haematologica
Journal of the Ferrata Storti Foundation

Mechanisms of blood-induced joint disease in hemophilia and potential novel targets for interventions

by Leonard A. Valentino and Yesim Dargaud

Received: May 1, 2026.
Accepted: July 16, 2026.

Citation: Leonard A. Valentino and Yesim Dargaud.
Mechanisms of blood-induced joint disease in hemophilia and potential novel targets for interventions.
Haematologica. 2026 July 23. doi: 10.3324/haematol.2026.301186 [Epub ahead of print]

Mechanisms of blood-induced joint disease in hemophilia and potential novel targets for interventions

Leonard A. Valentino[1] and Yesim Dargaud[2,3]

[1]Rush University Medical Center, Chicago, Illinois, USA,

[2] French Reference Centre for Hemophilia, Louis Pradel Hospital and University of Lyon 1, Lyon, France

[3] UR4609 Hemostase et Thrombose, Université Claude Bernard Lyon 1, Lyon, France

ORCID and email address
LAV: https://orcid.org/0000-0002-7927-6418 whybloodclots@gmail.com
YD: https://orcid.org/0000-0002-4888-1463 ydargaud@univ-lyon1.fr

Correspondence to: Leonard A. Valentino whybloodclots@gmail.com

Key words: Hemophilic arthropathy, Iron-driven inflammation, Synovial remodeling, TNF-α / ADAM17 signaling, AKT-mediated chondrocyte survival

Author contributions: LAV conceptualized and wrote the first draft of this manuscript and provided overall supervision. LAV and YD equally contributed to the methodology, investigation, revision and editing and both approved the final version.

No funding was used to support this work.

Disclosures:
LAV received honoraria and travel support from Regeneron Pharmaceuticals, Inc. for scientific presentations at conferences and also received consulting fees from: Inovio

Pharmaceuticals, Inc,, Novartis, Pharmaceuticals, Inc,, Regeneron Pharmaceuticals, Inc,, Sanofi, and Takeda Pharmaceuticals, Inc. LAV is Editor-In-Chief, Expert Review of Hematology and an Entrepreneur-In-Residence for the Pathway to Cures Venture Fund of the National Bleeding Disorders Foundation.
YD has no conflict of interest to declare

Abstract (word count: 248)

Hemophilic joint disease, or blood-induced joint disease (BIJD), a rises from recurrent or even subclinical hemarthroses that initiate a cascade of iron-driven synovial and cartilage pathology. When blood enters the joint, iron deposition triggers oxidative stress, generating IL-1β and TNF-α–mediated inflammation, synovial hyperplasia, and persistent angiogenesis. Foundational studies show that iron exposure activates oncogene-like programs in synovial fibroblasts, including c-MYC and MDM2, promoting proliferative and anti-apoptotic behavior that contributes to villous hypertrophy. Contemporary single-cell analyses reveal heterogeneous fibroblast, endothelial, and mast cell populations that orchestrate iron handling, inflammation, and vascular remodeling. Notably, endothelial cells exposed to repeated bleeding exhibit ferroptosis signatures, increased permeability, and signaling through ferritin light chain (FTL) to SCARA5-positive fibroblasts, forming an iron-responsive stromal circuit that may predispose joints to rebleeding. Macrophages propagate osteochondral injury through iRhom2/ADAM17-dependent TNF-α shedding, linking hemarthrosis to bone loss. Cartilage injury occurs rapidly: even short exposures to low concentrations of blood induce irreversible chondrocyte apoptosis, reduced proteoglycan synthesis, and matrix degradation driven by ROS and iron. Recent discoveries identify TNXB–AKT signaling as a critical cartilage-protective pathway lost in hemophilic arthropathy. Despite advances in systemic and non-factor therapies, joint bleeding and arthropathy persist, highlighting the need for mechanism-based interventions beyond hemostasis. Potential targets include iron/ROS modulation, endothelial ferroptosis inhibition, mast-cell stabilization, TNF-α pathway blockade, fibroblast state reprogramming, and activation of AKT-mediated cartilage survival pathways. An integrated model positions hemarthrosis as the initiating event and iron-centered inflammatory amplification as the driver of chronic joint degeneration, underscoring opportunities for joint-directed therapeutic innovation.

Introduction

Hemophilia is an inherited bleeding disorder due to deficiency of the action of coagulation factor (F) VIII (FVIII) or FIX activity.(1) Bleeding into joints (hemarthrosis) is the cardinal manifestation and the trigger for the development of hemophilic arthropathy (HA) . Despite decades of progress in the development of therapeutics to treat and prevent bleeding,(2, 3) joint bleeding persists and its complications remain a major cause of morbidity in people with hemophilia (PwH) .(4) Darg aud, et al., outlined the persistent gaps in the diagnosis and management of HA including incomplete bleed prevention, uncertain long-term joint outcomes of novel therapies, limited tools for early/subclinical damage, inadequate pain strategies, and the lack of interventions that specifically target synovium or cartilage. Among the processes that are thought to lead to HA (Figure 1A) (5-11) are iron-triggered 1) o xidative stress leading to IL-1β and TNFα mediated inflammation, (12, 13) 2) angiogenesis and increased vascular permeability, (14-17) and 3) chondrocyte apoptosis and suppressed matrix synthesis.(18, 19) Here, we discuss these biological processes that lead to blood-induced joint disease (BIJD) in PwH and the potential direct interventions that might be explored to address BIJD in PwH. We will provide the reader with a review of historical and modern data on the pathobiology of this disorder and synthesize this information into conceptualized, multi-system cascade of events involving multiple cell types and signaling pathways impacted by iron.

1) Hemarthrosis as the inciting event: blood and iron set the stage for BIJD

Blood entering the closed synovial space initiates synovitis and sets up a feed-forward loop involving iron deposition, oxidative stress, and proliferative synovial remodeling that ultimately destroys cartilage and bone. The seminal review of Valentino (5) framed BIJD as a blood-initiated disease in which iron/heme and other blood components are central drivers of inflammation, synovial hyperplasia, and degenerative change, establishing a conceptual throughline still used today. In contemporary updates, reviews reiterate that even subclinical hemarthroses can lead to arthropathy (6) a nd that prevention of bleeding through novel extended half-life FVIII or FIX products and non-factor drugs (3, 4, 20) has not eliminated joint disease, emphasizing the need to target intra-articular pathogenic mechanisms downstream of bleeding. (21)

A robust experimental foundation underpins these concepts. A murine model of factor VIII deficiency created by Valentino and colleagues recapitulated human hemophilic synovitis and enabled mechanistic dissection. Repeated or single induced bleeds produced synoviocyte hypertrophy/hyperplasia, neovascular responses, hemosiderin deposition, and progressive cartilage/bone changes mirroring human pathology and providing a platform for testing interventions.(22, 23) Together, these models situate

blood as the instigator and iron-laden synovium as the biological “reactor” that converts bleeding events into chronic joint pathology.

Two additional lines of evidence underscore the primacy of blood as the driver. First, localized intra-articular replacement of the deficient factor (e.g., FIX) p revents synovitis in hemophilia B mice, even in the absence of detectable circulating FIX demonstrating that joint-directed control of bleeding can interrupt BIJD at its source.(24) Second, aspiration or rapid clearance of blood from the joint is mechanistically rational: in vitro and in vivo work shows that even brief exposures of cartilage to blood can initiate persistent metabolic injury, while thresholds of concentration (≥10% v/v) a nd duration (≥2 days) a re sufficient to produce irreversible alterations in chondrocyte function.(19, 25)

Key pathobiological themes: Iron deposition → oxidative stress → synovial proliferation. Iron is a molecular linchpin, it accumulates as hemosiderin, catalyzes reactive oxygen species (ROS), a nd reprograms synovial and cartilage cells toward proliferative and apoptotic fates.(26-30) We next review how iron and other blood-derived signals engage specific synovial and joint-resident cell types to propagate BIJD.

2) Synovial lining and fibroblasts: iron-oncogene signaling, heterogeneity, and crosstalk

2.1 Iron drives oncogene programs and synovial hyperplasia

Pioneering studies showed that iron can directly induce proliferation of human synovial fibroblasts and activate proto-oncogenic pathways.(22, 31) In vitro, ferric iron (ferric citrate) increases c-myc expression and increases growth of primary human synovial fibroblasts, effects that could be reversed by the pro-apoptotic sphingolipid ceramide.(31) This work linked iron to c-myc–dependent growth control, proposing a tumor-like proliferative phenotype of hemophilic synovium. Extending these observations, hemarthrosis in vivo and iron in vitro upregulated MDM2, a p53-binding protein, in synovium. Iron exposure caused an ~8-fold rise in MDM2 expression in normal human synovial cells.(22) M DM2 up-regulation provides a mechanistic path to anti-apoptosis and synovial overgrowth, aligning with histologic villous hypertrophy observed after bleeds.

These oncogene-like changes are not merely cell culture artifacts. In the murine hemophilia A model, a single hemarthrosis induces villus formation, synovial hyperplasia, and angiogenesis, with a strong correlation between synovial and vascular hyperplasia, underscoring the angiogenic dependency of expanding inflamed synovium.(22) Re inforcing the model’s fidelity, the histologic grading system

emphasizes vascularity and hemosiderin discoloration as hallmarks of iron-driven synovitis.(32)

2.2 Synovial fibroblast states and pathogenic specialization

Contemporary single-cell and spatial analyses in inflammatory arthritis have redefined the “fibroblast” as a family of phenotypically and functionally distinct states, varying by location (lining vs sub-lining), e pigenetic imprinting, and microenvironmental cues. While this work was pioneered in rheumatoid arthritis (RA) , it provides a conceptual scaffold to interpret synovial fibroblast heterogeneity in hemophilia. Machado & Firestein summarize at least four fibroblast states in RA with distinct effector repertoires, some catabolic/invasive, others chemokine-rich and emphasize their plasticity under cytokine/growth-factor influence.(33) Such a framework is directly relevant to BIJD, in which repeated exposure to blood and iron could skew fibroblast states toward angiogenic, matrix-degrading, or iron-handling phenotypes.

Recent single-cell RNA-seq of hemophilic synovium by Lin et al. validates this idea in the hemophilia context.(34) They showed that following exposure to iron and activation of hemeoxygenase (HMOX), endothelial cells can induce scavenger receptor class A (SCARA) 5 fibroblast subpopulations by interacting with the Ferritin Light Chain (FTL) from stressed endothelium that appears linked to ferroptosis responses in the synovial niche. This iron-handling fibroblast state situates iron metabolism within stromal crosstalk, extending classic iron-oncogene observations into a microenvironmental circuit.

Insights from osteoarthritis (OA) single-cell atlases further illustrate fibroblast functional bifurcation: Cartilage Intermediate Layer Protein (CILP) p ositive fibroblasts associate with non-damaged chondrocytes and exhibit reparative transcriptomes, whereas Periostin/Osteoblast Specific Factor (POSTN) e xpressing fibroblasts interact with damaged chondrocytes and display catabolic/inflammatory signatures.(35) These programs suggest that stromal context and crosstalk with cartilage drive fibroblast specialization toward protective vs injurious roles—templates that plausibly translate to hemophilic synovium under iron and bleed stress.

To summarize, iron skews synovial fibroblasts toward proliferation (c-myc/MDM2) a nd, through vascular and endothelial signals and toward iron removal (Scavenger Receptor Class A Member 5, SCARA5) states, the balance of which can influence cartilage health and outcomes.

3) Synovial macrophages and cytokine axis: TNF-α, ADAM17/iRhom2, and osteo-immune crosstalk

Macrophages are pivotal in translating blood-borne iron and damage signals into cytokine milieus that remodel bone and cartilage.(36) In a murine study, blood or its

components triggered iRhom2/ADAM17-dependent shedding of TNF-α from macrophages; genetic or pharmacologic disruption of TNF-α or iRhom2 reduced osteopenia and synovitis after induced hemarthrosis in FVIII-deficient mice.(37) This defines a blood → macrophage → TNF-α axis as a core pathway of blood-induced bone loss and synovial inflammation in HA. The mechanistic chain links blood exposure to TNF-Alpha Convertase Enzyme (TACE) or Disintegrin And Metalloproteinase Domain-Containing Protein (ADAM) 17 activity and TNF-α release, providing actionable targets for protecting periarticular bone.

Beyond TNF-α, broader cytokine networks (IL-1β, IL-6, chemokines) contribute to BIJD, as reviewed comprehensively by Wojdasiewicz et al..(38) B lood-derived iron and cell debris amplify innate immune activation, while synovial lining cells perpetuate matrix metalloproteinase production and angiogenesis reinforcing a pro-inflammatory synovial ecosystem. Clinical-pathologic reviews further connect these cytokine circuits with chronic pain and structural decline, underscoring unmet needs in mechanism-directed therapies beyond hemostasis.(39)

To summarize, targeting TNF-α processing (iRhom2/ADAM17) a nd associated cytokine nodes early after bleeding may interrupt bone catabolism and synovial inflammation after bleeds, complementing factor prophylaxis.(40)

4) Endothelial cells and mast cells: vascular damage, ferroptosis, and permeability phenotypes

Vascular elements are central actors in BIJD. Histologic and morphometric data show that synovial hyperplasia tightly correlates with vascular expansion,(41) im plying that progression depends on endothelial proliferation and nutrient supply. Single-cell data now add mechanistic depth. Lin et al., demonstrate that repeated bleeding fosters iron deposition and a ferroptosis signature in synovial endothelium, including an $HMOX1^{+}$ endothelial disposition indicative of oxidative stress and heme catabolism.(34) Endothelial ferroptosis is postulated to injure microvasculature and drive endothelial migration, altering permeability (42, 43) and potentially predisposing to re-bleeding.(44, 45)

It is recognized that iron accumulation from hemarthroses drives synovitis and cartilage damage in HA. Ferroptosis has been experimentally demonstrated as a key mechanism of chondrocyte death under iron-rich conditions, including models of hemophilic arthropathy, where inhibition of ferroptosis attenuates cartilage damage. (46, 47) Preclinical studies show that D-penicillamin and deferoxamine reduce synovial iron and inflammation, while clinical experience with iron chelators confirms effective systemic iron depletion but not the iron within the synovial tissues. Translational studies highlight that timing and joint delivery are critical, as on-demand chelation has not yet demonstrated disease modification. (48) Testing of agents that reduce lipid ROS

accumulation such as the ferroptosis inhibitors ferrostatin-1 and liproxstatin-1 should be undertaken. (46, 49)

Endothelial–fibroblast crosstalk links vascular stress to stromal reprogramming. (50, 51) Iron-stressed endothelial cells signal via ferritin light chain (FTL) to SCARA5 on fibroblasts, promoting a SCARA5^{+} fibroblast subset with iron-handling features; this circuit connects iron biology to stromal heterogeneity and potential matrix outcomes. (34) The FTL to SCARA5 endothelial -to-fibroblast circuit is a direct mechanistic link between iron biology and stromal remodeling that may be operative in HA.

The proinflammatory milieu following bleeding results in iron retention and disturbed lymphangiogenesis.(52) T he findings of the von Drygalski laboratory suggest a link between iron homeostasis and lymphangiogenesis. (52, 53) Intact lymphatics are necessary for the removal of iron-laden macrophages, a process that is dependent on FVIII. In the absence of FVIII, vascular changes including expression of α-Smooth Muscle Actin (SMA), Endoglin (CD105) and vascular endothelial growth factor result in changes in vascular remodeling and changes in joint perfusion.(44) In FVIII deficient mice, hemarthrosis provokes defective lymphangiogenesis, synovial iron retention, and iron-laden macrophages which can be mitigated by therapeutic FVIII to restore lymphatic remodeling, accelerates iron clearance, and improves cartilage health. These dynamics parallel HA single-cell evidence of HMOX1^{+} endothelial ferroptosis and FTL→SCARA5 signaling inducing SCARA5^{+} fibroblasts, connecting iron handling to stromal remodeling and outcomes in HA.

A further link to the importance of endothelial cells is activated protein C (APC), which is generated when thrombin binds thrombomodulin on endothelial cells which is classically considered an anticoagulant (inactivates factors Va and VIIIa). Following acute hemarthrosis, APC binds to the endothelial protein C receptor (EPCR) to exert a protective effect through protease activated receptor 1 (PAR-1) signaling, stabilizing endothelial barriers and reduce vascular permeability. Importantly, APC reduces leukocyte adhesion and also functions as an inflammatory modulator inhibiting NF-κB activation to reduce the synthesis of TNF-α, IL-1β and IL-6. These anti-inflammatory effects are easily overwhelmed by repeated bleeding events and dysregulated APC activates matrix metalloproteinases to degrade collagen. Inhibition of APC activity increases the permeability and fragility of synovial endothelial cells increasing the propensity for re-bleeding. APC therefore emerges at the intersection of inflammation, endothelial biology, and synovial remodeling.

An unexpected and disease defining finding in hemophilic synovium is the presence of activated perivascular mast cells, more prominent than in OA or RA comparators. Lin

et al. identify activated mast cells as a distinctive immune population in HA, suggesting roles in vascular permeability, bleeding propensity, and early inflammatory amplification.(34) Heme iron activates mast cells, to release algogenic and pruritogenic mediators, and neutrophils, inducing the formation of extracellular traps, nano tubes and extracellular vesicles in addition to the secretion of proteases. (46, 47) Candidate mediators released by mast cells include tryptases (e.g., TPSB2), consistent with mast cell effects on endothelial junctions and matrix.(48, 49) The enrichment of activated perivascular mast cells, which may establish a paracrine mast cell–Angiopoietin (ANGPT)-2 axis whereby mast cell–derived mediators (such as TNF-α, IL-1β, IL-6, various proteases, and VEGF) activate the endothelium, inducing ANGPT-2 upregulation; in this inflammatory milieu, (50) ANGPT-2—an endothelial-derived regulator of the ANGPT/Tie-2 pathway that antagonizes ANGPT-1 while synergizing with VEGF—might thereby act as a central driver of angiogenesis and neovessel formation in HA. (51) Inhibitor of mast cell function is being used in a variety of diseases and Worrall and Reber provide an excellent summary of mast cell targeted therapies. (52)

Implications: A mast cell–endothelium axis may modulate vascular leakage and inflammatory tone, while endothelial ferroptosis and FTL–SCARA5 signaling organize stromal iron responses, suggesting novel targets (e.g., ferroptosis regulators, mast cell mediators) to limit re-bleeding and synovitis (figure 3).

5) Chondrocytes and cartilage matrix: apoptosis, AKT/TNXB signaling, and iron-ROS toxicity

5.1 Direct effects of blood on cartilage viability and matrix

A pivotal observation is that cartilage damage can occur independent of overt synovitis with even short exposures to blood: human cartilage co-cultured with 50% blood (or RBCs with mononuclear cells) shows profound, durable inhibition of proteoglycan synthesis and tripling of apoptotic chondrocytes; caspase inhibitors partially rescue synthetic activity, linking apoptosis to functional decline.(19, 25) Mechanistically, oxidative stress mediates these effects: ROS scavengers and antioxidant interventions attenuate blood-induced injury, and iron from RBCs catalyzes reactive species that damage chondrocytes. Threshold studies demonstrate that 2 days of exposure to 10% (v/v) blood suffices to cause prolonged damage in vitro, with poor reversibility, data often cited to support rapid treatment of hemarthrosis and/or evacuation of hemarthrosis.

5.2 Epigenetic signaling in HA cartilage: TNXB → AKT → chondroprotection

Contemporary human/mouse translational work has identified Tenascin-XB (TNXB) as a cartilage-centric regulator of BIJD. TNXB organizes and maintains tissue structure by regulating the production and assembly of certain types of collagens and its deficiency

is a cause of Ehlers-Danlos syndrome.(54) Chen et al. found aberrant DNA methylation and down-regulation of TNXB in HA cartilage; TNXB deficiency in FVIII deficient mice promotes matrix degradation, subchondral bone loss, and chondrocyte apoptosis.(18) Mechanistically, TNXB loss suppresses AKT (name derived from thymoma tissue isolated from the AK mouse strain, also known as Protein Kinase B) phosphorylation, and AKT supplementation rescues chondrocyte survival and matrix integrity, suggesting the TNXB–AKT axis as a therapeutic lever in HA cartilage degeneration. These findings build on the older BIJD paradigm(55) by placing a specific extracellular matrix gene and survival pathway at the center of blood-induced cartilage injury, linking epigenetics of HA cartilage to signal-transduction vulnerability under iron/ROS stress.

The implications of these observations are that blood exposure triggers chondrocyte apoptosis and diminishes matrix synthesis through ROS/iron injury and TNXB down-regulation further reduces AKT survival signaling, compounding matrix loss (figure 3A). Activation of the AKT pathway is chondroprotective in preclinical systems and may be a target for intervention clinically. Multiple intervention points may mitigate hemophilic cartilage degeneration (figure 3B). Following joint bleeding, rapid removal of intra-articular blood (56, 57) or joint-targeted hemostasis (24) to reduce iron exposure and synovial activation have been proposed. Targeting iron-driven oxidative injury, endothelial ferroptosis, and mast-cell–mediated vascular permeability may interrupt upstream inflammatory amplification may be beneficial. Enhancing FVIII availability to restore lymphangiogenesis and accelerate iron clearance may improve post-bleed recovery and reduce the likelihood or re-bleeding. Blockade of iRhom2/ADAM17-dependent TNF-α release may further limit synovial and osteochondral damage. Finally, preserving TNXB expression or augmenting AKT signaling provides direct chondroprotection against apoptosis and matrix loss. Imaging-based detection of subclinical bleeding may enable timely intervention, however the minimum exposure time to trigger pathology is not known and therefore prevention of bleeding(6) and chondroprotective prophylaxis are the optimal interventions.

Preventing bleeding alone is necessary but insufficient; true chondroprotection requires modifying intra-articular biological responses to blood, iron, synovial inflammation, and cartilage-intrinsic stress. Physiological compression activates the integrin-PI3K-AKT signaling pathway providing protective signals to cartilage but conversely, abnormal loading following joint bleeding inhibits PI3K and mitigate these protective pathways, suggesting rest and limited weight bearing is necessary following joint bleeding.(20, 58, 59)

Chondroprotective prophylaxis can be accomplished in several ways (figure 3B). All are predicated on the activation of phosphatidylinositol 3-kinase (PI3K) which is central to this mechanism (figure 3B). PI3K is activated when its regulatory subunit (p85) binds

phosphotyrosine motifs or when its catalytic subunit (p110) is engaged by upstream membrane signals. Therefore, PI3K can be activated by supplying the ligands to one of its receptor tyrosine kinases including insulin-like growth factor (IGF)-1 receptor (IGF-1R),insulin receptor (IR), platelet-derived growth factor (PDGF) receptor (PDGFR), epidermal growth factor (EGF) receptor (EGFR), vascular endothelial growth factor (VEGF) receptors (VEGFR1/2), or fibroblast growth factor (FGF) receptors (FGFRs), by activation of chemokine-linked G-protein–coupled receptors, by initiation of JAK/STAT signaling pathway following ligation of the IL-2, IL-4, IL-6 family receptors or the TNF-family of receptors, or following engagement of integrins activating the focal adhesion kinase-Src pathway.

6) Bone and osteochondral unit: TNF-α pathway and structural consequences

Periarticular bone is not spared in BIJD. In FVIII-deficient mice, bleeding triggers osteopenia adjacent to the affected joint. Mechanistically, blood components activate macrophage iRhom2/ADAM17, elevating TNF-α in the joint; blocking TNF-α or iRhom2 mitigates both synovitis and bone loss. This situates TNF-α, processed by ADAM17, at the nexus of osteo-immune damage post-bleed and offers path-specific targets to preserve bone architecture in HA.(37)

Clinically, arthropathy remains prevalent despite hemostatic advances,(3, 4, 20) reinforcing the need to address post-hemorrhagic biological cascades in the osteochondral unit. Reviews highlight persistent joint remodeling, pain, and eventual joint replacement in some patients,(21) calling for mechanism-based adjuncts (anti-cytokine strategies, antioxidants, ferroptosis modulators, synovial-targeted approaches) alongside prophylaxis.(4)

7) Integrative model: from blood to multi-cellular pathology

Bringing together classic and modern data, BIJD can be conceptualized as a multi-system cascade:

1. Hemarthrosis introduces blood/iron into the joint.
2. Synovial iron loading drives ROS and proliferative/anti-apoptotic programs (c-myc, MDM2), promoting hyperplasia and angiogenesis; vascular expansion supports ongoing synovial growth.
3. Endothelial cells undergo ferroptosis-like stress ($HMOX1^+$ states), altering permeability and favoring re-bleeding; mast cells amplify vascular leak and inflammation.
4. Endothelial–fibroblast FTL→SCARA5 signaling induces a $SCARA5^+$ fibroblast subset, embedding iron handling within stromal crosstalk.

5. Macrophages exposed to blood signal via iRhom2/ADAM17 to shed TNF-α, propagating synovitis and bone resorption.
6. Chondrocytes suffer apoptosis and matrix synthetic failure from direct blood/iron/ROS exposure; TNXB epigenetic loss disables AKT survival signaling, accelerating ECM degradation; AKT activation is protective.

This integrated view reconciles historical “chemical injury” models with an immune-vascular-stromal network identified by single-cell genomics and highlights therapeutic nodes beyond mere hemostasis.

8) Therapeutic implications and disease modification

8.1 Hemostasis remains foundational, but site-directed strategies matter

Prophylaxis and prompt bleed control are indispensable for preventing the initiating insult, blood and iron. Yet elegant experiments show that intra-articular factor delivery (protein or AAV-mediated) can suppress synovitis even without systemic factor, underscoring the joint-autonomous nature of BIJD and supporting local or joint-targeted adjuncts. Reviews emphasize that despite non-factor therapies and extended half-life products, arthropathy persists in subsets, motivating mechanistic adjuvants.

8.2 Mechanism-informed adjuncts

Methotrexate is the primary first-line therapy for RA due to its broad anti-inflammatory and immunomodulatory effects.(60) It suppresses immune-cell proliferation, shifts cytokine profiles toward anti-inflammatory activity, promotes adenosine release, disrupts pathogenic cell interactions, modulates NF-κB and JAK-STAT pathways, and reduces tissue-damaging enzymes. Methotrexate is highly effective and has a well-defined safety profile but lacks specificity. Newer immunomodulatory agents such as TNF-α inhibitors, IL-6 blockers and anti-angiogenic agents are more effective and have a wider margin of safety than methotrexate.(61) In addition, specific mechanisms to target (e.g., iron and ROS) in HA may be advantageous. These possibilities will be explored below.

8.2.1 Iron/ROS modulation. Data linking iron to c-myc/MDM2, chondrocyte apoptosis, and endothelial ferroptosis suggest exploring iron chelation, antioxidants, or ferroptosis regulators in joint-directed formats.

8.2.2 Cytokine processing blockade. The iRhom2/ADAM17/TNF-α axis offers a genetically validated target to protect bone and synovium post-bleed; translation will require safety studies in the context of hemostasis.

8.2.3 Mast-cell modulation. Discovery of activated mast cells in HA synovium suggests testing mast-cell stabilizers or tryptase inhibitors to reduce permeability and inflammatory amplification.

8.2.4 Stromal state reprogramming. Lessons from RA and OA indicate that fibroblast states are malleable; strategies that tilt hemophilic synovium away from catabolic/invasive programs (e.g., POSTN-like) and toward reparative programs (CILP-like) may aid cartilage preservation.

8.2.5 Cartilage survival signaling. The TNXB–AKT axis represents a cartilage-intrinsic pathway; AKT agonists or epigenetic interventions to preserve TNXB expression merit investigation as chondroprotective adjuncts after bleeds.

9) The "two-hit" frame and disease course

A helpful conceptualization is the two-hit hypothesis: (1) chemical injury caused by iron/heme and ROS causing chondrocyte apoptosis and synovial proliferation, followed by (2) inflammatory amplification where the synovial membrane becomes a reservoir of inflammatory mediators, transforming acute chemical damage into chronic inflammatory disease which then leads to structural damage to the osteochondrum. (62) The single-cell discoveries in HA operationalize this such that iron-driven endothelial ferroptosis (chemical hit) engages mast cells, fibroblast reprogramming, and macrophage TNF-α (inflammatory hit), culminating in self-sustaining pathology across synovium, cartilage, and bone. Collectively, the data presented in this review substantiate the proposed pathways with histological, biochemical, and genomic convergence.

10) Clinical translation and future directions

Preventive administration of hemostatic agents (repeatedly or as a one-time treatment) to restore thrombin generation and normalize hemostasis thereby preventing bleeding remains the cornerstone of modern hemophilia management. Despite optimal prophylaxis, evidence indicates that clinically recognized as well as subclinical joint bleeding persists in a subset of PwH even with modern-day agents and regimens, pointing to the need for joint-specific, pathway-directed adjuncts to prevent arthropathy. Furthermore, global access to factor products, non-factor therapies and gene and cell therapies are limited to high income countries and more than 80% of the world's hemophilia population do not have access to diagnosis and treatment. Based on the data above, testable hypotheses include:

10 .1 Ferroptosis modulation in endothelium to protect microvasculature and reduce the propensity for rebleeding. Mitigating the detrimental effect of iron in the joint has been explored with the use of steroids(63) and iron chelators.(64) Glucocorticoids protect tissues from iron-induced injury primarily by suppressing inflammation, reducing ROS generation, stabilizing vascular barriers, and limiting synovial and stromal responses to iron, rather than by acting directly on iron-handling pathways(65). A 1970 publication from Kisker and Burke(63) showed that a 5-day

course of oral corticosteroids improved outcomes after acute hemarthrosis in boys receiving standard replacement therapy for that era. Intra-articular steroids have been used to relieve pain and reduce inflammation.(66) The ability of chelators such as deferasirox to enter the joint appears limited.(48) Heme oxygenase-1 (HMOX1) protects cells against oxidative stress including that induced by iron. Agents that induce HMOX1 by upregulating nuclear factor erythroid 2-related factor 2(67) such as sulforaphane, bardoxolone methyl, or dimethyl fumarate(68) may be beneficial and warrant exploration. Alternatively, preventing iron-induced lipid peroxidation with activators of glutathione peroxidase-4 (GPX4) pathway would be explored. Selenium supplementation, administration of N-acetylcysteine, coenzyme Q10 or glutathione have not been studied. Activators of the AKT pathway (69) may also be beneficial but have not been evaluated in HA. Testing of these agents in a prophylactic setting or intra-particularly after bleeds might be studied. Finally, promotion of lymphangiogenesis favoring the physical removal of iron-filled macrophages may also have beneficial effects on cartilage. Chondrocyte regeneration has been demonstrated in animal models and may be possible in humans. The sex-determining region on the Y chromosome (SRY)-type high mobility group box-9 (SOX9) can promote chondrocyte regeneration and survival by influencing multicellular differentiation and antiapoptotic signaling. (70)

10 .2 Mast-cell stabilization with drugs such as cromolyn or its analogues) or with tryptase inhibitors(71) to reduce microvascular leakage and early inflammatory amplification. Although neither studied in animal models of hemophilia nor PwH, hemophilia synovial single-cell sequencing(34) performed by Lin, et al., identified activated perivascular mast cells as contributors to vascular permeability, inflammatory amplification and bleeding susceptibility providing a mechanistic basis for why mast cell stabilizers might be evaluated further in HA.

10 .3 ADAM17/iRhom2/TNF-α axis blockade following joint bleeding may limit synovitis and preserve cartilage and bone. TNF inhibitors have been used to treat inflammatory conditions such as rheumatoid arthritis, psoriatic arthritis, juvenile idiopathic arthritis, inflammatory bowel disease, ankylosing spondylitis and psoriasis as well as other inflammatory conditions. Zhang, et al., studied TNF inhibition in a mouse model and in PwH and chronic arthropathy and showed decreased synovial thickness and vascularity suggesting that anti-TNF may provide adjuvant protection in HA.(40) The TNF inhibitors approved by the United States Food and Drug Administration include infliximab, adalimumab, etanercept, golimumab, and certolizumab. These drugs should be studied both in a prophylaxis setting (prior to the onset of bleeding) and for treatment after bleeding has occurred. People with hemophilia have increased levels of circulating inflammatory markers compared to age- and weight-matched controls, with elevated

levels of IL-6, C-reactive protein, and LPS-binding protein(72) providing rationale for their use in a preventative setting in the absence of bleeding.

10 .4 Stromal state reprogramming—leveraging RA/OA insights to nudge fibroblasts toward reparative phenotypes (CILP-like) and away from injurious programs (POSTN-like) phenotypes.

10 .5 Cartilage survival therapies targeting the TNXB–AKT axis (AKT agonists, epigenetic modulators that preserve TNXB expression) as chondroprotective strategies after hemarthrosis.

11) Conclusion

Research over two decades has transformed BIJD in hemophilia from a descriptive entity to a multi-cellular, multi-organ process initiated by blood and orchestrated by iron-centered molecular injury. The cascade unfolds across synovial fibroblasts as a result of (iron inducing c-myc and MDM2 gene expression, macrophages triggering the iRhom2-ADAM17-TNF-α axis, mast cells activating perivascular endothelial cells and increasing permeability increasing the likelihood or re-bleeding, endothelial cells injured by iron and signaling through the FTL-SCARA5 pathway, chondrocytes responding to ROS and undergoing apoptosis in response to TNXB–AKT activation, and bone osteopenia as a result of TNF-α-mediated effects. This integrated view provides a map of actionable nodes- iron/ROS handling, ferroptosis, mast-cell mediators, ADAM17/TNF-α processing, fibroblast state reprogramming, and TNXB–AKT cartilage survival, that can be layered on top of thrombin-generating therapies to prevent or attenuate joint damage in PwH. As precision biologicals and delivery systems advance, joint-targeted, mechanism-based interventions hold potential to convert hemarthrosis from a chronic driver of degeneration into a manageable, non-progressive event, improving long-term function and quality of life for people with hemophilia.

References

1. Mannucci PM, Tuddenham EG. The hemophilias--from royal genes to gene therapy. N Engl J Med. 2001;344(23):1773-1779.

2. Mancuso ME, Mahlangu JN, Pipe SW. The changing treatment landscape in haemophilia: from standard half-life clotting factor concentrates to gene editing. Lancet. 2021;397(10274):630-640.

3. Valentino LA, Santaella ME, Carlson SA, Recht M. Contemporary approaches to treat people with hemophilia: what's new and what's not? Res Pract Thromb Haemost. 2025;9(1):e102696.

4. Dargaud Y, Lobet S, Roussel N, Valentino LA. Unmet needs in hemophilic arthropathy. Blood Rev. 2025;74:101304.

5. Valentino LA. Blood-induced joint disease: the pathophysiology of hemophilic arthropathy. J Thromb Haemost. 2010;8(9):1895-1902.

6. Valentino LA. The imperative to prevent joint bleeding in everyone living with hemophilia. Res Pract Thromb Haemost. 2024;8(2):102383.

7. Valentino LA, Hakobyan N. Histological changes in murine haemophilic synovitis: a quantitative grading system to assess blood-induced synovitis. Haemophilia. 2006;12(6):654-662.

8. Valentino LA, Hakobyan N. Assessment of blood-induced arthritis causes friction. Haemophilia. 2011;17(1):e242-243.

9. Valentino LA, Hakobyan N, Enockson C. Blood-induced joint disease: the confluence of dysregulated oncogenes, inflammatory signals, and angiogenic cues. Semin Hematol. 2008;45(2 Suppl 1):S50-57.

10. Valentino LA, Hakobyan N, Enockson C, et al. Exploring the biological basis of haemophilic joint disease: experimental studies. Haemophilia. 2012;18(3):310-318.

11. Leuci A, Dargaud Y. Blood-induced arthropathy: a major disabling complication of haemophilia. J Clin Med. 2023;13(1)225.

12. Srivastava A. Inflammation is key to hemophilic arthropathy. Blood. 2015;126(19):2175-2176.

13. van Vulpen LF, Schutgens RE, Coeleveld K, et al. IL-1beta, in contrast to TNFalpha, is pivotal in blood-induced cartilage damage and is a potential target for therapy. Blood. 2015;126(19):2239-2246.

14. Acharya SS, Kaplan RN, Macdonald D, Fabiyi OT, DiMichele D, Lyden D. Neoangiogenesis contributes to the development of hemophilic synovitis. Blood. 2011;117(8):2484-2493.

15. Cooke EJ, Zhou JY, Wyseure T, et al. Vascular permeability and remodelling coincide with inflammatory and reparative processes after joint bleeding in factor viii-deficient mice. Thromb Haemost. 2018;118(6):1036-1047.

16. Cooke EJ, Wyseure T, Zhou JY, et al. Mechanisms of vascular permeability and remodeling associated with hemarthrosis in factor VIII-deficient mice. J Thromb Haemost. 2019;17(11):1815-1826.

17. Caliogna L, Berni M, Torriani C, et al. Pathogenesis of osteoarthritis, rheumatoid arthritis, and hemophilic arthropathy: the role of angiogenesis. Haemophilia. 2024;30(6):1256-1264.

18. Chen J, Zeng Q, Wang X, et al. Aberrant methylation and expression of TNXB promote chondrocyte apoptosis and extracullar matrix degradation in hemophilic arthropathy via AKT signaling. Elife. 2024;13:RP93087.

19. Hooiveld M, Roosendaal G, Wenting M, van den Berg M, Bijlsma J, Lafeber F. Short-term exposure of cartilage to blood results in chondrocyte apoptosis. Am J Pathol. 2003;162(3):943-951.

20. Mancuso ME, McLaughlin P, Forsyth AL, Valentino LA. Joint health and pain in the changing hemophilia treatment landscape. Expert Rev Hematol. 2024;17(8):431-444.

21. Gualtierotti R, Solimeno LP, Peyvandi F. Hemophilic arthropathy: current knowledge and future perspectives. J Thromb Haemost. 2021;19(9):2112-2121.

22. Hakobyan N, Kazarian T, Jabbar AA, Jabbar KJ, Valentino LA. Pathobiology of hemophilic synovitis I: overexpression of mdm2 oncogene. Blood. 2004;104(7):2060-2064.

23. Hakobyan N, Kazarian T, Valentino LA. Synovitis in a murine model of human factor VIII deficiency. Haemophilia. 2005;11(3):227-232.

24. Sun J, Hakobyan N, Valentino LA, Feldman BL, Samulski RJ, Monahan PE. Intraarticular factor IX protein or gene replacement protects against development of hemophilic synovitis in the absence of circulating factor IX. Blood. 2008;112(12):4532-4541.

25. Jansen NW, Roosendaal G, Bijlsma JW, Degroot J, Lafeber FP. Exposure of human cartilage tissue to low concentrations of blood for a short period of time leads to prolonged cartilage damage: an in vitro study. Arthritis Rheum. 2007;56(1):199-207.

26. Dabbagh AJ, Trenam CW, Morris CJ, Blake DR. Iron in joint inflammation. Ann Rheum Dis. 1993;52(1):67-73.

27. van Vulpen LF, Roosendaal G, van Asbeck BS, Mastbergen SC, Lafeber FP, Schutgens RE. The detrimental effects of iron on the joint: a comparison between haemochromatosis and haemophilia. J Clin Pathol. 2015;68(8):592-600.

28. van Vulpen LFD, van Bergen EDP, Pulles AE, Mastbergen SC. The detrimental effects of iron to the joint; aggravation by inflammation. Haemophilia. 2023;29(1):365-366.

29. Hooiveld MJ, Roosendaal G, van den Berg HM, Bijlsma JW, Lafeber FP. Haemoglobin-derived iron-dependent hydroxyl radical formation in blood-induced joint damage: an in vitro study. Rheumatology (Oxford). 2003;42(6):784-790.

30. Roosendaal G, Vianen ME, Wenting MJ, et al. Iron deposits and catabolic properties of synovial tissue from patients with haemophilia. J Bone Joint Surg Br. 1998;80(3):540-545.

31. Wen FQ, Jabbar AA, Chen YX, Kazarian T, Patel DA, Valentino LA. c-myc proto-oncogene expression in hemophilic synovitis: in vitro studies of the effects of iron and ceramide. Blood. 2002;100(3):912-916.

32. Joseph BC, Whisenant TC, Cooke EJ, et al. Correction: Synovial gene expression after hemarthrosis differs between FVIII-deficient mice treated with recombinant FVIII or FVIII-Fc fusion protein. PLoS One. 2025;20(9):e0331108.

33. Machado CRL, Firestein GS. Fibroblast multiplicity in RA: a synovial state of affairs. Nat Rev Rheumatol. 2023;19(10):609-610.

34. Lin K, Fan S, Yang X, et al. Heterogeneity of active mast cells, endothelial cells, and fibroblasts in hemophilic arthritis defined by synovial single-cell sequencing. Sci Rep. 2025;15(1):43350.

35. Liu Z, Sun Y, Pan J, Guo K, Tang Z, Wang X. Single-cell profiling uncovers synovial fibroblast subpopulations associated with chondrocyte injury in osteoarthritis. Front Endocrinol (Lausanne). 2024;15:1479909.

36. Weivoda MM, Bradley EW. Macrophages and bone remodeling. J Bone Miner Res. 2023;38(3):359-369.

37. Haxaire C, Hakobyan N, Pannellini T, et al. Blood-induced bone loss in murine hemophilic arthropathy is prevented by blocking the iRhom2/ADAM17/TNF-alpha pathway. Blood. 2018;132(10):1064-1074.

38. Wojdasiewicz P, Poniatowski LA, Nauman P, et al. Cytokines in the pathogenesis of hemophilic arthropathy. Cytokine Growth Factor Rev. 2018;39:71-91.

39. Gualtierotti R, Tafuri F, Arcudi S, et al. Current and Emerging Approaches for Pain Management in Hemophilic Arthropathy. Pain Ther. 2022;11(1):1-15.

40. Zhang F, Xu M, Yang Q, et al. A translational study of TNF-alpha antagonists as an adjunctive therapy for preventing hemophilic arthropathy. J Clin Med. 2019;9(1)75.

41. McClain R, Hakobyan N, Valentino LA. Vascularization of the synovial membrane in hemophilic synovitis. Blood. 2006;108(11):4018.

42. Yuan W, Xia H, Xu Y, et al. The role of ferroptosis in endothelial cell dysfunction. Cell Cycle. 2022;21(18):1897-1914.

43. Dixon SJ, Olzmann JA. The cell biology of ferroptosis. Nat Rev Mol Cell Biol. 2024;25(6):424-442.

44. Bhat V, Olmer M, Joshi S, et al. Vascular remodeling underlies rebleeding in hemophilic arthropathy. Am J Hematol. 2015;90(11):1027-1035.

45. Vols KK, Kjelgaard-Hansen M, Ley CD, Hansen AK, Petersen M. In vivo fluorescence molecular tomography of induced haemarthrosis in haemophilic mice: link between bleeding characteristics and development of bone pathology. BMC Musculoskelet Disord. 2020;21(1):241.

46. Bieri S, Moller B, Amsler J. Ferroptosis in arthritis: driver of the disease or therapeutic option? Int J Mol Sci. 2024;25(15)8212.

47. Wagener N, Hardt S, Pumberger M, Schomig F. Cartilage destruction by hemophilic arthropathy can be prevented by inhibition of the ferroptosis pathway in human chondrocytes. J Clin Med. 2024;13(2)559.

48. Pulles AE, van Vulpen LFD, Coeleveld K, Mastbergen SC, Schutgens REG, Lafeber F. On-demand treatment with the iron chelator deferasirox is ineffective in preventing blood-induced joint damage in haemophilic mice. Haemophilia. 2021;27(4):648-656.

49. Scott C, Arora G, Dickson K, Lehmann C. Iron chelation in local infection. Molecules. 2021;26(1)189.

50. Xie M, Li X, Chen L, et al. The crosstalks between vascular endothelial cells, vascular smooth muscle cells, and adventitial fibroblasts in vascular remodeling. Life Sci. 2025;361:123319.

51. Nicin L, Wagner JUG, Luxan G, Dimmeler S. Fibroblast-mediated intercellular crosstalk in the healthy and diseased heart. FEBS Lett. 2022;596(5):638-654.

52. Cooke EJ, Joseph BC, Nasamran CA, Fisch KM, von Drygalski A. Maladaptive lymphangiogenesis is associated with synovial iron accumulation and delayed clearance in factor VIII-deficient mice after induced hemarthrosis. J Thromb Haemost. 2023;21(9):2390-2404.

53. Chumappumkal Joseph B, De Pablo-Moreno JA, Falah N, Lora Cacho M, von Drygalski A. Defective lymphangiogenesis and iron removal after hemarthrosis in FVIII-deficient mice are rectified with therapeutic fviii administration - implications for joint health. J Thromb Haemost. 2026;24(1)88-98.

54. Lindor NM, Bristow J. Tenascin-X deficiency in autosomal recessive Ehlers-Danlos syndrome. Am J Med Genet A. 2005;135(1):75-80.

55. Jansen NW, Roosendaal G, Bijlsma JW, DeGroot J, Theobald M, Lafeber FP. Degenerated and healthy cartilage are equally vulnerable to blood-induced damage. Ann Rheum Dis. 2008;67(10):1468-1473.

56. De la Corte-Rodriguez H, Rodriguez-Merchan EC, Alvarez-Roman MT, Martin-Salces M, Romero-Garrido JA, Jimenez-Yuste V. Accelerating recovery from acute hemarthrosis in patients with hemophilia: the role of joint aspiration. Blood Coagul Fibrinolysis. 2019;30(3):111-119.

57. Manners PJ, Price P, Buurman D, Lewin B, Smith B, Cole CH. Joint Aspiration for Acute Hemarthrosis in Children Receiving Factor VIII Prophylaxis for Severe Hemophilia: 11-year Safety Data. J Rheumatol. 2015;42(5):885-890.

58. Forsyth AL, Rivard GE, Valentino LA, et al. Consequences of intra-articular bleeding in haemophilia: science to clinical practice and beyond. Haemophilia. 2012;18 Suppl 4:112-119.

59. Valentino LA, Forsyth AL. Mouse trials and human triumphs: recovery after hemarthrosis? J Thromb Haemost. 2026;24(1):49-51.

60. Cronstein BN, Aune TM. Methotrexate and its mechanisms of action in inflammatory arthritis. Nat Rev Rheumatol. 2020;16(3):145-154.

61. Sebba A, Bingham CO, Bykerk VP, et al. Comparative effectiveness of TNF inhibitor vs IL-6 receptor inhibitor as monotherapy or combination therapy with methotrexate in biologic-experienced patients with rheumatoid arthritis: an analysis from the CorEvitas RA Registry. Clin Rheumatol. 2023;42(8):2037-2051.

62. Calcaterra I, Iannuzzo G, Dell'Aquila F, Di Minno MND. Pathophysiological role of synovitis in hemophilic arthropathy development: a two-hit hypothesis. Front Physiol. 2020;11:541.

63. Kisker CT, Burke C. Double-blind studies on the use of steroids in the treatment of acute hemarthrosis in patients with hemophilia. N Engl J Med. 1970;282(12):639-642.

64. Nieuwenhuizen L, Roosendaal G, Mastbergen SC, et al. Deferasirox limits cartilage damage following haemarthrosis in haemophilic mice. Thromb Haemost. 2014;112(5):1044-1050.

65. Zielinska KA, Van Moortel L, Opdenakker G, De Bosscher K, Van den Steen PE. Endothelial response to glucocorticoids in inflammatory diseases. Front Immunol. 2016;7:592.

66. Poenaru D, Sandulescu MI, Cinteza D. Intraarticular management of chronic haemophilic arthropathy (Review). Biomed Rep. 2023;19(3):59.

67. He F, Ru X, Wen T. NRF2, a Transcription Factor for Stress Response and Beyond. Int J Mol Sci. 2020;21(13)4777.

68. Dinkova-Kostova AT, Copple IM. Advances and challenges in therapeutic targeting of NRF2. Trends Pharmacol Sci. 2023;44(3):137-149.

69. Kumar HB, Manandhar S, Rathi E, et al. Identification of potential Akt activators: a ligand and structure-based computational approach. Mol Divers. 2024;28(3):1485-1503.

70. Lu H, Zeng C, Chen M, Lian L, Dai Y, Zhao H. Lentiviral vector-mediated over-expression of Sox9 protected chondrocytes from IL-1beta induced degeneration and apoptosis. Int J Clin Exp Pathol. 2015;8(9):10038-10049.

71. He S, Aslam A, Gaca MD, et al. Inhibitors of tryptase as mast cell-stabilizing agents in the human airways: effects of tryptase and other agonists of proteinase-activated receptor 2 on histamine release. J Pharmacol Exp Ther. 2004;309(1):119-126.

72. Knowles LM, Wolter C, Menger MD, et al. Activation of the acute-phase response in hemophilia. Thromb Haemost. 2023;123(9):867-879.

Figure legends

Figure 1. Blood-initiated mechanisms underlying hemophilic arthropathy.

(A) Schematic overview of blood-initiated joint disease in hemophilia. Recurrent joint bleeding (hemarthrosis) triggers a cascade of pathogenic processes that extend beyond the acute bleeding event and are incompletely prevented by contemporary hemostatic therapies, including extended half-life clotting factors and non-factor agents. Intra-articular blood exposure leads to iron deposition, oxidative stress, and synovial remodeling, creating a pro-inflammatory microenvironment within the joint. These interrelated mechanisms promote progressive synovitis and drive downstream structural damage. Over time, persistent inflammatory and catabolic signaling culminates in the characteristic outcomes of hemophilic arthropathy, including fibrotic synovium, cartilage destruction, bone damage, and irreversible joint dysfunction. The figure was conceptualized and designed by one of the authors (LAV) and created with the assistance of artificial intelligence using Microsoft 365 Co-Pilot (Microsoft. (2026). Copilot (GPT-4) [Large Language Model]. https://copilot.microsoft.com/)

(B) Cellular and molecular mechanisms contributing to the progression of hemophilic arthropathy. Blood exposure within the joint initiates endothelial injury and ferroptosis, accompanied by synovial proliferation and mast-cell activation. Activated macrophages (both tissue resident type A synovial cells and blood derived monocyte/macrophages) serve as central amplifiers of inflammation, producing cytokines such as tumor necrosis factor (TNF) that disrupt homeostatic signaling pathways, including AKT-dependent survival pathways, and downregulate extracellular matrix–stabilizing components. Crosstalk between macrophages, fibroblasts, endothelial cells, and chondrocytes accelerates chondrocyte apoptosis and matrix degradation, ultimately leading to cartilage loss. Parallel effects on subchondral bone contribute to skeletal damage. Together, these interconnected vascular, inflammatory, and degenerative pathways explain how hemarthrosis evolves into chronic hemophilic arthropathy despite effective control of overt bleeding. The figure was conceptualized and designed by one of the authors (LAV) and created with the assistance of artificial intelligence using Microsoft 365 Co-Pilot (Microsoft. (2026). Copilot (GPT-4) [Large Language Model]. https://copilot.microsoft.com/)

Figure 2. Iron-driven mechanisms of synovial remodeling in hemophilic arthropathy.

Schematic representation of pathways linking intra-articular blood and iron exposure to chronic synovial remodeling. Hemarthrosis results in iron accumulation, triggering endothelial cell injury and ferroptosis, accompanied by induction of iron-responsive stress pathways, including HMOX1. Endothelial dysfunction and altered fibroblast phenotypes promote synovial expansion and remodeling. A distinct $SCARA5^{+}$ fibroblast

subset contributes to intracellular iron handling and ferritin light chain (FTL) accumulation. Concurrently, impaired lymphangiogenesis limits iron clearance from the joint, reinforcing local iron toxicity and inflammation. Together, these processes perpetuate synovial pathology and drive progression of hemophilic arthropathy. The figure was conceptualized and designed by one of the authors (LAV) and created with the assistance of artificial intelligence using Microsoft 365 Co-Pilot (Microsoft. (2026). Copilot (GPT-4) [Large Language Model]. https://copilot.microsoft.com/)

Figure 3. Disruption and therapeutic modulation of PI3K–AKT signaling in hemophilic cartilage degeneration.

(A) Schematic representation of PI3K–AKT pathway dysregulation following intra-articular bleeding. Blood exposure within the joint alters receptor-mediated signaling and is associated with suppression of tenascin-X (TNXB), a matrix protein implicated in cartilage integrity. Reduced TNXB expression leads to impaired activation of the PI3K–AKT axis, characterized by diminished phosphatidylinositol-3-phosphate (PIP3) generation and downstream AKT signaling. Attenuation of AKT-dependent cellular responses compromises chondrocyte survival, stress resilience, and matrix homeostasis, thereby promoting hemophilia-associated cartilage degeneration. The figure was conceptualized and designed by one of the authors (LAV) and created with the assistance of artificial intelligence using Microsoft 365 Co-Pilot (Microsoft. (2026). Copilot (GPT-4) [Large Language Model]. https://copilot.microsoft.com/)

(B) Conceptual framework highlighting diagnostic and therapeutic opportunities to preserve cartilage in hemophilia by restoring or supporting AKT signaling. Potential interventions include improved prevention and detection of subclinical joint bleeding, control of iron-mediated oxidative stress, targeting of mast-cell–mediated inflammation, and blockade of pro-inflammatory cytokines such as tumor necrosis factor-α and supporting AKT signaling to protect and preserve cartilage. Early detection of subclinical joint disease using sensitive imaging or biomarkers may enable timely intervention. Strategies aimed at supporting PI3K–AKT signaling are proposed to counteract chondrocyte apoptosis and matrix degradation, complementing hemostatic therapy and addressing non-hemostatic drivers of hemophilic arthropathy. The figure was conceptualized and designed by one of the authors (LAV) and created with the assistance of artificial intelligence using Microsoft 365 Co-Pilot (Microsoft. (2026). Copilot (GPT-4) [Large Language Model]. https://copilot.microsoft.com/)

Figure 4. Integrated mechanisms of blood-induced joint disease leading to cartilage degeneration. Schematic overview of how intra-articular blood and iron drive progressive joint damage in hemophilic arthropathy. Iron accumulation within the synovium induces endothelial dysfunction and activates macrophages and fibroblasts, promoting inflammatory signaling mediated in part by iRhom2-dependent ADAM17

activity and tumor necrosis factor-α release. Concurrent suppression of tenascin-X (TNXB) results in impaired AKT signaling, reducing chondrocyte survival and extracellular matrix maintenance. The combined effects of inflammation, AKT pathway disruption, chondrocyte apoptosis, and matrix degradation culminate in irreversible cartilage damage characteristic of chronic hemophilic joint disease. The figure was conceptualized and designed by one of the authors (LAV) and created with the assistance of artificial intelligence using Microsoft 365 Co-Pilot (Microsoft. (2026). Copilot (GPT-4) [Large Language Model]. https://copilot.microsoft.com/)

A

Prevention of Bleeding

- Extended half-life factors
- Non-factor drugs →

Repeated joint bleeding ⟶ Hemarthrosis

Pathogenic Mechanisms of Hemarthrosis

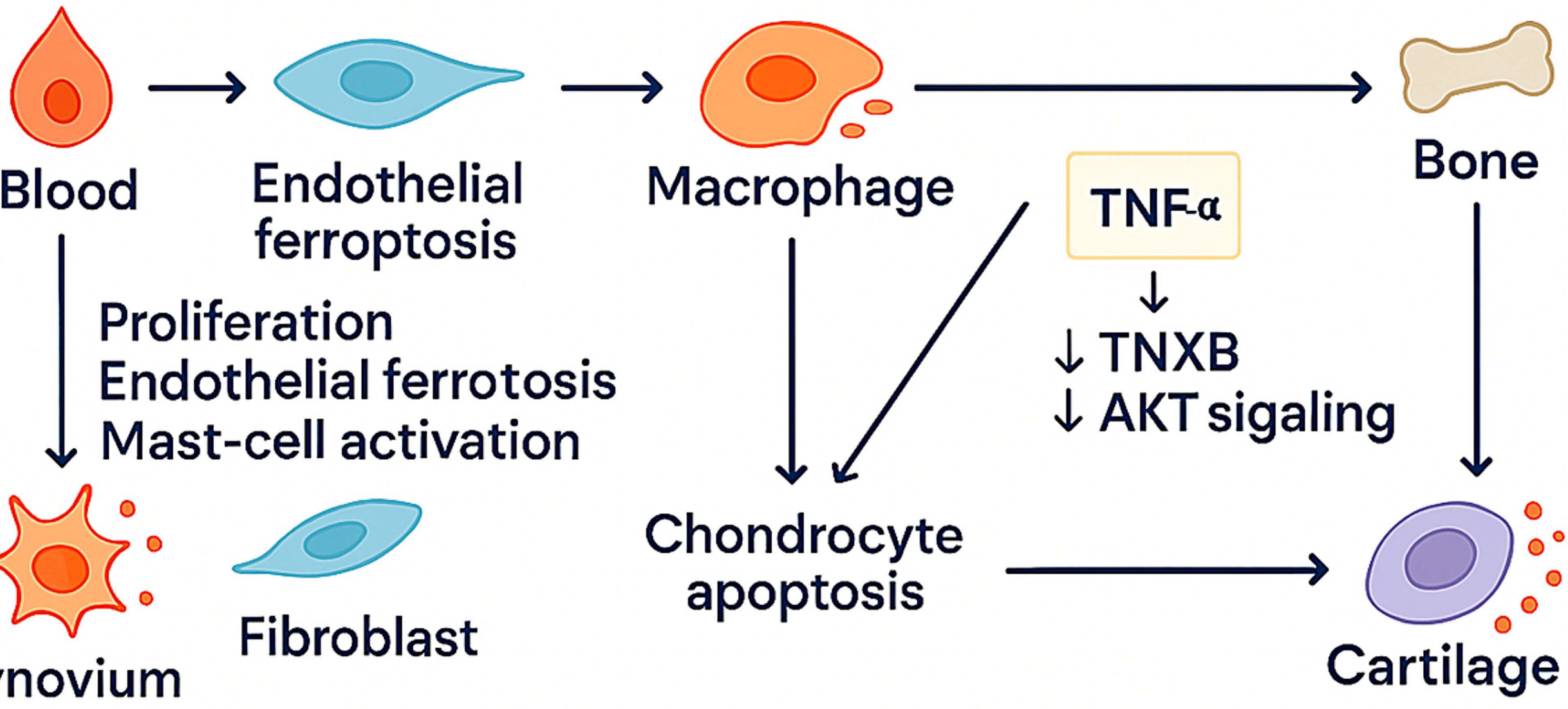
- Iron deposition
- Oxidative stress
- Synovial remodeling

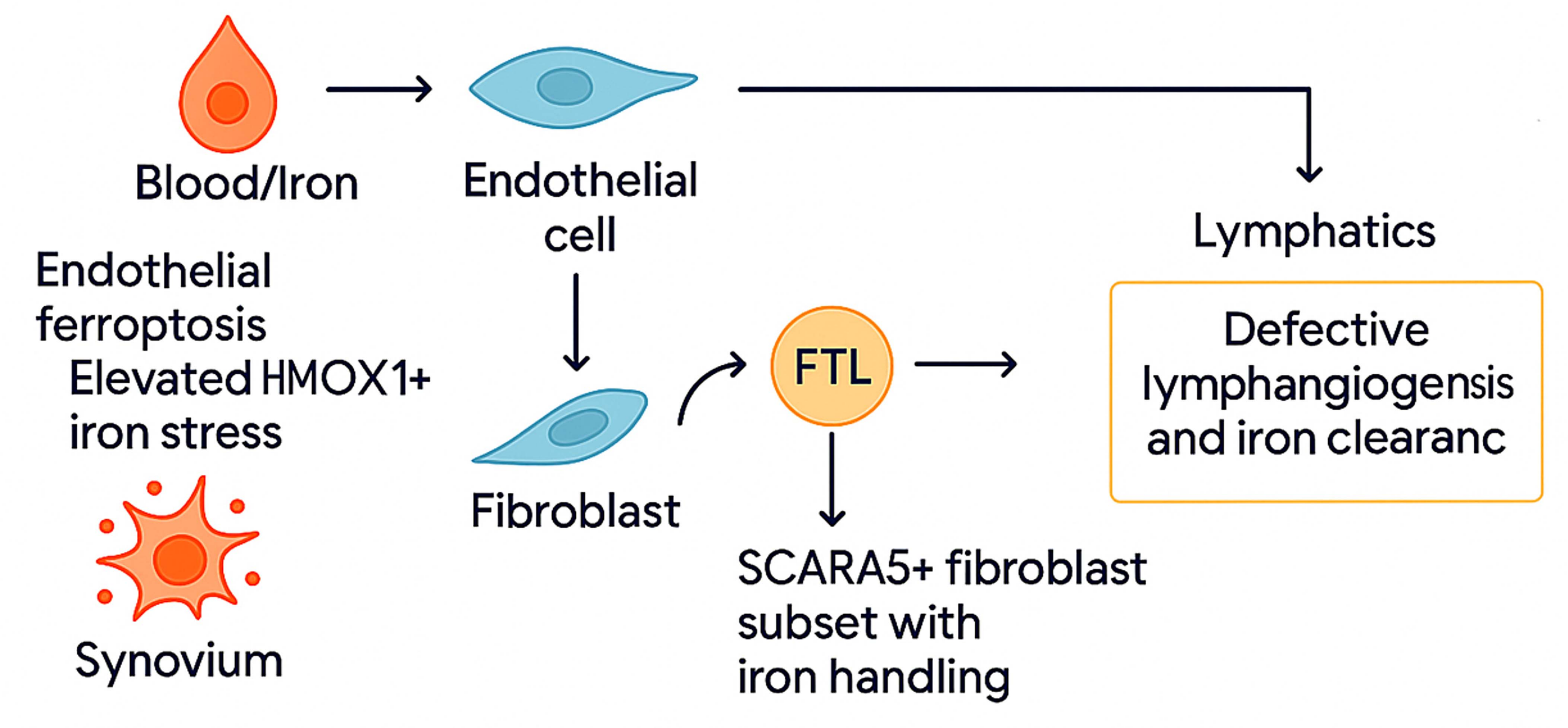
Outcomes

- Cartilage destruction
- Bone damage

Arthropathy

B




Blood/Iron
Endothelial cell
Lymphatics
Endothelial ferroptosis
Elevated HMOX1+ iron stress
FTL
Defective lymphangiogensis and iron clearanc
Fibroblast
Synovium
SCARA5+ fibroblast subset with iron handling

A

Blood in joint

Receptor

PI3K

PIP3

TNXB suppression
↓ AKT signaling

AKT

Hemophilic cartilage degeneration

Cellular responses

B

Blood iron

Iron/ROS control

Target mast cells

Detect subclinical disease

PIP3

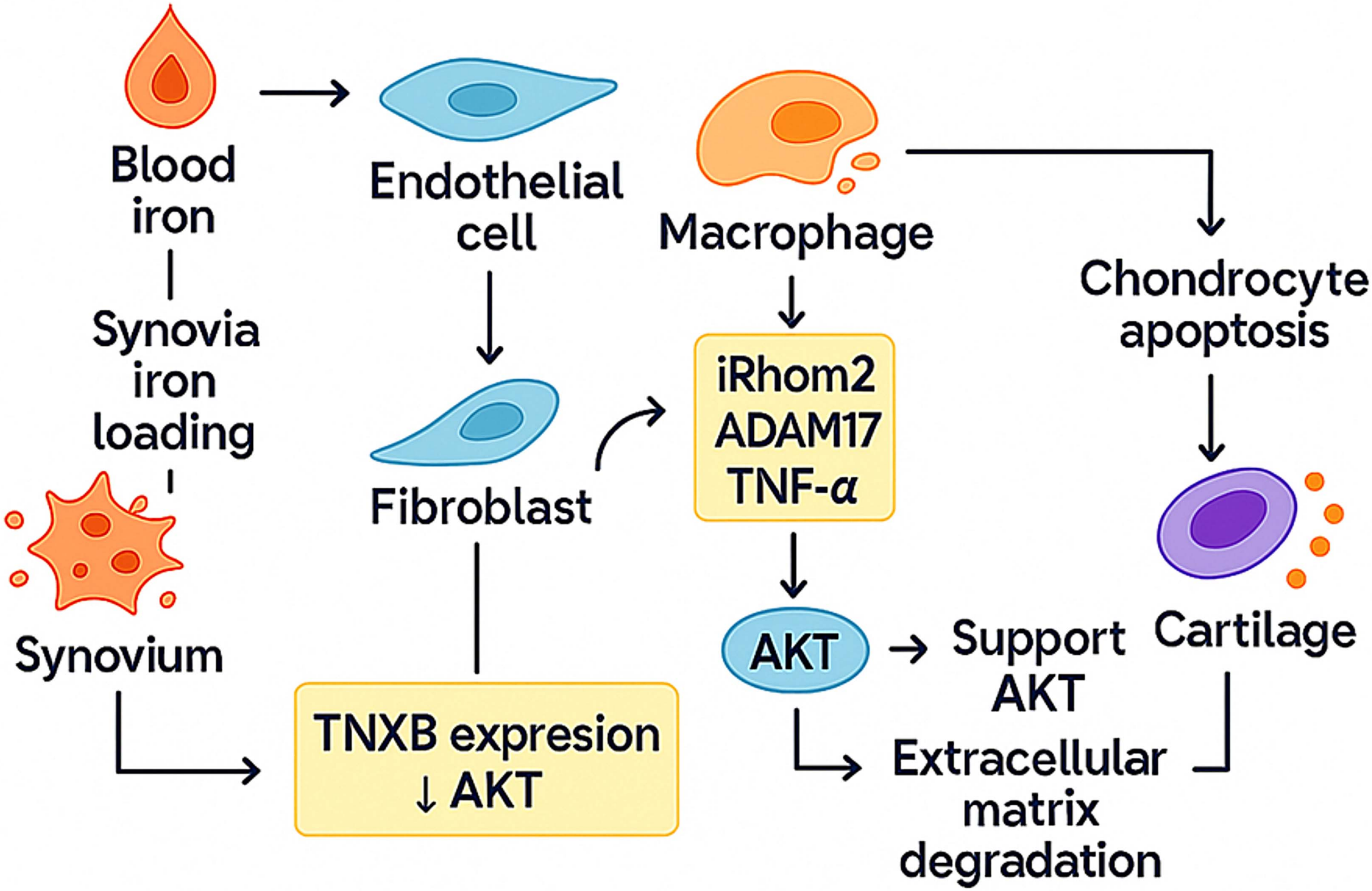
Block TNF-α

Prevent subclinical bleeding

AKT

Support AKT

Prevent cartilage degradation


Blood iron
Endothelial cell
Macrophage
Chondrocyte apoptosis
Synovia iron loading
Fibroblast
iRhom2
ADAM17
TNF-α
Synovium
AKT
Support AKT
Cartilage
TNXB expresion
↓ AKT
Extracellular matrix degradation