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VON WILLBRAND DISEASE: CONTRIBUTIONS AND CHALLENGES OF BIOLOGICAL DIAGNOSIS

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SUMMARY
Willebrand disease is the most common constitutional bleeding disorder, affecting approximately 0.6 to 1.3% of the population. It is characterized by a deficiency in Willebrand factor, a protein that plays a crucial role in primary hemostasis and, indirectly, in coagulation. Transmitted autosomally, this disease affects both men and women, most often dominantly. It presents in various clinical forms, ranging from severe to mild, or even asymptomatic. Diagnosis is based on the results of hemostasis tests. An initial assessment, generally orientative, must be supplemented by more specialized examinations in case of doubt. Once the diagnosis has been established and confirmed, characterizing the type of Willebrand disease becomes crucial, as it guides the choice of therapeutic interventions.

KEYWORDS: Von Willebrand disease, Von Willebrand factor, Biological diagnosis, Factor VIII.

INTRODUCTION

Willebrand disease (VWD) is the most common constitutional abnormality of hemostasis. Initially described in 1926 by Erik von Willebrand in several members of a family from the Aaland archipelago in Finland.[2] Its prevalence is estimated between 0.6% and 1.3% of the general population, placing it ahead of hemophilia.[3] In Morocco, the frequency of Willebrand disease is not known, mainly due to the discretion of clinical signs, which leads to underdiagnosis. This hemorrhagic disease, transmitted in an autosomal mode, often dominantly, affects both sexes and results from a quantitative or qualitative abnormality of the Willebrand factor (VWF), leading to disturbances in both primary and secondary hemostasis of coagulation. VWD presents a great heterogeneity in its clinical, phenotypic, genetic and biological expression. The biological characterization of this disease is an essential step to guide an appropriate therapeutic choice.

ROLE OF THE WILLEBRAND FACTOR

VWF is a high molecular weight multimeric glycoprotein whose gene located on the short arm of chromosome 12 is expressed in endothelial and megakaryocytic cells, which are the sites of synthesis and storage of VWF (Figure 1). The secretion of stored VWF multimers occurs in response to physiological (increased shear forces during a vascular breach) or pharmacological (desmopressin) stimuli. In addition, VWF regulation involves mechanisms influenced by environmental factors such as age, stress, and an inflammatory syndrome that can lead to increases in VWF levels. Genetic factors, including ABO blood type, also influence these levels, which are generally lower in blood type O subjects.[4] In addition, hormonal factors, such as pregnancy, can lead to a significant increase in this factor from the second trimester onwards. In pathology, certain chronic conditions such as hyperthyroidism, renal failure, diabetes, liver failure, and neoplasias are associated with an elevation of VWF levels.[5],[1],[3]

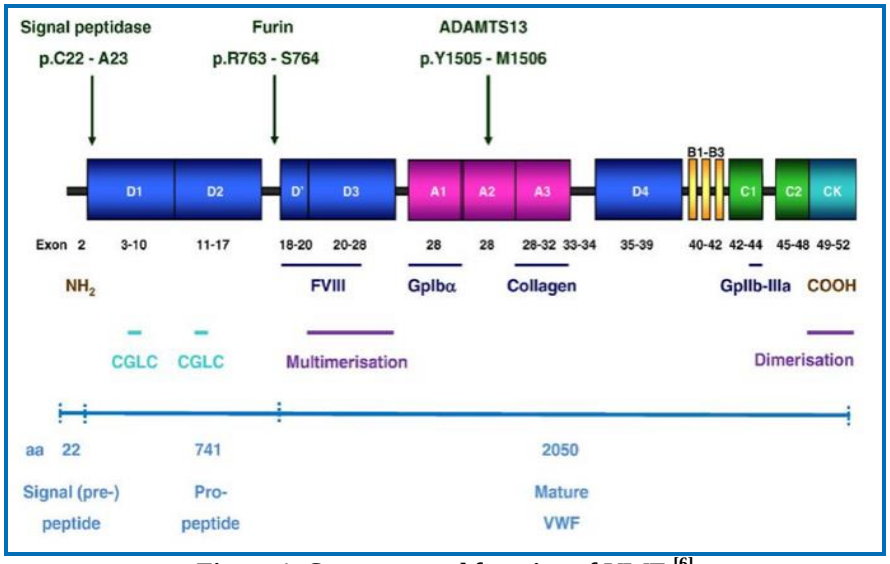

Figure 1: Structure and function of VWF.[6]

VWF plays two essential roles in hemostasis

- **Primary hemostasis:** In the event of a vascular breach, VWF binds to subendothelial collagen in a globular form. Upon vasoconstriction, its multimers adopt an elongated conformation, exposing binding sites for platelet glycoprotein Ib (GpIb) receptors on the surface. This leads to platelet adhesion to the subendothelium via VWF. After adhesion, platelets become activated, expose GpIIbIIIa receptors, bind fibrinogen and VWF, thereby promoting platelet aggregation and the formation of the platelet plug that occludes the vascular breach.
- **Secondary hemostasis:** VWF binds to Factor VIII (F VIII), an essential cofactor for the generation of activated factor X. Thus, by forming this complex, VWF stabilizes FVIII, facilitates its transport to the vascular lesion, reduces its clearance, and protects it from plasma proteolysis. Therefore, the level of FVIII depends on the level of VWF in the circulation[5] (Figure 2).

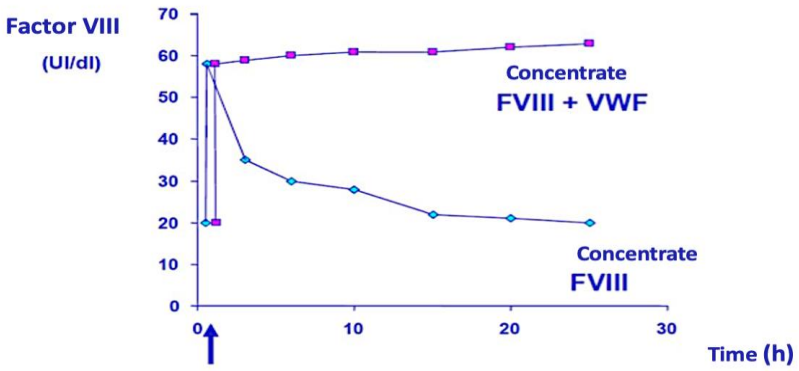

Figure 2: Role of VWF in its binding to FVIII.[7]

CLASSIFICATION OF WILLEBRAND'S DISEASE

The classification established by the International Society on Thrombosis and Haemostasis (ISTH) describes three types of VWD phenotypes different[1] (Figure 3):

- **Type 1:** The most common form (70 to 80%), is characterized by a partial quantitative deficiency of VWF. Patients are classified according to the degree of VWF deficiency, which can be mild, moderate, or severe. Transmission of this form is autosomal dominant.
- **Type 2:** Representing 15 to 20% of patients, this form is distinguished by a qualitative anomaly of VWF, and groups together several subtypes:
  - Subtype 2A: Decreased affinity of VWF for platelets, associated with the absence of high molecular weight multimers.
  - Subtype 2M: Decreased VWF affinity for platelets, not related to an abnormality of VWF multimers.
  - Subtype 2B: Increased affinity of VWF for platelets.
  - Subtype 2N: Decreased affinity of VWF for factor VIII (FVIII).
- **Type 3:** Characterized by a total quantitative deficit in VWF. This form is severe, very rare and its transmission is recessive.

Type	Subtype	Pathophysiology	Inheritance	Frequency
1		Partial quantitative deficiency of vWF	Dominant	50 to 75%
2		Qualitative deficiency of vWF	Dominant	20 to 45%
	2A	Decreased affinity of vWF for platelets with absence of high and intermediate molecular weight multimers	Dominant	
	2M	Decreased affinity of vWF for platelets without multimer abnormalities	Dominant	
	2B	Increased affinity of vWF for platelets	Dominant	
	2N	Decreased affinity of vWF for FVIII	Recessive	
3		Total quantitative deficiency of vWF	Recessive	< 5%

Figure 3: Classification of VWD according to ISTH.[1]

CLINICAL MANIFESTATIONS OF WILLEBRANDS DISEASE

The diagnosis of VWD is based on a detailed interview and a careful clinical examination, then leading to biological analyses. As in all anomalies of primary hemostasis, the hemorrhagic symptomatology is essentially cutaneous-mucosal, with clinical manifestations, either spontaneous or caused by trauma (dental avulsion, surgical procedure) even minimal, and include ecchymosis for minimal trauma, prolonged bleeding after cuts, bilateral epistaxis, gingivorrhagia, menometrorrhagia, and gastrointestinal hemorrhages.

VWD hemorrhagic syndrome is inconsistent and of variable intensity depending on the disease subtype, including gastrointestinal angiodysplasias in types 2 and 3, while joint hemorrhages, specific to type 3, are associated with severe factor VIII deficiency. The history assesses the severity of symptoms according to various criteria.

BIOLOGICAL DIAGNOSIS OF WILLEBRANDS DISEASE

The biological exploration of VWD has a dual purpose: on the one hand, to highlight the deficiency and, on the other hand, to identify the type and subtype of the anomaly. Different biological tests are useful to establish the diagnosis and specify the type of VWD. The interpretation of the results must take into account the clinical context, age and ABO blood group. The biological tests will not be detailed technically. Our work focuses primarily on their place and their value in the different stages of VWD diagnosis.

Routine screening biological tests

First-line biological tests play a crucial role in the screening of VWD but their usefulness may be limited in certain cases. They are mainly carried out during the exploration of a hemorrhagic syndrome, a preoperative assessment or in the presence of a family history of bleeding.[1]

- **Platelet count**: Systematically performed, it is part of the blood count and allows the number of platelets circulating in the blood to be determined. Generally normal except in subtype 2B where there may be moderate thrombocytopenia, often intermittent, which increases with age.
- **Bleeding time (BT):** is a global test that assesses primary hemostasis in vivo, simultaneously exploring the blood vessel, platelets and VWF. It is usually performed using the Ivy method, involving an incision in the forearm. Although often prolonged, BT may remain normal in minor forms such as type 1 and type 2N of VWD. In severe forms, BT may be significantly prolonged (>30 min). It is important to note that prolonged BT may indicate not only the presence of VWD, but also other conditions such as thrombopathies or thrombocytopenias. However, a normal BT does not exclude the diagnosis of VWD. Despite its usefulness, BT has limitations, including low reproducibility, lack of specificity, operator dependence and a high risk of false negatives.[8]
- **Occlusion time (OT) measurement:** measured by the PFA-200 corresponds to the time required for platelet plug formation. This measurement reproduces the primary hemostasis process and facilitates rapid detection of its alterations by simulating in vitro the hemodynamic conditions of platelet adhesion and aggregation. Citrated whole blood is drawn into a capillary by a vacuum system, generating a shear force similar to that encountered in small capillaries. The blood flows through a collagen-coated capillary, to which either ADP or epinephrine (agents causing VWF-dependent platelet adhesion) is added. Upon contact with the membrane, platelets adhere, become activated, secrete the contents of their granules, and finally aggregate. The PFA-200 measures the TO, corresponding to the time required for flow to stop across the membrane. In case of deficit, the TO will be prolonged. Despite the high sensitivity of PFA-200 for the detection of VWF deficiencies of

approximately 90% for all types combined and 100% for types 2A and 3, it lacks specificity while retaining great interest as a screening test.[9],[10]

- **Partial thromboplastin time with activator (PTT):** is the clotting time of recalcified deplateleted plasma in the presence of phospholipids (cephalin) and an activator (kaolin) of the contact coagulation system. Sensitive only to FVIII deficiency. It is always prolonged in type 3 and type 2N, and often normal in other types with qualitative VW abnormality.[1]

Specialized examinations

To establish the diagnosis of VWD, three specific biological tests must be carried out on the same sample without being dissociated (Figure 4).[11],[12],[13]

- **VWF immunoassay (VWF:Ag):** This test is performed using specific antibodies, either by immunoelectrophoresis, immunoradiometry (IRMA) or enzyme-linked immunosorbent assay (ELISA), the latter being the reference method. It allows the measurement of the amount of circulating plasma VWF, regardless of its functionality, hence the interest in supplementing it with VWF:RCo. Thus, in type 3 patients, VWF:Ag is undetectable in plasma and platelets, in type 1, the plasma VWF:Ag level is more or less reduced. While type 2 patients may have normal VWF:Ag levels.
- **Functional assay of VWF (vwF: RCo): The** ristocetin test, considered the most sensitive and specific, is currently the first-line reference test, although new promising tests are available on the market. It assesses the functional quality of VWF by measuring its ability to bind to platelet GpIb. This test, performed in the presence of fixed platelets, ristocetin (an antibiotic with a unique property allowing in vitro interaction of vWF with the platelet) and different dilutions of plasma, allows the determination of vWF activity, reduced in types 1, 2A and 2B, and is undetectable in type 3.[14]
- **Coagulant FVIII (FVIII:C) assay:** Specific assay used in routine practice for the diagnosis of FVIII deficiency, the level of which decreases in parallel with the VWF:Ag level. In the particular case of type 2N VWD, the FVIII deficiency is clear and dissociated from the VWF levels. Furthermore, it is reduced in moderate forms of the disease and greatly reduced in severe forms (2-5%), while it can remain normal in mild forms.

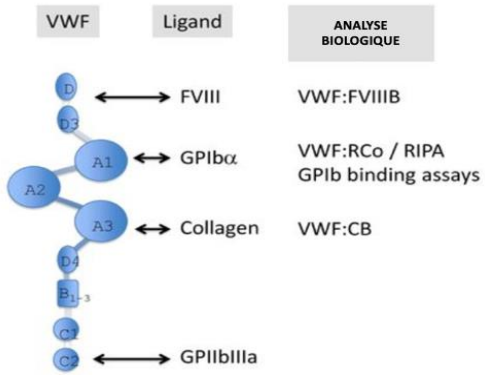

Figure 4: Indicative diagrams of specialized biological examinations.[15]

Finally, the calculations of the VWF:RC0/ VWF:Ag and FVIII/ VWF:Ag ratios are of great diagnostic interest. A ratio close to 1 suggests a quantitative anomaly while a ratio <0.7 indicates a qualitative anomaly.

Discriminative and specialized tests

These analyses, carried out in specialized laboratories, require special expertise for their interpretation. Although they are not always essential for making a diagnosis, they are useful for classifying the disease according to its types and subtypes, as well as for characterizing certain rare forms.[3],[11],[12],[16]

- **Platelet aggregation in the presence of ristocetin (RIPA):** This test measures aggregation in the presence of various concentrations of ristocetin . It is particularly useful for detecting increased affinity of VWF for platelet GpIb, particularly in type 2B VWD. This method can detect aggregation at very low doses of ristocetin (<0.8 mg/ mL), which do not cause aggregation in a normal individual (Figure 5).

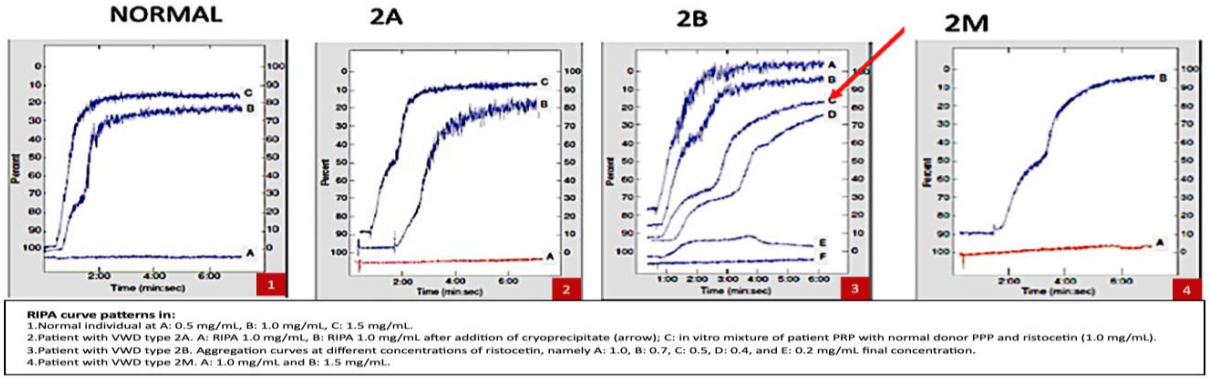

Figure 5: Platelet aggregation patterns in the presence of ristocetin.[17]

- **VWF-FVIII Binding Study:** This test measures the binding of purified exogenous FVIII to the patient's VWF. It is of crucial biological importance in the diagnosis of subtype 2N, in which this function is absent or greatly reduced, and allows the differentiation of VWD type 2N from hemophilia A where the affinity is preserved (Figure 6). It is systematically performed in the presence of a reduced FVIII:C/VWF:Ag ratio (< 0.5).

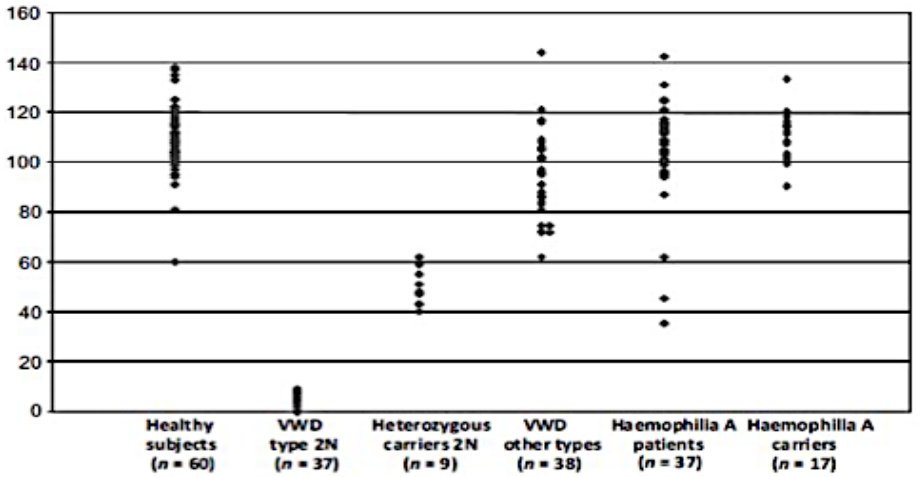

Figure 6: Analysis of VWF binding to FVIII.[7]

- **Analysis of VWF multimeric distribution**: electrophoretic method allows to separate and quantify the different multimeric forms of VWF, and allows to differentiate VWD of types 2M from 2A (Figure 7).

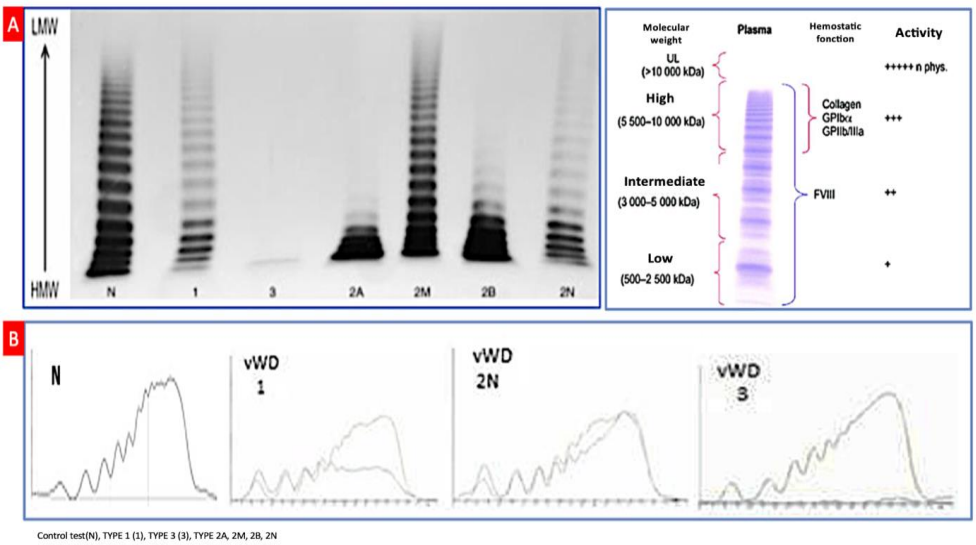

Figure 7: Analysis of VWF multimeters by 1.5% agarose gel electrophoresis (A); and densitometric evaluation (B).[7]

- **D etermination of the propeptide,** a marker of VWF synthesis, makes it possible to demonstrate accelerated clearance of VWF observed in certain forms of type 1 VWD or in acquired Willebrand syndrome.
- **Anti-VWF antibody testing:** These are polyclonal antibodies that inhibit the binding of VWF to platelets and can be measured in patients with type 3 VWD who are likely to develop anti-VWF alloantibodies (7-10% of patients). This assay is also performed in cases of suspected acquired von Willebrand syndrome, as some forms of this syndrome are associated with anti-VWF autoantibodies.
- **Study of VWF binding to platelets:** Allows not only to distinguish patients whose VWF has a high affinity with GPIb (type 2B) from those for whom it is reduced (type 2A or 2M) but also to discriminate type 2N from pseudo-Willebrand disease;

- **Study of VWF binding to Collagen:** This test is the only one that allows, thanks to the ELISA technique, the screening of very rare variants presenting a molecular anomaly in the collagen binding domain. Although it is complementary to VWF: Rco, this analysis lacks standardization and is highly dependent on VWF multimerization. It is abnormal in most types of von Willebrand disease.
- **Genetic analysis**: The search for molecular abnormalities at the level of the VWF gene is carried out using sequencing and enzymatic amplification (PCR) techniques from platelet DNA (Figure 8). This approach is reserved for patients who meet the biological criteria defined by the CRMW, in particular those presenting a phenotypic profile of VWD type 2 or 3, or type 1 with a VWF:Ag level < 30 IU/ dL.

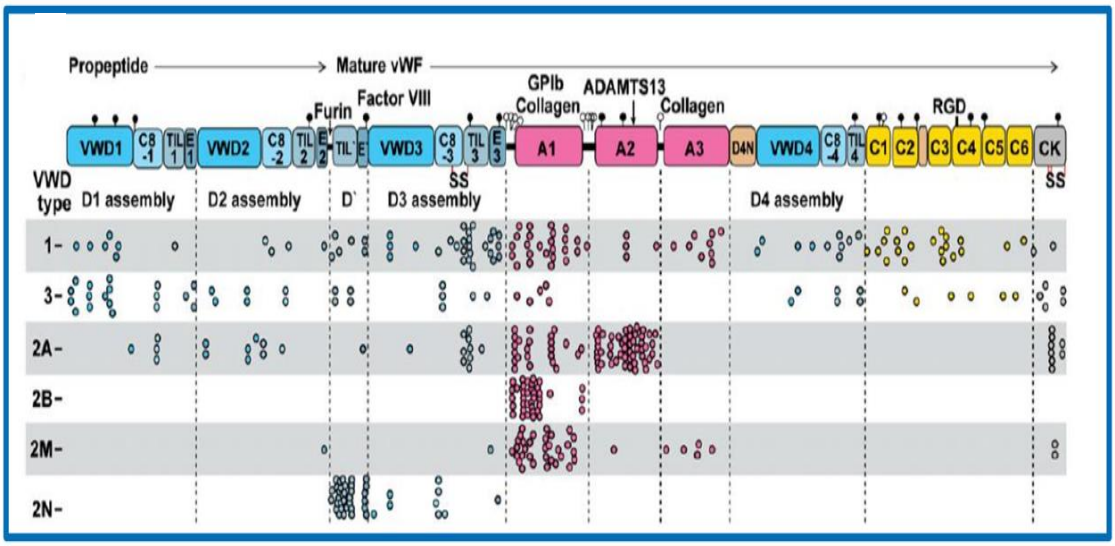

Figure 8: Representation of the sites of the main genetic anomalies according to the type of VWD.

Finally, all these biological explorations can be synthesized in a summary table accompanied by a decision tree, thus making it possible to establish a diagnostic strategy and optimal patient management (Figure 9).

	Type 1	Type 3	Type 2A	Type 2M	Type 2B	Type 2N
BT or PFA-100	↑ (or N)	↑↑	↑ (or N)	↑ (or N)	↑ (or N)	N
Platelets	N	N	N	N	↓	N
aPTT	↑ (or N)	↑↑	N (or ↑)	N (or ↑)	N (or ↑)	↑↑
FVIII	↓ (or N)	↓↓↓ (≤ 5%)	N (or ↓)	N (or ↓)	N (or ↓)	↓↓
VWF:Ag	↓ (< 50%)	↓↓↓ (< 1%)	N (or ↓)	N (or ↓)	N (or ↓)	N (or ↓)
VWF:RCo	↓ (< 50%)	↓↓↓ (< 1%)	N (or ↓)	N (or ↓)	N (or ↓)	N (or ↓)
VWF:Ag/VWF:RCo	N	Not relevant	↓	↓	↓	N
VWF:Ag/FVIII:C	N	N	N	N	N	↓
Multimeric Profile	Loss of all multimers	Absence of all multimers	Absence of HMW and intermediate MW multimers	Absence of HMW multimers	Absence of HMW multimers	N
RIPA at low dose (0.5 mg/mL)	-	-	-	-	+	-

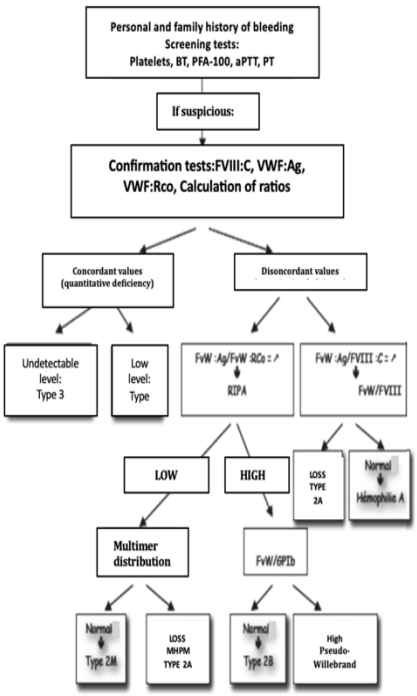

Figure 9: Summary table and decision tree for biological examinations during VWD.

CONCLUSION

Although VWD was first described over 90 years ago, it remains a constantly evolving field of research. In Morocco, its frequency is unknown, mainly due to the subtlety of clinical signs, which leads to underdiagnosis. Therefore, the development and evaluation of simple and standardized tools to assess the amount of bleeding could facilitate the diagnostic process and improve the identification of patients requiring treatment. On the diagnostic level, the introduction of new tests could potentially improve diagnostic performance.

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