

DIAGNOSTICA DI LABORATORIO IN EMOSTASI E
TROMBOSI

PLATELETS MATTER: THROMBIN GENERATION IN PLATELET-RICH PLASMA VERSUS PLATELET-POOR-PLASMA UNDER NON-REPLACEMENT THERAPIES IN HAEMOPHILIA

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Background and aims. Non-replacement therapies have transformed the management of Hemophilia A (HA) and B (HB). Emicizumab (Emz) is a subcutaneously administered, recombinant, humanized bispecific monoclonal antibody that mimics the function of missing activated FVIII (FVIIIa), thereby restoring activation of FX, downstream thrombin generation, and propagation of the coagulation cascade. Concizumab (Concz) is an anti-tissue factor pathway inhibitor (TFPI) monoclonal antibody that enhances thrombin generation by inhibiting the TFPI anticoagulant pathway, thus compensating for the deficiency of both FVIII and FIX. While their mechanism of action is well established, their potential interaction with cellular components of coagulation, such as platelets, remains poorly characterized. Platelets play a crucial role in coagulation by providing a procoagulant phospholipid surface and amplifying thrombin generation; however, their contribution in patients receiving non-replacement therapies has not been fully elucidated. This study aimed to assess whether platelets contribute to hemostatic potential in patients treated with Emz or Concz by comparing thrombin generation assay (TGA) parameters in platelet-rich plasma (PRP) and platelet-poor plasma (PPP).

Methods. Among 21 patients on Emz prophylaxis and 2 patients on Concz prophylaxis (in Novo Nordisk study NCT04082429) followed at our Centre, blood sampling was obtained from 15 patients with severe HA, with and without inhibitors, on stable Emz prophylaxis, and 2 patients receiving Concz (one with severe HA and one with severe HB). Thrombin generation was evaluated in both PRP and PPP using the ST Genesis® (Stago, Asnières-sur-Seine, France): frozen PPP samples were analyzed using the standard protocol. PRP samples were analyzed within 2 hours of collection using an in-house validated protocol. The following parameters

were recorded: Lag Time, Peak Height, Time to Peak, Endogenous Thrombin Potential (ETP), Velocity Index, and Start Tail. PRP and PPP results were compared to evaluate the impact of platelets on thrombin generation.

Results. Six samples were excluded due to instrument malfunction. A comparison of the results obtained in PRP and PPP in the 11 evaluable patients is shown in Table 1. A consistent trend was observed across all pts: in PRP, there was an increase in Lag Time, Time to Peak, ETP and Start Tail, and a decrease in Velocity Index; instead, Peak Height did not demonstrate a uniform trend across all pts. However, no appreciable differences in the thrombin generation profile were observed between patients treated with Concz and those treated with Emz.

Conclusions. A consistent difference between PPP- and PRP-based TGA was observed across all patients. Specifically, PRP curves exhibited a delayed onset of thrombin generation and a prolonged time to peak, alongside a higher total amount of thrombin generated and greater endogenous thrombin potential. These findings suggest that PRP-based TGA may provide a more comprehensive assessment of the hemostatic profile in patients receiving non-replacement therapies, capturing the contribution of platelet-dependent coagulation processes.

Increase or decrease between PPP and PRP			
Parameter	Emicizumab (Hemophilia A)	Concizumab (Hemophilia A)	Concizumab (Hemophilia B)
Lag Time	+151,80%	+133,19%	+109,06%
Peak Height	+19,53%	-0,62%	-46,81%
Time to Peak	+126,33%	+82,30%	+137,88%
ETP	+51,00%	+27,97%	+43,67%
Velocity Index	-33,49%	-62,73%	-82,04%
Start Tail	+33,47%	+43,94%	+157,67%