

Peer Review File

Article information: <http://dx.doi.org/10.21037/atm-21-747>

1. Reviewer A:

PS. The following references are just the reviewer's suggestions. It's the authors' own choice whether it is necessary to add these references.

Thank you very much for the appreciation of our manuscript, as well as for the important suggestions. We have carefully taken into consideration all the suggestions and now feel the manuscript is much better, more suitable for publication.

1) The paper needs a NEW SECTION on TREATMENT OF PAIN. Let me suggest the following reference and abstract.

Rodriguez-Merchan EC. Treatment of musculo-skeletal pain in haemophilia.

Blood Rev. 2018 Mar;32(2):116-121. doi: 10.1016/j.blre.2017.09.004. Epub 2017 Sep 19. PMID: 28943040

In acute hemarthroses pain treatment must continue until total disappearance (checked by ultrasonography) and include hematologic treatment, short-term rest of the involved joint, cryotherapy, joint aspiration and analgesic medication (paracetamol in mild pain, metamizole for more intense pain, and in a few precise patients, soft opioids such as codeine or tramadol). In the circumstance of intolerable pain we should use morphine hydrochloride either by continual infusion or a patient-controlled analgesia (PCA) pump, determined by the age, mental condition and grade of observance of the patient. Epidural blocks utilizing bupivacaine and fentanyl may be very efficacious as well.

Three main strategies to alleviate chronic musculoskeletal pain secondary to hemophilic arthropathy (joint degeneration) exist: pharmacologic management, physical medicine and rehabilitation, and intra-articular injections. As for pharmacologic management, NSAIDs (ibuprofen, diclofenac, celecoxib, rofecoxib) are better than paracetamol. The advantages of tramadol or tramadol/paracetamol and non-tramadol opioids are scanty. With respect to physical medicine and rehabilitation, there is insufficient confirmation that a brace has supplementary favorable effect compared with isolated pharmacologic management. Land-based curative exercise and watery exercise have at the minimum a tiny short-run benefit. Curative ultrasound can be helpful (poor quality of evidence). The efficacy of transcutaneous electrostimulation (TENS) for pain mitigation has not been proved. Electrical stimulation treatment can procure notable ameliorations. With respect to intra-articular injections, viscosupplementation appears to

be a useful method for pain alleviation in the short-run (months). The short-run (weeks) advantage of intra-articular corticosteroids in the treatment of joint pain has been shown.

Thank you very much for the appreciation of our manuscript, as well as for the important suggestions. We have taken into consideration the suggestion and added that:

“Therapy of pain.

Pain is of key important for patients with hemophilia. Spontaneous joint bleeds cause the accumulation of intraarticular blood, resulting in swelling, impaired mobility, and severe acute pain. Repeated hemarthrosis progressively contribute to irreversible joint degeneration and later development of chronic hemophilic arthropathy, characterized by joint deformity, disability, and chronic pain. Pain in haemophilia can be either acute (hemarthrosis) or chronic (hemophilic arthropathy), or occur concurrently, thereby posing unique challenges to pain assessment and management. Pain is yet suboptimally treated, underlining the need to address this concern within the hemophilia comprehensive care setting. Though it is consensual that a thorough pain assessment is the basis for optimal pain management, the lack of specific and validated pain tools for hemophilia is also acknowledged, in spite of the abundance of disease-specific questionnaires for other painful conditions. Haemophilia-related pain has been assessed using distinct measures, from unidimensional Visual or Numerical Rating Scales to multidimensional pain questionnaires like the McGill Pain Questionnaire or the Brief Pain Inventory.

Efficient pain therapy is important to increase the quality of life in patients. For hemarthrosis, the following five steps are efficient to properly manage acute pain, as according to Rodriguez-Merchan *et al*, intravenous infusion of FVIII/FIX, within 2 hours from the beginning of joint bleeding, till a plasma level not < 30–50% of the insufficient factor is attained, short-run repose of the painful articulation, local cryotherapy, joint aspiration of blood, and analgesic medication. The main step is substitution therapy with the insufficient coagulation. Factor, preferably in the first 2 hours after the pain started. The aim is to achieve corrective coagulation factor levels of 30–50 IU dL. After replacement therapy is achieved, rest is of crucial importance. Cryotherapy may reduce swelling and decrease pain by its vasoconstricting effect, but arthrocentesis (joint aspiration) should be carried out in 2 days to preclude long-run joint impairment. When there is doubt as to whether hemarthrosis is present, ultrasonography is the next step for a good clinical diagnosis. The choosing of a particular analgesic treatment will be determined by the severity of pain and on the peculiarities of the patient. For stronger pain, metamizole is an option. In particular cases, soft opioids such as codeine or tramadol may be employed. For atrocious pain, the optimal medication must be morphine hydrochloride, either continuous infusion or a PCA pump, based on the age, mental condition, and grade of acceptance of the patient. Epidural blocks with bupivacaine and fentanyl may be very effective.

For chronic pain, reported recommendations are separated over the ability of acetaminophen and non-steroidal anti-inflammatory medications (NSAIDs) as first-line pharmacologic treatment of osteoarthritis. Tramadol is more frequently used for the treatment of osteoarthritis. Thus, in contrast to NSAIDs, tramadol does not cause gastrointestinal bleeding or renal complications, and does not cause injury to the articular cartilage. Opioids can be a good alternative if patients with knee osteoarthritis have intense pain or if other analgesic medication is contraindicated. Still, the data related to their efficacy and security are inconsistent. Fransen *et al* stated that land-based therapeutic exercise is beneficial for people with osteoarthritis in terms of reduced joint pain for at least two six months. Regarding the intensity of exercise Brosseau *et al*, evaluated the effectiveness of therapeutic exercise of differing intensities on objective and subjective measures of disease activity in people with osteoarthritis.

Ultrasonography, apart from its diagnostic value, is of potential use therapeutically. Ultrasound is one of several physical therapy modalities suggested for the management of pain and loss of function due to osteoarthritis. Rutjes *et al*, compared therapeutic ultrasound with sham or no specific intervention in terms of effects on pain and function safety outcomes in patients with osteoarthritis. “

2) The paper needs a NEW SECTION on THE ROLE OF ULTRASONOGRAPHY

Querol F, Rodriguez-Merchan EC. The role of ultrasonography in the diagnosis of the musculoskeletal problems of haemophilia. *Haemophilia*. 2012 May;18(3):e215-26. doi: 10.1111/j.1365-2516.2011.02680.x. Epub 2011 Nov 2. PMID: 22044728

Ultrasonography is highly valuable for the diagnosis of musculoskeletal diseases in hemophilia. It is a fast, effective, safe, available, comparative, real-time technique that can help us confirm the clinical examination. It is particularly important in acute hemarthrosis, as it can be used to objectively identify the presence of blood in the joints, measure its size, pinpoint its location, assess its evolution and confirm its complete disappearance.

De la Corte-Rodriguez H, Rodriguez-Merchan EC, Alvarez-Roman MT, Martin-Salces M, Martinoli C, Jimenez-Yuste V. The value of HEAD-US system in detecting subclinical abnormalities in joints of patients with hemophilia. *Expert Rev Hematol*. 2018 Mar;11(3):253-261. doi: 10.1080/17474086.2018.1435269. Epub 2018 Feb 15. PMID: 29383965

The value of HEAD-US system in detecting subclinical abnormalities in joints of patients with hemophilia is great. In a reported series, 14% of patients exhibited HEAD-US (Hemophilia Early Arthropathy Detection with Ultrasound) signs of incipient arthropathy in joints with no history of bleeding and with a HJHS 2.1 score of 0. The most severely involved joint was the right ankle. Synovitis, articular cartilage and subchondral bone damage scores in joints with subclinical findings were slower than in joints with previous hemarthroses or HJHS 2.1 > 1. This study

demonstrated that HEAD-US is better than hemarthrosis records and the HJHS 2.1 scale in detecting the early signs of joint damage in people with hemophilia.

De la Corte-Rodriguez H, Rodriguez-Merchan EC, Jimenez-Yuste V. Point-of-care Ultrasonography in Orthopedic Management of Hemophilia: Multiple Uses of an Effective Tool. HSS J. 2018 Oct;14(3):307-313. doi: 10.1007/s11420-018-9604-x. Epub 2018 Mar 5. PMID: 30258338

One useful tool, point-of-care ultrasonography (POC-US), offers diverse diagnostic and therapeutic possibilities. These include early diagnosis of joint damage, differential diagnosis of articular pain, follow-up of joint injury, and guidance for both arthrocentesis and intra-articular injection. Studies show that for patients with hemophilia, POC-US enhances diagnostic accuracy and targeted treatments.

Thank you very much for the appreciation of our manuscript, as well as for the important suggestions. We have taken into consideration the suggestion and added that:

“ **The role of ultrasonography in diagnosis and monitoring.**

Imaging and diagnostics offer an objective assessment of joint structural outcome with earlier changes of hemophilic arthropathy best assessed with either ultrasound (US) or MR imaging. Both are able to detect and quantify the most relevant biomarkers of disease activity and degenerative damages by means of scoring scales of increasing disease severity. With Doppler imaging, US may detect synovial hyperemia, defined as intrasynovial detection of blood flow signals. In other chronic inflammatory disorders such as rheumatoid arthritis, the use of Doppler techniques is a mean to monitor disease activity. Intrasynovial hyperemia at Doppler imaging is uncommonly observed in hemophilic patients and, in the rare positive cases, only a few blood flow signals are visualized, suggesting mild hypervascularity that cannot be considered relevant enough to redirect treatment and patient management. Low volume blood flow signals in the synovium from tiny intrasynovial vasculature and capillary circulation remain beyond the threshold of sensitivity of the Doppler systems. Also, a high variability in the interpretation of Doppler images, the need for high-end machines to get better performance and high interequipment variability is expected. Thus, the use of Doppler imaging as a key tool to better predict the risk of hemorrhage and identify active disease seems to be problematic. US is not able to provide a complete evaluation of the cartilage and subchondral bone, especially at the level of the weight-bearing areas, due to problem of access of the US beam. Medullary bone changes and subchondral cysts are not revealed with this technique. Owing to the diffuse

osteocondral involvement of the disease, however, such a limited evaluation does not seem impacting significantly on the sensitivity of the method to detect the occurrence and assess the severity of hemophilic arthropathy. If we refer to the osteochondral surfaces that are exposed to the US beam, this technique has proved able to detect subtle echo textural changes, partial thickness losses through extensive cartilage derangement with spatial resolution even higher than surface-coiled MR imaging.

In Madrid, de la Corte-Rodriguez *et al* conclude that point-of-care US (POC-US) used for patients with hemophilia enhances diagnostic accuracy and targeted treatments. Still, further research is required into the most efficient use of POC-US and the training needed to develop clinicians' skills. The attributes of POC-US should be understood more fully to enable its widespread application. The same group, after properly understanding the POC-US, used the Hemophilia Early Arthropathy Detection with Ultrasound' (HEAD-US) protocol to detect abnormalities in joints without history of hemarthrosis and clinically asymptomatic joints of hemophilic patients. They analyzed almost 1000 joints from routine practice over a 3-year period and analyzed based on history of hemarthrosis and results of clinical (HJHS 2.1) and HEAD-US examinations, showing that HEAD-US is better than hemarthrosis records and the HJHS 2.1 scale in detecting the early signs of joint damage in patients with a hemophilia diagnosis. Thus, they concluded that US is highly valuable for the diagnosis of musculoskeletal diseases in haemophilia, as it is a fast, effective, safe, available, comparative, real-time technique that can help confirm the clinical examination. “

3) Line 189: add the three following two references at the end of the sentence “... as soon as possible”

Rodriguez-Merchan EC, Jimenez-Yuste V, Aznar JA, Hedner U, Knobe K, Lee CA, Ljung R, Querol F, Santagostino E, Valentino LA, Caffarini A. Joint protection in haemophilia. *Haemophilia*. 2011 Sep;17 Suppl 2:1-23. doi: 10.1111/j.1365-2516.2011.02615.x.PMID: 21819491

Rodriguez-Merchan EC. Articular Bleeding in Hemophilia. *Cardiovasc Hematol Disord Drug Targets*. 2016;16(1):21-24. doi: 10.2174/1871529x16666160613114506.PMID: 28049407

Thank you very much for the appreciation of our manuscript, as well as for the important suggestions. We have added the two references.

4) Line 278: After the sentence “fractures [100]” add the following comment and references:

In acute hemarthroses treatment should ideally be administered intensively (enhanced on-demand treatment) until the resolution of symptoms. Joint aspiration plays an important role in acute and profuse hemarthroses. Ultrasonography is an appropriate diagnostic technique to assess the evolution of acute hemarthrosis in hemophilia,

Rodriguez-Merchan EC, De la Corte-Rodriguez H, Jimenez-Yuste V.

Joint aspiration of acute tense knee haemarthroses in adult haemophilia A patients. *Thromb Res.* 2013;132(6):778-9. doi: 10.1016/j.thromres.2013.09.025. Epub 2013 Sep 26. PMID: 24119293

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 Apr;30(3):111-119. doi: 10.1097/MBC.0000000000000803. PMID: 30958454

Thank you very much for the appreciation of our manuscript, as well as for the important suggestions. We have added the two references and phrase.

5) Line 309: add the following sentence and reference at the end of the sentence “bleeding episode onset [101,103,104]”

Arthrocentesis must be carried out immediately after diagnosis of acute hemarthrosis in liquid phase. Patients must be infused with the deficient coagulation factor and instructed to observe relative rest until resolution of hemarthrosis.

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 Apr;30(3):111-119. doi: 10.1097/MBC.0000000000000803. PMID: 30958454

Thank you very much for the appreciation of our manuscript, as well as for the important suggestions. We have added the reference and phrase.

6) Line 321: add the three following references at the end of the sentence “it is the first treatment used for chronic synovitis”

De la Corte-Rodriguez H, Rodriguez-Merchan EC, Jimenez-Yuste V. Radiosynovectomy in hemophilia: quantification of its effectiveness through the assessment of 10 articular parameters.

J Thromb Haemost. 2011 May;9(5):928-35. doi: 10.1111/j.1538-7836.2011.04246.x.PMID: 21352468

Rodriguez-Merchan EC, De la Corte-Rodriguez H, Jimenez-Yuste V. Radiosynovectomy in haemophilia: long-term results of 500 procedures performed in a 38-year period. Thromb Res. 2014 Nov;134(5):985-90. doi: 10.1016/j.thromres.2014.08.023. Epub 2014 Sep 6. PMID: 25240555

Rodriguez-Merchan EC, Valentino LA. Safety of radiation exposure after radiosynovectomy in paediatric patients with haemophilia. Haemophilia. 2015 Jul;21(4):411-8. doi: 10.1111/hae.12668. Epub 2015 Apr 9. PMID: 25854422

Rodriguez-Merchan EC. Radiosynovectomy in haemophilia. Blood Rev. 2019 May;35:1-6. doi: 10.1016/j.blre.2019.01.002. Epub 2019 Jan 25. PMID: 30704767

Thank you very much for the appreciation of our manuscript, as well as for the important suggestions. We have added the 4 references.

7) Lines 338: Add the two following references at the end of the sentence “patients [113]”

Rodriguez-Merchan EC. Total knee replacement in haemophilic arthropathy. J Bone Joint Surg Br. 2007 Feb;89(2):186-8. doi: 10.1302/0301-620X.89B2.18682. PMID: 17322432

Rodríguez-Merchán EC. Total Knee Arthroplasty in Hemophilic Arthropathy.

Am J Orthop (Belle Mead NJ). 2015 Dec;44(12):E503-7. PMID: 26665252

Thank you very much for the appreciation of our manuscript, as well as for the important suggestions. We have added the 2 references.

8) Lines 340: Add the two following references at the end of the sentence “acid injection following capsule closure [115-117]”

Rodriguez-Merchan EC, Romero-Garrido JA, Gomez-Cardero P. Multimodal blood loss prevention approach including intra-articular tranexamic acid in primary total knee arthroplasty for patients with severe haemophilia A. *Haemophilia*. 2016 Jul;22(4):e318-20. doi: 10.1111/hae.12942. Epub 2016 May 26.PMID: 27227798

Rodriguez-Merchan EC, Encinas-Ullan CA, Gomez-Cardero P. Intra-articular Tranexamic Acid in Primary Total Knee Arthroplasty Decreases the Rate of Post-operative Blood Transfusions in People with Hemophilia: A Retrospective Case-Control Study. *HSS J*. 2020 Oct;16(3):218-221. doi: 10.1007/s11420-019-09711-0. Epub 2019 Aug 9.PMID: 33088236

Thank you very much for the appreciation of our manuscript, as well as for the important suggestions. We have added the 2 references.

9) Line 343: Add the following reference at the end of the sentence “extensive open synovectomy”

Merchan EC, Galindo E, Magallon M, Gago J, Villar A, Sanjurjo MJ. Resection of the radial head and partial open synovectomy of the elbow in the young adult with haemophilia: long-term results. *Haemophilia*. 1995 Oct;1(4):262-6. doi: 10.1111/j.1365-2516.1995.tb00086.x.PMID: 27214634

Thank you very much for the appreciation of our manuscript, as well as for the important suggestions. We have added the reference.

10) Line 503: Add the following reference at the end of the sentence “inhibitors [169]”

Rodriguez-Merchan EC, Valentino LA. Emicizumab: Review of the literature and critical appraisal. *Haemophilia*. 2019 Jan;25(1):11-20. doi: 10.1111/hae.13641. Epub 2018 Nov 15.PMID: 30431213

Thank you very much for the appreciation of our manuscript, as well as for the important suggestions. We have added the reference.

Reviewer B

The review includes a balanced view of the research on the management of hemophilia.

However the English language could be improved in many places. The manuscript should be corrected by an English-speaking person.

Thank you very much for the review of our manuscript. We have carefully revised the manuscript and now feel it is much better, more suitable for publication, should the reviewer decide to endorse its publication.

It would have been interesting to give in the introduction the number of hemophilia patients in Romania (if it is known).

Thank you very much for the review of our manuscript. The number of patients with a diagnosis of hemophilia in Romania is 850. Still, we are now upgrading the registry and will upgrade the actual number of patients with hemophilia and rare bleeding disorders. Should the reviewer decide, we can include the actual number of hemophilia patients in the manuscript.

line 45 : I do not understand why the authors evoke serum level. FVIII or FIX levels are plasma levels.

Thank you very much for the review of our manuscript. We have corrected this mistake in the revised manuscript.

line 46-47 : errors in the scale of concentration. Mild disease is defined as 5–40 IU/dL (ie, 5–40% of normal), moderate hemophilia as factor levels of 1–5 IU/dL (ie, 1–5% of normal) and Severe hemophilia displays factor level of less than 1 IU/dL (ie, <1% of normal).

Thank you very much for the review of our manuscript. We have corrected this mistake in the revised manuscript.

Legend of the Fig 1 is not clear:

Why do the authors speak about plasminogen?

what do you mean in line 54 « Both, the activation of factor XII and activation of factor XI. » ??

It should be better to write FXa instead of activated factor X.

The term FIII for TF is obsolete.

The explanation of the coagulation cascade is not correct. This cascade is initiated by the exposure of the extravascular protein Tissue Factor (TF) to blood, allowing the formation of the TF-factor VIIa (FVIIa) complex. This complex is able to activate small amounts of FIXa and FXa before it is rapidly inhibited by TF pathway inhibitor (TFPI). These small quantities of FXa promote the generation of thrombin. Although no sufficient amounts of thrombin are produced to allow fibrin formation, thrombin amplifies its own production by inducing a positive feedback loop via activation of FXI and the protein cofactors FV and FVIII. This feedback activation is crucial for the formation of the FIXa/FVIIIa complex (also known as the tenase complex), which is needed to generate adequate amounts of FXa and thrombin to permit fibrin formation. Now, it is known that the contact pathway (or intrinsic pathway) is not needed for normal hemostasis in vivo.

The development of allo-antibodies against FVIII as a serious adverse effect of factor injection is not developed as well as immune tolerance treatment.

Thank you very much for the review of our manuscript. We have reorganized the Figure 1, as well as the Figure legend, which now is:

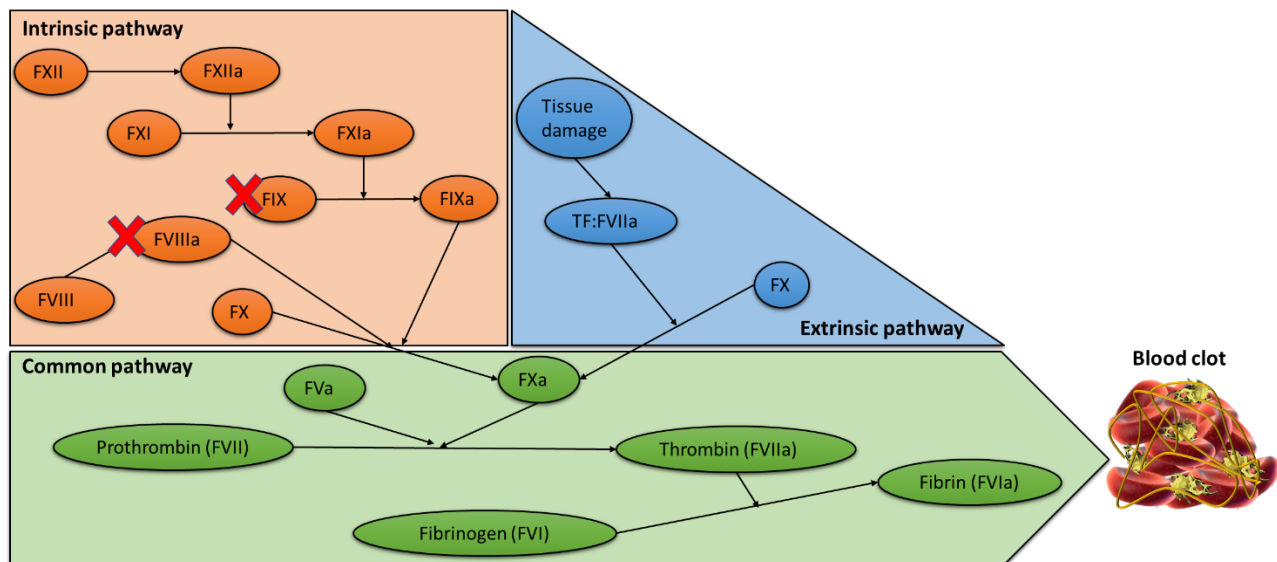


Figure 1. This cascade is initiated by the exposure of the extravascular protein Tissue Factor (TF) to blood, allowing the formation of the TF-factor VIIa (FVIIa) complex. This complex is able to activate small amounts of FIXa and FXa before it is rapidly inhibited by TF pathway inhibitor (TFPI). These small quantities of FXa promote the generation of thrombin. Although no sufficient amounts of thrombin are produced to allow fibrin formation, thrombin amplifies its own production by inducing a positive feedback loop via activation of FXI and the protein cofactors FV and FVIII. This feedback activation is crucial for the formation of the FIXa/FVIIIa complex (also known as the tenase complex),

which is needed to generate adequate amounts of FXa and thrombin to permit fibrin formation. Now, it is known that the contact pathway (or intrinsic pathway) is not needed for normal hemostasis *in vivo*.

Figure 3 and its legend was also slightly corrected, which now is:

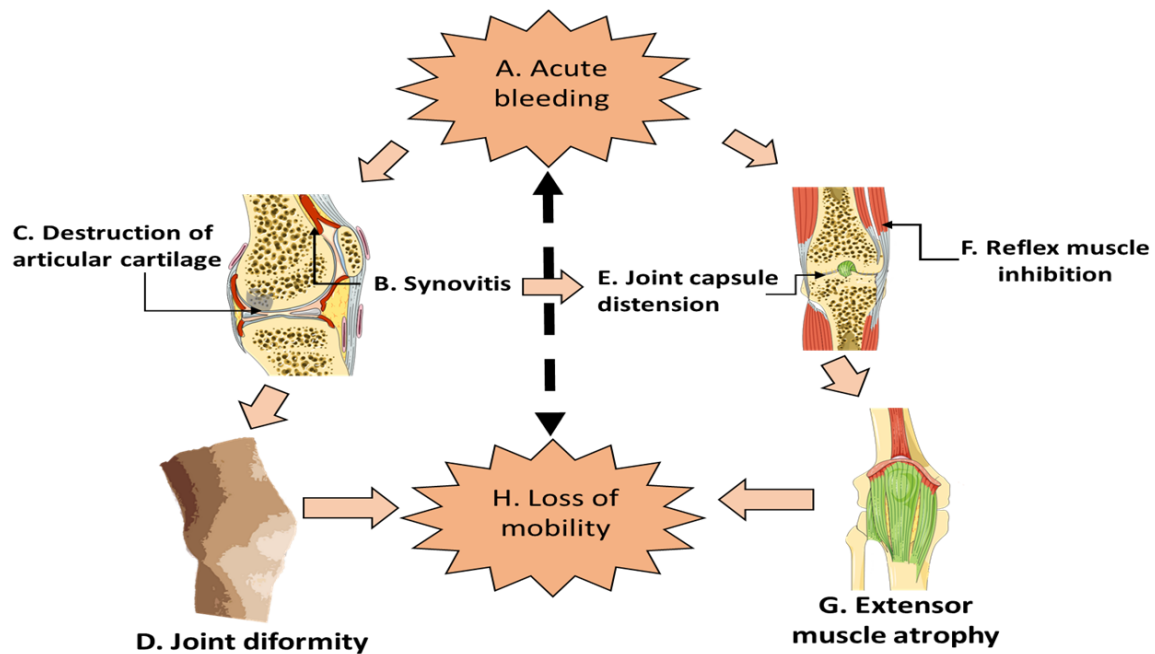


Figure 3. The **cascade of events** leading to hemophilic arthropathy. A. The acute bleeding in the joint space, results first in B. synovitis, which is followed by C. destruction of articular cartilage, which ultimately results in permanent D. joint deformity. Secondly, A. Acute bleeding causes E. Joint capsule distension, a condition which can also be the result of synovitis. The E. Joint capsule distension is followed by F. reflex muscle inhibition and G. Extensor muscle atrophy. All of these events ultimately lead to the H. Loss of mobility in the respective joint, which causes even more frequent episodes of acute bleedings.

Reference 56 is an old one. More recent Guidelines for the management of hemophilia have been published since.

Thank you very much for the review of our manuscript. We have replaced the references, with a recent publication.

- It is not clearly explained that prophylactic treatment is recognized as the optimum therapy for hemophilia, as it prevents the vast majority of joint and muscle damage and allows the child or person with hemophilia to lead a normal quality of life.

Thank you very much for the review of our manuscript. The reviewer is indeed correct and thus we added in the Conclusion section the phrase:

“Prophylactic treatment is recognized as the optimum therapy for hemophilia, as it prevents the vast majority of joint and muscle damage and allows the child or person with hemophilia to lead a normal quality of life. “

Strategies to improve prevention therapy in hemophilia

-guiding principle of this paragraph is not clear

Thank you very much for the review of our manuscript. The reviewer is correct. Thus, we have deleted all the un-necessary sentences and just added the previously suggestion paragraph:

“Prophylactic treatment is recognized as the optimum therapy for hemophilia, as it prevents the vast majority of joint and muscle damage and allows the child or person with hemophilia to lead a normal quality of life. “

Now, this subchapter is more fluent, easier to understand and more suitable for publication, should the reviewer decide to endorse its publication.

- Fig.4: what is the scale?

Unfortunately, we cannot add a scale to the arthroscopic images because the structures within the images have different depths. As a result, the structures closer to the camera would need a different scale compared to the structures farther. A scale can only be added to images with a fixed depth (e.g., an object on a table - where the object is at the maximum depth represented by the table)

line 334 « based on our experience » repeated twice.

Thank you very much for the review of our manuscript. We have deleted this extra 4 words.

Inhibiting antithrombotic pathways paragraph:

-It should be specified that serpinPC is an engineered serpin that specifically inhibits APC.

Thank you very much for the review of our manuscript. We have corrected this paragraph and added that “SerpinPC is an engineered serpin that specifically inhibits APC (activated protein C) [29884816]. “.

The sentence (lines 489-490) is not clear : « This approach would have the advantage of extending the long-term half-life of antibodies compared to conventional FVIII molecules ». Do you mean the Emicizumab has a longer half-life than recombinant FVIII ?

Thank you very much for the review of our manuscript. We have deleted this sentences, which indeed was very unclear.

-You forgot to mention the Kunitz-type protease inhibitor “tissue factor pathway inhibitor” (TFPI). Several aspects have been worked out to target its function for the sake of hemophilia treatment.

Thank you very much for the review of our manuscript. We have added that:

“Replacement therapy with missing factor VIII or IX in hemophilia patients for bleed management and preventative treatment or prophylaxis is standard of care. Restoration of thrombin generation through novel mechanisms is the focus of innovation to overcome limitations imposed by protein replacement therapy. Tissue factor pathway inhibitor (TFPI) is a multivalent Kunitz-type serine protease inhibitor that regulates tissue factor-induced coagulation through a FXa-dependent feedback inhibition of the TF-FVIIa complex in plasma and on endothelial surfaces. Inhibition of TFPI reverts the coagulation process to a more primitive state evolutionarily, whilst regulation by other natural inhibitors is preserved. Concizumab is a monoclonal, humanized antibody, specific for the second Kunitz domain of TFPI that binds and inhibits FXa, abolishing the inhibitory effect of TFPI. A trend towards decreasing bleeding tendency was observed and this preventative effect is being studied in phase II clinical trials, with additional data gathered to improve our understanding of the therapeutic window and potential for thrombosis. “

Reviewer C

This is a very comprehensive attempt to review a rapidly changing field, and does a good job in capturing the breadth of the important points. There are some grammatical and English language issues throughout that need some editing. I'll provide some specific comments on the content in the different sections:

-Introduction: It's generally Hemophilia A or B, and not 'type A' or 'type B'.

Thank you very much for the review of our manuscript and for the very good comments. We have corrected this, in the revised manuscript.

There are some broad generalizations in this section that are covered much better in subsequent sections, and it would be best to keep this as a concise introduction. The discussion of severity is overly focused on the factor levels with broad generalizations about the bleeding phenotype, which is not always accurate.

Thank you very much for the review of our manuscript and for the very good comments. We have deleted all the unrequired information.

Discussion of treatment is also overly simplified and would be best to simply define 'on demand' and 'prophylaxis' with the broad goals of therapy.

Thank you very much for the review of our manuscript and for the very good comments. We have taken into account every single one of them and have corrected the manuscript as such, in red, in the revised manuscript. Thus, we added that “On demand treatment is where factor concentrate is infused when a bleed occurs. Prophylaxis is the regular infusion of clotting factor concentrates in order to prevent bleeding. “.

The paragraph discussing the pathophysiology of coagulation should be earlier in the introduction, to set the stage for the disordered clotting in hemophilia.

Thank you very much for the review of our manuscript and for the very good comments. We have re-organized the introduction and moved this paragraph.

-Diagnostic tools: There is too much detail on the mechanics of the tests, which detracts from the value of those tests (or lack in PT and TT). Better to describe the tests and their limitations. Of note, mixing studies can also be done with the PT to investigate deficiencies of F.VII, X, and V.

Thank you very much for the review of our manuscript and for the very good comments. We have added all these details following the suggestions of the other reviewers. Still, should the reviewer decide, we are more than happy to simplify the manuscript and delete all the overwhelming technical information. We have also added in the paragraph which describes the mixing study that “Mixing studies can also be done with the PT to investigate deficiencies of factor VII, X, and V. “

-Genetic testing: Again, there is a lot of detail on the actual process of the genetic testing which is not helpful and detracts from the value of the test result itself. You've already stated that the family should receive genetic counselling, who would discuss the tests in detail. For the hematologist, it is important to be aware of the different options and when they are relevant. I do appreciate the comment towards the end of the section about the current state of care and how that would impact decisions.

Thank you very much for the review of our manuscript and for the very good comments. Again, we have added all these details following the suggestions of the other reviewers. Still, should the reviewer decide, we are more than happy to simply the manuscript and delete all the overwhelming technical information.

-Treatment strategy: Do not use 'prevention' - call it prophylaxis consistently.

Thank you very much for the review of our manuscript and for the very good comments. We have replaced the word “prevention” with “prophylaxis”.

The regimens suggested at the end of the first paragraph are too simplistic, which you do elaborate on later in the section in discussion of PK variability. This first part just calls for a good definition of the two strategies and the big picture risks and benefits. The paragraph describing PK variability has a lot of detail on PK principles which do not add to the value of the discussion. It would be better to remove those extra details and include the information from the final paragraph discussing PK determination in the patients. The paragraph on bleeding phenotype between these two paragraphs should move higher in this section, and probably ahead of the paragraph that begins with "As follows, the age and the bleeding phenotype...". This would improve the logical flow and allow the PK discussion to fit together.

Thank you very much for the review of our manuscript and for the very good comments. Again, we have added all these details following the suggestions of the other reviewers. Should the reviewer decide, we are more than happy to simply the manuscript and delete all the overwhelming technical information. Still, we have moved the paragraph on bleeding phenotype higher, to have a better, more natural flow of the manuscript.

-Surgical intervention: I like figure 3 but it is not really a circle - more of a progression to a common endpoint. Figure 4 does not add much to the discussion given the subtlety of the findings. The discussions of the surgical procedures themselves could be shortened to focus on the key considerations for the hemophilia provider and patient, mainly risks and benefits.

Thank you very much for the review of our manuscript and for the very good comments. Again, we have added all these details following the suggestions of the other reviewers. Should the reviewer decide, we are more than happy to simply the manuscript and delete all the overwhelming technical information.

-Treatment options: The discussion of SIPPET contradicts itself - I can see that it is meant to highlight the ongoing controversy, but it does not flow well for people less familiar with the study. The third generation concentrates (and now fourth generation) do not use HUMAN proteins, which is an important distinction. You also have generic names for some but not all of the products listed.

Thank you very much for the review of our manuscript and for the very good comments. The reviewer is correct and thus, we have decided to delete the paragraph referring to the SIPPET study.

In the EHL section, it would be better to describe the method of prolongation and then list the relevant products - ie. Fc fusion for Alprolix/Eloctate, PEG for Adynovate, Esperoct, Rebinyn. You have a very long description of PEG with almost no discussion of Fc fusion or Albumin. You also start with a brief sentence about Fc VIII and then a more details description of Fc IX at the end. The organization becomes confusing, and the key message is the mechanism of prolongation as used in either VIII or IX.

Thank you very much for the review of our manuscript and for the very good comments. We have added in the revised manuscript that “In order to extend half-lives, techniques like fusion to protein conjugates (Fc part of IgG₁ or albumin), chemical modification (PEGylation), and protein sequence modification are implemented. With these techniques, it is possible to extend half-lives of factor IX products 4- to 6- fold, while half-life extension of factor VIII products is limited to 1.5- to 2-fold due to their interaction with von Willebrand factor. Methods to extend half-lives include fusion to protein conjugates, chemical modification, or protein sequence modification. For Fc-Fused FVIII, a single molecule of a B-domain-deleted recombinant FVIII (rFVIII; human cell line) is covalently fused to the Fc domain of IgG₁ (rFVIII-Fc or efmoroctocog alfa). Efmoroctocog alfa (marketed as Elocta® in Europe) has been evaluated for safety, efficacy, and pharmacokinetics in two pivotal phase 3 studies. Terminal half-life of rFVIII-Fc was extended 1.5-fold as compared to non-EHL rFVIII. Several PEGylated FVIII products have been developed. They differ from each other by the PEGylation sites and the molecule length of FVIII. Rurioctacog alfa pegol (Adynovi®) is created through controlled PEGylation of a full-length, unmodified rFVIII (synthesized in Chinese hamster ovary cells), in which approximately 60% of PEG chains are localized at the B-domain. BAX855 was evaluated in 138 PTPs with severe hemophilia A aged 12-65 years. For Fc-Fused FIX, rFIX-Fc (eftrenonacog alfa; Alprolix®) was the first EHL approved by a regulatory body (US FDA) in March 2014. Eftrenonacog alfa is composed of a single recombinant FIX molecule (human cell line) fused to the dimeric Fc domain of IgG₁. In the pharmacokinetics subgroup rFIX-Fc exhibited a prolonged terminal half-life of 82 hours, the half-life of conventional FIX being 17 hours. “.

For bypassing agents, FEIBA is an activated prothrombin complex concentrate, and not a non-activated FVII. You don't mention Novo-Seven except in passing.

Thank you very much for the review of our manuscript and for the very good comments. We have corrected the mistake on FEIBA. Regarding Novo-Seven, we have added that “Recombinant factor VIIa (rFVIIa), also known as eptacog alfa (Novo-Seven®) is indicated for the treatment of bleeding episodes and peri-operative management in adults and children with hemophilia A or B with inhibitors, congenital Factor VII (FVII) deficiency, and Glanzmann's thrombasthenia with refractoriness to platelet transfusions, with or without antibodies to platelets

and for the treatment of bleeding episodes and peri-operative management in adults with acquired hemophilia. “.

And I would suggest the section on Emicizumab should follow this section, and come before antithrombin inhibitors. That product is available, and of most benefit in inhibitor patients on bypassing agents.

Thank you very much for the review of our manuscript and for the very good comments. We have switched the places of the paragraph on emicizumab.

The antithrombin discussion is interest, and you have shown that it's all clinical trials at this time.

Thank you very much for the review of our manuscript and for the very good comments. We now feed that with your help, the manuscript is much better, more suitable for publication, should you decide to endorse i's publication.

For FVIIIa mimetics, the data you've included on Emicizumab is incomplete. You should be including at least the full Haven series (Haven 3 is completely absent) and also newer studies such as HOHOEMI and STASEY with the open-label extension data from Haven.

Thank you very much for the review of our manuscript and for the very good comments. We have added that “HAVEN 3 clinical trial was published by Mahlangu *et al*, and randomly assigned 152 patients in a 2:2:1 ratio, participants 12 years of age or older who had been receiving episodic treatment with factor VIII to receive a subcutaneous maintenance dose of emicizumab of 1.5 mg per kilogram of body weight per week (group A) or 3.0 mg per kilogram every 2 weeks (group B) or no prophylaxis (group C). It concluded that emicizumab prophylaxis administered subcutaneously once weekly or every 2 weeks is associated with a significantly lower bleeding rate than no prophylaxis among persons with hemophilia A without inhibitors. Also, more than half the participants who received prophylaxis had no treated bleeding events. In an intraindividual comparison, emicizumab therapy led to a significantly lower bleeding rate than previous factor VIII prophylaxis. When looking at all HAVEN 1-4 studies, with data from 401 pediatric and adult HA patients with/without factor VIII inhibitors enrolled, as well as a 970.3 patient-years of exposure, emicizumab prophylaxis maintained low bleed rates and remains well tolerated, with no new safety concerns identified.

The HAVEN data was confirmed in Japan, in the the open-label study (HOHOEMI) study, evaluating the efficacy, safety and pharmacokinetics of emicizumab in pediatric patients aged <12 years with severe haemophilia A without factor VIII (FVIII) inhibitors, confirmed that emicizumab administration with four loading doses of 3 mg/kg every week followed by maintenance doses of 3 mg/kg every 2 weeks or 6 mg/kg every 4 weeks was efficacious and safe in pediatric patients with severe haemophilia A without inhibitors. “.

You also need to mention the guidance on FEIBA that came from the TMA in Haven 1 - the association of TMA with higher FEIBA dosing was conclusive, and the recommendations led to prevention of the complication in all future studies. That needs a fuller discussion here.

Thank you very much for the review of our manuscript and for the very good comments. We have added that “In the HAVEN 1 trial, thrombotic angiopathy was associated with higher FEIBA dosing, this recommendation preventing this complication in all future studies. Oldenberg *et al* concluded that thrombotic microangiopathy or thrombosis was reported only in patients having received high cumulative doses of activated prothrombin complex concentrate for breakthrough bleeding while receiving emicizumab prophylaxis. Thus, the limiting use of FEIBA in patients who have bleeding events while receiving emicizumab prophylaxis. “.

-Gene therapy: Generally a good discussion. I think your statement about the unjustified risk of gene therapy (line 562-564) might be too bold.

Thank you very much for the review of our manuscript and for the very good comments. The reviewer is correct. Thus, we decided to delete this statement.

-Conclusion: Generally a good recap, although the alternative therapies are completely absent. Emicizumab in particular needs to be mentioned by name as a licensed agent.

Thank you very much for the review of our manuscript and for the very good comments. The reviewer is correct. We have added that “For patients with/without factor VIII inhibitors, emicizumab prophylaxis maintains low bleed rates in HA patients of all ages and remains well tolerated, with no new safety concerns identified. Alternative therapies are also of good practical use, improving the quality of life in patients with hemophilia and rare bleeding disorders. “.