Device-Induced Hemostatic Disorders in Mechanically Assisted Circulation

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Abstract
Mechanically assisted circulation (MAC) sustains the blood circulation in the body of a patient undergoing cardiac surgery with cardiopulmonary bypass (CPB) or on ventricular assistance with a ventricular assist device (VAD) or on extracorporeal membrane oxygenation (ECMO) with a pump-oxygenator system. While MAC provides short-term (days to weeks) support and long-term (months to years) for the heart and/or lungs, the blood is inevitably exposed to non-physiological shear stress (NPSS) due to mechanical pumping action and in contact with artificial surfaces. NPSS is well known to cause blood damage and functional alterations of blood cells. In this review, we discussed shear-induced platelet adhesion, platelet aggregation, platelet receptor shedding, and platelet apoptosis, shear-induced acquired von Willebrand syndrome (AVWS), shear-induced hemolysis and microparticle formation during MAC. These alterations are associated with perioperative bleeding and thrombotic events, morbidity and mortality, and quality of life in MCS patients. Understanding the mechanism of shear-induce hemostatic disorders will help us develop low-shear-stress devices and select more effective treatments for better clinical outcomes.

Keywords
cardiopulmonary bypass, extracorporeal membrane oxygenation, ventricular assist device, mechanical shear stress, hemostatic disorder

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Introduction
Mechanically assisted circulation (MAC) includes all methods that can provide hemodynamic support for patients whose heart cannot provide adequately function due to cardiac failure or cardiopulmonary failure. MAC has been implemented in cardiopulmonary bypass (CPB), extracorporeal membrane oxygenation (ECMO) or extracorporeal life support (ECLS) and mechanical circulatory support (MCS). CPB is a technique in which the artificial blood pump and gas exchange device (oxygenator) completely take over the function of the heart and lung, and bypass both of them during aortic cross-clamping of cardiac surgery. ECMO or ECLS is a modified CPB technique in which the blood pump and oxygenator may partially or completely support the heart and/or lung for days to weeks for the management of patients in critical care suffering severe respiratory and cardiac failure. Ventricular assist devices (VADs) are used to provide MCS to patients who suffer from cardiac failure only. A VAD can be used to support left ventricle (LVAD) or right ventricle VAD (RVAD) or 2 VADs (BiVAD) for both ventricles for weeks to years. The total artificial heart (TAH) is a special form of MCS as a bridge to transplantation or to be used for destination therapy for specific patients with biventricular heart failure.1 It is estimated that over 400,000 (USA), 100,000 (Europe), and 36,000 (UK) cardiac surgeries are performed each year. Most of these procedures are performed on hearts under CPB support.2 More than 10,000 patients have been treated with ECMO every year since 2016 around the world.3 And, more than 2,500 LVADs have been implanted annually over the last decade.4 The only FDA-approved TAH—Syncardia has been implanted almost 1800 times in the United States, Canada, and Europe.5

No matter which type of MAC, blood is constantly exposed to non-physiological shear stress (NPSS) due to mechanical

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pumping action and non-biological, artificial surfaces of the circuit components, such as blood pump, hollow fiber membrane oxygenator, filters, arterial and venous cannulas, tubing and connectors. These 2 factors have been shown to cause blood cell damage and protein activation, leading to activation of the contact system, coagulation cascade, fibrinolytic system, complement system and possibly weakened immune defense system.\(^6\)-\(^9\) Although different anticoagulation strategies are used in patients with CPB or on ECMO or VAD support to prevent thrombosis, bleeding and thrombotic events in patients or circuitry remain frequent despite increasing knowledge of their pathogenesis and improvements in pump and oxygenator design, and the use of heparin-bonded surfaces.\(^10\)-\(^12\) The bleeding and thrombotic complications cannot be explained by type or severity of illness or the individual anticoagulation regimen. From the perspective of biomedical engineering, the non-physiological mechanical force acting on the blood’s formed elements and proteins could induce structural and functional changes of these blood elements and proteins in varying degrees, including platelet activation, platelet receptor shedding, platelet apoptosis, von Willebrand factor (VWF) fragmentation, hemolysis, microparticle generation, impaired blood adhesion and aggregation. Platelet dysfunction, VWF fragmentation and hemolysis may contribute to both thrombosis and bleeding. In this article, we briefly review prevalence of thrombotic and bleeding complications in patients with MAC and discuss platelet physiology and acquired hemostatic dysfunction during MAC, including structural alterations and dysfunction of blood elements relevant to hemostatic function.

**Prevalence of Bleeding and Thrombotic Complications**

The prevalence of bleeding complications in patients undergoing cardiac surgery with CPB and on ECMO or LVAD support is summarized in Table 1. Excessive post-operative bleeding continues to be a concern for cardiac surgeons and port is summarized in Table 1. Excessive post-operative bleeding and 10 patients had mild (601-800 ml) and moderate (801-1000 ml) insignificant bleeding (<600 ml within 12 h), nearly 40 patients experience re-exploration after cardiac surgery, which is associated with the need of transfusion and increased mortality (Table 1).\(^10\),\(^14\) The exact etiology of postoperative bleeding in cardiac surgery has not been elucidated completely, but postoperative bleeding and the need for blood product replacement to compensate for blood loss are associated with significant postoperative adverse events.

Bleeding in patients on ECMO is one of the most frequent complications, including bleeding at the gastrointestinal (GI), surgical site, peripheral/mediastinal cannula site, intracranial, and pulmonary hemorrhage. There were reports that 60% of V-A ECMO adult patients and 80% of V-V ECMO patients suffered from a bleeding event,\(^11\) and 70% of children with ECMO had a bleeding event.\(^15\) According to the Extracorporeal Life Support Organization (ELSO) registry in January 2020, the surgical site bleeding is the most common in all ECMO patients (cardiac/pulmonary support: neonatal 18%/6%, pediatric 17.9%/7.7% and adult 13.8%/6.4%).\(^3\) The other common bleeding complications include the mediastinal cannulation site bleeding during cardiac support (Neonatal/pediatric: 6.9%/6.6%), the pulmonary hemorrhage during pulmonary support (Neonatal/pediatric: 4.4%/5.8%) in neonatal and pediatric patients, and GI hemorrhage in all adult ECMO patients (Cardiac/pulmonary support: 4.1%/4.9%). The ECMO circuit clots are the main thrombotic events (6.6-30%). Bleeding during ECMO forces patients to receive a significant amount of blood transfusions and has negative impacts on patient outcomes (Table 1).

For patients on LVAD support, thromboembolic and bleeding events are common as well.\(^16\),\(^17\) Bleeding is the most common adverse event for LVAD patients. It has been reported that 20%-60% of LVAD patients experience bleeding complications after implantation.\(^12\),\(^18\) Bleeding and thrombotic complications continue to be associated with increased morbidity and mortality in patients with MCS, resulting in rise of hospital admission, procedure burden, transfusion requirement, morbidity, mortality and costs (Table 1).\(^14\),\(^15\),\(^19\),\(^20\) Bleeding (41.3%) is also the most common adverse event in the early phase (≤ 3 months) of the TAH implantation, and the incidence of GI hemorrhage is about 20% in patients with 6 months of TAH implantation.\(^21\)

**Shear Stress and Shear Rate Associated With MAC**

Fluid shear stress refers to the stress coplanar component along with a fluid cross section. It arises from the shear force, the component of force vector parallel to the material cross section. Shear stress is a function of the local velocity gradient (shear rate 1/s) and local blood viscosity. For blood flowing in a circular tube, the shear stress is small at the center line of the vessel and the largest adjacent to the vessel walls because the velocity gradient is small at the center line but the largest at the wall.\(^3\) Blood is a non-Newtonian fluid, and its viscosity is dependent on the local shear rate. However, the non-Newtonian characteristic is insignificant when shear rate is higher than 1000/s where blood flows under the normal physiological conditions.\(^38\)-\(^41\)

The physiological shear rates of veins, large arteries and arterioles are about 20-200/s, 300-800/s, and 500-1600/s.\(^42\) Accordingly, the highest level of physiological shear stresses in the circulation is about 6.4 Pa (64 dyne/cm\(^2\)) for blood with a viscosity of 0.004 N·s/m\(^2\). However, NPSS is inevitably created due mechanical pumping action with MAC. The conventional roller pump used during CPB procedures can generate up to 900 Pa of instantaneous shear stress,\(^43\) while the shear stresses in adult CPB oxygenators are much smaller (between 2.10-4.35 dyne/cm\(^2\)).\(^44\) NPSS also exists within the arterial cannula, depending on the cannula tip design and flow rates.\(^45\) In contemporary ECMO and LVAD systems, continuous-flow rotary
| MCS   | Duration          | Cases | Type                               | Bleeding (%) | Transfusion threshold | Transfusion | Mortality |
|-------|-------------------|-------|------------------------------------|--------------|-----------------------|-------------|-----------|
| CPB   | 1998.1-2007.12 (van Straten AH, et al.22) | 10,025 | Adult CABG                         | 5.3%         | N/A                   | Donor blood 3.0, 2.1, 1.1, 0.9 | 2.3% (30days), 11% (>30days) |
|       | 2000.1-2010.1 (Vivacqua A, et al.23)      | 18,891 | Adult cardiac surgery              | 3%           | N/A                   | Intraoperative: PRBC 29.4%, platelet 7.9% | 1% |
|       | 2004.1-2007.12 (Mehta RH, et al.24)       | 528,686 | Adult CABG                         | 2.4%         | N/A                   | Blood products 59.2%, PRBC 54%, platelet 22% | 2.3% |
|       | 2010.10.1-12.31 (Colson PH, et al.25)     | 4,904  | Adult cardiac surgery              | 2.6%         | N/A                   | Blood products 95%, PRBC 88%, platelet 48% | Incidence of death in ICU 7% |
|       | 2010.1-2011.3 (Klingele M, et al.26)      | 215    | Adult cardiac surgery              | 2.6%         | N/A                   | Need for transfusion 83.3% | 8.8% |
| ECMO  | 2001.1-2012.12 (Doyle AJ, et al.27)       | 402    | VV-ECMO 100%                       | 17.7%, intracranial hemorrhage 14.2% | Hgb 8-9 g/dl | Total RBC 2.296 units, 0.66 unit/patient | 27.6% (6 months) |
|       | 2005.1-2016.12 (Guimbretière G, et al.28) | 509    | VA-ECMO 81%                        | Thrombotic /hemorrhagic events 55.6% | Hgb 8 g/dl, platelets $5 \times 10^7$/L | PRBC 82.8%, 1.14 unit/patient; platelet 56.7%, 3.0 unit/patient | 55.2% (6 months) |
|       | 2006.9-2015.11 (Santiago Mj, et al.29)    | 100    | VA-ECMO pediatric                  | 76%, 52% (first 24 h), reoperation 39% | Hgb 10 g/dl, platelets $100 \times 10^7$/L | First 24 h: PRBC 85%, platelets 78%, plasma 83% | 48% |
|       | 2010-2017 (Lansink-Hartgring AO, et al.30) | 164    | VA-ECMO 61%                        | 45%          | Hgb 6.9 g/dl, Platelets $50 \times 10^7$/L | RBC 85% | 51% |
|       | 2012-2014 (Muszynski JA, et al.31)       | 514    | VA-ECMO 83.9%                     | Bleeding requiring a transfusion 65.8% | Hgb 10 g/dl | Platelet 43% | PRBC 29.4ml/kg, platelet 12.1ml/kg | 45.1% (in-hospital) |
| LVAD  | 2004.10-2009.5 (Schaffer JM, et al.32)   | 86     | HM II 100%                         | Reoperation for bleeding 20%, GIB 28% (1 year) | Hgb 8-10 g/dl, Platelet 100 $\times 10^7$/L | Intraoperative: 11.6 unit, Postoperative: 12.6 unit in 48 h, 15.6 unit in first week | 51% (1 year) |
|       | 2004.7-2014.6 (Quader M, et al.33)       | 666    | HM II (83%), HV HDAD (5.1%), HM XVE (3.9%) | Reoperation for bleeding 22% | Intraoperative: HCT 18%, postoperative: HCT 21% | Blood products 92%, intraoperative 71.8%, postoperative 73% | 13.2% |
|       | 2005.3-2006.5 (Miller LW, et al.34)      | 133    | HM II 100%                         | Bleeding requiring surgery 31% | N/A                   | 53% (Requiring ≥2 unit PRBC) | 32% (1 year) |
|       | 2006.12-2017.1 (Muslem R, et al.35)      | 83     | HM II 77%, HM 3 23%                | Bleeding event 54%, reoperation 47% | Hgb < 5 mmol/L, platelet $>100 \times 10^7$/L in bleeders | Bleders vs. non-bleeders: PRBC 7.8 vs. 1.3, platelets 0.6 vs. 0.03 unit | 17% (1 year) |
|       | 2012.1-2016.6 (Malik S, et al.36)        | 205    | HM II 100%                         | GIB 28% | N/A                   | GIB vs. Non-GIB: Blood products 29 vs. 13, PRBC 21 vs. 8, platelet 2 vs. 1 unit | 37.6%, GIB vs. Non-GIB: 42% vs. 36% |
| TAH   | 1993.1-2002.9 (Copeland JG, et al.37)    | 81     | SynCardia                          | 102 bleeding events | N/A                   | 18 events: 3 PRBC, 13 events: >8 PRBC | 21% before transplantation >8 PRBC |
|       | 2006.6-2017.4 (Arabía FA, et al.38)      | 450    | SynCardia                          | 41.3%/7.1% (≤3 />3 months) | N/A                   | N/A | 34.1% (1 year) |

CABG: Coronary artery bypass surgery; GIB: Gastrointestinal bleeding; HCT: hematocrit; HM: HeartMate; HV: HeartWare; N/A: Not available; PRBC: Packred red blood cells.
Platelet Physiology

Platelets are released from megakaryocyte cells in the bone marrow and lungs, with size of 2–4 μm in diameter, lifespan of 7–10 days, and normal count of about 150-450 /μl in the human bloodstream, requiring a continuous platelet clearance and production of 100 billion platelets (70 million/min) daily to maintain a physiological platelet concentration. Although platelets are revealed to be versatile and significantly contribute to many other physiological and pathological processes such as tumor metastasis, anti-inflammatory and immune regulatory functions, the main function of platelets is to form hemostatic thrombi after vascular injury to prevent blood loss and maintain vascular integrity. Due to their small size, platelets circulate in blood and are pushed by larger red blood cells aside near the outer edge of the blood flow, allowing a majority of platelets in close proximity to the vessel wall and to arrive first and rapidly respond after the vascular damage.

1. Platelet Granules

Platelet has 3 main secretory granules—alpha (α) granules, dense granule and lysosomes. The α-granules (50-80 per platelet) mainly contain fibrinogen, fibronectin, von Willebrand factor (VWF), platelet factor 4 (PF4), P-selectin, plasminogen activator inhibitor-1 (PAI-1). The dense granules (3-8 per platelet) have adenosine triphosphate (ATP), adenosine diphosphate (ADP), P-selectin, calcium, and others. The lysosomes (0-3 per platelet) contain some acid hydrolases. Upon activation, platelets secrete contents of these granules, which play an important role in inflammation, angiogenesis, hemostasis and thrombosis. When the endothelial cells of a blood vessel are damaged by injuries or pathological alterations, the subendothelial structures, including basement membrane, collagen and microfibrils are exposed and trigger sudden platelet activation and adhesion. Subsequent to platelets activation, for example, α-granule secretion results in P-selectin expression on the platelet surface, releases of VWF and coagulation factors. ADP is secreted from dense granules to further amplify platelet activation. Various soluble stimuli released from platelets strengthen platelet adhesion and recruit more platelets into the growing thrombus, leading to platelet aggregation.

2. Membrane Glycoproteins

Platelet membrane consists of at least 9 glycoproteins (GP), named GPI to GPIX. Among them, GPIb-IX-V complex is the platelet receptor for VWF, the second most abundant receptor complex on the human platelet surface. As the mediator of the interaction of platelets with the subendothelial collagen, VWF tethers platelets via the GPIb-IX-V complex to the site of vascular injury and initiates both hemostasis and pathological thrombosis. This is the first step of platelet adhesion at the site of vascular injury, and also facilitates fibrinogen binding to GPIIb/IIIa to help platelet aggregation. But at low shear stress, platelet adhesion is independent of GPIb-IX-V complex. High shear stresses change the conformation of VWF, leading to a high-affinity ligand between VWF and GPIb-IX-V complex. Because primary hemostasis is initiated by VWF tethering platelets via GPIb-IX-V complex, a dysfunction in either VWF or platelets could increase the risk of bleeding.

GPVI is a receptor for collagen. The interaction of platelet GPVI with collagen results in platelet activation and adhesion as well. The initial interaction between VWF and GPIb-IX-V is rapidly reversible and insufficient for stable adhesion. Firm platelet adhesion on collagen under high shear requires intracellular signaling from GPVI, and is reinforced by release of soluble mediators, such as ADP and thromboxane A2 (TXA2), and by the generation of thrombin.

GPIIb/IIIa is a receptor for fibrinogen, the most abundant platelet adhesion receptor on the platelet surface. The unactivated GPIIb/IIIa can bind only immobilized fibrinogen, but the activated GPIIb/IIIa can also capture soluble fibrinogen and other ligands, resulting in platelet aggregation. Platelet GPIIb/IIIa is usually activated via inside-out and/or outside-in signaling mechanisms. The common inside-out activation cascade is triggered by binding of thrombin to proteinase-activated receptor 1 (PAR1) on the platelet membrane, leading to increased affinity of GPIIb/IIIa to fibrinogen and other ligands. Thrombin is generated by the exposure of tissue factor to the coagulation system on the vessel injury area. The outside-in activation of GPIIb/IIIa is initiated by interactions of the GPIIb/IIIa extracellular domain with fibrinogen and other
ligands, leading to the generation of a complex cascade of intracellular signaling processes that mediate irreversible stable adhesion, spreading, aggregation of platelets, and subsequent thrombus growth. Platelet aggregation through platelet GPIIb/IIIa binding to fibrinogen is the final step in platelet activation. Platelet activation leads to a remarkable increase in the affinity of GPIIb-IIIa for fibrinogen. The active form of GPIIb/IIIa can be quantified by measuring the binding of the antibody PAC-1 (phosphatase of activated cells).

Shear-Induced Platelet Adhesion and Aggregation

Platelet adhesion, activation and aggregation play a pivotal role in thrombus formation at sites of vascular injury. Circulating platelets become adherent and activated at the site of damaged or diseased vessel wall, and form aggregations that promote coagulation and prevent blood loss after injury. Three different pathways trigger this complex process, including exposure of subendothelial matrix proteins (collagen and VWF), exposure of tissue factor, and mechanically-induced platelet activation. Among them, shear-dependent platelet activation is possibly initiated by binding of plasma VWF to platelets through GPIbα, leading to platelet secretion and GPIIb/IIIa-dependent aggregation. A recent study showed that shear stress can induce prominent procoagulant activity with increased phosphatidylserine externalization (PSE) and thrombin generation, but without GPIIb/IIIa activation. The latter may result from platelet cross-activation by biochemical agonists, such as ADP and thrombin. Shear stress may directly activate platelets without the help of exogenous agonists.

Human platelets can express a mechanosensitive Ca²⁺ entry pathway that is activated by arterial shear stress in vitro. The phosphoinositide 3-kinases (PI3 K)/protein kinase B (Akt) signaling pathway is also involved with shear-induced platelet activation. However, platelets may be damaged by shear stresses ranging from 10 Pa to 50 Pa.

When blood comes in contact with foreign substances by extracorporeal circuitry, the protein adsorption rapidly occurs in a few seconds; next, platelets may adhere to adsorbed proteins within the first minute, and then platelets aggregate leading to blood coagulation and thrombus formation. The protein adsorption includes albumin, fibrinogen, VWF, thrombin, factor XII, etc. depending on physicochemical properties of both surfaces and proteins. Platelets can adhere to an artificial surface through platelet interaction with these surface adsorbed proteins. Fibrinogen is the key protein in the platelet adhesion. Thus, non-activated platelets can become activated through protein adhesion and aggregation. The latter also occurs through the adsorption of proteins and other byproducts of contact/ complement system activations. Platelet aggregation is mainly through the interaction between platelet GPIIb/IIIa and fibrinogen, ultimately forming a firm platelet plug that can prevent bleeding.

Beside hemodilution, hypothermia and blood loss during CPB procedures or ECMO support, thrombocytopenia is common in patients undergoing CPB due to complete bypassing lungs in which more than 50% of total platelet production take place. CPB also causes structural and biochemical changes in platelets due to shear forces created by blood pumps. These changes could exacerbate platelet clearance and consumption in the circulation. In ECMO patients, platelet consumption also occurs, resulting in platelet count drop. Platelets adhere to the fibrinogen which is initially absorbed by the circuit surfaces, resulting in a time-dependent platelet activation and persistent and progressive platelet dysfunction during ECMO. The impaired secretion of α- and dense granules caused relevant platelet dysfunction in patients with V-V ECMO. The reduced platelet adhesion, decreased platelet activation, and reduced platelet aggregation were reported in adult patients during ECMO treatment. In simulated LVAD systems, our in-vitro studies demonstrated that high shear stress induced platelet activation, increased the platelet adhesion on fibrinogen, reduced platelet adhesion capacity with VWF and collagen, and reduced both ristocetin- and collagen-induced platelet aggregation. Some clinical studies also showed severely impaired ristocetin-induced platelet aggregation, which may contribute to an increased tendency of bleeding in the LVAD patients. However, high shear rates (≥10,000/s) can cause vWF-mediated activation-independent platelet adhesion and aggregation, followed by stable aggregation in LVADs. Thus, acquired platelet dysfunction contributes to risks of bleeding and thrombosis during MCS.

Shear-induced platelet activation, adhesion, aggregation and platelet-mediated thrombosis relative to MCS devices may be simulated by computational fluid dynamics (CFD) using multi-scale strategies with particle-based methods, helping researchers investigate and predict device thrombogenicity. The device thromboresistance optimization methodology can further optimize the thromboresistance performance of any MCS device during the research and development (R&D) stage, thereby reducing the R&D costs and shortening the development cycle.

Shear-Induced Platelet Receptor Shedding

The receptor shedding is a process of irreversible removal of transmembrane cell surface receptors by proteolysis of the receptor at a position close to the extracellular surface of the plasma membrane. It releases a soluble ectodomain fragment and preserves a membrane-associated remnant fragment. Ectodomain shedding is a ubiquitous control mechanism for rapid and irreversible downregulation of receptor expression and regulating cellular transmembrane proteins. Metalloproteinase-mediated ectodomain shedding of platelet receptors has been recently recognized as a mechanism for regulating platelet function. GPIIbα and GPVI are the 2 key platelet receptors that bind vWF and collagen, respectively, and initiate hemostasis at sites of vascular injury. Several biochemical pathways that induce shedding of GPIIbα and/or GPVI have been identified. While these pathways might be dependent or independent of platelet activation, the loss of these functional
receptors may result in defective platelets. Defects affecting normal expression and function of GPIbα-IX-V (Bernard-Soulier syndrome) or GPVI result in mild or more severe bleeding. Linking these defects to the bleeding is complicated by co-existing abnormalities in these patients. Animal studies demonstrated that genetic disruption of normal GPIbα and/or GPVI or inhibition using antibodies or other inhibitors impaired adhesion capacity of platelets on subendothelial matrix (vWF or collagen) and attenuated thrombotic propensity of platelets.

The role of shear stress in platelet receptor shedding has not been well defined. Only a few studies have been carried out to examine the shear-mediated platelet receptor shedding either in the context of platelet-surface interaction or direct shear effect. NPSS induces not only platelet activation and hemolysis, but also the shedding of platelet receptors. Activated platelets in the circulation increase the risk of thrombosis. But shedding of platelet receptors could significantly affect initiation and development of the thrombus. An in-vitro study showed that induction of GPV cleavage caused specific down-regulation of collagen-induced platelet aggregation. Loss of the platelet GPIba and GPVI may contribute to ablated platelet adhesion/aggregation. This may be a protective mechanism for down-regulating platelet adhesiveness during elevated shear stress, thereby reducing the level of platelet activation and thrombus formation.

NPSS has been considered to contribute to the shedding of 3 specific platelet receptors—GPIbα, GPVI, and GPIIb/IIIa. Platelet receptor shedding is regulated by endogenous membrane-associated proteinases—platelet sheddases. The major sheddases for GPIbα-IX-V and GPVI are members of the a disintegrin and metalloproteinase (ADAM) family—ADAM17 and ADAM10. ADAM17 cleaves GPIbα, ADAM10 primarily cleaves of GPVI, and both ADAM10 and ADAM17 cleave GPV. Plasmin can proteolyze GPIIa. shear-induced GPIbα shedding is dependent on GPIbα-VWF interaction, calpain, and metalloproteinase activations. Moreover, shear-induced GPVI shedding did not require VWF interaction with GPIbα, GPIIb/IIIa engagement, or platelet activation, and is metalloproteinase-dependent after exposure of platelets to NPSS in vitro. Beside ADAM-induced GP shedding, direct mechanical damage is partially responsible for NPSS-induced receptor shedding. Because the ADAM inhibition couldn’t completely suppress the NPSS-induced loss of the GPIbα and GPVI. This phenomenon could be explained as resulting from both the NPSS-induced GP shedding by mechanical forces and ADAM-induced glycoprotein shedding by triggering an intracellular signaling.

Loss of the platelet surface receptors GPIbα and GPVI has been reported in heart failure patients. Some early clinical studies showed that GPIb levels decreased during 120-min CPB and then gradually return to pre-bypass level. But no significant change in platelet GPIIb/IIIa expression throughout CPB, and a significant decrease in levels soon after CPB was reported. These may indirectly suggest the existence of platelet receptor shedding by CPB procedure. A recent clinical study confirmed a decrease in platelet GPIb surface expression after adult CPB. Our results also showed CPB-associated platelet activation and concomitant receptor shedding in pediatric patients with CPB. Similarly, increased platelet GPIbα and GPVI shedding is already present in patients before ECMO placement and remain significantly elevated after on ECMO support. The increase in soluble GPV levels was found in CF-VAD and ECMO patients as compared with healthy donors, indicating device-related shedding in patients on LVAD or ECMO support. For LVADs, in-vitro studies demonstrated that NPSS induce both activation and shedding of the GPIbα, GPVI and GPIIb/IIIa on the platelet surface occur simultaneously and increase with increasing shear stress level and exposure time. Plasma soluble GPIIb/IIIa also increased with increasing shear stress level. Platelet receptor shedding may lead platelet dysfunction and influence the coagulation system. In a study on LVAD patients, platelet GPIbα shedding was observed to be a preexisting condition and increase post-implantation in those patients who experienced bleeding events. Loss of the platelet receptors GPIbα and GPVI in LVAD patients may contribute to hemorrhagic complications. Therefore, on the one hand LVADs can induce platelet activation and enhanced platelet adhesion, which may lead to an elevated risk of thrombosis, but on the other hand they can also cause the loss of platelet receptors and reduced platelet adhesion capacities, which may lead to hemostasis dysfunction and elevated propensity of bleeding.

Shear-Induced Platelet Apoptosis

Apoptosis is a physiologic process of cell death to control the number of cells and eliminate unwanted cells. Platelet apoptosis can be induced by physiologic compounds (such as phosphatidylserine (PS) and thrombin), chemical stimuli, NPSS (mechanical rheological forces) and platelet storage. NPSS (117–388 dynes/cm²) were reported to induce not only platelet activation but also apoptosis. It’s believed that mechanoreceptors on platelets transmit activation and apoptotic signals to the cell interior, including P-selectin express on the platelet surface, depolarization of mitochondrial membrane potential, activation of cytosolic enzyme caspase-3, microparticle formation and PS translocation from the inner to the outer plasma membrane leaflet. The damaged platelets by NPSS may be recognized as “unwanted” cells by the reticuloendothelial system and then are removed from the circulation. Additionally, the GPIbα–VWF interaction initiates platelet adhesion and thrombus formation under pathophysiologic flow conditions, but it also induces apoptotic events in human platelets which occurs independently of platelet activation. GPIbα may act as one of mechanoreceptors transmitting apoptotic signals inside the platelet. Our study confirmed platelet apoptosis existed in continuous-flow-LVAD patients who developed bleedings within 1 month post-implantation and was associated with endogenous reactive oxygen species (ROS) generation triggering the intrinsic pathway of platelet apoptosis.
Shear-Induced Acquired von Willebrand Syndrome (AVWS)

VWF is a multimeric plasma glycoprotein, synthesized by endothelial cells and megakaryocytes in a multimemerized form from identical single-chain subunits into disulfide-linked multimers. Each subunit has a single A1 domain that is the binding site for platelet GPIbα and collagen IV, a A3 domain that binds to collagens I and III, and a C4 domain that is binding site for the platelet-integrin zIIb3 (glycoprotein GPIIb/IIIa). \(^{126,127}\) VWF is stored at the Weibel-Palade bodies in the endothelial cells and the z-granules in platelets. Endothelial cells secrete VWF to maintain basal levels of VWF in plasma (approximately 10 \(\mu\)g/mL in humans). After release into blood, Ultra-large VWF multimers (up to 20,000 kDa) bonded to the endothelial cell surface are rapidly cleaved by the metalloprotease ADAMTS-13 into smaller forms and release into the circulation. Soluble VWF cannot interact with platelets in the absence of injury. \(^{65,128}\) But, it can noncovalently link with blood clotting factor VIII to protect factor VIII from degradation. \(^{126,129}\) The absence of VWF results in low activity level of the factor VIII. A majority of VWF is cleared by macrophages in the liver, spleen and vascular endothelial cells and its circulating half-life ranges from 6 to 24 h. \(^{95,130}\) VWF plays a major role in primary hemostasis by acting as a bridging molecule between vascular injury and circulating platelets in blood, recruiting platelets to injured vessel wall. Upon vascular damage, subendothelial collagen is exposed. VWF can bind with exposed collagen at the A3 domain and become tethered to the vessel wall. Its A1 domain then binds to the platelet receptor GPIbα–IX-V complex, simultaneously triggering intracellular signaling cascades leading to platelet aggregation and thrombus formation. \(^{64}\) The adhesiveness of VWF is determined by its multimeric size. High-molecular-weight (HMW) multimers (5000–10,000 kDa) are the most effective to interact with collagen and platelet receptors. \(^{131}\) The GPIbα–VWF interaction also induces apoptotic events in human platelets. \(^{124}\)

Under normal circulation conditions, VWF exists in a compact globular (collapsed) conformation. It is essentially inert at the low shear rates of veins and normal arteries, and does not stimulate platelet adhesion. \(^{132}\) VWF multimers circulating in blood also do not bind the platelet GPIb-IX-V complex but can be induced by elevated shear stress. Platelet adhesion is strongly dependent on VWF at shear rates >1,000/s. \(^{126}\) Shear-induced conformational changes in VWF may contribute to the regulation of VWF binding to platelet GPIb, \(^{126,133}\) Force-sensing mechanisms of VWF are the key to its function in hemostasis and its role in thrombosis. \(^{134}\) NPSS can change VWF multimers from a globular shape to an elongated rope-like structure. \(^{135}\) Structural changes of VWF molecule induced by NPSS expose the A2 domain \(^{136,137}\) and promote proteolytic cleavage by ADAMTS-13 and multimeric binding to platelets GPIbα through A1 domain. \(^{138}\) The VWF-platelet binding also increases proteolytic cleavage by ADAMTS-13. \(^{139}\) As a result, VWF multimers are degraded into smaller multimers. The loss of large multimers of plasma VWF may increase the bleeding tendency associated with AVWS by down-regulation of VWF’s hemostatic potential. \(^{126}\)

AVWS became a clinical concern in 1990s after realizing the association between GI bleeding and aortic stenosis. \(^{140}\) The severity of the stenosis (transvalvular gradients) was negatively correlated with the percentages of the HMW-VWF multimers. \(^{141}\) NPSS across the stenotic valve lead to increased fragmentation of HMW-VWF multimers. \(^{142}\) Aortic valve replacement reverses the bleeding disorder. A similar AVWS is observed in LVAD patients. Nearly all patients with continuous flow LVADs suffer from AVWS diagnosed by the decrease in or absence of HMW vWF multimers. \(^{143-146}\) Therefore, AVWS is considered as one of the reasons for increased bleeding complications, such as epistaxis, GI and non-surgical hemorrhage, in patients with VAD. The loss of large multimers reduced collagen binding capacity (VWF: CB) and ristocetin cofactor activity (VWF: RCo) in LVAD patients early in the postoperative period after LVAD implantation, persistent during LVAD support, and resolves after LVAD explantation or heart transplantation. \(^{144,147,148}\) Similarly, gastrointestinal bleeding can stop after removal of the LVAD. \(^{137,149}\) ECMO patients also showed a decreased collagen binding capacity to VWF antigens, a decreased VWF: RCo/VWF: Ag ratios, a decreased VWF: CB/VWF: Ag ratios, and loss of HMW-VWF multimers. \(^{90,113,150,151}\) AVWS is also reversible in ECMO patients after ECMO removal. \(^{150}\) It is clear that the designs of contemporary LVADs or ECMO systems and long-term support affect hemostatic factors. \(^{93,147,152}\) It is known that artificial surface of the ECMO and LVAD systems plays an important role in the activation of platelets. It is possible that artificial surfaces could disrupt HMW-VWF multimers. It is widely considered that NPSS, generated around the impellers of rotary pumps, in the arterial/venous cannulas and in the circuits for hemoconcentration or continuous renal replacement therapy (CRRT) during MCS, can result in loss of HMW-VWF multimers, subsequently leading to AVWS and increase risk of bleeding complications. A reduced pump speed cannot effectively decrease VWF degradation. \(^{153}\) Supplementation with factor VIII/vWF concentrate is necessary in life-threatening bleeding episodes during ECMO and LVAD. \(^{154}\) Device explantation or transplantation can completely resolve the AVWS. However, AVWS is universal to all patients on ECMO and LVAD, possibly CPB, we believe that AVWS is a factor to increased bleeding, but not determining factor. Platelet defects are the key factor to bleeding in these patients.

Shear-Induced Hemolysis

Hemolysis was a key issue during the early stage of development and clinical use of MCS and is associated with increased blood product transfusion requirements, increased incidence of acute kidney injury, increased mortality and morbidity, impending oxygenator failure in ECMO and pump thrombosis in ECMO and LVAD. \(^{155,156}\) Although sub-clinical hemolysis is still generated by contemporary devices, hemolysis has become less an issue in patients on LVAD or ECMO support unless...
Shear-Induced Microparticle Formation

Microparticles in blood are small membrane vesicles derived from a variety of cell types, sizing 0.1-1 micron in diameter. Blood pumps used in CPB, ECMO and LVAD systems can generate high shear stress that may lead to the production of microparticles from shear-sensitive cells, such as platelets. Platelet-derived microparticles (PMP) are strongly procoagulant because they contain PS and tissue factor (TF). There was a report that cardiac surgery on CPB induced an increase amount of PMPs in systemic blood and pericardial blood. An in-vitro study showed that PMPs generation with an increasing trend over time in neonatal ECMO system with a centrifugal pump compared with a roller pump, perhaps because centrifugal pumps have a dynamic shear stress. Our in-vitro experiment also demonstrated PMPs generation with an increasing trend over time in the pediatric ECMO system. Patients with LVAD had significantly increased microparticles from platelets, leukocytes, and endothelial cells, which may increase risk of developing thromboembolic complications. LVAD patients had higher level of PS$^+$ microparticles and patients who developed an adverse event had significantly higher PS$^+$ microparticles than patients with no events.

Conclusions

MAC saves lives of millions of patients who suffer heart failure, respiratory failure, and cardiopulmonary failure or who undergo cardiac surgery. In these patients, the survival rate increases, and the post-operative complications decrease year after year. However, postoperative bleeding and thrombotic complications remain biggest challenges for clinicians and patients. The exposure of blood to artificial surfaces of the circuit and NPSS can induce platelet activation, which increases the risk for thrombotic events, but NPSS also causes platelet receptor shedding, AVWS and platelet apoptosis, which lead to hemostasis dysfunction. In addition, anticoagulation is must to prevent the circuit from clotting. So, patients undergoing MCS simultaneously face high risks of thrombosis and bleeding. The whole key is finding the balance between preventing clotting due to the circuit without inducing the risk for hemorrhagic complications by NPSS. Therefore, based on knowledge of shear-induced hemostatic disorders, it is necessary to modify circuit material and design for bioengineers, develop low-shear-stress blood pumps, oxygenators and cannulas for manufacturers, optimize anticoagulation therapy and strengthen clinical management for clinicians, aiming to reduce bleeding and thrombotic complications and improve clinical outcomes in patients with MCS.
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