

Diagnostica di laboratorio in emostasi e trombosi

FLOW-BASED WHOLE-BLOOD THROMBUS PROFILING DISCRIMINATES ANTITHROMBOTIC DRUG EFFECTS AND UNCOVERS PLATELET-DEPENDENT COAGULATION DEFECTS AFTER CARDIOPULMONARY BYPASS

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Background and Aims. Conventional coagulation tests and viscoelastic assays assess isolated enzymatic pathways or fibrin-weighted clot mechanics and often fail to capture thrombus formation as a dynamic process arising from platelet–coagulation interactions under flow. Most methods also rely on exogenous activators or inhibitors that may alter physiological responses. These limitations are relevant in complex settings such as cardiopulmonary bypass (CPB), where coagulopathy is multifactorial and not fully reflected by routine assays. We evaluated a flow-based whole-blood approach to quantify integrated thrombus formation under native conditions and assess its sensitivity to antithrombotic therapies and CPB-related hemostatic alterations.

Methods. Whole blood from healthy donors (n=62), patients on antiplatelet therapy (ASA n=24; $P2Y_{12}$ inhibitors n=11; DAPT n=32), anticoagulants (DOACs n=18; VKAs n=12), and CPB patients (Pre-CPB n=21; Post-CPB t_0 (immediately after protamine infusion) n=5; Post-CPB ICU (within ≈ 1.5h from protamine) n=21) was analyzed. Pre-CPB patients received low-dose aspirin (75–100 mg/day). Recalcified citrated blood was perfused over collagen at arterial shear ($\dot{\gamma}$=300 s^{-1}) without exogenous activators or inhibitors. Dual-channel epifluorescence imaging enabled real-time quantification of platelet aggregation and fibrin(ogen) formation. Time-series of integrated density (ID) were modeled to derive pseudo-volumes (AUC ID) for platelets, fibrin(ogen), and total thrombus. Platelet aggregate size was assessed at early (50–100 s) and late (200–450 s) phases; fibrin lag time was assessed by Kaplan–Meier analysis.

Results. The assay resolved distinct pharmacodynamic signatures. ASA reduced fibrin(ogen) and total thrombus more than platelet signal, whereas $P2Y_{12}$ inhibition markedly impaired platelet-dependent thrombus growth; DAPT showed the strongest inhibition. Anticoagulants mainly reduced fibrin(ogen) and total thrombus, with greater suppression in VKAs at higher INR. In CPB patients (Table 1), platelet (−66%), fibrin(ogen) (−71%), and total thrombus (−70%) pseudo-volumes were markedly reduced at ICU despite near-normal INR and aPTT. Early platelet adhesion (50–100 s) was preserved, indicating intact initial interaction with collagen. In contrast, aggregate growth was impaired in the late phase (200–450 s), when thrombin normally amplifies platelet recruitment and fibrin formation. Consistently, fibrin lag time was prolonged (219→332 s). These findings indicate that, despite preserved adhesion, insufficient development of a platelet-dependent procoagulant surface limits thrombin generation, resulting in reduced fibrin formation and smaller thrombus structures.

Conclusions. Flow-based whole-blood thrombus profiling discriminates functional signatures of anticoagulant and antiplatelet therapies and identifies a platelet-dependent coagulation defect after CPB not detected by conventional assays. The dissociation between preserved early adhesion and impaired thrombus growth reflects defective support of thrombin generation during the propagation phase of coagulation. By integrating platelet and fibrin dynamics under physiological flow without exogenous substances, this approach provides a mechanistic assessment of hemostatic function and may improve identification of clinically relevant bleeding risk in complex settings.

Table 1. Flow-Dependent Thrombus Parameters Before and After Cardiopulmonary Bypass

Parameter	Statistic	Pre-CPB (ASA) (n=21)	Post-CPB ICU (n=21)	Δ vs pre-CPB	Interpretation
INR	Range	1.00–1.41	1.03–1.76	Minimal change	Plasma pathways preserved
aPTT ratio	Range	0.80–1.39	0.76–1.67	No consistent prolongation	Intrinsic pathway preserved
Platelet AUC ID [×10⁷]	Mean ± SD	3.06 ± 1.56	1.04 ± 0.70	↓ 66%	Reduced platelet contribution under flow
Fibrin(ogen) AUC ID [×10⁷]	Mean ± SD	3.33 ± 1.47	0.96 ± 0.78	↓ 71%	Reduced thrombin-driven fibrin formation
Aggregate Size at 50 s (µm²)	Mean ± SD	17.7 ± 14.1	13.4 ± 5.6	↓ 24% (ns)	Initial adhesion preserved
Aggregate Size at 100 s (µm²)	Mean ± SD	13.3 ± 3.2	13.0 ± 4.5	↓ 2% (ns)	Early adhesion preserved
Aggregate Size at 200 s (µm²)	Mean ± SD	32.2 ± 9.9	18.6 ± 7.0	↓ 42%	Impaired thrombin-independent consolidation
Aggregate Size at 450 s (µm²)	Mean ± SD	131.7 ± 80.4	55.9 ± 37.0	↓ 58%	Reduced thrombus expansion
Fibrin Lag Time (s)	Median (K–M)	219	332	↑ 52%	Delayed thrombin onset
Total Thrombus AUC ID [×10⁷]	Mean ± SD	6.37 ± 2.72	1.94 ± 1.41	↓ 70%	Markedly reduced global thrombus capacity