

## Transfusion Medicine 2

### Platelet transfusions

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Ever since platelet transfusions were shown to reduce mortality from haemorrhage in patients with acute leukaemia in the 1950s, the use of this therapy has steadily grown to become an essential part of the treatment of cancer, haematological malignancies, marrow failure, and haematopoietic stem cell transplantation. Today, more than 1·5 million platelet products are transfused in the USA each year, 2·9 million products in Europe. However, platelet transfusion can transmit infections and trigger serious immune reactions and they can be rendered ineffective by alloimmunisation. There are several types of platelet components and all can be modified to reduce the chances of many of the complications of platelet transfusion. Transfusion practices, including indications for transfusion, dose of platelets transfused, and methods of treating alloimmunised recipients vary between countries, and even within countries. We review commonly used platelet components, product modifications, transfusion practices, and adverse consequences of platelet transfusions.

#### Introduction

Platelet transfusions were shown to reduce mortality from haemorrhage in patients with acute leukaemia in the 1950s,<sup>1,2</sup> and the use of the therapy has steadily grown since then. The procedure has become an essential part of the treatment of cancer, haematological malignancies, marrow failure, and haematopoietic stem cell transplantation. Despite the procedure's medical importance, it can trigger serious side-effects, and modifying platelet components to reduce potential complications is vital.

More than 1·5 million components of platelets are transfused each year in the USA<sup>3</sup> and 2·9 million in Europe.<sup>4</sup> Three different platelet preparations are used in the two regions; we review the main features of each preparation, and highlight clinically relevant differences. We also discuss the most important biochemical indices of platelet quality during storage, as well as technical and operational issues related to the collection and production of platelets.

#### Technological advances

A key step in the development of methods for preparing platelet products—often called platelet concentrates or components—for transfusions was the change from glass collection bottles to disposable multiple plastic bag sets that are still used for the collection of the standard unit of 450–500 mL of whole blood.<sup>5</sup>

The change from glass bottles to disposable plastic bag sets for the collection of blood made it possible to collect and prepare platelets within a closed system. This not only greatly reduced the risk of bacterial contamination but also facilitated the implementation of a simple, two-step differential centrifugation platelet preparation protocol. The first step in this protocol involves centrifuging whole blood at a slow speed—a soft-spin—that sediments the red and white cells and concentrates most platelets in the supernatant plasma, also called the platelet-rich plasma (PRP). In the second

step, the PRP is centrifuged at a higher speed—a hard-spin—which sediments the platelets. The supernatant or platelet-free-plasma is removed and the sedimented platelets are re-suspended in 50–70 mL of plasma. Despite its simplicity, an important limiting step of this method is the need to pool several platelet concentrates to achieve an appropriate platelet dose for most adults (300–600×10<sup>9</sup> platelets, which corresponds to 4–8 concentrates).

A second important advancement was the development in the 1970s of blood-cell separators that allowed the selective collection of large numbers of platelets in pre-defined volumes of donor plasma, using a procedure termed apheresis.<sup>6–8</sup> Although this procedure is more expensive than PRP centrifugation, it carries the inherent advantages of automation and of decreasing the number of donors to which a recipient is exposed, and thus the recipient's risk of acquiring a transfusion-transmitted infectious disease.

In the same decade, investigators began removing leucocyte-rich and platelet-rich buffy coats from red-cell concentrates, to use the white cells for interferon production in the pre-recombinant technology era and to reduce leucocyte-related transfusion side effects.<sup>9</sup> The regular use of this procedure yielded large numbers of routinely produced buffy coats, which in turn led to the development of a novel whole-blood procedure for the preparation of platelet concentrates, named the buffy coat method.<sup>10,11</sup>

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#### Search strategy and selection criteria

We searched PubMed for English-language articles with the keywords "platelet", "platelet transfusion", "platelet refractoriness", "alloimmunization", and "platelet components" published between 1960 and 2006. Priority was given to prospective clinical studies published in journals with a high impact factor.

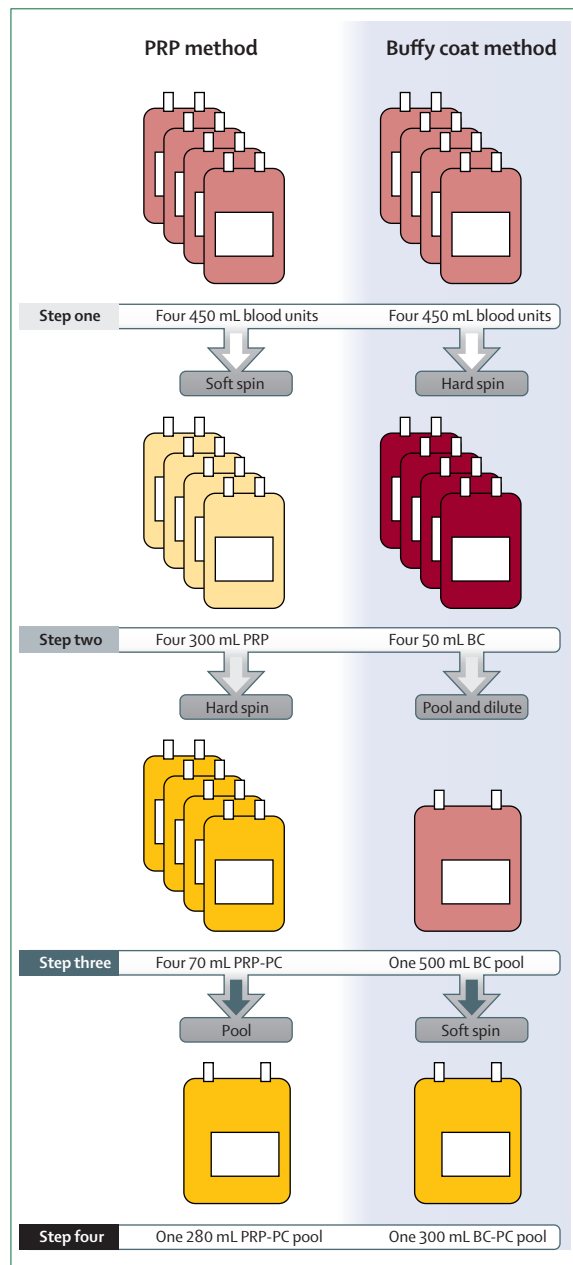


Figure 1: Comparison of platelet production by the platelet rich plasma (PRP) and buffy-coat (BC) methods

Both the buffy coat and PRP procedures use a two-step differential centrifugation process, but the sequence of the steps for the buffy coat method is reversed; its first step is a hard-spin of whole blood that leads to the sedimentation of all cells, including platelets. The platelets and leucocytes sediment on top of the red cells forming the buffy coat. Four to eight buffy coats of the same ABO/Rh group are collected, pooled, and diluted in autologous plasma or in a crystalloid solution. The pooled buffy coats are centrifuged (soft-spin) and the platelet-rich supernatant is retained as the platelet concentrate while the sedimented red and

white cells are discarded (figure 1). An important feature of the buffy coat method is the ability to select the optimal platelet storage additive solution as the diluent, thus decreasing side-effects from the infusion of large volumes of plasma, and improving platelet metabolism during storage. Studies have shown that PRP platelet concentrates can be easily adapted to incorporate an additive solution and pooled storage, and support the long-standing evidence that pooled storage is not detrimental to platelet quality.<sup>12</sup>

It has long been a principle of blood component production that they should contain as few white cells as possible, since leucocytes increase the risk of untoward complications. Because removal of white cells by post-storage filtration does not remove biologically active substances released by white cells during storage<sup>13</sup>, leucocyte reduction is now achieved by pre-storage filtration of platelet concentrates or by apheresis protocols which use size and density differences between platelets and white cells to remove white cells during platelet collection.<sup>14–16</sup>

Although the in-vivo and in-vitro properties and effectiveness of buffy coat, PRP, and apheresis platelets are similar,<sup>17–19</sup> the USA and Europe have different standards on the platelet concentrates (table 1). A multicentre analysis of blood components prepared by both methods summarises the composition of these products.<sup>22</sup> Routine haematology cell counters, however, which are frequently used for quality assurance of blood components, are not specifically designed nor calibrated for this purpose. Thus, such data in scientific reports should be interpreted carefully.

### Storage and transportation

Lack of oxygen is detrimental to platelet metabolism, so manufacturers of platelet storage bags have developed special plastic containers with volume-to-surface ratios that allow sufficient gas exchange between the internal volume and the external ambient air.<sup>23,24</sup> Moreover, current standards require that stored platelets are continually gently agitated to prevent the platelet sedimentation that makes oxygen inaccessible to a proportion of platelets.

The complex platelet subcellular anatomy suffers at temperatures below 18°C. These temperatures damage micro-canalicular structures and induce the clustering of platelet receptors. These clustered receptors are easily recognised by macrophages, which rapidly remove the previously “chilled” platelets from the circulation.<sup>25</sup> Galactosylation can prevent the clearance of platelets chilled for 2 h,<sup>26</sup> but has no effect when platelets have been stored for 48 h or longer.<sup>27</sup> The standard temperature for platelets storage is 20–24°C but this is associated with an increased risk of bacterial growth in the small fraction of platelet concentrates that harbour microbes in the suspension media.<sup>28</sup>

Although it is common to try to maintain platelets in agitated suspension during transportation, recent studies have challenged the need for this requirement, particularly for products with low platelet concentrations.<sup>29</sup>

	Source	Total platelet count	Total leucocyte count	Total leucocyte count in leucocyte-reduced products	pH at end of allowable storage
PRP platelets from one unit of whole blood	AABB	$>55 \times 10^9$ in $\geq 90\%$ of units	NA	$<0.83 \times 10^6$ in $\geq 95\%$ of units	$>6.2$ in $\geq 90\%$ of units
PRP platelets from one unit of whole blood	CoE	$>60 \times 10^9$ in $\geq 75\%$ of units	$<200 \times 10^6$	$<0.2 \times 10^6$ in $\geq 90\%$ of units	6.4–7.4
Buffy coat platelets from one unit of whole blood	CoE	$>60 \times 10^9$ in $\geq 75\%$ of units	$<50 \times 10^6$	$<0.2 \times 10^6$ in $\geq 90\%$ of units	6.4–7.4
Apheresis platelets	AABB	$>300 \times 10^9$ in $\geq 90\%$ of units	NA	$<5 \times 10^6$ in $\geq 95\%$ of units	$>6.2$ in $\geq 90\%$ of units
Apheresis platelets	CoE	$>200 \times 10^9$ in $\geq 90\%$ of units	NA	$<1 \times 10^6$ in $\geq 90\%$ of units	6.4–7.4

NA=not available.

**Table 1: Standards for platelet and white-cell counts and pH values of platelet concentrates as required by the AABB<sup>30</sup> and the Council of Europe (CoE)<sup>21</sup>**

It is possible that less stringent, more practical, agitation rules will be developed if conclusive supporting evidence is obtained.

### Platelet quality

Determination of pH is a simple laboratory procedure that has been traditionally used to determine the quality of platelets in vitro. Many standards quote a “safe” pH range, which is allegedly associated with good in-vivo recovery and function. But several studies have challenged the validity of this approach because of the weak correlation between in-vitro pH and post-transfusion in-vivo function.

An even simpler test to determine quality is “swirling”. “Swirling” is a visual effect caused by defraction when platelets are manually re-suspended and held up to a strong light (figure 2). The presence of swirling indicates the suspension contains high-quality, discoid platelets. Swirling provides a reasonable correlation with in vivo data. An important advantage of the swirling test is that it can not only be done in a laboratory setting such as a blood bank, but also immediately before transfusion in the clinic, on the ward, or at the bedside. Training staff to perform this test is simple, since the visual image produced by 1-day old platelets (usually showing very good swirling) can be easily compared with that of expired platelets stored in a refrigerator for several hours. Old platelets do not show any swirling since their morphology changes from discoid to spherical, a shape that does not diffract light.

### Detecting bacteria in platelets

Several methods have been used to screen for contaminated platelets, including microscopic examination of gram stains of platelets,<sup>30</sup> measurement of glucose levels and pH, and swirling, but these methods are insensitive (table 2).<sup>31,32</sup> Point-of-transfusion bacteria detection systems involving solid-phase laser cytometry and dielectrophoresis are available in Europe, but the assays require at least 30 mins and are technically demanding,<sup>34</sup> making this technology impractical in some settings.

In 2003, some US blood centres began to use automated liquid media cultures capable of detecting very low levels of bacteria. Testing is usually done 24 h or more after

platelets collection to allow any contaminating bacteria to grow and increase the sensitivity of the assay.<sup>35,36</sup> Another system involves the sterile removal of 2–3 mL of platelets 1 or more days after collection, and measurement of oxygen levels after incubating for 24 hours at 35°C.<sup>37,38</sup> A fall in oxygen tension indicates the presence of bacteria. These methods have reduced, but not eliminated, the risk of bacterial contamination.

### Indications for transfusion

#### Transfusion trigger

Traditionally, platelets were administered prophylactically when a patient's platelet count fell below 20 000 platelets per  $\mu\text{L}$ .<sup>39</sup> This practice was challenged in 1991 when Gmür and colleagues<sup>40</sup> reported their 10-year transfusion study in 103 leukaemic patients. Stable patients were transfused prophylactically at a count of 5000 platelets per  $\mu\text{L}$  or less; patients with fresh minor haemorrhage or body temperature higher than 38°C were transfused at 6000–10 000 platelets per  $\mu\text{L}$ ;



Figure 2: Swirling to judge platelet quality

	Threshold for detection (CFU per mL)
Fall in pH <sup>*31,32</sup>	$\geq 10^7$ – $10^8$
Fall in glucose level <sup>*31,32</sup>	$\geq 10^7$ – $10^8$
Loss of swirling <sup>31</sup>	$\geq 10^7$ – $10^8$
Gram stain <sup>30</sup>	$\geq 10^5$ – $10^6$
Chemiluminescence detection of ribosomal RNA <sup>33</sup>	$\geq 10^4$ – $10^5$
Dielectrophoresis <sup>34</sup>	$\geq 10^3$ – $10^5$
Fall in oxygen tension <sup>35</sup>	$\geq 10^2$ – $10^3$
Solid-phase laser cytometry <sup>34</sup>	$\geq 10$ – $10^3$
Automated bacterial culture <sup>34</sup>	$\geq 10$

CFU=colony forming units. \*Measured with a dipstick.

**Table 2: Methods for detecting bacteria in platelets**

those with coagulopathy or heparin therapy, or both, and before bone-marrow biopsy or lumbar puncture were transfused at 11 000–20 000 platelets per  $\mu\text{L}$ ; and patients with major bleeding complications or about to undergo minor surgical procedures were transfused at counts of >20 000 platelets per  $\mu\text{L}$ . That Gmür and colleagues recorded evidence of only three fatal haemorrhages suggested that the traditional transfusion trigger of 20 000 platelets per  $\mu\text{L}$  could be safely decreased to 10 000 platelets per  $\mu\text{L}$  in stable patients with cancer or blood disorders. Since then, several other prospective and retrospective studies<sup>41–45</sup> have confirmed these findings. A transfusion trigger of 10 000 platelets per  $\mu\text{L}$  is now widely recommended,<sup>46–49</sup> and widely adopted in clinical practice.<sup>50</sup>

It is important to point out that the patient's platelet count is just one element that needs to be considered. The cornerstone of platelet transfusion therapy is careful monitoring of the patient for the early detection of signs and symptoms of increased haemorrhagic risk and, when appropriate, increasing the transfusion threshold. Factors that indicate the patient is at increased risk of bleeding include raised body temperature, rapid decrease in platelet count, and sepsis. If a prophylactic transfusion trigger of 10 000 platelets per  $\mu\text{L}$  is used for stable patients, the clinical automated cell counter used to monitor the patient's platelet count must have the power to discriminate very low platelet counts.<sup>51</sup>

A therapeutic platelet transfusion strategy is also being investigated by several groups of researchers. In this approach, stable patients are given platelets only for clinically relevant bleeding. This strategy was judged safe in a study of autologous peripheral blood stem-cell transplant patients,<sup>52</sup> and preliminary results from a multiple-centre randomised study support this finding.<sup>53</sup>

#### Patients undergoing surgery

The threshold for prophylactic platelet transfusions is generally higher for surgery patients. For most procedures, a platelet count of >50 000 platelets per  $\mu\text{L}$  is

considered adequate. In CNS procedures, however, the threshold is >100 000 platelets per  $\mu\text{L}$ .<sup>46</sup> Patients with normal preoperative platelet counts might need platelet transfusion if surgical blood loss is great and large quantities of erythrocytes are transfused. Platelet counts fall during surgery because of haemodilution, but the quantity of blood loss that leads to thrombocytopenia varies greatly between patients.<sup>54</sup>

#### Exceptions

Transfusion triggers and indications differ for some patients and clinical conditions and these exceptions are thoroughly reviewed elsewhere.<sup>46,49</sup> However, it is worth noting transfusion practices for patients undergoing cardiopulmonary bypass (CPB) surgery, those with thrombotic thrombocytopenic purpura (TTP), heparin-induced thrombocytopenia (HIT), and immune thrombocytopenic purpura (ITP), and for neonates.

CPB is associated with a reduction in platelet counts because of haemodilution and transient platelet function impairment.<sup>55</sup> In the past, CPB patients were routinely transfused with platelets during or after the procedure, but prospective randomised studies have shown this to be ineffective, suggesting that prophylactic platelet transfusion is inappropriate in this setting.<sup>56,57</sup>

Thrombocytopenic neonates are at increased risk for intracranial haemorrhage; although the threshold for prophylactic transfusions is higher as a result, there is no consensus on the number. A threshold of 30 000 platelets per  $\mu\text{L}$  for prophylactic transfusions has been recommended by some, with a threshold of 50 000 platelets per  $\mu\text{L}$  for neonates at increased risk of bleeding—especially those weighing less than 1000 g.<sup>58,59</sup> Since neonates are at risk for cytomegalovirus (CMV) disease, transfusion-associated graft-versus-host disease, and volume overload, platelets transfused to neonates should be CMV-safe, gamma-irradiated and, in some cases, volume-reduced.

Several clinicians have reported sudden clinical deterioration and death immediately after transfusion of platelets to TTP patients.<sup>46,60–61</sup> However, such patients undergoing plasma exchange therapy have received platelet transfusion without adverse effects.<sup>62,63</sup> Until more data are available, it seems prudent to avoid platelet transfusions in TTP patients unless they are at serious risk of bleeding. Patients with HIT are at risk for arterial and venous thrombosis, and platelet transfusion is not recommended.<sup>46</sup> Platelet transfusion can be effective in ITP patients,<sup>64</sup> but it is generally reserved for life-threatening bleeding.

#### Transfusing platelets

##### Transfusion dose

The optimum platelet dose has not yet been defined, and is controversial. The general consensus is that therapeutic transfusions should increase the transfusion recipient's platelet count to a level that provides adequate

haemostasis.<sup>65,66</sup> There is less agreement on the transfusion of platelets to prevent bleeding. On the basis of convention, rather than evidence from robust clinical studies, most centres use a standard platelet dose of about  $300\text{--}600 \times 10^9$  platelets which works out to be  $50\text{--}100 \times 10^9$  platelets per 10 kg of recipient bodyweight.<sup>39,46,67</sup> In practice, the dose of platelets transfused falls in the extremely wide range of  $1.7\text{--}294.2 \times 10^9$  platelets per 10 kg.<sup>67</sup>

The prophylactic transfusion of both high and low doses has been investigated. A study comparing a standard dose transfusion with a lower dose (25% fewer platelets) showed no difference in numbers of bleeding episodes.<sup>68</sup> These results suggest that smaller, but more frequent, doses could be as efficient and cost effective. However, since the intravascular lifespan of platelets is shortened at low platelet counts,<sup>69</sup> higher doses could have advantages. Comparisons of high dose with standard dose transfusions indicate that high-dose transfusions result in greater post-transfusion platelet count increments and increased intervals between transfusions, but do not result in an increase in the quantity of platelets transfused.<sup>70–72</sup> The increased interval between high dose transfusions could lead to fewer total transfusion events for each patient, which might cut the cost of administering the platelets and would be more convenient for the transfusion recipient. However, higher post transfusion platelet counts could suppress endogenous thrombopoietin production and slow the recovery of the recipient's platelet production.

Higher platelet doses have been produced for possible clinical trials by giving donors a form of thrombopoietin—pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF)—to increase platelet counts and apheresis collection yields.<sup>73,74</sup> But because some donors produced autoantibodies to thrombopoietin, and developed clinical thrombocytopenia, PEG-rHuMGDF is no longer used.<sup>75</sup> However, if another thrombopoietin preparation proves safe, it is possible it will be given to platelet donors.

### Assessing effectiveness

Several methods and criteria have been used to assess the effectiveness of platelet transfusions. Most methods

use platelet counts measured at 60 mins and 18–24 h after the transfusion. For the convenience of the recipient and clinical care staff, platelet counts are often measured 10 minutes after the transfusion rather than after 60 minutes. In practice, platelet counts are measured once at either 10 or 60 min after transfusion.<sup>76</sup>

One method is to compare the difference in platelet counts before and after transfusion—the absolute platelet count increment (API)<sup>77</sup> (table 3). Since the API depends on the quantity of platelets in the transfused product and the patient's size, it is difficult to set an API criteria for an effective transfusion. The corrected count increment and percent platelet count increment make adjustments for the dose of platelets transfused. When platelet count increments are low, the patient is considered to be refractory to transfusions (table 3).

Whole-blood measurements of platelet function before and after transfusion have also been used to assess the effectiveness of platelet transfusions.<sup>82</sup> Measures of clinical bleeding are sometimes used in clinical trials that compare the effectiveness of various platelet components.<sup>83</sup>

### Refractoriness to transfusion

Refractoriness to platelet transfusions is most likely to be due to non-immune factors, although immune factors can sometimes be responsible. In refractory patients with cancer or haematological diseases, non-immune factors are present in 72–88% and HLA antibodies in 25–39%.<sup>84–86</sup> Non-immune factors associated with decreased post-transfusion platelet count increments include clinical conditions such as splenomegaly and drugs such as vancomycin.<sup>87–91</sup> (panel).

Platelets express HLA-A, HLA-B, and human platelet antigens (HPA). There is a strong association between the presence of HLA antibodies in the transfusion recipient and platelet refractoriness, but the relation between platelet-specific antibodies and refractoriness is weaker.<sup>89,90</sup> Before the widespread use of leucocyte-reduced blood components to prevent alloimmunisation, 45–70% of chronically transfused patients developed antibodies to HLA class I antigens.<sup>92–95</sup> Chronically transfused patients become alloimmunised to platelet-specific antigens less commonly. The proportion

Calculation		Criteria for an adequate response*	
		10–60 min post-transfusion	18–24 h post-transfusion
Absolute platelet increment (API)	Post-transfusion minus pre-transfusion platelet counts	NA	NA
Corrected count increment (CCI)	(Post-transfusion minus pre-transfusion platelet counts) × (patient's body surface area)/number of platelets transfused	>4500 platelets per m <sup>2</sup>	>2500 platelets per m <sup>2</sup>
Percent platelet increment (PPI)	Observed/expected platelet count increment†	>20%	>10%

Adapted from references 49, 78, and 79. NA=not available. \*Generally, two or three consecutive transfusions must be ineffective before a patient is considered refractory to platelet transfusions;<sup>78</sup> some centres require the transfusion of fresh ABO-compatible platelets for assessing platelet refractoriness since count increments can be lower for older<sup>80</sup> or ABO-incompatible platelets.<sup>31</sup> †The expected change in platelet counts is a value based on the number of platelets transfused and the recipient's blood volume.

**Table 3: Methods used to assess the effectiveness of platelet transfusions**



of patients with antibodies to platelet-specific antigens varies, but ranges from 2% to 17%.<sup>84,86,94,96,97</sup>

Platelets also express blood group A and B antigens,<sup>98</sup> and ABO-compatible platelets are usually transfused. However, when platelet inventories are low or when platelets from HLA-matched donors are required, ABO-incompatible platelets might be transfused. Repeated transfusions of ABO-incompatible platelets could increase the titres of the recipient's anti-A and anti-B, and lead to a fall in post-transfusion platelet count increments by about 30%.<sup>81</sup>

### Preventing alloimmunisation

Removal of contaminating leucocytes from erythrocyte and platelet components prevents alloimmunisation.<sup>94,99</sup> The treatment of platelets with ultraviolet B irradiation is also effective at preventing alloimmunisation,<sup>94</sup> but this method is not widely used. While these methods are highly effective, alloimmunisation remains an important impediment to effective transfusion. Antibodies to HLA class I antigens can be found in 14% of women who have had one or two pregnancies, and in 26% who have had three or more pregnancies.<sup>100</sup> Although in some countries, all blood components are

leucocyte-reduced, in many countries leucocyte-reduced blood products are either unavailable or are only used in some cases.

### Transfusing alloimmunised patients

Two main strategies have been used to transfuse alloimmunised patients: matching donor-recipient HLA antigens and crossmatching platelets. HLA-matching involves identifying the HLA type of the recipient and transfusing platelets from donors with matched antigens.<sup>101</sup> HLA matching requires the availability of large numbers of HLA-typed donors. A registry of about 18 000–25 000 HLA-typed people is needed to provide at least five HLA-A and HLA-B matched donors for 80% of white patients.<sup>102</sup>

Since maintaining a registry of this size is expensive and difficult, alloimmunised patients are often transfused with platelets from donors that are only partially matched.<sup>101</sup> Systems have been developed to match donor and recipient by assigning HLA-A and HLA-B antigens with shared public epitopes to clusters called cross-reactive groups (CREGs). When non-matched platelets are to be transfused, the donor is selected so that the antigens of donor and recipient belong to the same CREG. When one or two mismatches of HLA-A or HLA-B antigens in CREGs is permitted, a pool of 1000–3000 donors will meet the transfusion needs of most white patients.<sup>103</sup> However, transfusion with platelets from partially matched donors is not as effective as that with all four antigens matching.<sup>93,104</sup>

Another approach to finding HLA-compatible donors is the selection of donors with “acceptable” antigen mismatches. Patient plasma is tested against a panel of screening cells from several people; HLA-A and HLA-B antigens on the screening cells that give negative reactions are considered acceptable. The alloimmunised patient is transfused with platelets from donors expressing HLA-A and HLA-B identical or acceptable antigens.

A molecular-based computer algorithm called HLA-Matchmaker can be used to find HLA compatible platelet donors.<sup>105,106</sup> This algorithm is based on the principle that short three-aminoacid sequences or triplets, characterise polymorphic sites of the HLA molecules, and are the critical components of allo-sensitising epitopes. The selected HLA alleles will be compatible since they do not contain any epitope absent in the recipient. A retrospective study<sup>107</sup> has shown that platelets selected with this algorithm result in higher post-transfusion count rises than those selected using traditional HLA matching strategies.

A commonly used alternative to HLA-matched platelets is the transfusion of crossmatch-compatible platelets.<sup>77,108,109</sup> Crossmatching tests plasma from an alloimmunised patient against platelets available for transfusion or aliquots of platelets from potential donors that have been frozen or refrigerated.<sup>109</sup>

#### Panel: Factors associated with refractoriness to platelet transfusions or reduced post-transfusion platelet responses

##### Non-immune factors

###### Clinical factors

Splenomegaly<sup>88–90</sup>

Infection<sup>90,91</sup>

Fever<sup>88,90</sup>

Bleeding<sup>90</sup>

Disseminated intravascular coagulation<sup>89,90</sup>

###### Drugs

Amphotericin<sup>88–90</sup>

Vancomycin<sup>88,91</sup>

Ciprofloxacin<sup>88</sup>

Heparin<sup>90</sup>

###### Patient factors

Sex (male)<sup>87,90</sup>

Increased weight<sup>87,90</sup>

Increased height<sup>87,90</sup>

Previous pregnancies<sup>90</sup>

Previous transfusions<sup>90</sup>

##### Immune factors

###### Antibodies

HLA<sup>89,90</sup>

Platelet-specific<sup>89</sup>

Erythrocyte<sup>81,90</sup>

##### Other

Platelet product

Age<sup>90</sup>

HLA-specific and platelet-specific antibodies in the patient's plasma react with platelets expressing incompatible antigens. Only platelet components that are compatible are transfused. Several methods have been used to crossmatch patient samples including commercial kits and automated systems.

HLA-matched and crossmatch-compatible platelets are equally effective.<sup>77,108</sup> The decision over which to use mostly depends on the resources available. Since providing HLA-matched platelets requires the recruitment of specific donors, such platelets can only be obtained by apheresis. For crossmatching, apheresis, buffy coat, or the PRP method can be used.

For highly alloimmunised patients and those with rare HLA types, finding compatible platelets can be difficult. Several immune-modulatory therapies have been used to try to overcome alloimmune platelet refractoriness: intravenous immune globulin;<sup>110,111</sup> cyclosporin A;<sup>112,113</sup> vinblastine;<sup>114</sup> staphylococcal protein A;<sup>115</sup> removal of HLA antigens with citric acid.<sup>116,117</sup> Despite anecdotal positive outcomes, these strategies are usually not successful or practical.

### Adverse effects

Platelets, like most blood products, can transmit blood-borne pathogens including HIV, hepatitis B virus (HBV), hepatitis C virus (HCV), and CMV. Testing of donors for HIV, HBV, and HCV, and preventing people at risk of infection with these viruses from donating blood has greatly reduced the incidence of transmission of these agents by transfusion. However, infections still occur as do other complications (table 4).

### Infectious complications

#### Cytomegalovirus

CMV resides in peripheral blood leucocytes, and infection can cause serious morbidity in immune-compromised patients. The transfusion of platelets from CMV-seronegative donors is effective in preventing infection.<sup>118</sup> However, because less than 50% of blood donors are CMV-seronegative, it is not always possible to provide CMV-seronegative platelets. The transfusion of leucocyte-reduced blood components can also prevent infection,<sup>119</sup> and leucocyte-reduced platelets are widely used when CMV-safe blood is required. However, one analysis suggests that CMV-seronegative components might be slightly more effective at preventing virus transmission.<sup>120</sup>

#### Bacterial contamination

Since platelets can be stored at 20–24°C for up to 5 days in Canada, Europe, Korea, and the USA, bacterial levels in contaminated platelets can become very high. Bacteria usually enter the platelet concentrate by the blood-collection needle entering the vein through skin that has been ineffectively disinfected. Rarely, platelets are contaminated as a result of donor bacteraemia from

asymptomatic infections<sup>121,122</sup> or occult colon cancer.<sup>123</sup>

Although bacterial contamination affects a small proportion of platelet concentrates (about 1 in 3000 units),<sup>34</sup> it is often fatal, particularly in immuno-compromised patients with cancer or blood disorders.

The risk of severe transfusion reactions because of bacterial contamination increases with longer storage periods, and platelet storage is limited to 3 days in Japan.<sup>124</sup> However, platelet quality can be maintained beyond 5 days, and some US centres are extending the maximum storage time to 7 days for platelets that have been tested for bacteria with an automated culture system 24 h after collection.

### Pathogen inactivation

Systems are now available to reduce the levels of microbes in platelets. One system, Intercept, that is being used in Europe but is not available elsewhere, involves crosslinking pathogen DNA and RNA by adding a psoralen, S-59, and exposing the platelets to ultraviolet A light.<sup>125</sup> This and other systems under development<sup>126</sup> inactivate viruses, bacteria, and parasites.<sup>127</sup> Although

	Cause	Prevention
<b>Infectious</b>		
AIDS	HIV-infected donor	Donor screening and testing Pathogen inactivation
Hepatitis	Hepatitis B or hepatitis C virus infected donor	Donor screening and testing Pathogen inactivation
CMV disease	CMV-infected donor	Donor testing Leucocyte reduction Pathogen inactivation
Sepsis or septic shock	Contamination from the platelet donor's skin or from an occult or asymptomatic donor bacteraemia	Culture product 24 or more hours after collection Test for bacteria shortly before transfusion Pathogen inactivation
<b>Immunological</b>		
Alloimmunisation	Leucocytes in platelets	Leucocyte reduction UVB irradiation
Febrile reactions	HLA antibodies in transfusion recipient and IL-1 $\beta$ and IL-6 in platelets	Leucocyte reduction
TRALI	Leucocyte antibodies, bioactive lipids, or CD40L in platelets	Exclude donors with leucocyte antibodies
Anaphylaxis	Antibodies in patients reacting with IgA, haptoglobin, antibodies, or other plasma antigens	IgA-deficient platelet donors Washed platelets
GVHD	Engraftment of donor leucocytes in an immunosuppressed recipient	Gamma irradiation of platelets (25 Gy) Possibly pathogen inactivation
RhD alloimmunisation	Transfusion of platelets from RhD-positive donors to RhD-negative recipients	Administer Rh immune globulin within 48 h of transfusion
Haemolysis	Anti-A and anti-B in donor's plasma	Exclude donors with high titres of anti-A or anti-B
Hypotension	Generation of bradykinin by the bedside filtration of platelets in a patient taking angiotensin-converting enzyme (ACE) inhibitors	Pre-storage or in laboratory leucocyte reduction

Table 4: Adverse consequences of platelet transfusions

the treatment of platelets with the Intercept system results in the loss of some platelets,<sup>128</sup> the quality of the remaining platelets is the same as control platelets.<sup>129</sup> Pathogen-inactivation systems have the advantage of protecting transfusion recipients from pathogens known to cause clinically important infections, and the potential to protect recipients from emerging pathogens. In addition to the loss of platelets, pathogen inactivation procedures are limited by the possible exposure of blood-processing personnel and the recipient to toxic substances, possible environmental contamination, and added cost to the final product.<sup>127</sup>

### Non-infectious complications

#### Febrile transfusion reactions

Patients with HLA antibodies often have febrile reactions after transfusion of leucocyte-rich platelets,<sup>92</sup> which can be prevented by transfusing leucocyte-reduced platelets. Soluble cytokines in platelet components can also cause febrile reactions. Immediately after collection, soluble cytokine levels are very low, but during room temperature storage of leucocyte-rich platelets, the levels of cytokines IL-1 $\beta$  and IL-6 rise.<sup>130</sup> The transfusion of platelet products with elevated concentrations of IL-1 $\beta$  and IL-6 is

associated with fever, chills, rigours, and nausea.<sup>130</sup> Increased cytokine levels and transfusion reactions can be prevented by removing leucocytes during or immediately after collection.<sup>130,131</sup>

#### Rh alloimmunisation

Although platelet products contain small quantities of erythrocytes, almost always less than 1 mL, the transfusion of platelets from RhD-positive donors to RhD-negative recipients can result in RhD alloimmunisation.<sup>132,133</sup> This is particularly problematic for women of childbearing age or younger since fetal or newborn children of women with anti-D are at risk of haemolytic disease. The administration of anti-D immunoglobulin within 48 h of the transfusion of RhD-positive platelets prevents alloimmunisation.<sup>134</sup> One dose can prevent alloimmunisation from multiple incompatible platelet transfusions.<sup>135</sup>

#### Graft-versus-host disease

The transfusion of platelets to patients with congenital immune diseases and those undergoing immuno-suppressive therapy can result in lethal graft-versus-host disease (GVHD).<sup>136,137</sup> GVHD can be prevented by irradiating blood components with gamma rays or x-rays at 25 Gy—high enough to prevent lymphocyte proliferation.<sup>138,139</sup> Since the threshold of the quantity of transfused lymphocytes that can cause GVHD is unknown, even leucocyte-reduced platelets are irradiated. Pathogen-inactivation techniques that target nucleic acids have the potential to inactivate lymphocytes in treated platelets and prevent GVHD.<sup>127</sup>

#### Transfusion-related acute lung injury

The transfusion of plasma containing blood products can cause severe pulmonary reactions known as transfusion-related acute lung injury (TRALI).<sup>140</sup> About 1 in 1500 to 1 in 10 000 transfusions cause TRALI, and 5–15% of these reactions are fatal;<sup>140,141</sup> TRALI is now the leading cause of transfusion related deaths.<sup>142</sup> The causes of the injury are controversial, but the transfusion of neutrophil-specific antibodies, HLA antibodies, the bioactive lipid lysophosphatidylcholine, and soluble CD40 ligand have been implicated.<sup>143–145</sup>

The UK is attempting to transfuse only fresh frozen plasma collected from men since plasma from men is much less likely to contain leucocyte antibodies than would plasma from women. Transfusing plasma only from men reduces the proportion of products that contain leucocyte antibodies, and thus reduce the incidence of TRALI.<sup>142</sup> However, this is not always possible and it is less practical to collect platelets only from men. Platelets stored in additive solutions are less likely to cause transfusion reactions than those stored in plasma;<sup>146</sup> it is also possible that platelets stored in additive solution are less likely to cause TRALI, but this has not yet been investigated.

Issue	Controversy
Platelet product	Are there any clinical differences between buffy-coat, PRP, and apheresis platelets?
Measurement of platelet quality	What in-vivo or in-vitro indices correlate best with the effectiveness of transfused platelets?
Leucocyte reduction	Should some or all platelets be leucocyte-reduced?
Pathogen inactivation	Should all platelets be treated to inactivate viruses and bacteria?
Transfusion in oncology and haematology patients	Should platelets be transfused prophylactically or therapeutically?
Dose of platelets transfused	Should the dose be increased, decreased, or left unchanged?
Transfusing refractory patients	Which method is best, HLA matching or crossmatching? If HLA matching is used, how should compatible platelets be selected?

Table 5: Controversies in the collection and manufacture of platelet products and transfusion

Issue	Difference
Donors	Most, but not all nations forbid the payment of blood donors <sup>152</sup>
Blood procurement	Many European countries and Canada have national blood procurement organisations, but in other European countries and in the USA, blood is collected by hospitals or private organisations <sup>153</sup>
Type of platelets transfused	The buffy-coat method is used to produce platelets from whole blood in Europe and Canada, but the PRP method is used in the USA <sup>153</sup>

Table 6: International differences in the collection and manufacture of platelet products and transfusion

Development	Purpose
Stealth platelets	Removal of HLA antigens for transfusion to alloimmunised patients <sup>117</sup>
Extended platelet storage	Extended liquid room temperature storage of platelets found not to be contaminated with bacteria <sup>154</sup>
Lyophilised platelets	Freeze-dried platelets for prolonged storage <sup>155</sup>

Table 7: Latest developments in the collection and manufacture of platelet products and transfusion



## Other immune reactions

Transfusions can cause a wide variety of allergic reactions. The most serious are anaphylactic reactions—antibodies to IgA in patients who lack IgA are the most common cause.<sup>147</sup> Patients with anti-IgA antibodies should be given washed platelets or platelets collected from IgA-deficient donors.<sup>148</sup> Rarely, platelet transfusions can cause mild haemolysis because of the transfusion of anti-A or anti-B<sup>149,150</sup> or hypotensive reactions due to bradykinin when using bedside leucocyte reduction filters.<sup>151</sup> (table 4).

## Differences in guidelines and practices

In the developing world, platelet transfusions are either available on a limited basis or not at all and, in general, national policies and regulations on platelet collection, processing, and transfusion do not exist. Whereas platelets are routinely transfused in the developed world, several issues related to platelets and their transfusion are still controversial.

The optimal indications for transfusion, platelet transfusion dose, and methods to transfuse refractory patients are still unknown. Moreover, the platelet preparation technologies used to overcome transfusion complications and adverse effects differ between countries because of differences in the availability of resources, the general organisational framework of blood procurement, national policies, and national regulations. A comparison of regulations in 17 European countries identified many basic differences, including the exclusion of remunerated donors and the absence of regulations on the use of blood products.<sup>151</sup> We summarise key controversies and international differences in tables 5 and 6. Differences between countries limit progress toward identifying the best transfusion practices and, in some cases, prevent the provision of the optimum platelet product. Platelet production technology continues to evolve (table 7), but it is likely that even though new platelet products might be developed, it will be many years before they are available in all countries. More unified national regulations and policies are needed.

## Conclusion

Platelet transfusions are an important therapy, and their use will probably continue to increase. Although transfusion practices are variable and in some cases the best practices are not fully known, greater harmonisation of national policies and regulations might promote the use of optimum platelet products and development of the best transfusion policies.

### Conflict of interest statement

DFS has no conflict of interest. PR has been an advisory board member for Cerus Corporation and a consultant for Navigant Biotechnology.

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