review
Haematologica 1994; 79:460-474

PLATELET SUPPORT OF PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

Paolo Rebulla

Centro Trasfusionale e di Immunologia dei Trapianti, Ospedale Maggiore Policlinico, Milano, Italy

ABSTRACT

The most significant advances in platelet transfusion therapy for oncology-hematology patients can be summarized as follows: 1) *prophylaxis versus treatment of hemorrhage.* Usual practice is based on hemorrhage prophylaxis. Debate is still open on the transfusion trigger, which is traditionally set at 20×10^9/L platelets: some authors suggest it could safely be decreased in stable patients to 10 or 5×10^9/L platelets; 2) *preparation of platelet concentrates.* Platelets prepared from platelet-rich plasma or buffy-coats obtained from multiple bag donations should be used as the first-choice for all patients, while apheresis platelets, which have a significantly higher cost of production, should be reserved for patients refractory to random donor support. The final choice, however, of a prudent strategy must also consider logistic aspects, such as product availability, distance from site of production to site of use, etc; 3) *leukocyte reduction.* Filtration is the method of choice to prepare leukocyte-reduced platelets. Leukocyte-reduced platelets can be used to prevent transmission of CMV in selected patient groups for whom this is indicated. When leukocyte reduction is used for the prevention of NHFTR, it should be performed with fresh platelets and reserved for patients developing more than 1 reaction. Routine leukocyte reduction for all oncology-hematology patients cannot be recommended at this time, in the absence of definitive information on the cost-effectiveness of this approach; 4) *quality control.* Studies are under way to check whether evaluation of the swirling phenomenon, that is produced by good quality platelets when inspected with the naked eye against a strong light source is a useful and inexpensive test for quality control; 5) *correction of refractoriness to random donor platelet support.* Effective platelets for refractory patients can be obtained through HLA typing and/or platelet cross-matching. Although HLA typing can be very effective, cross-matching seems to be equally effective, simpler and less expensive.

Key words: platelets, blood transfusion, leukemia

Platelet transfusion is a cornerstone of treatment in many malignant hematological disorders.[1] The aim of platelet transfusion is to prevent morbidity and mortality secondary to the hemorrhage that can occur in patients when chemotherapy or the natural history of the disease cause severe thrombocytopenia.

Most commonly platelets are administered prophylactically, with the aim of maintaining the patient's platelet count above 20×10^9/L, usually regarded as a *safety level* in stable patients. Other protocols suggest transfusing platelets only for the treatment of hemorrhagic episodes.[2]

Approximately one third of chronic recipients of red blood cells (RBC) and platelet concentrates (PC) develop antibodies reactive against platelet and leukocyte antigens, most frequently antigens of the HLA system. This event, caused by white cells present in standard blood components, can decrease post-transfusion platelet count increment and *in vivo* platelet survival. As a consequence, resolution of hemorrhagic

Correspondence: Dr. Paolo Rebulla, Centro Trasfusionale e di Immunologia dei Trapianti, Ospedale Maggiore Policlinico, via Francesco Sforza 35, 20122 Milan, Italy.
Acknowledgements: the author is grateful to Prof. G. Sirchia, Prof. A. Zanella and Dr. D. Prati for manuscript discussion and to Drs. D. Riccardi, F. Marangoni, L. Porretti, F. Bertolini and N. Greppi for their contribution to the development of the platelet transfusion policy followed at Ospedale Maggiore Policlinico and described in this review.
Received March 22, 1994; accepted June 28, 1994.

episodes is more difficult in alloimmunized patients.

This condition, defined as refractoriness to platelet support, exposes the patient to the risk of life-threatening hemorrhagic episodes. Providing effective platelets to refractory recipients requires considerable organizational and laboratory efforts.

The aim of this review is to discuss different platelet transfusion protocols and different methods for the preparation of platelet concentrates. In addition, strategies aimed at providing effective platelets to refractory patients will be presented.

Platelet transfusion protocols in blood malignancies: prophylaxis vs treatment of hemorrhage

As indicated above, it is common policy to administer one PC, containing on average 70×10^9 platelets, per 10 kg of body weight, when the platelet count falls below 20×10^9/L.[1,3] This practice is based on data published about 30 years ago by Gaydos and coworkers,[4] who reported that hemorrhagic episodes in acute leukemia were more important and frequent when the platelet count was below 5×10^9, while they occurred only in 8 and 4% of days if the platelet count exceeded 10 and 20×10^9 respectively.

Whether to use this policy or the possible alternative of limiting platelet transfusion to the treatment of hemorrhagic episodes is still under debate. In fact, although the prophylactic approach is undoubtedly effective in preventing unexpected hemorrhagic episodes,[3] it also entails the use of a larger number of concentrates, which theoretically increases the risk of the recipient developing transfusion-associated infections and refractoriness. In 1987, the Consensus Development Conference on platelet transfusion of the US National Institutes of Health did not make definitive recommendations on this issue, although it was remarked that "[*the level of 20×10^9 platelets*] *might safely be lower for some patients based on clinical judgement and close observation*".[2,5]

This possibility was already suggested by data published in 1978 by Slichter and Harker, who measured blood loss in the stools of 20 thrombocytopenic patients. While at platelet counts of $5\text{-}10\times10^9$ blood loss in stools was 9±7 mL, below 5×10^9 it rose to 50±20 mL.[6] In the same year Solomon and coworkers published a study in adult leukemics,[7] comparing two policies: a) transfusion in the case of clinically significant hemorrhage or in the presence of a platelet count below 20×10^9; b) as in a), but only in cases when a platelet count below 20×10^9 was preceded by more than a 50% decrease in the previous 24 hours. These authors concluded that routine prophylactic transfusion at 20×10^9 did not offer advantages over treatment b) above, which, conversely, had lower costs and less risk of transfusion-transmissible infections. Similar conclusions were drawn in pediatric patients by Murphy et al.,[8] who showed that the prophylactic approach was not associated with improved survival, percentage of complete remissions or fewer hemorrhage-related deaths. In addition, some patients in the prophylaxis arm of the trial experienced refractoriness and untreatable hemorrhage.

Further data on this topic have been collected more recently by Gmür et al.,[9] who studied over 10 years 102 leukemic patients treated according to the following protocol: a) transfusion was given in all cases when the morning platelet count was below 5×10^9; b) at values between 6 and 10×10^9 prophylactic transfusion was limited to the cases with recent minor hemorrhages or body temperature above 38°C; c) at values between 11 and 20×10^9 platelets were given only to patients with coagulopathy, treated with heparin or undergoing marrow biopsy or lumbar puncture. In addition, platelets were given for major hemorrhages and to patients undergoing extensive surgery. The 102 patients developed 28 non-fatal and 3 fatal (3%) hemorrhagic episodes.

This incidence is similar to that (3.2%) reported by Bayer et al.,[1] who evaluated a protocol of prophylactic platelet transfusion at a threshold level of 15×10^9 platelets in 31 patients with acute leukemia. In addition to lacking advantages in terms of survival, this protocol required three times more PC than that of Gmür et al.[9] Since the latter authors used pre-

dominantly apheresis platelets transfused within 6 hours of collection in their study, it has been pointed out that their conclusions could not be immediately applicable in settings using standard PC stored for up to 5 days.[10] Gmür and Schaffner, however, reported that in their more recent experience the use of standard PC stored for 3-4 days did not determine an increase of hemorrhagic episodes.[11]

The bulk of these data suggest that in stable patients a more restrictive policy based on specific and detailed indications offers advantages over the current practice of administering platelets in all cases when the platelet count falls below 20×10^9. On the basis of these data, a possible lowering of the threshold in stable patients to 10^{12} or even 5×10^9 platelets[9,13] has been suggested.

In this regard, it must be pointed out that lower thresholds require more careful observation of the time interval between platelet count determination and the start of platelet transfusion. Moreover, a critical aspect of these protocols concerns the accuracy of the patient's platelet count, which decreases with the number of platelets. In one study very low counts determined with automated counters were checked with manual microscopic methods,[9] a probable sign of the belief that at very low levels platelet manual counts are more accurate than automated ones.

However, notwithstanding the extreme accuracy of modern automated counters,[14,15] we are unaware of extensive studies reporting the relative accuracy of manual versus automated counts below 20×10^9 platelets.[16,17]

Preparation and storage of platelet concentrates

Platelet-rich-plasma methods

The availability of multiple plastic bag systems allowed the development of easily standardizable methods for the preparation of PC. A number of these methods are based on initial centrifugation of a 450 mL unit of whole blood at low speed to concentrate the platelets in approximately 200-250 mL of supernatant plasma. The platelet-rich plasma (PRP) is then transferred into a satellite bag, which is centrifuged at high speed in order to concentrate the platelets at the bottom of the bag. Finally, the supernatant platelet-poor plasma is transferred into a new satellite bag, except for 50-70 mL that are used to resuspend the platelet button after 90 minutes of undisturbed platelet rest to favor their spontaneous disaggregation.[18,19]

Buffy-coat methods

Other methods have been developed, based on the use of the buffy-coat (BC), a fraction of 40-50 mL collected at the red cell/plasma interface formed after centrifugation of whole blood.[20] In the method currently in use at the author's Institution, whole blood units are centrifuged at high speed in order to concentrate the platelets in the BC, which is then transferred into a satellite bag.[21] BC contain approximately 10% red cells, 10% plasma, 60-70% white cells and 70-80% of the platelets originally present in the whole blood unit. After gentle resuspension, 4-7 BC are pooled in a bag containing 350 mL of a simple glucose-free crystalloid medium (Plasmalyte A, Baxter, Deerfield, IL, USA), commonly used in surgery as a crystalloid replacement fluid. The pool of BC suspended in this medium (composed after the admixture of approximately 30% plasma and 70% Plasmalyte A) is then centrifuged at low speed to concentrate the platelets in the supernatant, which is ultimately transferred into a storage bag. The platelet concentrate from buffy-coat (BC-PC) is used within 6 hours if the bags have been entered during pooling. Alternatively, storage can be extended to 5 days if the whole procedure is performed in a closed system with the aid of a sterile connection device (SCD 312, Terumo, Tokyo, Japan).[22] In the latter case, the storage bag must have optimal gas exchange characteristics to maintain sufficient O_2 influx and CO_2 efflux, and to preserve platelet aerobic metabolism. In fact, increased quantities of lactic acid (resulting from anaerobic metabolism) and of CO_2 can cause a significant pH drop, which is associated with irreversible platelet damage.[23] While the traditional and inexpensive PVC containers are adequate for BC-PC storage for 1 day, more expensive and more gas-permeable polyolefin containers are necessary for 5-day storage.[24]

Some studies suggest that the quality of BC-PC is equal or superior to that of PRP-PC.[25-27] Compared with PRP-PC, platelets in BC-PC are less activated possibly as a result of more gentle centrifugation. Moreover, leukocyte contamination of concentrates is also inferior in BC-PC.[21] Although it is not known if this decrement is of clinical significance, it seems wise to aim at obtaining low white cell counts in blood components in general, given the important side effects that the infusion of allogeneic leukocytes can cause in recipients.[28] Finally, the use of a crystalloid medium eliminates the need to consider plasma incompatibility between donor and recipient, since the donor's ABO agglutinins are diluted to a titer with no clinical effect.[26]

Because of their good quality and of the practical advantages of this method of preparation, BC-PC have become the routine platelet product in several Centers in Europe.[26,27,29,30]

Apheresis

A different approach to platelet procurement is platelet apheresis. In this procedure a number of platelets corresponding to that contained in 5-8 whole blood donations can be collected in about one hour from a blood donor connected to a blood separator. Detailed results, advantages and disadvantages of different machines and procedures are reported in the literature.[31-34] The chief disadvantages of platelet apheresis are the cost of the blood separator and its disposables and the potential inconvenience caused to the donor by extracorporeal circulation. Along with these disadvantages one must consider the advantage of the smaller number of donors necessary for each patient. In this regard, a number of studies provided no conclusive evidence that the use of single-donor apheresis platelets can determine a lower incidence of HLA alloimmunization and refractoriness.[35-37] Accordingly, the routine use of single-donor platelet transfusion to reduce the frequency of anti-HLA alloimmunization is still controversial.[5]

Also of note is the theoretical advantage of a decreased risk of acquiring transfusion-transmissible infections.[38] In this regard, it is difficult to determine if the risk decrease is clinically relevant, since the current risk of acquiring transfusion-transmitted infections is quite low following the implementation of reliable assays for the identification of donors at risk of transmitting the causative agents of hepatitis B and C and of AIDS.[39] Although in some settings PC from standard donations can be insufficient to meet the patients' needs, and procurement through apheresis may be necessary, the use of random PC from standard donations as first-choice product for all patients has been recommended, while single-donor PC, collected by apheresis, are necessary to obtain effective support in patients refractory to random donor transfusion.[3,5,40]

Storage

PC prepared from PRP or BC or obtained through apheresis must be stored at 22±2°C under continuous gentle agitation,[41] since storage at 4°C causes platelet activation.[42] This activation determines good hemostatic activity immediately after infusion, but also decreases platelet survival in circulation. Even short periods of storage at temperatures below 20°C before fractionation of whole blood units can induce substantial damage and decrease *in vivo* viability and *in vitro* properties.[43]

PC should not be stored for more than 5 days in order to reduce the risk of bacterial contamination and septic shock in recipients. The main cause of bacterial contamination is inadequate asepsis of the donor's arm at the time of venipuncture.[44] Bacterial contamination is usually limited to a very low number of concentrates, and is generally caused by microorganisms of little or no clinical significance. However, great attention must be paid to this problem, particularly by not exceeding the maximum storage time of 5 days, since most fatal septic complications following platelet transfusion have been reported after the use of PC during their 4th-5th day of storage.[45] In addition, older platelets also carry an increased risk of causing transfusion reactions. In fact, in a recent series of 2,707 platelet transfusions given in our hospital to 192 patients, we found that the frequency of transfusion reactions reported after transfusion of 5-day-old PC was more than ten times that with

1-2-day-old PC (Riccardi et al, 1994, unpublished observations). This is possibly due to cytokine release from white cells contained in PC during storage (see *Leukocyte reduction* below).

Cryopreservation

A number of methods have been developed to permit frozen platelet storage. The vast majority of protocols require deep freeze storage capability and involve the use of DMSO as a cryoprotectant,[46] although glycerol, glucose and other reagents have also been used.[47-49] In spite of some interesting *in vivo* results obtained with certain protocols, in particular for autologous use,[50-52] no method has produced results that would promote routine use of cryopreserved platelets. To the best of our knowledge, the average *in vivo* effectiveness of platelets cryopreserved in experienced Centers corresponds to 50% of that of fresh PC.

Leukocyte reduction

An important aspect of platelet production is the removal of contaminating leukocytes. With current filtration techniques it is possible to obtain PC containing less than 1% of the original number of white cells.[53] These technical developments prompted a number of studies aimed at discovering whether the use of leukocyte-reduced blood components can prevent HLA alloimmunization and platelet refractoriness. In general, these studies have shown that the frequency of alloimmunization can decrease from 20-50% to 10-30%.[54-57] However, it has not been clearly shown yet whether this decline in the frequency of HLA alloimmunization is paralleled by reductions in patient refractoriness to random donor support, morbidity and mortality. Different opinions exist in this regard;[58,59] but in consideration of the high cost of red cell and platelet filters, a generalized use of leukocyte-reduction filters in blood malignancy has not been recommended.[60,61] A recent study supporting the cost-effectiveness of white-cell reduction filters in adult acute myelogenous leukemia, as well as similar investigations in other patient categories might help to change this recommendation in the future.[62]

Other techniques aimed at preventing HLA alloimmunization, including PC irradiation with UV light,[63] are currently under evaluation. Several pending questions will hopefully be answered upon completion of a well-designed, sufficiently large controlled clinical trial currently being carried out in the US, called TRAP (*Trial to Reduce Alloimmunization to Platelets*).

As for virus transmission, several studies indicate that leukodepleted blood components do not transmit CMV.[64-66] However, some concern has been raised by a recent report from a large study performed in patients following bone marrow transplantation.[67] In this study, the probability of any CMV event, including the median time of onset, was reported not to be significantly different in recipients of blood products from seronegative donors vs recipients of blood filtered through Pall filters (3 of 246 vs 5 of 241, respectively, p=0.33). In spite of this finding, 5 patients in the *filtered blood* arm were initially reported to have developed CMV disease, and 4 of the 5 died of CMV pneumonia, whereas no recipient of seronegative blood products developed CMV disease (p=0.02). From this finding the authors concluded that filtered blood should be reserved as a second choice when seronegative blood is not available for patients undergoing bone marrow transplantation. As reported by Lane in a recent review on leukoreduction,[57] somewhat different conclusions from this study were presented orally at the American Society of Hematology Meeting, December 6, 1993, St. Louis, MO, "*apparently based on a more thorough analysis of the data than was possible at the time the abstract was submitted*". In fact, "*since two of the five events in the study patients and one event in the control patients occurred within 21 days of the start of the study, they were considered unrelated to blood component use. The investigators concluded that there was no significant difference between the use of blood from CMV seronegative donors and leukocyte-reduced blood components, and that the choice of one vs the other technique should be made on the basis of cost*".

The prevention HTLV transmission, another leukotropic virus, although probable,[68] has not been demonstrated conclusively. Conversely, it

has been clearly shown that the transmission of non leukocyte-restricted viruses such as HIV is not prevented by leukocyte reduction by filtration.[69]

In regard to the prevention of non-hemolytic, febrile transfusion reactions (NHFTR) to platelets, filtration is not as effective as it is in red cell transfusion.[70-73] This is suggested by recent studies performed both in the general population of PC recipients and in groups of patients with previous NHFTR (reactors). Table 1 reports a summary of some comparative studies together with previous data obtained by Décary et al. in recipients of standard PC pools.[74] The data indicate that leukocyte reduction by filtration, although capable of slightly reducing the incidence of NHFTR in some studies, was inferior to single donor (apheresis) PC in one study,[75] and was associated with a paradoxically increased occurrence in others.[72,76] It must be pointed out that in most studies patients given unfiltered PC were different from those treated with filtered PC, and this can create some difficulty in the interpretation of these data. However, these studies clearly indicate that the efficacy of filtration leukocyte reduction of stored platelets in decreasing the frequency of NHFTR in recipients is not as impressive as that of RBC.

A clue to interpreting these findings comes from recent studies showing that cytokines are released by white cells into the platelet suspending medium during PC storage,[71,77,78] and that early white cell removal can prevent cytokine release.[79] It is known that certain cytokines, including interleukin-1b, interleukin-6, interleukin-8 and tumor necrosis factor, can mediate the onset of symptoms frequently reported during NHFTR.[79,80] The observation that the release of these cytokines in PC is particularly evident from the third to the fifth day of storage[78] is in accordance with our findings that reactions occur more frequently towards the end of the platelet storage period.

Definitive conclusions on the role of PC leukocyte reduction in the prevention of NHFTR to platelets cannot be drawn at the moment. The current level of information supports the need for further study in this area. For the time being, we believe that, if filtration is used with the goal of preventing NHFTR, platelet filters should be used only with PC

Table 1. Frequency of reactions reported after the transfusion of different types of filtered and non-filtered PC to general patient populations (A) and to patients with previous reactions when transfused with standard PC (reactors) (B).

Author	Year	Type of PC	Number of transfusions	Number of reactions	Transfusion reaction rate (%)
(A) General population					
Décary	1984	Standard PC	795	22	2.8
Goodnough	1993	Standard PC	1901	32	1.7
		Filtered PC	1704	90	5.3
Riccardi	1993	Buffy-coat PC	1476	7	0.47
		Filtered buffy-coat PC	2014	24	1.19
(B) Reactors					
Chambers	1990	Standard PC	583	125	21.4
		Single donor PC	438	37	8.4
Mangano	1991	Standard PC	202	55	27.2
		Filtered PC	206	40	19.4
Goodnough	1993	Standard PC	152	20	13
		Filtered PC	152	15	10

stored for not more than 2-3 days, and that the filtered PC should be administered only to patients with anti-HLA antibodies in the serum, showing repeated NHFTR also occurred with fresh platelets.

Irradiation

Transfusion-associated graft versus host disease (TA-GVHD) is a rare but almost unvariably fatal complication of the transfusion of cellular blood components. The small number of residual white cells present in leukodepleted blood components prepared with some current, highly effective filters is still capable of causing TA-GVHD.[81] It appears that donor HLA homozygosity can play a role in determining TA-GVHD.[82] Immunoincompetent patients are at greater risk of developing TA-GVHD than other categories, although this complication has also been reported in immunocompetent subjects.[83-85] Therefore, in selected patient categories gamma irradiation is used to abolish lymphocyte reactivity in blood components completely and to prevent TA-GVHD. A recent report on successful treatment of TA-GVHD supports the use of OKT3 (5 mg/d), cyclosporin A (250 mg/d) and granulocyte colony-stimulating factor (250 μg/d) in such cases.[86]

Discordant data are present in the literature concerning the damage induced in platelets by gamma irradiation. Although it is commonly accepted that irradiated PC can be stored for 5 days,[87] some authors suggest limiting post-irradiation storage to 3 days.[88] More detailed information on irradiation protocols used in different institutions is presented and discussed in the excellent papers by Anderson et al.[89,90]

In vitro quality control

The Standards of the American Association of Blood Banks require that at least 75% of PC contain 55×10^9 platelets or more.[19] According to current Italian law on blood transfusion[91] and to the Council of Europe,[92] the limit that should be met by at least 75% of units is 60×10^9 platelets/unit. Notwithstanding this requirement, a recent survey performed by the *Cooperative Group on Quality of Blood Components of the Italian Society of Blood Transfusion* (SIITS/AICT) indicates that only 43% of routine PC prepared in 15 Italian Centers that volunteered to participate in the study contained more than 60×10^9 platelets per unit.[93] This may be due to several factors, including blood collections below 405 mL in some of these Centers, in contrast to the 450 mL ± 10% volume required by the current Italian law,[91] or incomplete optimization of procedures for PC preparation. Since several reports indicate that it is not difficult to exceed 70×10^9 platelets per PC,[18,19] action should be taken to reach such levels even under routine conditions.

Besides quantitative aspects, a number of assays have been proposed for *in vitro* quality control of platelet concentrates, which is regulated by many agencies.[19,92] Nonetheless, clear evidence of correlation of any *in vitro* assay or combination of assays with *in vivo* results is lacking.[94] This notwithstanding, tests designed to evaluate platelet morphology seem more informative than biochemical or functional (aggregation) assays.[94] Of the morphological assays, the very simple determination of the swirling phenomenon (a particular shimmering effect produced when discoid, good quality platelets are observed in agitation with the naked eye against a strong light source) seems promising.[95] International multicenter studies are currently being performed by the BEST (*Biomedical Excellence for Safer Transfusion*) Working Party of the International Society of Blood Transfusion to elucidate if swirling evaluation can replace the battery of cumbersome and expensive tests traditionally employed in PC quality control.

Future directions

This brief overview on methods of PC preparation, storage and quality control would be incomplete without a mention of some research programs aimed at producting semi-artificial platelets.[96-98] Some of these studies aim at the insertion of platelet membrane glycoproteins, which carry the most important receptors involved in platelet function, into artificial or natural membranes, such as liposomes or red cell membranes. In spite of the interest generat-

ed by these research programs, at the time of this writing it is difficult to determine whether they will find practical applications in transfusion medicine in the near future.

Evaluation of in vivo effectiveness of platelet transfusion

A platelet transfusion is deemed effective if it results in correction of hemorrhage or if it determines a significant increment in the platelet count of the recipient. Effectiveness can also be determined by correction of a prolonged bleeding time. Since the evaluation of bleeding time is not sufficiently practical, in routine conditions the post-transfusion platelet increase remains the cornerstone for determining effectiveness.

The recommended dose of 70×10^9/L platelets (one average PC) per 10 kg of body weight should, theoretically, determine a posttransfusion rise of approximately 100×10^9 platelets. In fact, platelets contained in 7 concentrates administered to a 70 kg patient (total approximately 500×10^9/L platelets) are diluted into a blood volume of approximately 5 L (70 mL/kg). However, in clinical practice increments are much lower. The causes for this have been identified in studies showing that approximately one third of transfused platelets are immediately sequestered by the spleen, and that up to 60% of platelets may be unable to circulate as a result of damage induced during storage.[43,99] Moreover, a number of clinical factors such as fever, infection, and the administration of certain drugs further reduces effectiveness.[100,101] On the basis of the experimental evidence of the above mentioned studies, a platelet transfusion is commonly considered effective if 1 hour after transfusion it determines a platelet count increase equal to or greater than 40% of that expected. The expected increment, which takes into account splenic sequestration of approximately 1/3 of transfused platelets, is calculated with the following formula:

$$\text{Expected increment} = \frac{\text{Number of platelets administered} \times 2/3}{\text{Patient's body weight} \times 70\ \text{mL}}$$

The actual increment is computed by the difference between the pretransfusion count and that obtained 1 hour after the end of transfusion. The following formula is thus used to determine the effectiveness:

$$\text{Effectiveness} = \frac{(\text{Post} - \text{pre-transfusion count}) \times \text{Patient's body weight} \times 70\ \text{mL} \times 3/2}{\text{Number of platelets administered}}$$

This formula is known as the *Milwaukee formula*, from the center first proposing its use in 1976.[102]

An apparently different formula derived from a similar approach is known as CCI (corrected count increment). In this computation as well post-transfusion increment is corrected to allow meaningful comparisons between patients of different size. However, body surface area (BSA) is used as a correction factor instead of blood volume, and the correction factor for splenic pooling is not considered. The following formula is used to determine the CCI:

$$\text{CCI} = \frac{(\text{Post- pretransfusion platelet count}) \times \text{BSA}}{\text{Number of platelets administered}} \times 100$$

As clearly indicated by their structure, the *Milwaukee formula* and the CCI are different modalities of expressing the same evaluation. In fact, the same variables are used in the computation (platelet increment and dose), while corrections for patient size are made with blood volume and BSA, which are related to each other.[103,104]

Hogge et al. determined the CCI in different conditions. They found that mean CCI calculated at 1 hour and at 24 hours after the end of transfusion in patients without lymphocytotoxic antibodies in the serum were 16.1×10^9/L and 12×10^9/L platelets, while in patients with lymphocytotoxic antibodies they were 5.6 and 2.600×10^9/L, respectively.[105] These data suggested that the effectiveness of platelet transfusion should be considered acceptable if the 1-hour CCI exceeds 10×10^9/L, while repeated values below 5×10^9/L indicate refractoriness.[106] Other authors use slightly or even significantly different levels.[103]

The post-transfusion platelet count used in the computation of CCI is traditionally determined one hour after the end of transfusion, although counts performed after 10 minutes produce similar results.[107] If the 1-hour count cannot be determined, it is common practice to rely upon the 24-hour count, which is often coincident with the complete blood count determined on the day following the transfusion. The 24-hour CCI corresponds to approximately 2/3 of the 1-hour or 10-minute CCI.[100,103]

Therefore, routine patient monitoring is sufficiently accurate if it is based on the determination of the 24-hour CCI, while the 1-hour determination can be limited to patients showing unsatisfactory 24-hour CCI. It has been claimed that low 1-hour CCI are determined by the presence of platelet-reactive antibodies in the recipient, while good 1-hour CCI followed by insufficient values at 24 hours suggest that the causes of ineffectiveness are mainly related to clinical factors, although recent experimental data do not support this view.[100,101,103]

In 1990 we evaluated the effectiveness of 2432 platelet transfusions prepared with the PRP method and given in 1988-1989 in our Institution to 189 unselected hematological recipients (approximately 90% of patients were affected by acute leukemia). The median 1-hour effectiveness, determined with the *Milwaukee formula* was 42% of expected. It was 58% and 22% in patients without and with anti-HLA antibodies in the serum respectively. The latter were approximately 1/3 of the total number of patients. In addition to anti-HLA alloimmunization, an important detrimental factor associated with reduced effectiveness was body temperature above 38.5°C.

Strategies for providing effective transfusions to refractory patients: HLA selection or cross-match?

Because refractoriness to random donors is often caused by anti-HLA antibodies,[105,108] transfusion of HLA compatible platelets is often effective in refractory patients.[109] However, this strategy is expensive and cumbersome, since it requires the availability of several thousand typed donors.[110,111] In addition, approximately one quarter of HLA compatible platelet transfusions are ineffective.[3] Besides clinical factors capable of decreasing the effectiveness of platelet support, other reasons for ineffectiveness include the presence of non-HLA (platelet specific) antibodies capable of reacting with transfused platelets, and an incomplete HLA match. Moreover, selection on the basis of HLA type can exclude donors with an HLA type different from that of the recipient, but potentially effective for some recipients alloimmunized to different antigenic determinants. These considerations prompted the development of a different strategy, based on a cross-match between recipient and donor, that is currently recommended as an alternative to HLA typing.[112] The cross-match can be performed with donor lymphocytes or platelets. While employing the lymphocytes as target cells offers the advantage of relying upon the lymphocytotoxicity test, a well-known, standardized assay, the use of platelets can offer other important advantages. Firstly, purified donor platelets are easier to obtain than purified donor lymphocytes. Secondly, some HLA specificities are weakly and variably expressed on platelets. It is thus possible that antibodies reacting *in vitro* with lymphocytes might prove unreactive or less reactive *in vitro* as well as *in vivo* to platelets from the same donor. These considerations are supported by a large number of recent studies treating the use of platelets as a target in the cross-match.[113-123]

An excellent review by von dem Borne et al. on theoretical and practical aspects of platelet cross-matching[124] reports a comparison of results obtained in 9 studies using 5 different techniques (immunoradiometric assays with polyclonal and monoclonal antibodies, ELISA, immunobead assays, immunofluorescence, solid phase). In the 9 studies, 601 platelet transfusions selected by cross-match were administered to 141 patients refractory for immunological reasons. The overall efficiency of the assays, expressed as the ratio of [true positive+true negative] to the total number of tests, was 87%. In view of these data von dem Borne and coworkers recommended the use of platelet cross-match for refractory patients. These rec-

PATIENT ID: 64643
NAME: _____
ABO/Rh: B+

DIAGNOSIS: AML

CENTER: Policlinico
FROM: 01-03-94
TO: 10-04-94

DATE	TR	POOL TYPE	AGE days	PLT ABO	DOSE	PRE	POST 1H	POST 24H	%EFFICACY 1H	%EFFICACY 24H	CCI 1H	CCI 24H	DF	ABS
2/3/94	R	35M	1	B	280	6	32		43		11760			NEG
4/3/94	R	35M	2	B	360	7	50		55		15127			
7/3/94	R	35S	4	B	330	5	22		24		6524*		F	
9/3/94	R	35M	2	O	420	7	63		61		16886			
14/3/94	R	35M	2	O	310	3	28		37		10213			
16/3/94	R	35M	2	B	300	8	29		31		8543			NEG
18/3/94	R	35M	1	B	290	8								
21/3/94	R	35S	2	B	300	5	32		39		10984			
23/3/94	R	35M	2	B	380	12	49		42		11883			
28/3/94	R	35M	2	O	450	5	56		49		13831			
1/4/94	R	35M	2	O	390	8	39		35		9701			NEG
5/4/94	R	35M	3	B	360	7	56		47		13221			
8/4/94	R	35S	2	B	400	10	43		47		13119			
MEAN			2.1		350	7	42		42		11816			
SD			0.7		50	2.3	12		9.9		2752			
MIN			1		280	3	22		24		6524			
MAX			4		450	12	63		61		16886			
No.			13		13	13	12		12		12			

Figure 1. PLATELET report from patient 64643, showing adequate CCI values during March-April 1994. TR: Type of transfusion (R: random; C: crossmatched; H: HLA-typed); DOSE: Number of platelets administered ($\times 10^9$); PRE and POST: pre- and post-transfusion platelet counts ($\times 10^9$/L); %EFFICACY: efficacy of platelet transfusion, expressed as "% of expected" and computed with the "Milwaukee" formula (see text); CCI: corrected count increment; DF: clinical and pharmacological detrimental factors capable of decreasing CCI (F: fever; S: splenomegaly; D: DIC; A: administration of amphotericin B). An asterisk identifies 1-hour CCI values below 7.5×10^9/L.

ommendations are also supported by a recent report by Friedberg et al.,[125] who performed a study on 220 platelet transfusions given to a well-characterized cohort of refractory patients free from non immune detrimental factors. In this study cross-match-compatible platelets selected with a solid-phase assay provided similar post-transfusion increments, independently of the HLA match. In addition, it was found that "*none of 31 cross-match-incompatible platelets transfused provided an adequate increment, including 13 that were ordered as HLA-matched platelets*". In addition to these clinically important findings, the advisability of platelet cross-matching vs HLA type is also supported by economic considerations.[126,127]

The system in use since 1991 at the author's Institution is based on a platelet cross-match performed with immunofluorescence against platelets of ABO compatible, non HLA-typed donors. Several hundred platelet samples of donors readily accessible and potentially available to undergo a plateletpheresis procedure if compatible are stored in DMSO in microtiter plates at –80°C. Platelet-specific and HLA antigens remain stable under these conditions for at least 12 months.[128] Test results are read with a FACScan cytofluorimeter (Becton Dickinson, Mountain View, CA, USA). Cross-matching one plate, which includes 40 donor platelet samples, requires 3-4 hours. Other methods, such as solid phase, are currently under evaluation.

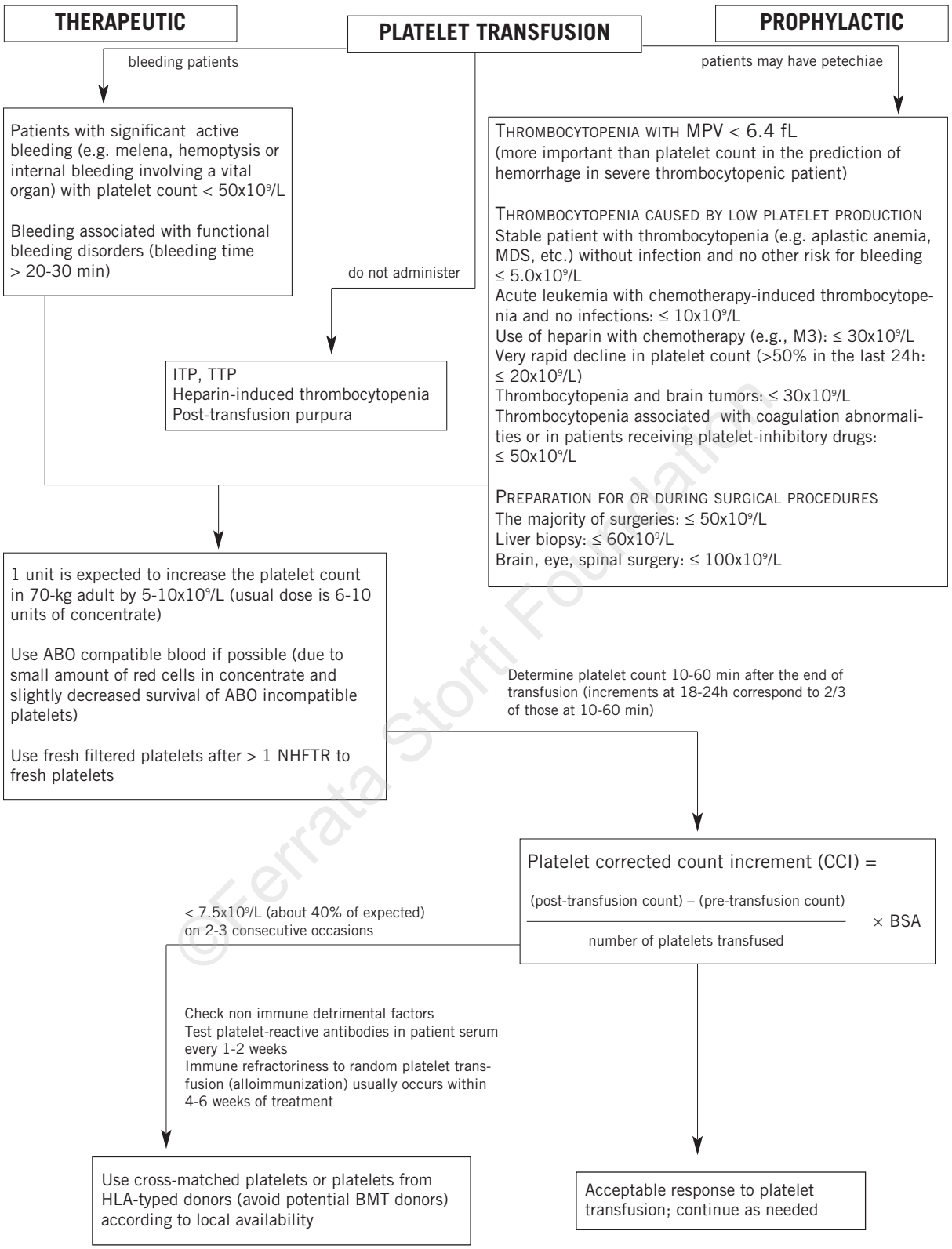

Figure 2. An algorithmic approach to platelet transfusion [modified from Djulbegovic (ref. #130)].

Continuous and timely patient monitoring is facilitated by the use of PLATELET, an MS-DOS compatible piece of software developed in cooperation with V. Sirelson (Optimark Corporation, Randwick, NY) for managing patient data, that is currently under final validation and field evaluation.[129] PLATELET automatically performs the calculations for platelet transfusion effectiveness and provides patient reports. An example of a patient report is given in Figure 1.

Serum samples from all platelet recipients are screened for the presence of platelet reactive antibodies every 2 weeks, or more frequently if inadequate CCI are obtained. A physician from the transfusion service is responsible for coordinating this program.

So far, 52 platelet transfusions selected with the system described above for 19 refractory patients have given 1-hour CCI above 7.5×10^9/L in 65% of cases. These findings support similar data in the literature,[127] which indicate that random donor cross-matching is effective in approximately two thirds of transfusions given to refractory patients.

Conclusions

Significant advances regarding platelet support in oncology-hematology patients have been made in recent years. A compact view of these advances is provided by the algorithmic approach to platelet transfusion in oncology-hematology recently designed by Djulbegovic[130] that summarizes the most recent guidelines issued on this topic,[2,13] and can be used as an educational tool for clinicians and blood bankers. In Figure 2 we present a slightly modified version of this algorithm that adheres more strictly than the original to the concepts presented in this review. Apart from minor details, the main differences regard (a) the use of leukocyte-reduction filters, which is recommended in the original algorithm if prolonged platelet use is suspected. We choose not to recommend this before the conclusion of current multicenter clinical trials that should fully balance the advantages and disadvantages of this approach. Moreover, we have added (b) a step before implementing immunological platelet selection through HLA typing or cross-match for refractory patients, since it is important to ascertain whether detrimental clinical factors (fever, infection, splenomegaly, drugs, etc) do or do not play a major role in decreasing the effectiveness of platelet support. Finally, the original algorithm does not include (c) a strategy for the prevention of NHFTR, which has been added in our version. This strategy supports the use of fresh PC when leukoreduction filtration procedures are performed.

References

1. Bayer WL, Bodensteiner DC, Tilzer LL, Adams ME. Use of platelets and other transfusion products in patients with malignancy. Semin Thromb Haemost 1992; 18:380-91.
2. National Institutes of Health Consensus Conference. Platelet transfusion therapy. Transfus Med Rev 1987;1:195-200.
3. Slichter SJ. Principles of platelet transfusion therapy. In: Hoffman R, Benz EJ Jr, Shattil SJ, Furie B, Cohen HJ, eds. Hematology. Basic principles and practice. New York: Churchill Livingstone, 1991: 1610-22.
4. Gaydos LA, Freireich EJ, Mantel N. The quantitative relation between platelet counts and hemorrhage in patients with acute leukemia. N Engl J Med 1962; 266:905-9.
5. Heyman MR, Schiffer CA. Platelet transfusion therapy for the cancer patient. Semin Oncol 1990; 17:198-209.
6. Slichter SJ, Harker LA. Thrombocytopenia: mechanisms and management of defects in platelet production. Clin Haematol 1978; 7:523-8.
7. Solomon J, Bofenkamp T, Fahey J, Chillar RK, Beutler E. Platelet prophylaxis in acute non-lymphoblastic leukaemia. Lancet 1978; 1:267.
8. Murphy S, Litwin S, Herring LM, et al. Indications for platelet transfusion in children with acute leukemia. Am J Hematol 1982; 12:347-56.
9. Gmür J, Burger J, Schanz U, Fehr J, Schaffner A. Safety of stringent prophylactic platelet transfusion policy for patients with acute leukaemia. Lancet 1991; i:1223-6.
10. Seymour J. Prophylactic platelet transfusion in acute leukaemia. Lancet 1992; ii:120.
11. Gmür J, Schaffner A. Prophylactic platelet transfusion in acute leukaemia. Lancet 1992; 339:120-1.
12. Murphy WG. Prophylactic platelet transfusion in acute leukaemia. Lancet 1992; 339:120.
13. Fresh-frozen plasma, cryoprecipitate, and platelets administration practice guidelines development Task Force of the College of American Pathologists. Practice parameters for the use of fresh-frozen plasma, cryoprecipitate, and platelets. JAMA 1994; 271:777-81.
14. Dacie JV, Lewis SM. Practical haematology. 7th ed., Edinburgh: Churchill Livingstone, 1991.
15. Lord RA, Smith GA, Nightingale MJ, Boulton FE. Platelet counting using plasma platelet concentrate samples. Transfus Med 1992; 2:201-5.
16. Cornbleey JP, Kessinger S. Accuracy of low platelet counts on the Coulter S-Plus IV. Am J Clin Pathol 1985; 83:78-80.
17. Mayer K, Chin B, Magnes J, Thaler T, Lotspeich C, Baisley A. Automated platelet counters. A comparative evaluation of the latest instrumentation. Am J Clin Pathol 1980; 74:135-50.

18. Slichter SJ, Harker LA. Preparation and storage of platelet concentrates. I. Factors influencing the harvest of viable platelets from whole blood. Br J Haematol 1976; 43:395-402.
19. Widmann FK. Standards for blood banks and transfusion services. 15th ed. Bethesda, American Association of Blood Banks, 1993.
20. Prins HK, de Bruijn JCGH, Henrichs HJP, Loos JA. Prevention of microaggregate formation by removal of "buffy coats". Vox Sang 1980; 39:48-51.
21. Bertolini F, Rebulla P, Riccardi D, Cortellaro M, Ranzi ML, Sirchia G. Evaluation of platelet concentrates prepared from buffy coats and stored in a glucose-free crystalloid medium. Transfusion 1989; 29:605-9.
22. Kothe FC, Platenkamp GP. The use of the sterile connecting device in transfusion medicine. Transfus Med Rev 1994; 8:117-22.
23. Bertolini F, Murphy S, Rebulla P, Sirchia G. Role of acetate during platelet storage in a synthetic medium. Transfusion 1992; 32:152-6.
24. Bertolini F, Rebulla P, Porretti L, Murphy S. Platelet quality after 15-day storage of platelet concentrates prepared from buffy coats and stored in a glucose-free crystalloid medium. Transfusion 1992; 32:9-16.
25. Bertolini F, Rebulla P, Porretti L, Sirchia G. Comparison of platelet activation and membrane glycoprotein Ib and IIb-IIIa expression after filtration through three different leukocyte removal filters. Vox Sang 1990; 59:201-4.
26. Rebulla P, Bertolini F, Riccardi D, Smacchia C, Sirchia G. Platelet concentrates prepared from pooled buffy-coats and stored in a glucose-free crystalloid medium. The Milan experience. Transfus Sci 1990; 11:357-62.
27. Pietersz RNI, Loos JA, Reesink HW. Platelet concentrates stored in plasma for 72 hours at 22°C prepared from buffy-coats of citrate-phosphate-dextrose blood collected in a quadruple-bag saline-adenine-glucose-mannitol system. Vox Sang 1985; 49:81-5.
28. Rebulla P, Bertolini F, Parravicini A, Sirchia G. Leukocyte-poor blood components: a purer and safer transfusion product for recipients? Transfus Med Rev 1990; 4(suppl 1):19-23.
29. Kretschmer V, Biermann E, Loh H. Separation of platelet concentrates from buffy coats using the bottom and top drainage system. Transfus Sci 1990; 11:363-6.
30. Eriksson L, Shanwell A, Gullikson H, et al. Platelet concentrates in an additive solution prepared from pooled buffy coats. Vox Sang 1993; 64:133-8.
31. Puig LI, Mazzara R, Gelabert A, Castillo R. Plateletpheresis: a comparative study of six different protocols. J Clin Apheresis 1986; 3:129-38.
32. Glowitz RJ, Slichter SJ. Frequent multi-unit plateletpheresis from single donors: effects on donors' blood and the platelet yield. Transfusion 1980; 20:199-205.
33. Kenney DM, Peterson JJ, Smith JW. Extended storage of single-donor apheresis platelets in CLX blood bags: effect of storage on platelet morphology, viability, and *in vitro* function. Vox Sang 1988; 54:24-33.
34. Burgstaler EA, Pineda AA, Brecher MA. Plateletpheresis: comparison of platelet yields, processing time, and white cell content with two apheresis systems. Transfusion 1993; 33:393-8.
35. Kakaiya RM, Hezzey AJ, Bove JR, et al. Alloimmunization following apheresis platelets vs pooled platelet concentrate transfusion. A prospective randomized study (abstract). Transfusion 1981; 21:600.
36. Sintnicolaas K, Vriesendorp HM, Sizoo W, et al. Delayed alloimmunization by random single donor platelet transfusions. A randomised study to compare single donor and multiple donor platelet transfusions in cancer patients with severe thrombocytopenia. Lancet 1981; 223:750-4.
37. Gmür J, vonFelten A, Osterwalder B, Frick P. Delayed alloimmunization using random single donor platelet transfusions: a prospective study in thrombocytopenic patients with acute leukemia. Blood 1983; 62:473-9.
38. Rebulla P, Baroni L, Bertolini F, Sirchia G. The risk of acquiring transfusion-transmissible infections. Vox Sang 1988; 55:186-7.
39. Perkins HA. The safety of the blood supply: making decisions in transfusion medicine. In: Nance SJ, ed. Blood safety: current challenges. Bethesda: American Association of Blood Banks, 1992: 125-50.
40. Schiffer CA, Slichter SJ. Platelet transfusions from single donors. N Engl J Med 1982; 307:245-8.
41. Murphy S, Sayar SN, Gardner FH. Storage of platelet concentrates at 22°C. Blood 1970; 35:549-57.
42. Murphy S, Gardner FH. Platelet preservation. Effect of storage temperature on maintenance of platelet viability - Deleterious effect of refrigerated storage. N Engl J Med 1969; 280:1094-8.
43. Moroff G, Holme S, George VM, Heaton WA. Effect on platelet properties of exposure to temperatures below 20°C for short periods during storage at 20 to 24°C. Transfusion 1994; 34:317-21.
44. Blajchman MA, Ali AM. Bacteria in the blood supply: an overlooked issue in transfusion medicine. In: Nance SJ, ed. Blood safety: current challenges. Bethesda: American Association of Blood Banks, 1992: 213-28.
45. Morrow JF, Braine HG, Kickler TS, Ness PM, Dick JD, Fuller AK. Septic reactions to platelet transfusions. A persistent problem. JAMA 1991; 266:555-8.
46. Melaragno AJ, Abdu WA, Katchis RJ, Vecchione JJ, Valeri CR. Cryopreservation of platelets isolated with the IBM 2997 blood cell separator: a rapid and simplified approach. Vox Sang 1982; 43:321-6.
47. Herve P, Potron G, Droule C, et al. Human platelets frozen with glycerol in liquid nitrogen: biological and clinical aspects. Transfusion 1981; 21:384-90.
48. Taylor MA. Cryopreservation of platelets: an *in-vitro* comparison of four methods. J Clin Pathol 1981; 34:71-5.
49. Borzini P, Assali G, Riva MR, Bramante M, Sciorelli G. Platelet cryopreservation using dimethylsulfoxide/polyethylene glycol/sugar mixture as cryopreserving solution. Vox Sang 1993; 64:248-9.
50. Schiffer CA, Aisner J, Wiernik PH. Frozen autologous platelet transfusion for patients with leukemia. N Engl J Med 1978; 299:7-12.
51. Lazarus HM, Kaniecki-Green EA, Warm SE, Aikawa M, Herzig RH. Therapeutic effectiveness of frozen platelet concentrates for transfusion. Blood 1981; 57:243-9.
52. Mulder POM, Maas A, de Vries EGE, et al. Bleeding prophylaxis in autologous bone marrow transplantation for solid tumors. Comparison of cryopreserved autologous and fresh allogeneic single-donor platelets. Haemostasis 1989; 19:120-4.
53. Lutz P, Dzik WH. Large-volume hemocytometer chamber for accurate counting of white cells (WBCs) in WBC-reduced platelets: validation and application for quality control of WBC-reduced platelets prepared by apheresis and filtration. Transfusion 1993; 33:409-12.
54. Andreu G, Dewailly J, Leberre C, et al. Prevention of HLA immunization with leukocyte-poor packed red cells and platelet concentrates obtained by filtration. Blood 1988; 72:964-9.
55. Meryman HT, Mincheff M. Transfusion-induced immune tolerance. In Smit Sibinga CTh, Das PC, The TH, eds. Immunology and blood transfusion. Dordrecht: Kluwer Academic Publishers, 1993: 11-9.
56. Heddle NM. The efficacy of leukodepletion to improve platelet transfusion response: a critical appraisal of clinical

studies. Transfus Med Rev 1994; 8:15-28.
57. Lane TA. Leukocyte reduction of cellular blood components. Effectiveness, benefits, quality control, and costs. Arch Pathol Lab Med 1994; 118:392-404.
58. Reesink HW, Nydegger UE, Brand A, et al. Should all platelets concentrates issued be leukocyte-poor? Vox Sang 1992; 62:57-64.
59. Lane TA, Anderson KC, Goodnought LT, et al. Leukocyte reduction in blood component therapy. Ann Intern Med 1992; 117:151-62.
60. Schiffer CA. Prevention of alloimmunization against platelets. Blood 1991; 77:1-4.
61. The Royal College of Physicians of Edinburgh. Consensus conference. Leukocyte depletion of blood and blood components. Edinburgh, 1993.
62. Balducci L, Benson K, Lyman GH, et al. Cost-effectiveness of white cell-reduction filters in treatment of adult acute myelogenous leukemia. Transfusion 1993; 33:665-70.
63. Andreu G, Boccaccio C, Klaren J, et al. The role of UV radiation in the prevention of human leukocyte antigen alloimmunization. Transfus Med Rev 1992; 6:212-24.
64. Gilbert GL, Hayes K, Hudson IL, James J, and the Neonatal Cytomegalovirus Infection Study Group. Prevention of transfusion-acquired cytomegalovirus infection in infants by blood filtration to remove leucocytes. Lancet 1989; i:1228-31.
65. Bowden RA, Slichter SJ, Sayers MH, Mori M, Cays MJ, Meyers JD. Use of leukocyte-depleted platelets and cytomegalovirus-seronegative red blood cells for prevention of primary cytomegalovirus infection after marrow transplant. Blood 1991; 78:246-50.
66. Eisenfeld L, Silver H, McLaughlin J, et al. Prevention of transfusion-associated cytomegalovirus infection in neonatal patients by the removal of white cells from blood. Transfusion 1992; 32:205-9.
67. Bowden RA, Cays M, Schoch G, et al. Comparison of filtered blood (FB) to seronegative blood products (SB) for prevention of cytomegalovirus (CMV) infection after marrow transplant (Abstract). Blood 1993; 82(suppl.1):204a.
68. Okochi K, Sato H. Transmission of adult T-cell leukemia virus (HTLV-I) through blood transfusion and its prevention. AIDS Res 1986; 2(suppl 1):S157-S161.
69. Rawal BD, Busch MP, Endow R, et al. Reduction of human immunodeficiency virus-infected cells from donor blood by leukocyte filtration. Transfusion 1989; 29:460-2.
70. Mangano MM, Chambers CA, Kruskall MS. Limited efficacy of leukopoor platelets for prevention of febrile transfusion reactions. Am J Clin Pathol 1991; 95:733-8.
71. Mintz PD. Febrile reactions to platelet transfusions. Am J Clin Pathol 1991; 95:609-11.
72. Goodnough TL, Riddell JIV, Lazarus H, et al. Prevalence of platelet transfusion reactions before and after implementation of leukocyte-depleted platelet concentrates by filtration. Vox Sang 1993; 65:103-7.
73. Rebulla P, Bertolini F, Moggi G, Sirchia G. Transfusion reactions in thalassemia. A survey from the Cooleycare Programme. Haematologica 1990; 75(suppl.5):122-7.
74. Décary F, Ferner P, Giavedoni L, et al. An investigation of nonhemolytic transfusion reactions. Vox Sang 1984; 46:277-85.
75. Chambers LA, Kruskall MS, Pacini DG, Donovan LM. Febrile reactions after platelet transfusion: the effect of single versus multiple donors. Transfusion 1990; 30:219-21.
76. Riccardi D, Raspollini E, Rossini G, et al. Reactions reported to red blood cells without buffy-coat and to platelet concentrates prepared from buffy-coats. Transfusion 1993; 33(suppl):42S.
77. Muylle L, Joos M, Wouters E, De Bock R, Peetermans ME. Increased tumor necrosis factor α (TNFα), interleukin 1, and interleukin 6 (IL-6) levels in the plasma of stored platelet concentrates: relationship between TNFα and IL-6 levels and febrile transfusion reactions. Transfusion 1993; 33:195-9.
78. Stack G, Snyder EL. Cytokine generation in stored platelet concentrates. Transfusion 1994; 34:20-5.
79. Muylle L, Peetermans ME. Effect of prestorage leukocyte removal on the cytokine levels in stored platelet concentrates. Vox Sang 1994; 66:14-7.
80. Dzik WH. Is the febrile response to transfusion due to donor or recipient cytokine? (Letter). Transfusion 1992; 32:594.
81. Akahoshi M, Takanashi M, Masuda M, et al. A case of transfusion-associated graft-versus-host disease not prevented by white cell-reduction filters. Transfusion 1992; 32:169-72.
82. Kruskall MS, Alper CA, Awdeh Z, Yunis EJ. HLA-homozygous donors and transfusion-associated graft-versus-host disease. N Engl J Med 1990; 322:1005-6.
83. Arsura EL, Bertelle A, Minkowitz S, Cunningha JN Jr, Grob D. Transfusion-associated graft-versus-host disease in a presumed immunocompetent patient. Arch Intern Med 1988; 148:1941-4.
84. Capon SM, DePond WD, Tyan DB, et al. Transfusion-associated graft-versus-host disease in an immunocompoetent patient. Ann Intern Med 1991;114:1025-6.
85. Juji T, Takahashi K, Shibata Y, et al. Post-transfusion graft-versus-host disease in immunocompetent patients after cardiac surgery in Japan. Lancet 1989; ii:56.
86. Yasukawa M, Shinozaki F, Takaaki H, et al. Successful treatment of transfusion-associated graft-versus-host disease. Br J Haematol 1994; 86:831-6.
87. Moroff G, George VM, Siegl AM, Luban NLC. The influence of irradiation on stored platelets. Transfusion 1986; 26:453-6.
88. Duguid JKM, Carr R, Jenkins JA, Hutton JL, Lucas GF, Davies JM. Clinical evaluation of the effects of storage time and irradiation on transfused platelets. Vox Sang 1991; 60:151-4.
89. Anderson KC, Weinstein HJ. Transfusion-associated graft-versus-host disase. N Engl J Med 1990; 323:315-21.
90. Anderson KC, Goodnough LT, Sayers M, et al. Variation in blood component irradiation practice: implications for prevention of transfusion-associated graft-versus-host disease. Blood 1991; 77:2096-102.
91. Caratteristiche e modalità per la donazione del sangue ed emoderivati. Gazzetta Ufficiale della Repubblica Italiana 1991, Serie generale, n. 20:6-11.
92. Council of Europe. Guide to the preparation, use and quality assurance of blood components. Council of Europe Press, Strasbourg, 1992.
93. Rebulla P, Mulas G for il Gruppo di Studio SIITS/AICT Controllo di qualità degli emocomponenti. Metodi di preparazione, volume e contenuto di emoglobina, piastrine e leucociti negli emocomponenti prodotti in 15 centri trasfusionali italiani (Abstract). 31st Italian Congress of Transfusion Medicine, Genova, June 6-10, 1994.
94. Murphy S, Rebulla P, Bertolini F, et al. for the BEST (Biomedical Excellence for Safer Transfusion) Task Force of the International Society of Blood Transfusion. *In vitro* assessment of the quality of stored platelet concentrates. Transfus Med Rev 1994; 8:29-36.
95. Murphy S, Bertolini F for the BEST Working Party of the International Society of Blood Transfusion. Assessment of swirling and percent discs by oil, phase microscopy as quality control procedures for platelet concentrates (abstract). Transfusion 1993; 33(suppl):10s.
96. Agam G, Livne AA. Erythrocytes with covalently bound fibrinogen as a cellular replacement for the treatment of thrombocytopenia. Eur J Clin Invest 1992; 22:105-12.
97. Coller BS, Springer KT, Beer JH, et al. *In vitro* studies of a potential autologous, semi-artificial alternative to platelet transfusions. J Clin Invest 1992; 89:546-55.

98. Rybak ME, Renzulli LA. A liposome based platelet substitute, the plateletsome, with hemostatic efficacy. Biomat Art Cells & Immob Biotech 1993; 21:101-18.
99. Harker LA. The role of the spleen in thrombokinetics. J Lab Clin Med 1971; 77:247-56.
100. Bishop JF, Matthews JP, McGarth K, Yuen K, Wolf MM, Szer J. Factors influencing 20-hour increments after platelet transfusion. Transfusion 1991; 31:392-6.
101. Murphy S. Amphotericin B and platelet transfusion. Transfusion 1992; 32:7-8.
102. Filip DJ, Duquesnoy RJ, Aster RH. Predictive value of cross matching for transfusion of platelet concentrates to alloimmunized recipients. Am J Hematol 1976; 1:471-9.
103. Bishop JF, Matthews JP, Yuen K, McGrath K, Wolf MM, Szer J. The definition of refractoriness to platelet transfusions. Transfus Med 1992; 2:35-41.
104. Rebulla P. Formulae for definition of refractoriness to platelet transfusion. Transfus Med 1993; 3:91-3.
105. Hogge DE, Dutcher JP, Aisner J, Schiffer CA. Lymphocytotoxic antibody is a predictor of response to random donor platelet transfusion. Am J Hematol 1983; 14:363-70.
106. Daly PA, Schiffer CA, Aisner J, Wiernik PA. Platelet transfusion therapy. One hour posttransfusion increments are valuable in predicting the need for HLA-matched preparations. JAMA 1980; 243:435-8.
107. O'Connell B, Lee EJ, Schiffer CA. The value of 10-minute post-transfusion platelet counts. Transfusion 1988; 28:66-7.
108. Godeau B, Fromont P, Seror T, Duedari N, Bierling P. Platelet alloimmunization after multiple transfusions: a prospective study of 50 patients. Br J Haematol 1992; 81:395-400.
109. Yankee RA, Grumet FC, Rogentine GN. Platelet transfusion therapy. The selection of compatible platelet donors for refractory patients by lymphocyte HLA-typing. N Engl J Med 1969; 281:1208-11.
110. Schiffer CA, Keller C, Dutcher JP, Aisner J, Hogge D, Wiernik PH. Potential HLA-matched platelet donor availability for alloimmunized patients. Transfusion 1983; 23:286-8.
111. Bolgiano DC, Larson EB, Slichter SJ. A model to determine required pool size for HLA-typed community donor apheresis programs. Transfusion 1989;29:306-10.
112. Petz LD. Platelet cross-matching. Am J Clin Pathol 1988; 90:114-5.
113. Waters AH, Minchinton RM, Bell R, Ford JM, Lister TA. A cross-matching procedure for the selection of platelet donors for alloimmunized patients. Br J Haematol 1981; 48:59-68.
114. Myers TJ, Byung KK, Steiner M, Baldini MG. Selection of donor platelets for alloimmunized patients using a platelet-associated IgG assay. Blood 1981; 58:444-50.
115. Hecht T, Wolf JL, Mraz L, Scott EP, Petz LD. Platelet transfusion therapy in an alloimmunized patient. The value of crossmatch procedures for donor selection. JAMA 1982; 248:2301-3.
116. Yam P, Petz LD, Scott EP, Santos S. Platelet crossmatch tests using radiolabelled staphylococcal protein A or peroxidase anti-peroxidase in alloimmunized patients. Br J Haematol 1984; 57:337-47.
117. Kakaiya RM, Gudino MD, Miller WV, et al. Four crossmatch methods to select platelet donors. Transfusion 1984; 24:35-41.
118. van der Velden KJ, Sintnicolaas K, Lowenberg B. The value of a 51Cr platelet lysis assay as crossmatch test in patients with leukaemia on platelet transfusion therapy. Br J Haematol 1986; 62:635-40.
119. Heal JM, Blumberg N, Masel D. An evaluation of crossmatching, and ABO matching for platelet transfusion to refractory patients. Blood 1987; 70:23-30.
120. McFarland J, Aster RH. Evaluation of four methods for platelet compatibility testing. Blood 1987; 69:1425-30.
121. Brubaker DB, Duke JC, Romine M. Predictive value of enzyme-linked immunoassay platelet crossmatching for transfusion of platelet concentrates to alloimmunized recipients. Am J Hematol 1987; 24:375-87.
122. Kickler TS, Ness PM, Braine HG. Platelet cross-matching. A direct approach to the selection of platelet transfusions for the alloimmunized thrombocytopenic patient. Am J Clin Pathol 1988; 90:69-72.
123. Rachel JM, Summers TC, Sinor LT, Plapp F. Use of a solid phase red blood cell adherence method for pretransfusion platelet compatibility testing. Am J Clin Pathol 1988; 90:63-8.
124. von dem Borne AEGKr, Ouwehand WH, Kruijpers RWAM. Theoretic and practical aspects of platelet crossmatching. Transfus Med Rev 1990; 4:265-78.
125. Friedberg RC, Donnelly SF, Mintz PD. Independent role for platelet crossmatching and HLA in the selection of platelets for alloimmunized patients. Transfusion 1994; 34:215-20.
126. Freedman J, Gafni A, Garvey MB, Blanchette V. A cost-effectiveness evaluation of platelet crossmatching and HLA matching in the management of alloimmunized thrombocytopenic patients. Transfusion 1989; 29:201-7.
127. Welch HG, Larson EB, Slichter SJ. Providing platelets for refractory patients. Prudent strategies. Transfusion 1989; 29:193-5.
128. Porretti L, Marangoni F, Rebulla P, Sirchia G. Frozen platelet plates for platelet antibody detection and cross-match. Vox Sang 1994; 67:52-7.
129. Rebulla P, Bertolini F, Porretti L, et al. Platelet concentrates from buffy-coats: improved conditions for preparation and evaluation in routine clinical use. Transfus Sci 1993; 14:41-6.
130. Djulbegovic B, Beganovic S. Blood transfusions. Red blood cells and platelet transfusion. In: Djulbegovic B, ed. Reasoning and decision making in hematology. New York: Churchill Livingstone, 1992:239-42.