

## Shear flow-based assay with HUVECs shows von Willebrand Factor (VWF) strings anchored to the endothelial surface and platelet aggregation in acute immune-mediated Thrombotic Thrombocytopenic Purpura

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### INTRODUCTION

In the absence of functional ADAMTS13, ultralarge VWF multimers and platelets lead to the formation of platelet-rich microthrombi within small arterioles inducing immune-mediated Thrombotic Thrombocytopenic Purpura (iTTP).

Under physiological conditions, VWF is secreted from endothelial cells as ultralarge multimers, and ADAMTS13 progressively reduces the size of these VWF multimers as they circulate in the blood.

### AIM

We studied the impact of VWF in platelet-dependent primary haemostasis in reduced/absence ADAMTS13 activity under flow conditions in acute thrombotic iTTP patients.

### METHOD

A shear flow-based assay with Human Umbilical Vein Endothelial Cells (HUVECs) was developed.

After histamine stimulation of endothelial cells, plasma with the addition of platelets at a concentration of  $0.5-1 \times 10^9$  /mL was perfused through gelatin coated  $\mu$ -slides VI (Ibidi, Germany) at a constant wall shear stress of 2.5 dynes/cm<sup>2</sup>.

Cells were then immunelabelled for VWF and CD41 after being fixed under flow with 4% formaldehyde.

Platelet recruitment and VWF coverage were quantified.

### RESULTS

We investigated twenty acute iTTP presentation patients with severe deficiency (<10%) of ADAMTS13 activity.

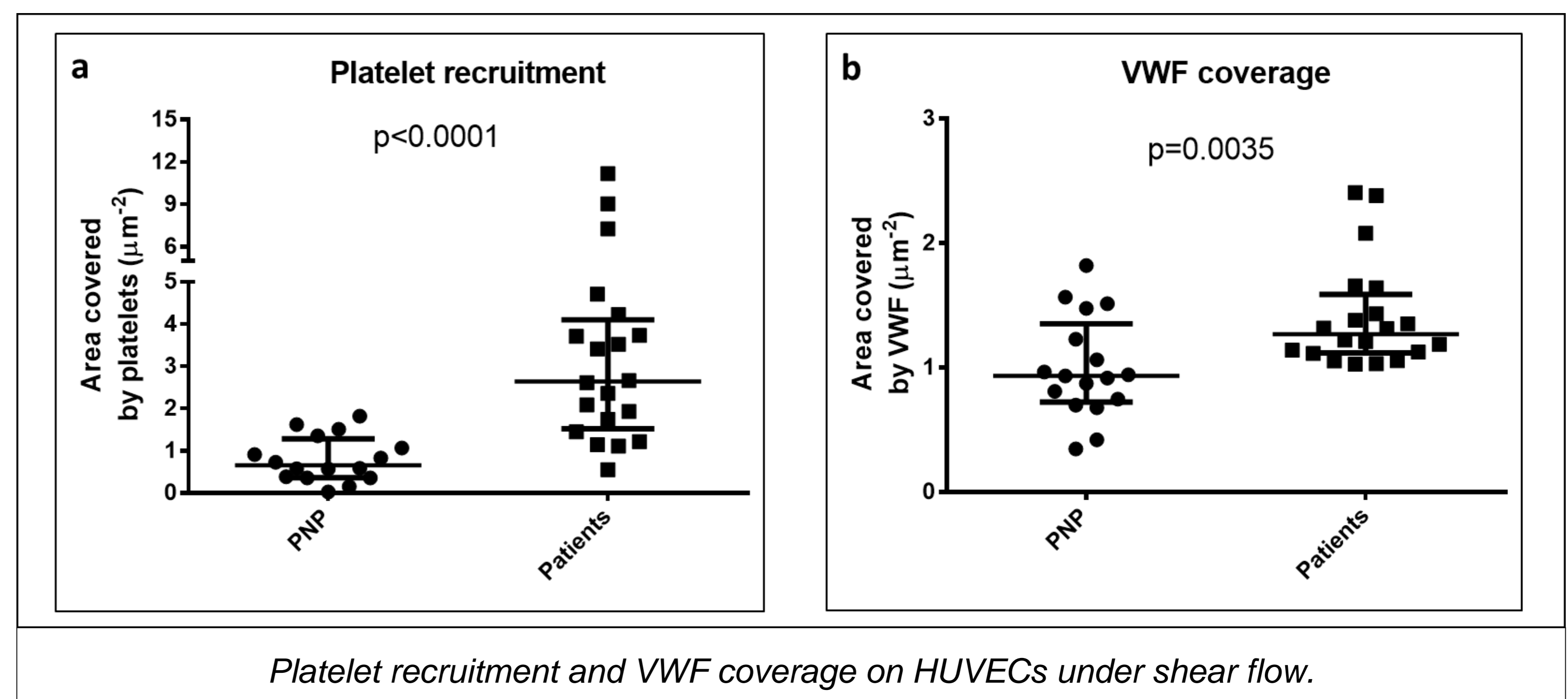
Median presenting anti-ADAMTS13 antibodies levels was 67% (range, 2%-140%) and median presenting ADAMTS13 antigen levels was 3.0% (range, 0.7%-47.6%).

Pre-treatment median levels of VWF antigen and VWF activity were respectively 1.71IU/mL (range, 0.72 IU/mL-3.16 IU/mL) and 0.84 IU/mL (range, 0.15 IU/mL-2.10 IU/mL).

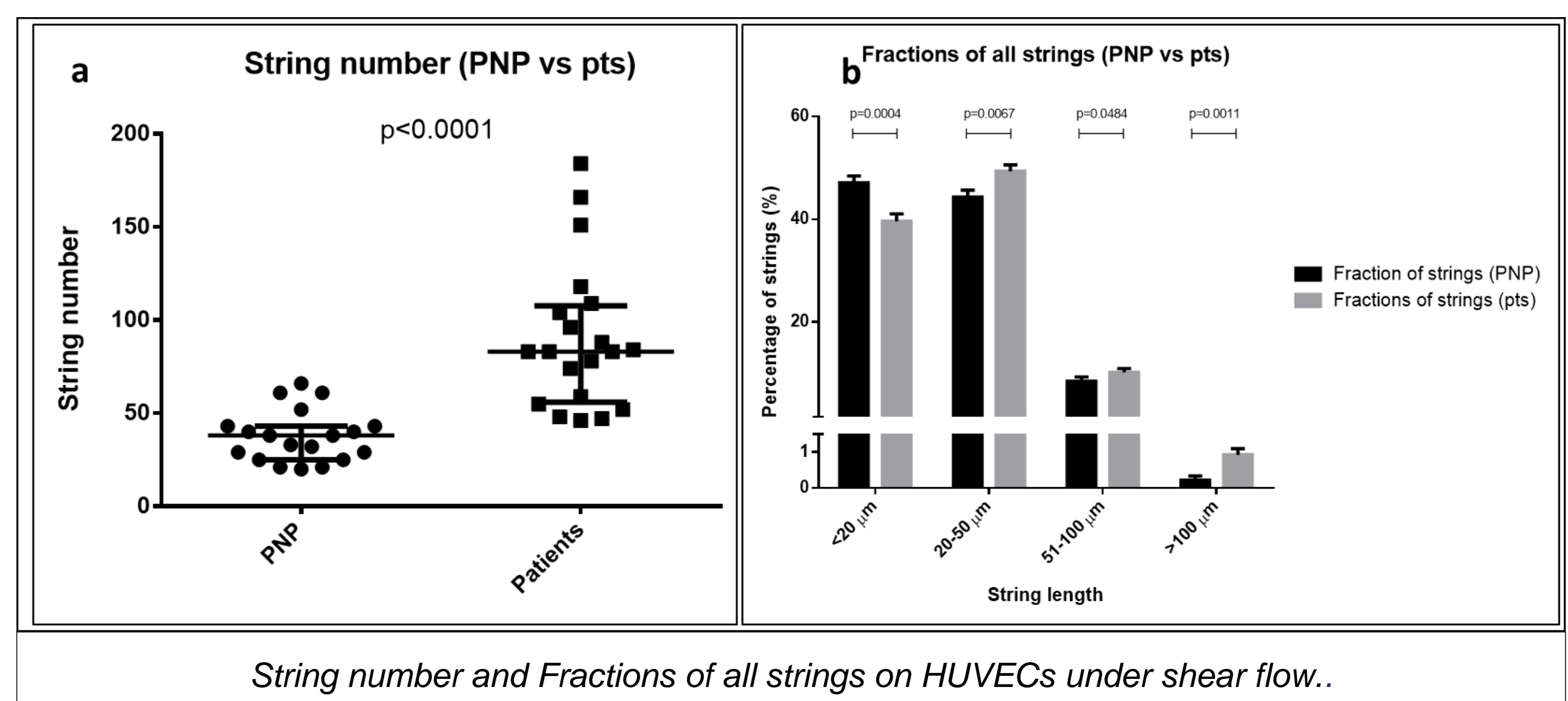
With the shear flow-based assay with HUVECs, significant increase of platelet recruitment ( $p < 0.0001$ ) and VWF coverage ( $p = 0.0035$ ) were observed in patients compared to pool normal plasma.

Significant increase in median string number was reported in patients ( $n = 83$ , range 46-184) compared to pool normal plasma ( $n = 38$ , range 20-66) ( $p < 0.0001$ ).

Reduced ADAMTS13 activity increased the percentage of the longest (>100 $\mu$ m) strings in patients ( $p = 0.0011$ ).



Platelet recruitment and VWF coverage on HUVECs under shear flow.



String number and Fractions of all strings on HUVECs under shear flow..

### CONCLUSIONS

Investigation of the interaction between VWF and platelets on HUVECs under shear flow may help to understand the pathophysiological mechanism of iTTP and could contribute in disease severity evaluation.

### REFERENCES

Sadler JE. *Hematol. Am. Soc. Hematol. Educ. Progr.* 2015;2015:631-636.

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