HAEMOSTASIS AND THROMBOSIS

Original article

Acquired von Willebrand syndrome in patients with monoclonal gammopathy of undetermined significance investigated using a mechanistic approach

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Background - Acquired von Willebrand syndrome (AVWS) has been reported to occur in association with monoclonal gammopathy, usually of undetermined significance (MGUS). It may present as a type 1 or type 2 von Willebrand factor (VWF) defect depending on the patient's representation of large VWF multimers.
Materials and methods - The mathematical model by Galvanin *et al.*, already employed for studying inherited von Willebrand disease (VWD), was used to explore the pathogenic mechanisms behind MGUS-associated AVWS.
Results - The patients studied showed significantly reduced VWF levels and function; an increased VWF propeptide to VWF antigen ratio; and all VWF multimers present but in reduced quantities, with the low-molecular-weight VWF forms being significantly more represented than those of higher molecular weight. Our mathematical model revealed a significantly increased VWF elimination rate constant, with values similar to those of type Vicenza VWD. An even more increased VWF proteolysis rate constant was observed, with values one order of magnitude higher than in type 2A VWD but, in contrast, no loss of large multimers. The model predicted the same elimination rate for high- and low-molecular-weight VWF multimers, but proteolysis of the high-molecular-weight forms also contributes to the pool of low-molecular-weight oligomers, which explains why they were relatively over-represented.
Discussion - In MGUS-associated AVWS the increase of both clearance and proteolysis contributes to the circulating levels and multimer pattern of VWF, with a phenotype that appears to be a combination of type Vicenza and type 2A VWD. Hence, the mechanisms behind the onset of AVWS seem to differ from those of inherited VWD.

Keywords: *von Willebrand factor, VWF survival, DDAVP, theoretical model.*

INTRODUCTION

Acquired von Willebrand syndrome (AVWS)[1] is a bleeding disorder resembling inherited von Willebrand disease (VWD) that occurs in patients with no personal or family history of bleeding. The acquired syndrome is strikingly similar to the inherited disorder in terms of diagnostic laboratory parameters, but it is not caused by any genetic defects in

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the von Willebrand factor (VWF) gene[2]. VWF is a large multimeric glycoprotein involved in the first phase of primary haemostasis, mainly promoting platelet binding to injured vessel walls, and platelet plug formation[2]. It also serves as a carrier and stabiliser of factor VIII (FVIII)[3].

AVWS may be the result of many different underlying pathological conditions, such as lympho- and myeloproliferative disorders, solid tumours, immune diseases, cardiovascular disorders, hypothyroidism, diabetes, and infectious diseases, or the side effects of drugs[1]. In most cases, total circulating VWF levels are lower than normal, or there is a loss of high-molecular-weight VWF multimers, which are the haemostatically most efficient form of VWF[4]. Low circulating VWF levels and/or the loss of only the high-molecular-weight multimers may be due to: (i) specific autoantibodies against FVIII/VWF; (ii) non-specific antibodies that form circulating complexes with VWF, cleared by Fc-bearing cells; (iii) VWF absorption on malignant cell clones; (iv) increased proteolytic degradation of VWF; or (v) loss of high-molecular-weight VWF multimers under high shear stress conditions[5-11].

About half of the reported cases of AVWS (48%) are caused by lymphoproliferative disorders, the most common being monoclonal gammopathy of undetermined significance (MGUS), which accounts for about 23% of all cases of AVWS in the International Registry of the Subcommittee on von Willebrand factor[1,12]. MGUS is a premalignant clonal plasma cell disorder characterised by the presence of a monoclonal protein in the plasma, no more than 10% of clonal plasma cells in the bone marrow, and no lymphoplasmacytic malignancies[12]. This condition is found in 3% of the general population over 50 years old, and only 0.3% of younger people, with a higher risk and earlier age of onset in blacks than in whites[6]. AVWS associated with MGUS has many features in common with type Vicenza VWD, a variant of the disease characterised by a very short VWF half-life despite normal synthesis of the protein[13]. Type Vicenza VWD has an autosomal dominant inheritance, and features very low levels of plasma VWF, a normal platelet VWF content, and the presence of ultra-large multimers (that are, however, haemostatically not more efficient than the high-molecular-weight multimers)[14]. The exact mechanism behind the aberrant VWF clearance caused by Vicenza-type mutations is still not fully understood. What we do know is that the clearance process occurs in the liver, and involves binding and uptake of VWF by macrophages and subsequent degradation of the internalised protein[15]. Measuring the levels of VWF propeptide (VWFpp), which is involved in VWF multimerisation, then cleaved and released separately in the blood flow, has been proven useful for identifying patients with type Vicenza VWD[16].

In this study, we applied a mathematical model to shed light on the pathogenic mechanism underlying MGUS-associated AVWS, demonstrating that increases in both clearance and proteolysis contribute to the reduced circulating VWF levels.

MATERIALS AND METHODS

Patients and controls were studied after obtaining their written informed consent in accordance with the Declaration of Helsinki.

Haemostatic tests

Blood samples were anticoagulated using sodium citrate (3.2%, 1:10, vol/vol). Platelet-rich and platelet-poor plasma were prepared, platelet function was analysed with a PFA100, and ristocetin-induced platelet aggregation (RIPA) was measured, as reported elsewhere[17]. Blood samples were anticoagulated with 50 mM EDTA, 50 IU/mL Trasylol, 10 mM leupeptin, and 60 mM N-ethylmaleimide as protease inhibitors. FVIII was measured using a one-step method with cephaloplastin as activated cephalin. Plasma and platelet VWF antigen (VWF:Ag) were measured using a home-made enzyme-linked immunosorbent assay (ELISA) with a horseradish peroxidase (HRP)-conjugated anti-VWF antibody (Dako, Glostrup, Denmark). VWF collagen binding (VWF:CB) was assessed with an ELISA method using type III collagen (Sigma, Milan, Italy), as explained elsewhere[18]. VWF ristocetin cofactor (VWF:RCo) was measured with normal washed, formalin-fixed platelets and 1.0 mg/mL of ristocetin in a Chronolog aggregometer. VWFpp was measured with a home-made ELISA using antibodies CLB-Pro 35 and CLB-Pro 14.3-HRP (Sanquin, the Netherlands). The values obtained were expressed in U/dL, taking as 100 the first dilution of the reference curve consisting of a pool of normal plasma samples[19]. Anti-VWF antibodies were detected by ELISA, as described elsewhere[20]. VWF multimer analysis was performed on high-gelling-temperature agarose containing 0.1% sodium

dodecyl sulfate, using 1.6% agarose gel to obtain low-resolution conditions. VWF multimers were detected by autoradiography with a sodium iodide ^{125}I-labeled purified anti-VWF antibody. The photographic plate was digitalised with an Epson Ds 50000 scanner (Seiko Epson Corporation, Suwa, Japan), and the images were analysed using the Fiji distribution of the ImageJ software (NIH, Bethesda, MA, USA)[21]. The multimer pattern was divided into four areas by molecular weight (low [1-5mer], intermediate [6-10mer], high [11-20mer] and ultra-large [>20mer]: LMW, IMW, HMW and ULMW, respectively) for further analyses.

1-desamino-8-D-arginine vasopressin (DDAVP) (Emosint, Kedrion, Castel Vecchio Pascoli, Italy) was administered subcutaneously at a dose of 0.3 µg/kg, and blood samples were collected before the infusion, then 15, 30, 60, 120, 180, 240, 360, and 480 minutes, and 24 hours afterwards. After administering 2,000 U of Haemate P (Behring GMBH, Hattersheim am Main, Germany), blood samples were collected at 4, 15, 30, 60, 120, 180, 240, 360, and 480 minutes, and 24 hours. A similar sampling schedule, but with the addition of a 1 minute time point, was used for the infusion of 2,000 U of Wilfactin (Kedrion).

Pharmacokinetic parameters and mathematical model

The time courses of the post-DDAVP plasma VWF concentrations were analysed: with a one-compartment model, using first-order input and output kinetics[22]; and with a two-compartment, physiology-based model proposed by Galvanin *et al.*[23], which is capable of characterising the mechanisms of VWF release, proteolysis and clearance, and its multimer distribution[23]. The latter model comprises a system of differential and algebraic equations, in which each subject is characterised by three main pharmacokinetic constants: the VWF release rate constant k_o; the proteolysis rate constant k_1; and the elimination rate constant k_e, which is assumed to be the same for UL+HMW multimers as for LMW multimers. The model is based on the assumptions: (i) that HMW and LMW multimers are present, in the basal state and/or after DDAVP; (ii) that UL and HMW multimers can be cleaved to form LMW multimers; (iii) that we can judge the quantities of UL + HMW + LMW multimers from VWF:Ag measurements; and (iv) that VWF:CB gives us a measure of the quantity of UL + HMW multimers.

Genetic analysis

DNA was extracted from peripheral blood leucocytes using the Maxwell® 16 Blood DNA Purification Kit, and a Maxwell 16 Instrument (Promega, Madison, WI, USA). Polymerase chain reaction amplification and sequencing of the *VWF* gene was performed as previously described[24].

RESULTS

Patients

The three patients studied were two men and one woman, all over 70 years old (**Table I**). They were referred to our centre for the study of haemostatic disorders because they had severe bleeding symptoms but no personal or family history of haemorrhagic disorders (one patient had even been a blood donor for years). All three had an IgG-kappa MGUS, so an acquired bleeding disorder was suspected, and the patients were studied accordingly. Twenty-five healthy subjects, nine with type Vicenza VWD, and three with type 2A VWD were enrolled as controls when applying the mechanistic approach. Their pertinent data are included in the *Online Supplementary Content*, **Table SI**.

Table I - *Main haemostatic parameters of the patients studied*

N.	Sex/ age	ABO blood group	PT %	aPTT sec	PFA100 sec	Platelet number ×10^3/µL	RIPA %	FVIII:C (U/dL)	VWF:Ag (U/dL)	VWF:CB (U/dL)	VWF:CB/Ag ratio	VWF:RCo (U/dL)	VWF:RCo/Ag ratio	Platelet VWF:Ag (U/dL)
1	M/75	O	95.8	47.9	>300	358	0	16.1	9.0	4.4	0.49	4.8	0.53	109.7
2	F/71	O	94	45.7	>300	304	0	10.9	11.0	6.8	0.62	6.6	0.60	71.3
3	M/81	A	72	46.3	>237	246	0	18	7.2	2.6	0.36	nd	nd	45.6
Normal range			70-100	30-40	94-193	150-450	60-84	60-160	60-160	65-150	>0.75	60-130	>0.75	70-140

PT: prothrombin time; aPTT: activated partial thromboplastin time; PFA: Platelet Function Analyzer; RIPA: ristocetin-induced platelet aggregation at 1.2 mg/mL; FVIII:C: factor VIII coagulant activity; VWF: von Willebrand factor; Ag: antigen; CB: collagen binding; RCo: ristocetin cofactor; nd: not determined.

Haemostatic findings

All patients showed (**Table I**) prolonged activated partial thromboplastin time, no PFA100 closure, no ristocetin-induced platelet aggregation, significantly reduced VWF:Ag (9 U/dL, 11 U/dL, and 7.2 U/dL for patients 1, 2 and 3, respectively), and even more reduced VWF:CB and VWF:RCo, with significantly lower than normal VWF:CB/VWF:Ag and VWF:RCo/VWF:Ag ratios (called the VWF:CB ratio and the VWF:RCo ratio, respectively, from now on). Platelet VWF content was normal in patients 1 and 2, and slightly lower than normal in patient 3 (45.6 U/dL *vs* normal 70-140 U/dL) (**Table I**). The search for anti-VWF antibodies was negative for all three patients. VWF multimer analysis showed that all oligomers were present, but in significantly reduced quantities (**Figure 1**). Most of the patients' VWF molecule was represented by LMW multimers (86.2±5.5% of all multimers, as opposed to 41.9±8.3% in normal plasma), while the IMW and HMW multimers accounted for just 9.3±2.3% and 3.4±2.3%, respectively (*vs* 31%±4.8 and 24.9±1.78% in normal plasma). VWFpp was measured to determine whether a shorter survival might explain the patients' low plasma VWF levels. It was normal for all three patients (suggesting normal VWF synthesis), but the VWFpp ratios were extremely high (11.9 for patient 1, 8.9 for patient 2, and 9.9 for patient 3, *vs* a normal range of 0.77-1.25) (*Online Supplementary Content*, **Table SI**), as seen in our type Vicenza VWD patients, whose VWFpp ratios range from 7.0-11.2.

Genetic analysis

To rule out any *VWF* gene mutations, the coding exons, splicing junctions, and 3' and 5' untranslated regions of the *VWF* gene were sequenced for all three patients. No mutations or suspicious single nucleotide polymorphisms were found, not even in patient 3, whose slightly reduced platelet VWF content might have been suggestive of a genetically determined defect of VWF synthesis.

Endogenous and exogenous VWF half-life and multimer pattern

In patient 1, a 24-hour DDAVP test showed a very short half-life for VWF:Ag (2.01 h *vs* normal 14±6.9 h), and an even shorter one for VWF:CB (0.91 h *vs* normal 10.6±3.9 h), as calculated with a one-compartment model. A similar reduction in the half-life of exogenous VWF was seen in patient 1 after the infusion of 2,000 U of Haemate P or 2,000 U of Wilfactin and in patient 2 after the infusion of 2,000 U of Haemate P. In short, both endogenous and exogenous VWF were quickly removed from these patients' circulation, further pointing to the absence of any inherited VWF defect.

Post-DDAVP multimer analysis for patient 1 (**Figure 2**) showed a significant increase in VWF multimers associated with the appearance of UL multimers at 15 min, which persisted until 60 min. The HMW and UL oligomers then started to decrease at 120 min, and at 360

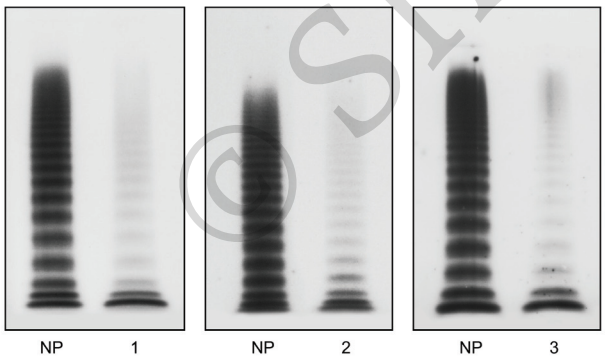

Figure 1 - von Willebrand factor multimer pattern observed in three patients (1-3) with monoclonal gammopathy of undetermined significance
Electrophoresis was performed under non-reducing conditions, using 1.6% agarose gel. The oligomers were detected with a ^{125}I anti-VWF antibody. High multimers are at the top, low ones at the bottom. NP: normal plasma; VWF: von Willebrand factor.

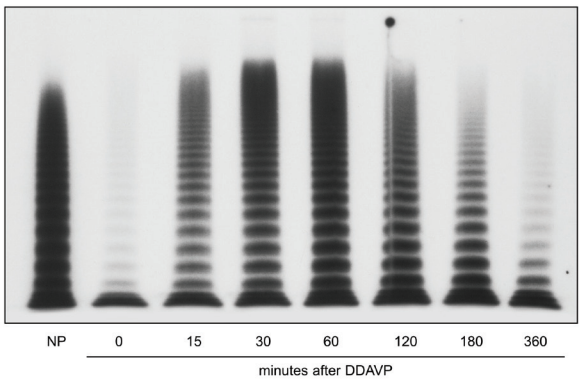

Figure 2 - von Willebrand factor multimer pattern observed in patient 1 before (0), and at various times after DDAVP infusion
For multimer representation, see legend to **Figure 1**. At 15 min, the increase in multimers is associated with the presence of ultra-large oligomers. Starting from 120 min, ultra-large and large multimers start to decline. At 360 min, the multimer pattern is much the same as before DDAVP infusion. DDAVP: 1-desamino-8-D-arginine vasopressin; NP: normal plasma.

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min the overall multimeric pattern was almost the same as before the DDAVP infusion. Administering Haemate P in patient 1 prompted a sudden increase in LMW and IMW multimers (at the first observation after 4 minutes), while no HMW VWF multimers were ever detectable at any time point (**Figure 3A**). The increase in LMW and IMW multimers persisted at 15 minutes, but after 60 minutes the IMW multimers started to decrease (**Figure 3A**), and by 240 minutes after administering Haemate P most of the infused VWF was no longer detectable. One minute after the infusion of Wilfactin in patient 1, the amount of LMW and IMW multimers appeared to increase, but no HMW forms were detectable at any time, suggesting that these forms might be quickly removed from (or might not persist in) the circulation (**Figure 3B**). The same was seen in patient 2 after administering Haemate P (*data not shown*).

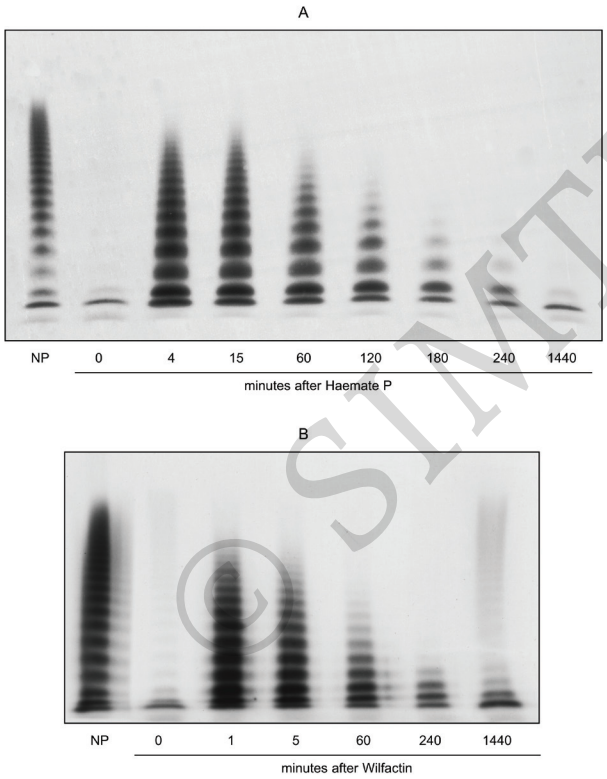

Figure 3 - von Willebrand factor multimer pattern seen in patient 1 after the infusion of 2,000 U Haemate P (panel A) and 2,000 U Wilfactin (panel B)
Note that large VWF multimers never appear in the circulation, even right after infusing the concentrate, and the other VWF oligomers seem to be quickly removed from the bloodstream.
VWF: von Willebrand factor; NP: normal plasma.

A mathematical model for exploring VWF half-life

The mathematical model proposed by Galvanin *et al.*[23] in VWD was used to explore DDAVP-induced VWF release, proteolysis and elimination in patient 1, in an effort to establish the main mechanism(s) behind the patient's AVWS. The results were compared with those of a pool of healthy subjects, and patients with type Vicenza or type 2A-II VWD. Representative results of the model's application are shown in **Figure 4**, and the pertinent statistics in the *Online Supplementary Content*, **Table SII**.

Post-DDAVP VWF elimination in patient 1, as expressed by the k_e parameter, which measures the rate at which the phenomenon occurs, was significantly faster than in the group of healthy subjects (9×10^{-3} min^{-1} *vs* 1.17×10^{-3} min^{-1}, respectively) (**Figure 4A**), consistently with a very short VWF half-life (*Online Supplementary Content*, **Table SII**). The patient's k_e (9×10^{-3}) was higher than in type 2A-II VWD (1.26×10^{-3}), and similar to the value seen in type Vicenza VWD (8×10^{-3}). The k_1 value - which quantifies the rate of conversion of large VWF multimers into smaller ones, *i.e.* the rate of proteolysis - was much higher in patient 1 than in the control group (1.13×10^{-2} *vs* 4.59×10^{-4}) or in type Vicenza VWD (1.5×10^{-3} min^{-1}), or even in type 2A-II VWD (4.23×10^{-3}) (**Figure 4B**). Indeed, the patient's k_1 value was one order of magnitude higher than in type 2A-II VWD, the variant characterised by a greater susceptibility of VWF to ADAMTS13. A slightly reduced VWF release rate (k_o), compared to that of the control group, was also apparent (**Figure 4C**). The k_1 value in patient 1 clearly fits with the rapid disappearance of HMW VWF multimers seen after DDAVP, as documented by the densitometer analysis (**Figure 4D**). After DDAVP, the proportion of LMW multimers (90%), relatively more represented than in normal subjects, decreased rapidly until 30 minutes after the injection (dropping to a minimum level of 31.3%), while the IMW and HMW forms increased, and a small quantity of UL forms appeared (**Figure 4D**). The situation was reversed at 60 minutes after DDAVP, when the proportion of HMW multimers started to decline and the LMW forms increased, up to 360 minutes. This behaviour was also predicted by the mathematical model that, using the VWF:Ag and VWF:CB values, showed an inversion in the respective proportions of HMW and LMW VWF multimers over time after DDAVP (**Figure 4D**, solid and dashed lines). The mathematical model was also able to

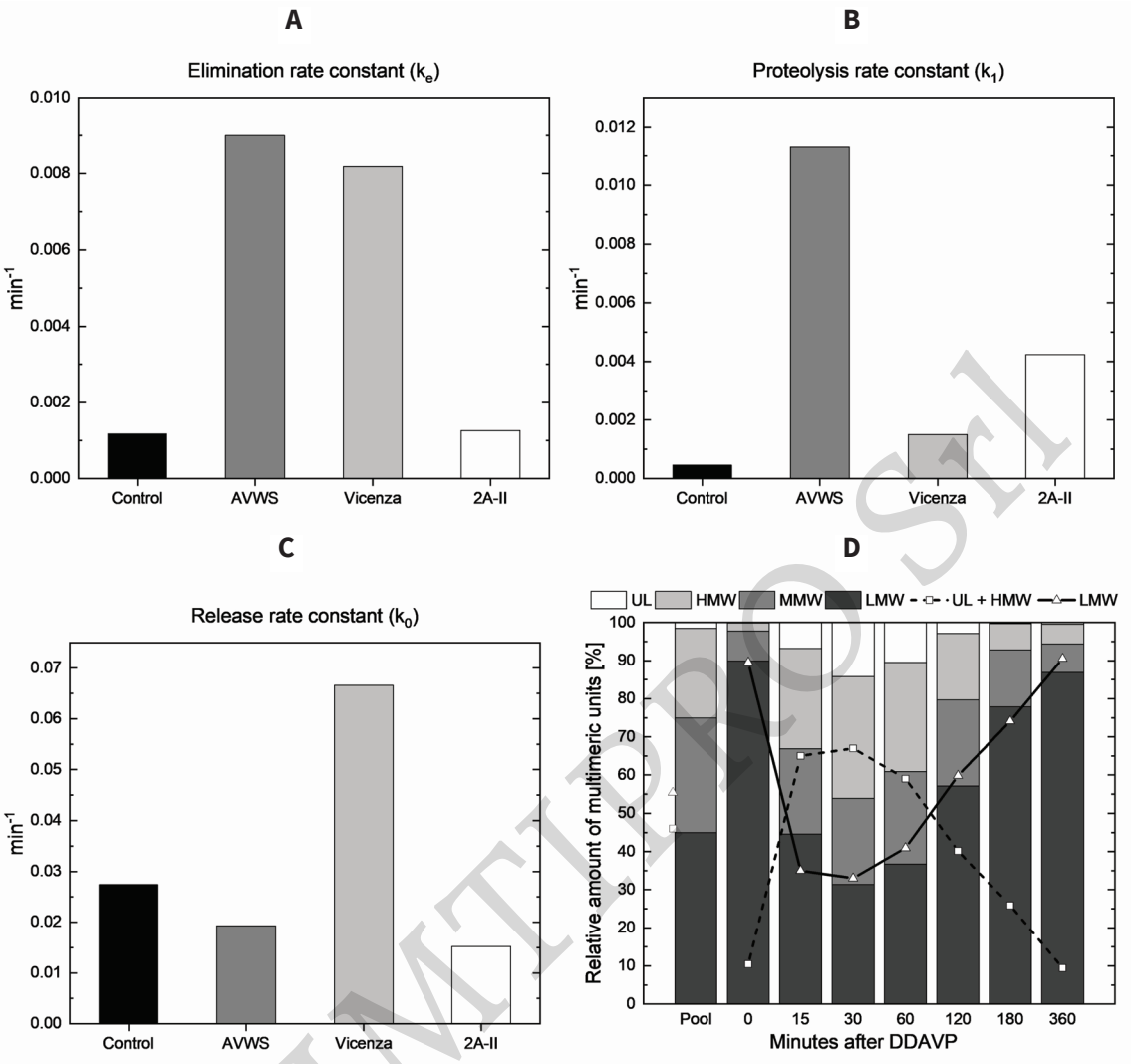

Figure 4 - Pharmacokinetic values: k_e (A), k_1 (B) and k_0 (C) estimated with the mathematical model in patient 1 with acquired von Willebrand syndrome, healthy controls, and patients with type Vicenza and type 2A-II von Willebrand disease (D)
Relative amounts of multimeric units over time after DDAVP challenge as quantified with ImageJ software from the photographic plate (stacked bars), and as predicted by the mathematical model in terms of UL+HMW *vs* LMW multimers (solid and dashed lines). Before the infusion, the proportion of the AVWS patient's LMW multimers was significantly larger than that of the MMW and HMW multimers, unlike the picture seen in normal plasma. DDAVP infusion drastically reversed the relationship between LMW and HMW von Willebrand factor multimers.
AVWS: acquired von Willebrand syndrome; DDAVP: 1-desamino-8-D-arginine vasopressin; UL: ultralarge, HMW: high-molecular-weight, MMW: intermediate-molecular-weight, LMW: low-molecular-weight.

quantify the patient's VWF multimers over time after administering DDAVP by comparison with the means for patients with type Vicenza and type 2A-II VWD, and healthy controls (**Figure 5**). The AVWS patient (**Figure 5A**) shares much the same increased multimer elimination rate as type Vicenza patients (**Figure 5B**) - with both UL + HMW and LMW multimers being eliminated completely after approximately 6 and 10 hours, respectively - but he differs in that the peak amount of LMW multimers is much higher. At the same time, he clearly shows a faster conversion of UL + HMW multimers into smaller ones than in 2A-II patients (**Figure 5C**), consistent with the patient's higher rate of proteolysis, followed by a rapid LMW multimer elimination. The peak amount of LMW

multimers was reached just 180 minutes after starting the DDAVP infusion, as opposed to 420 minutes in patients with type 2A-II VWD. Such a difference is due to the faster VWF elimination rate seen in the MGUS patient, compared with cases of 2A-II VWD. The relative distribution of the AVWS patient's multimers predicted by the model was consistent with the densitometric analysis of the multimeric patterns (**Figure 5D**): 6 hours after the DDAVP infusion, 90% of the multimers in the AVWS patients were LMW forms.

On the whole, applying the mathematical model to MGUS patients demonstrated that, once VWF had entered the circulation, it underwent increased proteolysis, and there was generally faster elimination of all VWF multimers, especially the higher-molecular-weight ones.

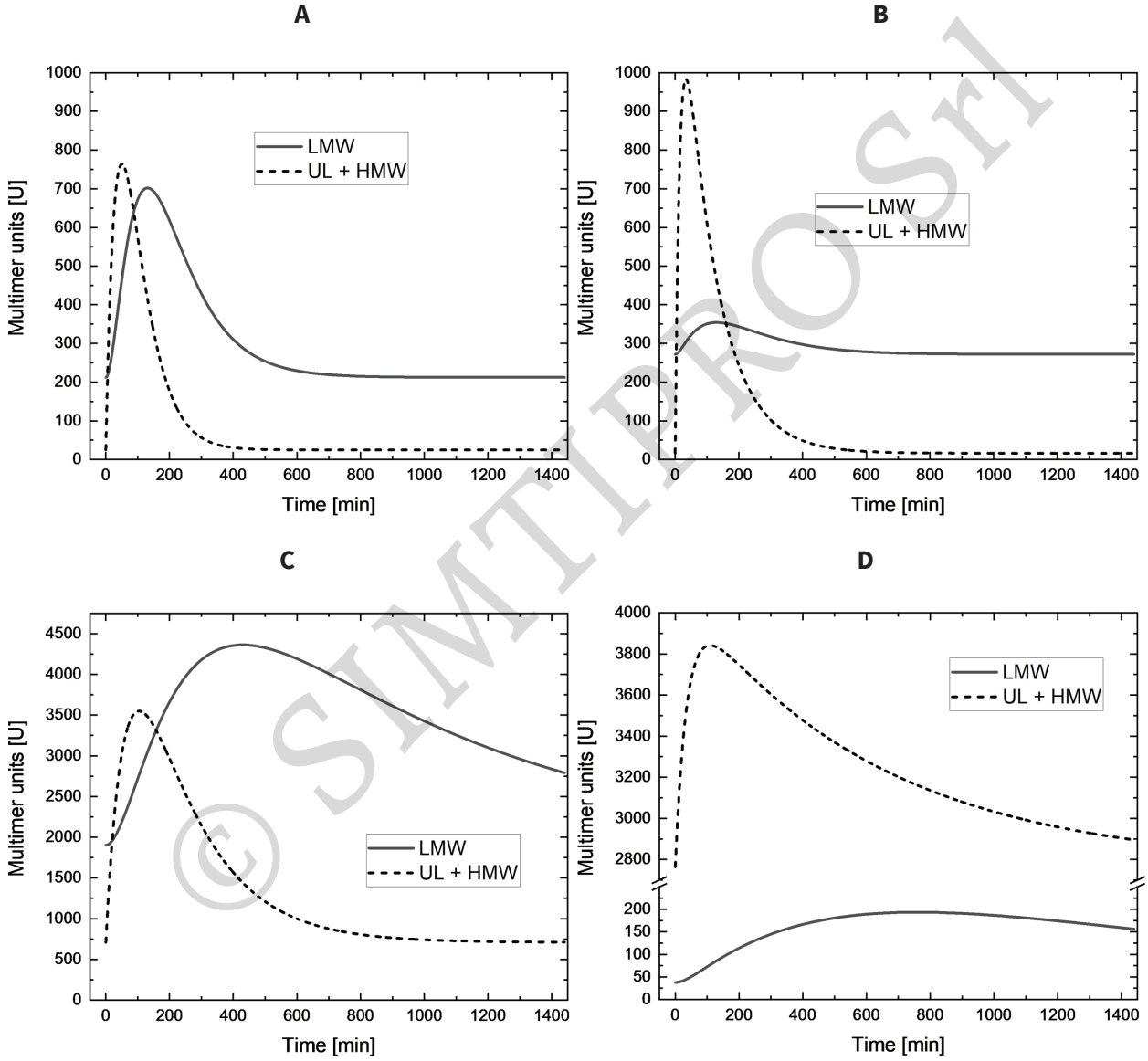

Figure 5 - Time course of multimeric units after DDAVP infusion as quantified by the model for patients with acquired von Willebrand wyndrome (A), type Vicenza von Willebrand disease (B), type 2A-II von Willebrand disease (C) and healthy controls (D)

UL: ultralarge; HMW: high molecular weight; MMW: intermediate molecular weight; LMW: low molecular weight.

DISCUSSION

The mechanistic approach to study VWD proposed by Galvanin *et al.*[23] was used to explore the abnormal biochemical pathways responsible for MGUS-associated AVWS. It enabled us to demonstrate that both accelerated elimination and, especially, increased proteolysis of VWF contribute to the onset of AVWS in patients with MGUS.

Our three patients had the same phenotype, characterised by significantly reduced VWF level and function, not associated with any loss of HMW VWF multimers, a picture resembling a moderate-to-severe VWD. Although all multimers were present, they were in different proportions from those in normal plasma, since most of the VWF molecule was represented by LMW oligomers, a feature that would suggest an increased proteolysis of large VWF multimers. No specific anti-VWF antibodies were found, ruling out auto-immune processes as a cause of the unbalanced multimer composition and VWF functional abnormalities.

The MGUS patients' AVWS was characterised by a rapid elimination of VWF from the circulation (as suggested by their very high VWFpp ratios), and a much shorter post-DDAVP VWF survival (calculated with a one-compartment model), as already seen in patients with type Vicenza VWD (the variant with the shortest VWF half-life among all inherited forms of VWD)[22]. The rapid elimination of exogenous VWF confirms that the shorter VWF half-life in these patients was not due to any intrinsic VWF defects.

The post-DDAVP multimer pattern showed a significant increase in VWF levels, and the appearance of UL VWF oligomers –as happens in normal subjects– which did, however, quickly decline or disappear. The post-DDAVP time course analysis showed an inverse relationship between the quantities of UL/HMW and LMW multimers, hinting at an enhanced VWF proteolysis contributing to the causes of AVWS in these patients. The two-compartment, physiology-based mathematical model proposed by Galvanin *et al.*[23] was used to shed light on the underlying mechanisms. This model has already proven useful for analysing the post-DDAVP time course of VWF:Ag and VWF:CB values in the process of diagnosing and characterising inherited VWD variants[23,25,26]. The model is robust enough not to be influenced by the number of patients studied. It is capable of exploring the release of VWF from endothelial cells, its proteolysis and its clearance, *i.e.* the biochemical pathways regulating plasma VWF levels in both inherited and acquired defects. Unlike other models, it enables us to investigate VWF proteolysis, thus providing information about the kinetic behaviour of the large VWF multimers and their relative representation compared to the smaller ones, even where no multimer analysis is available. Using Galvanin's model, we found that the pharmacokinetic values of our patient's VWF featured a very high k_e constant (a measure of the VWF elimination rate), the value of which was the same as in type Vicenza VWD. Using the k_1 rate, the model also identified a markedly increased VWF proteolysis, which was at least one order of magnitude higher than in patients with type 2A-II VWD, the inherited VWD variant in which increased proteolysis of VWF is responsible for the loss of HMW VWF multimers[25]. Our AVWS patients thus shared with type Vicenza VWD a very short VWF half-life, but differed from them in that they also had a very high rate of VWF proteolysis, even more pronounced than in type 2A-II VWD patients. On the other hand, they differed from type 2A-II VWD patients because of the shorter VWF half-life and the presence of HMW VWF multimers.

The results obtained by the mathematical model were confirmed by the post-DDAVP behaviour of the patient's VWF multimers, which offered a solid demonstration that MGUS-associated AVWS is a combination of type Vicenza and type 2A-II VWD. In fact, both HMW and LMW VWF multimers are quickly removed from the circulation, as seen in type Vicenza VWD - but in AVWS a significant proteolysis of HMW oligomers also contributes to the amount of LMW multimers, as observed in type in 2A-II VWD, although with no loss of large VWF multimers

The diagnosis of AVWS may be a difficult matter[27], because routine laboratory diagnostic flow charts and findings are the same for inherited or acquired VWD[28], and also because of the different origin of the acquired VWF defect. Thanks to our mathematical model, we now know that, in addition to the already described increased VWF elimination rate[29], another factor is involved in MGUS-associated AVWS, *i.e.* proteolysis of VWF. This should be taken into consideration when we predict the bleeding risk of these patients and decide on how best to treat them[30], especially when long-lasting haemostatic coverage is required.

CONCLUSIONS

Taking a mechanistic approach to investigate patients with MGUS-associated AVWS allowed us to reveal a complex pathogenic mechanism involving both faster elimination and enhanced proteolysis of VWF, via mechanisms that differ from those of inherited variants of VWD. Studying AVWS patients with a combination of routine laboratory VWF tests, VWFpp measurements and our mechanistic approach will help to better characterise the haemostatic profile and bleeding risks of such patients.

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AUTHORSHIP CONTRIBUTIONS

EG performed the haemostatic tests and wrote the paper; FG did the mathematical modelling; VD conducted the genetic analysis and analysed the data; AC designed the research and wrote the paper.

The Authors declare no conflicts of interest.

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Table SI - *Main haemostatic parameters of patients with type Vicenza and type 2A-II von Willebrand disease and healthy subjects*

Subjects	Age	Blood group O/non-O	aPTT sec	RIPA (%)	FVIII:C (U/dL)	VWF:Ag (U/dL)	VWF:CB (U/dL)	VWF:CB /Ag ratio	VWF:RCo (U/dL)	VWF:RCo/Ag ratio	VWFpp (U/dL)	VWFpp ratio	VWF mutations
Type Vicenza VWD	26-59	2/6	42.5±5.3	15.6±23.6	22.2±12.0	10.5±4.7	10.5±4.5	0.98±0.12	6.9±4.8	0.83±0.22	77.4±49.2	9.53±1.9	p.R1205H, p.M740I + p.R1205H
Type 2A-II VWD	56	0/1	41.4	0	64	39.4	6.25	0.15	11.6	0.29	70.9	1.8	p.L1562P
Healthy	19-52	17/25	34.8±3.3	NP	101.2±40.8	96.3±46.4	99.4±45.9	1.02±0.09	NP	NP	84.2	096±0.12	
Normal range			30-40	60-84	60-160	60-160	65-150	>0.75	60-130	>0.75	60-160	≥0.74	

aPTT: activated partial thromboplastin time; RIPA: ristocetin-induced platelet aggregation at 1.2 mg/mL; FVIII:C: factor VIII coagulant activity; VWF: von Willebrand factor; Ag: antigen; CB: collagen binding; RCo: ristocetin cofactor; pp: propeptide; VWD: von Willebrand disease; NP: not performed

Table SII - *Estimated pharmacokinetic parameters and D values including statistics obtained after parameter estimation for patient 1 with acquired von Willebrand syndrome, patients with type 2B, 2A-II, and Vicenza von Willebrand disease and a control group*

PK param.	AVWS		2B		2A-II		Vicenza		Control
	Estimate (SD)	t-value	Mean (SD)	p-value	Mean (SD)	p-value	Mean (SD)	p-value	Mean (SD)
k_0 [min^{-1}]	1.93E-02 (4.46E-03)	2.16	1.77E–02 (7.02E–03)	3.76 E–03	1.52E–02 (-)	-	6.66E–02 (3.60E–02)	9.82 E–03	2.74E-02 (8.41E-03)
k_1 [min^{-1}]	1.13E-02 (2.24E-03)	2.52	4.71E–03 (6.79E–03)	3.17 E–03	4.23E–03 (-)	-	1.50E–03 (2.99E–03)	7.21 E–01	4.59E-04 (6.40E-04)
k_e [min^{-1}]	9.00E-03 (2.23E-03)	2.01	3.23E–03 (1.18E–03)	6.09 E–05	1.26E–03 (-)	-	8.18E–03 (1.72E–03)	3.20 E–06	1.17E-03 (6.88E-04)
D [U/dL]	1.44E+03 (4.21E-04)	1.71	5.97E+02 (3.32E+02)	9.68 E–01	6.49E+02 (-)	-	2.71E+02 (1.80E+02)	1.93 E–03	5.07E+02 (1.51E+02)

Mean values and statistics are shown. The standard deviation (SD) is indicated in parentheses; 95% confidence t-values for parameters failing the t-test are indicated in bold (reference t-value is t^{ref} = 1.75). PK: pharmacokinetic; AVWS: acquired von Willebrand syndrome.