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Platelets: versatile effector cells in pneumonia and sepsis

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The role of platelets in sepsis

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Abstract

Platelets are small circulating anucleate cells that are of crucial importance in haemostasis. Over the last decade, it has become increasingly clear that platelets play an important role in inflammation and can influence both innate and adaptive immunity. Sepsis is a potentially lethal condition caused by detrimental host response to an invading pathogen and activation of the coagulation system. Platelets, major effector cells in both haemostasis and inflammation, are involved in sepsis pathogenesis and contribute to sepsis complications. Platelets catalyse the development of hyperinflammation, DIC and microthrombosis, and subsequently contribute to multiple organ failure. Inappropriate accumulation and activity of platelets are key events in the development of sepsis-related complications such as acute lung injury and acute kidney injury. Platelet activation readouts could serve as biomarkers for early sepsis recognition; inhibition of platelets in septic patients seems like an important target for immune-modulating therapy and appears promising based on animal models and retrospective human studies.

Introduction

Sepsis is a life-threatening condition that arises when the body's response to an infection injures its own tissues and organs. In the United State alone the number of sepsis cases exceeds 750,000 annually and its incidence is growing¹. The pathogenesis of sepsis involves a series of complex regulatory interactions, with concomitant and often antagonistic processes, resulting in a dysregulated host response with both exaggerated inflammation and immune suppression^{1,2}. The proinflammatory response to sepsis leads to activation of the coagulation system with concurrent inhibition of anticoagulant mechanisms and fibrinolysis³. Early after infection, local activation of coagulation contributes to host defence against infectious agents in an attempt to trap and kill the invading microorganisms^{3,4}. An uncontrolled procoagulant response however, can lead to the clinical syndrome known as disseminated intravascular coagulation (DIC) characterized by both microvascular thrombus formation and haemorrhage³. In sepsis, triggering of inflammatory and coagulation cascades, together with endothelial damage, invariably leads to activation of platelets, which can be further stimulated by direct interactions with pathogens^{5,6}. Platelets are traditionally considered essential components of primary haemostasis. Platelets adhere and aggregate at sites of vascular injury to form a plug, which, together with activation of the coagulation system, safeguards vessel integrity and prevents haemorrhage. More recent investigations have revealed an additional role for platelets, namely in immunity. Excellent reviews summarizing data on platelets and the immune continuum have been published in recent years^{5,7-9}. This review focuses on platelet activation and their functional role in the context of sepsis.

Platelet activation during sepsis

Owing to their high numbers and sensitivity to environmental changes, platelets are uniquely positioned to perform sentinel tasks in our circulatory system. It is therefore thought that platelets are one of the first responding cells during the development of sepsis, when pro-inflammatory and pro-coagulant mechanisms derail. Consequently, platelet activation readouts have been suggested as biomarkers for the development of sepsis complications and have been related to prognosis. Platelet biomarkers could be platelet secreted products, platelet P-selectin expression, platelet-leukocyte complex formation or platelet functionality assays (Table 1).

Observational studies have documented marked platelet activation in sepsis patients, as reflected by an increase in P-selectin expression on the platelet surface^{10,11} and increased plasma levels of alpha granular released products such as soluble P-selectin¹², triggering receptor expressed on myeloid cells-like (TREM-like) transcript-1 (sTLT-1)¹³ or platelet factor

Table 1: Platelet activation read outs during sepsis

Human studies			
What	In	Associated with	
Thrombocytopenia	Critical illness	Mortality	21
Thrombocytopenia	Critical illness	Mortality	20
Thrombocytopenia	Sepsis, ALI	Mortality	120
IPF	Critical illness	Sepsis progression	23
Impaired platelet function	Sepsis	-	18
Impaired platelet function	Sepsis	MODS	17
Impaired platelet aggregation	Sepsis	Sepsis progression and outcome	16
Impaired platelet aggregation	Critical illness	Sepsis progression	121
Platelet P-selectin expression / Platelet-neutrophil adhesion	Sepsis	MODS	11
Platelet P-selectin expression / Platelet-neutrophil adhesion	Sepsis	MODS and poor clinical outcome	10
Spliced tissue factor mRNA	Sepsis	-	57
Granzyme B upregulation platelets	Sepsis	-	60
sP-selectin	Sepsis	ALI	12
sTLT-1	Sepsis	DIC	13
Platelet derived MPs	Sepsis, severe trauma	-	24
Animal studies			
Altered proteomic pattern	Sepsis	-	58
Plasma PF4	Pneumonia/sepsis		14

ALI = acute lung injury, IPF = immature platelet fraction, MODS = multiple organ dysfunction syndrome, sTLT-1 = triggering receptor expressed on myeloid cells-like (TREM-like) transcript-1, DIC = diffuse intravascular coagulation

(PF)4 in mice¹⁴. TLT-1 is an orphan receptor expressed only by the platelet and megakaryocyte lineage, and moved to the platelet surface upon activation with thrombin, collagen or LPS¹⁵. Patients diagnosed with sepsis have dramatically increased levels of sTLT-1 in their blood, and this level correlates with the clinical manifestation of DIC¹³. As such, sTLT-1 levels could be used as an early predictor the development of DIC. TLT-1 was additionally shown to augment platelet aggregation, suggesting a role for TLT-1 as a regulator of haemostasis during sepsis via autocrine stimulation of platelet aggregation¹³. Platelet functionality, measured by whole blood impedance aggregometry, was recently suggested as a biomarker for diagnosis and prognosis of severe sepsis. High platelet function was associated with a mortality of 10%, while mortality was 40% when platelet function was low¹⁶. This phenomenon was also seen in some other studies, in which the severity of sepsis correlated to platelet aggregation defects^{17,18}.

Thrombocyte counts may be useful as a directive for prognosis in critically ill patients. A decrease in platelet counts can indicate pathologic coagulation activation, which contributes

to complications such as DIC and multiple organ failure¹⁹. Irrespective of the cause, thrombocytopenia is an independent predictor of mortality in the ICU¹⁹. Thrombocytopenia in critically ill patients has a biphasic pattern that is different in patients who do or do not survive. Platelet counts drop after admission to the ICU in both patient groups, an increase relative to admission counts however was only present in survivors²⁰. A drop in platelet counts of 30% or more independently predicts death²¹. Whether thrombocytopenia represents platelet activation and consumption as a primary pathologic event or merely serves as a marker for disease severity is unknown. This uncertainty is a consequence of the multitude of conditions known to influence circulating platelet numbers in ICU patients. Critically ill individuals may have diminished platelet production due to medication effects, bone marrow suppression, nutritional deficiencies and infection²². Measuring immature platelet fractions (IPF%) distinguishes between thrombocytopenia due to increased platelet destruction and thrombocytopenia related to bone marrow failure. Increased IPF% has been related to thrombotic events including DIC in sepsis. Indeed, IPF% increase before sepsis becomes clinically manifest in patients with systemic inflammation, and might also be useful as a prognostic marker of this condition²³.

Platelet microparticle formation during sepsis

In thrombotic conditions such as sepsis, increased concentrations of microparticles (MPs) have been reported^{24,25} and related to sepsis prognosis²⁵. MPs are fragments of the cell membrane ranging from 50 nm to 1000 nm shed from almost all cell types, typically following apoptosis, and reflect the antigenic content of the cells from which they originate²⁶. MPs positive for platelet markers are a normal component of circulating blood, derived from mature platelets upon activation and from a constitutive production by megakaryocytes in the bone marrow²⁷. MPs are considered as a distributed storage pool of bioactive effectors, exerting proinflammatory and prothrombotic properties in the immediate microenvironment of their formation²⁸. Functional differences between megakaryocyte-derived MPs and MPs generated from activated platelets may exist. The MP types differ in their expression of platelet activation markers such as P-selectin²⁷; megakaryocyte-derived MPs may be less capable of supporting inflammatory responses and complement activation^{27,29}. Platelet-derived MPs may contribute to myocardial dysfunction in sepsis, as they induced a decrease in myocardial contractility in isolated heart and papillary muscle preparations *in vitro*³⁰. Moreover, a particular subset of platelet-derived MPs from septic patients induced apoptotic death of endothelial and smooth muscle cells *in vitro* by superoxide release^{25,31}. Platelet-derived MPs are important for primary haemostasis upon endothelial injury and provide a catalytic surface for the assembly of vitamin K-dependent clotting factors (factors VII, IX, and X), which - relative to intact platelets - offers approximately 50-100-fold more procoagulant activity³². In addition, MPs are the most important source for blood-borne TF.

Mechanisms for platelet activation in sepsis

In an intact circulatory system, platelets circulate at high shear rate and are maintained in an inactive state by prostacyclin and nitric oxide secreted by endothelial cells. During sepsis, inflammation-induced coagulation results in excessive thrombin formation⁶. Thrombin is an important platelet agonist via protease activated receptor (PAR)1, PAR3 and PAR4, present on the platelet membrane³³. The pathogenesis of sepsis is furthermore characterised by endothelial activation, which leads to subendothelial collagen exposure and von Willebrand Factor (vWF) and tissue factor (TF) expression on endothelial cells. Collagen and vWF can bind to platelet GPIIb/IIIa and GPIIb/IIIa-GPIIb/IIIa respectively (Figure 1)³⁴ and TF can further initiate the extrinsic pathway of coagulation resulting in more thrombin - all initiating additional platelet activation and recruitment of platelets and immune cells (Figure 1). The complement system is also activated in overwhelming bacterial infections that lead to sepsis³⁵. The complement system is one of the key players in host defence. Its activation during the innate immune response leads to the generation of several proteins that contribute to the lysis and opsonization of microorganisms, regulate inflammatory reactions and bridge innate immunity with the subsequent adaptive immune response. Complement component C1q has been shown to additionally be able to activate platelets via the C1q receptor (C1qR) (Figure 1)³⁶.

Several bacteria have been shown to mediate platelet activation. Platelet-bacterial interactions can be direct or indirect, mediated via plasma proteins such as fibrinogen, vWF, complement and immunoglobulins. Recently, FcγRIIa was shown to play a critical role in platelet activation by several *Streptococcal* strains and *Staphylococcus aureus*. Induction of FcγRIIa is dependent on IgG and GPIIb/IIIa, after which feedback agonists ADP and TXA2 are mandatory for platelet aggregation³⁷. Additionally, PF4 binds to bacteria and reduce lag time for aggregation³⁷. Of note, mouse platelets do not express FcγRIIa. Other platelet receptors that mediate platelet-bacterial interactions are GPIIb/IIIa, PAR1, complement receptor C1qR and toll-like receptors (TLRs) (Figure 1). Extensive research has been done on bacterial species specific platelet interactions (reviewed in^{38,39}).

TLR expression on human and mouse platelets is a relatively recent observation now documented by many laboratories⁴⁰⁻⁴³ (Figure 1). TLRs are a family of pattern recognition receptors that are critical for microbial surveillance and regulation of inflammatory and immune responses⁴⁴. Several research groups have confirmed expression TLR1-7 and 9 on human and mouse platelets⁵ and functional roles for some platelet TLRs have been described - indicating that they are not residual receptors conserved from their bone marrow precursors⁴⁰⁻⁴³. Sepsis is associated with increased cell death, after which histones are released in the circulation. Histones promote thrombin generation via platelet TLR2 and TLR4, contributing to a pro-coagulant phenotype⁴⁵. LPS infusion caused profound thrombocytopenia in WT mice, but not in *tlr4* deficient mice, in which platelets did not accumulate in the lungs⁴¹. Platelet TLR4 activation additionally induced platelet binding to adherent neutrophils,

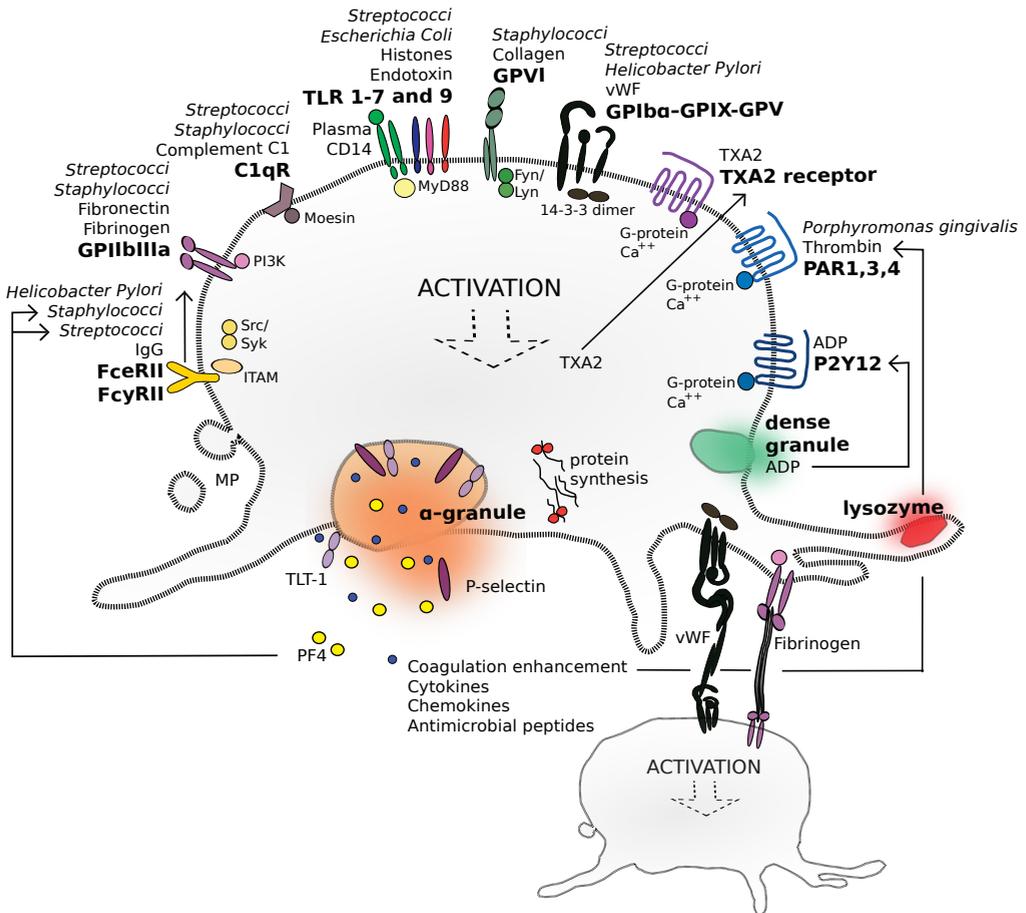


Figure 1: Mechanisms of platelet activation and platelet response during sepsis. Inflammation-induced activation of the coagulation cascade results in thrombin formation and platelet activation via PARs. Endothelial cell damage leads to subendothelial collagen exposure and vWF and TF expression on endothelial cells which bind to platelet GPIb and GPIIb/IIIa respectively. Complement C1q activates platelets via C1qR. Several bacteria have been shown to mediate platelet activation in a direct or indirect manner through induction of the indicated receptors. Activation via FcγRIIa is dependent on IgG and GPIIb/IIIa. Additionally, PF4 binds to bacteria and reduce lag time for aggregation. Other platelet receptors that mediate platelet-bacterial interactions are GPIIb/IIIa, PAR1, complement receptor C1qR and toll-like receptors (TLRs) (reviewed in detail in ^{38,39}). Circulating pathogens and released pathogen-associated and damage-associated molecular patterns such as histones are likely to play a superfluous role in platelet activation during sepsis through TLR signalling. Activated GPIIb/IIIa also mediates platelet activation by its ability to bind soluble fibrinogen, which bridges other platelets. Activated platelets enhance further platelet activation via catalysation of the coagulation cascade, TXA2 and ADP release. Platelet activation can result in shape change, aggregate formation, release of granular content and MP shedding. Furthermore, platelets respond to physiological stimuli using biosynthetic processes using megakaryocyte derived mRNA.

which leads to robust neutrophil activation and formation of neutrophil extracellular traps (NETs) (NETs are discussed in more detail below)^{43,46}. *In vitro* data of platelet activation by TLR ligands is conflicting^{43,47-49}. TLR-agonist concentrations that have been described to activate platelets *in vitro*, are 10 to 100-fold higher than concentrations needed for leukocyte activation. Other cell types are therefore more likely to respond to physiologic levels of TLR agonist concentrations *in vivo* and platelet activation is therefore more likely the result of the subsequent inflammatory reaction. Possibly, induction of platelet TLRs by lower TLR agonist concentrations could be a priming event^{49,50} preceding hyperactive response to other platelet stimuli.

Haemostatic and inflammatory function of platelets during sepsis

Platelet activation can result in shape change, platelet-platelet aggregation, platelet-leukocyte complex formation and the release of granular content (Figure 1). Platelets contain three types of granules: alpha- and dense granules, and lysosomes, of which the alpha granules are most abundant. The utilisation of proteomic techniques has recently demonstrated that platelets have the ability to express more than 300 different proteins following activation with thrombin^{51,52} including coagulation factors, chemokines, adhesive proteins, mitogenic factors and regulators of angiogenesis⁵². These molecules are heterogeneously organised into distinct subpopulations of alpha granulae, which undergo differential patterns of release during platelet activation⁵³. The following section discusses how sepsis influences platelet content and the regulatory role for platelets during sepsis.

Platelets as inflammatory cells in sepsis

Sepsis decreases the haemostatic function of platelets, while platelets maintain adhesion molecule expression, secretion capability and growth factor production¹⁸. Recently, genome-wide expression analysis has provided a foundation for the identification of IL-27 as a novel candidate diagnostic biomarker for predicting bacterial infection in critically ill children⁵⁴. Platelets were shown to release IL-27 upon thrombin receptor stimulation *in vitro*, potentially contributing to increased plasma IL-27 levels and immune dysregulation during sepsis⁵⁵. Platelets are not only containers that stockpile bioactive mediators, but have also been shown to respond to physiological stimuli using biosynthetic processes that are regulated at the level of translation, demonstrating a functional role for platelet mRNA^{56,57} (Figure 1). Already 12 hours after induction of sepsis, proteins associated with platelet activation, cytoskeleton structure, energy production and acute phase proteins were upregulated in circulating platelets in an animal model of coecal ligation and puncture⁵⁸. Platelet formation by megakaryocytes is an active process, in which the megakaryocyte transportation system delivers mRNA actively into the proplatelet extensions⁵⁹; evidence that megakaryocytes equip platelets with a different mRNA and protein set in health and disease is now accumulating

^{60,61}. Acidosis, which is one of the hallmarks of tissue damage, promotes platelet functions involved in amplifying neutrophil-mediated inflammatory response, while it downregulates haemostatic functions ⁶². On the contrary, a number of inflammatory mediators, among which IL-6, has been found to increase platelet production by megakaryocytes, and the newly formed platelets are more thrombogenic ⁶³.

Platelets are directly bactericidal through the release of platelet antimicrobial peptides (PMPs or defensins) (Figure 2). The vast majority of these proteins are of a cationic nature and this is thought to be crucial in allowing them to bind and disrupt bacterial membranes ⁶⁴. For example, platelet released PMPs have been found to reduce viability of *Staphylococcus*

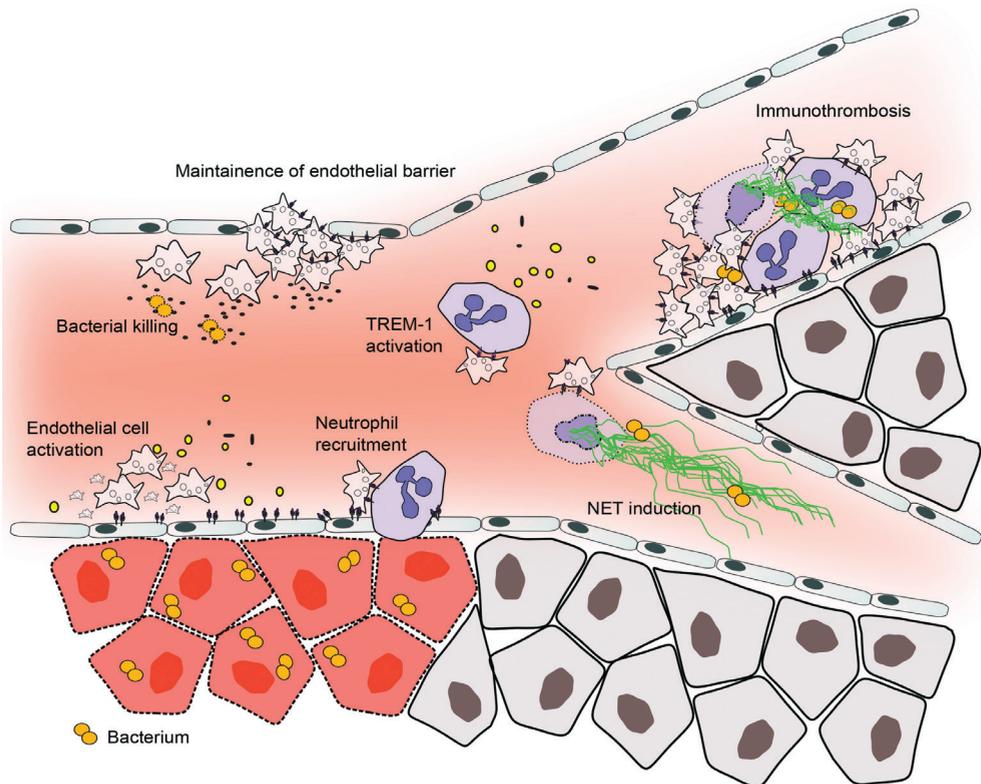


Figure 2: Platelets as immune regulators during sepsis.

Activated platelets secrete several regulatory proteins such as cytokines, chemokines, coagulation mediators and antimicrobial peptides. Platelets play an important role in maintenance of the endothelial barrier, accounting for massive platelet recruitment and activation on the surface of the damaged endothelium. Endothelial cells are activated by platelet derived CD40L and platelet derived IL1 β positive microparticles, after which endothelial cells will secrete adhesion molecules and TF. Platelets bound to endothelium support adhesion of neutrophils following selectin-mediated tethering and rolling, hereby guiding neutrophils to transmigrate. Engagement of neutrophil TREM-1, for which platelets express a ligand, results in increased expression of proinflammatory chemokines and cytokines and amplifies the inflammatory response. Platelets additionally function as a threshold switch for the secretion of NETs by neutrophils, resulting in bacterial entrapment in microvasculature. Platelets are also involved in immunothrombosis in which platelets and products of the coagulation pathway regulate the effector function of innate immune cells, and recruit additional cells.

aureus when incubated in large platelet-to-*S. aureus* ratio⁶⁵, and pure platelet rich plasma (P-PRP) inhibited growth of several oral cavity microorganisms⁶⁶. While platelets store most antimicrobial proteins within their alpha-granules, there are exceptions such as human β -defensin (hBD)-1. hBD-1 is basally localized in submembrane cytoplasmic domains and released when platelets are lysed as a terminal event that discharges intracellular content in the extracellular milieu. This release via a mechanism distinct from traditional secretory pathways may guard against inappropriate release of PMPs, which could have unwarranted cytotoxicity⁶⁷.

Platelets and the endothelium in sepsis

One of the hallmarks of sepsis is microvascular dysfunction, in which endothelial cell activation and debilitation play a pivotal role⁶. Platelets are important for maintenance of the endothelial barrier under physiologic conditions. During sepsis, components of the bacterial cell wall activate endothelial cells, as well as several host derived mediators such as cytokines, chemokines, coagulation factors and components of the complement system. Platelets are additionally involved in endothelial cell activation by several mechanisms. Engagement of platelet GPIIb/IIIa upregulates CD40 ligand (CD40L) expression on the platelet membrane, stimulating endothelial cells to express adhesion molecules and TF⁶⁸⁻⁷⁰. Platelet secreted MPs have been shown to contain newly synthesized mature IL-1 β upon LPS stimulation *in vitro*⁷¹; these IL-1 β -rich MPs were additionally potent in endothelial cell activation. The endothelium responds with the expression of several adhesion molecules, promoting neutrophil recruitment and extravasation and serving as a procoagulant surface; endothelial (dys)function during sepsis is reviewed in detail in⁶. An important feature of endothelial dysfunction in sepsis is increased vascular permeability, resulting in redistribution of body fluid and oedema⁶. These circumstances account for massive platelet recruitment and activation on the surface of the damaged endothelium¹⁹ (Figure 2), however insufficient to restore vascular barrier function during sepsis as the condition is characterized by hypotension, massive extravascular oedema and tissue swelling¹.

Platelet leukocyte interaction in sepsis

Activated platelets correlatively interact with peripheral blood mononuclear cells (PBMCs), and are capable of triggering expression of activation markers of PBMC subsets, such as T- and B-cells and monocytes when they are co-cultured⁷². Platelets bound to thrombogenic surfaces or injured endothelium have been shown to support adhesion of neutrophils following selectin-mediated tethering and rolling, hereby guiding neutrophils to transmigrate^{73,74} (Figure 2). During sepsis, there is an increase in circulating platelet-neutrophil complexes^{10,11}. More specifically, platelet-neutrophil complexes were shown to initially rise during sepsis, and subsequently decrease when multiple organ failure develops – indicating peripheral sequestration and a possible causal relation⁷⁵. Neutrophils in complex with

platelets represent a subpopulation of neutrophils with a more activated adhesion molecule profile, and a greater capacity for phagocytosis and toxic oxygen metabolite production ⁷⁶. Efficient neutrophil phagocytosis of periodontopathogens has been shown to be dependent on the presence of plasma factors as well as platelets ⁷⁷. Platelets have recently been shown to express a ligand for Triggering Receptor Expressed on Myeloid Cells (TREM)-1 expressed by neutrophils and monocytes ⁷⁸. Engagement of TREM-1 results in increased expression of proinflammatory chemokines and cytokines and amplifies the inflammatory response (Figure 2). Although this likely aids improved detection and elimination of pathogens during early infection, excessive production of cytokines and oxygen radicals can also severely harm the host in sepsis and blocking of TREM-1 holds considerable promise by blunting excessive inflammation ⁷⁹. TLR4 activated platelets have been shown to bind to neutrophils and function as the threshold switch for their secretion of nuclear content, forming NETs ⁴⁶ (Figure 2). NETs are web-like structures of DNA with proteolytic activity that can trap and kill microbes in tissue microvasculature ⁸⁰. This occurs primarily in small vessels like the liver sinusoids and pulmonary capillaries, however at the expense of tissue damage and organ dysfunction. In *Escherichia (E.) coli* induced sepsis, this NET-induced bacterial killing significantly contributed to bacterial clearance, as disruption of NETs by depletion of platelets or intravenous administration of DNase resulted in profoundly elevated bacteraemia ⁴⁶. To the contrary, platelets in complex with macrophages have been shown to inhibit the secretion of inflammatory mediators during sterile and bacterial systemic inflammation in a cyclooxygenase type (COX)1/2 dependent fashion ⁸¹.

Platelets and microthrombosis

Vascular endothelial cell activation, platelet adhesion and activation, innate immune cell recruitment, NET formation and fibrin deposition, all contribute to an increased propensity of thrombosis in sepsis ^{7,46}. Microthrombi act as antimicrobial matrices that mediate host protection against pathogens, by forming a physical barrier to the pathogen and by the generation of a distinct compartment that concentrates antimicrobial strategies of resident and recruited immune cells ⁴. A new concept of coagulation in the immune response has recently been launched by Engelmann et al: immunothrombosis (reviewed in ⁴). In immunothrombosis, innate immune cells generate the procoagulant surface with local delivery of TF and degradation of anticoagulant proteins. The subsequent recruitment of platelets further catalyses clot growth and the induction of NETs ^{43,46}. Within this thrombus, platelets and products of the coagulation pathway regulate the effector function of innate immune cells, and will recruit additional cells (Figure 2).

Platelet contribution to sepsis complications

While platelets can have a beneficial role in host response to an invading pathogen, during sepsis, platelet activation contributes to the development of complications such as DIC, multiple organ failure, acute lung injury (ALI) and acute kidney injury (AKI) (figure 3).

Platelets and haemostasis in sepsis

DIC is the result of pathologic overstimulation of the coagulation system⁸². During sepsis, coagulation is initiated by endothelial cell disruption and collagen exposure. Furthermore, monocytes or endothelial cells are stimulated to produce and secrete vWF, TF and cytokines in response to injury. Physiologic haemostasis subverts into pathologic DIC when the prothrombotic response exceeds coagulation inhibitors and the fibrinolytic system. Thrombin is then free to convert fibrinogen into fibrin and to activate platelets⁸². Additionally, histones released from dying cells, such as is prevalent in sepsis, have been shown to promote plasma thrombin generation in a platelet dependent manner⁴⁵. Platelet activation further catalyses development of hypercoagulation and DIC, as activated platelets

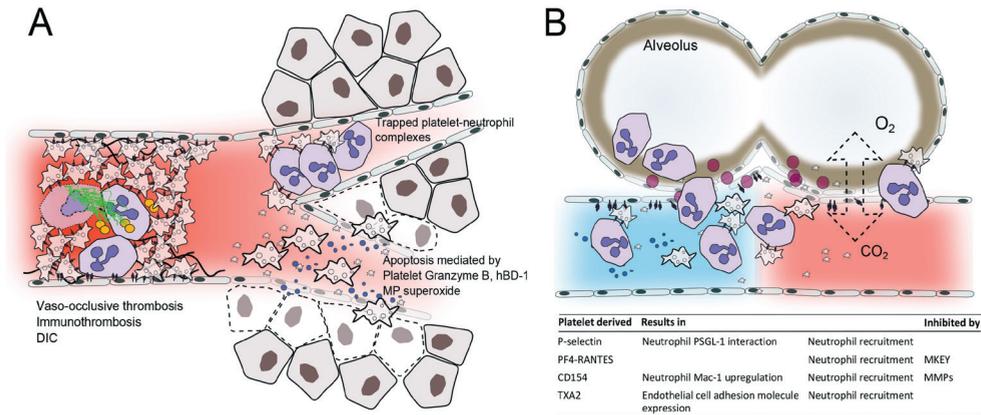


Figure 3: Platelets contribute to sepsis complications. Although platelets can have a beneficial role in the initial host response, platelet activation during sepsis contributes to the development of complications such as DIC, multiple organ failure, AKI and ALI. (A) The inflammatory and coagulation dysbalance in sepsis induces derailment of initial host defence strategies such as immunothrombosis, immune cell recruitment and circulating platelet-leukocyte complexes. Platelets play a role in the development of vaso-occlusive thrombi in capillary vascular beds by their contribution to immune cell recruitment and catalysation of hyperinflammation and DIC. Platelets can additionally have direct cytotoxic effects by release of Granzyme B, hBD-1 and cationic antimicrobial proteins. Platelet derived microparticles can be cytotoxic and contain superoxide. (B) Platelet and neutrophil recruitment to the lungs is a key event in the development of ALI in which there is vascular permeability, fluid leakage and impaired oxygenation. Platelets aid neutrophil recruitment to the lungs by formation of platelet-neutrophil aggregates via P-selectin and the secretion of chemoattractants such as PF4-RANTES heterodimer and CD40L; platelet TXA2 release results in expression of several adhesion molecules, polymerization of actin, and contraction by endothelial cells. Granule proteins derived from recruited and activated neutrophils are implicated in the degradation of surfactant proteins, epithelial cell apoptosis and induction of coagulation.

provide a suitable phospholipid surface on which the maintenance phase of coagulation is propagated. Complexes of activated coagulation factors assemble on the platelet membrane, thereby catalysing the generation of thrombin several-folds and rendering the coagulation system less susceptible to protease inhibitors⁸³. Platelet derived MPs enable both local and disseminated amplification of the haemostatic response to endothelial injury, through exposure of TF and coagulation factor binding sites²⁸. The result of DIC is consumption of coagulation factors and platelets, leading to decreased platelet counts²⁰. Patients with severe forms of DIC can present with manifest thromboembolic disease or clinically less apparent microvascular occlusion, which predominantly presents as multiple organ dysfunction. Alternatively, bleeding can be the leading symptom, although simultaneous bleeding and thrombosis also occurs^{82,83}. Thrombocytopenia per se does not cause bleeding in experimental animal models⁸⁴; in the absence of platelets however there is massive bleeding in inflamed organs^{84,85}. It appears that during inflammation - such as in sepsis - platelets preserve physiologic organ function by maintaining the organ's vascular integrity.

Platelets contribute to multiple organ failure

Animal studies of sepsis have demonstrated platelet accumulation in lungs, spleen, liver and intestine⁸⁶⁻⁸⁸. Platelets are proposed to have a major role there in the development of multiple organ failure by three mechanisms: 1) by contributing to immune cell recruitment and hyperinflammation, 2) by propelling the development of vaso-occlusive thrombi in capillary vascular beds and 3) by direct cell toxic effects of platelets and platelet derived MPs. Under physiologic conditions, neutrophils have a pivotal role in defence against bacterial infections. Overwhelming activation of neutrophils is however known to elicit tissue damage⁸⁹. Platelets are important in immune cell recruitment, and leukocytes attached to platelets form a vigilant subpopulation, with high binding potential and increased toxicity⁷⁶. Moreover, platelets function as a threshold for the induction of NETs. Intravascular NETs contribute to host defence during bacterial sepsis, albeit at the expense of tissue damage and organ dysfunction⁴⁶. The pro-coagulant state in sepsis, as well as the formation of NETs and microthrombi as a defence strategy, increase the risk of the development of vaso-occlusive thrombi (Figure 3A)^{4,46}. During DIC, thrombocytopenia does not protect from thrombosis⁸². There is a linear correlation between stopped-flow capillaries and platelet adhesion per millimetre, indicating that platelet adhesion in capillaries predicts 90% of capillary blood flow stoppage.

Many of the failing organs in sepsis show increased apoptosis^{90,91}. Platelets have been shown to be important mediators in the induction of apoptosis in the spleen and at least in one non-lymphoid organ: the lung⁹². During sepsis, alterations in the megakaryocyte-platelet transcriptional axis result in strongly cytotoxic platelets via serine protease granzyme B⁶⁰. Moreover, lysed platelets release hBD-1 from submembrane cytoplasmic domains, which can be toxic to pathogens but also damaging to host tissues (Figure 3A)⁶⁷.

Platelets in AKI

Acute renal failure occurs in approximately 19% of patients with moderate sepsis; this number increases to 51% when there is septic shock and blood cultures are positive⁹³. Platelet P-selectin, not endothelial P-selectin, is key in the development of ischaemic AKI⁹⁴. Platelets attached to endothelium recruit leukocytes to the kidneys and platelet-leukocyte aggregates get trapped in narrow peritubular capillaries, both contributing to damage and obstruction of flow⁹⁵ (Figure 3A). Septic patients with renal dysfunction demonstrated an increase in MPs. The levels of platelet derived MP levels correlated with serum blood urea nitrogen (BUN) and creatinine concentrations, suggesting a role for these platelet MPs in the development of renal failure⁹⁶ (Figure 3A).

Platelets in ALI

ALI is an important complication in sepsis. The term ALI incorporates a continuum of clinical and radiographic changes that affect the lungs with the acute respiratory distress syndrome (ARDS) representing the more severe end of this continuum. ALI is characterized by an increased permeability of the alveolar-capillary barrier, resulting in lung oedema, with protein-rich fluid and consequently impaired arterial oxygenation (Figure 3B). Inappropriate accumulation and activity of leukocytes and platelets are key events in the development of ALI⁹⁷. Granule proteins derived from recruited and activated neutrophils such as azurocidin, α -defensin and elastase are implicated in the degradation of surfactant proteins, epithelial cell apoptosis and coagulation⁹⁸. Early studies have found excessive platelet deposition within damaged pulmonary microvasculature in postmortem series and by ultrastructural examination of lungs of patients with ARDS²². There is a direct correlation between the degree of pulmonary injury and platelet-specific alpha-granule proteins in bronchoalveolar lavage fluid in ARDS patients⁹⁹. Neutrophil recruitment into the lungs is platelet dependent. Platelets directly interact with neutrophils via P-selectin¹⁰⁰ and release cytokines such as PF4-RANTES⁹⁸, CD40L^{101,102} and TXA2¹⁰³, which further enhance inflammatory processes, resulting in increased adhesion molecule expression, actin polymerisation and contraction of endothelial cells (Figure 3B). Platelet depletion results in an improved phenotype in several mouse models of ALI^{98,104}. Specifically, inhibition of P-selectin¹⁰⁴ and disruption of the platelet derived PF4-RANTES heterodimer⁹⁸ significantly improved outcome. Inhibiting matrix metalloproteinases (MMPs) might be another attractive target in abdominal sepsis, as MMPs reduce CD40L shedding from platelets as well as formation of CXC chemokines in the lung¹⁰⁵.

Platelet inhibitors in sepsis

Experimental studies

Several experimental studies have shown beneficial effects of antiplatelet therapy in sepsis models (Table 2). GPIIb/IIIa blockade with abciximab, eptifibatide or tirofiban prevents platelet aggregation. Blockade of this receptor resulted in a decreased mortality in rabbits with *E. coli* endotoxin induced shock¹⁰⁶, and protection from the development of microangiopathic haemolysis and renal insufficiency in *E. coli* sepsis in baboons¹⁰⁷. Treatment with eptifibatide prevented contact-mediated platelet induced apoptosis and resulted in less severe sepsis and extended survival in a coecal ligation and puncture model in mice⁹².

Clopidogrel irreversibly inhibits the platelet membrane P2Y₁₂ receptor, thereby preventing ADP activation. Administration of clopidogrel reduced platelet secretion of adhesive ligands and blocked the formation of platelet-leukocyte conjugates, resulting in decreased leukocyte activity^{22,108}. Clopidogrel pre-treatment attenuated the drop in platelet counts and improved end organ damage in a polymicrobial sepsis model in mice¹⁰⁹. In contrast, clopidogrel treatment did not result in significant differences in outcome parameters in *E. coli* endotoxin infused pigs¹¹⁰.

Table 2: Platelet inhibitory therapy in experimental studies

Experimental studies				
What	Model	Species	Outcome	
AZ-1, anti GPIIb/IIIa antibody	Endotoxin shock	Rabbit	Decreased mortality	¹⁰⁶
7E3 F(ab') ₂ , anti GPIIb/IIIa antibody	<i>E. coli</i> and c4b binding protein infusion	Baboon	Protection against haemolysis and renal insufficiency	¹⁰⁷
Eptifibatide	CLP	Mice	Prevention of apoptosis and extended survival	⁹²
Clopidogrel	Polymicrobial sepsis (peritoneal human faeces injection)	Mice	Prevented thrombocytopenia, improved end organ damage	¹⁰⁹
Clopidogrel	<i>E. coli</i> endotoxin infusion	Pig	No effect	¹¹⁰
MKEY	LPS, acid and sepsis induced ALI	Mice	Impaired neutrophil influx, improved lung damage	⁹⁸
Antagonist to P-selectin and GPIIb/IIIa	LPS induced ALI	Mice	No effect	⁹⁸
Antibodies to P-selectin	Acid and sepsis induced ALI	Mice	Improved gas exchange, decreased neutrophil, prolonged survival	¹⁰³
ASA	Human endotoxaemia	Human	Decreased platelet plug formation	¹¹¹

GPIIb/IIIa = glycoprotein IIb/IIIa CLP = coecal ligation and puncture, LPS = lipopolysaccharide, ALI = acute lung injury, ASA = acetylsalicylic acid

Platelet depletion is protective for the development of ALI^{98,104}. While in one study P-selectin blockade equally improved outcome¹⁰⁴, another other study observed no effect after treatment with antagonists to P-selectin and glycoprotein (GP)IIb/IIIa⁹⁸. Here, antibodies to both the platelet derived chemokines PF4 and RANTES dramatically decreased ALI development. More specifically, MKEY, a newly developed inhibitor of PF4-RANTES heterodimer formation, decreased neutrophil infiltration and improved lung tissue damage, emphasizing the importance of this heterodimer in ALI pathogenesis⁹⁸.

The use of acetylsalicylic acid (ASA) has been associated with decreased levels in C-reactive protein and inflammatory cytokine levels²². ASA inhibits platelet function through blocking of COX-1. In human endotoxemia, ASA attenuated platelet plug formation, without influencing sP-selectin or vWF-levels¹¹¹.

Clinical studies

Platelet inhibiting drugs such as such as clopidogrel or ASA are widely used in the secondary prevention of cardiovascular, cerebrovascular and peripheral arterial thrombosis. The effect or anti-platelet therapy has been investigated in hindsight in sepsis patient cohorts (Table

Table 3: Platelet inhibitory therapy in sepsis

Retrospective studies				
What	Study design	Inclusion	Outcome/association	
ASA	Retrospective cohort study	Critically ill	Reduced mortality	112
ASA, clopidogrel, anagrelide	Population-based historical cohort study	Patients admitted to ICU	Reduced incidence of ALI	113
ASA	Multicentre observational study	Hospital patients with ALI risk factor	No significant association with ALI	114
ASA or clopidogrel	Retrospective cohort study	Patients admitted to ICU	Reduced incidence of ALI	115
ASA or clopidogrel	Retrospective study	Hospital admission for CAP	Reduced ICU treatment, shorter hospital stay	116
clopidogrel	Cohort study	Adult Medicaid beneficiaries	Increased CAP incidence	118
clopidogrel	Meta-analysis	ICU sepsis patients	May reduce sepsis severity	118
ASA, clopidogrel, ticlopidine, dipyridamole	Retrospective cohort study	Patients admitted to ICU	Reduced incidence of ALI, no association with mortality	117
Ticagrelor VS clopidogrel	Post-hoc analysis PLATO study	Patients with acute coronary syndrome	Reduced adverse events and mortality in the ticagrelor treated group	119

ASA = acetylsalicylic acid, ICU = intensive care unit, ALI = acute lung injury, CAP = community acquired pneumonia

3). Retrospective studies show a beneficial effect of pre-existing therapy with an anti-platelet drug with respect to organ failure, development of ALI, duration of ICU- and hospital stay, and mortality in critically ill patients ¹¹²⁻¹¹⁷. A strong association has been found between ASA and survival of non-infectious critical illness and sepsis, in which pre-existent treatment with ASA reduced the odds ratio for mortality by nearly five-fold ¹¹², although another study found no effect of antiplatelet therapy on mortality ¹¹⁷. Additionally, the incidence of ALI/ARDS was lower in patients who were taking antiplatelet medications at the time of hospital admission, compared with patients who were not on antiplatelet medications ^{113,116,117}. The aforementioned findings were apparent despite the fact that the patients receiving antiplatelet medications usually were older, had more advanced disease and had greater severity of illness at the time of hospitalisation. In one study however, no statistically significant association between pre-hospitalization ASA therapy and ALI remained after adjusting for the propensity to receive ASA ¹¹⁴. Notably, patients with clopidogrel prescriptions were found to have an increased risk to be hospitalized for pneumonia and sepsis. Among pneumonia patients, those with active clopidogrel prescriptions had a higher incidence of sepsis than those with no clopidogrel prescriptions. Although these associations were largely attributable to differences in comorbidities between the groups, risk factors were reduced but not eliminated after risk factor adjustment ¹¹⁸. In the PLATO trial ¹¹⁹, with over 18,000 patients included, ticagrelor or clopidogrel treated patients were compared with respect to pulmonary infection or sepsis adverse events. Ticagrelor inhibits adenosine reuptake, in addition to P2Y₁₂ receptor blockade. Fewer on-treatment pulmonary adverse events occurred in the ticagrelor group compared to the clopidogrel group, and there were fewer deaths following these adverse events and fewer deaths attributable to sepsis. Taken together, these results support the role for platelets in host defence in the initial encounter with a pathogen. During sepsis however, platelet inhibition might have beneficial effects. Carefully designed prospective intervention studies are needed in order to elucidate the possibilities of platelet inhibitory therapy during sepsis.

Conclusions

Platelets are important modulators of the host response during infection, serving as sentinels in our circulatory system. In situations of extreme immune activation and coagulation dysbalance such as in sepsis, however, platelet activation is involved in disease progression. Activated platelets shed platelet products and MPs enhance inflammatory and coagulation responses. Platelets catalyse the development of hyperinflammation, DIC and microthrombosis, and subsequently contribute to multiple organ failure. Moreover, inappropriate accumulation and activity of platelets are key events in the development of sepsis-related complications such as ALI and AKI. Inhibition of platelets in septic patients seems like an important target for immune-modulating therapy and appears promising

in animal models and retrospective human studies. This area of research will continue to evolve in both translational and clinical arenas, with urgent need for further evaluation with prospective randomized intervention studies of the effect of platelet inhibition on mortality of patients with sepsis.

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