Full title: Alternatives to allogeneic platelet transfusion

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Abstract

Allogeneic platelet transfusions are widely used for the prevention and treatment of bleeding in thrombocytopenia. Recent evidence suggests platelet transfusions have limited efficacy and are associated with uncertain immunomodulatory risks and concerns about viral or bacterial transmission. Alternatives to transfusion are a well-recognised tenet of Patient Blood Management, but there has been less focus on different strategies to reduce bleeding risk by comparison to platelet transfusion. Direct alternatives to platelet transfusion include agents to stimulate endogenous platelet production (thrombopoietin mimetics), optimising platelet adhesion to endothelium by treating anaemia or increasing von Willebrand factor levels (desmopressin), increasing formation of cross-linked fibrinogen (activated recombinant factor VII, fibrinogen concentrate or recombinant factor XIII), decreasing fibrinolysis (tranexamic acid or epsilon aminocaproic acid) or using artificial or modified platelets (cryopreserved platelets, lyophilised platelets, haemostatic particles, liposomes, engineered nanoparticles or infusible platelet membranes). The evidence base to support the use of these alternatives is variable, but an area of active research. Much of the current randomised controlled trial focus is on evaluation of the use of thrombopoietin mimetics and anti-fibrinolytics. It is also recognised that one alternative strategy to platelet transfusion is choosing not to transfuse at all.

Introduction

Platelet transfusions are widely used for the prevention or treatment of bleeding in thrombocytopenia (Van Hoeven*, et al* 2015, Whitaker 2015). Concerns over their efficacy and the risks of platelet transfusion means that alternatives to transfusion are needed. There has been considerable research into alternatives to red cell transfusion as part of Patient Blood Management (Clevenger and Richards 2015, Kotze*, et al* 2015) but the investigation of alternatives to platelet transfusion is more limited. This review will summarise the evidence for a number of potential alternatives to platelet transfusion including: thrombopoietin mimetics, antifibrinolytic agents, treatment of anaemia, DDAVP (also known as desmopressin or 1-deamino-8-D-arginine vasopressin), fibrinogen concentrate, recombinant factor VIIa, recombinant factor XIII and artificial platelets (Figure 1a and 1b; Table 1). Although described as alternatives to transfusions of platelets, these agents may also be very effective adjuncts to infusions of platelets, which may be important given the limited efficacy of platelet transfusions to reduce bleeding in patients with haematological malignancies (Stanworth*, et al* 2013) or to improve outcomes in patients on anti-platelet drugs with intracranial bleeding (Baharoglu*, et al* 2016).

Why is there a need for alternatives to platelet transfusion?

Two million, four-hundred thousand units of platelets are transfused each year in Europe (Van Hoeven*, et al* 2015) and 1.3 million units in the United States (Whitaker 2015). Platelet components are derived from donations of whole blood or apheresis and are then stored (with a short expiry of 5 to 7 days) at room temperature prior to administration. However, only recently have randomised controlled trials been undertaken which have addressed fundamental questions about reduction of bleeding with platelet transfusions. These trials reported limited evidence of efficacy in the prevention of bleeding following platelet transfusion to thrombocytopenic patients with haematological malignancies and also emphasised the importance of factors other than platelet transfusions to reduce bleeding risk (Stanworth*, et al* 2013). Specifically, all platelet transfusions trials in patients with haematological malignancies have indicated high rates of bleeding despite prophylactic platelet transfusion policies.

Platelet transfusions are associated with diverse risks such as transfusion reactions, bacterial contamination and viral transmission, which may include emerging infections such as Zika virus, which are hard to detect (Musso*, et al* 2014). Platelet transfusions require compatibility testing, and may take time to request and obtain for administration (not all hospital blood banks have a ready supply of platelets for all blood groups). There is a relative lack of standardisation between platelet

units with variability in platelet reactivity between donors (Garner*, et al* 2008) and differences in platelet function following storage (Rosenfeld*, et al* 1995). Even in the emergency setting of major traumatic bleeding when policies for timely use of blood components should be optimised, the average time to administration of platelets has been reported to be 2 to 3 hours (Stanworth*, et al* 2016). Alternative agents that could be given more quickly may allow procedures to go ahead more rapidly, or arrest bleeding sooner. Patients who refuse blood, such as Jehovah's witnesses, would also benefit from alternatives to platelet transfusion that do not contain blood components. Another group who may benefit from alternatives to platelet transfusion are those who are refractory to platelet transfusions. Lastly, in the developing world, access to safe blood components may be limited. The prevalence of transfusion-transmitted infections in each blood transfusion is much higher in low income countries than in high income countries (HIV 0.85% versus 0.002%, hepatitis B 3.59% versus 0.02% and hepatitis C 1.07% versus 0.02%) (World Health Organization 2015), and safe, cheap alternatives to platelet transfusion would be appealing.

No platelet transfusion

Thrombocytopenia

A key question before considering alternatives to transfusion is whether prophylactic platelet transfusion is necessary at all. A systematic review synthesising the data from six trials of prophylactic platelet transfusion versus on demand transfusion for patients undergoing myelosuppressive chemotherapy or stem cell transplantation found that clinically significant bleeding was less common amongst patients treated with prophylactic platelet transfusions than those receiving on demand therapy (Crighton*, et al* 2015). The occurrence of life-threatening bleeding is rare in this patient group and there is insufficient evidence to determine whether there is an increase in the risk of severe or life-threatening bleeding if platelet transfusion is only given on demand, although it seems likely that other patient factors may be more relevant to explain risk of major bleeding. From the largest of these trials to date (TOPPS) (Stanworth*, et al* 2013), the authors reported in subgroup analyses that in recipients of autologous haematopoietic stem cell transplantation, there was no difference in bleeding between those on the two different platelet transfusion strategies (Stanworth*, et al* 2014). It is also important to consider that non-bleeding patients with chronic bone marrow failure do not benefit from prophylactic platelet transfusion (Padhi*, et al* 2015) and platelet transfusion may be harmful in the setting of thrombotic thrombocytopenic purpura and heparin-induced thrombocytopenia (Goel*, et al* 2015). The evidence base for platelet transfusion before interventional procedures or surgery is very limited. There are no completed randomised controlled trials of platelet transfusion thresholds in this setting to date.

There is broader recognition in guidelines that some interventional procedures such as bone marrow aspirate and trephine, or long line insertions, may not require 'cover' with platelet transfusions (Padhi*, et al* 2015). However, many audits have documented widespread use of platelet transfusions pre-procedure (Charlton*, et al* 2014, Qureshi*, et al* 2007).

Reversal of antiplatelet agents

Transfusion of two to three units of platelets to reverse antiplatelet agents in the event of major bleeding or emergency surgery is recommended in several guidelines (Ferraris*, et al* 2012, Makris*, et al* 2013). These recommendations are largely based on studies demonstrating that impaired platelet aggregation due to antiplatelet agents can be corrected *in vitro* with the addition of donor platelets (Li*, et al* 2012, Vilahur*, et al* 2007); and a single arm clinical trial that administered two units of platelets pre-operatively to patients on dual antiplatelet therapy (Thiele*, et al* 2012). This strategy has been called into doubt by a recent trial examining the use of platelet transfusions for reversal of antiplatelet agents in the setting of spontaneous intracranial haemorrhage (Baharoglu*, et al* 2016). Patients were randomised to receive standard care or platelet transfusion (one unit for those taking aspirin or two units for those taking other antiplatelet agents). There was no difference in bleeding between the groups but those transfused with platelets had a significant increase in the risk of mortality or a poor functional outcome at three months.

Thrombopoietin mimetics

Agents that stimulate platelet production, rather than replace platelets as transfusions, are one potential alternative. Thrombopoietin (TPO) mimetics licensed for use in the UK at present are romiplostim and eltrombopag. These agents act by stimulating megakaryopoiesis resulting in increased thrombopoiesis. TPO mimetics have been used to treat thrombocytopenia in a large number of settings, most notably immune thrombocytopenic purpura (ITP). In the setting of ITP they have been shown to increase platelet counts but have not been found to reduce the incidence of serious bleeding events compared to placebo (Zeng*, et al* 2011). Patients with liver disease on antiviral therapies have also been examined, although in this group, an unacceptable increased risk of both bleeding and thrombosis occurred following TPO mimetic administration (Afdhal*, et al* 2014). There was early promise for the use of TPO mimetics in the treatment of myelodysplastic syndrome (MDS) where their administration improved the exposure-adjusted bleeding rate (Prica*, et al* 2014). Previous trials in the setting of haematological malignancy undergoing intensive chemotherapy found no difference in the duration of thrombocytopenia but did note a reduction in platelet transfusions. These trials were halted, as the TPO mimetic in question, pegylated recombinant

human megakaryocyte growth and differentiation factor (PEG-rHuMGDF) was found to cause anti-TPO antibodies in both patients and volunteers and was withdrawn from further development (Li*, et al* 2001). TPO mimetics may cause an increase in blast count in this setting but there is no evidence for an increased risk of progression to acute myeloid leukaemia (Prica*, et al* 2014). Bleeding was only reported in one of these trials with an overall reduction in clinically significant bleeding from 50% to 25% with TPO mimetics, although this trial was underpowered for both this outcome and the more important outcome of preventing serious or life-threatening bleeding (Geissler*, et al* 2003). Five further trials of TPO mimetics in haematological malignancy are presently underway, which are aiming to recruit 424 patients in total. These agents are expensive and in some settings, have been found to be associated with an increased risk of thrombosis (Afdhal*, et al* 2014). The administration of TPO mimetics for patients with aplastic anaemia is of particular interest, as in addition to their effects in improving the platelet count; TPO mimetics may also have a disease-modifying effect in this setting (Desmond*, et al* 2014).

Antifibrinolytic agents

Tranexamic acid and epsilon aminocaproic acid are synthetic lysine analogues which block the lysine binding sites on plasminogen, preventing it from interacting with fibrin and thus from being converted to plasmin (the final enzyme in the fibrinolytic pathway) and from cleaving its substrate (Ortmann*, et al* 2013). These agents result in resistance to clot lysis and increased clot strength. The formation of plasmin may have other effects outside the clotting system such as immune and endothelial activation (Syrovets*, et al* 2012) and cleavage of von Willebrand factor (vWF) (Tersteeg*, et al* 2014). It is unclear if these antifibrinolytic agents exert part of their effect through mechanisms other than inhibition of clot lysis.

There are limited specific data on use of antifibrinolytic agents for preventing bleeding for patients with thrombocytopenia. This approach is appealing, as these agents are cheap and have been shown to be effective in a number of other patient settings (not necessarily characterised by thrombocytopenia such as trauma) with minimal adverse events (Ker*, et al* 2012, Shakur*, et al* 2010). Platelet poor clots are more vulnerable to lysis than platelet rich clots (Fay*, et al* 1994) and platelets are a source of plasminogen activator inhibitor 1 (PAI-1), a key inhibitor of fibrinolysis (Mutch*, et al* 2007) and factor (F)XIII (which is essential for cross-liking alpha-2-antiplasmin (A2AP) to fibrin to prevent fibrinolysis (Mitchell*, et al* 2014). A recent systematic review focussing on the use of tranexamic acid in patients with haematological malignancies (Estcourt*, et al* 2016) identified three trials examining tranexamic acid and one trial examining epsilon-aminocaproic acid, all of which were of low quality, and small sample size. Three large trials are presently underway, one of epsilon

aminocaproic acid (PROBLEMA) and two of tranexamic acid (TREATT and ATREAT) for the prevention of bleeding in thrombocytopenic patients undergoing intensive chemotherapy or stem cell transplantation. These trials in total are expected to recruit 1276 patients and are expected to be completed by December 2020.

Treatment of anaemia

Red cells play a direct role in haemostasis through a number of pathways. Axial flow of red cells through blood vessels pushes platelets towards vascular walls, promoting contact with vWF (Chen*, et al* 2013). Murine studies have found that red cells adhere to the vascular endothelium faster than platelets following vascular injury and the red cell structures then go on to recruit platelets (Barr*, et al* 2013). Additionally the red cell surface acts as a site of thrombin generation and may contribute one third of the phospholipid surface necessary for thrombin generation (the other two thirds is provided by platelets) (Whelihan*, et al* 2012). The contribution of red cells to clots alters their structure; with red cell-rich clots having greater resistance to lysis (Wohner*, et al* 2011).

Consequently, an important question is whether treatment of anaemia can prevent bleeding or risk of re-bleeding. To date, trials comparing liberal transfusion of red cells to more restrictive practice have reported mixed findings. In some settings, there was no significant difference in bleeding risk in several settings: bleeding following intensive chemotherapy for haematological malignancy (71% liberal, 75.9% restrictive) (Webert*, et al* 2008); bleeding after cardiac surgery (1.7% liberal, 1.2% restrictive) (Murphy*, et al* 2015); severe bleeding in septic shock (10.6% liberal, 6.1% restrictive) (Holst*, et al* 2014); re-bleeding following gastrointestinal bleeding (16% liberal, 10.1% restrictive) (Villanueva*, et al* 2013) and 8.1% liberal, 5.1% restrictive (Jairath*, et al* 2015); and bleeding in paediatric intensive care (0% liberal, 0.9% restrictive) (Lacroix*, et al* 2007). It should be noted of interest that the balance of risk for bleeding risks appears to favour restrictive use of red cell transfusions, more clearly apparent in the two recent trials of acute upper gastrointestinal bleeding (Jairath*, et al* 2015, Villanueva*, et al* 2013). Large volume red cell transfusions are associated with a dilutional thrombocytopenia (Hess*, et al* 2008) and may increase portal venous pressure, increasing the risk of re-bleeding following acute variceal haemorrhage (Villanueva*, et al* 2013). Other methods for preventing bleeding by treating anaemia, such as correction of iron deficiency, may have more promise than red cell transfusion (Ho 1998). More data are needed before a recommendation on the use of red cell transfusions to prevent bleeding can be made.

DDAVP

DDAVP acts by stimulating release of FVIII and vWF from Weibel-Palade bodies in endothelium. It also has some effects in boosting platelet activity (Svensson*, et al* 2014). This is a potentially appealing treatment for thrombocytopenia, as vWF is necessary for the initial adhesion of platelets to the endothelial surface. This may increase the number of platelets that adhere to damaged endothelium by increasing the concentration of vWF, optimising the function of the platelets that are present. It is associated with adverse events including facial flushing, antidiuresis, hypotension, hyponatraemia and rarely seizures. Tachyphylaxis occurs after repeated administration of DDAVP with 30% less increase in vWF antigen when it is re-administered 24 hours after an initial dose (Mannucci*, et al* 1992). One randomised trial has compared DDAVP to platelet transfusion for treatment of cirrhotic patients with platelet counts between 30 and $50x10^9$/l undergoing dental extractions. This trial was too small to demonstrate efficacy, as only a single bleeding event occurred (Stanca*, et al* 2010). One single arm trial assessing the efficacy of DDAVP for 15 bleeding patients (platelet count range 2 to 71 $x10^9$/l) with haematological malignancies found that bleeding was arrested in all cases, although it was most effective for those with a platelet count ≥$30x10^9$/l (Castaman*, et al* 1997). Another single arm trial examining the effects of DDAVP for 5 children with haematological malignancies and one with ITP (platelet count range 10 to 34 $x10^9$/l) found that bleeding stopped in all cases following administration of DDAVP (Kobrinsky and Tulloch 1988). Neither trial included a comparator arm limiting assessment of efficacy. By contrast another study of 15 patients (platelet count range 1 to 53 $x10^9$/l) found that DDAVP made no difference to bleeding time compared to placebo, although it should be noted that baseline vWF levels were high in this study, which may have limited the effectiveness of DDAVP (Mannucci*, et al* 1986).

Targeting fibrinogen

A normal clot consists of both a platelet plug and cross-linked fibrin strands. A potential strategy to treat, or prevent, bleeding for thrombocytopenic patients would be increasing the concentration of cross-linked fibrinogen. This could potentially be done by increasing thrombin generation (for instance through the use of recombinant FVIIa), administration of fibrinogen concentrate or administration of recombinant FXIII.

Recombinant factor VIIa

FVIIa is essential for triggering thrombin generation in combination with tissue factor. The recombinant version of FVIIa (rFVIIa; eptacog alfa) has been used in a number of settings to control bleeding. It has a short half-life of 2.7 to 3.5 hours and consequently repeated infusions are necessary. A trial of three doses of intravenous rFVIIa to control bleeding post-stem cell transplant

found that 40µg/kg and 120µg/kg made no difference to the risk of severe or life-threatening bleeding, whereas, there was a reduction in bleeding using a dose of 80µg/kg. The event rate for severe bleeding is low and these results are likely to be imprecise. rFVIIa was associated with an increased risk of thromboembolic events in this trial, which are difficult to manage in the setting of thrombocytopenia (Pihusch*, et al* 2005). Intrapulmonary rFVIIa has been used following allogeneic stem cell transplantation to control bleeding from alveolar haemorrhage but no clear benefit has been demonstrated (Elinoff*, et al* 2014). The limited efficacy, side effect profile and expense of this agent limit its use as an alternative to platelet transfusion (Simpson*, et al* 2012).

Fibrinogen concentrate or cryoprecipitate

In vitro work combining administration of fibrinogen concentrate and rFVIIa demonstrated normalisation of whole blood clot formation in an *in vitro* model of ITP and suggested the two agents may have a synergistic effect (Larsen*, et al* 2013) and trials administering fibrinogen concentrate to thrombocytopenic pigs demonstrated an improvement in maximum clot firmness (Velik-Salchner*, et al* 2007). No trials of fibrinogen concentrate have been performed for treatment of thrombocytopenia. We are not aware of any trials using cryoprecipitate for the treatment of thrombocytopenia but it is likely that it would have similar effects to fibrinogen concentrate.

Recombinant factor XIII

FXIII is partially secreted by platelets (Mitchell*, et al* 2014), so thrombocytopenia limits delivery of FXIII to sites of bleeding. Recombinant FXIII is used for the treatment of congenital FXIII deficiency (Inbal*, et al* 2012). It has also been used empirically in cardiothoracic surgery with mixed results for control of blood loss (Godje*, et al* 2006, Karkouti*, et al* 2013) and in cancer surgery where a pilot trial demonstrated an improvement in clot firmness and a small reduction in bleeding (Korte*, et al* 2009). A single randomised controlled trial of FXIII supplementation for patients with acute leukaemia from 1982 found no difference in the risk of bleeding after administration (Rasche*, et al* 1982).

Alternative platelet storage methods

Platelet haemostatic and secretory function diminishes within hours of blood storage and by 5 to 7 days, there is a marked reduction in agonist-stimulated aggregation of liquid-stored platelets regardless of their storage medium (Pidcoke*, et al* 2013, Ringwald*, et al* 2006). Different methods of preparing and storing active components or fractions of platelets such as refrigeration (Pidcoke*, et al* 2013), cryopreservation (Slichter*, et al* 2014) or lyophilisation (Fitzpatrick*, et al* 2013) have potential advantages over standard platelets.

They will have a prolonged shelf-life, may be more readily stored for immediate administration to patients, and could potentially have a better safety profile with a lower risk of bacterial contamination (Milford and Reade 2016).

Refrigerated platelets

Platelets are usually stored at 22 ± 2°C, because refrigeration of platelets at 4°C has been associated with a shorter *in vivo* survival time (Murphy and Gardner 1969). Factors contributing to accelerated clearance include changes to glycosylation and signalling functions of the von Willebrand receptor, Glycoprotein Ibα (Rumjantseva and Hoffmeister 2010, van der Wal*, et al* 2010). However refrigerated platelets have good preservation of haemostatic activity (Reddoch*, et al* 2014) and are more haemostatically active after storage than those stored at 22°C (Pidcoke*, et al* 2013). Refrigeration of platelets reduces the production of reactive oxygen species and preserves respiratory capacity of platelet mitochondria: features that are associated with greater aggregation responses to collagen (Bynum*, et al* 2016).

In view of the haemostatic benefits of refrigerated platelets and considering the recent evidence that balanced ratios of red cells, platelets and plasma are most effective in trauma resuscitation, there has been a corresponding interest in refrigerated whole blood (Pidcoke*, et al* 2013). Data has recently been shared from a volunteer study of the *in vivo* viability of platelets prepared from autologous whole blood stored at 4°C, compared with fresh autologous platelets. Platelets produced from blood stored with continuous rotation at 4°C for 15 days Showed acceptable post-transfusion recoveries with significantly reduced platelet survival (Slichter*, et al* 2015). Trials of refrigerated platelets to control bleeding are in prospect in a number of countries, making them a promising potential future alternative.

Cryopreserved platelets

Cryopreserved platelets (CPPs) have been used by the Dutch Military since 2001 (Lelkens*, et al* 2006). CPP are preserved with glycerol and frozen at -196°C in liquid nitrogen. They can potentially be stored for up to 5 years, compared to 5 to 7 days for standard platelets. Recently, a thorough review has been published of *in-vivo* studies performed with CPPs concluding that the platelets appear haemostatically active with no significant adverse effects (Slichter*, et al* 2014). They are scheduled for use by the U.S. Army, subject to satisfactory safety and efficacy being demonstrated in a US clinical trial due to finish in 2016 (Dumont*, et al* 2013). In Australia, a trial is ongoing to assess the efficacy of CPPs in controlling coagulopathy and haemorrhage in patients bleeding after cardiac

surgery (CLIP Trial (Royse*, et al* 2015)). Associated laboratory work has attributed haemostatic capacity of CPPs partly to microparticles (Johnson*, et al* 2014).

Lyophilized platelets

A stable method of freeze-dried platelet production dates back to 1978, when Brinkhous and Read used limited paraformaldehyde fixation to stabilise platelets for lyophilisation (Brinkhous and Read 1978). This preparation retained various haemostatic properties *in vitro* (Read*, et al* 1995) and *in vivo* (Bode*, et al* 2008), although trials in swine revealed thrombotic complications associated with lyophilised human, but not swine, platelets (Hawksworth*, et al* 2009). More recently, a trehalose-based stabilising agent has been developed commercially to produce lyophilized, Group O pooled platelets (Fitzpatrick*, et al* 2013). When applied to a swine model of liver injury, such lyophilized human platelets actually enhanced intraoperative blood loss, whilst those prepared from swine decreased it over the 48h observation period. A clinical, dose-escalation trial, to evaluate safety and immunogenicity of autologous, lyophilised platelets, is scheduled to report in 2016 (Slichter 2014).

Recently, a membrane-permeable trehalose-based compound has been developed for potential application in stem cell preservation (Abazari*, et al* 2015). Such developments may be used in the future to better preserve lyophilised platelets.

Artificial platelets

Artificial platelets have been used in some early clinical trials but predominantly in animal models. These agents could potentially be easy to store, could be rapidly accessed and have a better safety profile than platelets. However haemostatic particles of the current generation are designed to augment platelet aggregation but not to perform the other necessary functions of the platelet in clot evolution. Platelets also have a number of important non-haemostatic functions, including an important role in maintenance of endothelial integrity (Gros*, et al* 2014) - properties that have been measured in stored components (Baimukanova*, et al* 2016). Once the necessary factors are identified, these may be included into synthetic particles for which endothelial targeting strategies are also being developed, by incorporating surface ligands and modifying their geometry (Kowalski*, et al* 2014, Muro*, et al* 2008).

More preclinical research needs to be done to ensure the safety, and dosing accuracy, of haemostatic particles. Safety developments include a swine model to monitor for complement-activation related pseudoallergy (Szebeni 2014), which will inform particle surface chemistry design (Lashof-Sullivan*, et al* 2013). Dose monitoring that relies on the standardisation of submicron particle counting is still some way off (Mooberry and Key 2016), despite clinical studies correlating

bleeding control with cellular microvesicles in that size range (Matijevic*, et al* 2014). This is also the case for potential haemostatic monitoring, for although studies are improving the discrimination of platelet and plasma components by elastography (Kornblith*, et al* 2014, Moore*, et al* 2015), their interpretation is equivocal (Hunt*, et al* 2015).

Haemostatic (platelet-bridging) particles

Various formulations of synthetic, haemostatic particles have been developed to the pre-clinical stage. All have been designed to augment the aggregation of platelets. Aggregation occurs when the conformation of platelet surface fibrinogen receptors (Glycoprotein IIb/IIIa) is switched; rendering them adhesive to plasma fibrinogen, a dimer through which they become bridged (Mosesson 2005). Synthetic, haemostatic particles display fibrinogen, or its peptide motifs recognised by the receptor – RGD and HHLGGAKQAGDV (H12) - chemically coupled to their surfaces.

A number of attempts were made in the 1990s to produce such agents. Initially, red cells were coated with RGD-containing peptide sequences (Thromboerythrocytes; (Coller*, et al* 1992)). Later, albumin microcapsules coated with fibrinogen (3.5 to 4.5μm diameter, compared to platelets of 2 to 5μm) were shown to reduce bleeding in severely thrombocytopenic rabbits (e.g. Synthocytes (Levi*, et al* 1999)) and were adopted commercially but are currently not in active development.

Liposomes

For more than a decade, research groups in Japan have developed synthetic liposomes as an infusible haemostatic agent (Okamura*, et al* 2005). These particles (approximately 300nm diameter) are coated with the H12 peptides and contain adenosine 5'-diphosphate that is released in an aggregation-dependent manner to enhance platelet activation (Okamura*, et al* 2009). Their biodistribution and haemostatic properties have been characterised in a number of pre-clinical animal models (Hagisawa*, et al* 2015).

Engineered Nanoparticles

Haemostatic nanoparticles (300 to 500nm), based on the biocompatible, synthetic polymer poly(lactic *co*-glycolic acid) (PGLA) and coated with an RGD-containing peptide, were shown to reduce bleeding time upon intravenous administration in a rat model of major trauma (Bertram*, et al* 2009) and to improve survival after 1 hour in a recently developed murine model of blast injury (Lashof-Sullivan*, et al* 2014).

Although spherical nanoparticles are rapidly cleared from the circulation which may be advantageous to prevent longer term side-effects, the recognition and clearance by the endocytic

machinery of macrophages can now be modulated by controlling particle geometry during synthesis (Modery-Pawlowski*, et al* 2013).

Infusible platelet membranes

Microparticles formed from platelets have a procoagulant effect and this finding has led to investigation of whether preparations of membranes from out-dated platelet units could be used as an alternative to platelets. Infusible platelet membranes were the subject of some commercial development and a small clinical trial in the late 1990s, with more recent pre-clinical work confirming some haemostatic properties but development has been limited due to difficulties demonstrating the efficacy of this approach (Nasiri 2013).

Platelets generated from stem cells (blood pharming)

A future strategy may be generation of laboratory grown platelets. Recent work has demonstrated that ectopic expression of the transcription factors GATA, FLI1 and TAL1 in human pluripotent stem cells induces them to differentiate into megakaryocytes that can be expanded and banked as frozen cell lines from which (currently few) functional platelets can be generated (Moreau*, et al* 2016). Stem cells can also be modified to create HLA-compatible or HLA-silenced platelets for treatment of patients with anti-HLA antibodies (Figueiredo and Blaszczyk 2014). This approach has considerable future promise and may reduce platelet shortages and reduce transfusion-transmitted infections and transfusion reactions.

Other factors

Any discussion of the role of alternative strategies to platelet transfusions to reduce bleeding risk should acknowledge the importance of other patient factors in determining the need for interventions. We advise that non-specialists consult a haematologist to optimise the bleeding risk for these patients. Fever is reported as an independent risk factor for bleeding in patients with haematological malignancies (Stanworth*, et al* 2015) and inflammation has been shown to contribute towards bleeding in animal models (Hillgruber*, et al* 2015) suggesting that recognition and treatment of sepsis may reduce the risk of bleeding. Maintaining a normal calcium, pH and temperature is essential for normal haemostasis (Lier*, et al* 2008). Vitamin K deficiency is common, especially in the critically ill (Chakraverty*, et al* 1996) and is a readily reversible bleeding risk.

A practical approach to managing thrombocytopenia for patients who refuse blood components

A single centre cohort study from the United States of 125 Jehovah's witnesses undergoing stem cell transplantation reported strategies used to reduce the risks of bleeding, as these patients would not receive red cell or platelet transfusions. The protocol described by the authors therefore may provide a template for how different interventions can be applied to critically ill patients. Proton pump inhibitors, stool softeners, and progestational agents in menstruating women were used to minimise blood loss and all patients received vitamin K. DDAVP 0.3µg/kg was used as required and recombinant interleukin-11 was used initially but then discontinued as the trial progressed due to failure to demonstrate improvement in platelet engraftment and unacceptable side effects; particularly cardiac toxicity. Bleeding events occurred in 14.4% patients and severe or life-threatening bleeding occurred in 1.6% (Ford*, et al* 2015). It should be noted that the incidence of severe or life-threatening bleeding in this cohort is higher than that seen in studies using prophylactic platelet transfusion where rates are typically less than 1% (Stanworth*, et al* 2013).

Conclusion

There is considerable scope to expand our understanding of the role of alternative agents to platelet transfusion. This must be considered in the context of the limited efficacy of platelet transfusions to reduce bleeding risk in patients with haematological malignancies, which have been most extensively studied to date. The results of trials of TPO mimetics for patients undergoing intensive chemotherapy or stem cell transplantation, or who have MDS or aplastic anaemia are awaited. There is no adequate evidence for the effectiveness of antifibrinolytic agents in thrombocytopenia at present although several large trials are underway which will inform future practice. It remains to be seen whether the size of these trials will provide adequate information on safety, given previous research has highlighted risks of alternatives, namely aprotinin and rVIIa when used more widely. There is insufficient evidence for the use of DDAVP, fibrinogen concentrate, rFVIIa or rFXIII for reducing blood loss in thrombocytopenic patients, and these agents require further research evaluation. Arguably, the best evidence for an alternative treatment is for the decision not to transfuse platelets prophylactically in patients with haematological malignancies if the platelet count is greater than 10 $x10^9$/l or before low–risk procedures (Padhi*, et al* 2015). Future treatments may include laboratory-grown platelets and artificial platelet substitutes. Alternatives to platelet transfusions (with the exception of TPO mimetics for ITP) should in many cases only be considered in a clinical trial setting, and future studies should also recognise the need to assess cost-effectiveness.

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Conflicts of interest

MD, SS and LE are investigators for trial of DDAVP for thrombocytopenia. SS and LE are investigators for a trial of tranexamic acid for thrombocytopenic patients undergoing intensive chemotherapy or stem cell transplantation. No financial conflicts of interest to declare.

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Figures

***Figure 1a. Mechanisms of action of alternatives to platelet transfusion: platelet, von Willebrand factor and endothelial interaction*.** Following endothelial injury, subendothelial collagen and von Willebrand Factor (vWF) are exposed. Under shear forces, vWF uncoils and binds to exposed collagen supporting the adhesion of circulating platelets. DDAVP (1-deamino-8-D-arginine vasopressin) promotes vWF release from endothelial Weibel-Palade bodies, increasing plasma vWF concentrations (Svensson*, et al* 2014). Rapid treatment of sepsis helps to maintain endothelial integrity (Stanworth*, et al* 2015). Thrombopoietin mimetics directly increase platelet numbers by stimulating megakaryopoiesis (Prica*, et al* 2014). Red cell flow through blood vessels pushes platelets into proximity with the endothelium (and also collagen and vWF) promoting platelet adhesion (Chen*, et al* 2013). Red cells also provide approximately one third of the phospholipid surface for thrombin generation (Whelihan*, et al* 2012) and red cell incorporation into clots increases their resistance to lysis (Wohner*, et al* 2011). Alternatives to platelet transfusion are marked in red.

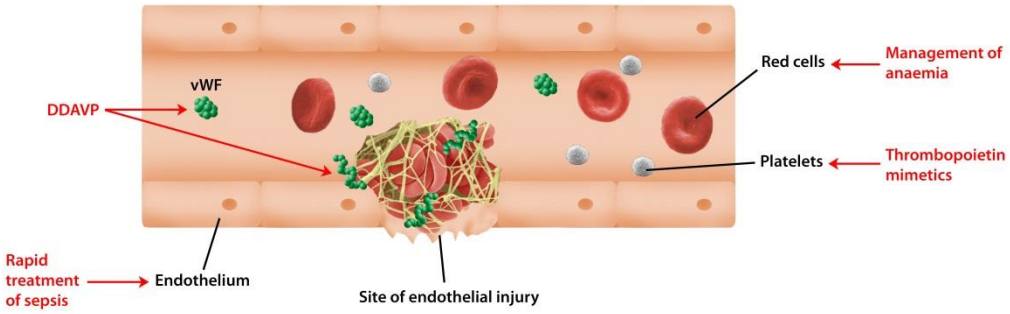
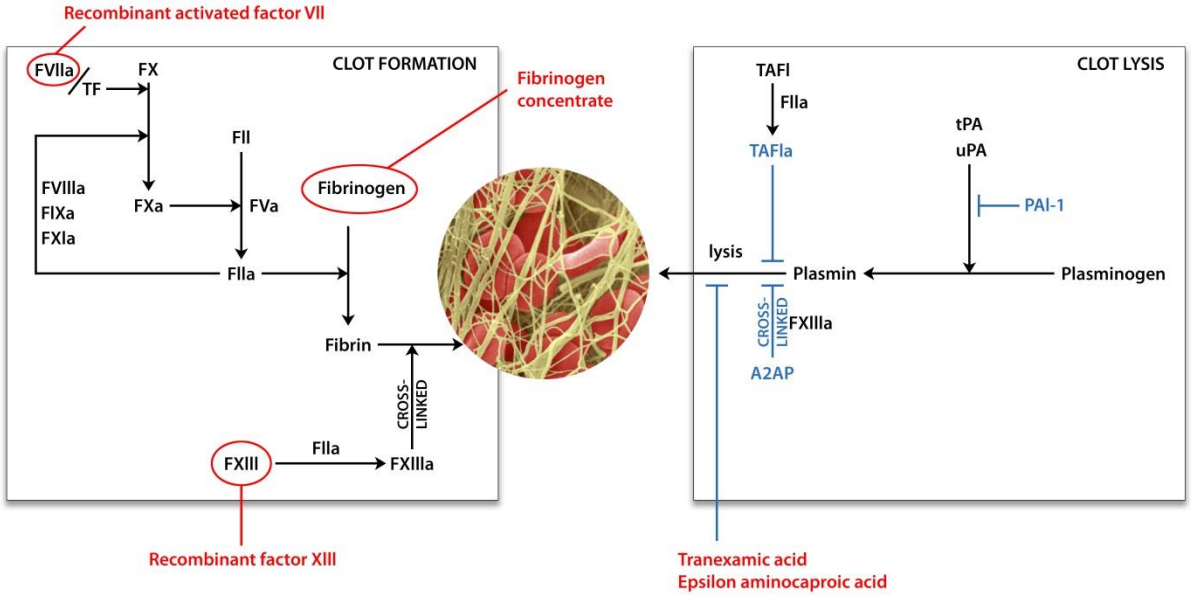


***Figure 1b. Mechanisms of actions of alternatives to platelet transfusion: fibrin formation, cross-linkage and fibrinolysis*.** *Clot formation (left box)*. Following endothelial injury, release of tissue factor (TF) and activated (a) factor (F) VII triggers thrombin formation. Thrombin (FIIa) is the key enzyme in clot formation resulting in conversion of fibrinogen to fibrin as well as providing a feedback loop, further amplifying thrombin generation through FVIIIa, FIXa and FXIa. Thrombin also promotes conversion of FXIII to FXIIIa and thrombin activatable fibrinolysis inhibitor (TAFI) to TAFIa, and can directly activate platelets. Formation of fibrin from fibrinogen is the final key step in stabilising the clot and fibrin is further strengthened by cross-linkage by FXIIIa. *Clot lysis (right box).* The key enzyme in clot lysis is plasmin. Conversion of plasminogen to plasmin is driven by tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA). There are three main inhibitors of fibrinolysis, alpha-2-anitplasmin (A2AP) (which is cross-linked to plasmin by FXIIIa) (Fraser*, et al* 2011), TAFIa (which is converted from TAFI by thrombin) and plasminogen activator inhibitor (PAI)-1 (which inhibits the activity of tPA and uPA) (Mutch*, et al* 2007). Black arrows

represent activation and blue arrows inhibition. Alternatives to platelet transfusion are in red text and where appropriate, their site of action is circled.



Platelet transfusion alternative	**Stage of development**
No platelet transfusion (Stanworth, *et al* 2013)	In clinical use. RCT evidence in setting of haematological malignancy undergoing intensive chemotherapy
Thrombopoietin mimetics (Afdhal, *et al* 2014, Geissler, *et al* 2003, Prica, *et al* 2014, Zeng, *et al* 2011)	In clinical use in some types of thrombocytopenia. RCTs in ITP, haematological malignancy undergoing chemotherapy, MDS and liver disease
Antifibrinolytics (Estcourt, *et al* 2016)	In clinical use largely outside the setting of thrombocytopenia. Limited RCT evidence in setting of haematological malignancy. Several large ongoing RCTs at present.
Treatment of anaemia (Webert, *et al* 2008)	In clinical use. Limited RCT evidence for red cell transfusion for patients with haematological malignancies undergoing intensive chemotherapy.

	Several ongoing RCTs at present.
DDAVP (Svensson*, et al* 2014)	In clinical use largely outside the setting of thrombocytopenia. Clinical case series and RCT evidence with laboratory outcomes in the setting of thrombocytopenia.
Fibrinogen concentrate (Larsen*, et al* 2013)	In clinical use largely outside the setting of thrombocytopenia. *In vitro* data in setting of thrombocytopenia. RCT evidence in other settings. Not licensed in most countries outside the setting of congenital hypofibrinogenaemia.
Recombinant factor VIIa (Simpson*, et al* 2012)	In clinical use largely outside the setting of thrombocytopenia. Limited RCT evidence in setting of stem cell transplantation
Recombinant factor XIII (Rasche*, et al* 1982)	In clinical use largely outside the setting of thrombocytopenia. Limited RCT evidence in setting of acute leukaemia undergoing induction chemotherapy
Refrigerated platelets (Slichter*, et al* 2015)	Autologous platelet recovery and survival study completed. Clinical trials currently underway
Cryopreserved platelets (Slichter*, et al* 2014)	In clinical use in some countries. RCT in healthy volunteers underway.
Lyophilised platelets (Slichter 2014)	Not in clinical use. Dose escalation study in healthy volunteers underway.
Haemostatic particles (Coller*, et al* 1992, Levi*, et al* 1999)	Not in clinical use. Animal trials only
Liposomes (Hagisawa*, et al* 2015)	Not in clinical use. Animal trials only

Engineered nanoparticles (Lashof-Sullivan*, et al* 2014)	Not in clinical use. Animal trials only
Infusible platelet membranes (Nasiri 2013)	Not in clinical use. Limited RCT evidence in thrombocytopenic patients
Platelets generated from stem cells (Moreau*, et al* 2016)	Not in clinical use Animal trials only

Table 1. Stages of development for alternatives to platelet transfusion. DDAVP – 1-deamino-8-D-arginine vasopressin, ITP – immune thrombocytopenic purpura, MDS – myelodysplastic syndrome, RCT – randomised controlled trial