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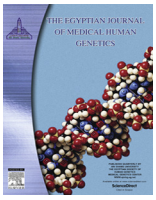

Review

Pathophysiology of bleeding diathesis in haemophilia-A: A sequential and critical appraisal of non-FVIII related haemostatic dysfunctions and their therapeutic implications

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ABSTRACT

Haemophilia-A is characterized by deficiency of FVIII, but the bleeding diathesis is not a mere reflection low FVIII activity. The pathophysiology of haemophilic bleeding diathesis is a complex interplay between defective procoagulant function and up-regulated fibrinolysis. Moreover, haemophilic bleeding diathesis is frequently compounded by treatment-related and infective complications such as FVIII inhibitors, hepatitis, HIV infection, non-steroidal anti-inflammatory drugs (NSAID) induced gastritis, and infective mucosal injuries such as H pylori gastritis and intestinal and urinary helminthiasis. Hence, pathophysiology of haemophilic bleeding is multi-factorial, encompassing both FVIII and non-FVIII haemostatic defects. Currently available literature on pathophysiologic roles of non-FVIII haemostatic defects in haemophilia is fragmented. This articles is aimed at providing a composite and comprehensive review of the roles of non-FVIII haemostatic defects and their therapeutic implications in haemophilic bleeding diathesis, which will enable a holistic approach towards clinical management of the bleeding diathesis. This is necessary because FVIII therapy alone maybe insufficient in managing complicated haemophilic bleeding unless compounding non-FVIII-related haemostatic dysfunctions and comorbidities are identified, targeted and treated. This will necessitate appropriate use of non-FVIII therapeutic modalities, which may include anti-fibrinolytic agents, FVIII by-passing agents, immune modulation, and anti-microbial agents. Lots of work has been done in the areas of non-FVIII agents and FVIII by-pass therapy in the management of haemophilia, but more research is needed to validate many of these targeted therapeutic techniques. Meanwhile, healthcare personnel must consider the roles of both FVIII and non-FVIII haemostatic defects when evaluating haemophilic bleeding diathesis for the purpose of choosing appropriate and optimal treatment options.

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1. Introduction

Haemophilia-A is inherited as an X-linked recessive disorder characterized by lifelong bleeding diathesis due to deficiency of FVIII, which leads to insufficient formation of tenase, reduced generation of thrombin, impaired fibrin deposition and poor clot formation [1]. Although low FVIII level is the fundamental initiator of bleeding diathesis in haemophilia-A, the pathophysiology of the haemophilic bleeding diathesis is not a simple reflection of poor clot formation due to low level of FVIII. The pathophysiology of the haemophilic bleeding diathesis is in fact a complex interplay between a defective procoagulant function initiated by low FVIII activity and several non-FVIII related haemostatic dysfunctions. These non-FVIII related haemostatic dysfunctions include general inability of the extrinsic pathway to compensate for low thrombin generation by the intrinsic defect, local attenuation of the extrinsic pathway by local inhibitors such as tissue factor pathway inhibitor (TFPI) in the joints [2], general up-regulation of the fibrinolytic pathway due to low thrombin burst with inadequate activation of thrombin activable fibrinolysis inhibitor (TAFI) [3], and local up-regulation of the fibrinolytic pathway by local activators of fibrinolysis such as urokinase-plasminogen activator (uPA) and tissue-plasminogen activator (tPA) within the urinary tract [4,5] and the oral cavity [6]. Moreover, the haemophilic bleeding diathesis is frequently compounded by incidentally acquired pro-haemorrhagic treatment-related complications that range from FVIII inhibitors [7] to chronic liver disease due to viral hepatitis [8], HIV-associated thrombocytopenia [9], and non-selective non-steroidal anti-inflammatory drugs (NSAIDs) induced gastritis and thrombocytopathy [10] on the one hand, and the acquisition of environmentally ubiquitous pro-haemorrhagic infectious diseases that range from Helicobacter pylori gastritis [11] to tropical diseases such as intestinal helminthiasis [12] and urinary schistosomiasis [13] on the other hand. There is therefore the need for haemophilia healthcare providers to have comprehensive understanding of all of these non-FVIII related issues and appreciate the pathophysiologic mechanisms through which they contribute to the haemophilic bleeding diathesis. This is necessary because only proper understanding of these haemostatic defects will enable a holistic approach towards solving the lifelong bleeding problems of the haemophilia patients. This is necessary because FVIII therapy alone maybe insufficient in the management of complicated haemophilic bleeding diathesis unless compounding non-FVIII related haemostatic dysfunctions and comorbidities are identified, targeted and treated. The currently available literature on non-FVIII related haemostatic dysfunctions in haemophilia and their relationship to targeted management strategies are fragmented. Hence, the need for an updated critical appraisal to harmonize these important issues in a composite review.

Therefore, the aim of this review is twofold. The primary aim is to conduct a comprehensive harmonized review of various components of the haemophilic bleeding pathophysiology in a stepwise sequential manner starting from physical triggers of bleeding to FVIII deficiency and proceeding sequentially to subsequent haemostatic dysfunctions beyond FVIII deficiency (the non-FVIII related issues). The secondary aim is to review, where appropriate, the pathophysiologic relationships between individual components of non-FVIII related haemostatic dysfunctions and targeted management strategies for haemophilia. Hence, in this review we conducted a broad but concisely critical appraisal of the various non-FVIII related pathophysiologic mechanisms that act singly or in concert in the causation and/or aggravation of haemophilic bleeding diathesis with special reference to functional perturbations of normal haemostasis within the context of traumatic and infective tissue damage and vascular injury, primary and secondary haemostasis, and the fibrinolytic system as outlined in Table 1.

2. Triggers of haemophilic bleeding: Spontaneous versus non-spontaneous

The severity of haemophilia is largely determined by the extent to which different mutations abolish functional FVIII production [14]. Consequently, the clinical severity of the disease and bleeding rates significantly correlates with residual FVIII levels, which is classified as severe (FVIII level < 1%), moderate (FVIII level 1–5%) or mild (FVIII level 6–40%) [14]. Severe haemophilia is typically associated with the null mutations, and is clinically characterized by high frequency of bleeding episodes that occur spontaneously [14]. Whereas mild and moderate haemophilia are associated with less severe non-null mutations, and are clinically characterized by less frequent bleeding episodes that are usually provoked by traumatic events [14]. The conventional terms ‘spontaneous bleeding’ and ‘trauma-induced bleeding’, have for a long time been accepted terminologies in haemophilia literature. Nonetheless, every bleeding episode is almost certainly triggered by some type and degree of trauma [15]. Therefore, in reality every haemophilic bleeding is triggered either by significant trauma (referred to as trauma-induced bleeding) or insignificant trauma (referred to as spontaneous bleeding) [15]. Hence, a recent study has revealed that the so-called ‘spontaneous bleedings’ are actually triggered by trivial trauma incurred during normal routine physical activities [15].

3. Trauma, tissue damage and vascular Injury: Simultaneous activation of primary, secondary and tertiary haemostasis

The physiological consequences of trauma, tissue damage and vascular injury include bleeding and activation of haemostatic

Table 1
Pathophysiology of haemostatic abnormalities of bleeding diathesis in haemophilia-A.

Haemostatic stages and events	Pathophysiologic implications in haemophilic bleeding diathesis
Trauma, tissue damage and vascular injury	(1) Vascular endothelial injury causes collagen exposure (2) Tissue damage releases TF and tPA.
Primary haemostasis	Triggered by collagen contact activation of platelet. But platelet function maybe partially impaired due to: (1) Low thrombin-mediated platelet activation (2) Effect of immune complexes and prostacyclin on platelets
Secondary haemostasis: Intrinsic pathway	(1) Triggered by collagen contact activation of FXII (2) But cascade is blocked by FVIII deficiency causing: (a) Decreased tenase formation (b) Decreased prothrombinase formation (c) low thrombin generation
Secondary haemostasis: Extrinsic pathway	Triggered by interaction of TF and FVII. But overall extrinsic pathway activity is insufficient to compensate for the low thrombin generation by intrinsic pathway
Compensatory failure by extrinsic pathway	Persistent low thrombin generation causes low thrombin burst
Low thrombin burst	Consequences of low thrombin burst include: (1) Inadequate activation of platelets (2) Inadequate activation of FV: low prothrombinase production (3) Inadequate activation of FXIII: uncross-linked loose fibrin (4) Inadequate activation of TAFI: de-regulation of fibrinolysis
Tertiary haemostasis: fibrinolysis	There are two important fibrinolytic pathways: (1) Collagen contact activation pathway produces FXIIa from FXII (note: FXIIa is fibrinolytic) (2) tPA pathway produces plasmin from plasminogen Fibrinolytic effects of both (1) and (2) above are deregulated by inadequate activation of TAFI due to low thrombin burst
Naturally occurring aggravators of haemophilic bleeding diathesis	There are three areas of clinical Significance: (1) TFPI Production in Joints Aggravates Haemarthrosis (2) Fibrinolytic activity in saliva aggravates oral bleed (3) Fibrinolytic activity in urine aggravates urinary tract bleed
Treatment-related complications that aggravate haemophilic bleeding diathesis	Immunologic, infective and haemostatic issues: (1) FVIII Inhibitors aggravate all types of Bleed (2) CLD due to viral hepatitis aggravates all types of bleed (3) HIV-thrombocytopenia aggravates all types of bleed (4) NSAID thrombocytopathy may Aggravates all Types of Bleed (but more effect on Gastrointestinal tract bleed)
Infection-related mucosal injuries that aggravate haemophilic bleeding diathesis	Microbes that damage mucosa and/or secrete anticoagulants: (1) *H Pylori* bacteria: aggravates gastrointestinal bleed (2) Intestinal helminths: aggravates gastrointestinal bleed (3) Urinary schistosomes: aggravates urinary tract bleed

TF = Tissue Factor; TFPI = Tissue Factor Pathway Inhibitor; tPA = Tissue Plasminogen Activator; TAFI = Thrombin Activable Fibrinolysis Inhibitor; CLD = Chronic Liver Disease; NSAID = Non-steroidal Anti-inflammatory Drug.

pathways at the site of injury. The local tissue damage and vascular injury expose tissue factor, tPA, subendothelial collagen and microfibrils to the blood, which lead to simultaneous activation of the primary pathway (via contact activation of platelets) [16], the intrinsic pathway (via contact activation of FXII) [17], the extrinsic pathway (via FVII activation by tissue factor) [18] and the fibrinolytic pathway (via plasminogen activation by tPA) [19]. Moreover, the activity of fibrinolytic pathway is augmented by contact-activation of FXII because FXIIa is a pro-fibrinolytic factor and an important component of the fibrinolytic pathway [20] (see also Section 10 below). The pathophysiology of bleeding diathesis in haemophilia-A is significantly affected by the extent to which these haemostatic pathways are inhibited or potentiated in the process of platelet activation and platelet plug formation (primary haemostasis), thrombin generation and fibrin deposition (secondary haemostasis), and plasminogen activation and fibrin degradation (tertiary haemostasis) as highlighted in subsequent paragraphs.

4. Primary haemostasis within the context of haemophilia

Primary hemostasis is generally presumed to be normal in haemophiliacs. But this presumption may not be entirely true. However, haemophilia is characterized by insufficient generation of thrombin that is needed for platelet activation and aggregation, both of which are important in primary platelet plug formation [21], exposure of platelet membrane-associated procoagulant phospholipids [22], and the assemblage of tenase and prothrombinase complexes of the secondary haemostatic pathways [23]. Therefore, low thrombin generation which is a haemostatic hallmark of haemophilia [1], should be expected to adversely affect both primary (reduced platelet activation and aggregation) and secondary (reduced tenase and prothrombinase formation) haemostasis. Accordingly, some studies have suggested that diminished thrombin mediated platelet activation may contribute to the haemophilic bleeding diathesis [24]. It is therefore not surprising that some studies also reported that severe haemophiliacs with higher levels of platelet procoagulant activity had milder clinical phenotypes in comparison with severe haemophiliacs who had relatively lower platelet procoagulant activity [25]. The exact mechanism responsible for elevation of platelet procoagulant activity as reported in some haemophiliacs [25] is not precisely known, but it is presumed to be related to the so-called 'compensatory platelet activation' that is thought to occur during haemophilic bleeding events [26–28]. However, other studies have refuted the compensatory platelet activation hypothesis and found no differences in the levels of markers of platelet activation between severe haemophiliacs and controls in both clinical [29] and experimental [30] settings. In related research developments, some researchers investigated the roles of so-called 'coated' or 'super-activated' platelets in reducing haemophilic bleeding tendencies [31,32]. These 'coated' or 'super-activated' platelets refer

to subpopulations of platelets that express high amounts of negatively charged membrane procoagulant phospholipids that are thus reportedly associated with fewer bleeding episodes and milder clinic phenotypes [31,32]. Again, in contrast to these findings, another study found no difference in levels of 'coated' platelets between severe hemophiliacs with mild and severe bleeding phenotypes [33].

Studies on bleeding time in haemophiliacs also yielded inconsistent results. Earlier studies have reported that bleeding time was prolonged in a significant proportion of haemophiliacs, including children and young adults [34,35]. These two studies suggested that the prolongation in bleeding times in haemophiliacs might be due to some unidentified intrinsic platelet defects and malfunctions [34,35]. But a subsequent study suggested that circulating immune complexes and high levels of vascular prostacyclin (an anti-platelet aggregation agent), rather than intrinsic platelet defects might be linked to the prolonged bleeding times observed in some haemophiliacs [36]. The origin of these circulating immune complexes has not been precisely identified, but they are probably induced by humoral immune response to antigenic impurities contained in FVIII concentrates [36]. Hence, it was suggested that prostacyclin-induced defective vascular function mediated by the circulating immune complexes might be the pathophysiologic mechanism underlying the prolongation of bleeding time in some haemophilia patients [36].

It therefore appears that platelet functions may not be entirely normal in haemophilia [37]. However, the inconsistencies that exist in the literature [37] regarding the associations between platelet functions, bleeding time, bleeding tendencies and clinical phenotypes in haemophiliacs call for more research in this area of study.

5. Secondary haemostasis: Intrinsic pathway within the context of haemophilia

The physiological activation of the intrinsic pathway is initiated by vascular endothelial injury with exposure of subendothelial collagen [38]. The intrinsic pathway is thus triggered by contact activation of FXII by collagen [17], which is facilitated by the participation of high molecular weight kininogen and prekallikrein in the contact activation process [38]. FXIIa catalyzes the conversion of FXI to FXIa, which subsequently converts FIX to FIXa in the presence of calcium ions [38]. The haemophilic haemostasis proceeds normal up till this stage. However, the next stage requires the provision of catalytic anionic phosphotidylserine-rich phospholipid on membrane surfaces of activated platelets for the assemblage of intrinsic tenase complex (FIXa, FVIIIa, Ca, Phospholipids) [39]. Unfortunately, the deficiency of FVIII militates against adequate tenase formation, which is the first and main haemostatic blockade in the pathophysiology of haemophilia-A [1]. This perturbation of tenase formation in haemophilia has a number of collateral effects including decreased prothrombinase formation and low thrombin generation that ultimately lead to poor fibrin plug formation, which is the principal cause of the haemophilic bleeding diathesis [1]. The most rational therapeutic option for the management of haemophilic bleeding with respect to uncomplicated haemostatic blockade created by FVIII deficiency is logically FVIII concentrate infusion.

6. Secondary haemostasis: Extrinsic pathway within the context of haemophilia

The extrinsic coagulation cascade is physiologically initiated by exposure of blood to tissue factor (TF), which is the cellular receptor and cofactor for FVII [18]. Binding of FVII to the TF receptor in the presence of calcium leads to rapid activation and formation FVIIa, which subsequently undergoes auto-catalytic reactions leading to more production of FVIIa and TF-FVIIa complexes [18]. The TF-FVIIa complex (extrinsic tenase) drives the cascade through the common haemostatic pathway by activating FX leading to optimal production of prothrombinase (FXa, FVa, Ca, Phospholipids) with adequate thrombin generation and effective fibrin deposition [38]. Hence, the extrinsic pathway cascade does not appear to be blocked by FVIII deficiency. But the key question is why then does the extrinsic pathway fail to compensate for the failure of the intrinsic pathway in haemophilia?

7. Compensatory failure by extrinsic pathway to boost thrombin burst in haemophilia

In accordance with the conventional coagulation cascade, the TF-FVIIa complex can activate FX, produce prothrombinase, generate thrombin and deposit fibrin [38]. Hence, the extrinsic pathway should theoretically be able to compensate for the lack of thrombin generation by the intrinsic pathway as seen in the case of FXII deficiency in which there is no bleeding diathesis despite prolongation of clotting time in-vitro [40]. If this compensation is extended to the deficiencies of other intrinsic clotting factors, the extrinsic pathway would successfully prevent bleeding diathesis in other intrinsic pathway deficiencies diseases such as haemophilia-A (FVII) and haemophilia-B (FIX). Unfortunately this compensation does not occur in haemophilia A and B. The reason for this compensatory failure by the extrinsic pathway in haemophilia is elucidated in a solitary study using an in-vitro continuous-flow coagulation reactor model, which revealed that adequate concentrations of FVIII and FIX are required as essential 'cofactors' for optimal FXa production by the extrinsic pathway, this being the reason why the extrinsic pathway cannot therefore compensate for thrombin generation and prevent the bleeding diathesis in both haemophilia-A and B [41]. Hence, the authors of this solitary publication curiously referred to 'haemophilia' as a 'defect of the tissue factor pathway of blood coagulation' in the title of their interesting publication [41]. The failure by the extrinsic pathway to compensate for intrinsic pathway thrombin production and the inability to remedy the haemophilic defect leads to persistence of low thrombin generation with lack of essential thrombin burst. Low thrombin burst in haemophilia is associated with certain haemostatic consequences as outlined in subsequent paragraphs.

8. Haemostatic consequences of low thrombin burst in haemophilia

Because of the significance of thrombin in haemostasis, low thrombin generation in haemophilia has a number of pathophysiologic consequences on the primary, secondary and tertiary (fibrinolytic) haemostatic pathways as follows:

8a. Inadequate activation of platelets: Reduced platelet aggregation

The normal physiological response of platelets to thrombin is mediated by a subset of membrane receptors known as protease-activated receptors [42]. These receptors are activated on cleavage by thrombin, initiating the intracellular signaling events needed to transform mobile, non-adhesive platelets into adhesive cells that can undergo release reaction and aggregation, and ultimately participate in the growth of an immobile haemostatic plug at the site of vascular injury [42]. Thus, the recruitment of platelets into a growing haemostatic plug at the site of vascular injury requires the accumulation of thrombin, which is unfortunately low in haemophilia [1]. Hence, a laboratory model of whole blood platelet aggregation stimulated by endogenously generated thrombin suggests that the

impaired thrombin generation in haemophilia reduces platelet activation [43]. The findings of this study have thus expanded the spectrum of haemophilic haemostatic defects and diathesis to include the primary haemostatic pathway. Therefore, in addition to the mechanism of immune complex mediated prostacyclin-induced vascular dysfunction [36] (as detailed in Section 4 above), low thrombin generation may be an important contributory factor in the pathogenesis of prolongation of bleeding time as earlier demonstrated in haemophilic laboratory models [43].

8b. Inadequate activation of FV: Reduced production of prothrombinase

The prothrombinase complex (FXa, FVa, Ca, Phospholipid) is assembled on an anionic phospholipid membrane [44]. FVa is thus an integral part of the prothrombinase complex wherein it functions both as a receptor for FXa and a positive effector of FXa catalytic efficiency in the conversion of prothrombin to thrombin [44]. The activation of FV to FVa is an essential reaction that occurs early in the process of coagulation and is basically mediated by thrombin [45]. Since the initial production of thrombin is not robust, thrombin-catalyzed activation of FV is an essential positive feedback reaction aimed at amplifying the production of prothrombinase and hence thrombin within the blood clotting system [45]. However, inadequate production of tenase (FIXa, FVIIIa, Ca, Phospholipid) due to low FVIII level in haemophilia causes reduced production of FXa with a resultant low production of prothrombinase complex. This scenario leads to persistent low production of thrombin, which attenuates thrombin-mediated FV activation in the positive feedback amplification, thereby causing further stagnation in the production of prothrombinase. Therefore, low endogenous production of prothrombinase complex due to reduced thrombin-mediated activation of FV is an important factor in the pathogenesis of haemophilic bleeding. This is consistent with the fact that augmentation of FVa activity within the prothrombinase complex was associated with improved haemostasis in haemophilia as supported by experimental and clinical evidences [46,47]. Experimental evidence suggested that infusion of an artificially engineered protein-C-resistant mutant FVa (referred to as super FVa) increased thrombin generation in FVIII-deficient plasma in-vitro, and reduced the severity of bleeding events in FVIII-deficient mice in-vivo [46]. Similarly, clinical evidence suggested that coinheritance of the naturally occurring mutant FV-Leiden in haemophiliacs leads to relatively high FVa-Leiden activity, which enhances the prothrombinase complex, and subsequently shifts the thrombo-haemorrhagic balance in favour of greater thrombin generation with a significant reduction in bleeding rates [47]. Thus, the genetically engineered mutant FVa may have potential therapeutic benefits as a positive modifier of bleeding phenotype in severe haemophiliacs without inhibitors and in those with inhibitors in whom it may serve as a possible FVIII by-passing agent [46]. There is therefore the need for further work and clinical validation in this regard.

The activated protein-C (APC) pathway serves as a major physiological system for controlling thrombosis by neutralizing excess FVa and FVIIIa [48]. The essential components of APC pathway include thrombin, thrombomodulin, protein-C, and protein-S [48]. Thrombomodulin functions as a cofactor in the thrombin-induced activation of protein-C in the anticoagulant pathway by forming a 1:1 stoichiometric complex with thrombin, thereby speeding-up the protein-C activation several fold [49]. It is therefore possible to speculate that inhibiting the APC pathway may be a future strategy in developing next-generation therapeutic targets for haemophilia treatment. This speculation is corroborated by the work of some researchers who designed anti-APC serpins that led to significant reduction in the neutralization of FVa, resulting in increased thrombin generation, restoration of fibrin deposition and normalization of bleeding in haemophilic mice [50]. In a similar development, in-vitro use of FV-stabilizing Fab fragments resulted in enhanced thrombin generation and clotting times in the blood of severe haemophilia-A patients [51]. However, additional studies are needed to further characterize how modulating the APC pathway may prove beneficial in developing new and safe therapeutic models for uncomplicated severe haemophiliacs and also as a potential by-passing strategy for cases complicated by FVIII inhibitors.

8c. Inadequate activation of FXIII and TAFI: Reduced fibrin stability and increased fibrin degradation

Besides formation of fibrin clot, thrombin also prevents clot lysis in two ways. First, thrombin activates FXIII that mediates transamidation reactions leading to ligation of adjacent fibrin monomers and the production of cross-linked fibrin, which is more resistant to plasmin degradation [52]. Second, thrombin causes activation of TAFI, which is a potent inhibitor of fibrinolysis and promoter of clot stability [3]. Therefore, thrombin burst is important in protecting fibrin clot against premature lysis by the dual effect of facilitating cross-linkage of fibrin and activating TAFI. Hence, lack of sufficient thrombin burst in haemophilia would be expected to cause insufficient cross-linkage of fibrin resulting in ease of lysis [52] on the one hand, and sub-optimal activation of TAFI resulting in defective down regulation of fibrinolysis with high rate of fibrin degradation [3] on the other hand. Accordingly, a study has demonstrated that supra-physiological concentrations of FXIII stabilizes clots in in-vitro blood samples obtained from haemophiliacs by improving fibrin resistance to tPA-induced fibrinolysis even at very low concentrations of FVIII [53]. This study had shown that the addition of FXIII significantly corrected the fibrin clot structure, reduced clot permeability and facilitated fibrin generation [53]. Hence, the result of this study suggested that FXIII may be a useful adjunct in the treatment of hemophilia-A [53]. Indeed, an earlier study had reported that in addition to being a useful adjunct in the treatment of haemophilia, FXIII therapy reduces FVIII concentrate consumption and is thus a cost-effective regimen for haemophiliacs [54]. This is because FXIII has a relatively long half-life of about 9 days, which will be associated with less frequent dosing, high patient convenience with greater compliance, and lesser therapy-related expenditure, all of which are hugely advantageous for patients with lifelong bleeding diathesis such as haemophilia [54]. There is therefore the need for further research to standardize and validate the use of FXIII in clinical management of haemophilia.

9. Helping the extrinsic pathway to compensate for thrombin production in haemophilia: Targeting the TFPI

As earlier mentioned thrombin generation in haemophilia is doubly jeopardized by FVIII deficiency in the intrinsic pathway and the compensatory failure of the extrinsic pathway to adequately boost thrombin generation. Nonetheless, it is conceivable that a fraction of the total amount of thrombin generated in haemophilia might still be generated via the TF pathway extrinsic tenase (FVIIa, TF, Ca, Phospholipids), which is strictly regulated by TFPI [18]. Hence, it can be presumed that haemophilic bleeding can be reduced by inhibiting the negative modulatory effect of TFPI [55]. This presumption is supported by the discovery and characterization of the anticoagulant TFPI, which has led to the realization that inhibition of TFPI activity could restore thrombin generation and functional hemostasis through the extrinsic pathway even in the absence of optimal levels of FVIII or FIX [55]. There are currently several investigational models and therapeutic

agents that can theoretically and potentially inhibit TFPI and treat hemophilia in the future [55]. Meanwhile, some researchers have already preliminarily demonstrated that monoclonal antibodies against TFPI can restore hemostasis in haemophilic mouse model [56] and in volunteer human haemophilia patients [57]. The potential clinical usefulness of monoclonal anti-TFPI for the treatment of haemophilia is thus under active investigation.

10. Tertiary haemostasis: Fibrinolysis within the context of haemophilia

As expected from normal physiological perspective, the local tissue damage and vascular endothelial injury at the site of bleeding would activate the fibrinolytic pathway in two ways. First, local tissue injury would release tPA, which would then convert plasminogen to plasmin [19]. Second, vascular injury would expose sub-endothelial collagen, which would lead to contact activation of FXII to FXIIa [17], noting that FXIIa is a pro-fibrinolytic factor and an important component of the fibrinolytic pathway [20]. Therefore, the fibrinolytic pathway in haemophiliacs is fully triggered via the activation of both tPA and FXII in the usual physiological manner [17,19]. However, the physiologically important thrombin-mediated activation of TAFI, which is a potent inhibitor and negative modulator of fibrinolysis and promoter of clot stability [3], does not occur at sufficient level in haemophilia because of inadequate thrombin generation [3] as previously described in more detail (see also Section 8c above). Therefore, the fibrinolytic process is poorly modulated and undesirably up-regulated in haemophilia patients [3]. Thus localized hyper-fibrinolysis of blood clot at the site of injury is an important contributor to the haemophilic bleeding diathesis [3].

In order to improve clot stability, a number of anti-fibrinolytic agents including epsilon amino caproic acid and tranexamic acid have been tried in the management of haemophilia [58]. However tranexamic acid, which reversibly binds the lysine-binding site on the plasminogen molecule and inhibits the conversion of plasminogen to plasmin is more potent than other anti-fibrinolytic agents [58]. Hence, tranexamic acid has been widely and successfully used alone or in combination with FVIII or other anti-haemophilic agents in the prevention and management of various haemophilic bleedings due to accidental or surgical trauma in haemophilia patients with and without FVIII inhibitors [58,59]. Thus we believe it is theoretically possible that a combination of anti-fibrinolytic agent such as tranexamic acid (an inhibitor of plasmin production) and FXIII therapy (an augmenter of fibrin resistance to plasmin) (see also Section 8c above) may act synergistically to produce greater blockade of the fibrinolytic process in the management of severe haemophiliacs especially in those complicated by inhibitors with frequent severe bleeding. Nonetheless, we reckon that this dual targeting and blockade of the fibrinolytic process must be carefully assessed against the risk of developing intravascular thrombosis, hence, the need for more studies in this area.

11. Naturally occurring aggravators of haemophilic bleeding diathesis

There are at least 3 areas of the body where naturally occurring aggravators of haemophilic bleeding diathesis exist. These include TFPI in the joints and plasminogen activators in the oral cavity and the urinary tract as described below.

11a. TFPI production in joints aggravates haemarthrosis

The TFPI is the specific and exclusive inhibitor of tissue factor pathway [60]. The TFPI is a potent and direct inhibitor of FXa and FXa-dependent inhibitor of the FVIIa-TF complex [60]. The microvascular endothelium is thought to be the main source of TFPI [61]. In addition to endothelial cells, it has been shown that many other vascular wall cells such as mesangial cells, smooth muscle cells, monocytes, fibroblasts, and cardiomyocytes can also synthesize TFPI to a lesser extent [62]. After synthesis, TFPI becomes externalized and attached to the surface of the endothelium where it serves an important physiological function of maintaining normal endothelial thrombo-regulatory mechanisms for the prevention of excessive thrombotic tendencies within blood vessels [60]. Moreover, the synovial cells and chondrocytes have also been shown to be significant producers of TFPI, which is undesirable in the haemophilic patients [2]. This local production of TFPI leads to localized intra-articular attenuation of the extrinsic pathway with resultant aggravation of bleeding tendency within the haemophilic joints [2]. Hence, the high frequency of haemarthrosis, which is pathognomonic of severe haemophilia, is due to local intra-articular production of TFPI [2]. However, there is a strong optimism that currently ongoing research on the haemostatic effects of monoclonal anti-TFPI that is being tested in experimental [56] and clinical [57] haemophilic models (see also Section 9 above) may eventually reduce the occurrence of crippling haemarthrosis and arthropathy in haemophilia patients in the very near future.

11b. Fibrinolytic activity in saliva aggravates oral bleeding

Salivary fibrinolysis due to the presence of plasminogen activators in the saliva is usually strongly associated with blood sucking animals such as the vampire bats [63]. However, the human saliva is to a lesser extent also known to have significant fibrinolytic potential that may aggravate bleeding even in haemostatically normal (non-haemophilic) persons after accidental or surgical trauma within the oral cavity [6]. It is therefore not surprising that oral cavity bleeding may pause special challenge in the haemophiliac because the salivary fibrinolytic activity causes undue clot instability that aggravates bleeding [64]. Hence, down-regulating the salivary fibrinolysis by local (mouth wash) or systemic use of anti-fibrinolytic agents have proven to be very effective in controlling oral cavity bleeding in haemophiliacs [64].

11c. Fibrinolytic activity in urine aggravates urinary tract bleeding

The human urinary tract has long been recognized as major source of plasminogen activators. It has been demonstrated that uPA is synthesized by human glomerular epithelial cells and secreted into the urine [4], while the cellular distribution of tPA within the prostate gland suggests that the central zone of the prostate is the selective site of synthesis of tPA, from which it is secreted into the urine [5]. Functional deductions strongly suggest that both uPA and tPA are essential in the maintenance of patency within the urinary tract, wherein they mitigate obstructive pathology by catalyzing extracellular proteolysis (to prevent protein precipitation) and facilitate fibrinolysis (to prevent blood clot formation) [4,5]. However, this localized up-regulation of the fibrinolytic pathway by uPA and tPA within the urinary tract is undesirable in haemophiliacs because it causes clot instability and aggravate bleeding from even trivial injuries within the urinary tract. However, unlike in the case of haemophilic oral bleeding that can be treated by down-regulating salivary fibrinolysis through the use anti-fibrinolytic agents [64], urinary tract bleeding cannot be treated in the same way because of the possible risk of obstructive uropathy that may occur as a result of enhancement of fibrin clot formation by anti-fibrinolytic agents [64]. Hence, FVIII replacement therapy is safer than anti-fibrinolytic agents in treating urinary tract bleeding in haemophilia.

12. Treatment-related complications that aggravate haemophilic bleeding diathesis

These aggravators of haemophilic bleeding diathesis refer to acquired aggravators that are only incidentally found in haemophiliacs. But when they occur, they may negatively affect systemic haemostasis and cause general increase in the rate of all types of bleeding without any special predilection for specific regions or organs of the body. These acquired aggravators are manifestations of immunological, infective and haemostatic complications of the treatment received by haemophilia patients as described below.

12a. Immunological complications: FVIII inhibitors

The development of FVIII inhibitors is the most devastating complication of FVIII therapy in haemophilia. The clinical significance of FVIII inhibitors is linked to their ability to neutralize FVIII as a result of which affected patients have increased risk, frequency and severity of bleeding, with a concomitant sub-optimal response to factor replacement and a corresponding increase in the demand for factor concentrate that ultimately raises the intensity and cost of treatment [7]. The risk of developing FVIII Inhibitor is mainly related to the type of FVIII mutation [14]. It has been demonstrated that FVIII gene mutations that are associated with the absence of the gene product (null mutations) confer higher risk for inhibitor development, while mutations that are associated with the presence of the gene product (non-null mutations), confer a lower risk for inhibitor development [14]. Interestingly, the type of FVIII mutation also significantly influences the therapeutic response to immune tolerance induction (ITI) therapy [65]. It appears that there is an inverse correlation between the risk of inhibitor development and success rate of ITI therapy [65]. This is because it has been demonstrated that FVIII mutations that confer higher risk of inhibitor development were also significantly associated with lower success rates of ITI therapy than that seen in patients with lower-risk FVIII mutations [65]. Apart from FVIII gene mutations, other genetic factors also contribute to the risk of developing inhibitors. These factors include a positive family history of inhibitors, ethnicity, and certain polymorphisms in immune modulatory genes that may increase (eg IL-10 and TNF-alpha genes) or decrease (eg CTLA-4 gene) the risk of developing inhibitor [14,66]. The relatively higher incidence of polymorphisms in the immune modulatory genes for IL-10 and TNF-alpha may partly explain the higher risk of inhibitor development in blacks and other non-Caucasian haemophiliacs [67]. Moreover, the role of ethnicity and FVIII haplotype variation between donor and recipient in the development of FVIII inhibitors has been studied among black hemophiliacs living in Caucasian communities [68]. The study suggested that mismatched FVIII replacement therapy is a risk factor for the development of FVIII antibodies in black patients receiving human FVIII derived from Caucasian plasma or rFVIII derived from genetic template obtained from Caucasian genomes [68]. Moreover, the ABO blood groups play important roles in the risk of inhibitor development as the inheritance of non-O blood groups appear to confer a higher relative risk of developing FVIII inhibitor, while blood group-O appears to be a negative regulator of in-vivo immunogenicity of infused FVIII and hence a protector against the development of inhibitors [69].

Non-genetic factors are also important in the development of FVIII inhibitors. It has been suggested that exposure to FVIII in the presence of immune modulators such as active infections or vaccinations, as well as tissue damage due to accidental or surgical trauma have the potential to generate signals that would activate the antigen-presenting cells and increase the risk of developing FVIII inhibitors [70]. Moreover, treatment-related cofactors, such as early age at first exposure to FVIII, type of FVIII product and its inherent immunogenicity, as well as the intensity of treatment, especially when administered by continuous infusion may significantly increase the risk of inhibitor development [70–72].

The management of FVIII inhibitors is both complex and costly. There are basically three main ways of managing inhibitors viz: flooding (high dose infusion), immune tolerance induction, and FVIII by-pass therapy. The feasibility and success of 'flooding' therapy depends on the level of inhibitor and intensity of anamnestic response after re-exposure to FVIII [73]. Patients with low responding inhibitors (inhibitor level lower than 5 BU/mL) usually have fewer clinical problems because haemostasis can usually be ensured by saturating the inhibitor through the administration of higher 'flooding' doses of FVIII [73]. In contradistinction, high responding (inhibitor level higher than 5 BU/mL) inhibitors are not amenable to 'flooding' therapy with high doses of FVIII, and hence, alternative haemostatic agents including by-passing agents are required in such cases [73]. For these reasons, the primary aim of treatment in such cases is the permanent eradication of the inhibitor by ITI therapy, which will enable effective replacement therapy and make prophylaxis feasible [73]. However, ITI therapy is costly and fails in about one third of patients [73]. Nonetheless, the possibility of using Rituximab as an independent immune tolerance inducer or an adjunct that may increase response rate in ITI therapy is being explored [74]. Meanwhile, by-passing agents such as activated prothrombin complex concentrates (aPCC) and recombinant activated factor VII (rFVIIa) are currently the treatment of choice for the management of bleeding in patients with high titre inhibitors that cannot afford or have failed ITI therapy [73]. Newer innovative ways of by-passing FVIII function that awaits further standardization involves the use of bi-specific recombinant humanized IgG4 antibody (Emicizumab) [75]. This novel antibody simultaneously binds factor IX and factor X and bring them into close proximity, thus providing some of the scaffold–cofactor function of FVIIIa with a resultant reduction in bleeding rates in haemophiliacs with FVIII inhibitors as demonstrated in a randomized clinical trial [75]. There is no doubt that Emcizumab has the potential to positively impact haemophilia therapy in the very near future.

12b. Infective complications: Viral hepatitis, chronic liver disease and HIV infection

Transfusion of blood or blood products is associated with the risks of contracting blood born infectious agents such as hepatitis (B and C) and HIV. The risks of acquiring these infections in the developed countries have been greatly minimized as a result of modernization of blood safety protocols with efficient donor screening procedures, effective viral inactivation techniques and production and use of recombinant blood products [76]. However, infection risks are particularly high in the tropics where the prevalence of blood born infections is high, donor screening procedures are inadequate, viral inactivation techniques are virtually absent, and recombinant blood products are unavailable [77]. Both chronic viral hepatitis and HIV infections can negatively affect the bleeding rates and clinical phenotypes of infected haemophiliacs by causing chronic liver disease with multiple coagulation factor deficiencies [8] and HIV-associated thrombocytopenia [78] respectively. The liver is the most important source of coagulation factors in the human body. The hepatocyte is the main producer of all coagulation factors, except FVIII and vWF that are produced by the hepatic sinusoidal cells [79]. Therefore, acquisition of chronic liver disease essentially transforms haemophilia from a 'single factor' deficiency disorder into a 'multi-factor' deficiency disorder, that would not respond optimally to FVIII therapy alone. Hence, in addition to FVIII

concentrate, haemophiliacs with liver disease may require supplementary use of concentrates that contain multiple clotting factors such as cryoprecipitate [80] and anti-viral immune modulators such as alpha interferon [81]. Similarly, acquisition of thrombocytopenia jeopardizes primary haemostasis and aggravates bleeding in haemophilia [78]. Therefore, HIV-associated thrombocytopenia should be treated adequately with anti-retroviral agents in conjunction with immuno-suppressants or immune modulators [78]. However, in view of their prothrombotic side effects, HIV protease inhibitors [82] may be particularly advantageous for HIV-infected haemophiliacs. This is because the protease inhibitors will certainly treat the HIV infection and also predictably reduce the haemophilic bleeding rate as a paradoxical 'benefit' of their prothrombotic side effects [82]. Hence, physicians involved in the management of HIV infections may consider taking this point into perspective when 'tailoring' drug regimen for HIV-infected haemophiliacs especially for cases that are complicated by thrombocytopenia.

12c. NSAID-induced gastritis and thrombocytopathy

Non-steroidal anti-inflammatory drugs are potential aggravators of bleeding in haemophilia for two reasons. First, NSAIDs are known to induce gastritis and increase the risk of gastrointestinal bleeding even in haemostatically normal persons [83], hence it is not surprising that NSAIDs are associated with increased risk of gastrointestinal bleeding in haemophiliacs [84]. Second, NSAIDs cause acquired thrombocytopathy by down-regulating the primary haemostatic pathway through the inhibition of *cyclo*-oxygenase, reduction of thromboxane-A2 production, and attenuation of platelet activation and aggregation [85]. Moreover, because NSAID-induced thrombocytopathy is a systemic haemostatic problem, some NSAIDs have also been associated with increased risk of very serious non-gastrointestinal bleeding complications such as haemorrhagic stroke [86]. Therefore, while the haemorrhagic risk of NSAID consumption may be higher in the gastrointestinal tract, other organs of the body are not exempted from this risk. Localized ulcerative effect of NSAIDs on the gastrointestinal tract could be reduced by proton pump inhibitors [83], however there are no specific antidotes for the potential haemorrhagic risk that may arise as a result of systemic thrombocytopathic effect of NSAIDs on the primary haemostatic pathway [85,86].

These potential bleeding risks call for caution when contemplating the use of NSAIDs for the management of pain and inflammation in relation to haemophilic arthropathy or any other inflammatory condition in haemophilia patients. Studies have shown that cyclooxygenase-2 (COX-2) inhibitors have comparable analgesic effect to the traditional non-selective NSAIDs but with less bleeding risks [87]. Therefore, COX-2 inhibitors provide a suitable option for treatment of haemophilic arthropathy [87]. Despite their relative safety with respect to bleeding, it should be appreciated that COX-2 inhibitors have an increased risk of thrombotic cardiovascular complications [88]. Although haemophiliacs have lower risk of thrombotic complications as compared to general population, nonetheless they are not completely protected from the cardiovascular risks of COX-2 inhibitors [88]. Therefore, it seems reasonable to prudently use the lowest possible dose of COX-2 inhibitors for the shortest period in conjunction with a proton pump inhibitor in order to further minimize the risk of gastrointestinal ulceration and bleeding [87].

13. Infection-related mucosal injuries that aggravate haemophilic bleeding diathesis

These aggravators of haemophilic bleeding diathesis refer to environmentally ubiquitous global and tropical infectious diseases that are incidentally found in haemophiliacs. These infections specifically aggravate bleeding in the gastrointestinal and urinary tracts by causing mucosal injury and in some cases by manipulating the blood coagulation system as described below:

13a. Globally ubiquitous pro-haemorrhagic bacterial Infections: Helicobacter pylori induced gastrointestinal bleeding

H pylori is a highly ubiquitous bacterial pathogen with global distribution and approximately half of the world population has been estimated to be infected, but the risk of infection is higher in developing countries [89]. When *H pylori* is ingested, it survives in the acidity of the gastric fluid, propagates towards the gastric epithelial cells by flagella-mediated motility, attaches to the epithelial cells via receptor mediated interaction, and releases toxins that cause inflammatory epithelial damage culminating in peptic ulceration, which increases the risk of gastrointestinal bleeding even in haemostatically normal persons [89]. *H pylori* infection is therefore highly undesirable in haemophiliacs in whom it significantly raises the risk, frequency and severity of upper gastrointestinal bleeding [11]. For this reason it is pertinent that haemophiliacs who present with recurrent symptoms of gastritis and upper gastrointestinal bleeding should be screened for *H pylori* infection, and positive cases should be promptly treated with appropriate antibiotics in order to heal the gastric epithelium, stop any active bleeding, and reduce future risks of gastrointestinal bleeding [11]. It must be appreciated that FVIII infusions alone, without antibiotics, would not be therapeutically sufficient in these cases. Moreover, continuous infusions without antibiotics in these cases may even promote the development of FVIII inhibitors since active infections and inflammations are important non-genetic risk factors for inhibitor development in haemophiliacs [70].

13b. Tropical pro-haemorrhagic parasitic Infestation: Intestinal and urinary helminthiasis

Tropical intestinal parasites cause gastrointestinal bleeding by inducing mucosal injuries leading to a wide spectrum of chronic or intermittent blood losses that range from occult bleeding to melena and frank bleeding in association with soil transmitted helminths such as hook worms [90,91], *A lumbricoides* [92,93], and *T trichiura* [94]. In addition to mucosal injuries, some intestinal helminths have the capacity to manipulate the host haemostatic system by actively producing anti-coagulants such as anti-FX and anti-FIX as in the case of hook worms [95]. Therefore, intestinal helminthiasis is an important risk factor for gastrointestinal bleeding even in haemostatically normal individuals [90–94]. Hence, it is easy to predict that the haemostatic abnormality in the haemophiliac would create a highly pro-haemorrhagic host-parasite relationship, which would increase the vulnerability of haemophilia patients to the haemorrhagic effects of intestinal helminths. However, this potentially significant vulnerability have not been adequately studied because the vast majority of publications on haemophilia arose from developed nations where parasitic diseases are not endemic. A solitary study conducted in Nigeria on the subject matter revealed that haemophiliacs with intestinal helminthiasis had significantly higher frequency of gastrointestinal bleeding and iron deficiency than their counterparts without helminthiasis [12]. This study suggests that intestinal helminthiasis is an important risk factor for frequent and severe gastrointestinal bleeding in haemophiliacs. Therefore, healthcare personnel in the tropics should ensure that all haemophiliacs are regularly screened and treated for intestinal helminthiasis in order to attenuate the risk and frequency of gastrointestinal bleeding. This is pertinent since helminthiasis-induced gastrointestinal haemorrhage in the

haemophiliacs cannot be successfully managed by mere infusion of FVIII until and unless appropriate anti-parasitic chemotherapy is instituted.

Urinary schistosomiasis is yet another tropical pro-haemorrhagic parasitic disease caused by *Schistosoma haematobium*. The parasites preferentially settle in the vesical plexus wherein they reproduce, deposit eggs and trigger inflammation that damages the epithelium and cause haematuria [96]. In addition to epithelial injuries, *S. haematobium* parasite is known to secrete serine protease inhibitors with anti-thrombin properties that manipulate the host haemostatic system and escalate the severity of haematuria [97]. Consequently, urinary schistosomiasis is an important cause of haematuria and iron deficiency even in haemostatically normal persons in the tropics [96,97]. It can therefore be easily deduced that the haemostatic abnormality associated with haemophilia would create a highly pro-haemorrhagic host-parasite relationship, which would make the haemophiliacs particularly vulnerable to the haemorrhagic effects of urinary schistosomiasis. In similarity with intestinal helminthiasis, the significance of urinary schistosomiasis is not adequately studied because the vast majority of publications on haemophilia originated from developed countries in which parasitic diseases are not endemic. However, a solitary study from Nigeria had revealed that haematuria is not uncommon in haemophiliacs and it is mainly caused by spontaneous haemorrhage or trauma but urinary schistosomiasis was responsible for a significant proportion of cases [13]. Moreover, the study revealed that schistosomal haematuria was severer and caused significant anaemia in contradistinction to spontaneous and traumatic haematuria that were milder and did not cause significant anaemia [13]. This study suggests that urinary schistosomiasis is an important risk factor for frequent and severe of urinary tract bleeding in haemophiliacs infected by the parasite. The result of this study underscores the need for healthcare personnel in the tropics to ensure that all haemophiliacs presenting with haematuria are properly investigated by urinalysis for early detection and prompt treatment. This is important because schistosomal haematuria would not remit even with the use of FVIII infusion unless the underlying parasitic infection is eradicated by appropriate anti-schistosomal chemotherapy. Moreover, continuous infusions without anti-parasitic drugs in these cases may even induce the development of FVIII inhibitors since active infections and inflammations are known to be important non-genetic risk factors for the development inhibitors in haemophilia patients [70].

14. Conclusion

The bleeding diathesis in haemophilia-A is not merely a reflection of low FVIII activity. The pathophysiology of haemophilic diathesis is multi-factorial, encompassing both FVIII and non-FVIII related haemostatic defects. This fact underscores the need for healthcare providers to have comprehensive understanding of all of these aetiologic factors and the pathophysiologic mechanisms through which they contribute to the bleeding diathesis. This is pertinent because only proper understanding of these complex and inter-related haemostatic issues will enable a holistic approach within the context of currently available treatment options and therapeutic choices towards solving the lifelong bleeding problems of the haemophilia patients. It should therefore be appreciated that FVIII therapy alone may be insufficient in the management of complicated haemophilic bleeding diathesis unless compounding non-FVIII related haemostatic comorbidities are identified, targeted and treated with the appropriate non-FVIII therapeutic modalities, which may include anti-fibrinolytic agents, FVIII by-passing agents, immunotherapy, immune-modulation, and anti-microbial agents. Therefore, haemophilia healthcare personnel must take into consideration non-FVIII-related pathophysiologic dysfunctions when clinically evaluating haemophilic bleeding diathesis for the purpose of choosing optimal and appropriate treatment options.

15. Authorship contributions

SGA: Conceptualized the study and gave intellectual interpretation of literature and citations accrued from web search; UAI: Conducted web search, collected and collated relevant citations from the literature on the web, and synchronized the literature and citations for each section of the manuscript; SGA and UAI: Conducted clerical formatting and verified the intellectual accuracy of all citations within the manuscript.

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