. Overall, preliminary evidence suggests potential clinical utility for selected biomarkers, although their roles vary substantially across biomarker classes and clinical contexts.

3. Conclusion

A notable shift in focus from controlling bleeding episodes to managing longitudinal musculoskeletal health is reflected in this review, which summarizes findings from numerous studies of varying scope supporting the utility of biomarkers in hemophilia.

While traditional coagulation factor assays remain the mainstay of diagnostic and treatment monitoring, data suggest that certain biochemical markers of inflammation, cartilage turnover, and bone metabolism provide useful real-time information about joint health and disease that imaging studies may not capture.

More precisely, inflammatory markers respond to acute events, whereas tissue turnover markers are used to predict structural damage before it becomes permanent. Still, it must be remembered that most of these biomarkers, including CRP and COMP, are not hemophilia-specific and are influenced by comorbidities, age, and systemic factors. This is why these are best described as candidate markers that require further testing, rather than as definitive markers. Ultimately, the combination of these biological signatures, clinical scores, and imaging may be the beginning of a more tailored prophylaxis, one that is not only based on factor levels but also on the precise biological condition of the patient's joints.

3.1. Periodization

The most important pathways to be addressed to translate evidence into clinical practice concern biomarkers. Data on the association of C-reactive protein (CRP) and vascular endothelial growth factor (VEGF) with inflammatory flares suggest that these biomarkers can be prospectively validated as adjuncts to the identification and monitoring of acute joint bleeding. For chronic joint damage and hemophilic arthropathy, cartilage turnover markers CTX-II and COMP demonstrate the most robust and reproducible associations with joint structural changes. They should be prioritized for longitudinal studies to define prognostic thresholds. The microRNA and genetic polymorphism biomarker families have, for the most part, remained largely unexplored. They offer promising mechanistic insights and are potential candidates for precision medicine, but they lack standardized assays and validated cutoffs. They also need robust multicenter studies, which are lacking and remain in the exploratory stage; these should be pursued as such rather than implemented.

4. Limitations

In the explanation of the review process, several limitations warrant mention. The review is purely narrative; the selection of studies is subjective, and it is not conducted according to a firm PRISMA protocol. The search was limited to studies in English, thereby excluding potentially relevant studies in other languages. Additionally, no risk-of-bias assessment, such as QUADAS-2 or ROBINS-I, was used; therefore, the authors are unable to provide a subjective or objective measure of the strength of evidence supporting the biomarkers. Lastly, the literature is limited to small observational studies because hemophilia is a rare disorder, which likely introduces publication bias. This is where literature with positive relationships is more likely to be published than literature that is negative or inconclusive.

4.1. Recommendations and Considerations for Biomarkers Integration

This review can be interpreted as a step toward hemophilia biomarker evaluation and characterization, especially since most studies didn't focus on biomarkers only as a diagnostic or prognostic tool. However, the results in this review should be interpreted with caution due to the small sample size and limited data on specific biomarkers, given that hemophilia is a rare X-linked recessive disorder. Most studies included small sample sizes and also examined other potential confounders, such as BMI, treatment adherence, and prophylactic use.

In conclusion, the identification and validation of biomarkers specifically with patient-reported quality-of-life and joint-health outcomes hold great promise for improving various aspects of patient care, including diagnosis, prognosis, and treatment selection. The field of biomarker research in hemophilia is still evolving, and further validation and standardization are necessary before widespread clinical implementation. However, this process is hindered by significant financial and logistical barriers, underscoring the need for WHO-referenced standards and cost-effectiveness frameworks. Large-scale multicenter studies, aligned with established ISTH and WFH biomarker adoption frameworks and involving diverse patient populations, are needed to confirm the reliability and generalizability of the identified biomarkers. Specific biomarker panels, such as inhibitor-risk genotypes combined with baseline Bethesda inhibitor assays and thrombin generation profiles for patients on non-factor therapies, warrant prospective multicenter validation to establish their diagnostic and monitoring accuracy. Additionally, future studies should also standardize biomarker reporting, including specimen type, timing relative to bleeding episodes, and analytic platform, to improve reproducibility. To move these findings toward clinical application, there is a need for a clear research pathway that includes:

- Multicenter diagnostic accuracy studies with predefined thresholds.
- External validation of existing prognostic and predictive models.
- Economic assessments comparing biomarker-guided prophylaxis with standard care.

Integrating these efforts with patient-reported quality-of-life and joint-health outcomes may further strengthen the clinical relevance and overall value of biomarker use in hemophilia.

Conflicts of Interest

The authors declare that they have no competing interests.

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Ethical approval

Not applicable. This article is a narrative review of previously published studies and did not involve direct research on human or animal subjects.

Large Language Model

None.

Author Contributions

This review was conceptualized and designed by DA. The manuscript was written by DA, FE, AM, EM, MH, MM, HE, and MM, with critical revision by ME and DA.

Data Availability

No new datasets were generated or analyzed during this study. This article is a narrative review based on previously published literature, and all data supporting the findings are included in the article and its referenced sources.

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