Supplementary Material to Sambola et al. "Increased von Willebrand factor, P-selectin and fibrin content in occlusive thrombus resistant to lytic therapy" (Thromb Haemost 2016; 115.6)

Turbidimetric assays and fibrinolysis *in vitro*

Ex vivo plasma fibrin clot turbidimetry and effectiveness of fibrinolysis were determined using r-TPA following a previously described method in 44 patients undergoing p-PCI to verify sensitivity to fibrinolysis. Fibrin clot structure was evaluated by turbidimetric assays in citrated plasma in duplicate as previously described [(8)]. In a plasma sample, clot formation was induced using thrombin plus CLCA2 according to a method described previously. The interassay coefficients of variation (CV) ranged from 9% to 21%. After thrombus formation was achieved, r-TPA was added at concentrations of 1, 2.2, 3.3 and 100 mg/ml (the 2.2 mg/ml concentration is the most similar to that received by patients).

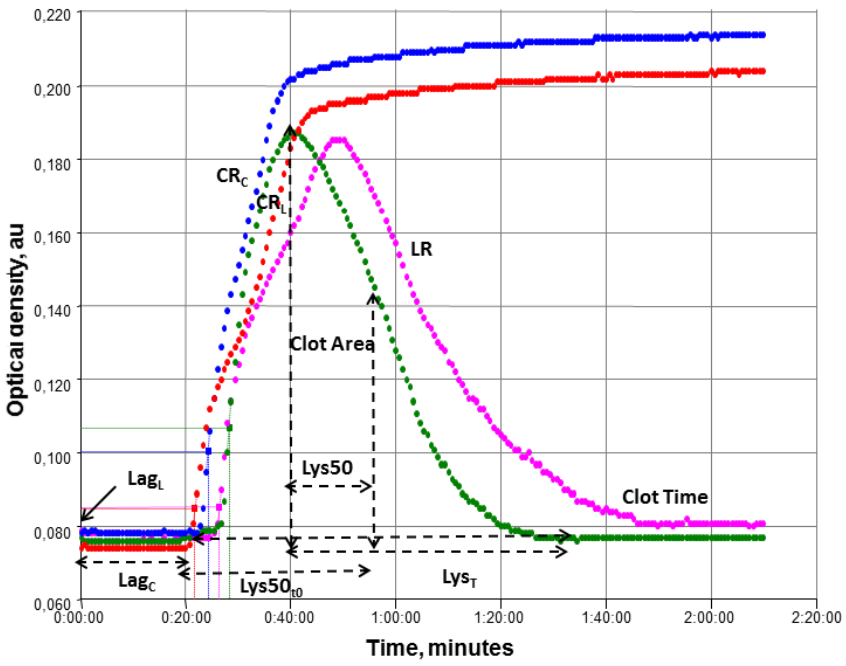
Formation clot fibrinolysis in plasma and r-TPA

The following key parameters were recorded to assess resistance to fibrinolysis: a) clot maximum absorbance (arbitrary units), evaluating fibrin fiber thickness and network density; b) fibrinolysis time (min) corresponding to the time from maximum clot formation to 50% fibrinolysis, turbidity at 405 nm (t 50%) induced by 1 mg/ml of r-TPA, as described previously; and c) fibrinolysis area, which reflects the balance between clot formation and fibrinolysis [(8)]. The interassay CVs were from 8% to 22%. (Figures S1. A, B, C)

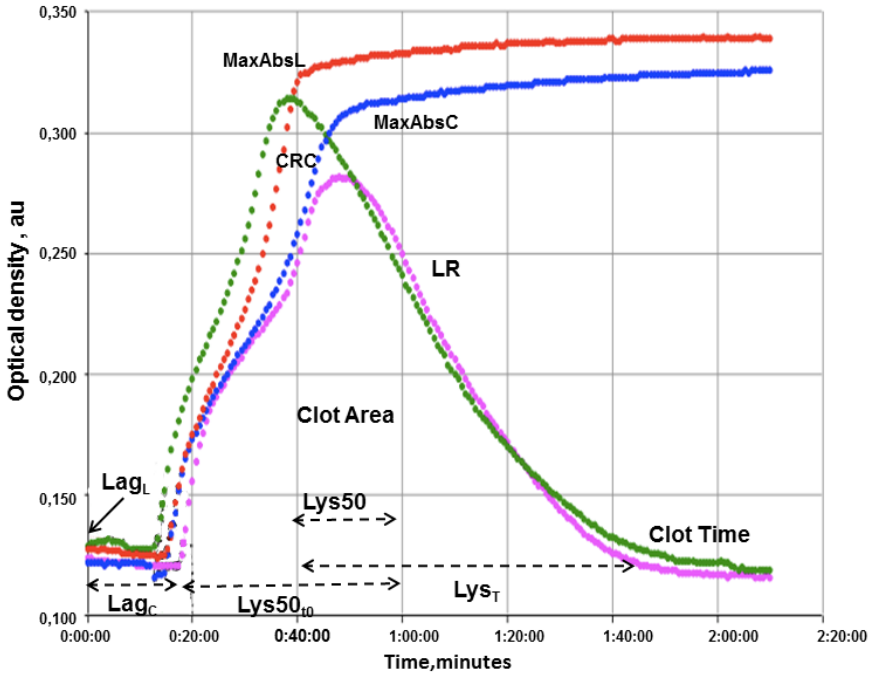
Figure S1. Illustration of turbidimetric clotting and fibrinolysis variables in: A) a patient sensitive to fibrinolysis; B) a healthy subject; C) a patient resistant to fibrinolysis. A, Turbidimetric clotting assay: lag time (LagC); maximum absorbance (ODC); clot rate (CRC). B, Turbidimetric fibrinolysis assay: lag time (LagL); maximum absorbance (ODL); clot rate

(CRL); fibrinolysis times Lys50t0, Lys50MA, LysT; fibrinolysis rate (LR); area clot. Red and blue lines represent clot formation, while green and pink represent clot fibrinolysis.

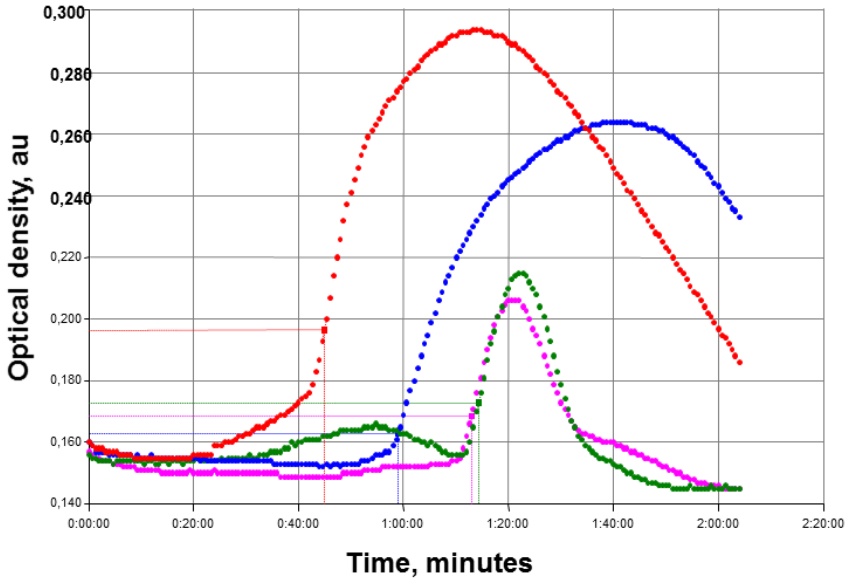
maxim absorbance to lysis; $MaxAbs_c$; Clot maximum absorbance (arbitrary units (au). evaluating fibrin fiber thickness and network density; **$MaxAbs_L$** the highest absorbance value adjusted for Lag_L; **CR_c**; crude rate of clot formation ;**CR_L** crude rate of clot formation; **Clot time; Lysis 50_{t0}**: the time from initiation of clot formation to the time at which a 50% fall in absorbance from $MaxAbs_L$ occurred; **Lysis Time, $t_{50\%}$**: corresponding to the time from maximum clot formation to 50% lysis, turbidity at 405 nm (t 50%) induced by 1 g / ml of r-TPA, as described previously**, Lys_T**: the time from and crude lysis rate; **AUC:** reflects the balance between clot formation and clot lysis.



Supplementary Figure 1A



Supplementary Figure 1B



Supplementary Figure 1C

Suppl. Table 1. Comparison of turbidimetric and fibrinolysis measures of clot between patients undergoing primary PCI and healthy subjects.

	Sensitive to fibrinolysis N=20	**Healthy donors** N=20	**p**
Lag time without t-PA, (Lag_{c}), min	17.86 ± 7.94	19.48 ± 6.12	0.65
OD max without t-PA ($MaxAbs_{c}$), min	0.15 ± 0.07	0.18 ± 0.02	0.41
Crude rate clot formation CR_{c}, min	9.25 ± 3.87	11.16 ± 6.59	0.38
Clot time, min	84.97 ± 23.96	91.16 ± 19.35	0.57
Lag time with t-PA, (Lag_{L}), min	19.26 ± 10.15	18.35 ± 5.30	0.84
OD max with t-PA ($MaxAbs_{L}$), min	0.13 ± 0.75	0.17 ± 0.02	0.29
Crude rate clot lysis, with t-PA CR_{L}, min	9.66 ± 6.42	14.42 ± 6.84	0.13
Lysis Time 50%, min	29.88 ± 2.58	32.80 ± 8.49	0.33
Lysis 50_{t0}, min	51.88 ± 13.30	47.07 ± 8.50	0.45
Lysis total, min (Lys_{T})	66.05 ± 23.07	71.80 ± 14.52	0.58
Clot area (AUC)	0.01 ± 0.007	0.01 ± 0.07	0.32

PCI: percutaneous coronary intervention; Lag_{c}: Time at which sufficient protofibrils have formed to enable lateral aggregation in the absence of tPA (i.e. initiation of clot formation); Lag_{L}: Time elapsed from the addition of tPA to maximal lysis; **MaxAbsC**: Highest absorbance value of the clot (arbitrary units), an index of fibrin fiber thickness and network density; **MaxAbsL**: highest absorbance value after the addition of tPA; **CRc:** crude rate of clot formation; **CR_{L}** crude rate of clot lysis after the addition of tPA; **Clot time:** total time elapsed from the initiation of clot formation to final clot lysis **; Lysis t50**: time elapsed from maximal lysis absorbance (MaxAbsL) to the moment at which 50% fall in absorbance occurred; **Lysis Time, $t_{50\%}$**: Time elapsed from maximum clot formation to 50% lysis; **Lys_{T}**: Total time elapsed from the initiation of clot formation to final clot lysis Time from and crude lysis rate; **AUC:** area under the curve, reflects the balance between clot formation and clot lysis. Turbidity is quantified at at 405 nm and lysis is induced by 1 g/ml of r-TPA.