Alloantibodies in von Willebrand disease

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The development of alloantibodies against von Willebrand factor (VWF) represents a rare but serious complication of treatment of von Willebrand disease (VWD), occurring in ~5% to 10% of type 3 VWD patients. Affected patients can present with a range of symptoms, including lack or loss of hemostatic response to infused VWF concentrates up to anaphylactic reactions in rare cases. It is classically reported in multitransfused patients and occurs most frequently in patients with partial or complete *VWF* gene deletions. A positive family history of anti-VWF antibodies also appears to be a risk factor. There is a lack of standardization of laboratory methods for antibody identification and characterization. Issues of variability in laboratory approaches as well as the rarity of the complication act as a barrier to future studies. Recombinant factor VIII as well as bypassing agents and immune tolerance have been reported as effective treatments; however, aside from case reports, little exists in the literature to guide management. The imminent clinical availability of recombinant VWF has prompted a resurgence of interest in this area. Additional study is warranted to address the deficiencies in our understanding of this treatment complication. (*Blood*. 2013; 122(5):636-640)

Introduction

Von Willebrand disease (VWD) is generally considered the most common inherited bleeding disorder known in humans, with a population prevalence of ~1% and a symptomatic prevalence of ~1 in 1000.[1-3] It was originally described in 1926 by Erik von Willebrand in a Finnish medical journal.[4] In this landmark publication, a young woman was reported to have bled to death at the time of her fourth menstrual period. Since then, significant advances have been made in our understanding of the disease, including the underlying pathophysiology, molecular basis, and potential complications of treatment.

VWD is caused by deficiency or dysfunction of the multimeric glycoprotein, von Willebrand factor (VWF), and is clinically characterized by excessive mucocutaneous bleeding as well as musculoskeletal bleeding in type 3 VWD, the most severe form. At present, VWD classification includes 3 types: type 1 is a partial quantitative deficiency of functionally normal VWF, type 2 VWD encompasses 4 qualitative variants, and type 3 is a virtual absence of VWF.[5] Treatment options include the infusion of concentrates of VWF (which also usually contain factor VIII [FVIII]) given to prevent or treat bleeding episodes. In 1974, Sarji et al first reported a case of an alloantibody against VWF in a multitransfused patient.[6] This was followed quickly by additional reports from Sweden and Italy, including the description of a precipitating anti-VWF antibody by Mannucci et al.[7-9] In such cases, treatment with VWF concentrates is rendered ineffective, and anaphylaxis with subsequent exposures has been described.[10,11] In this review, we will discuss the epidemiology of alloantibodies against VWF including what is known about underlying risk factors, the challenges facing laboratory characterization, and the clinical presentation and treatment options.

Epidemiology

In 1984, a cross-sectional study was published describing the results of a survey of severe VWD in Western Europe and Israel, with all laboratory results confirmed in a centralized laboratory. One hundred and six patients were included from 21 countries; of those, 8 were found to have alloantibodies, resulting in a prevalence of 7.5%.[12] These results are generally consistent with results of other studies, which showed prevalence estimates ranging from 5.8% to 9.5%.[13,14] Thus, alloantibodies against VWF are a rare complication. It is important to highlight that all reported cases have occurred in severe or type 3 VWD; there are no reports of VWF alloantibody development in either type 1 or type 2 VWD.

Interesting comparisons can be made with the hemophilias in terms of alloantibody prevalence. In hemophilia A, the overall prevalence across the spectrum of disease (including severe, moderate, and mild hemophilia A) of inhibitory alloantibodies to FVIII is ~6%, with the majority occurring in severe, hemophilia A (prevalence ~15%). The incidence of inhibitors in severe hemophilia A is ~25%, but many are transient. The development of these antibodies greatly complicates treatment.[15,16] Affected patients have to be treated with bypassing agents during acute bleeding episodes, and costly immune tolerance regimens are not universally effective in eradicating inhibitors.[17] Anaphylaxis has been described very rarely in patients with hemophilia A receiving FVIII, although the inciting antigen in these cases is not always clear and has been hypothesized to be concentrate components other than the FVIII molecule.[18-20] Further complicating the issue, the reports of anaphylaxis do not always temporally relate to the development of an inhibitor, leaving unresolved questions about the underlying pathophysiology.

In hemophilia B, inhibitor prevalence (and incidence) is ~4%.[21,22] In contrast to inhibitors in hemophilia A, anaphylaxis is reported much more frequently (in nearly all cases) and typically coincides with the development of the inhibitory alloantibody. This certainly affects the clinical management of a hemophilia B patient with an inhibitor and necessitates that any future reexposure to factor IX occurs in a monitored setting.[23] In both hemophilia A and

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Table 1. Characteristics of patients reported with anti-VWF antibodies

Lead author	Year	Patient ID	Sex	Age	Precipitation Formation	Anaphylaxis	Mutation
Sarji,[6] Stratton[7]	1974 1975	vWF-(FM)	M	38	NA	No	NA
Egberg,[8] Zhang[25]	1976	AKP	F	29	NA	No	Nonsense
	1992	AM	F	10	NA	No	NA
Mannucci[9]	1976	GS	M	9	Yes	No	Deletion
Ruggeri[26]	1979	GE	M	6	Yes	No	Deletion
Shelton-Inloes,[27] Ngo[49]	1981	GT	F	4	Yes	No	Deletion
	1987	SG	M	10	Yes	No	Deletion
	1988						
Shoa'i[50]	1977	19	F	25	Yes	No	NA
Lenk[51]	1978	DW	M	1.5	Yes	No	NA
Bloom,[28] Peake[52]	1979	IV6	M	28	No	No	Deletion
	1990	(S1)					
Maragall,[29] Lopez-Fernandez,[39]	1979	AR	F	21	Yes	No	Deletion
Mancuso[53]	1988	(HU2)					
	1994						
Miller[54]	1983	EE			No		NA
		AA			No		NA
		KA			No		NA
Mannucci,[12] Shelton-Inloes[27]	1984	CK	F	8	Yes		Deletion
	1987						
Mannucci[10]	1987	CK	F	16	Yes	Yes	NA
Mancuso[48]	1994	JF	F	34	Yes	n/a	Deletion
Lopez-Fernandez,[39] Batlle[40]	1988	HU1 (GF)	F	24	Yes	No	Deletion
Mancuso[48]	1994	BM	F	26	NA	NA	Deletion
	1997						
Bergamaschini[10]	1995	CK				Yes	
Tout[38]	2000	1	F	26	NA	No	Deletion
		2	M	12	NA	No	Nonsense
Lak[55]	2000	10/385 Iranian patients reported developed alloantibodies				8/10	Deletions or nonsense
Baronciani[30]	2003	21	F	52			Nonsense
		36	F	28			Deletion
Mohl[31]	2008	14	M	14	NA	No	Frameshift
Pergantou[42]	2012	NA	M	9	NA	No	Nonsense
Bowman	In preparation	T018	F	28	NA	No	Frameshift/ nonsense
Halimeh[42]	2011	NA	F	7	NA	No	NA

B, inhibitors tend to develop early in the course of treatment and the underlying gene mutation is a risk factor with deletions conferring the highest risk.[16,24]

Molecular pathology

The majority of the early reports of anti-VWF antibodies were all from patients with partial or complete *VWF* gene deletions; however, there was a patient reported in the 1970s who was subsequently determined to have a nonsense mutation.[9,25-29] Subsequent publications reported patients with additional nonsense mutations as well as a frameshift plus more cases with partial or complete *VWF* gene deletions.[30,31] A summary of reported cases and their characteristics can be found in Table 1. In many instances, individuals who developed anti-VWF antibodies were related, suggesting a heritable risk for anti-VWF antibodies, although complete penetrance of the immune phenotype is not observed in these families.[26] Furthermore, not all cases of type 3 VWD caused by partial gene deletions develop alloantibodies against VWF. Mohl et al[32] described 25 Hungarian type 3 VWD patients of which 5 were homozygous for a large partial gene deletion. None of these 5 patients developed anti-VWF antibodies as a complication of treatment, raising unanswered questions about additional genetic or environmental modifiers.[32]

Laboratory identification and antibody characteristics

There is no standard laboratory approach for the identification of anti-VWF antibodies. In general, the available assays are based on the principle of a mixing study to demonstrate the inhibition of the platelet-dependent function of VWF, although recommendations to evaluate VWF function more broadly (including collagen-binding and FVIII-binding) exist.[33] The anti-VWF antibodies do not demonstrate time and/or temperature dependence and the assay is typically done at 37°C with an incubation time between 15 minutes and 2 hours. The antibody titer is reported in Bethesda units.[34] Negative results from mixing studies do not necessarily rule out the presence of an anti-VWF antibody, because it may be directed against nonfunctional epitopes. More recently, some laboratories have used an enzyme-linked immunosorbent assay (ELISA) approach and, although these assays appear highly sensitive, there is concern about the rate of false positivity.[35-37] Based on these

issues, a strong case can be made for centralized testing in an experienced laboratory familiar with both the screening ELISA and the functional mixing studies.

When immunoglobulin (Ig) characterization has been performed, anti-VWF antibodies are most commonly polyclonal IgG and exhibit a wide range of epitope recognition on VWF.[38] IgG subclasses 1–4 have been identified, with IgG4 being the most common, as is also the case with anti-FVIII antibody responses.[7,26,39] Interestingly, when an inhibitory immune response develops in either VWD or hemophilia, IgG4 subclass antibodies predominate. This low abundance IgG subclass (<5% of total IgG) does not activate complement and is recognized to undergo antigen binding domain switching, often resulting in monovalent and sometimes bispecific antibodies. However, the significance of these properties in the context of the anti-VWF (or FVIII) immune response is unknown. As mentioned previously, a striking feature of some, but not all, of the anti-VWF antibodies identified is their ability to precipitate VWF in normal plasma.[9] In an Italian study, only high-titer antibodies precipitated with VWF; this phenomenon was not observed with lower-titer antibodies.[26]

A consistent feature of all anti-VWF antibodies to date is their specificity for VWF and lack of activity against FVIII. Interactions with plasma FVIII are possible, but likely as a result of the bound antibody causing steric hindrance of the FVIII binding site on VWF.[6,7,40] This distinction is clinically relevant in terms of treatment options for patients with anti-VWF antibodies.

The lack of standardization of laboratory approaches for the identification of anti-VWF antibodies is a barrier to further advances of our understanding of this complication. A coordinated approach to this problem would be beneficial, perhaps organized by the VWF Scientific and Standardization Committee of the International Society on Thrombosis and Hemostasis. Given the rarity of both this disease and this complication, this would of course need to be a multinational, multicenter study and could involve both the evaluation of laboratory protocols on shared samples of previously identified cases and the prospective assessment of VWF alloantibody incidence and pathophysiology. Such a study is currently under way under the leadership of Federici and Mannucci, entitled 3WINTERS-IPS (Type 3 von Willebrand International Registries Inhibitor Prospective Study). This study provides the much-needed opportunity to identify correlations between antibody characteristics and clinical presentation; clinicopathologic correlations that are currently missing from the literature.

Clinical presentation and treatment

The clinical presentation of an anti-VWF antibody usually involves lack of, or loss of, hemostatic response to infused concentrates of VWF.[8] Lower than expected VWF recoveries can also be seen.[13] With regard to prophylaxis, a recently published retrospective study reports that of 59 severe VWD patients, only 1 developed an inhibitor during the reporting period.[41] In another recent study reporting 32 patients receiving secondary prophylaxis for VWD, 1 developed an inhibitor following intensive exposure for an ankle bleed.[42] As previously mentioned, some patients, particularly those with high-titer anti-VWF antibodies, can experience severe or life-threatening anaphylactic reactions when reexposed to VWF. These cases are characterized by complement activation as well as immune complex formation.[10,11] In a detailed investigation of a patient with severe VWD, IgG alloantibodies against VWF and a history of transfusion anaphylaxis failed to identify the presence of IgE against VWF.[11] As a result of these reports, and our lack of ability to definitively predict who will experience anaphylaxis with subsequent exposure, products containing VWF should be avoided or reexposure only attempted in carefully monitored settings.

Recombinant FVIII has been used successfully for hemostatic therapy in patients with anti-VWF antibodies. The resultant plasma FVIII half-life can be expected to be short (<2 hours in a reported case), because of the lack of stabilization by VWF, but by using higher doses given by continuous infusion, bleeding has been avoided in high-risk situations such as major abdominal surgery.[13] Importantly, there is a case report published in 2008 of a 35-year-old woman with type 3 VWD and an alloantibody successfully treated with recombinant FVIII (rFVIII), who upon exposure to Recombinate developed an allergic response as well as an increase in antibody titer, which subsequently decreased. The authors hypothesize that the reaction was the result of trace VWF found in the final formulation of Recombinate because of the coexpression of the VWF gene with FVIII in the cell culture system used to synthesize this rFVIII product.[43]

Additional therapeutic options include bypassing agents such as activated FVII (rFVIIa), factor VIII inhibitor bypassing activity, and platelet infusions.[44,45] The experience with rFVIIa and factor VIII inhibitor bypassing activity is much greater in patients with hemophilia A and inhibitors, and extrapolation of that experience to this setting seems reasonable; however, the lack of experience in patients with anti-VWF antibodies warrants a cautious approach. Some clinicians advocate the use of rFVIII alternating with rFVIIa, and no reports exist of thrombosis occurring with either product in this patient population. Given that the available evidence exists only as case reports and expert opinion, additional study is needed to determine the optimal approach. The rationale for the use of transfused platelets is that in contrast to the patient's own platelets, which are devoid of VWF,[46,47] VWF will be present in the α granule of the transfused platelets in normal amounts. This localized storage will protect the VWF from the alloantibody in the plasma, which when released at the site of vascular injury might have some local hemostatic benefit before the alloantibody has a chance to bind. This rationale is supported by the relatively mild bleeding phenotype seen in patients with acquired severe von Willebrand syndrome secondary to the development of autoantibodies to VWF; however, the efficacy and safety of this approach in type 3 VWD patients requires clinical evaluation.

Again extrapolating from the experience in hemophilia A and the use of immune tolerance induction to eradicate inhibitors, immune tolerance to VWF has been attempted. In 2012, the case of a 9-year-old boy whose anti-VWF antibody was successfully eradicated with immune tolerance induction was published.[45] Whether or not this approach is safe, feasible, or effective in all patients with anti-VWF antibodies remains to be seen.

Source of VWF

The first patients reported with anti-VWF antibodies were sensitized by exposure to cryoprecipitate. Plasma-derived concentrates of FVIII containing VWF have also been implicated in the development of this complication. Although the data are not conclusive, significant differences in prevalence rates of anti-VWF antibodies between the 2 types of concentrate are not apparent. Currently, recombinant human VWF is in clinical trials and preliminary results regarding anti-VWF antibody development have been published in abstract form.[48] Reported patients were studied using a VWF-binding ELISA plus inhibitory assays evaluating VWF

platelet-dependent function, collagen binding, and FVIII binding. Three of 39 subjects had preexisting high titer nonneutralizing antibodies identified before exposure to recombinant human VWF. One of these patients was excluded from further study because inhibitory activity against the collagen binding function of VWF was identified at a titer of 1.3 Bethesda units. The remaining 2 patients participated in the study; 1 showed evidence of a decreased VWF half-life but neither went on to develop neutralizing antibodies. It is difficult to predict whether or not these results will change clinical practice, and certainly more data are necessary to determine if there are differences in rates of antibody development between plasma-derived and recombinant forms of VWF.

Conclusions

The development of anti-VWF antibodies in type 3 VWD is a rare complication of a rare disease, and many unanswered questions remain. What are the underlying modifiers that lead only some patients with *VWF* gene deletions to develop anti-VWF antibodies? What are the determinants of antibody specificity and how should these be best characterized in the laboratory? Why is there such variability in the clinical presentation of affected individuals? And finally, what is the best treatment strategy for an affected patient? It is only through coordinated, international study that we will be able to begin to address these questions.

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References

1. Rodeghiero F, Castaman G, Dini E. Epidemiological investigation of the prevalence of von Willebrand's disease. *Blood.* 1987;69(2):454-459.
2. Werner EJ, Broxson EH, Tucker EL, et al. Prevalence of von Willebrand disease in children: a multiethnic study. *J Pediatr.* 1993;123(6):893-898.
3. Bowman M, Hopman WM, Rapson D, et al. The prevalence of symptomatic von Willebrand disease in primary care practice. *J Thromb Haemost.* 2010;8(1):213-216.
4. Von Willebrand EA. Hereditary pseudohaemophilia. *Haemophilia.* 1999;5(3):223-231, discussion 222.
5. Sadler JE, Budde U, Eikenboom JCJ, et al; Working Party on von Willebrand Disease Classification. Update on the pathophysiology and classification of von Willebrand disease: a report of the Subcommittee on von Willebrand Factor. *J Thromb Haemost.* 2006;4(10):2103-2114.
6. Sarji KE, Stratton RD, Wagner RH, et al. Nature of von Willebrand factor: a new assay and a specific inhibitor. *Proc Natl Acad Sci USA.* 1974;71(8):2937-2941.
7. Stratton RD, Wagner RH, Webster WP, et al. Antibody nature of circulating inhibitor of plasma von Willebrand factor. *Proc Natl Acad Sci USA.* 1975;72(10):4167-4171.
8. Egberg N, Blombäck M. On the characterization of acquired inhibitors to ristocetin induced platelet aggregation found in patients with von Willebrand's disease. *Thromb Res.* 1976;9(5):527-531.
9. Mannucci PM, Meyer D, Ruggeri ZM, et al. Precipitating antibodies in von Willebrand's disease. *Nature.* 1976;262(5564):141-142.
10. Mannucci PM, Tamaro G, Narchi G, et al. Life-threatening reaction to factor VIII concentrate in a patient with severe von Willebrand disease and alloantibodies to von Willebrand factor. *Eur J Haematol.* 1987;39(5):467-470.
11. Bergamaschini L, Mannucci PM, Federici AB, et al. Posttransfusion anaphylactic reactions in a patient with severe von Willebrand disease: role of complement and alloantibodies to von Willebrand factor. *J Lab Clin Med.* 1995;125(3):348-355.
12. Mannucci PM, Mari D. Antibodies to factor VIII-von Willebrand factor in congenital and acquired von Willebrand's disease. *Prog Clin Biol Res.* 1984;150:109-122.
13. Mannucci PM, Federici AB. Antibodies to von Willebrand factor in von Willebrand disease. *Adv Exp Med Biol.* 1995;386:87-92.
14. Iorio A, Oliovecchio E, Morfini M, et al; Association of Italian Hemophilia Centres Directors. Italian Registry of Haemophilia and Allied Disorders. Objectives, methodology and data analysis. *Haemophilia.* 2008;14(3):444-453.
15. Lusher JM, Arkin S, Abildgaard CF, et al; Kogenate Previously Untreated Patient Study Group. Recombinant factor VIII for the treatment of previously untreated patients with hemophilia A. Safety, efficacy, and development of inhibitors. *N Engl J Med.* 1993;328(7):453-459.
16. Dimichele D, Rivard G, Hay C, et al. Inhibitors in haemophilia: clinical aspects. *Haemophilia.* 2004;10(Suppl 4):140-145.
17. Hay CRM, DiMichele DM; International Immune Tolerance Study. The principal results of the International Immune Tolerance Study: a randomized dose comparison. *Blood.* 2012;119(6):1335-1344.
18. Helmer RE III, Alperin JB, Yunginger JW, et al. Anaphylactic reactions following infusion of factor VIII in a patient with classic hemophilia. *Am J Med.* 1980;69(6):953-957.
19. Bove JR, McIntosh S. Anaphylactic reaction to purified anti-hemophilic factor concentrate. *Transfusion.* 1988;28(6):603.
20. Shopnick RI, Kazemi M, Brettler DB, et al. Anaphylaxis after treatment with recombinant factor VIII. *Transfusion.* 1996;36(4):358-361.
21. Kamiya T, Takahashi I, Saito H. Retrospective study of inhibitor formation in Japanese hemophiliacs. *Int J Hematol.* 1995;62(3):175-181.
22. Sultan Y; French Hemophilia Study Group. Prevalence of inhibitors in a population of 3435 hemophilia patients in France. *Thromb Haemost.* 1992;67(6):600-602.
23. Franchini M, Lippi G, Montagnana M, et al. Anaphylaxis in patients with congenital bleeding disorders and inhibitors. *Blood Coagul Fibrinolysis.* 2009;20(4):225-229.
24. Thorland EC, Drost JB, Lusher JM, et al. Anaphylactic response to factor IX replacement therapy in haemophilia B patients: complete gene deletions confer the highest risk. *Haemophilia.* 1999;5(2):101-105.
25. Zhang ZP, Lindstedt M, Falk G, et al. Nonsense mutations of the von Willebrand factor gene in patients with von Willebrand disease type III and type I. *Am J Hum Genet.* 1992;51(4):850-858.
26. Ruggeri ZM, Ciavarella N, Mannucci PM, et al. Familial incidence of precipitating antibodies in von Willebrand's disease: a study of four cases. *J Lab Clin Med.* 1979;94(1):60-75.
27. Shelton-Inloes BB, Chehab FF, Mannucci PM, et al. Gene deletions correlate with the development of alloantibodies in von Willebrand disease. *J Clin Invest.* 1987;79(5):1459-1465.
28. Bloom AL, Peake IR, Furlong RA, et al. High potency factor VIII concentrate: more effective than cryoprecipitate in a patient with von Willebrand's disease and inhibitor. *Thromb Res.* 1979;16(5-6):847-852.
29. Maragall S, Castillo R, Ordinas A, et al. Inhibition of Willebrand factor in von Willebrand disease. *Thromb Res.* 1979;14(2-3):495-500.
30. Baronciani L, Cozzi G, Canciani MT, et al. Molecular defects in type 3 von Willebrand

disease: updated results from 40 multiethnic patients. *Blood Cells Mol Dis.* 2003;30(3): 264-270.

31. Mohl A, Marschalek R, Masszi T, et al. An Alu-mediated novel large deletion is the most frequent cause of type 3 von Willebrand disease in Hungary. *J Thromb Haemost.* 2008;6(10): 1729-1735.
32. Mohl A, Boda Z, Jager R, et al. Common large partial VWF gene deletion does not cause alloantibody formation in the Hungarian type 3 von Willebrand disease population. *J Thromb Haemost.* 2011;9(5):945-952.
33. Berntorp E, Peake I, Budde U, et al. von Willebrand's disease: a report from a meeting in the Åland islands. *Haemophilia.* 2012;18(Suppl 6):1-13.
34. van Genderen PJ, Vink T, Michiels JJ, et al. Acquired von Willebrand disease caused by an autoantibody selectively inhibiting the binding of von Willebrand factor to collagen. *Blood.* 1994; 84(10):3378-3384.
35. Tiede A, Priesack J, Werwitzke S, et al. Diagnostic workup of patients with acquired von Willebrand syndrome: a retrospective single-centre cohort study. *J Thromb Haemost.* 2008;6(4):569-576.
36. Stewart MW, Etches WS, Shaw AR, et al. vWf inhibitor detection by competitive ELISA. *J Immunol Methods.* 1997;200(1-2):113-119.
37. Siaka C, Rugeri L, Caron C, et al. A new ELISA assay for diagnosis of acquired von Willebrand syndrome. *Haemophilia.* 2003;9(3):303-308.
38. Tout H, Obert B, Houllier A, et al. Mapping and functional studies of two alloantibodies developed in patients with type 3 von Willebrand disease. *Thromb Haemost.* 2000;83(2):274-281.
39. López-Fernández MF, Martin R, López-Berges C, et al. Further specificity characterization of von Willebrand factor inhibitors developed in two patients with severe von Willebrand disease. *Blood.* 1988;72(1):116-120.
40. Batlle J, Lourés E, Vila P, et al. Alloantibody from a patient with severe von Willebrand disease inhibits von Willebrand factor-FVIII interaction. *Ann Hematol.* 1997;75(3):111-115.
41. Abshire TC, Federici AB, Alvarez MT, et al. Prophylaxis in severe forms of von Willebrand disease: results from the von Willebrand's Disease Prophylaxis Network (VWD PN). *Haemophilia.* 2013;19(1):76-78.
42. Halimeh S, Krümpel A, Rott H, et al. Long-term secondary prophylaxis in children, adolescents and young adults with von Willebrand disease. Results of a cohort study. *Thromb Haemost.* 2011;105(4):597-604.
43. Franchini M, Gandini G, Giuffrida A, et al. Treatment for patients with type 3 von Willebrand disease and alloantibodies: a case report. *Haemophilia.* 2008;14(3):645-646.
44. Ciavarella N, Schiavoni M, Valenzano E, et al. Use of recombinant factor VIIa (NovoSeven) in the treatment of two patients with type III von Willebrand's disease and an inhibitor against von Willebrand factor. *Haemostasis.* 1996;26(Suppl 1):150-154.
45. Pergantou H, Xafaki P, Adamtziki E, et al. The challenging management of a child with type 3 von Willebrand disease and antibodies to von Willebrand factor. *Haemophilia.* 2012;18(3): e66-e67.
46. Mannucci PM. Platelet von Willebrand factor in inherited and acquired bleeding disorders. *Proc Natl Acad Sci USA.* 1995;92(7):2428-2432.
47. Sultan Y, Bouma BN, de Graaf S, et al. Factor VIII related antigen in platelets of patients with Von Willebrand's disease. *Thromb Res.* 1977;11(1): 23-30.
48. Suiter TM, Mannucci PM, Kempton CL, et al. Detection of non inhibitory binding antibodies to von Willebrand factor affecting the clearance of VWF:Ag in von Willebrand disease. *Blood* (*ASH Annual Meeting Abstracts).* 2011;118(21): 2275.
49. Ngo KY, Glotz VT, Koziol JA, et al. Homozygous and heterozygous deletions of the von Willebrand factor gene in patients and carriers of severe von Willebrand disease. *Proc Natl Acad Sci USA.* 1988;85(8):2753-2757.
50. Shoa'i I, Lavergne JM, Ardaillou N, et al. Heterogeneity of von Willebrand's disease: study of 40 Iranian cases. *Br J Haematol.* 1977;37(1): 67-83.
51. Lenk H, Weissbach G, Domula M. An inhibitor in the Willebrand-Jürgens syndrome and its effect on the factor VIII molecule properties [in German]. *Folia Haematol (Frankf).* 1978; 105(6):826-834.
52. Peake IR, Liddell MB, Moodie P, et al. Severe type III von Willebrand's disease caused by deletion of exon 42 of the von Willebrand factor gene: family studies that identify carriers of the condition and a compound heterozygous individual. *Blood.* 1990;75(3):654-661.
53. Mancuso DJ, Tuley EA, Castillo R, et al. Characterization of partial gene deletions in type III von Willebrand disease with alloantibody inhibitors. *Thromb Haemost.* 1994;72(2):180-185.
54. Miller CH, Bussel J, Hilgartner M. Characteristics of inhibitors in severe von Willebrand's disease. *Thromb Haemost.* 1983;50(34).
55. Lak M, Peyvandi F, Mannucci PM. Clinical manifestations and complications of childbirth and replacement therapy in 385 Iranian patients with type 3 von Willebrand disease. *Br J Haematol.* 2000;111(4):1236-1239.