Disordini emorragici congeniti: aspetti diagnostici e clinici

IN VITRO CORRECTION OF SEVERE FACTOR V DEFICIENCY USING A NOVEL PLASMA-DERIVED FV CONCENTRATE: A WHOLE BLOOD THROMBIN GENERATION STUDY

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Background and Aims. Severe factor V (FV) deficiency is a rare bleeding disorder currently managed with fresh frozen plasma. A novel plasma-derived FV concentrate has been developed as a targeted potential replacement therapy. We evaluated the *in vitro* ability of this concentrate to restore global hemostatic function in patients with severe FV deficiency using the whole blood thrombin generation assay.
Methods. Whole blood samples from 10 patients with severe FV deficiency were spiked *in vitro* with increasing FV concentrations (0%, 25%, 50%, and 100%). Due to limited sample availability, 2 patients were not tested at 25%. Thrombin generation was assessed in whole blood using 1 pM tissue factor, without addition of exogenous phospholipids, by measuring lag time, peak thrombin, and endogenous thrombin potential (ETP). In addition, viscoelastic testing by rotational thromboelastometry (ROTEM; INTEM, EXTEM, FIBTEM) was performed. Statistical analysis was performed using a mixed-effects model (REML), followed by Dunnett's multiple comparisons test versus baseline (0%). Healthy control samples were used to define the interquartile range, which served as reference for lag time, peak thrombin, and ETP.
Results. FV supplementation significantly affected all thrombin generation parameters. Lag time was reduced ($F(1.003, 8.361)=20.06$, $p=0.0018$), with all concentrations showing shorter lag time compared to baseline (25%, $p=0.0059$; 50%, $p=0.0032$; 100%, $p=0.0027$). Peak thrombin was increased ($F(1.576, 13.13)=17.96$, $p=0.0003$), with differences versus baseline at all concentrations (25%, $p=0.0126$; 50%, $p=0.0026$; 100%, $p=0.0037$). ETP showed significant increases at 50% ($p=0.0006$) and 100% ($p=0.0009$), while the increase at 25% did not reach statistical significance ($p=0.0624$). As shown in Figure 1A-B, at baseline lag time was prolonged in all patients and returned within the interquartile range of healthy controls in all cases already at 25% FV. In contrast, peak thrombin was normal or increased in half of cases (Figure 1C), while ETP was within or above the interquartile range of healthy controls in most patients (Figure 1D); in the five patients with low baseline peak thrombin values, levels at 25% FV exceeded the interquartile range of healthy controls (Figure 1C). Consistently, ROTEM showed prolonged clotting times at baseline. Both INTEM and EXTEM-clotting time normalized after FV supplementation at 25% in most patients, although a minority required 50% FV to reach normal values. Parameters reflecting clot propagation and strength were largely within the normal range at baseline and FV supplementation had no relevant effect.
Conclusions. Whole blood thrombin generation and ROTEM suggest that delayed coagulation initiation is the predominant functional abnormality in severe FV deficiency, while thrombin and fibrin-generating capacity is variably preserved. In our study, the novel plasma-derived FV concentrate effectively corrected this defect, restoring lag and clotting times within the interquartile range of healthy controls already at 25% FV. These findings support the potential clinical utility of whole blood thrombin generation as a global assay to evaluate treatment response.

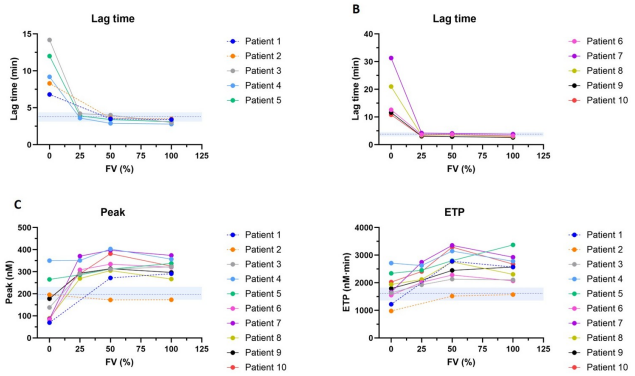


Figure 1. Effect of a novel plasma-derived factor V (FV) concentrate on whole blood thrombin generation parameters in patients with severe FV deficiency. (A–B) Lag time, (C) peak thrombin, and (D) endogenous thrombin potential (ETP) at FV concentrations of 0%, 25%, 50%, and 100% following supplementation with the FV concentrate in individual patients (n=10). Patients 1-5 are shown in panel A and patients 6-10 in panel B for lag time; panels C and D include all patients. Each line represents an individual patient. The dashed horizontal line indicates the median, and the shaded area the interquartile range, of healthy controls. Dashed lines for patients 1 and 2 indicate missing data at 25% FV.