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INVITED REVIEW

Platelet functionality in cold-stored whole blood

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Whole blood is currently being reintroduced as a blood product to be used in massive bleeding situations because it affords plasma, red cells and platelets in a balanced ratio and in a logistical advantageous way. Questions concerning the haemostatic potential of the platelets have arisen, especially in cold-stored whole blood, as this is the major whole blood product in use. When reviewing current knowledge on this, there is an abundance of publications demonstrating that *in vitro*, platelets in cold-stored whole blood have a haemostatic capacity up to 14 days, and even after 21 and 35 days of storage depending on type of additive solution. There is a paucity of data on clinical trials of cold-stored platelets, whereas there is an abundance of previous clinical experience with whole blood, both cold-stored and fresh, as an efficacious and safe product for use in pre- and in-hospital patients with life-threatening bleeding.

Key words: cold storage, massive transfusion, platelet function, platelets, transfusion strategy, whole blood

Introduction

Whole blood (WB) as a blood product for direct patient use has virtually been absent for 40 years in the practice of transfusion medicine in high-income countries of the world. It is now back in vogue particularly in clinical situations of damage control resuscitation and haemostatic resuscitation [1–3]. With this revival renewed concerns on its use have arisen, namely availability, blood group to be utilized, haemolytic impact of antibodies in plasma, leucoreduction, infections, red cell and platelet functionality. To minimize risks of untoward reactions, group 0 WB with a low titre of anti-A and anti-B is recommended [4–7]. Some also remove leucocytes, but spare blood platelets [8]. Concerns have been raised about lack of platelet-mediated haemostatic function in whole blood, particularly after prolonged cold storage, but conversely, also worries about increased thrombotic potential.

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When addressing platelet function the term 'viability' of platelets is frequently used, usually pertaining to the survival or recovery of transfused donor platelets in the patient. It may also refer to platelet longevity in the blood bank storage facility as measured by *in vitro* aspects like metabolic activity, secretion of cytokines, aggregation ability or shape change. However, when considering platelet function in case of bleeding patients, this term should refer to the actual *in vivo* haemostatic ability.

This review highlights platelet functionality in whole blood as measured by laboratory experiments and clinical investigations.

WB defined

Whole Blood is collected from a donor by phlebotomy of 450–-500 ± 50 ml blood into a plastic bag containing an anticoagulant solution and stored under varying conditions, see Table 1. Cold-stored whole blood, CWB, can be stored at 1–6°C for up to 21 days if the anticoagulant is CPD, or 35 days if the anticoagulant contains adenine (e.g. CPDA-1), however, the storage time is defined by investigations of red cell survival and not platelet function. WB for transfusion is used without further processing; however, leucocyte-depleted whole blood (WB-LD) is preferred within some regulatory domains (e.g. Europe < 1·0 x 10^6/l leucocytes) to

Table 1 Variants of whole blood for transfusion

	Storage time	Storage temp°C	Leucoreduction	Anticoagulant additive
WFWB	8 h	20	Optional depending on anticoagulant additive	CPD/CP2D/CPDA-1
	Followed by 16 h at	4		
CWB	21 days	4	Frequently[a]	CPD/CP2D
	35 day	4	No	CPDA-1
CFWB	48 h	4	Optional depending on anticoagulant additive	CPD/CP2D/CPDA-1

CFWB, cold-stored fresh whole blood; CPD, citrate-phosphate-dextrose; CP2D, citrate-phosphate-double dextrose; CPDA-1, citrate-phosphate-dextrose-adenine; CWB, cold-stored whole blood; WFWB, warm fresh whole blood.
[a]Leucoreduction with platelet-sparing filter.

reduces risk of WBC-induced transfusion reactions [9]. Leucoreduction can be performed by use of a platelet-sparing (PS) or non-platelet-sparing (NPS) filter. Using a NPS filter has the obvious disadvantage of loosing the platelets essential for the haemostatic effects of WB, whereas using a PS filter retain platelet concentration and function within required limits (J Sivertsen, H Braathen, THF Lunde, et al., submitted) [10]. Currently, only one PS filter is commercially available (IMUFLEX® WB-SP, Terumo BCT; Lakewood, CO, USA). According to European Guidelines, WB should contain a minimum of 45 g haemoglobin (43 g for WB-LD) and show less than 0.8% haemolysis at the end of storage [9].

If CWB is used before 48 h following collection, it may be called cold 'fresh' WB (CFWB). In some situations, WB may be used shortly after donation and is then called warm fresh WB, WFWB. WFWB may be stored at room temperature (20°C) for up to 8 h, and subsequently at 4°C for 16 h [11]. These time restraints are probably more related to the subsequent harvest of plasma than functional aspects of WFWB [12]. WFWB has traditionally been used in neonatal open-heart surgery or in remote austere settings where other blood products are unavailable.

The transfusion of WB is subject to risk of the same kind of adverse transfusion-related reactions as when transfusing red cell concentrates. Likewise, as for plasma and platelet concentrate transfusions, blood group antibody and citrate toxicity must be considered when WB is transfused. When WB is used fresh, the risk of transfusion-associated graft-versus-host disease (TA-GVHD) is perceived to be higher than when transfusing red cell or platelet concentrates. TA-GVHD has been reported in one military patient receiving WB, although HLA-typing of the patient and donor did not seem to imply the WB [13]. TA-GVHD may be mitigated by irradiation [14].

PLT functionality defined

'Viability' is the term usually coined when describing the function of platelets. The term refers to the ability of transfused platelets to remain in circulation [15], which is an important factor in prophylactic platelet transfusions. The ability to circulate is, however, of less interest in clinical situations with ongoing bleeding. In the latter, the immediate haemostatic ability of platelets is of primary interest. Platelet haemostatic function involves several phenomena; extent of shape change, aggregation, activation, receptor expression and binding of coagulation factors, to name some. The sum of these phenomena defines the platelet functionality. This can be measured by a multitude of *in vitro* laboratory methods such as aggregometry (e.g. Chrono-log, Multiplate), viscoelastic tests (thromboelastography (TEG) and thromboelastometry (RoTEM)), flow cytometry (expression of different surface markers either spontaneous or agonist-induced), measures of membrane integrity (phosphatidylserine externalization, cytosolic esterase induced fluorescence), electron microscopy, heat-shock resistance, shear-stress (PFA-100), shape changes and microvesicle formation. For several of these tests, the actual platelet concentration will influence the results. The different types of platelet functional tests have been reviewed elsewhere [16,17].

However, even though the *in vitro* tests result in precise and quantifiable parameters, they do not translate directly to clinical efficacy. Measurements of platelet effects *in vivo* include assessments of bleeding grade by standardized bleeding scores, standardized measurements of bleeding time, quantification of bleeding volume, total use of blood products, survival and recovery of radiolabelled platelets. The *in vivo* efficacy of platelets should be evaluated in prospective, preferably randomized, clinical trials. Although these are time-consuming and expensive to perform, they yield high-quality data. When evaluating the clinical effect of platelet transfusion, several blood component and patient-related factors might influence the measured haemostatic effects of transfusion. The platelet component factors include the content of platelet and plasma, storage duration, and pre-storage production methods that may influence on platelet function. Patient-related factors may include concentration of red cells and leucocytes in the circulation, the interaction with the

endothelial lining, and the clinical condition (ongoing bleeding, coagulation disorders, anti-platelet drugs, fever, etc.).

Functionality of platelets *in vitro* and *in vivo*

The *in vitro* functionality of CWB platelets

The physiology of cold-stored platelets has recently been reviewed [18]. Storing platelets in the cold causes structural, molecular, and metabolic changes, which is often referred to as the cold platelet storage lesion [18]. Cold-stored platelets show signs of activation and are thought to be 'primed' and ready to participate in the haemostatic process. The *in vitro* functions of platelets in CWB have been evaluated by many researchers, and they generally confirm that significant haemostatic functionality remains at least beyond 14 days of storage and for some laboratory test even longer (J Sivertsen, H Braathen, THF Lunde, et al., submitted) [8,10,11]. The results are, however, dependent on method of preparation, additive solution (CPD or CPDA-1) and type of test used (J Sivertsen, H Braathen, THF Lunde, et al., submitted) [8,19].

When subjecting CWB to thromboelastographic/-metric analysis (TEG/RoTEM) researcher have generally found that most test parameters stay within normal ranges after 14 and even 21 days of cold storage [8,10,20,21]. This is also the case for stored CWB that was leucoreduced using a platelet-sparing filter (IMUFLEX® WB-SP, Terumo BCT, US)[8,10,11,21,22], while TEG tracings of WB-NonPS were grossly abnormal due to the absence of platelets [21,23]. This has been corroborated in blood collection bags with adenine added to the anticoagulant solution (CPDA-1) [11]. The IMUFLEX® WB-SP leucoreduction filter seems to perform similarly for both CPDA-1 and CPD with respect to the removal of leucocytes and also for the haemostatic function of platelets (J Sivertsen, H Braathen, THF Lunde, et al., submitted); [10] however, the effects of leucoreduction on platelet function are under debate [24]. When evaluated in pathogen reduced WB by use of the Mirasol System with or without leucocyte removal, pathogen reduction, especially in combination with leucocyte removal, reduces haemostatic function [24,25]. The effect of different types of anticoagulants (CPD, CP2D, CPDA-1) in CWB on platelet function have been investigated revealing little impact on haemostatic functionality [19]. Interestingly, mixing CWB at expiry date with fresh whole blood improve coagulation responses and platelet function as measured by RoTEM [19].

Platelet aggregation in CWB evaluated by shear forces (PFA-100) also maintained aggregation for 14 days when leucoreduced with platelet-sparing methods but not when platelets were removed [21]. Aggregation evaluated by agonist-induced aggregation is reduced, but persistent, beyond day 10 [10,20,24–26]. Using a novel flow cytometry-based platelet aggregation method Sperling *et al*. [20] found that platelets from CWB (non-leucoreduced) stored for up to 21 days performed at least as well as both buffy coat and apheresis platelets (room-temperature storage for 7 days) when subjected to a panel of various platelet stimulators. Pidcoke *et al*. [25] compared warm and cold storage of WB with or without pathogen reduction for 21 days and found that for CWB shear-induced aggregation was increased compared to WFWB.

As room-temperature platelet concentrates are stored using continual horizontal agitation in order to avoid aggregation, concerns have been raised pertaining to the mechanical storage conditions of CWB. Groups have looked at daily manual mixing, continual rotational mixing vs. no mixing. Some publications show platelet counts to decrease during storage by up to 50%, whereas others show no significant differences on platelet numbers even at day 21 [10,22,27]. This discrepancy may reflect differences in pre-sampling manipulation. However, little differences on *in vitro* platelet functional tests were found [10,22,26–28].

The *in vivo* functionality of CWB platelets

The haemostatic function of platelets in CWB has been reported from case studies, retrospective studies, observational prospective studies and randomized clinical trials. The transfusion of CWB to massively bleeding patients started more than 100 years ago [29,30], and after a period of absence is now returning to military medicine [5,31,32], indeed as the preferred military transfusion product [33–35]. Descriptive reports on the effect of fresh and stored whole blood transfusion have been published, mainly from military operations [36–40],but also civilian reports are being published [41–45].

A randomized controlled trial performed by Manno and colleagues in paediatric patients undergoing cardiac surgery showed reduced blood loss and improved platelet function by aggregometry after transfusion with 24–48 h cold-stored whole blood when compared to component-based therapy with room-temperature platelets [46]. By radiolabelling studies, Slichter and colleagues have shown that CWB stored for up to 15 days have similar post-storage platelet viability [47] as the FDA approved 3-day-old cold-stored platelet concentrates [48]. The authors conclude that platelet yields, post-storage platelet recoveries and survivals, and platelet function should be sufficient to support the short-term haemostatic needs of traumatized patients [27].

Warm fresh whole blood is considered to have full haemostatic function [46], especially when given early in

resuscitation [39] and is considered to be superior to CWB [49] and room-temperature-stored platelets (RTP) [50,51]. It has routinely been used in open-heart surgery of the newborn [46], although it may not confer any advantage over reconstituted blood (packed red blood cells and fresh frozen plasma) in priming the cardiopulmonary bypass circuit [52]. Currently, its use is mostly limited to situations when other blood products are unavailable, either in austere remote situations, or where blood bank inventory (particularly of platelet concentrates) is depleted [42]. Time constraints may necessitate the use of WFWB from pretested donors with test for transfusion transmittable infectious diseases performed after use as recently reported [42].

The functionality of cold-stored platelets in plasma or platelet additive solutions

There is an abundance of publications, old and more recent, on cold-stored platelet, CSP, concentrates either in plasma of platelet additive solutions from both *in vitro* and *in vivo* perspectives. It was early recognized that platelet survival and recovery was decreased when stored cold [15], but the superior aggregation potential of CSPs was also recognized early [53–55]. Valeri demonstrated the greater potential of CSP to reverse aspirin-induced platelet defect in healthy subjects compared to RTP. The *in vitro* CSP haemostatic potential has more recently been confirmed and elaborated using an expanding palette of methods by several groups [56–64], and has even been found on prolonged storage up to 18–21 [57,63]. The importance of maintaining a minimum level of glucose has been demonstrated raising the question to increase the plasma concentrations in CSP or perhaps store platelets in WB [57,65]. Data from *in vitro* experiments indicate that platelet haemostatic ability is better in WB than in WB reconstituted from components [49], and may also outperform platelet concentrates [54], even after extended storage [20].

The potential functions and use of CSP was recently reviewed elsewhere [62],but recent *in vivo* studies of CSP are still few. Clinical use of 3 days CSP has started in the US in 2015, but did initially have challenges with aggregates in the CSPs probably due to lack of PAS[66]. Preliminary results from a recently concluded randomized pilot trial by our group comparing CSP to RTP, either stored for up to 7 days in PAS, indicate that CSP is at least as effective in treating postoperative bleeding in patients undergoing complicated open cardiac surgery with no increased risk of adverse events [67] The outcome measures of the study were postoperative bleeding and platelet haemostatic functions (aggregation and thromboelastography/metry), but also number of blood products transfused. Encouraged by these findings, we have extended the study with an arm of 14 days CSP storage [68].

Discussion

Based on the experiences from recent military conflict most civilian trauma programs to day include the use of massive transfusion protocols [69,70]. Early balanced transfusion is recommended, and most centres still use components to mimic as closely as possible a 1:1:1 ratio. However, concerns have been raised on the probable inferiority of a reconstituted product when it comes to platelet activity, coagulation factor loss, haemoglobin concentration and the significant dilatory effect of additives [71]. Therefore, more civilian trauma centres are switching to CWB as their preferred blood product in massively bleeding patients (D Jenkins, personal communication, 2019) [1,72–74]. CWB has been in uninterrupted routine use in the developing low to medium income economies around the world, even though the targeted use of blood components is increasing, particularly packed red cells used for normovolaemic patients with severe anaemia. However, as clinical situations with massive bleeding (especially traffic accidents, shooting, stabbings and peripartum haemorrhage) is common in many low- to medium income countries of the world, a complete conversion to a component-based transfusion practice is not advantageous neither from a medical nor from an economic perspective. Providing a balanced transfusion in massive bleeding situations requires more resources with a component strategy compared to using WB, especially in providing platelets. Realizing this, the Blood Transfusion Service of Zanzibar, Tanzania, has implemented a Massive Transfusion Protocol that includes the forward storage and resupply of several units uncrossmatched low titre group 0 WB in the operating theatres of the maternity ward for use in critical obstetric bleeding situations [75]. This intervention has so far assisted in saving numerous lives and serves as an example for obstetrical wards and trauma centres elsewhere.

Conclusions

For decades, CWB was the only platelet-containing product available for the treatment of massive bleeding and was considered an effective and safe intervention, especially when using only group 0 WB. *In vitro* tests of platelet functionality in WB uniformly demonstrate that within commonly used storage and time constraints, platelets are highly functional. Publications on the *in vivo* functionality of platelets in CWB are few, but those that are leaves little doubt of the effectiveness of platelets.

But, we do not know how long platelet haemostatic functionality is preserved in the clinical setting. And, although logistically superior, we still need to find out whether WB improves outcome in a hospital setting compared to a well-balanced component transfusion practice. In the aftermath of the re-introduction of CWB in both pre-hospital as well as in-hospital protocols of massive transfusion, more evidence will emerge in the near future.

Conflict of interests

The authors declare no conflict of interests.

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