

AIMS

- The analysis of von Willebrand factor (vWF) multimers is necessary for the classification of hereditary and acquired forms of von Willebrand disease (vWD).
- Only a few specialized laboratories are skilled enough to perform this analysis due to the complexity of the method itself and to its very slow turnaround time (2-3 days).
- In fact, vWF multimers are usually separated in “home-made” discontinuous SDS agarose gel (difficult to produce) followed by a western blotting step and multimers are then identified by immunofluorescence or other staining techniques.
- We present here the use of a new commercial method (Hydragel 5 von Willebrand multimers) for multimer analysis, which is rapid (6 hours), easy to perform (no western blot), reproducible (ready to use SDS agarose gel). This method assesses the overall size distribution of vWF multimers (low, intermediate, high and very high molecular weight).

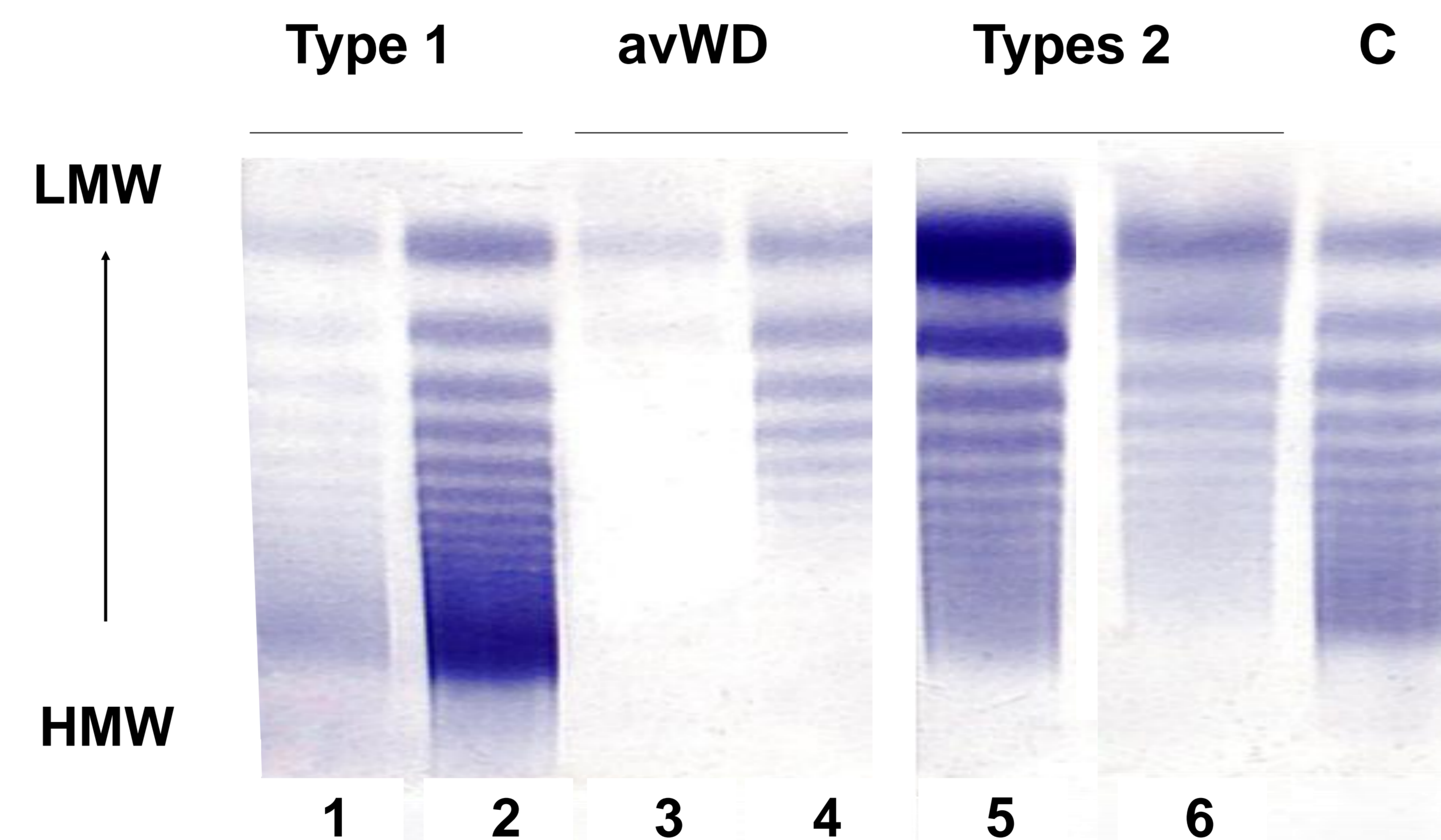
PATIENTS

- 4 patients with suspected vWD using conventional assays
- Global primary haemostasis was evaluated using a PFA-100 analyzer (Siemens) were > 300 sec in basal conditions (PFA-ADP and PFA-Epi cartridges)
- vWF:antigen (vWF:Ag) and ristocetin cofactor activity (vWR:Co) were quantified using Instrument Laboratories reagents on an ACL 500 analyzer
- Platelet counts were performed on Advia 2120 analyzers (Siemens) and were in the normal range

METHOD

- Plasma were loaded and separated in continuous SDS agarose gel system (no stacking and running gel) within 110 mn.
- Multimers were probed in gel by immunofixation using horse-radish peroxidase (HRP) conjugated to a rabbit anti-vWF (90 min).
- Visualisation of multimers was achieved by colorimetry using commercially available TTF1/TTF2 reagents.
- Curves were produced using the manufacturer’s gel scanner and interpretation software

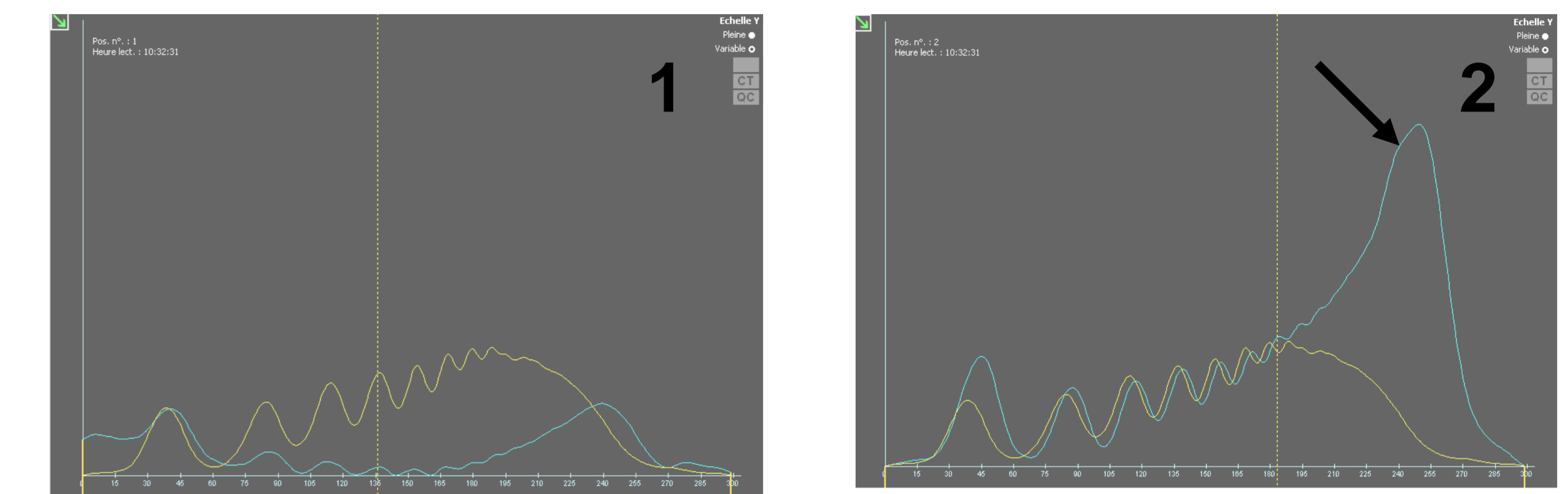
RESULTS



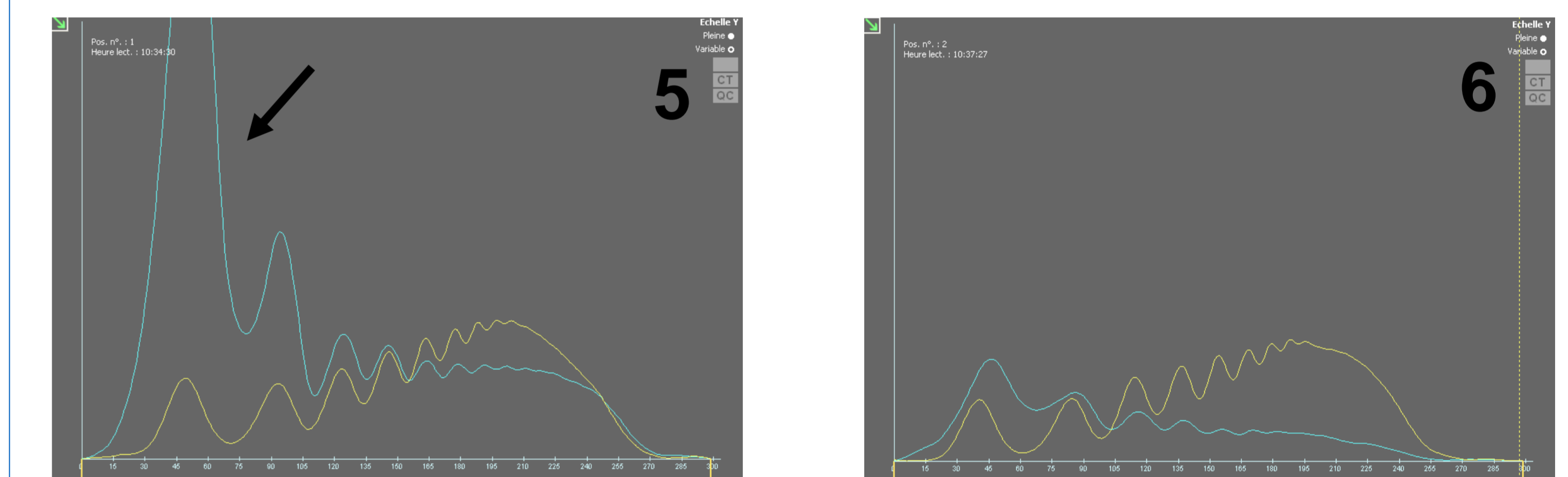
Treatment	Abs.	dDAVP	Abs	vWF Conc.	Abs	Abs	Abs
vWF:Ag (%)	49	178	49	101	225	132	102
vWR:Co (%)	47	188	28	58	86	77	99
Ratio Co/Ag	0.96	1.06	0.57	0.57	0.38	0.58	0.97

Abs: absence; dDAVP: desmopressin; Conc: concentrates

RESULTS



Scanning of the line of plasma 1 (blue) shows a weaker coloration than in control (yellow), with the presence all the multimers corresponding to a type 1 vWD, and the release of very high molecular weight (arrow) after desmopressin infusion (line 2)



Scanning of the line of plasma 5 (blue) shows an excess of low molecular weight monomers (arrow) suggesting a type 2:C vWD, while the absence of high molecular weight multimers in line 6 suggests an type 2:A

Conclusion

This method for vWF multimer analysis is :

- simple to carry out,
- produces results within 6 hours,
- performed on a commercially available instrument (nom)

It is particularly valuable in emergency situations such as preterm labor or urgent surgery, when the patient has no document specifying its vWD type.