

Hemophilia A and B are X-linked recessive hemorrhagic disorder due to deficiency of factor VIII and IX respectively. However, 1/3<sup>rd</sup> cases are due to spontaneous mutations. These patients present with bleeding. The platelet count, BT and PT are normal but APPT is deranged. Correction of APTT on mixing with normal plasma suggests intrinsic pathway defect. Normal factor levels are between 50% -150%. Hemophilia is defined as mild (factor levels >5% to 40%), moderate (1-5%) and severe (<1%).

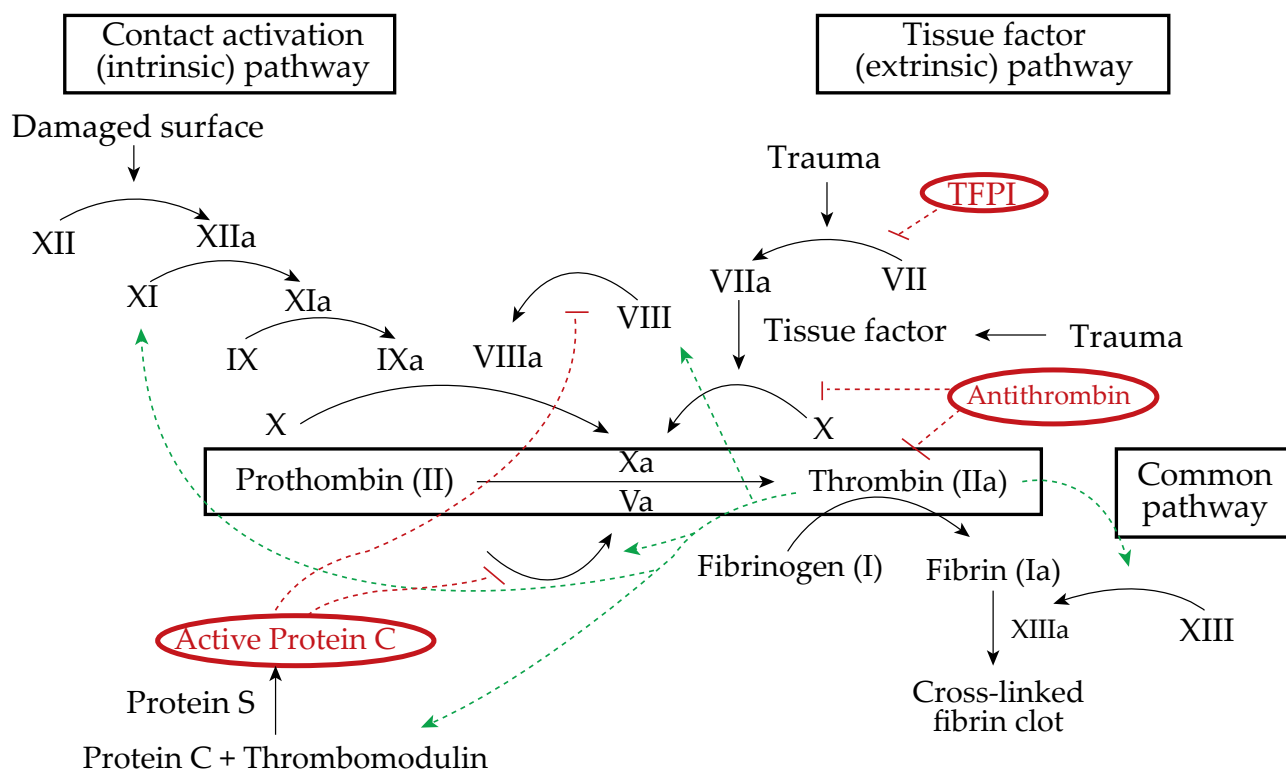
**PRESENT STANDARD OF CARE**

Over the year's treatment changed from giving whole blood, plasma transfusion, cryoprecipitate and later factors plasma derived followed by recombinant ones. This lead to decrease in transfusion transmitted infections and increase in inhibitors. Initial treatment begins with PRICE therapy- protection (splint), rest, ice, compression, and elevation. For mild bleeds desmopressin and antifibrinolytics are used for moderate to severe disease each unit of FVIII/ FIX per kg iv will raise the plasma FVIII and FIX level to 2 IU/dl and 1IU/dl respectively. Depending upon the type of bleed the factor level can be raised to desired level. Problems with existing factor

therapy are need for daily infusions, risk of TTI's and development of inhibitors.

**NEWER THERAPEUTICS FOR PATIENTS WITH INHIBITORS**

1. Role of factor VIII bypassing agent- Presently patients with inhibitors are treated with either Factor VII or factor eight inhibitor bypassing agents (FEIBA).
2. OBIZUR- Due to variations in the amino acid sequence between human and porcine FVIII, there is reduced reaction between inhibitors and porcine FVIII. As a consequence, porcine FVIII can be used to treat bleeding episodes in patients with inhibitors. However, it was found to transmit parvo virus in one of the studies. This lead to development of a recombinant B-domain truncated porcine FVIII (OBI-1). This was found to be well tolerated in trials, however it is not yet approved for the treatment.
3. Alb-rFVII a-FP- Alb-rFVIIa-FP is a fusion protein (FP) linking human coagulation Factor VIIa and human albumin. A single recombinant gene



**Fig. 1: Coagulation cascade**

construct is expressed in Chinese hamster ovary cells yielding a recombinant human albumin attached to the C-terminus of rFVIIa via a flexible glycine-serine linker. It has a half-life of 8.5 hrs and can be used in patients with inhibitors.

4. TheraPEG-Factor VII a-TheraPEG is a molecule in which PEG is conjugated to recombinant human clotting factors at a site remote from the active immunogenic site so as to decrease immune response. The goal is to retain clotting activity while reducing immunogenicity, prolonging the plasma half-life and allowing small-volume s/c administration of clotting factors. It is still under development.

### NEWER THERAPEUTIC TARGETS FOR HEMOPHILIA

#### A. Longer Acting Factor VIII and IX – Once Weekly

Protein modifications are done to increase the half-life of factors by decreasing their clearance. This includes linkage of recombinant clotting factor to various molecules such as fragment crystallizable (Fc) region of human antibodies, to recombinant albumin and to polyethylene glycols (PEGs) of various sizes (Pegylation). Some of the extended half-life (EHL) factor IX clotting factor products are as follows:

#### EHL-FIX Agents

1. rFIX:Albumin-Fusion to albumin prolongs drug half-life and reduces renal clearance of large molecules. rFIX:Albumin is produced in Chinese hamster ovary cells with albumin linked to the cleavage site i.e. C-terminus of rFIX. The linker between the albumin and the cleavage site is designed in such a way that it allows the albumin removal once the FIX gets activated and does not prolong coagulation. This rFIX:Albumin is approved by the US FDA in 2016 for prophylaxis or treatment of bleeding in individuals with hemophilia B. The half-life is five to six fold longer than unmodified factor IX products.
2. Eftrenonacogalfa: It is a newer agent in which one molecule of rFIX is covalently fused directly to the dimeric Fc of IgG1 (rFIX:Fc). It is currently approved for use in the USA. This fusion shields FIX from intravascular degradation proteases. In one study the annualized bleeding rates (ABRs) of prophylaxis regimens once weekly (Group 1) or initially once every 10 days (Group 2) were compared with on-demand therapy (Group 3, episodic). Low ABRs were achieved in both prophylaxis regimens. No bleeding was seen in 23% and 42.3% of group I and II respectively. Efficacy of acute bleed treatment was 90.4% with a single dose and 97.3% with more than one dose. No inhibitors or vascular thrombotic events were detected. However, 10.9% had at least one serious adverse event.
3. Nonacog beta pegol: GlycoPEGylated rFIX

(rFIX:PEG). Here rFIX is produced in Chinese hamster ovary cells and glycoPEGylation is performed at the activation peptide site. PEGylation within the activation peptide domain maintains the catalytic activity and increases the half-life to fivefold. In a recent Phase III study rFIX:PEG weekly IV prophylaxis at 10 IU/kg or 40 IU/kg was compared with on-demand dosing. Lower ABRs were seen in both the prophylaxis arm than on-demand with the 40 IU/kg having the lowest ABR. In the 40 IU/kg group the bleeding episodes, 99% resolved with one dose as compared to 84% with 10 IU/kg dose. No inhibitors, deaths and thromboembolic events were seen. However adverse events were experienced by 81% of patients.

EHL-FVIII Agents - Similar to the lines of FIX some of the EHL FVII are pegylated factor VIII (PEGylated rFVIII), GlycoPEGylation of rFVIII and TheraPEG (given s/c)

#### B. Factor 8 Mimetics

1. Monoclonal humanized bispecific antibody Emicizumab (ACE 910) has been developed recently as a conformational replica of factor VIII. It binds to Factor IX and X and helps in progression of the coagulation cascade leading to thrombin generation. Its greatest advantage is that it is given subcutaneously once a week. In one study<sup>5</sup> by Naoki Uchida et al. involving 64 subjects with hemophilia A who had bleeding despite prophylactic or on-demand therapy, once-weekly subcutaneous administration of emicizumab (1mg/kg) markedly decreased bleeding rates. There were no serious adverse events, anaphylactic reactions and thrombotic complications. No neutralizing anti-emicizumab antibodies developed in any of the patients. The estimated FVIII equivalent activity of emicizumab is around 10–30 IU/dL at dose of 1–3 mg/Kg/wk.

#### C. Targeting Tissue Factor Pathway Inhibitor (TFPI)

It has been seen that patients with factor VIII and IX deficiency or with inhibitors to both can maintain their hemostasis if Factor VII is given i.e. maintaining the activated factor X (FXa) by extrinsic pathway. TFPI is single-chain polypeptides which can reversibly inhibit FXa on the membrane, the formed FXa-TFPI complex can subsequently also inhibit TF/FVIIa and thus controlling the excess coagulation. A close interaction between the TFPI KPI-2 (Kunitz type protease inhibitor) domain and the FXa active site is essential for this inhibitory action of TFPI and further FXa generation. Scientists have now invented Anti-TFPI agents such as:

1. NN-7415 (concizumab/mAB2021) (anti-TFPI). Concizumab is an anti-TFPI antibody that binds to the K2 domain of TFPI. The aim is to decrease the inhibitor activity of TFPI against the tissue

factor pathway and thus increase the ability of this pathway to support hemostasis. By doing so, the need for intrinsic pathway support of activated Factor X (FXa) production is reduced. In a first-in-human, Phase I, multicenter, randomized, double-blind, placebo-controlled trial, escalating single IV (0.5–9,000 µg/kg) or SC (50–3,000 µg/kg) doses of concizumab were given to healthy subjects (n=28) and hemophilia patients (n=24). No serious adverse events and antibodies were seen.

2. Non-anticoagulant sulfated polysaccharides (NASP) including Fucoidanare derived from seaweed. These NASP interact with the highly positively charged C-terminal of TFPI and inhibit it, however actual mode of action is unclear. Studies on Fucoidan AV513 are going on in hemophilic animals models.

3. A PEGylated version of the aptamer BAX499 blocked the inhibition by TFPI of the TF/FVIIa activity. BAX499 markedly improved clotting in hemophilia A and B plasmas and was able to reduce bleeding in a nonhuman primate model.

4. Specific peptides with high affinity for TFPI obtained by phage display technique represent yet another type of antagonist developed for the treatment of hemophilia patients. A PEGylated anti-human TFPI 20-mer peptide (JBT2329) was shown to improve survival of hemophilic mice.

#### D. RNAi Therapeutic Targeting Antithrombin

ALN-AT3 (Alnylam). It is an RNAi therapeutic agent (small interfering RNAs) which reduces the production of antithrombin-3proteins (AT3) thus preventing the inhibition of FXa. Since AT3 controls FXI, FIX, FII, and to some degree FVII, therefore inhibiting AT3 will have the potential to modify a variety of clotting factor deficiencies. In animal models, Alnylam yields potent (up to 100%), dose-dependent (1–30 mg/kg), and durable (30 days) knockdown of AT3. This silencing of antithrombin results in fourfold increase in thrombin generation. In a Phase I multiple ascending S/C dosing study in normal subjects (low dose only) and patients with hemophilia A or B, around 70% decrease in AT3 with concurrent 334% increase in thrombin generation was noted at a dose of 45 µg/kg given weekly for 3 weeks.

#### E. Gene Therapy

Apparently safe adeno-associated viral (AAV) vectors have been used for delivery of the FIX gene

to hematocytes in hemophilia B. Factor IX activity levels sufficient to reduce spontaneous bleeding have been obtained in patients with severe disease for more than 4 years in some patients. The use of high-activity variants of FIX may allow achieving higher factor levels. However this can have a problem in patients with preexisting antibodies to vectors which are capable of eradicating transduced cells. The progress in gene therapy for hemophilia A has been hampered to a significant degree by the size of the gene. While the FIX gene can be placed easily in AAV vectors, the FVIII gene barely fits. Therefore there is a need to modify the FVIII gene cassette to allow accommodation in AAV vectors or use of some other viral vectors such as lentiviral vectors that are capable of carrying the FVIII gene. The advantage of the lentivirus is that they integrate into the target genome, even if the cells are in quiescent phase and are not actively dividing. Recently use of stem cells have been tried in delivering the gene therapy. Gene therapy for hemophilia B is on the horizon, and gene therapy for hemophilia A is becoming feasible.

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