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REVIEW ARTICLE

Blood-induced joint disease: the pathophysiology of hemophilic arthropathy

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Summary. Arthropathy is a frequent and serious complication of repeated joint bleeding in patients with hemophilia, resulting in pain, deformity, and disability. Although the pathogenesis of hemophilic arthropathy has not been fully elucidated, it appears to have similarities with the degenerative joint damage that occurs in osteoarthritis and the inflammatory processes associated with rheumatoid arthritis. This article reviews the potential actions of various blood constituents on joint components that culminate in the development of hemophilic arthropathy.

Keywords: arthropathy, hemarthrosis, hemophilia, joint bleeding, joint damage.

Introduction

Blood-induced joint disease (BIJD) may follow acute joint injury [1] and is a key feature of hemophilia, with intra-articular bleeding (hemarthrosis) accounting for more than 90% of all serious bleeding events in patients with severe hemophilia [baseline factor (F)VII or FIX activity < 1%] [2]. Over time, recurrent bleeding into the same joint (a target joint) results in progressive damage and the development of hemophilic arthropathy. Despite advances in treatment and the delivery of comprehensive care at dedicated centers, joint bleeding and arthropathy remain among the most common complications of hemophilia and are major concerns of clinicians and patients.

This article reviews what is known about the mechanisms by which joint bleeding causes hemophilic arthropathy and potential new areas for investigation.

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The targets: joint components

Free-moving synovial joints are encapsulated by a fibrous sheath consisting of dense connective tissue (Fig. 1). The synovial fluid that lubricates the joint is contained within the joint capsule, and primarily consists of high concentrations of hyaluronic acid, albumin, and phagocytes. The bones that conjoin to create a synovial joint are encased in a thin (2 mm) layer of smooth hyaline or articular cartilage.

Articular cartilage

A number of mechanical (load and motion) and chemical (enzymatic) processes appear to be involved in the development of hemophilic arthropathy, and result in fibrosis of the synovial lining and disintegration of the hyaline cartilage [3]. Intra-articular bleeding may first impact on the normally avascular joint cartilage, as iron-catalyzed reactive oxygen intermediates (ROIs) induce chondrocyte apoptosis [4]. An analysis of two biomarkers of bone turnover – cartilage oligomeric matrix protein, a tissue-specific non-collagenous matrix protein, and type II collagen telopeptides – confirmed that the articular cartilage is an early target of for the development of BIJD [5–8] Next, the hemosiderin-laden synovium produces inflammatory mediators, thus creating an intra-articular cytokine storm that further damages cartilage and then bone. The cartilage matrix is degraded by connective tissue proteinases released from the hypertrophic synovium, chondrocytes, and pannus tissue [9–11].

Changes in the composition of the articular cartilage matrix are prominent in hemophilic arthropathy. Articular cartilage is composed mostly of water, electrolytes, and a solid matrix chiefly consisting of collagen and proteoglycans (glycosaminoglycans), and its viscoelastic properties result from water flow through the solid matrix [12]. The composition of the glycosaminoglycans across the articular surface varies according to location [13], patient age [13], and compressive load [14]. The continuous presence of blood in a joint increases cartilage compliance, possibly attributable to the loss of proteoglycans and production of degenerative enzymes from iron-laden synovial or subsynovial macro-

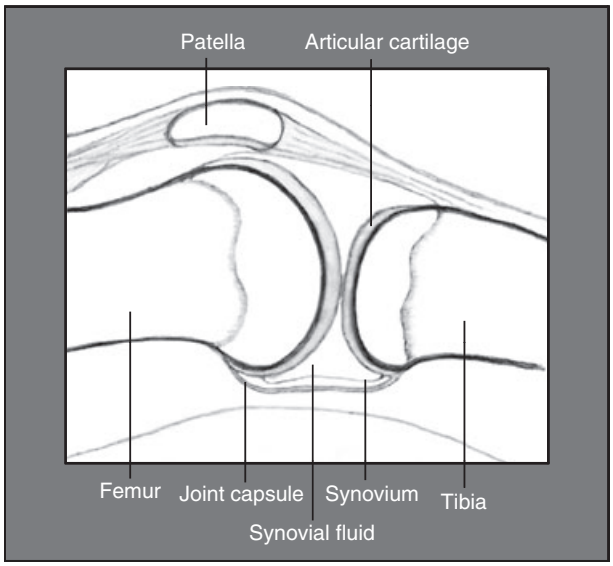

Fig. 1. Diagram of a normal synovial joint. Synovial joints allow movement of articulating bones. The articular cartilage covers the bone epiphyses and provides a cushion to resist the load of weight-bearing. A fibrous capsule that is continuous with the periosteum of bone contains many nerve fibers but lacks blood and lymph vessels, and encases the articulating surfaces. A clear, viscous, non-Newtonian fluid lubricates the joint and reduces friction. The synovial fluid consists of hyaluronic acid, lubricin, proteinases, and collagenases. The synovial membrane, which covers the non-cartilaginous surfaces within joints, is composed of two layers, a subintimal layer and intimal layer. Fibroblast cells within the intimal layer have a synthetic function, whereas macrophages provide a scavenger function, removing blood and other deleterious substances from the joint space. Beneath the intima there is a rich network of delicate blood vessels to provide oxygen, nutrients and growth factors to the synovium and the avascular cartilage.

phages [15]. Laboratory studies have shown that when cartilage is cultured in the presence of whole blood together with mononuclear cells and added erythrocytes or with monocytes/macrophages and added erythrocytes, the inhibition of proteoglycan synthesis is prolonged [16]. This activity is contained within the erythrocytes, as hemolysed erythrocytes in combination with interleukin (IL)-1β produce similar inhibition of proteoglycan synthesis (which can be reverses by using dimethylsulfoxide to remove the ROIs) [16]. Experimental evidence also suggests that IL-1 acts directly to mediate cartilage destruction [17].

Synovial membrane

Recurrent joint bleeding and the deposition of iron in the synovium increase synovial fibroblast DNA synthesis and synovial cell proliferation, resulting in synovial hypertrophy and chronic inflammation [18]. Iron-laden synovial tissue produces various inflammatory cytokines, including tumor necrosis factor (TNF)-α, interferon-γ, IL-1, and IL-6, which directly induces receptor activator of nuclear factor-kappaB ligand (RANKL) [19], that enhance the hypertrophic process [18] and induce *myc* expression by synovial fibroblasts [20]. In animal studies, *in vivo* joint bleeding and *in vitro* iron deposition resulted in increased expression of murine double min 2 (mdm2), the p53-binding protein [21].

In rheumatoid arthritis (RA), macrophages within the synovial membrane, but not infiltrating T or B lymphocytes, are correlated with the degree of joint erosion and synovial fluid levels of IL-6 [22]. Synovial monocytes/macrophages have also been implicated in the pathobiology of hemophilic arthropathy. An ultrastructural study of synovial membranes of hemophilia patients identified solitary iron-containing granules (siderosomes), compound siderosomes, and electron-dense particles within the cytoplasm of synovial intimal cells, subsynovial macrophages, and fibroblasts [23].

Blood vessels

Vascular development and angiogenesis are essential to both physiologic [24] and pathologic processes [25]. Just as angiogenesis is required for tumor growth, angiogenesis is also likely to be required for the synovium to expand beyond several millimeters in size [26]. The new capillaries formed during angiogenesis are composed of endothelial cells as well as pericytes, which are formed from differentiated intimal/subintimal smooth muscle cells [27]. Pericytes are enzymatically active cells that ensure vascular integrity [28] and contribute to ongoing blood vessel formation by fibrinolysis and proteolytic degradation of the extracellular matrix [29]. In biopsy samples of the synovial membranes of patients with thalassemia, iron oxide deposits were identified as microgranular intracellular inclusions in the cytoplasm of the vessel cells and pericytes [30]. The impact of iron on blood vessels may be inferred from experiments in which the intravenous administration of iron at a concentration sufficient to saturate transferrin induces hypervascularity and subsequent expansion of the synovial lining and subsynovial tissue [31]. Two hours after administration, a four-fold increase in synovial cell mitotic activity and pinocytosis by endothelial cells was observed. After 8–24 h, mature collagen appeared between endothelial cells, pericytes, and pericyte layers, and iron-containing mononuclear cells. Evidence of the direct effect of blood, specifically platelets, on vascular permeability was seen in an experiment in which blood was directly injected into the joints of rats [32]. A marked increase in the permeability of synovial venules was observed that persisted for up to 16 h.

Bone cells

As BIJD progresses, bone remodeling occurs, and osteoclastic (but not osteoblastic) activity is increased, as shown by increased levels of the biomarkers N-terminal cross-linking telopeptide of collagen type I, and tartrate-resistant acid phosphatase isoform-5b. The resultant decrease in bone mineral density leads to osteoporosis, which has been consistently associated with hemophilic arthropathy for more than four decades [33–40]. The severity of osteoporosis correlates with a patient's clinical and radiologic scores.

Joint hemorrhage and pathologic findings

Bleeding into a joint is the single most important risk factor for the development of hemophilic arthropathy [36]. In nearly half of all children with severe hemophilia, the initial hemarthrosis occurs during the first year of life [41], and 90% of youngsters who are severely deficient in FVIII or FIX experience at least one joint hemorrhage before the age of 4.5 years [42]. Eighty per cent of joint bleeding episodes involve the knees, elbows, and ankles [2]. and patients often develop multiple target joints.

Although blood is rapidly cleared from the joint space, the pathologic process continues [5], resulting in both radiographic and clinical changes. The Pettersson radiologic score increases by 1 point for every three joint hemorrhages occurring after 5 years of age [43]. Furthermore, as joint damage progresses, the patient experiences pain [44], reduced range of motion [45], muscle atrophy [46–48], ankylosis, osteoporosis [33,34,37–40], cartilage degeneration with collapse of the joint space [3,49–55], and cyst formation [56,57].

It is possible that the clinical and radiographic manifestations of BIJD are at least partially attributable to genetic polymorphisms unrelated to the hemophilia genotype. In the landmark US Joint Outcome Study, Manco-Johnson *et al.* found radiographic evidence of joint damage by age 6 years in some of the subjects who had no or only a few clinical evident hemarthroses (Fig. 2, green line) [58]. On the other hand, magnetic resonance imaging (MRI) showed no structural changes for some of the other subjects, despite each having experienced up to 16 joint bleeding events (blue line). In this latter group, there was no direct relationship (yellow line) between the number of joint hemorrhages and the MRI findings, and nor was there a threshold number of hemarthroses (red line) that predicted target joint formation and the development of arthropathy. Thus, it appears that some patients are more vulnerable to the effects of intra-articular bleeding (those who fall along the green line), others are resistant to such hemorrhages (those who fall along the blue line), and a third group of patients has an intermediate phenotype.

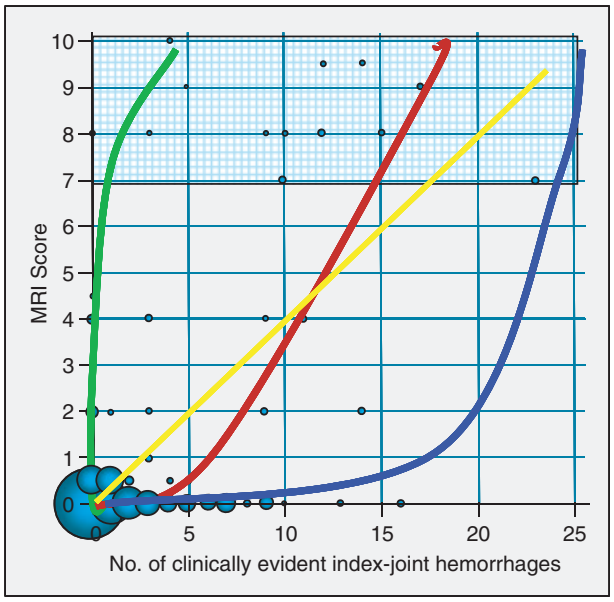

Fig. 2. Clinically evident joint hemorrhages in the US Joint Outcome Study [30]. The yellow line indicates that in some subjects there was a direct relationship between the number of joint hemorrhages and the magnetic resonance imaging (MRI) score, and in others there was a threshold number of hemarthroses needed to cause joint damage (red line). The green line indicates the MRI score of subjects with radiographic evidence of joint damage by age 6 years but who had no or only a few clinically evident hemarthroses, and the blue line indicates those subjects with a normal MRI score despite many clinically evident episodes of hemarthrosis. Original figure from Manco-Johnson, *et al.* [30], reproduced with permission, Copyright © 2007 Massachusetts Medical Society. All rights reserved.

Pathophysiology of joint disease

Biological processes typically follow a simple principle: an agent (stimulus) interacts with a responding element (target), resulting in a measurable effect (response) [59]. In the case of hemarthrosis, the stimulus is blood, the target is the joint, and the response is arthropathy. Whereas the concept of stimulus–target–response is simple, the actual process is often highly complex; in some instances, many of the details are incompletely understood. With regard to hemophilic arthropathy, it is unclear what constituent(s) in blood stimulates the interaction, what joint components are targets, and what signaling networks must be activated to produce the response. Despite these uncertainties, data generated from research into the degenerative joint damage that occurs with osteoarthritis (OA) and the inflammatory processes associated with RA (Tables 1 and 2) provide some clues that may be applicable to BIJD associated with hemophilia.

The stimulus: blood constituents

The presence of blood in a joint results in decreased range of motion secondary to pain and spasm, iron staining of the articular cartilage, hypertrophy of the synovial membrane with reactive blood vessels, and macrophages containing heme [60]. These changes are transient and, in the absence of repeated or massive bleeding, reversible. When joint bleeding is recurrent or severe, however, large amounts of hemosiderin, an iron-storage complex, are deposited into the synovial membrane [61], overwhelming the ability of the membrane to absorb the breakdown products and transfer them into the general circulation. As iron accumulates, the metabolic properties of the synovium are altered, setting the stage for BIJD [62].

Plasma enzymes, cytokines, chemokines, and growth factors By stimulating cell proliferation, mediating the inflammatory response to blood, and promoting proteoglycan release from cartilage, plasma enzymes, cytokines, chemokines and growth factors may play key roles in the pathophysiology of hemophilic arthropathy [4,63–65]. The enzyme thrombin, for example, which is produced by

Table 1 Blood constituents potentially responsible for hemophilic arthropathy

Plasma constituents
Enzymes
MMP-1
MMP-3
MMP-13
Thrombin
Tryptase/chymase
Elastase
Cathepsin
'PG-degrading activity'
Cytokines and chemokines
IL-1
IL-6
TNF-α
MCP-1
Growth factors
VEGF
PDGF
Cellular constituents
Erythrocytes
Hemoglobin
Iron
Leukocytes
Monocytes/macrophage
TIMP-1
CD3+ T cells, platelets
Growth factors

MMP, matrix metalloproteinase; PG, proteoglycan; IL, interleukin; TNF-α, tumor necrosis factor-α; MCP-1, monocyte chemoattractant protein-1; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; TIMP-1, tissue inhibitor of metalloproteinase-1.

Table 2 Joint components that are potential targets in the development of hemophilia arthropathy

Articular cartilage
Chondrocytes
Matrix
Bone cells
Osteoblasts
Osteoclasts
Synovial membrane
Fibroblasts
Synovial macrophages
Blood vessels
Endothelial cells
Smooth muscle cells
Pericytes

inflammatory cells, is a mitogen for synovial fibroblast-like cells, increasing levels of cyclic AMP [66]. This mitogenic activity increases the expression of mRNA of platelet-derived growth factor (PDGF) receptors (both PDGF-A and PDGF-B receptors), leading to synovial hyperplasia [67]. Using a mouse model of arthritis, Flick *et al.* demonstrated that thrombin-generated fibrin exacerbates the inflammatory response [68]. Conversely, less severe arthritis developed in mice lacking fibrinogen or the integrin fibrin(ogen) receptor $\alpha_M\beta_2$, and fibrin(ogen) was not necessary for leukocyte trafficking to joints. Synovial cells [69], the neutral proteinases that they produce (e.g. elastase) and other enzymes may degrade cartilage, thereby reducing its proteoglycan content [70–72].

Immunohistologic analysis combined with digital image analysis of synovial biopsy specimens from the inflamed knee joints of patients with RA show increased expression of CD68+ macophages, CD3+ T cells, matrix metalloproteinase (MMP)-1, MMP-3, MMP-13, IL-1, IL-6, TNF-α, and vascular endothelial growth factor (VEGF) [73,74]. Cytokines such as IL-1 and TNF-α, produced by activated synoviocytes, mononuclear cells, and articular cartilage, significantly upregulate expression of the MMP gene [75]. In a mouse model of hemarthrosis, the concentrations of IL-1β, IL-6, keratinocyte-derived chemokine and monocyte chemotactic protein-1 (MCP-1) were elevated in the bloody synovial fluid [76,77]. Cytokines also increase the uptake of transferrin-bound and non-transferrin-bound iron into monocytes and increase transferrin-bound iron uptake by synovial fibroblasts [78]. This increased iron uptake accelerates the vicious cycle of bleeding–synovitis–bleeding.

VEGF is involved in angiogenesis and chondrocyte metabolism [79]. Both VEGF and PDGF have been implicated in the development and progression of RA and OA, possibly through to an imbalance between cytokine-related cartilage degradation and maintenance of proliferative and synthetic cell responses associated with growth factor activity [80]. Synovial expression of VEGF is increased in persons with RA and OA as compared with unaffected controls. Among individuals with a recent diagnosis of polyarthritis, serum concentrations of VEGF, VEGF receptor, angiopoietin-1 and Fms-like tyrosine kinase-1 have been found to be related to inflammatory markers and bone destruction [81] VEGF may have similar activity in hemophilic arthropathy.

Cellular components In addition to producing enzymes, cytokines, chemokines, and growth factors, the cellular components of blood may directly influence the development of hemophilic arthropathy. Moderate amounts of protein-bound heme are essential for various biological processes. However, when heme is present in large quantities, as occurs in hemarthrosis, it is toxic, mediating oxidative stress and inflammation [82–90]. Chronic oxidative stress within the joint microenvironment leads to the generation of ROIs that contribute to the destruction of cartilage and bone [91].

In vitro, iron stimulates synovial cell proliferation [20], increases c-*myc* proto-oncogene expression [20], promotes DNA synthesis [18], and has an additive effect on cytokine activity [18]. Iron is required to support p21 expression [92]. Chelation of iron markedly increases mRNA levels of p21 (WAF1/CIP1), a cyclin-dependent kinase inhibitor involved in regulating the G(1)/S cell cycle checkpoint [92]. Iron depletion also increases p53 levels by phosphorylation of this protein at serine 15 and serine 37, preventing p53 degradation and its interaction with mdm2.

The increased intracapsular pressure caused by bleeding into a joint eventually exceeds capillary perfusion pressure, leading to repeated hypoxic–reperfusion injury and the generation of

ROIs [93] and superoxide radicals [94] by synoviocytes (but not chondrocytes) [95]. Heme oxidase enzyme activity may protect cells from oxidative injury caused by iron deposition and inflammation. Myc activation and exposure of ovarian carcinoma cells to proapoptotic ligands, including the fatty acid synthase ligand, or to TNF-related apoptosis-inducing ligand, induces heavy-chain ferritin expression. The resultant binding to intracellular iron and its depletion triggers apoptosis [96].

Proliferation of the synovial membrane and its infiltration with inflammatory cells creates a hypercapnic, acidic and hypoxic environment that adversely affects the membrane, chondrocytes, and underlying subchondral bone [97]. Hypoxia inhibits cell proliferation [98], alters the synthesis of matrix components in synovial tissue [98], and increases MCP-1 expression, thus promoting chemotaxis of monocytes into the synovial membrane [99]. Hypoxia also induces proangiogenic factors, such as the transcription factor hypoxia-inducible factor (HIF)-1 [100–112]. HIF-1A activity is controlled by the iron-dependent enzyme HIF-1A prolyl hydroxylase. Chelating iron from this enzyme inhibits its activity, stabilizing HIF-1A and its subsequent effects on downstream targets that are critical for angiogenesis.

The signals: from blood to joint destruction

The mechanisms and pathways by which blood in the joint space causes cartilage and bone destruction are unknown. However, members of the TNF receptor superfamily of cytokines – the receptor activator of nuclear factor-kappaB (RANK), its ligand, RANKL, and its natural decoy receptor, osteoprotegrin (OPG) – probably play major roles [113]. The balance of these proteins regulates osteoclast formation and activity, and they are the final effector proteins of osteoclastic bone resorption [114]. OPG colocalizes with P-selectin, thrombospondin, and von Willebrand factor within the Weibel–Palade bodies [115], and may also be involved in vascular injury, inflammation, and hemostasis [116].

Like RA, BIJD begins with inflammatory synovitis [117] and progresses to the cartilage and bone destruction that is characteristic of OA. The inflammatory milieu combined with local hypoxia promotes blood vessel formation and angiogenesis of the synovial membrane [46,91,101,118]. The poor quality of the blood vessels and increased permeability [32] further exacerbate bleeding. Additionally, the increased expression of RANK by normal neutrophils and RANKL by both normal and inflammatory neutrophils, together with the decrease in OPG, favor osteoclast differentiation and bone resorption [119] Hence, the arthropathy that follows joint bleeding probably results from a disruption in the physiologic bone remodeling process, which is normally characterized by a dynamic equilibrium between osteoclastic bone resorption, regulated by a number of cytokines, colony-stimulating factors, and calciotropic hormones [120], and osteoblastic bone formation, stimulated by bone morphogenic proteins [121–123].

Osteoclast precursors (OCPs) are derived from hematopoietic (monocyte) progenitors in the spleen and liver that migrate from blood into bone and fuse with one another to form multinucleated osteoclasts. Blood neutrophils in the joint create an inflammatory environment that produces numerous cytokines, including IL-1, IL-6, RANKL and TNF [124–127]. TNF increases the proliferation and differentiation of OCPs [128]. TNF also inhibits the production by bone marrow stromal cells of stromal cell-derived factor 1, which, in turn, increases the release of OCPs from the bone marrow [129]. RANKL, synthesized by reactive lymphocytes in the joint, binds to RANK, its signal-transducing receptor, and stimulates osteoclasts to resorb bone.

Conclusion

Hemophilic arthropathy, caused by blood in a joint, results in pain, deformity, and disability. Structural joint damage may occur following just a few (or no) hemarthroses in persons predisposed to arthropathy, or may develop after numerous joint bleeding episodes in those who appear to be protected from the adverse effects of blood. Data generated from research into the pathophysiology of OA and RA suggest that multiple blood constituents trigger the arthropathic process, and that several joint components are the targets. Further study is needed to elucidate the precise mechanisms by which bleeding induces hemophilic joint damage. Such knowledge is essential for the development of novel strategies to prevent or reverse this common and serious complication of hemophilia.

Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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