

Von Willebrand's Disease

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Von Willebrand factor is a single polypeptide chain with molecular weight of 300,000 dalton which is processed to 250,000 daltons. vWF monomers of 250,000 daltons oligomerize to form polymers of anywhere from 1,000,000 daltons to 20,000,000 daltons. Multimerization occurs in the last compartments of the Golgi apparatus and in the post-Golgi secretory vesicles in acidic conditions. The vWF propeptide plays an important role in multimerization and cleavage of the propeptide is the final step in the processing of von Willebrand factor. Plasma multimeric pattern depends on electrophoresis conditions. With low concentrations of agarose (0.65%) the multimeric pattern is maximized showing multimers in increments of ~600,000 daltons up to 20,000,000 daltons. With high concentrations of agarose (2-3%), each individual multimer can be resolved into up to three to five distinct bands.

There are two sites of vWF synthesis. The major site of vWF synthesis is in endothelial cells. Following synthesis, vWF is stored in Weibel-Palade bodies and secreted into the circulation by both constitutive and regulated mechanisms. Small molecular weight multimers are secreted constitutively into the circulation, where they circulate in a complex with coagulation factor VIII. Large molecular weight multimers are secreted from Weibel-Palade bodies in response to a variety of secretagogues. vWF is also synthesized in megakaryocytes and large molecular weight multimers stored in the α secretory granules of platelets along with other large adhesive glycoproteins such as fibrinogen, thrombospondin, and factor V.

vWF mediates the adhesion of platelets with components of the vessel wall, especially collagen. So when there is vascular injury, vWF participates in the very earliest hemostatic responses. Adhesion is mediated through a direct interaction of vWF with platelets through a membrane glycoprotein called GPIb on the surface of platelets. The largest molecular weight multimers of vWF, from 15,000,000-20,000,000, appear to be most active in mediating platelet adhesion to collagen. Carrier protein for coagulation factor VIII. In the absence of von Willebrand factor, the half-life of factor VIII in the circulation decreases from a normal of 8-12 hours to 2-3 hours.

Von Willebrand's disease is a hemorrhagic disorder caused by an inherited or, rarely, acquired deficiency or abnormality of plasma and/or cellular von Willebrand factor. Disease prevalence has been estimated to be as high as 1.6%, making it one of the most common genetic diseases in man. Is there some functional advantage for individuals with von Willebrand's disease, i.e. protection against atherosclerosis?

The diagnosis of von Willebrand's disease is based on the demonstration of an abnormality in one or more of the following tests:

- *Von Willebrand factor activity*: Also called ristocetin cofactor activity, vWF activity is measured by von Willebrand factor-supported agglutination of platelets by ristocetin, a glycopeptide derived from the Actinomycete *Nocardia lurida*. Activity can also be measured using Botrocetin, a functionally related compound from the venom of *Bothrops jararaca*. vWF activity can be falsely reduced by ristocetin-binding proteins in plasma such as Vancomycin.
- *Von Willebrand factor antigen*: Formerly referred to as factor VIII-related antigen, this is the antigenic measure of the von Willebrand factor protein. vWF:Ag is measured by Laurell immunoelectrophoresis technique or ELISA.
- *Factor VIII activity*: measured by one-stage coagulation assay using factor VIII-deficient plasma as substrate.
- *Bleeding time*: measured by Ivy bleeding time. The diagnosis based on finding reduced levels of vWF activity, vWF antigen, factor VIII and/or a prolonged bleeding time.

In the classical case, all four tests will be abnormal, but there is remarkable phenotypic diversity so that any combination of the above abnormalities maybe seen. Variability even exists within the same individual.

vWF activity, vWF antigen, and factor VIII are affected by blood type, with levels of each being reduced in individuals with type O blood, estrogens, epinephrine/vasopressin, and genetic factors.

Classification

The classification of von Willebrand's disease is based on determination of the multimeric composition of plasma von Willebrand factor.

Type 1 vWd: In type 1 vWD, there is a partial quantitative deficiency of vWF. This is the most commonly diagnosed type of vWD, accounting for approximately 70-80% of cases. The characteristic finding in type 1 vWD is the demonstration on multimer analysis that all multimers in plasma are present and in the same relative proportion as in normal plasma but are reduced. The reduction in multimers is variable among patients and may be variable on different occasions in any given patient. Plasma levels of factor VIII, vWF activity, and vWF antigen may be reduced, usually concordantly, but also may be normal, and the bleeding time may be either prolonged or normal. It is generally assumed that promoter mutations, nonsense mutations, frameshift mutations, and large deletions account for most type 1 defects. In some patients with type 1 disease, silent alleles that produce undetectable or greatly reduced amounts of mRNA for vWF have been demonstrated. Point mutations produc-

ing premature truncation of the vWF protein and missense mutations within functional domains of vWF have also been demonstrated.

Type 2 vWD: In type 2 vWD, there is a qualitative abnormality of vWF. Patients may have normal levels of vWF protein, but the protein is dysfunctional. These variants account for 15-30% of cases.

Type 2A vWD refers to a qualitative variant of vWD in which there is defective platelet-vWF interactions associated with the absence of the high- and middle-molecular-weight vWF multimers in plasma. Satellite multimer bands may or may not be normal. The hemostatic abnormality in type 2A vWD is due to the absence of the hemostatically effective large vWF multimers. Platelet aggregation in response to ristocetin is reduced in proportion to the deficiency of high-molecular-weight vWF multimers. At least two distinct mechanisms account for the usual type 2A phenotype: (1) abnormal cellular assembly of vWF multimers, and (2) increased susceptibility of vWF to proteolysis in vivo. Platelet vWF in patients with abnormal cellular assembly of vWF is similar to that in plasma and is normal in patients with increased susceptibility to proteolysis.

Type 2B vWD is caused by a qualitative abnormality of vWF in which there is increased platelet-vWF interaction due to an increased affinity of vWF for its platelet receptor, GPIb. Patients previously classified as IIB, I-New York, and Malmö are included in this category. In the usual form of type 2B vWD, there is an absence from plasma of only the highest molecular weight multimers. The middle and low molecular weight multimers are present in normal amounts and in the same relative proportions as in normal plasma. The hallmark of type 2B vWD is an enhanced aggregation of the patient's platelets in the presence of reduced concentrations of ristocetin. At a concentration of 0.3-0.5 mg/ml, ristocetin does not usually induce aggregation of normal platelets, but in type 2B disease, 0.3-0.5 mg/ml of ristocetin stimulates a full aggregation response. The high-molecular-weight multimers missing in plasma are present in platelets, suggesting that all of the vWF multimers are synthesized and that the absence of the high-molecular-weight forms is due to increased turnover of the high-molecular-weight multimers because of the increased affinity of vWF for GPIb. Patients with type 2B vWD often have mild thrombocytopenia.

Type 2M vWD refers to qualitative variants in which there is decreased platelet-vWF interaction that is not caused by the absence of high-molecular-weight multimers. Previous vWD types B, Vicenza, IC, and ID are grouped in this category. The vWF multimer distribution is typically normal and there may even be larger-than-normal multimers. The satellite bands may be abnormal. In vWD type B and type 2M^{Milwaukee-1}, the defect was shown to cause decreased binding of vWF to platelet GPIb.

Type 2N vWD, also termed Normandy type vWD, is a qualitative abnormality of vWF in which there is defective interaction with factor VIII. This form of vWD masquer-

ades as an autosomal form of hemophilia A. Patients with this variant have reduced levels of factor VIII, typically 5-15%, with normal levels of vWF and vWF:Ag. The bleeding time is normal. The molecular defect in vWD Normandy is in the region of vWF involved in binding factor VIII and results in impaired formation of the vWF/factor VIII complex. As a result, the half-life of factor VIII in the circulation is markedly shortened and factor VIII levels are reduced. The phenotypic expression of the Normandy variant only occurs if the person is homozygous for the defect or if the other allele is a second vWD allele with reduced or absent levels of normal vWF such that only the abnormal factor VIII binding allele is predominantly expressed.

Type 3 vWD is a severe form of vWD, with nearly complete deficiency of vWF. Usually vWF activity and vWF:Ag are undetectable, and factor VIII levels are typically markedly reduced (to <10%). The bleeding time is prolonged, usually to >20 minutes. Multimeric analysis of plasma from patients with type 3 disease shows essentially no multimers because of the marked reduction in vWF. This severe form of vWD may be the result of a homozygous defect or of compound heterozygosity.

Platelet-type, pseudo-vWD is a primary platelet disorder involving the platelet receptor for vWF. Phenotypically, patients with platelet-type, pseudo-vWD are similar to patients with type 2B disease: multimeric analysis reveals absence of the highest-molecular-weight multimers; factor VIII, vWF activity, and vWF:Ag are variably reduced; and the bleeding time is prolonged. Aggregation is enhanced in response to low concentrations of ristocetin (0.3-0.5 mg/ml), and mild thrombocytopenia is commonly present. However, in contrast to type 2B vWD, in which the abnormality is in the vWF itself, the defect in platelet-type, pseudo-vWD is in the platelet receptor for vWF, which has an increased affinity for normal vWF. The deficiency of the high-molecular-weight multimers is thought to occur as a result of increased utilization secondary to the increased affinity of platelets for vWF. Platelet-type pseudo-vWD can be distinguished from type 2B disease by addition of normal human cryoprecipitate to the patient's platelet-rich plasma. In platelet-type disease the cryoprecipitate will induce platelet aggregation, while in type 2B disease it will not. Platelet-type pseudo-vWD can also be distinguished from type 2B disease by the differential binding of the patient's plasma vWF to formalin-fixed platelets. Unlike the response to cryoprecipitate, this method can be performed with previously frozen plasma.

Acquired vWD may occur in people who were previously normal. In most cases, this is caused by antibodies to vWF that neutralize vWF activity. Patients present with new onset bruising and bleeding. vWF activity, vWF:Ag, and factor VIII are markedly reduced and the bleeding time is prolonged. Inhibitors may occur in otherwise healthy people, but often are associated with an underlying disorder such

as lymphoproliferative disorders, benign monoclonal gammopathies, and other diseases characterized by immunologic abnormalities. Acquired vWD may also occur in the absence of antibodies when there is increased utilization of vWF. For example, people with cardiac disease, particularly ventricular septal defect or valvular heart disease, may acquire a deficiency of the high-molecular-weight vWF multimers and present with a pattern consistent with type 2B vWD with decreased factor VIII, decreased vWF, decreased vWF:Ag, and an absence of high-molecular-weight multimers. Repair of the cardiac defect may result in normalization of vWF.

Clinical Manifestations

The clinical manifestations of vWD relate primarily to the bleeding tendency. Hemorrhagic manifestations are primarily from delicate mucous membranes—the nasal mucosa, the gastrointestinal tract, and in females the vaginal mucosa. Joint bleeds may occur in severe type 3 von Willebrand's disease but they are not common and do not occur in type 1 von Willebrand's disease unless there is attendant trauma. Clinical complications include the development of inhibitors in about 10% or less of patients with severe type 3 von Willebrand's disease. Mitral valve prolapse has been reported in all types of von Willebrand's disease, but it is not clear if these are linked disorders. Angiodysplasia are small telangiectasia that occur in the wall of the intestine in patients with von Willebrand's disease, typically in those over the age of 50 and with type 3 disease. Estrogen therapy may be helpful with angiodysplastic bleeding.

Treatment of von Willebrand's Disease

Treatment of von Willebrand's disease is aimed at correction of the bleeding time and the partial thromboplastin time. Since the bleeding time is sensitive to only the highest molecular weight multimers, the source of the von Willebrand factor and its multimeric composition is important therapeutically.

Type 1 vWD. DDAVP (1-deamino-8-D-arginine vasopressin) is the treatment of choice for most individuals with type 1vWD. DDAVP is a synthetic analog of vasopressin which has been engineered to remove the pressor activity. DDAVP increases von Willebrand factor activity, von Willebrand factor antigen, and factor VIII by indirect mechanisms that are thought to involve the release of vWF from storage sites. A trial administration should be performed to be sure that the patient will respond to DDAVP. Hormonal agents may be used to control menstrual bleeding in von Willebrand's disease.

Type 2A vWD. In most cases of type 2A vWD, DDAVP only results in the secretion of the abnormal von Willebrand factor and is therefore not useful for treatment. Humate-P, vWF concentrates, or cryoprecipitate is the treatment of choice for this form of vWD. Humate-P is a concentrate of

human plasma factor VIII that is of intermediate purity and is pasteurized to remove lipid coated and non-lipid coated viruses. All intermediate purity factor VIII concentrates contain von Willebrand factor but most lack the high molecular weight multimers and therefore do not effectively correct the bleeding time in von Willebrand's disease. Humate-P does not contain all the high-molecular-weight multimers, but it contains more of them than other factor VIII concentrates and can shorten the bleeding time. vWF concentrates, both plasma-derived and recombinant, are currently in development and promise to be useful agents. Cryoprecipitate contains all of the multimers, including the highest molecular weight multimers, of von Willebrand factor and is the best agent to correct the bleeding time in von Willebrand's disease, but cryoprecipitate is not processed to remove infectious blood born viruses and therefore has a risk of hepatitis and AIDS. Antifibrinolytic agents may be useful for epistaxis, gingival bleeding, and gastrointestinal bleeding.

Type 2B vWD. Humate-P, vWF concentrates, or cryoprecipitate is the treatment of choice for type 2B vWD. As with type 2A vWD, DDAVP results in the secretion of the abnormal von Willebrand factor and may cause an increase in the thrombocytopenia. For this reason, DDAVP is contraindicated in type 2B vWD.

Type 2M vWD. Humate-P, vWF concentrates, or cryoprecipitate is the treatment of choice.

Type 2N vWD. Concentrates of factor VIII can be used for short-term correction of the factor VIII level, but in the absence of correction of the von Willebrand factor, the biological half-life of the infused factor VIII will be short. Humate-P, vWF concentrates, or cryoprecipitate is the treatment of choice.

Type 3 vWD. Humate-P, vWF concentrates, or cryoprecipitate is the treatment of choice in type 3 vWD. It has been suggested for that to achieve complete correction of the bleeding time, combinations of Humate-P, vWF concentrates, or cryoprecipitate with DDAVP may be needed.

Platelet-type, pseudo-vWD. Platelet concentrates are used to treat platelet-type pseudo-vWD. The administration of Humate-P, vWF concentrates, cryoprecipitate, or DDAVP may increase the amount of von Willebrand factor to interact with the abnormal GPIb on the platelet surface and may exacerbate the thrombocytopenia.

Complications of treatment with DDAVP are primarily water retention with hyponatremia which may be severe and may cause seizures. Monitor serum sodium in individuals receiving multiple doses of DDAVP. Limit free water when concomitant intravenous fluids are administered. Cryoprecipitate and Humate-P: hepatitis A, B, or C and HIV infection with cryoprecipitate. Humate-P is viral safe but may not completely correct the bleeding time in patients.