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## Advances and Challenges in Hemophilic Arthropathy

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### Abstract

Hemophilic arthropathy is a form of joint disease that develops in secondary to joint bleeding and presents with synovial hypertrophy, cartilage and bony destruction. The arthropathy can develop despite clotting factor replacement and is especially disabling in the aging population. Pathobiological tissue changes are triggered by release of hemoglobin and iron deposition in the joint, but the sequence of events and the molecular mechanisms resulting in joint deterioration are incompletely understood. Treatment options other than clotting factor replacement are limited. Improvements in the treatment of hemophilia necessitate a better understanding of the processes that lead to this disabling condition and better diagnostic tools. Towards that end, studies of the molecular mechanisms leading to the arthropathy, as well as the development of sensitive imaging techniques and biomarkers are needed. These will pave the way to identify the cause of acute pain such as joint bleeding or synovitis, detect early, potentially reversible structural changes, and predict progression of disease. This review describes current imaging techniques and the development of high resolution musculoskeletal ultrasound with power Doppler to afford point-of-care diagnosis and management, the potential utility of diagnostic biomarkers, and summarizes our current knowledge of the pathobiology of hemophilic arthropathy.

### Introduction

Patients with severe Factor (F)VIII or FIX deficiency (Hemophilia A or B) suffer from spontaneous joint bleeding in childhood<sup>1</sup> that results in “target joints”, defined as joints with several consecutive bleeds within a 6 month period.<sup>2</sup> Target joints often progress to hemophilic arthropathy (HA)<sup>1, 2</sup> that is characterized by joint deformities, synovial hypertrophy, and cartilage and bone destruction. Compared to episodic treatment of joint bleeds, prophylactic clotting factor treatment can reduce joint bleeding and the development of HA dramatically.<sup>3</sup> There is compelling evidence that initiation of prophylaxis in early childhood (< age 2)<sup>4</sup> and higher intensity dosing regimens have beneficial effects on joint

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outcomes.<sup>5</sup> However, HA cannot be entirely avoided with clotting factor replacement, as shown by a high percentage of adults with hemophilia from industrialized countries (~30–50% of patients) presenting with clinical arthropathy despite access to prophylaxis since childhood.<sup>6–8</sup> These recent studies do not always provide detailed information regarding number of patients on uninterrupted prophylaxis, start or intensity of prophylaxis and adherence, all of which may influence joint outcomes. However, these studies do provide evidence that HA is highly prevalent in the aging population of patients with hemophilia. This is important since HA is a disabling condition that negatively impacts physical activity and quality of life.<sup>9, 10</sup> In the last century patients died at a relatively early age and age-related comorbidities were therefore of minor interest. This has changed with the advent of virally safe clotting factor. The life span of hemophilia patients has become comparable to that of the general population<sup>11</sup>, making diagnosis, prevention and treatment of HA a critical focus of hemophilia care. However, few treatment options are currently available outside of clotting factor replacement, and management chiefly comprises various synovial ablation techniques<sup>12, 13</sup> and surgical correction including joint replacement.<sup>14, 15</sup> Preventing or slowing the arthropathy will require targeted management, which explains the growing interest in this field. Current efforts mainly focus on three major areas, which are 1) to develop sensitive joint imaging modalities to detect early changes and diagnose the etiology of acute and chronic pain, 2) to explore diagnostic and predictive biomarkers, and 3) to understand the underlying pathobiology of HA. This review summarizes the current progress in all those areas, and aims to bring this information into clinical context.

## Novel Imaging Modalities

The desire to explore structural changes and disease burden of hemophilic joints dates back almost one century, when x-ray technology was developed and first applied for the diagnosis and staging of medical conditions.<sup>16</sup> From the 1960s to the 1980s, efforts focused on devising radiographic grading systems to document progression of joint disease.<sup>17</sup> Two main classification systems were introduced, the Arnold-Hilgartner scale,<sup>18</sup> and the Pettersson score during that time.<sup>19</sup> These systems differ in several respects. Arnold-Hilgartner scoring includes soft tissue changes and is progressive, whereby the worst imaging finding dictates the stage of arthropathy. Pettersson scoring excludes soft tissue changes and is additive, whereby each abnormality is assigned points until a maximum score is reached. Based on recommendations by the World Federation of Hemophilia, Pettersson scoring has become the most widely used radiographic staging system for several reasons that include ease of administration, exclusion of soft tissue changes that cannot be reliably assessed by x-rays, and a lesser ceiling effect as encountered with the progressive scale.<sup>17</sup> At the time, systems such as the Pettersson score were invaluable to document beneficial long-term effects of prophylactic clotting factor treatment on joint outcomes in observational cohorts.<sup>3, 20</sup>

Since radiography captures only irreversible late stage changes, several new scoring systems using magnetic resonance imaging (MRI) were proposed in the early 2000s.<sup>21</sup> MRI permits direct visualization of cartilage, soft tissue, hemosiderin deposits, joint effusions, bony cysts, osteopenia, and marrow edema, and therefore detection of early, possibly reversible, pathology. The increasing adoption of prophylactic rather than episodic clotting factor treatment in children in industrialized countries,<sup>8</sup> and the need to detect early, subtle changes

to evaluate treatment efficacy in young patients anticipated to have near-normal life spans,<sup>11</sup> spurred investigations as to which MRI scale would be most suitable. As with radiography, several progressive and additive scales were developed, and excellent reviews of their advantages, disadvantages and accuracy to quantify pathological tissue changes are provided elsewhere.<sup>21–23</sup> In 2012, the International Prophylaxis Study Group merged systems into one, attempting to enable easier comparisons across studies and populations, separating soft tissue and osteochondral changes for more detailed information.<sup>24</sup>

However, problems with MRI include the necessity to sedate children, cost, availability and inability to frequently scan multiple joints. This prompted investigation into alternative new imaging modalities, which led to the adoption of musculoskeletal ultrasound (MSKUS) for the imaging of hemophilic joints and its recent introduction into clinical practice.<sup>25–28</sup> Continued innovative development and wide-spread utilization for HA care are expected over the next few years. Advances in ultrasound technology permit high resolution imaging for detailed visualization of anatomy and pathology of the musculoskeletal system, including tendons, muscles, ligaments and fluid. With high-frequency transducers, spatial compounding and power Doppler, soft tissue resolution approaches, or is sometimes greater than, the sensitivity of routine MRI.<sup>29–31</sup> Comparison of MSKUS to MRI in hemophilia has demonstrated reliable detection of soft tissue and osteochondral abnormalities, cartilage destruction and effusions.<sup>32–34</sup> MSKUS has been demonstrated to be a critical tool to differentiate if bleeding is associated with acute and/or chronic pain and to determine if sprains, tendon/ligament tears or enthesopathies contribute to pain (Figure 1). MSKUS examination with sonopalpation is able to distinguish complex bloody from simple serous effusions with high accuracy as confirmed by needle aspiration.<sup>25, 35</sup> In addition, power Doppler permits rapid and dynamic assessment of synovial blood flow, thereby demarcating synovitis from fluid. In contrast, conventional MRI cannot easily differentiate joint fluid from synovitis without contrast administration,<sup>36</sup> nor can it easily distinguish bloody from non-bloody effusions (Figure 2). The MRI signals of acute/subacute blood products in the joint have not been studied, and the diagnosis of hemarthrosis with MRI depends heavily on the clinical context.

The ability to diagnose inflammatory soft tissue changes and synovitis with power Doppler<sup>25, 26, 33, 35</sup> is important in HA. As in rheumatoid arthritis (RA), synovial hypertrophy is often present and can be accompanied by synovitis characterized by increased synovial blood flow. Power Doppler is sensitive to slow blood flow in the microcirculation,<sup>37</sup> and therefore able to quantify synovial perfusion abnormalities. In rheumatic joints, the quantification of power Doppler signals has been validated to evaluate the response to anti-inflammatory agents by comparisons with histological specimens and contrast-enhanced MRI.<sup>37</sup> Based on new evidence in hemophilia, positive power Doppler signals in HA are associated with vascular remodeling and joint bleeding,<sup>38</sup> whereby bleeding risk increases significantly with increasing power Doppler signals. This observation adds a new dimension and may become an important consideration when monitoring hemophilic joints with power Doppler.

Compared with MRI, MSKUS is rapid, less costly, does not require contrast administration or sedation and is therefore an ideal point-of-care imaging modality.<sup>36, 39</sup> Imaging with

MSKUS has been validated for a wide spectrum of musculoskeletal pathology in rheumatology, orthopedics and sports medicine, and has been recommended by the American College of Rheumatology as the point-of care imaging modality to assess disease activity.<sup>40</sup> For hemophilia, this is of utmost importance. Rather than assuming that a painful joint represents bleeding, MSKUS can diagnose musculoskeletal conditions for which specific treatments other than clotting factor are available. MSKUS is also mobile, with a potential for use in patients' homes or work place, and it can monitor resolution of hemarthrosis or inflammation.<sup>27, 28</sup>

However, among the potential difficulties with ultrasound assessment of the joints are the nonvisualization of internal bone structure, bone edema, and the inability to penetrate deeper structures such as cruciate ligaments in the knee. There is no doubt that ultrasound is operator dependent with a learning curve for the inexperienced operator.<sup>36</sup> However, diagnostic ultrasound is now emerging rapidly in many medical disciplines including medical education.<sup>41</sup> It is expected that appropriate training will result in the prerequisite understanding of anatomy, pathology, tissue discrimination and planes. This will enable non-radiologists to use point-of-care ultrasound to improve diagnosis and inform treatment decisions for a variety of medical conditions in the future.

In addition to the utilization of MSKUS for point-of care imaging of painful hemophilic joints, MSKUS has been proposed for scoring joint health in analogy to radiographic or MRI scales.<sup>35, 42, 43</sup> The most recent published scoring system (HEAD-US) uses several defined transducer positions to semi-quantitatively evaluate synovium, cartilage and subchondral bone on low cost portable machines.<sup>43</sup> However, this scoring algorithm has not yet been validated, and while good to excellent inter- and intra-operator reliability were reported for experienced radiologists, the same has not yet been established for non-radiologists. Long-term monitoring of joint health with ultrasound holds great promise since the ease of scanning permits fast and frequent monitoring of all joints. In general, MSKUS is evolving rapidly into a promising tool to guide treatment decisions, although the impact of MSKUS-guided treatment decisions on outcomes remains to be documented in future studies.

## Biomarkers

As defined by the Food and Drug Administration, a biomarker can be "objectively measured and evaluated as an indicator of normal biologic process, pathogenic process, or pharmacologic response to a therapeutic intervention".<sup>44</sup> Compared to imaging, biomarkers have the advantage of rapidly capturing processes of tissue turnover that indicate structural and/or early changes at a molecular level. In hemophilia, where joint disease is currently treated with multiple self-infusions of costly clotting factor concentrates without knowing whether it is the most appropriate treatment for every patient, availability of biomarkers for diagnosis, prognosis and determination of treatment efficacy is therefore highly desirable. Biomarker analyses could identify patients for targeted treatment to improve outcomes and reduce cost, which has become an important paradigm in the era of precision medicine<sup>45</sup>.

The ideal properties of a biomarker will vary according to the underlying condition and clinical question being asked. However, to be clinically useful, biomarkers must have high

disease specificity, high correlation with disease burden, and be predictive of outcomes if used to gauge treatment success. Biomarker research in hemophilia is complicated by the largely unknown mechanisms that underlie the pathogenesis of HA, including processes associated with repeated joint bleeding. Studies in hemophilia published to date exclusively mirror efforts in osteoarthritis (OA) and RA that have traditionally focused on biomarkers of cartilage and bone turnover<sup>46, 47</sup> as a final common denominator of joint destruction. Generally speaking, OA is a degenerative disease of aging, RA an systemic inflammatory disease with a predilection for joints, and HA is caused by joint bleeding. Thus, although OA, RA and HA all result in progressive cartilage destruction, their pathogenesis appears distinct and this needs to be considered when developing markers of early disease activity. Since biomarker research in HA is currently in its infancy, it is relevant to provide a brief background of the studies of biomarkers in OA and RA.

The desire to determine effects of new disease modifying agents and to guide treatment decisions in OA and RA spurred efforts to identify biomarkers over the past decade. Both OA and RA show a patient-specific onset and time course of chronic progressive joint destruction, involving cartilage and bone degradation. Therefore, candidate biomarkers to determine disease activity and predict progression originated mostly at the level of tissue degradation where different pathways converge. Prototypic candidates are neoepitopes of cleaved cartilage matrix components or markers of bone or collagen turnover. It is felt that their levels in urine, serum or synovial fluid may reveal early molecular changes not yet detectable by imaging methods.<sup>48</sup> Selected commercially available candidates include C-terminal telopeptides of type I or II collagen (CTX-I, CTX-II), cartilage oligomeric matrix protein (COMP), hyaluronan, various cartilage cleavage products, procollagen type II N-terminal propeptide, chondroitin sulfate (CS)-846 or MMP-3.<sup>44</sup> Figure 3 depicts sources of candidate biomarkers explored in OA. Despite intense effort, no single biomarker or composite algorithms involving multiple biomarkers have been validated to be clinically useful.<sup>48-51</sup>

The identification of markers of synovitis and inflammation has been pursued more in RA than OA, since synovial inflammation is a hallmark of RA and associated with progressive joint deterioration. However, the fact that RA is a systemic inflammatory disease with immunologic abnormalities not only in joints but also other organs<sup>52</sup> has complicated the identification of useful biomarkers. Inflammatory markers such as C-reactive protein (CRP) may be elevated in RA, but reflect overall systemic inflammation and are not specific for synovitis or joint tissue status. This is underscored by the fact that systemic and joint tissue levels of inflammatory cytokines such as TNF- $\alpha$  and IL-1 $\beta$  do not always correlate in RA, making it difficult to distinguish synovial contributions from overall inflammation. Consequently, it is difficult to use inflammatory biomarkers for treatment decisions at the joint level.<sup>53, 54</sup> Therefore, biomarkers (for example CRP) are embedded into clinical algorithms to determine disease activity,<sup>55</sup> whereby the abnormal vascular perfusion associated with synovitis is often quantified by MSKUS.<sup>56</sup>

The anticipated availability of many new clotting factor products and treatments for hemophilia has spurred new efforts to identify tailored treatment approaches to individual patients, similar to efforts in OA and RA. The identification of specific biomarkers that

could be applied to customize the choice of treatment according to inter-individual bleeding propensity, personal clotting factor pharmacokinetics, subclinical bleeding and other variables, would be a major step forward. Biomarkers that could diagnose bleeding or joint destruction, or could assess response to treatment, would considerably advance hemophilia care. It has been shown that diagnosing joint bleeding by patient and/or physician perception is imprecise<sup>25</sup> and that current imaging techniques for cartilage damage, bone and soft tissue changes lack sensitivity for early changes following treatment intervention.<sup>57, 58, 59</sup> Hence, the identification of biomarkers, that are also predictive of long-term joint health, would permit point-of-care diagnosis and decisions for individual treatment, thereby avoiding delays in the detection of joint destruction.

Analysis of biomarkers in HA is currently limited to a few studies. A small cross-sectional analysis of markers of bone and cartilage degradation demonstrated that a composite score of levels of CTX-II, COMP and CS-846 correlated best with radiographic joint changes during bleed-free intervals, while CTX-I and cartilage cleavage products (C1, 2C and C2C) did not (Figure 4). CTX-II, COMP and CS-846 are all indicative of cartilage degradation, originating from different matrix components.<sup>46</sup> Interestingly, a significant rise of CTX-II and CS-846, but not COMP, was demonstrated within 5 days after self-reported joint bleeding in 10 adult hemophilia patients.<sup>47</sup> The individual percentage increase for CTX-II and CS-846 in response to bleeding ranged from ~25 to 500% and from ~80 to 120%, returning gradually to baseline during the following 10 days. The wide inter-individual initial rise of CTX-II may have been related to imprecision of self-reported bleeding as etiology of painful episodes,<sup>25, 26</sup> bleeding volume or individual joint responses to the blood. For CTX-II, similar findings were noted in a canine model when blood was injected into the joint.<sup>47</sup> In summary, these pilot studies suggest that some biomarkers of cartilage degradation correlate to some extent with radiographic joint destruction in hemophilia, and that joint bleeding can cause their acute, but reversible, rise. However, the predictive value of such biomarker levels for progression of HA is undetermined. VEGF as a proangiogenic mediator may be another interesting direction for synovial changes. VEGF is elevated in hemophilia patients with arthropathy compared to patients with other bleeding disorders without joint involvement.<sup>60</sup> VEGF is also elevated in RA and OA<sup>61, 62</sup> and levels appear to be related to disease activity and synovitis.<sup>60, 62, 63</sup> VEGF is thought to contribute to the pathogenesis of arthritis by various mechanisms, although its correlation with extent of synovitis, dynamic neoangiogenesis and long-term joint outcomes requires more study.<sup>64</sup> In general, the detection of early tissue changes and their meaning for progression of arthropathy require the discovery of new biomarkers specific for HA. Towards that end, defining the pathobiology of HA will become paramount and will pave the way for innovative biomarker research including proteomics, mass spectrometry and molecular signatures.

## The Pathobiology of Hemophilic Arthropathy

The pathobiology of HA is unique in that it is influenced by repeated release of hemoglobin and iron depositions in the joint. Although HA may resemble OA or RA, and some molecular changes may have common denominators such as inflammation or cartilage destruction,<sup>65</sup> HA does not fit either category and remains poorly understood. Whereas the

clinical findings of advanced HA are well documented, the pathobiology is not. In particular, there remains a large knowledge gap as to why bleeding occurs into joints, and what facilitates re-bleeding and the development of target joints sometimes despite clotting factor administration. Also, the heterogeneity of bleeding phenotypes and severity of arthropathy is not understood; especially genetic modifiers such as the presence of genetic risk factors for thrombosis in the general population (e.g. Factor V<sub>Leiden</sub> mutation) remain to be fully appreciated.<sup>66, 67</sup>

The current paradigm for HA proposes that recurrent bleeding induces hyper-reactive synovial changes including the formation of villi protruding into the joint space. Hypertrophic synovium is often associated with increased vascular perfusion, which is a hallmark of synovial inflammation, termed synovitis. Metabolic changes in cartilage and subchondral bone ensue and result in their destruction.<sup>65, 68, 69</sup> This current model of HA is based on studies of synovial and cartilage changes in response to joint bleeding using in vitro assays, ex vivo histology and animal models of experimental HA. However, the relative contributions of various molecular pathways involved and the proper sequence of events clearly need further definition. A view of our current understanding of HA is shown in Figure 5.

### Synovial hypertrophy

Erythrocytes in the joint are removed through their uptake by macrophages and synoviocytes. Iron released from erythrocytes is bound as hemosiderin and cleared.<sup>70-72</sup> However, with excessive and/or repeated joint bleeding the iron burden may exceed iron clearance capacity despite increased expression of iron regulators.<sup>73, 74</sup> Synovial hypertrophy is facilitated by the exposure to iron that triggers the upregulation of the oncogenes MDM2 and c-MYC, thereby promoting proliferation.<sup>75, 76</sup> Additionally, local synovial upregulation of inflammatory cytokines and chemokines such as interleukin(IL)-1 $\beta$ , IL-6, tumor necrosis factor alpha (TNF $\alpha$ ), interferon- $\gamma$ , monocyte chemoattractant protein-1 and IL-8, enhance the catabolic activity of chondrocytes to promote cartilage destruction.<sup>77-79</sup> The hypoxic environment ensuing synovial hypertrophy results in the expression of hypoxia-inducible factor (HIF)-1 $\alpha$ , HIF-2- $\alpha$ , and subsequent upregulation of vascular endothelial growth factor (VEGF)-A, stromal cell-derived factor 1 $\alpha$ , pro-matrix metalloproteinase (proMMP)3 and proMMP13, These processes fuel a vicious cycle of neoangiogenesis and enzymatic tissue destruction.<sup>60, 78</sup>

A worrisome and clinically relevant observation is that synovial neoangiogenesis may be perpetuated systemically in HA, a conclusion supported by significantly increased levels of VEGF-A and bone marrow-derived endothelial progenitor cells expressing VEGF-receptor 2 (VEGFR2/KDR) in the peripheral blood of patients with hemophilia.<sup>60</sup> Further support for systemic activation is provided by the fact that peripheral blood mononuclear cells from patients with hemophilia can induce VEGF-dependent synovial cell proliferation in vitro.<sup>60</sup> There is also new evidence in mice and humans that systemically-mediated angiogenesis and continued vascular remodeling may perpetuate bleeding and facilitate progression of HA.<sup>38</sup> Vascular abnormalities consisted of vascular architecture changes and highly abnormal joint tissue perfusion patterns, as well as pronounced expression of  $\alpha$ -Smooth Muscle Actin, a

marker of mesenchymal-type progenitor cells involved in vascular remodeling. These changes appeared unique to hemophilia, as they are not present in rheumatoid arthritis or osteoarthritis.<sup>38</sup> The mediators of these neoangiopoietic pathways in hemophilia remain to be determined, but may involve inflammatory cytokines because modulation of cytokine levels, whether by inhibiting pro-inflammatory cytokines (e.g. IL-6 receptor antagonist<sup>80</sup>) or by administering protective cytokines (e.g. IL-10<sup>81</sup> and/or IL-4<sup>82-84</sup>), alleviated the sequelae of experimental hemarthrosis.

The fibrinolytic system in the joint provides another important aspect in relation to hemarthrosis.<sup>85-87</sup> Extravascular fibrin deposition in the inflamed synovium is known to be deleterious in RA, OA and with joint trauma and is induced by the complex interplay between inflammation and hemostasis.<sup>88-91</sup> For instance, one interaction between inflammation and hemostasis is the direct engagement of fibrin(ogen) with the integrin receptor alpha(M)beta(2) on leukocytes, thereby regulating leukocyte function in the joint.<sup>92</sup> Modulation of fibrinolysis in the joint is clinically relevant since antifibrinolytics are often used in hemophilic bleeding<sup>93, 94</sup> without an understanding of the effect of such treatment on joint health. Studies in RA and OA have shown ongoing fibrin degradation that correlates with the degree of inflammation, involving increased expression of tissue-type plasminogen activator (t-PA) in OA<sup>89, 95</sup> and urokinase (uPA) in RA<sup>87, 89</sup>. In this context, plasmin generated from plasminogen is a central player. Plasmin promotes fibrin degradation, tissue disintegration and remodeling with direct detrimental effects on cartilage.<sup>96</sup> Plasmin facilitates proteoglycan release, potentially by plasmin-mediated activation of pro-MMPs as demonstrated on healthy human cartilage explants.<sup>97</sup> Consistent with this, systemic or intra-articular injection of  $\alpha_2$ -antiplasmin during experimental hemarthrosis led to improvement of histological scores of synovial inflammation and cartilage destruction in hemophilia mice.<sup>98</sup> Plasmin levels in the synovial fluid and the number of uPA-expressing synovial cells were shown to be elevated in hemarthrosis, although fibrinolysis inhibition was induced simultaneously as evidenced by elevated plasminogen activator inhibitor 1 (PAI-1) levels.<sup>97</sup> Enhanced PAI-1 expression, for example mediated by TNF $\alpha$  in RA, may on the other hand facilitate fibrin depositions,<sup>99</sup> which all together suggests that the de-regulation of fibrinolysis contributes to joint damage in various, not yet fully understood ways. This is further underlined by the fact that opposing effects of plasmin modulation were observed in a model of rheumatoid arthritis in the plasminogen-deficient mouse and depended on the type of joint.<sup>100</sup> In this model mice developed severe arthritis in the distal joints despite decreased MMP activity, whereas proximal joints were protected.<sup>100</sup> The extent to which fibrinolysis dysregulation comprising fibrin depositions, hyperfibrinolysis or reduced fibrinolysis may play a role for HA therefore remains an important open question. In hemophilia, increased fibrinolysis is known to contribute to bleeding due to defective thrombin-mediated activation of thrombin-activatable fibrinolysis inhibitor (TAFI).<sup>101-103</sup> Hence, it is conceivable that impaired TAFI activation may contribute to an imbalanced regulation of fibrinolysis in hemophilic joints. Interestingly, activated TAFI (TAFIa) not only impairs fibrinolysis, but also exerts anti-inflammatory effects<sup>104</sup> that are protective in RA and OA.<sup>105-107</sup> The anti-inflammatory properties result from inhibition of thrombin-cleaved osteopontin, bradykinin and the anaphylatoxins (complement C3a and C5a) by TAFIa.<sup>104</sup> In animal studies, synovial inflammation and arthritis were significantly increased in TAFI<sup>(-/-)</sup>



mice subjected to experimental RA due to decreased TAFI-dependent inactivation of C5a.<sup>108</sup> TAFI's important role in modulating inflammatory joint disease in humans is further supported by the fact that TAFI is expressed in synovium and in synovial fluid,<sup>90</sup> and that patients with RA with the enzymatically more stable TAFI polymorphism (rs1926447, Thr325Ile) are better protected against joint disease.<sup>108</sup> The extent to which TAFI may provide an important molecular link between joint inflammation, joint degeneration and hemostasis in HA is interesting and remains to be studied.

### Cartilage degradation

Cartilage is composed of chondrocytes and cartilage matrix, mainly containing collagen type II and proteoglycan (aggrecan) which are required for the tensile strength and resilience of the cartilage tissue. Joint hemorrhage may lead to cartilage deterioration in a number of ways. First, cartilage damage may be inflicted by hemophilic synovitis through the production of catabolic cytokines and tissue-destructive enzymes as mentioned above.<sup>77</sup> However, studies in the dog have verified early signs of cartilage erosion prior to or independent of synovitis after experimental hemothrosis, and those findings closely resembled the pathology of OA.<sup>109–111</sup> Therefore, and second, cartilage damage may also be induced by direct exposure to blood, which simultaneously decreases synthesis and increases release of cartilage proteoglycan-glycosaminoglycan (GAG). Hemoglobin-derived iron from erythrocytes and cytokines released from mononuclear cells were shown to be responsible for these effects.<sup>112</sup> Additionally, IL-1 $\beta$ -induced hydrogen peroxide release from activated monocytes/macrophages appears to affect GAG metabolism. Hydrogen peroxide reacts with hemoglobin-derived iron to form hydroxyl radicals that induce chondrocyte apoptosis and destruction of the cartilage matrix; this was partially prevented by the oxygen radical scavenger, N-acetylcysteine.<sup>112–114</sup> Taken together, cartilage destruction may be induced directly by contact with blood, but also indirectly through catabolic downstream effects during soft tissue inflammation or alteration.

### Summary

HA is emerging as a major morbidity of hemophilia as the average age of the patient with hemophilia increases. HA is distinct from other arthropathies in that it is propelled by repeated bleeding, rather than systemic inflammation as in RA, or age-related degeneration in OA. The pathobiological mechanisms that result in hypertrophic synovium, hypervascularity and cartilage destruction are incompletely characterized, but newest evidence suggests involvement of systemic neoangiogenesis in response to joint bleeding that is associated with vascular remodeling and perpetuated bleeding.<sup>38, 60</sup> To better address care of the hemophilic joint, novel strategies involving diagnostic biomarkers and point-of-care imaging techniques are being explored. These advancements will permit a shift from empiric diagnosis and treatment to precision medicine and targeted treatment to preserve or improve joint function especially for the aging generation of hemophilia patients.

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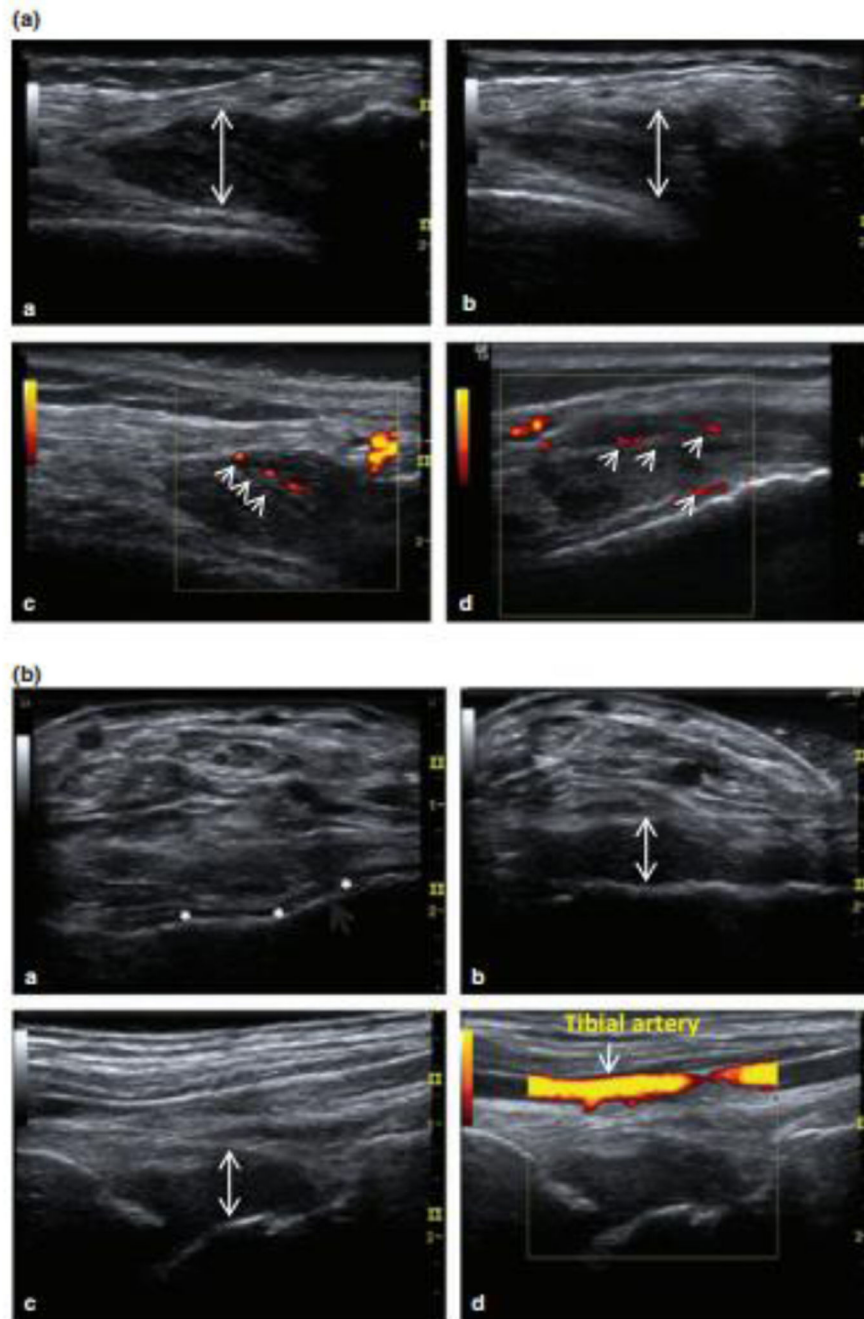
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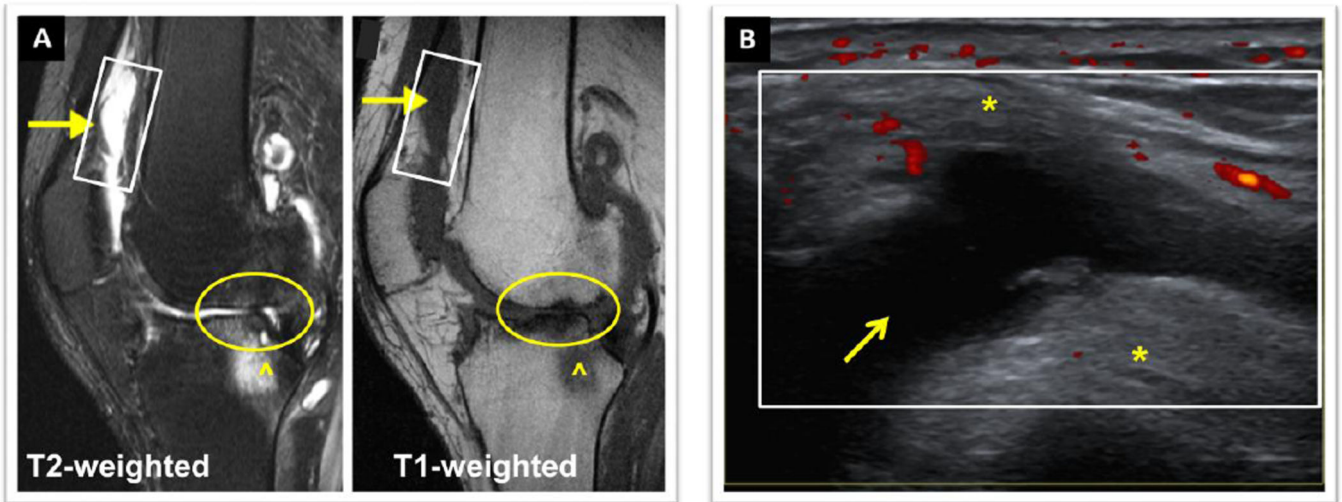


**Figure 1. Two case studies illustrating musculoskeletal ultrasound (MSKUS) in the diagnosis of bleeding and non-bleeding episodes during acute painful episodes**

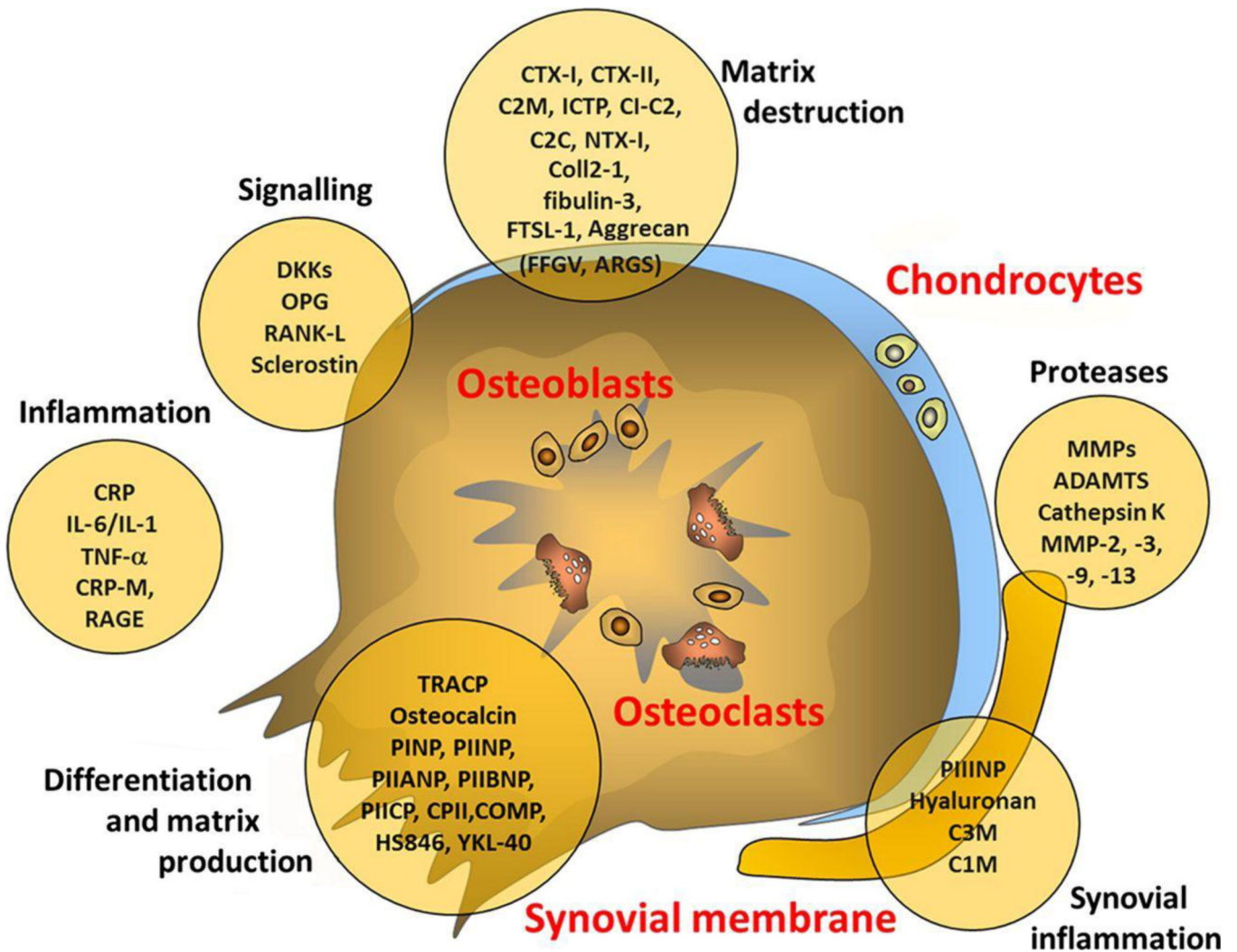
(A) 37-year-old man with severe haemophilia A presented with perceived joint bleeding. a: Axial view of the lateral gutter of the left knee showed heterogeneous hypoechoic intra-articular material (arrow). b: The intra-articular (i/a) material was hardly compressible and not displaceable (arrow) suggestive of thickened synovial tissue. c/d: power Doppler (PD) revealed vascularization of thickened hypervascular synovial tissue without evidence of sonographic effusion. This episode was reclassified as synovitis.



(B) 23-year-old man with severe haemophilia B presented with ankle pain and perceived “arthritis pain”. a: Baseline axial view of the ankle showed thin anechoic joint space in the tibiotalar joint (\*). b: Interval examination during pain episode showed marked volume increased tibiotalar synovial space (arrow). Complex echogenic pattern and compressibility of the synovial space were consistent with complex effusion//intra-articular bleed. c/d: Orthogonal view without and with power Doppler demonstrated absence of tissue vascularization. This episode was reclassified as acute haemarthrosis  
Ceponis, A. et al. Haemophilia 2013. 19: pp 790–798. Modified. With permission form the journal.



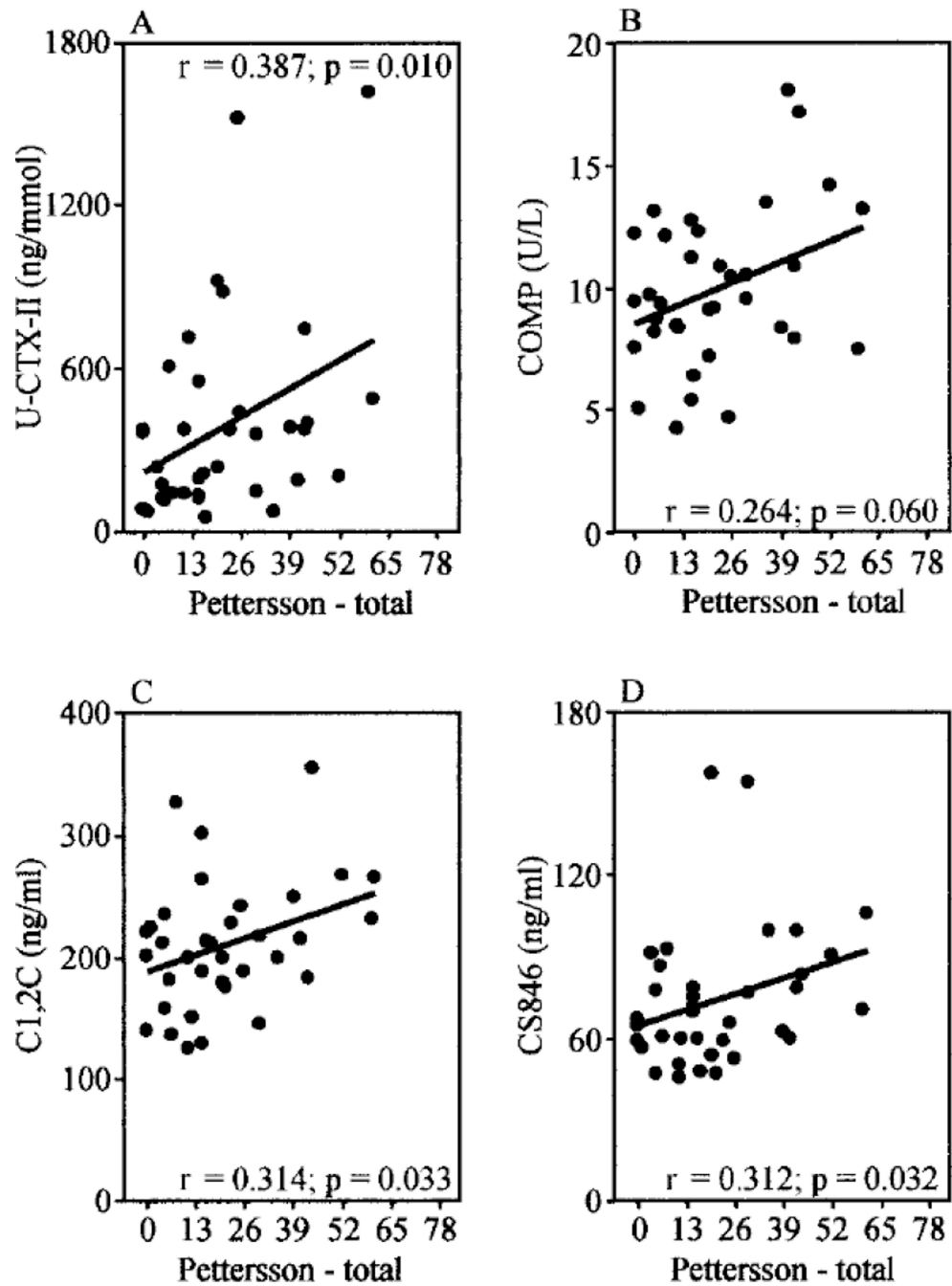
**Figure 2. Comparison of MRI and MSKUS of the knee of a 54 year old man with moderate Hemophilia A. Both imaging studies were performed on the same day**  
 The patient presented with persistent knee swelling and pain 2 weeks after a fall. A) MRI (sagittal view): ^ Marrow edema, O cartilage and meniscus destruction. The arrows (→) indicate areas in the suprapatellar bursa and patellar recesses where diagnostic distinctions of synovial hypertrophy/synovitis from effusion, and of bloody from non-bloody effusions were difficult by conventional MRI. The rectangle depicts the same area scanned with B) MSKUS. MSKUS demonstrated a simple non-bloody effusion (anechoic, compressible area (→)) surrounded by \*fat pad and \*synovium. Power Doppler (red dots) revealed areas of synovial perfusion. MRI, Magnet Resonance Imaging. MSKUS, Musculoskeletal Ultrasound. University of California San Diego Case Series, Images provided by Annette von Drygalski, MD. Patient provided written informed consent.



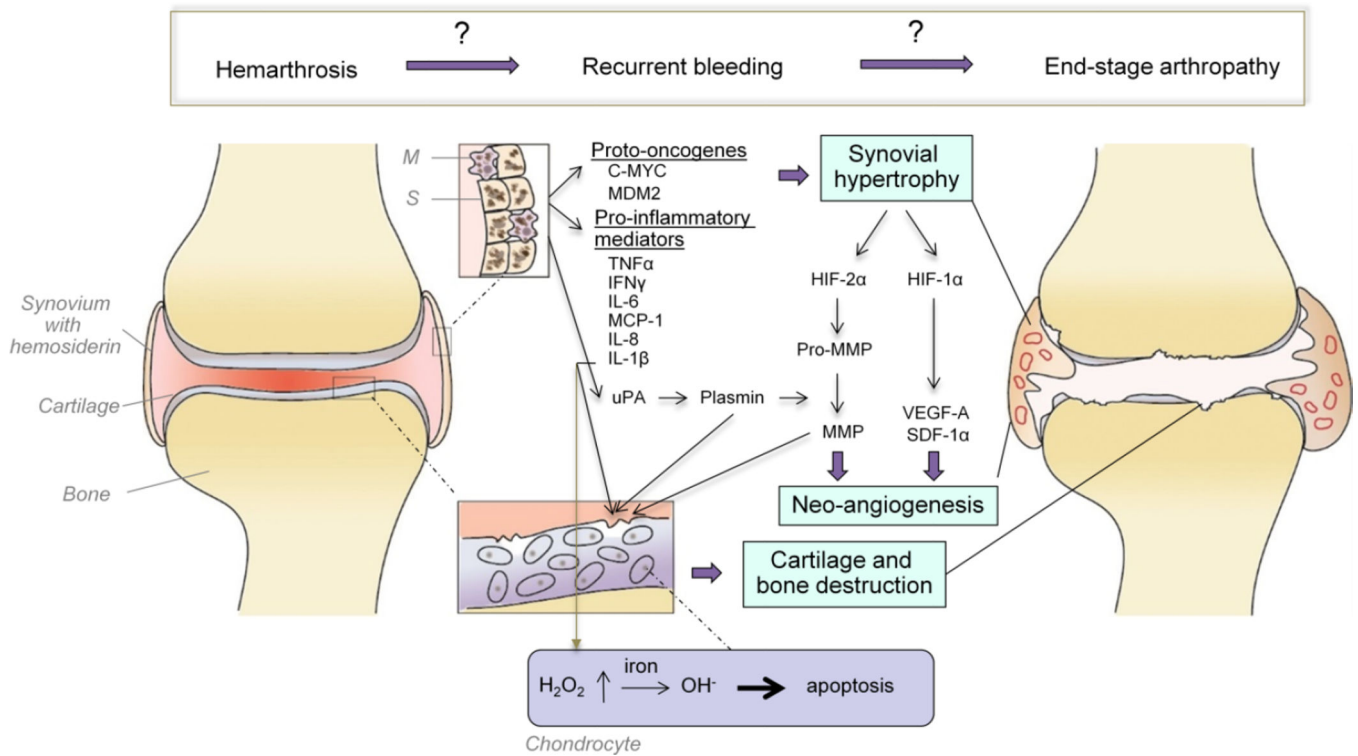
**Figure 3. Sources of possible biomarkers in osteoarthritis**

10–20 C2C, cleavage of type II collagen; C2M, collagen type II-specific neoepitope; C3M, collagen type III-specific neoepitope; Coll 2-1, 9-amino acid peptide of type II collagen (nitrated form Coll 2-1 NO<sub>2</sub>); COMP, cartilage oligomeric protein; CII, type II collagen propeptide; CRP, C reactive protein; CTX, C-terminal telopeptide of collagen; DKK, wnt inhibitor; FSTL-1, follistatin-like protein 1; ICTP, type I collagen-derived cross-linked carboxy-terminal telopeptide; IL, interleukin; MMP, matrix metalloproteinase; NTX, N-terminal telopeptide of collagen OPG, osteoprotegerin; PIIANP, N-propeptide IIA of type II collagen; PIIBNP, N-propeptide IIB of type II collagen; PIICP, C-propeptide of collagen type II; PINP, N-propeptide of type I collagen; PIINP, N-propeptide of type II collagen; RANK-L, receptor activator of nuclear factor  $\kappa$ B ligand; RAGE, receptor for advanced glycation endproducts; TNF, tumour necrosis factor.

Lotz M, et al. *Postgrad Med J* 2014; 90: pp 171–178. With permission from the Journal.



**Figure 4.** The correlation of radiographic joint damage with biomarker levels of cartilage turn-over with radiographic Pettersson scores in hemophilic arthropathy. Jansen N.W.D., et al. *Arthritis & Rheumatism* 2009; 60: pp 290–298. With permission from the Journal.



**Figure 5. Pathobiology of hemophilic arthropathy. A provisional scheme with many open questions**

Erythrocytes in the joint space are taken up by synoviocytes (S) and tissue macrophages (M), that store iron released from erythrocytes as hemosiderin. The expression of the oncoproteins C-MYC and MDM2 in response to iron results in synovial hypertrophy. Synovial hypertrophy creates a hypoxic environment causing a rise in hypoxia inducible factor (HIF)-1 $\alpha$  and HIF-2 $\alpha$ , which induce increased expression of the pro-angiogenic mediators vascular endothelial growth factor-A (VEGF-A) and stromal-cell derived factor 1 $\alpha$  (SDF-1 $\alpha$ ), as well as pro-matrix metalloproteinases (pro-MMP). VEGF-A stimulates synovial neoangiogenesis and plasmin-mediated conversion to MMPs results in glycosaminoglycan release from the cartilage matrix, and cartilage and subchondral bone destruction. Synoviocytes and tissue macrophages also release pro-inflammatory mediators, such as tumor necrosis factor alpha (TNF $\alpha$ ), interferon- $\gamma$ , interleukin (IL)-1 $\beta$ , IL-6, monocyte chemoattractant protein-1 (MCP-1) fueling these processes. Secreted IL-1 $\beta$  induces increased production of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in the chondrocytes, which upon reaction with iron (Fe<sup>2+</sup>) forms cytotoxic hydroxyl radicals (OH<sup>-</sup>) and prompts chondrocyte apoptosis. Neoangiogenesis and vascular remodeling are assumed to maintain a vicious cycle of re-bleeding that results in progressive hemophilic arthropathy, characterized by synovial hypertrophy, cartilage and bone destruction and joint deformities.