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DIE DRESDNER.

Diagnostic approach to von Willebrand disease with special consideration of von Willebrand factor propeptide: a new, rapid and specific vWFpp ELISA

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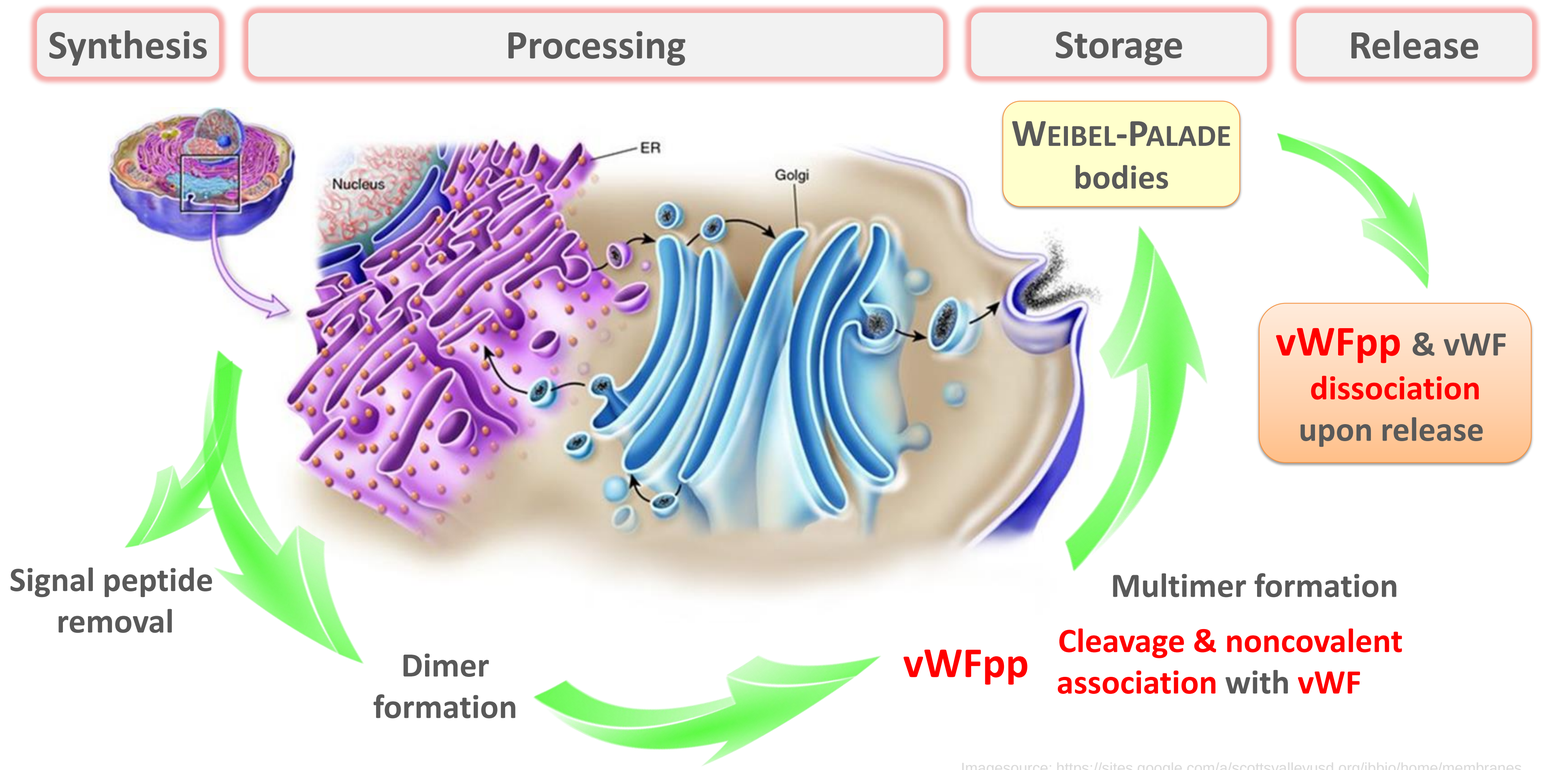
Background & Aim

In 2015 Sanders et al. reported, that severe type 1 von Willebrand disease (vWD) with very low von Willebrand factor (vWF) levels in patients who had previously been classified as type 3 vWD was successfully identified by quantification of von Willebrand factor propeptide (vWFpp) underlining the diagnostic significance of vWFpp in classifying vWD patients.[1] In 2018 O'Sullivan noted that enhanced vWF clearance may play an important role in the pathogenesis of vWD and has therapeutic relevance.[2]
Currently available colorimetric ELISA-based vWFpp assays are rather complicated and can not be completed within 24h. In contrast, a rapid, specific and simple ELISA that could be easily implemented into routine laboratory diagnostic procedures was recently developed.[3]

Life cycle of vWF & vWFpp (Endothelial cell)



Methodology of the new assay

A sandwich type ELISA was designed based on precoated ready-to-use strips with a total assay time of about 90 min. The new assay exhibits a detection limit of 1.7 mIU/ml and a broad measurement range (1.7 - 120.0 mIU/ml). The assay was calibrated against the SSC/ISTH Sec. Coagulation Standard Lot #4, 0.97 IU/ml vWFpp.

Assay procedure

- 50 µl sample
  + 50 µl antibody-POD conjugate
- **60 min**, 37 °C
- **4x** washing
  + 100 µl substrate (TMB)
- **15 min** (15–25 °C in the dark)
  + 100 µl stop solution
- OD reading (450/620 nm)

Assay characteristics

CV - within series (n=8)
0.1 % at 60.6 mIU/ml
8.1 % at 3.8 mIU/ml

CV - day to day (n=8)
0.6 % at 60.6 mIU/ml
9.8 % at 3.8 mIU/ml

Comparison reference assay vs. new assay:

Citrate anticoagulated plasma samples obtained from healthy controls (VWF:Ag >40 %) and VWD patients were analyzed with the new ELISA. Results were compared to the Sanquin VWFpp ELISA (figure 1).

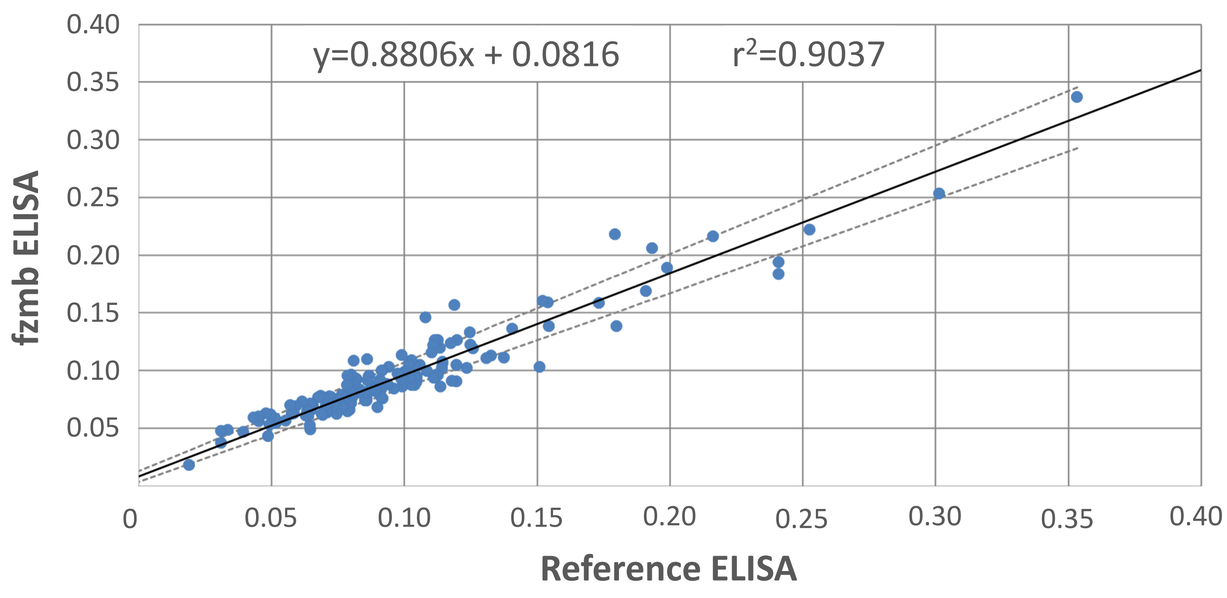


Figure 1: Method comparison according to Passing/ Bablok in 162 samples (normals, various VWD types)

Results

During a clinical evaluation period, the assay was applied on blood specimens of 17 vWD patients - low von Willebrand factor (LWF, n=5), vWD type 1 (n=7), vWD type 1C (n=3), vWD type 3 (n=2), acquired vWD (AQvWD, n=1).
vWFpp was below the limit of detection in the cases of vWD type 3. As expected, all vWD type 1C patients were characterised by a markedly increased vWFpp/vWF:Ag-ratio (9.7 - 16.7). Within the vWD type 1 group, three cases revealed a vWFpp/vWF:Ag-ratio < 2.0 and 4 cases a ratio > 2.0. In all LWF-cases the vWFpp/vWF:Ag-ratio was < 2.0. In the case of the severe AQvWD the vWFpp/vWF:Ag-ratio exceed 100.

Conclusion

The newly developed ELISA with a short assay and less hands-on time, ready-to-use microtiter strips, and good performance characteristics enables an integration of vWFpp into established vWD diagnostic work-up algorithms. vWFpp differentiates type 3 and severe type 1 vWD.[1]
The vWFpp/vWF:Ag-ratio appears to be helpful in discriminating patients with a markedly increased vWF clearance (acquired vWD or vWD type 1 Vicenza) from those with a modest increased clearance (majority of all vWD patients).[4,5]
Information about clearance defects may have direct therapeutic implications.[6] DDAVP is the treatment of choice in vWD type 1 patients, but increasing the endogenous vWF levels by regular DDAVP dosing may not be sufficient in patients with a major vWF clearance defect.[6]
Identification of vWD patients with enhanced vWF clearance may contribute to optimized therapeutic regimes.

References:
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