

The vWF/ADAMTS-13 Axis in Thrombosis-Hemostasis and Beyond

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Our Experience with vWF:antigen and ADAMTS-13:antigen (Relative Mass)

Methods used:

- ✓ **vWF:antigen**: latex particle-enhanced immuno-turbidimetric assay using an hemostasis automated testing system (ACL Top 3G, IL, USA) with inter- and intra-assay CVs <2% and 3%, respectively. Equal performance serum/plasma. **(IVD)**.
- ✓ **ADAMTS-13**: quantitative sandwich enzyme immunoassay technique (R&D Systems, USA.) with inter- and intra-assay CVs <5.7% and 3.7%, respectively. Best performance serum/heparin-plasma. **(RUO)**.
- ✓ **Calculation of ADAMTS-13/vWF:antigen**. Preferable logarithmic transformation of values. Molar ratio with assumption as the predicted MW of **145 kDa** differs from the observed molecular mass of purified plasma ADAMTS13 (ca. **190 kDa**), and this difference is likely due to its extensive glycozylation.

ADAMTS-13 Activity to Antigen Ratio in Physiological and Pathological Conditions Associated with an Increased Risk of Thrombosis

Condition	<i>n</i>	VWF:Ag	ADAMTS13 activity (%)	ADAMTS13 antigen (%)	Activity to antigen ratio
Healthy individuals					
Age, years <35	33	118 ± 35‡	117 ± 24	107 ± 17	1·10 ± 0·20
36–50	35	140 ± 54	110 ± 30	106 ± 19	1·05 ± 0·25
51–65	32	142 ± 46	113 ± 32	104 ± 19	1·10 ± 0·30
>65	32	149 ± 41	86 ± 20†	90 ± 15*	0·98 ± 0·24
Neonates					
Full term	41	112 ± 27	43 ± 20*	73 ± 9*	0·59 ± 0·25*
Pregnancy					
Third trimester	42	290 ± 102*	85 ± 36†	92 ± 14†	0·90 ± 0·32‡
Oral contraceptive intake					
–	33	141 ± 42	103 ± 26	97 ± 23	1·09 ± 0·26
Liver cirrhosis					
Child A	33	295 ± 80*	93 ± 41‡	94 ± 35	0·99 ± 0·29
Child B	32	319 ± 108*	89 ± 34†	90 ± 32†	1·05 ± 0·33
Child C	25	442 ± 262*	67 ± 38*	63 ± 35*	1·13 ± 0·39
Inflammatory bowel disease					
CRP <1	32	139 ± 49	99 ± 29	97 ± 15	1·04 ± 0·33
CRP >1	12	167 ± 66‡	82 ± 28†	82 ± 20†	1·02 ± 0·33
Cardiac surgery					
Baseline	30	146 ± 64	80 ± 24*	95 ± 15	0·87 ± 0·21*
During surgery	30	174 ± 65‡	53 ± 21*	67 ± 14*	0·81 ± 0·34*
After 4 d	30	350 ± 95*	48 ± 15*	72 ± 14*	0·71 ± 0·31*

**p* < 0·001, †*p* < 0·01, ††*p* < 0·05

AL-Amyloidosis

Table 1. Characteristics of the patients in the analysis

	N = 111 patients
Age, median (range)	66 (40-84)
Males/females (%)	60/40
Organ involvement (%)	
Cardiac	65
Renal	71
Liver	15
PNS	19
Soft tissue	21
Number of involved organs, (range)	2 (1-4)
NT-proBNP (pg/mL), median (range)	2297 (17.0-121 000)
hsTnT (ng/L), median (range)	42 (3-338)
Involved FLC (mg/L), median (range)	235 (9-900)
Mayo stage (%)	
I	20
II	49
III	31
Stage IIIB (Mayo stage III and NTproBNP >8500 pg/mL) (%)	19
Proteinuria g/day, median (range)	4.8 (0.5-39)
eGFR mL/min/1.73 m ² , median (range)	71 (8 to >150)
VWF U/dL, median (range)	203.0 (20.0-1046.0)
IQR (range)	127.0-235.0
ADAMTS-13 ng/mL, median (range)	1044 (770-1600)

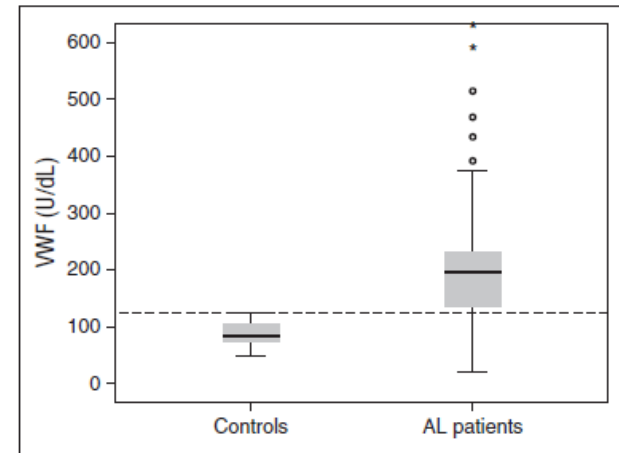


Figure 1. Levels of VWF in patients with AL compared with controls.

Table 2. Multivariate analysis for survival in 111 patients with AL amyloidosis

	<i>P</i> value	HR	95% CI for HR	
			Lower	Upper
VWF ≥230.0 U/dL	.011	2.173	1.193	3.957
SBP <100 mm Hg	.009	2.278	1.227	4.232
Mayo stage I		1		
Mayo stage II	.001	7.833	2.259	27.166
Mayo stage III	<.001	15.078	4.247	53.533

Main Results (VWF:Ag)

- ✓ The median serum level of VWF:Ag in patients with AL-amyloidosis was 203U/dL(range20-1046) and was significantly higher than that measured in healthy controls (median 84.0U/dL, range 48.0-124.0U/dL).
- ✓ 76% of patients with AL-amyloidosis had VWF:Ag levels higher than the upper level measured in healthy controls (124.0 U/dL).
- ✓ There was no significant association of VWF:Ag levels with:
 - Cardiac, nerve, or liver involvement.
 - Levels of NT-proBNP, free light chains or Mayo stage, and the degree of renal dysfunction.
- ✓ Significant association with presence of hypotension, an independent poor prognostic risk factor.

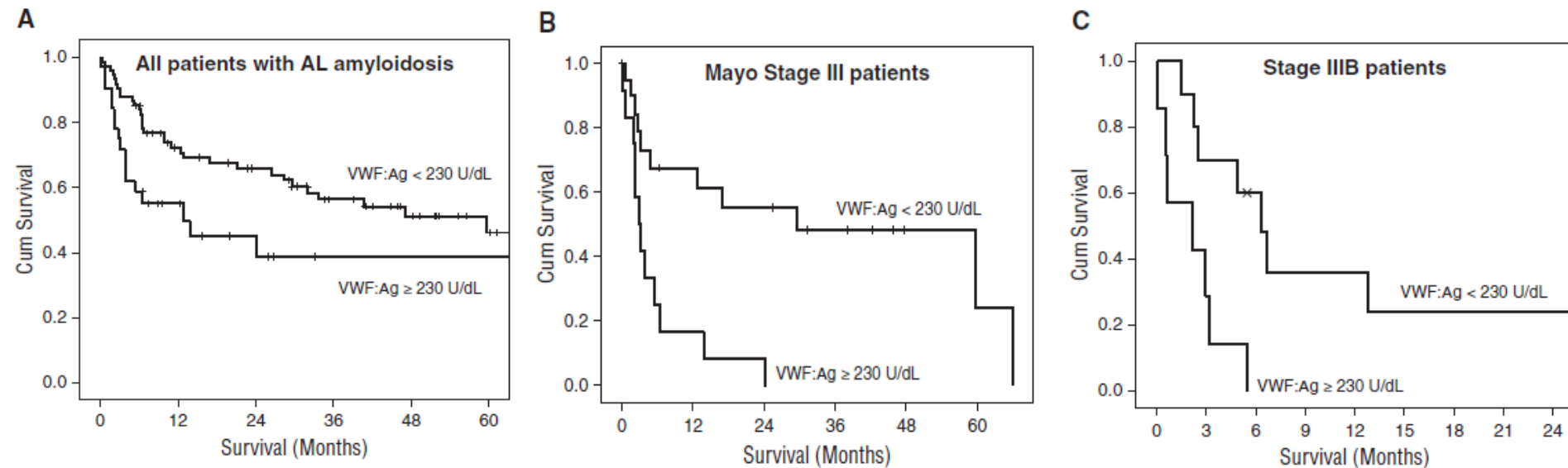
Main Results (ADAMTS-13 Ag)

- ✓ Median levels of ADAMTS-13:Ag were 1044 ng/mL (range 770-1600) and were not significantly different from the levels of healthy, age-matched controls (median 1170, range 745-1610)
- ✓ No correlation of the levels of ADAMTS-13:Ag with vWF:Ag
- ✓ ADAMTS-13:Ag levels had a significant inverse correlation with NT-proBNP levels.

Prognostic Importance of vWF:Ag and ADAMTS-13:Ag Levels

- ✓ vWF:Ag was independently associated with shorter OS. The median OS of patients in the highest, middle two, and lowest quartiles was 14, 28, 29, and 85 months, respectively.
- ✓ A vWF:Ag level >230 U/dL was associated with early death. This effect was independent of Mayo stage IIIb. Stage IIIb patients with vWF:Ag levels >230 U/dL had a median OS of 2 months vs 6 months in those with lower levels.
- ✓ On the other hand, no difference in ADAMTS-13 Ag levels was found among healthy controls and it had no independent prognostic effects despite an inverse correlation with NTproBNP.

Prognostic Importance of vWF:Ag



Survival of Patients with **vWF:Ag >230.0U/dL**.

All Patients (A); Patients with Mayo Stage III (B); Stage IIIB Disease (C).

Waldenström's Macroglobulinemia

Table 1 Patient Characteristics (n = 42 Patients With Symptomatic WM)	
Characteristic	%
Gender	
Male	54
Female	46
Age, y	
Median	65
Range	37-83
Anemia (<11.5g/dL)	78
Platelet count (<100 × 10 ⁹ /L)	17
β ₂ -Microglobulin >3 mg/dL	56
Lactate dehydrogenase >250 U/L	7.5
Serum albumin <3.5 g/dL	58
IgM, mg/dL	
Median	3340
Range	246-9563
IPSS risk	
Low	22
Intermediate	43
High	35
Symptoms	
Cytopenia	42
B-symptoms	15
Hyperviscosity	12
Neuropathy	10
Other	21
Rituximab-based primary therapy	93
Response (≥50% IgM reduction)	54

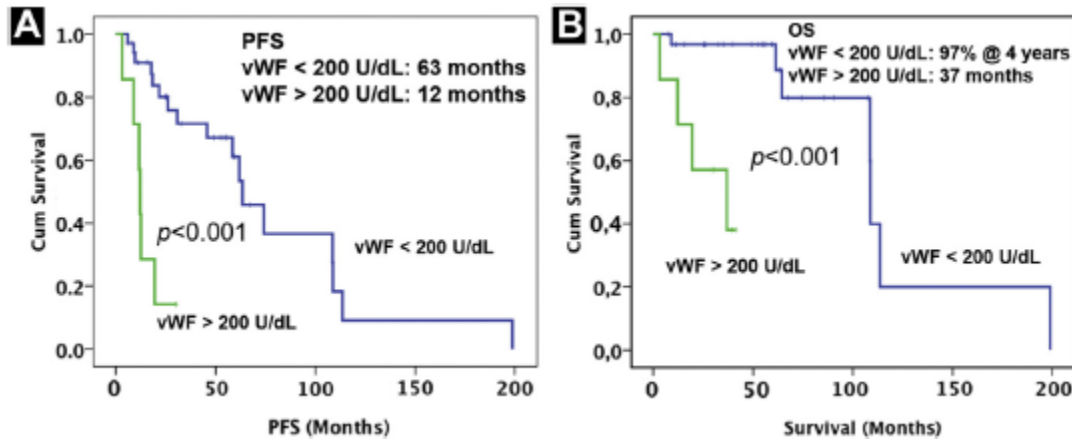
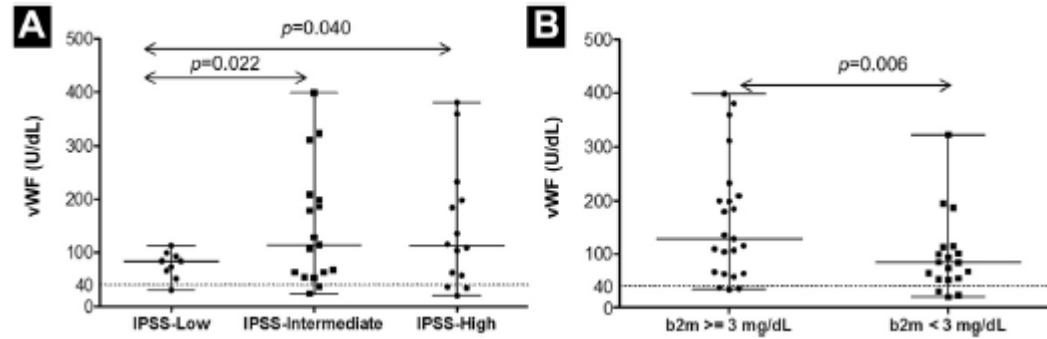
Waldenström's Macroglobulinemia

- ✓ The median serum level of vWF antigen was 101 U/dL (mean, 132.5 U/dL; range, 19.9-399 U/dL) and was slightly greater than the serum level of the healthy controls (median, 85 U/L; mean, 85 U/L; range, 48-124 U/L).
- ✓ However, 6 of the 42 patients (14%) had vWF antigen levels that were < 40 U/L. This finding could have been compatible with acquired vWF syndrome. An inverse correlation was found between the platelet count and vWF:antigen level.
- ✓ No correlation was found between the vWF:antigen levels and IgM levels or between the extent of bone marrow infiltration or other manifestations of the disease.

Waldenström's Macroglobulinemia

- ✓ Median vWF:antigen levels or greater were found less frequently in patients with low-risk (11%) versus intermediate-risk (59%) or high-risk (62%) disease using the IPSS and more frequently found in patients with levels β_2 -microglobulin levels > 3 mg/L.
- ✓ The median follow-up period for the symptomatic patients was 4 years. Patients with vWF:antigen levels within the upper quartile (ie, vWF:antigen, 200 U/dL) had a median progression-free survival of 12 months compared with 63 months for patients with a vWF antigen levels < 200 U/L.
- ✓ The median overall survival for patients with vWF antigen levels of 200 U/dL was 37 months (4-year survival, 29% vs. 97% for patients with vWF antigen levels < 200 U/L.

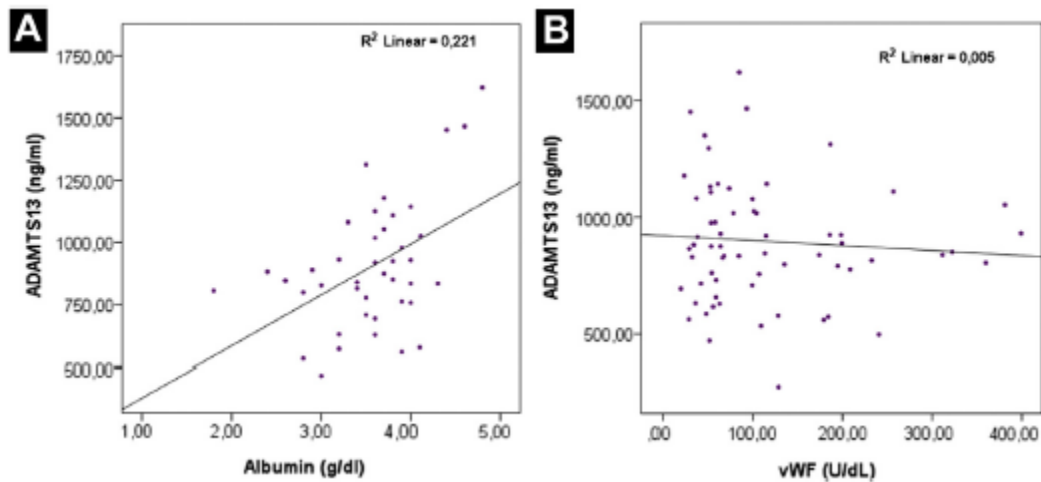
Waldenström's Macroglobulinemia



Waldenström's Macroglobulinemia

- ✓ The median ADAMTS-13 levels in the patients with symptomatic WM was 848 ng/mL (range, 471-1622 ng/mL) compared with 1170 ng/mL (range, 770-1598 ng/mL) in the healthy controls.
- ✓ Significant difference was found in the ADAMTS-13 levels between the patients with symptomatic WM and normal individuals. negative correlation between low levels of ADAMTS-13 and high levels of IgM, b2-microglobulin and infiltration by lymphoplasmacytic cells.
- ✓ A positive correlation between the ADAMTS-13 antigen levels and the serum albumin levels was found.
- ✓ In the patients who required therapy because of hyperviscosity syndrome, the serum ADAMTS-13 levels were lower. We found no association between the ADAMTS-13 and vWF antigen levels.

Waldenström's Macroglobulinemia



Waldenström's Macroglobulinemia: Key Points

- ✓ Elevated vWF:antigen levels correlate strongly with a poor prognosis.
- ✓ Also, the underlying mechanisms might indicate a coexisting endothelial injury. Our data strongly suggest the need for further validation of this marker.
- ✓ ADAMTS-13 antigen levels were lower in the patients with WM than those in the healthy controls, and these reduced levels inversely correlated with the IgM levels, b₂-microglobulin levels, and degree of bone marrow infiltration.
- ✓ The ADAMTS-13 levels did not increase in compensation for increased levels of vWF antigen. The underlying role of the endothelium and the possible biologic mechanisms in the pathophysiology of this phenomenon require further investigation.

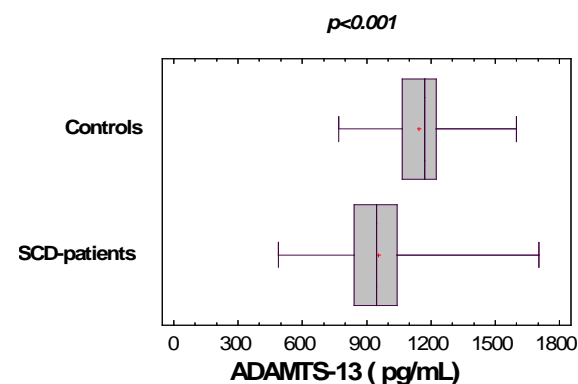
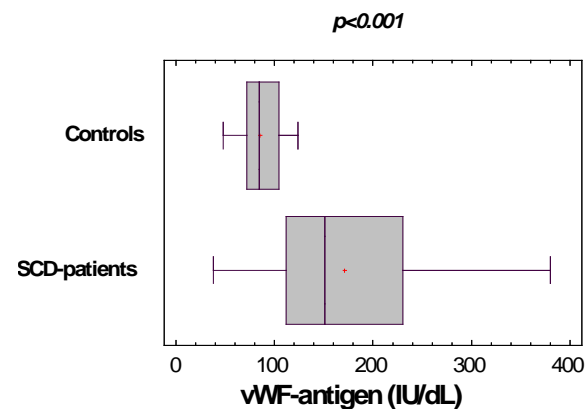
HbS/ β ^{thal}

Table 1

Hematologic and blood chemistry parameters in patients with HbS/ β ^{thal} and healthy controls.

	HbS/ β ^{thal} (HU +) N = 49	HbS/ β ^{thal} (HU -) N = 40	HbS/ β ^{thal} (ALL) N = 89	Controls N = 20
Hematologic parameters				
Hb (g/L)	94 ± 13	98 ± 17	96 ± 15	125–160 ^d
Hb A (%)	6.6 ± 6.0	9.9 ± 9.1	8.3 ± 7.9	> 96.5 ^d
Hb F(%)	20.4 ± 9.2	6.7 ± 5.9 ^c	14.2 ± 10.4	< 1.0 ^d
MCV (fL)	85.5 ± 3.1	68.1 ± 1.8 ^c	77.7 ± 12.3	85.0–100.5
Reticulocytes (%)	5.4 ± 2.7	4.6 ± 2.8	5.0 ± 2.8	0.5–1.2 ^d
Blood chemistry parameters				
Uric acid (mg/dL)	5.4 ± 1.3	5.2 ± 1.7	5.3 ± 1.5	4.8 ± 1.3
LDH (U/L)	657 ± 213	602 ± 240	632 ± 226	375 ± 65 ^d
Ferritin (µg/L)	422 ± 355	351 ± 699 ^c	447 ± 367	42 ± 22 ^d
Special chemistry parameters				
hs-CRP (mg/L)	7.5 ± 5.7	6.2 ± 4.8	6.9 ± 5.3	0.43 ± 0.10 ^d
vWF:antigen (IU/dL)	174 ± 72	168 ± 74	171 ± 78	85 ± 22 ^d
D-Dimers (pg/mL)	929 ± 255	983 ± 236	956 ± 245	340 ± 187 ^d
GDF-15 (pg/mL)	2478 ± 222	1520 ± 204	1980 ± 159	665 ± 50 ^c
Hepcidin-25 (ng/mL)	68.9 ± 9.1	46.1 ± 8.1 ^b	57.2 ± 6.2	67.3 ± 4.5 ^d
Hepcidin-25/ Ferritin (molar ratio)	31 ± 3	53 ± 10 ^a	43 ± 5	320 ± 38 ^d

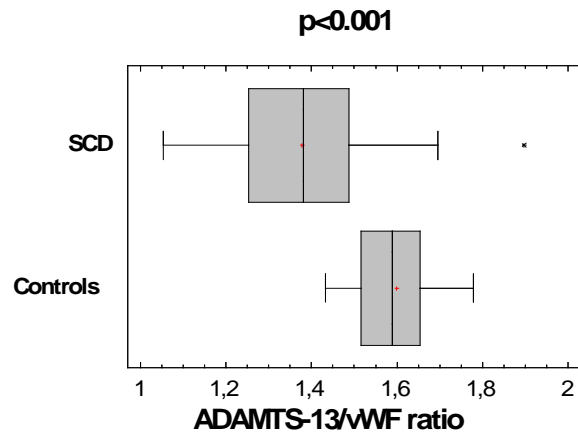
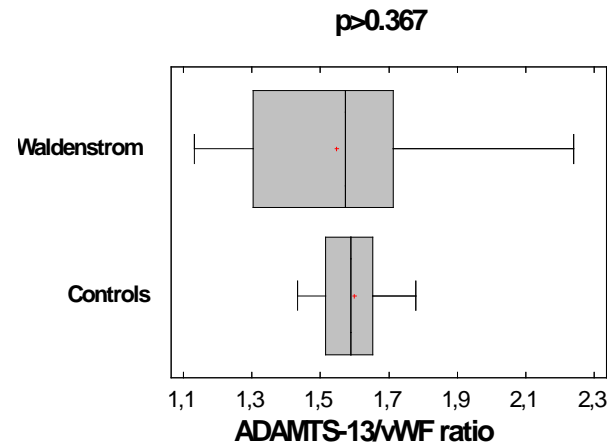
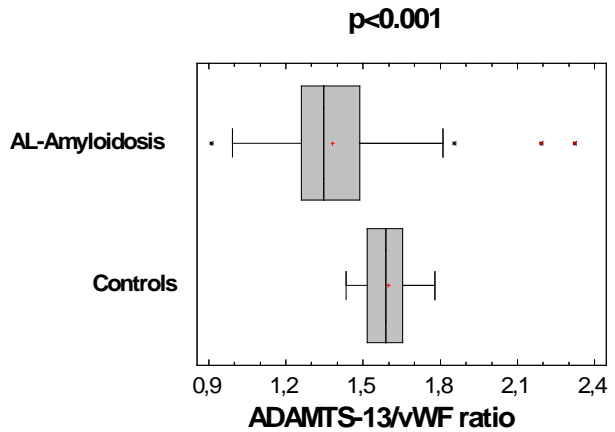
Significant differences: patients with HbS/ β ^{thal} HU + vs HU -, ^ap < 0.05; ^bp < 0.01; ^cp < 0.001, significant differences: patients with HbS/ β ^{thal} vs controls ^dp < 0.05-p < 0.001.

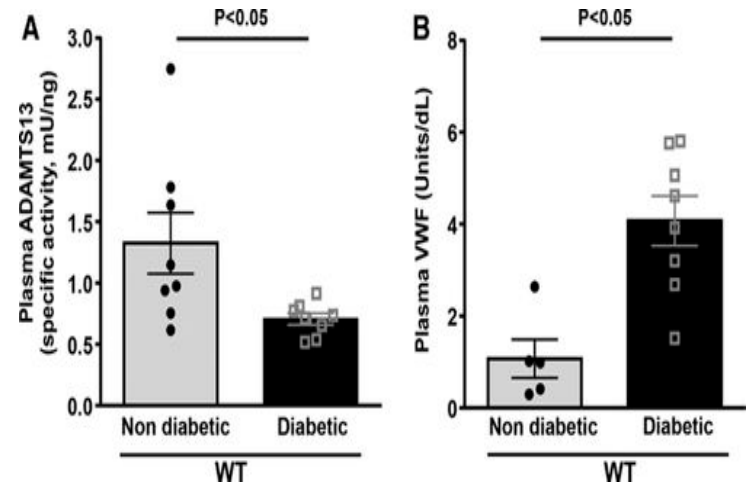
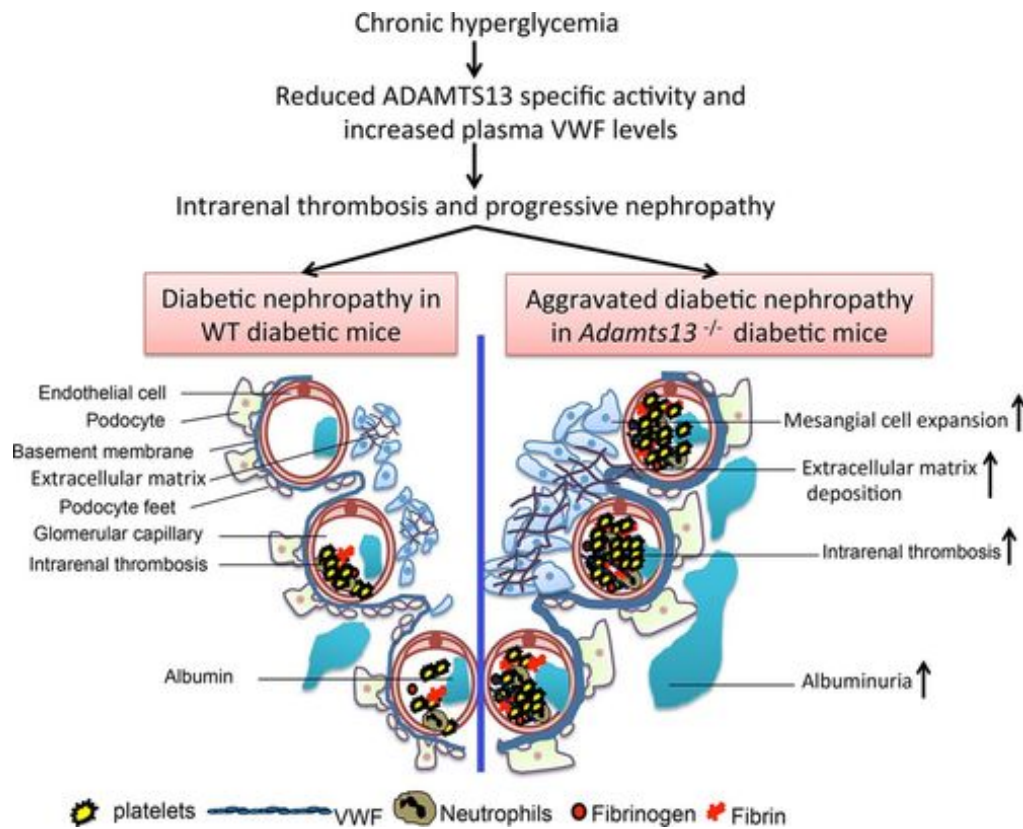


HbS/ β thal

- ✓ Both vWF:antigen and ADAMTS-13:antigen correlated positively with inflammation.
- ✓ vWF:antigen correlated with angiogenesis, hemolytic component, while no correlation found with iron burden, pulmonary hypertension and/or lipid metabolism.
- ✓ vWF:antigen correlated negatively with renal function, while ADAMTS-13:antigen correlated positively with renal function.

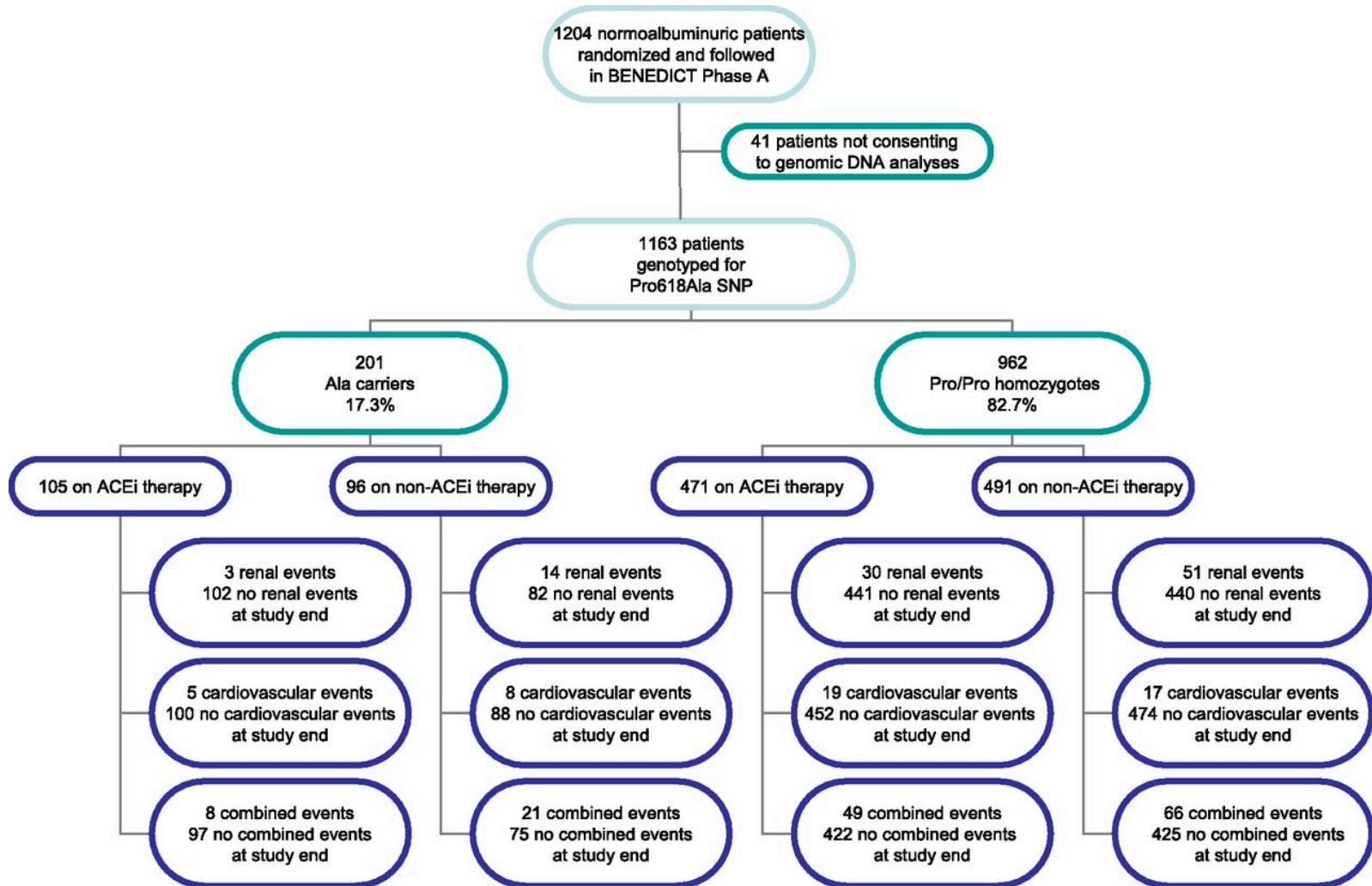
Log(vWF:antigen)/Log(ADAMTS-13)



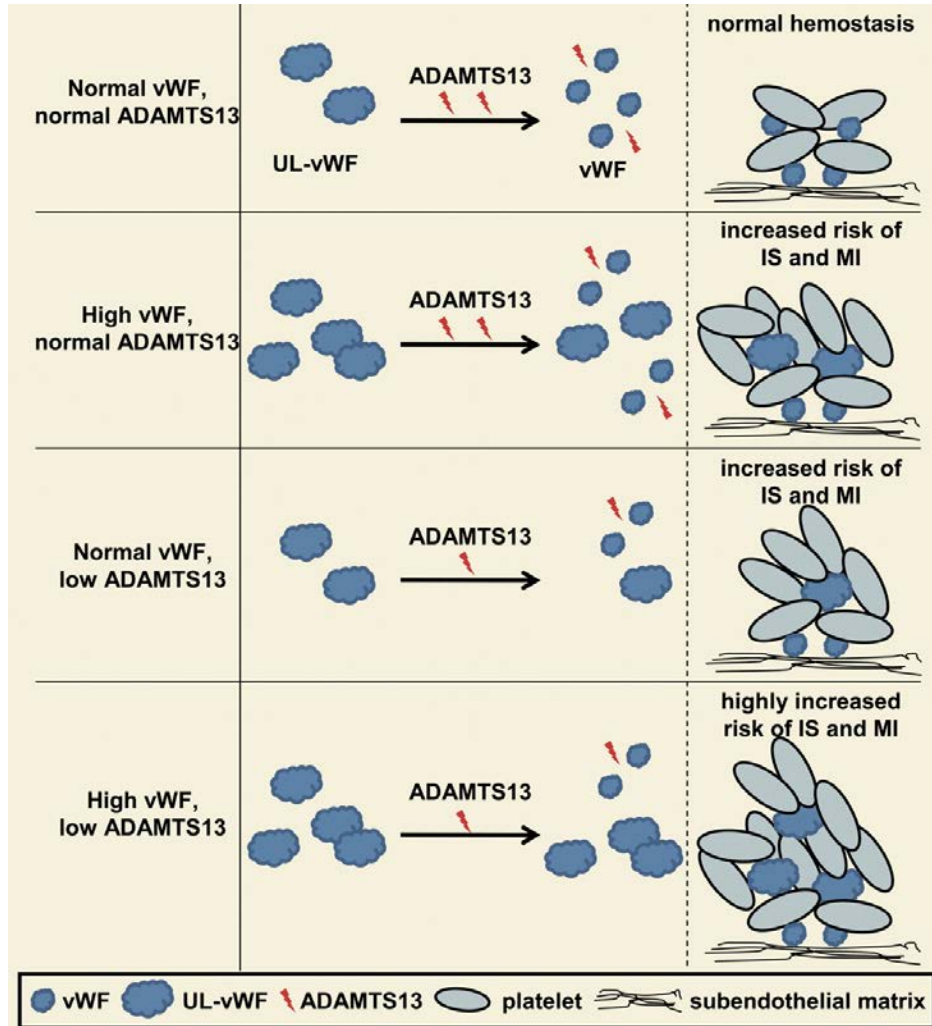


ADAMTS-13/VWF imbalance may causally contribute to thrombotic angiopathy in diabetic patients rather than simply serving as a biomarker of endothelial dysfunction and oxidative stress. Severe ADAMTS-13 deficiency exacerbates diabetic nephropathy, likely mediated by VWF-dependent increased intrarenal thrombosis. Alteration in ADAMTS-13/VWF balance may be one of the key pathophysiological mechanisms of increased thrombotic angiopathies observed in diabetic patients.

Schematic diagram of BENEDICT phase A type 2 diabetic patients screened for the Pro618Ala ADAMTS-13.



Simplified Model of VWF and ADAMTS13 Plasma Levels and Their Contribution to Thrombotic Events



***vWF* and *ADAMTS-13*:
Too Much or Too Little of A Good Thing?**