von Willebrand Factor as New Noninvasive Predictor of Portal Hypertension, Decompensation and Mortality in Patients With Liver Cirrhosis

Thesis

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List of Abbreviations

ALT ..... Alanine aminotransferase

AFP..... Alpha feto protien

AMP ..... Adenosine mono-phosphate

AST ..... Aspartate aminotransferase

CBC ..... Complete Blood Count

CK ..... Cystine knot

CLDs ..... Chronic liver diseases

CSPH ..... Clinically significant portal hypertension

CT ..... Computerize Tomography

DDAVP ..... Desamino-8-D-arginine vasopressin

ECM ..... Extracellular matrix

ECs ..... Endothelial cells

EIA ..... Enzyme immunoassay

FVIII ..... Factor VIII

GGT ..... Glutamyl transpeptidase

GP ..... Glycoprotein

HBV ..... Hepatitis B virus

HCC ..... Hepatocellular carcinoma

HCV ..... Hepatitis C virus

HDV ..... Hepatitis D virus

HGV ..... Hepatitis G virus

HMM ..... High molecular mass

HMWvWF ..... High molecular weight

List of Abbreviations (Cont...)

HSCs ..... Hepatic stellate cells

HVPG ..... Hepatic venous pressure gradiant

INR ..... International normalized ratio

IRMA ..... Immunoradiometric assay

IQR ..... Inter quartile range

MELD ..... Model for end stage liver disease

MRI ..... Magnetic resonance imaging

NASH ..... Non-alcoholic steatohepatitis

PFV ..... Portal flow velocity

PH ..... Portal hypertension

PT ..... Prothrombin time

PVD ..... Portal vein diameter

RCO ..... Restocetin cofactor

TAV ..... Time averaging flow velocity

TE ..... More recently, transient elastography

TIPS ..... Transjugular intrahepatic portosystemic shunt

v WF ..... von Willebrand factor

v WF-Ag ..... von Willebrand factor antigen

vWF: Ag II ..... vWF antigen II

vWFpp ..... vWF propeptide

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INTRODUCTION

Portal hypertension (PH) accounts for the major complications of liver cirrhosis, such as ascites, variceal hemorrhage and decompensation. Early diagnosis of PH is essential for the management of patients with cirrhosis ***(Thabut et al., 2011).***

Clinically significant portal hypertension (CSPH), {HVPG (hepatic venous pressure gradient) 10mmHg}, is associated with a higher risk of liver-related mortality, development of varices, and other PH related complications. An HVPG 12 mmHg is associated with a higher risk of bleeding from varices ***(Bosch et al., 2006).***

Normally, portal blood flows towards the liver (hepatopetal flow), In normal patients the mean PV (portal velocity) flow rate is 13 to 23 cm/sec but in patients with portal hypertension it tend to decrease, the mean PV (portal vein) velocity may vary depending on the presence and location of spontaneous shunts ***(Al-Nakshabandi et al., 2006).***

More recently, transient elastography (TE) was described as a non invasive tool for the diagnosis of PH in patients with liver cirrhosis, but the costs and availability of TE represent limiting factors in smaller hospitals. Thus, the recent Baveno V consensus conference on PH recommended to investigate and

identify further noninvasive markers for PH ***(De Franchis et al., 2010)***.

Hepatic decompensation is the most important predictor of prognosis and mortality in patients with liver cirrhosis, with several precipitating factors contributing to the first event of decompensation ***(D'Amico et al., 2006)***.

Endothelial cells dysfunction is considered as an important determinant of the increased intrahepatic vascular resistance in cirrhotic livers ***(Iwakiri et al., 2007)***.

von Willebrand factor antigen (v WF-Ag) is released by activated endothelial cells (ECs) and therefore represents an indicator of EC activation and plays a crucial role in high shear stress, depending on primary hemostasis. Furthermore, in patients with liver cirrhosis, elevated levels of vWF-Ag are frequently observed ***(Lisman et al., 2006).***

Although it is established that VWF-Ag is increased in patients with cirrhosis, no data on the association of vWF-Ag and portal pressure exist (***La Mura et al., 2011).***

Because vWF-Ag plays a crucial role in primary hemostasis and is an indicator of endothelial cells activation and development of thrombotic vascular obliteration, which are all discussed as possible mechanisms leading to PH, it is hypothesized that patients with CSPH have increased vWF-Ag levels, compared to patients without CSPH ***(Ferlitsch et al., 2012)***

AIM OF THE WORK

The aim of this work is to evaluate the diagnostic performance of vWF-Ag to detect clinically significant PH correlated by portal vein velocity in patients with liver cirrhosis and to evaluate vWF-Ag levels in the prediction of mortality and decompensation.

VON WILLEBRAND FACTOR

Over the last 20 years, investigators have gained much insight into the structure and function of vWF by studying its molecular and cellular biology. Such studies have helped us understand the clinical manifestations of vWF and develop assays for laboratory diagnosis, the classification of vWD and its variants, and the rationale for therapy ***(Colman et al., 2006).***

A) vWF structure

vWF is a large multimeric glycoprotein present in blood plasma and produced by the endothelium (in the Weibel-Palade bodies), megakaryocytes (α-granules of platelets), and subendothelial connective tissue ***(Zaverio, 2007).***

The vWF gene has been localized to chromosome 12, contains 52 exons, and has a length of about 178 kb. Transcription of the gene results in mRNA of approximately 8.7 kb ***(Baronciani et al., 2003).***

The primary product of the vWF gene is a 2813 amino acid protein made of a signal peptide of 22 amino acids (also called a pre peptide) synthesized by endothelial cells and megakaryocytes. Post translational processing cleaves the pro-vWF peptide into a large pro-peptide of 741 amino acids and a

mature vWF molecule of 270 kDa containing 2050 amino acids ***(Castaman et al., 2003).*** The pro-peptide (pre-pro-vWF) composed of four types of repeated domains (D1, D2, D′, D3, A1, A2, A3, D4, B1, B2, B3, C1, C2 from the N- to C-terminal region) of cDNA which are responsible for the different binding functions of the molecule (Figure 1) ***(Franchini and Lippi, 2007).***

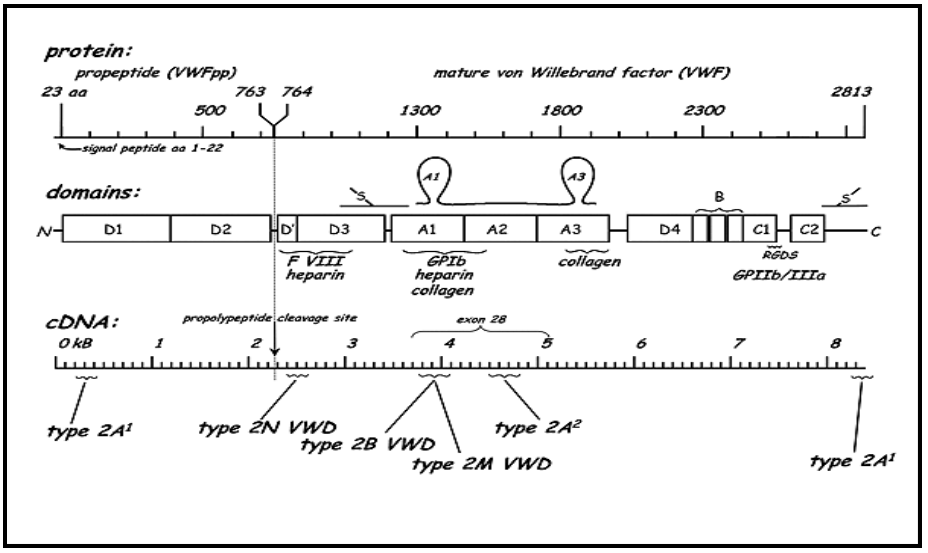

Fig. (1): Structure and domains of von Willebrand factor (vWF) and Type 2 mutations which are located in specific domains (regions) **(Nichols et al., 2008).**

Monomers of mature vWF are assembled into variable sized multimers via disulfide bridging of cysteine residues at both the amino and carboxy-terminal ends. Under physiological conditions, smaller complexes constitutively exit the cell and enter the circulation, where they bind and stabilize the

coagulation factor VIII or undergo reduction and proteolytic cleavage.The size of circulating vWF multimers is also regulated through processing by the metalloprotease ADAMTS13. Large complexes, with up to 100-fold activity of monomeric fragments, are essential for normal platelet adhesion and aggregation after vascular injury in rapid-flow, high shear stress vessels such as arterial capillaries ***(Kulkarni et al., 2000)***.

The basic vWF monomer (subunit) consists of 2050 amino acid. Every monomer contains a number of specific domains with a specific function:

- The D'/D3 domain, which binds to FVIII.
- The A1 domain also contains the binding site for the platelet glycoprotein GP Ib–IX–V complex, different matrix collagens.
- The A3 domains bind to different matrix collagens ***(Sadler et al., 2000).***
- The C1 domain, in which binds to the platelet.
- The C2 domain is close to the carboxy terminal region of vWF and interacts with the activated GP IIb–IIIa complex (Figure 2) ***(Ruggeri et al., 1998).***

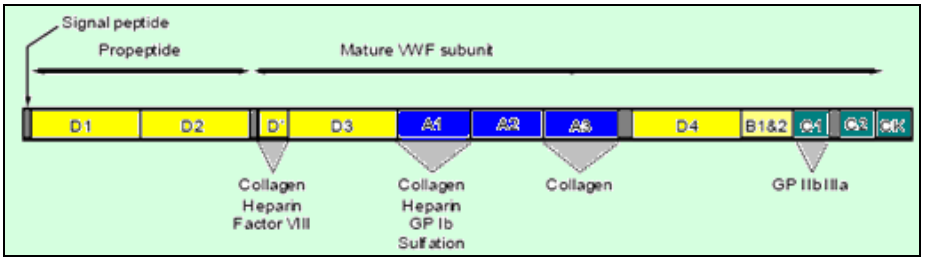

Fig. (2): The structure of vWF factor ***(Carol, 2008).***

The building block of vWF multimers is a dimer formed in the endoplasmic reticulum and made up of two single chain pro-vWF molecules, joined through disulphide bonds within their C-terminal region. The pro-vWF dimers are then transported to the Golgi apparatus where they are polymerized into very large molecules, with molecular weights of up to 20, 000 kDa, through disulphide bonds connecting the two N-terminal ends of each dimmer ***(Franchini and Lippi, 2007).***

B) vWF Biosynthesis:

vWF circulates in plasma at concentration ranging between 5 and 10 µg/ml. Some of these molecules are complexed with FVIII, apparently protecting FVIII against degradation ***(Volt et al., 2000).***

vWF is synthesized by vascular endothelial cells and megakaryocytes ***(Mannucci., 2000),*** and stored in the Weibel-Palade bodies of endothelial cells and in α-granules of platelets.

In physiologic conditions, vWF is mainly synthesized by the endothelial cell (Figure 3) ***(Mertens et al., 2006)***.

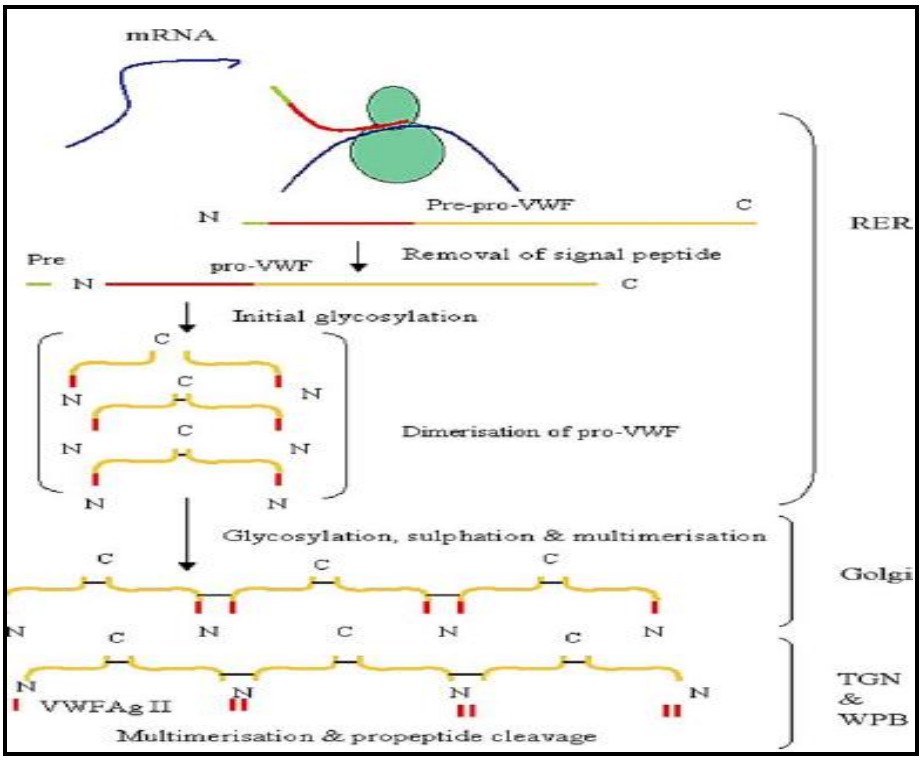

Fig. (3): Biosynthesis of vWF ***(Millar and Brown et al., 2006)***.

Small vWF multimers are secreted constitutively, and the large multimeric forms are stored in Weibel– Palade bodies of endothelial cells and in platelet α-granules ***(Fuchigami, 2008)***.

1. <u>Endothelial Synthesis:</u>

The primary translation product of the mRNA is a 2813 residue pre-pro-peptide, known as pre-pro- vWF. This consists