Platelets in haemostasis, thrombosis and inflammation after major trauma

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Abstract

Trauma currently accounts for 10% of the total global burden of disease and over 5 million deaths per year, making it a leading cause of morbidity and mortality worldwide. Although recent advances in early resuscitation have improved early survival from critical injury, the mortality rate in patients with major haemorrhage approaches 50% even in mature trauma systems. A major determinant of clinical outcomes from major injury is a complex, dynamic haemostatic landscape. Critically injured patients frequently present to the emergency department with an acute traumatic coagulopathy that increases mortality from bleeding, yet within 48-72 hours after injury will switch from a hypocoagulable to a hypercoagulable state with increased risk of venous thromboembolism and multiple organ dysfunction. This review will focus on the role of platelets in these processes. As effectors of haemostasis and thrombosis, they are central to each phase of recovery from injury, and our understanding of post-injury platelet biology has dramatically advanced over the past decade. This review describes our current knowledge of the changes in platelet behaviour that occur following major trauma, the mechanisms by which these changes develop, and the implications for clinical outcomes. Importantly, supported by research in other disease settings, this review also reflects the emerging role for thrombo-inflammation in trauma including cross-talk between platelets, innate immune cells and coagulation. We also address the unresolved questions and significant knowledge gaps that remain, and finally highlight areas that with further study will help deliver further improvements in trauma care.

Introduction - The disease of physical injury

Trauma is a leading cause of morbidity and mortality worldwide, accounting for 10% of the total global burden of disease.[1] Recent developments in early resuscitation of critically injured patients have yielded striking improvements in survival,[2] but the mortality rate in patients with major haemorrhage after trauma still approaches 50% even in mature trauma systems.[3] With the burden of injury projected to increase over the coming decades, delivering further improvements in trauma care is an important global health priority.

Many of the recent advances in treatment of severe trauma have been driven by a greater understanding of the human response to injury. One area of particular focus over the past two decades has been post-injury disturbances in haemostasis. In 2003, two separate investigations first identified an acute traumatic coagulopathy (ATC) in severely injured patients that develops within minutes of injury.[4,5] This endogenous coagulopathy develops as a direct response to tissue injury and haemorrhage, which may be compounded by dilution with non-haemostatic blood products and additional physiological insults such as surgical procedures. The combination of these endogenous and iatrogenic processes are referred to as trauma-induced coagulopathy (TIC); an umbrella term describing the complex, multifactorial failure of haemostasis after injury that confers a four-fold increase in mortality.[6] As such, targeting coagulopathy is now a fundamental pillar of contemporary trauma resuscitation.

Improvements in early resuscitation have resulted in increasing numbers of survivors from injuries that would previously have been fatal.[7] In patients who survive the initial bleeding phase and reach intensive care, a switch from a hypocoagulable to a hypercoagulable state

occurs (**Figure 1**). The precise timing of this switch is poorly defined, and is likely subject to considerable inter-individual variability, however a procoagulant state is generally thought to be established by 48 hours after injury. The most obvious manifestation of this state is an extremely high rate of in-hospital thrombotic events, particularly venous thromboembolism (VTE), which occurs in up to 35% of critically injured patients.[8] It is important to note that although aggressive thromboprophylaxis with low-molecular weight heparin and calf compression reduces the rate of these events, a significant number still occur in spite of this, particularly in patients developing pulmonary embolism.[9–11] This suggests that unappreciated mechanisms are at play in the pathogenesis of post-injury thrombosis that are not targeted by current therapeutic and prophylactic regimens.

In addition to macrovascular thrombotic events, multiple organ dysfunction (MODS) is an extremely common complication of severe injury and a major clinical challenge.[12,13] Post-traumatic MODS is generally held to result from a dysregulated immune response to injury, and the precise mechanisms and pathways involved have been subject to intense scrutiny.[14–17] Synchronized activation of coagulation and inflammation, a process termed thromboinflammation, has surged to prominence as a result of the COVID pandemic and appears central to the pathophysiology of organ failure in this condition.[18–20] Evidence for this phenomenon as a mechanism of organ failure in trauma patients is comparatively sparse, but patients with TIC have significant significantly higher rates of MODS,[21] and aggressive treatment of coagulopathy is associated with a reduction in the incidence and severity of organ failure.[22] These observations suggest that interplay between coagulation and inflammation after major injury are relevant to post-injury MODS, but the precise mechanisms involved in this crosstalk are still being elucidated.

Platelets are central effectors of haemostasis and thrombosis, and have been implicated both in trauma-induced coagulopathy and in the development of the subsequent prothrombotic switch (**Figure 2**). Whilst there have been major advances in our understanding of post-injury platelet biology over the past decade, significant knowledge gaps and unresolved questions remain. The aim of this review is to describe the changes in platelet behaviour that occur following major trauma, the mechanisms by which these changes develop, and the implications for clinical outcomes. We will also address some of the major 'unknowns' in the field, and highlight areas that warrant further study.

Platelets in trauma-induced coagulopathy

Changes in platelet function and number after major trauma

A number of studies have described profoundly impaired responsiveness of platelets from trauma patients to *ex vivo* agonist stimulation (**Figure 2**). Initial reports of this phenomenon used whole blood aggregometry, and described a global impairment in platelet aggregation following challenge with a range of classical agonists compared to reference ranges in healthy volunteers.[23] These blunted responses develop within the first two hours of injury, are present in over 40% of severely injured patients, and the degree of platelet hyporeactivity is a strong predictor of adverse clinical outcomes including mortality.[24,25] Platelet transfusions do not reverse these defects in *ex vivo* aggregation whilst bleeding is ongoing.[26] Interestingly, this state seems to persist for several days after the initial injury,[23] suggesting a process of ongoing inhibition that is not directly attributable to the circulating intravascular milieu in the immediate post-injury period. The underlying mechanisms for this persistent dysfunction are unclear, but may point to an intrinsic defect in circulating platelets. It is important to

recognise that impaired platelet aggregatory function in TIC occurs alongside a range of perturbations in haemostasis, including fibrinogen depletion and hyperfibrinolysis,[27,28] which themselves may influence *ex-vivo* assays of platelet function. Injury-induced consumption of von Willebrand factor (vWF), for example, has been shown to influence *ex vivo* platelet aggregation in patients with traumatic brain injury.[29]

Injury and patient-specific factors influence the degree and characteristics of trauma-induced platelet function. For example, traumatic brain injury may be associated with a distinct pattern of platelet dysfunction with particular defects in adenosine diphosphate and arachidonic acid induced-aggregation that occur independently of haemorrhagic shock.[30,31] In addition, major haemorrhage and resuscitation result in global ischaemia-reperfusion and oxidative stress, which can have profound effects on platelet activity.[32,33] Similarly, patient age, sex, medical history, and pre-injury antiplatelet therapy may also influence the pattern of coagulopathy that develops,[34] although detailed studies on how these factors independently influence platelet function are injury are lacking.

Importantly, these qualitative changes in platelet activity are not accounted for by thrombocytopenia, with platelet counts almost always maintained above critical levels in the acute phase after major trauma.[23] In this way TIC is distinct from disseminated intravascular coagulation (DIC), a condition in which thrombocytopenia is a near universal finding.[35] However, early reductions in platelet count are evident in trauma patients, albeit generally well within the normal range, and occur in proportion to injury severity.[36] Even reductions in platelet count within the normal range seem to confer an increased mortality risk.[25,37] This points to platelet consumption or destruction, or impaired production, that is present early

in the clinical course but does not cause thrombocytopenia. The mechanisms responsible for platelet depletion after major trauma have not yet been investigated in detail, and similarly the effects of trauma on the normal 7–10-day lifespan of platelets are unknown. Whilst dilution during resuscitation may be a factor, particularly at later timepoints, several studies[25,26,37] focus on admission blood samples that are taken before significant volume replacement has occurred indicating an endogenous contributor to this process.

Glycoprotein VI and the collagen response

Subsequent more targeted investigations have identified several potential mechanisms contributing to impaired platelet responses in TIC. In addition to blunted *ex vivo* aggregation, several studies have found that platelet adhesion to collagen is impaired in the acute phase after major trauma, with a more pronounced impairment in patients with TIC and among non-survivors.[38,39] This observation led to more detailed investigations of receptors involved in platelet-collagen interactions, specifically Glycoprotein (GP)1bα and GPVI. Surface expression levels of these receptors are reduced on platelets from trauma patients compared to healthy volunteers, while circulating levels of the soluble ectodomain fragment of GPVI are elevated, particularly in those with TIC (**Figure 2**). Addition of platelet-poor plasma from trauma patients to healthy platelets recapitulates these blunted collagen and CRP responses, pointing to a circulating factor causing GPVI-dependent platelet inhibition.[40,41]

Two potential candidates in this process are soluble fibrin and the fibrin degradation product D-dimer. Both are abundant in the plasma of patients with TIC as a consequence of systemic activation of coagulation and fibrinolysis[28] and both molecules interact with GPVI.[42] Fibrin induces aggregation and GPVI receptor shedding[43], and impaired-GPVI signalling.[44] D-dimer,

by contrast, has been reported to only facilitate spreading but can inhibit aggregation induced by fibrin and collagen.[45,46] However, numerous other molecules also interact with GPVI, including its primary ligand collagen as well as charged particles such as extracellular histones, which are present in high concentrations after major trauma.[47,48] It is therefore likely that multiple ligands are implicated in post-injury GPVI responses.

These mechanisms for platelet dysfunction represent a promising potential therapeutic target for novel rescue therapies in TIC. Montague *et al* demonstrated the ability of polyanionic molecules to block fibrin-GPVI interactions, resulting in inhibition of fibrin-dependent GPVI shedding.[49] Fibrin appears to bind to either a different epitope or confirmation of GPVI (monomer or dimer) than collagen[45] and this therefore represents an opportunity for targeted therapeutic blockade that would not disturb platelet activation in response to collagen exposure. Similarly GPVI shedding is principally mediated by the metalloproteinase ADAM10.[50] Therefore the advent of antibody based or more specific small molecules,[51] rather than non-selective metalloproteinase inhibitors, is an additional pharmacological opportunity.

Platelet 'exhaustion'

Other investigators have explored potential mechanisms for the apparent loss of responsiveness to numerous agonists. Several studies have found that platelets have elevated basal levels of surface activation markers despite reduced *ex vivo* responses to agonist stimulation.[38,52,53] This suggests a degree of 'pre-activation' that may develop as a consequence of intense stimulation by circulating stimuli, a state that has been described as 'platelet exhaustion'.[54] A recent report found that elevated circulating levels of platelet-

stimulatory catecholamines are associated with impaired platelet aggregation and overall clot strength in trauma patients.[55] *In vitro* studies with healthy platelets demonstrated reduced platelet responses following prolonged exposure to the catecholaminergic platelet agonists epinephrine and norepinephrine.[55] This observation may explain the previous findings that *ex vivo* platelet inhibition is evident in some patients with minor injuries,[56] in whom catecholamine release occurs without significant tissue damage or blood loss.[57] This general concept of sustained exposure to agonists causing desensitization to further stimulation is another potential mechanism explaining the global impairment of platelet function involving multiple separate pathways.

Procoagulant platelets

In addition to classically activated platelets, circulating procoagulant platelets have also been described in trauma patients.[58,59] This discrete platelet subset are characterised by ballooning morphology and translocation of phosphatidylserine to the outer membrane leaflet, which together act to amplify the thrombin-generating capacity on the surface of platelets[60] (**Figure 2**). This process occurs as a result of sustained agonist-induced elevations in cytosolic calcium, which triggers a process of cytoskeletal disassembly and water entry that results in cellular swelling and membrane ballooning.[60,61] A hallmark characteristic of the early phase of TIC is elevated markers of systemic thrombin generation despite normal or reduced levels of procoagulant factors in plasma.[28,62,63] The presence of circulating procoagulant platelet balloons during trauma haemorrhage provides an explanation for these apparently conflicting observations. The frequency of platelet ballooning has been found to correlate with the extent of injury, and severely injured patients exhibit both platelet hypofunction on *ex vivo* aggregometry occurs and elevated markers of systemic thrombin generation.[59] In view of

previous observations that procoagulant platelets are unable to contribute to so-called primary haemostatic processes of adhesion and aggregation,[64–66] this suggests that a 'switch' in function towards a procoagulant phenotype may contribute to platelet hyporesponsiveness in trauma. However, the relationship between procoagulant platelet ballooning and clinical outcomes after trauma has not yet been clearly defined and requires further study.

Combined stimulation with collagen and thrombin is a central mechanism by which procoagulant platelets develop *in vitro,*[67] which is intuitive in that these are the conditions present at the site of endothelial breach and therefore the location at which amplification of thrombin generation is most critical for haemostasis. A more recently identified alternative mechanism for procoagulant transformation is through the action of extracellular histones on platelets, which induce platelet-dependent thrombin generation[59,68] (**Figure 2**). When released into the extracellular space by damaged or necrotic cells, histones exert cytotoxic effects on platelets and trigger ballooning, which in turn amplifies thrombin generation by platelets. Histones are present in the bloodstream in massive quantities after major trauma[47] and are detectable on the surface of circulating platelet balloons.[59] This observation is an example of how changes in platelet behaviour after major trauma are directly linked to tissue injury and haemorrhage. Neutralisation of histone-platelet interactions and downstream emergence of procoagulant platelets is therefore a potential therapeutic target in TIC. However, a clearer understanding as to whether these interactions are injurious or protective during bleeding will also be required.

Platelet-derived extracellular vesicles

The process of cytoskeletal disassembly that occurs during procoagulant platelet transformation is associated with a surge in extracellular vesicle (EV) production.[69,70] These subcellular membrane-bound fragments are important in intercellular communication both within and beyond the vascular space[71] and have been implicated in the pathophysiology of a wide range of diseases.[72–74] A number of authors have described increased levels of circulating platelet-derived EVs after major trauma.[75,76] A substantial proportion of these EVs express phosphatidylserine on their outer membrane leaflet, implying that they originate from platelets undergoing or having undergone procoagulant transformation, which would explain the increased thrombin generating potential of platelet-poor plasma often described after trauma[77] (**Figure 2**). Non-survivors and patients with TIC have lower levels of circulating platelet-derived EVs compared to survivors[76] and those without TIC[78] suggesting that they may be protective during the acute bleeding phase by supporting coagulation. This has led some authors to suggest administration of purified platelet-derived EVs as a potential treatment for TIC.[79,80] However, as discussed below, platelet EVs have also been linked to thrombotic events after trauma[75,81] and therefore further evaluation of this approach is needed.

Measuring trauma-induced platelet dysfunction in clinical practice

Incorporation of these observations into clinical care is challenging, due to the logistical difficulties associated with timely platelet phenotyping. Resuscitation of bleeding trauma patients is an extremely dynamic process and the majority of deaths from haemorrhage occur within three hours of injury.[82,83] As a result, point-of-care tools such as rotational viscoelastometry (such as TEG and RoTEM) and multiple electrode aggregometry have

attracted considerable interest in the trauma setting because of their ability to generate rapid and therefore clinically valuable outputs. In particular, viscoelastic assays such as TEG and RoTEM have been used widely to profile platelet contributions to clot formation, which can then be used within massive transfusion protocols as a trigger for platelet transfusion.[84] However, conventional thromboelastometry is relatively insensitive to mild platelet dysfunction, whereas the use of TEG-platelet mapping can uncover defects in specific platelet activation pathways and therefore may be better suited to identify platelet dysfunction in the clinical setting.[85] The goal-directed approach has become established practice in many well-resourced trauma systems, although the evidence for this approach from randomised clinical trials is conflicting with one study suggesting a survival benefit[86] and another no difference in outcome.[87]

Platelet transfusions to counter trauma-induced platelet dysfunction

A full discussion of platelet transfusion in trauma is beyond the scope of this review, but there are strong signals for a mortality benefit associated with administering high-volumes of platelets and therefore they are now a cornerstone of most major haemorrhage protocols in well-resourced settings.[88–91] However, platelet transfusions do not appear to restore platelet aggregatory function during active bleeding,[26,89,92] and there is evidence that the apparent survival benefit may be attributable either to the dose of platelet-derived mediators, such as the antifibrinolytic PAI-1, or the EVs released during storage.[93] Questions have also been raised about the clinical efficacy of platelet transfusions, particularly in the context of brain injury and antiplatelet-associated intracerebral bleeding, where platelet transfusions have been implicated in worse clinical outcomes.[94,95] In patients with trauma-haemorrhage there is increasing interest in use of cold-stored platelets, which appear to have a greater

haemostatic benefit compared to platelets stored at room temperature.[96,97] Indeed, cold-stored platelets are now licensed for use by the Food and Drug Association in the USA and are in use at several major trauma centres in the USA. Prehospital administration of cold-stored whole blood (containing chilled platelets) is currently being evaluated in the ongoing SWiFT randomised control trial in the UK[98] which will add to the evidence regarding use of cold-stored platelets with traumatic haemorrhage.

Novel therapeutic approaches for treatment of platelet dysfunction

Platelet transfusions are a scarce resource with a short shelf-life and are associated with complications common to all allogeneic blood products. Several alternatives for treatment of trauma-induced platelet dysfunction have therefore been developed. Among these new approaches are liposome-based platelet-mimicking nanoparticles, which are decorated with peptides that confer site-selective aggregatory, adhesive and procoagulant properties.[99] This agent has been shown to reduce bleeding in animal models of traumatic-haemorrhage[100,101] and is a promising surrogate for platelet transfusion. Platelet-derived extracellular vesicles have also been investigated as a novel resuscitation fluid (referred to as thrombosome and platelet-enhanced plasma) which has been shown to have haemostatic efficacy both in vitro[80] and in animal models of bleeding.[102] Other strategies to augment platelet function in trauma that have been proposed include the use of desmopressin, which increases plasma vWF levels and enhances platelet aggregation in a rat model of traumatic haemorrhagic shock. In addition, there is some evidence that tranexamic acid, an antifibrinolytic that is widely used in major haemorrhage protocols worldwide, can improve platelet function.[103,104] However use of this agent to specifically treat trauma-induced platelet dysfunction has not been evaluated.

Platelets in post-injury inflammation and organ dysfunction

Beyond the first 24 hours after injury, the major causes of morbidity and mortality in severely injured patients are multiple organ failure and thrombotic events[12] (**Figure 1**). Compared to the large number of studies investigating platelet haemostatic function in TIC, there is a relative paucity of data regarding changes in platelet activity later in the clinical course and in relation to these outcomes. In particular, the immunological roles of platelets in the context of major trauma, and the extent to which these are linked to their roles in coagulation, are not fully understood. The COVID pandemic has further emphasised the extent to which platelets contribute to the interplay between coagulation and inflammation in disease, or thromboinflammation, and as such this is a highly topical issue.[105] Although elements of the pathophysiology of COVID, sepsis and other diseases characterised by thromboinflammation may overlap with major trauma, the specific mechanisms involved are distinct.[106] Moreover, the link between early changes in platelet behaviour during TIC and subsequent post-injury organ dysfunction is still being elucidated.

DAMPs and sterile inflammation

Major trauma results in a dramatic systemic sterile inflammatory response that is triggered by release of damage-associated molecular patterns (DAMPs) from injured or ischaemic tissues.[17] DAMPs are a diverse group of intracellular molecules that, when present in the extracellular space, act as a signal to innate immune cells that injury as occurred, triggering effector responses.[107] Platelets respond to several of the major DAMPs that are implicated in post-injury inflammation, including extracellular histones (as detailed above), High-Mobility

Group Box protein-1 (HMGB-1), and S100A8/9 as has been reviewed in detail previously[108] (**Figure 2**).

Platelet-DAMP interactions are highly topical given their relevance in the pathophysiology of COVID-19, exemplified by the identification of neutrophil-associated S100A8/9 as a trigger of platelet procoagulant transformation leading to thrombosis in this disease.[109] Similarly, platelet-dependent release of HMGB1, another archetypal DAMP, is elevated in the circulation of injured patients and implicated in clinical outcomes.[110] Upon activation, platelets express HMGB1 on their surface[111], and in circulating platelets of trauma patients there is significantly higher platelet surface expression of HMGB1 within 48 hours of injury, compared to healthy volunteers[112] (**Figure 2**). The release of platelet-derived HMGB1 into the extracellular space has been shown to act in a paracrine manner to induce platelet activation[113], and HMGB1-containing platelet EVs activate neutrophils to promote neutrophil extracellular trap (NET) release.[73,114] Collectively, increased extracellular levels of HMGB1 promote inflammation, organ injury and thrombosis[112,113] whereas platelet-specific deletion of HMGB1 significantly reduces microvascular thrombosis and immune cell activation in murine models of trauma-haemorrhage, and is protective against macrovascular thrombosis.[112] The role of histone-platelet interactions in altered platelet behaviour in TIC described above is another example of the relevance of DAMPs in modulating platelet function after major trauma[59]. However, the relative contributions of these different DAMPs to post-injury changes in platelet biology and the downstream consequences of these interactions in terms of the overall immune response to major trauma are still poorly defined.

In addition to DAMP release as a result of parenchymal cell death, neutrophils also extrude nuclear material in the process of extracellular trap (NET) production, known as NETosis.[115] Platelets are known drivers of NET production under inflammatory conditions,[116] and NETs are enriched in multiple DAMPs which can in turn induce platelet activation and procoagulant transformation.[117] This represents a classical example of the interplay between platelets and leukocytes in thromboinflammation. Trauma patients have evidence of NET production detectable within an hour of injury,[118] although the functional significance of NETosis and the potential role of platelet-neutrophil interactions in the development of inflammatory complications after trauma has not been examined in detail.

Platelet-leukocyte interactions in trauma

A principal mechanism by which platelets influence immune responses is through the formation of heterotypic aggregates with leukocytes. In general, the overall effect of platelet binding is a phenotypic switch towards a pro-adhesive, proinflammatory phenotype in innate leukocytes which augments their ability to access injured tissues and initiate the process of repair.[119,120] These interactions are undoubtedly important for effective recovery after injury, as platelet depletion in animal models of localised tissue injury prolongs recovery and worsens outcome.[121] Conversely, excessive or exaggerated platelet-dependent leukocyte responses appear to exacerbate organ damage in ischaemia-reperfusion syndrome and models of acute lung injury.[122,123] This suggests that although platelet-leukocyte interactions are host-protective in localised, contained insults, they may contribute to a dysregulated immune response and end-organ damage in systemic inflammation.

In trauma, crosstalk between platelets and leukocytes occurs from an early timepoint. Two separate studies found widespread interactions which principally involve neutrophils and monocytes[59,124] (**Figure 2**). The frequency of these interactions is proportional to injury severity, and is greater in patients who develop organ dysfunction.[59] Visualisation of platelet-leukocyte aggregates in trauma patients have revealed them to primarily involve platelet-derived EVs rather than whole platelets.[59,124] Moreover, platelet EV-leukocyte aggregates correlate with the proportion of ballooning platelets, suggesting a mechanistic link to platelet procoagulant transformation, which is associated with a surge in EV production.[125] The functional consequence of platelet-derived EV binding on leukocyte behaviour in trauma patients, and the downstream implications in terms of inflammation and organ dysfunction, are an area of active ongoing investigation.

Platelet derived cytokines

Platelets also influence immune responses through release of immunomodulatory moieties into the circulation upon activation. A plasma proteomic signature of platelet activation is an early feature of trauma and haemorrhagic shock, indicating that alpha degranulation occurs rapidly in response to major injury[126] (**Figure 2**). In humans, levels of the platelet-derived cytokine CD40L in the acute phase after injury are positively correlated with injury severity and elevated levels are associated with increased mortality.[127] CD40L induces migratory behaviour[128] and cytokine release by monocytes[129] and triggers adhesion molecule expression on endothelial cells,[130] representing a classical example of platelet-mediated links between haemostasis and vascular inflammation. Other platelet-derived cytokines including TGFβ, beta-thromboglobulin and Platelet Factor 4 are similarly increased in plasma after trauma,[126] although the relationships with clinical outcomes are less clear.

Platelets in post-injury thrombosis

As noted above, major trauma patients who survive to reach intensive care have an exceptionally high rate of venous thrombotic events.[8] The timing and anatomical pattern of venous thrombosis events after major trauma are unusual, and often occur in spite of early aggressive thromboprophylaxis with low molecular weight heparin and calf compression.[131] A recent large prospective observational study found that *de novo* pulmonary thrombi (PT) were more frequent than pulmonary emboli arising from deep vein thrombosis, and that many of these clots occur early in the clinical course.[10] These observations illustrate that the aetiology of venous thrombosis in trauma patients is heterogenous and does not always follow the classical thrombosis-embolus sequence. PT in particular may be associated more with degree of haemorrhage and the subsequent systemic inflammatory response than with venous stasis.[10,11,132]

Increasing evidence supports an important role for platelets in venous thrombosis through various mechanisms.[133,134] Crosstalk between platelets and monocytes appears to be important in the initiation and propagation of VTE, partly mediated by induction of tissue factor expression on the surface of monocytes following platelet binding.[135,136] Platelet-dependent generation of highly prothrombotic extracellular traps from neutrophils has also been implicated in the development of venous thrombosis.[134,137] Given that platelet-leukocyte interactions are frequent after major trauma, this may represent a mechanism of post-injury thrombosis that is not targeted by current prophylactic regimens.

Platelet-derived EVs are also implicated in the pathophysiology of post-injury thrombosis. EVs released by platelets after injury have also been shown to augment thrombin generation,[93,138] and circulating levels of platelet-derived EVs are higher in injured patients who go on to develop VTE.[75] In experimental models, adoptive transfer of platelet EVs harvested from mice subjected to trauma-haemorrhage amplify venous thrombosis in uninjured mice.[81] This serves as an example of how responses that are protective during the bleeding phase may also increase subsequent thrombosis, and illustrates the challenges inherent in developing therapies to modulate these processes in this complex patient population.[139]

In trauma patients, two separate studies have found that persistently impaired platelet aggregation is independently associated with VTE, and viscoelastic measurements of clot strength also show delayed recovery from TIC in patients who develop thrombosis.[24,140] The explanation for this apparently paradoxical observation is unclear, and is subject to further investigation. Given that basal pre-activation has been proposed as a mechanism for blunted *ex vivo* responses, this may indicate a persistent state of *in vivo* activation that predisposes to thrombosis. Another interlinked potential mechanism is that the switch towards a procoagulant platelet phenotype at the expense of aggregatory function during major haemorrhage persists over time, resulting in persistent elevation of thrombin generation and consequently a prothrombotic state despite an impairment in *ex vivo* responsiveness on aggregometry.

Future Directions

The field of post-injury platelet biology is unrecognisable from a decade ago and there have been major improvements in our understanding of the key alterations in platelet function

that occur after major trauma. These advances have been driven by more sophisticated experimental approaches and supported by increasing evidence from other disease settings. However, as is often the case, many new questions have arisen from this work and a number of key uncertainties remain.

A major challenge in trauma research is elucidating which observations reflect phenomena that are detrimental to outcome versus those that are protective. Adding to this challenge is the extremely dynamic nature of these patients, and the confounding influence of the various medical and surgical interventions that they receive. The switch from hypocoagulability to hypercoagulability exemplifies this challenge, as treatments to support haemostasis may be beneficial during the former but harmful during the later. Defining the mechanisms by which this occurs, and developing biomarkers to clearly guide therapeutic interventions, is an important research priority. Although it is difficult to propose an overarching theory of the mechanisms underlying trauma-induced platelet dysfunction given the multitude of candidate pathways involved, our opinion based on current literature is that DAMP-platelet interactions are likely to represent the primary driver of initial changes in platelet behaviour during TIC. We believe this occurs by inducing exhaustion of platelet haemostatic capacity, driving procoagulant transformation at the expense of aggregatory function, and by triggering release of platelet-derived EVs and granular contents. This is likely to be compounded by various additional pathways including catecholamine release, consumption of vWF, and systemic activation of coagulation. We postulate that the subsequent procoagulant state is a 'rebound' phenomenon that occurs due to persistent circulating procoagulant platelets and efflux of hyper-activated immature platelets from the bone marrow, but empiric evidence to support this hypothesis is required.

In our view, there are two principal limitations in the current literature that require addressing in further studies. First, the focus of most work has largely been on the role of platelets in coagulopathy and bleeding – this is of course warranted given their critical roles in haemostasis and the importance of TIC in clinical outcomes. However, more attention is now needed to changes in platelet activity later in the clinical course, and in particular investigations into how platelets mediate links between inflammation and thrombosis following major trauma. Second, the great majority of human data focuses on changes in the peripheral bloodstream, including circulating platelets, that may not reflect changes in biology at tissue level. Major strides in deciphering the pathophysiology of COVID-19 were made through autopsy and tissue-based assays, and similar advances may be possible in trauma with a comparable approach. Coupled with this, use of established animal models of trauma-haemorrhage with advanced imaging technologies such as intravital microscopy, a tool that has been used to identify several novel aspects of platelet behaviour in other disease states,[141,142] may clarify some of the key outstanding questions outlined in this review.

Conclusion

Major trauma results in profound changes in platelet behaviour. These changes develop rapidly, and are intrinsically linked to the body's response to tissue damage and blood loss. The release of DAMPs and catecholamines are just two of an unknown number of potential stimuli that appear capable of driving the global impairment of platelet haemostatic function, emergence of a sub-population of circulating procoagulant platelets, and production of platelet EVs. Critically, these apparent dysfunctional phenomena are not fully reversed by currently available treatments, and further research is required to guide the development of

targeted interventions during this phase. At later timepoints, platelets are also implicated in the development of organ dysfunction and thrombosis, the major causes of subsequent morbidity and mortality. Elucidating the pathways involved in these processes, and the extent to which thromboinflammation and dysregulated immune responses are linked after major trauma, are important questions as we seek advances in trauma care.

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Disclosures

None

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Highlights

- Major trauma results in rapid and profound changes in platelet function that contribute to trauma-induced coagulopathy in the acute post-injury period.
- Such changes are innately linked to tissue damage and release of damage-associated molecular patterns following injury.
- At later timepoints, platelets are also implicated in the switch to a hypercoagulable state characterised by development of organ dysfunction and thrombosis.
- Future research must address these later time points and elucidate the pathways by which platelets mediate links between inflammation and thrombosis after major trauma.

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Figure legends

Figure 1: *Coagulation profiles over time following major trauma.* An initial hypocoagulable phase, described under the umbrella term 'trauma-induced coagulopathy (TIC)' is associated with increased bleeding, transfusion requirements and mortality. During hypocoagulable phase, platelets are less responsive to stimuli with decreased aggregation and adhesion capability. Patients that survive through initial phase enter secondary hyper-coagulable phase characterised by increased incidence of multiple organ dysfunction (MODS) and thrombotic events. This is associated with rebound thrombocytosis and increased presence of platelet-leukocyte aggregates.

Figure 2: *Pathways to altered platelet function during major trauma*. Major trauma include haemorrhage, tissue damage, collagen exposure at sites of vascular injury and activation of coagulation pathways. Release of damage associated patterns (DAMPs), catecholamines and increased fibrin/D-dimers have all been implicated as initiators of altered platelet behaviour, including: 1. Granule secretion. 2. Membrane ballooning with associated pro-coagulant PS-exposure. 3. Vesicle release with ability to bind directly to leukocytes. 4. Receptor shedding, particularly GPVI and GPIbα. 5. Impaired response to stimuli including thrombin and pro-thrombotic mediators thromboxane (TXA_2) and adenosine diphosphate (ADP).