Acquired von Willebrand syndrome in myeloproliferative disorder

Case 6

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Summary
We present a woman (age: 57 years) with an excessive bleeding episode under acetylsalicylic acid after bone marrow puncture due to an acquired von Willebrand syndrome (avWS) in the context of a myeloproliferative disorder. The laboratory features showed a high platelet concentration and a qualitative defect of von Willebrand factor (vWF) with a low normal vWF ristocetin cofactor activity, a normal vWF antigen and a decrease of the larger vWF multimers in plasma.

The exact mechanism of avWS is still incompletely resolved. Myeloproliferative diseases are one of several underlying disorders that may cause avWS. The diagnosis of the underlying disease is important because its treatment may lead to an improvement of the avWS abnormality. For symptomatic treatment of bleeding, desmopressin, vWF concentrate infusion, intravenous immunoglobulin and/or fibrinolysis inhibitors can be tried.

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We present a 57-year-old woman, who was referred to our outpatient clinic because of an asymptomatic thrombocytosis. There were neither symptoms of a haemorrhagic diathesis nor thromboembolism in her personal history. The clinical examination was unrevealing, in particular neither palpable splenomegaly nor signs of mucocutaneous bleeding were found.

There were no signs of infection, chronic inflammatory disease, tumour or iron deficiency. Further results showed normal arterial oxygen saturation of her haemoglobin, an increased erythrocyte mass and plasma volume (by radioisotope dilution studies), persistent thrombocytosis, and an elevated alkaline leucocyte phosphatase. These results corresponded to two major and two minor criteria for the diagnosis of polycythaemia vera (13). Supporting the diagnosis, the level of erythropoietin was decreased and stem cell clonogenic assays from peripheral blood showed an increased spontaneous growth of the three cell lines. The BCR-ABL-transcript in peripheral blood cells was negative. Despite her negative history for thromboembolic complications a treatment with acetylsalicylic acid 100 mg daily was initiated.

Bone marrow biopsy and aspiration were performed at a platelet count of 1054 G/l and normal plasmatic coagulation tests. The patient was taking acetylsalicylic acid (100 mg/d). The local anesthesia was done with 10 ml lidocain 2% above the posterior iliac spine. Already the skin cut led to a diffuse subcutaneous bleeding. The aspiration and biopsy were done without any problem at the first attempt. As the bleeding continued after puncture a compression with a resorbable gelatine sponge was applied. After 30 min the patient sat up and bleeding immediately restarted. Neither local nor intravenous tranexamic acid (Cyklokapron® Pharmacia, Dübendorf, Switzerland) combined with a compression of about 2 h could stop the bleeding, which continued to be diffuse and subcutaneous.

As we suspected acquired von Willebrand syndrome (avWS), we finally administered von Willebrand factor (vWF) concentrate.

Diagnosis and treatment
The laboratory features at the first consultation showed

- a slightly elevated haematocrit of 0.46 (reference range 0.36-0.44),
- slight erythrocytosis (5.23 T/l; reference range 3.9-5.00 T/l) and
- reticulocytosis (84 G/l; reference range 20-80 G/l), and
- thrombocytosis of 977 G/l (reference range 140-380 G/l) as a major finding.
(Haemate® 2000 U intravenously; Aventis Behring GmbH, Marburg). Bleeding stopped about 4 h after bone marrow puncture. Acetylsalicylic acid was stopped and cytoreduction with hydroxyurea started.

The laboratory features confirmed the suspicion of avWS. At the day of the bone marrow examination the platelet count was 1054 G/l; factor VIII : C (FVIII : C) and vWF antigen (vWF : Ag) were within the reference values: FVIII : C 92% (reference range 50-150), vWF : Ag 105 % (reference range 42-136). VWF ristocetin cofactor activity (vWF : RCo) was 54% (reference range 50-150) and the calculated vWF : RCo/vWF : Ag ratio was 0.51 (reference range 0.60-1.50). The largest vWF multimers in plasma were decreased (patient: 30.2%; normal plasma: 38.1%) (6).

After the reduction of the platelet count to 502 G/l at the following consultation the vWF : RCo (102%), the vWF : RCo/vWF : Ag ratio (0.98) and the multimeric pattern of vWF (large multimers in patient plasma 37.7%, in normal plasma 38.1%) had normalized. No circulating inhibitor of vWF was found by ristocetin cofactor assay in mixtures of patient plasma and normal plasma at the time of low-normal vWF : RCo activity.

**Discussion**

Acquired von Willebrand syndrome (avWS) is a rare but probably underdiagnosed bleeding disorder. Clinical and laboratory presentations are similar to hereditary von Willebrand disease (vWD) but occur in patients without personal or familial history of bleeding diathesis (14, 15). AvWS results from an acquired defect in vWF, a large multimeric glycoprotein present in platelets, endothelial cells and plasma. The vWF is synthesized in megakaryocytes and endothelial cells (1). The subunits have a molecular mass of 250 kDa and are dimerized by disulfide bonds at their carboxyterminal sites. The multimerization is achieved by linear assembly of dimers linked by disulfide bonds at the aminoterminal of the subunits. In blood vWF circulates as an array of molecules from about 500 kDa to 20 000 kDa. More than 20 individual multimers can be distinguished by SDS agarose gel electrophoresis of plasma vWF (see case 9). With 20 000 kDa vWF is the largest known protein in blood. Its key role is mediation of the adhesion of platelets to the subendothelial structures of injured vessel walls. Additionally, vWF protects coagulation factor VIII from rapid proteolysis and clearance in the circulation by non-covalently binding the latter. Defects of vWF result in a bleeding disorder.

**Associated disorders and pathogenic mechanisms**

Several diseases may lead to avWS (14). Most frequently reported are:
- lymphoproliferative disorders, particularly monoclonal gammopathies including those of undetermined significance (MGUS), as well as myeloma and Waldenström’s macroglobulinaemia,
- myeloproliferative disorders (most often essential thrombocythaemia), and
- cardiovascular conditions (e. g. aortic stenosis, septal defects and angiodysplasia).

Other disorders associated with avWS are listed in Table 1 (5, 14).
The pathophysiology of avWS remains incompletely explored and is heterogeneous (5, 14). In association with hypothyroidism avWS appears to result from decreased biosynthesis of vWF, since the ratio vWF : RCo/vWF : Ag is usually normal and patients show a sustained response to desmopressin (4). In most cases of avWS, however, vWF synthesis by megakaryocytes and endothelial cells, as well as its release into the blood are normal, but the removal of vWF from plasma is accelerated. Three main mechanisms are discussed. None of them appears to be specific for an underlying disease and probably several mechanisms may be simultaneously responsible for the vWF deficiency.

The first mechanism involves circulating antibodies to vWF, which have been reported in MGUS and myeloma, as well as in immunological diseases such as systemic lupus erythematosus. These antibodies may be directed against either non-functional or functional domains of vWF. Their binding to vWF leads to the formation of immune complexes that are rapidly removed from the circulation by the reticulo-endothelial system. On rare occasions, antibodies were described which are directed against the collagen-binding site (24), or the binding sites for platelet glycoproteins GPIb-IX-V and GPIIb-IIIa (12), thus inhibiting the binding of vWF to collagen or to platelets. So far, no antibodies were found against the binding site for factor VIII on vWF (imitating a vWD subtype 2N phenotype).

The second mechanism is the selective adsorption of the vWF multimers onto malignant cells or platelets. Among tumour cells, those of lymphoproliferative diseases (myeloma, Waldenström’s macroglobulinaemia, non-Hodgkin’s lymphoma, hairy cell leukaemia) and adrenal cell carcinomas are the most frequently described. These observations are based on immunofluorescence studies using antibodies against vWF, that bound to tumour cells (18). With the help of flow cytometry, aberrant expression of GPIb- or GPIIb-IIIa-like receptors on malignant cell surfaces was demonstrated (19). The adsorption of vWF multimers to (activated) platelets is described in patients with myeloproliferative disorders and leads to an inverse relationship between platelet count and the concentration of the large multimers of vWF in plasma (7). A similar mechanism was postulated in aortic valve stenosis, where high shear rates may activate platelets leading to adsorption of vWF multimers onto their surface (16). Additionally, increased proteolysis of vWF in patients with aortic valve stenosis was described as well (17).

The third mechanism described for avWS is increased proteolysis of vWF in plasma. Different enzymes, such as platelet-derived calcium activated neutral protease (calpain), plasmin and elastase secreted by neoplastic cells, or ADAMTS-13 (see case 2) are held responsible for this proteolysis. Proteolytic fragments of vWF with a molecular mass of 140 and 176 kDa, corresponding to the cleavage fragments produced by ADAMTS-13, have been detected by electrophoresis of plasma vWF from patients with essential thrombocythaemia (22). Plasmin is suspected to be responsible for avWS associated with cardiac valve disease (11) and increased fibrinolytic activity (9).

In other diseases reported to be associated with avWS the mechanism remains unclear. For instance, a clear association of avWS with Wilm’s tumour has been described, but no antibodies or adsorption to the tumour cells were found, suggesting that other mechanisms, e. g. hyaluronic acid (3), play a relevant role.

**Laboratory features**

The laboratory diagnosis of avWS requires the demonstration of plasma vWF abnormality and the identification of a causal underlying disease or pathogenic mechanism. A defect in primary haemostasis is demonstrated by a prolonged in vivo bleeding time or by prolonged closure times of in vitro testing with the PFA-100® (Dade-Behring GmbH, Marburg) (see case 9).

Specific assays for plasma vWF usually show a normal or mildly decreased vWF : Ag and a (low) normal factor VIII clotting activity in contrast to a more markedly decreased vWF : RCo and collagen binding activity (vWF : CBA). Analysis of the multimeric pattern shows absence or decrease of large vWF multimers in plasma. Therefore, an avWS usually is associated with a diminished ratio of vWF : RCo/vWF : Ag (or vWF : CBA/vWF : Ag).

In myeloproliferative disorders, a clear inverse relationship exists between the vWF : RCo/vWF : Ag ratio and the platelet concentration, but not the leucocyte count, suggesting that platelets rather than granulocytes are involved in the pathogenesis of avWS (6, 7). In order to distinguish inherited vWD from avWS the quantitative analysis of plasma vWF propeptide was proposed (8, 21):

- Congenital vWD usually shows low levels of vWF propeptide due to a decreased synthesis.
- Normal or increased propeptide levels reflect an increased removal of vWF suggestive of avWS.

Among the antibodies directed against vWF only few of them may be detected as inhibitors in vitro. The detection of circulating immune complexes is not yet available in routine laboratories. Finally, the diagnosis of avWS requires the identification of a causal underlying disease (Table 1).

**Therapeutic options**

The successful treatment of the underlying disease associated with avWS (e. g. L-thyroxine replacement in hypothyroidism, normalisation of platelet count by cytoreductive agents in myeloproliferative disorders, surgery in an underlying neoplasia or in an aortic stenosis, chemotherapy in a lymphoproliferative disease or discontinuing a critical drug), improves the bleeding diathesis and the laboratory features (14). In cases where the underlying disease can not be controlled and/or active bleeding needs immediate correction of haemostasis, the following options can be evaluated:

- Desmopressin or vWF concentrate replacement are effective in hypothyroidism associated avWS (4) but show otherwise only a limited efficacy, because of excessive clearance of the vWF from the circulation.
Intravenous immunoglobulins can be helpful in some patients with lymphoproliferative disorders or monoclonal gammopathies. However, only those with monoclonal IgG may benefit from this treatment, but not those with monoclonal IgM (10). Intravenous immunoglobulins may result in a rapid improvement of bleeding diathesis and permit surgery up to 1-2 days following infusion. In chronic bleeding, intravenous immunoglobulins have been successfully applied every 21 days as a long-term therapy (10).

Especially in patients with high titers of vWF inhibitors, extracorporeal immunoadsorption may be an option (20).

However, there is no consensus regarding the management of avWS since the disorder is certainly heterogeneous and may need case-specific management. At our institute, we have successfully treated a patient with avWS due to MGUS and very severe gastrointestinal bleeding by the antifibrinolytic agent tranexamic acid (2).

In myeloproliferative diseases as encountered in our patient, especially in those with symptoms related to a high platelet count, acetylsalicylic acid is usually administered (see case 10). However, the prolongation of the bleeding time and clinical bleeding may be excessive in patients with preexisting disordered haemostasis (23). It is therefore important to carefully evaluate the indication for an antiplatelet agent and to exclude avWS and/or acquired platelet dysfunction before prescribing acetylsalicylic acid to a patient affected by a myeloproliferative disorder.

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References