Cover Page

Universiteit Leiden

The handle http://hdl.handle.net/1887/18951 holds various files of this Leiden University dissertation.

Author: Kerkhoffs, Jean-Louis Henri
Title: Efficacy of platelet transfusions
Issue Date: 2012-05-16

Chapter 1

Introduction

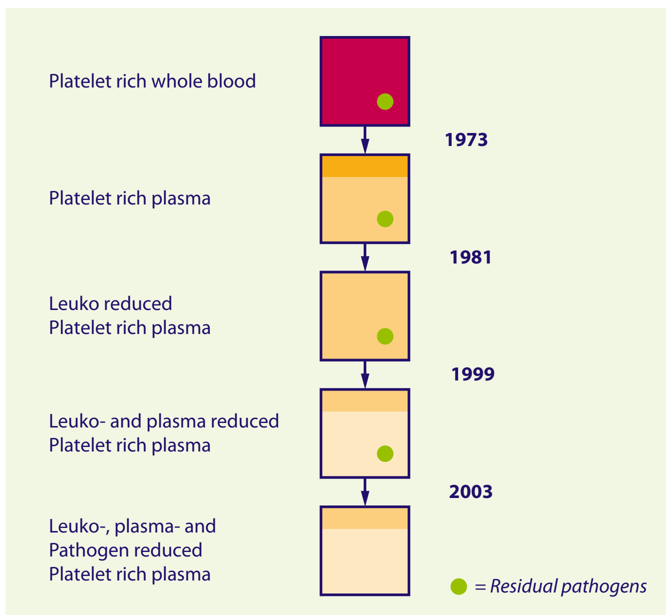
1

1 Introduction

PLATELET TRANSFUSIONS: PRODUCTS AND ENDPOINTS IN RANDOMIZED CONTROLLED TRIALS

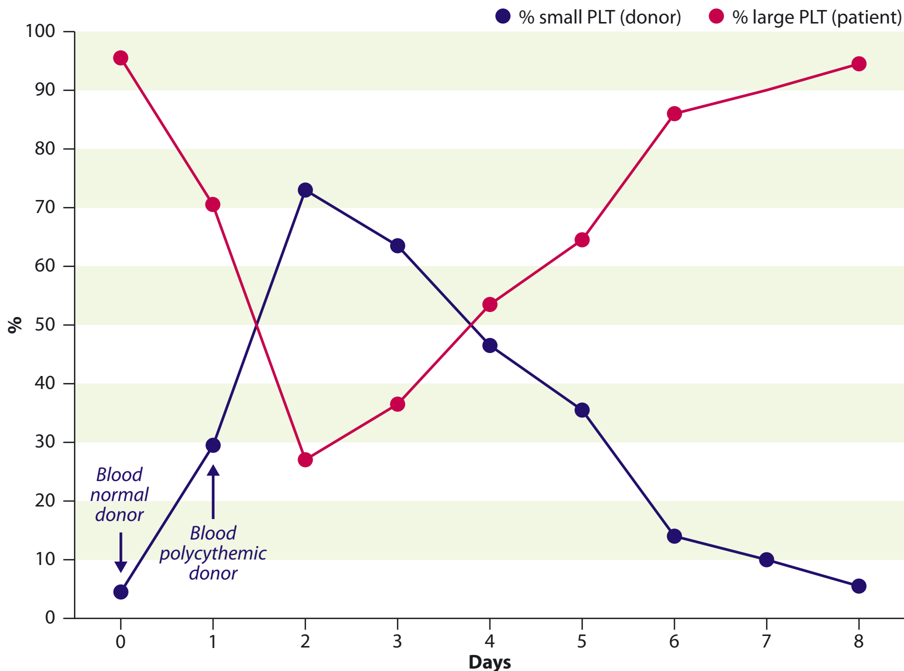
Under the assumption that thrombocytopenia correlates with bleeding complications, guidelines advise to institute a platelet transfusion policy to prevent and treat bleeding complications in haemato-oncology patients with thrombocytopenia due to myelosuppressive diseases and/or treatment.[1-4] At the start of the research for this thesis platelet transfusion therapy appeared to be a fact of life and a point of no return had been achieved, underlining the statement by Schiffer in 1992 "unfortunately, it will be scientifically impossible to perform studies 20 years after the horse is out of the barn to prove the value of this approach".[5] Meanwhile clinicians paid only limited attention to the quality of the platelet products as long as they were available on demand. The product we tested in our first randomized controlled trial (RCT), platelets stored in an additive solution, was just one of the last of several modifications since the systematic preventive use of platelets. Studies concerning platelet transfusions conducted between the early eighties until the late nineties mainly investigated product modifications to reduce adverse reactions, such as (non)-immunological refractoriness and febrile transfusion reactions. It was only in the last decade that clinical efficacy became an issue, even more to blood bankers than to clinicians.[6] The modifications which have been performed to improve the safety profile are typically characterised by removing one or more components after blood donation (figure 1). This introduction aims to recapitulate platelet transfusions from a historical perspective and the development of policy guidelines, mainly focussing on the clinical evidence for the several product modifications throughout time.

Figure 1: *Figure 1 shows the several general modifications in the production of platelet products. Not shown is the proces of pooling (possible since the introduction of sterile docking devices in the eighties) leading to multidonor Platelet Rich Plasma v.s. Buffy-coat platelet products.*



One of the first physicians investigating the fate of transfused platelets and a potential method to treat bleeding was Duke in 1911.[7] Using blood from a polycythemic donor Duke et al determined that donor platelets survived five to six days following transfusion in a young patient probably suffering from congenital thrombocytopenia. In contrast to the transfusion of blood of a normal donor, blood of the polycythemic donor resulted in a longer lasting rise of the platelet count and a reduction in bleeding time (figure 2).[8] A platelet survival study in 22 patients using a direct transfusion of polycythemic blood after a first transfusion using a normal donor to prevent "platelet hunger" suggested a survival of 48 – 72 hours, potentially influenced by active bleeding and an enlarged spleen. Bleeding time was shortened promptly after transfusion, a beneficial effect which lasted approximately 24 hours longer than the platelet survival.[9]

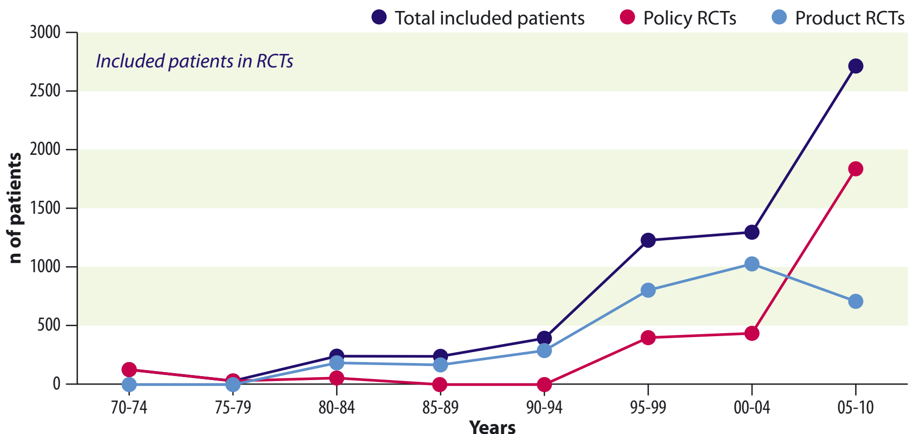
Figure 2: *Figure 2 shows one of the first in vivo experiments studying the survival of transfused platelets in a thrombocytopenic patient. PLT= Platelets*



Klein and Freireich et al developed a technique for platelet separation by differential centrifugation to obtain platelets for clinical use and a first study comparing fresh versus banked blood in the management of bleeding was performed.[10, 11] In one of the first reviews platelet transfusions were already considered as a part of the "total care" for patients with malignancies, however the difficulty of an objective evaluation of the "self-apparent" effects of platelet transfusions and the need for controlled studies was also emphasized.[12] A state-of-the-art review concerning the development and quality of platelet products as well as guidance for clinical use stated that the indication for platelet transfusions was haemorrhage associated with severe thrombocytopenia, defined as a platelet count less than 10 x 10^9/l.[13] Two autopsy studies showed that fatal haemorrhages are frequently encountered in patients with acute leukaemia and two of these studies

reported a dramatic reduction of fatal haemorrhages after the implementation of platelet transfusions.[14, 15] The first prospective landmark study in 92 patients with acute leukaemia by Gaydos et al showed a relationship between the platelet count as well as a decrease in platelet count and the risk of bleeding, although no threshold level could be observed. However, it was after this publication that the concept of prophylactic rather than therapeutic platelet transfusions emerged (figure 3).[16]

Figure 3: *Figure 3 shows the number of patients included in randomised trials studying platelet transfusions. Policy driven RCTs are trials investigating indication (prophylactic versus therapeutic), thresholds and doses. Product related RCTs classically compare an alternative platelet product, for instance using additive solutions, with conventional platelet products, usually platelets stored in plasma*



Since this publication, several strategies have been studied in clinical trials investigating the use of prophylactic transfusions as opposed to therapeutic platelet transfusions, platelet transfusion thresholds and platelet transfusion dose. In parallel with these trials the manufacturing of platelet products has constantly evolved with the development of several ways to harvest platelets, sterile docking device, storage bags and the introduction of additive solutions as well as pathogen reduction techniques, driven by emerging blood-borne infections and bacterial growth associated with prolonged storage of platelets at ambient temperature. Thus far 5 RCTs have studied prophylactic platelet transfusions as opposed to therapeutic transfusions.[17-21] Based on the three oldest studies, the combined relative risk for major or more severe bleeding complications was 0.49 (95%CI 0.28 – 0.87) favouring prophylactic platelet transfusions.[17, 19, 23, 22] More recently, a prophylactic versus therapeutic trial in patients with acute myeloid leukaemia reported also that bleeding complications were significantly increased in the therapeutic arm. There were 5 minor cerebral haemorrhagic complications and 2 lethal cerebral haemorrhages in the therapeutic arm as opposed to zero in the prophylactic arm.[21] Four RCTs and six observational studies compared different platelet transfusion thresholds for prophylactic platelet transfusions from as low as 5 x 10^9/l to 30 x 10^9/l.[27-32] Despite the heterogeneity of the studies, none of these studies has shown a difference with regard to bleeding complications, although lowering the platelet transfusion trigger led to a significant reduction in the mean number of platelet transfusions.[22] Before the recent publication of the PLADO and the STOP trial, 4 RCTs and 1 observational study

investigated platelet dose.[33-38] These trials were initiated to investigate the "optimal" platelet dose. Both economical as well as safety issues, i.e. minimizing donor exposure, are the main driving factors to reduce the transfusion threshold as well as the platelet dose. Both the PLADO as well as the STOP have made two important points. Firstly and most importantly, haemorrhagic complications are very frequent despite the use of prophylactic platelet transfusions (the % of patients with WHO ≥ 2 bleeding: PLADO 70%, STOP 50% without differences between the study arms with platelet doses reaching from ± 0.8 x $10^{11}/m^2$ (lowest dose in the STOP trial) to 4.4 x $10^{11}/m^2$ (highest dose in the PLADO trial) and secondly more transfusions are administered in patients receiving low-dose platelet transfusions.[37, 38] Although prophylactic platelet transfusions likely prevent severe haemorrhages, no definitive quantitative strategy with regard to threshold or doses can be made based on these trials.

In contrast to the straight forward search for an "optimal" platelet transfusion strategy, the approach to develop the "optimal" platelet product evolved more complicated by a diversity of ways to manufacture and store platelet products. Trials studying product modifications use an array of endpoints, including transfusion efficacy, haemostatic efficacy and the incidence of adverse transfusion reactions. Platelets can be prepared using aphaeresis (AP) or a whole-blood method (WBD), of which the platelet-rich plasma (PRP) method is mostly used in the United States, whereas the buffy-coat method (BC) is used in Europe. On top of these different collection and production methods, a large diversity in platelet products arises if we also include a variety of storage bag systems, methods for leukoreduction, gamma irradiation, variations in storage time and several additive solutions, which all potentially affect efficacy. A simple calculation results in over a thousand different currently clinically used platelet products. This diversity resulted in the need for defining which requirements should ideally be met by new platelet products as stated in a draft guidance of the FDA in 1999 (table 1).[39]

Table 1: *FDA Guidance for industry.*

	Test	Subcategory	Type of tests
A	**Paired in vitro studies**	Morphology	Different levels of resolution, including EM
		Biochemical	ATP, glucose, lactate, pH and LDH
		PLT activation	P-selectin (CD62), CD63, fibrinogen binding (PA-CI), B-thromboglobulin, Plateletfactor 3 and 4
		Physiological respons	HSR, aggregation and serotonin release
		Microparticles	
B	**Platelet survival**	Healthy volunteers	Radiolabeling of autologous platelets with ^{111}Indium and/ or ^{51}Chromium
C	**Clinical haemostatic efficacy**	Thrombocytopenic patients	Transfusion efficacy and haemostatic efficacy

PLT = Platelet; EM = Electron Microscopy; ATP = Adenosine Triphosphate; CD = Cluster of Differentiation; HSR = Hypotonic Shock Response; FDA = Food and Dug Administration

This manuscript mainly deals with efficacy, although most new platelet products or changes in production are driven by safety and economics. An optimal platelet product should be easy to produce with constant quality parameters, should be stored for preferably longer period than the current 5 – 7 days maintaining this quality, clinically efficient in terms of bleeding prevention and without adverse reactions such as febrile and allergic transfusion reactions, transfusion related acute lung injury (TRALI), infectious

complications and alloimmunization resulting in refractoriness. Moreover, preferably we would have in vitro measures to predict clinical safety and efficacy. However, to date there are no in vitro tests predicting viability in terms of recovery and survival or haemostatic function after transfusion in patients.[40] The suggested performance of recovery / survival studies is hampered by the fact that the use of radiolabeling in volunteers is not operational in many countries, performed studies are small showing huge variation and more important the efficacy in healthy volunteers does not reflect the clinical outcome in thrombocytopenic patients. From 1970 to 2005 thirty-four RCTs have been performed testing one or more platelet product modifications (table 2).

Table 2: *RCTs with different products and/or product modifications up to 2005.* [43-55, 57-65, 67-81]

Type of modification	Endpoints	N trials
Production (PRP, BC, AP)	Alloimmunsation	6
	Adverse TRF reactions	
	Post transfusion recovery	
Leukodepletion I inactivation	Alloimmunisation	11
	Post transfusion recovery	
Plasmareduction	Adverse TRF reactions	6
	Post transfusion recovery	
HLA or ABO matching	Post transfusion recovery	4
	Bleeding	
Preincubation	Post transfusion recovery	2
Storage	Post transfusion recovery	2
Photochemical pathogen reduction	Post transfusion recovery	3
	Bleeding	
Total		34

TRF = Transfusion; Note: all the trials have been counted once, although some trials could have been included in more than one category.

Apart from studies investigating the clinical effect of storage time or production method (PRP, BC, AP), in historical order the following modifications have been subject of study: leukoreduction (centrifugation, filtration and UV irradiation), plasmareduction (concentration, additive solutions) and pathogen reduction (Amotosalen / UVA) (see also figure 1).

The incentives for leukoreduction, by far the most studied topic in platelet product RCTs, were mainly the prevention of alloimmunisation, platelet refractoriness and febrile non-haemolytic transfusion reactions (FNHTR) and CMV transmission. As the use of prophylactic platelet transfusions started to increase exponentially, the induction of HLA-antibodies with refractoriness as a consequence posed a major problem. Observations in mice by Claas et al supported previous clinical observations that the antibody response to platelets was enhanced by contaminating leukocytes acting as professional antigen presenting cells directly stimulating recipient T cells.[41,42] A first non randomised study subsequently showed a significant decrease in the incidence of alloimmunization, from 93% to 24%, in patients receiving platelet concentrates, depleted from leukocytes below 2×10^7 by centrifugation.[42] The first RCT using leukocyte-depleted platelet products was published in 1983. This study failed to show a reduction of alloimmunisation with leukocyte-depleted platelet products using a centrifugation technique, although a trend was noticed in

patients who where previously exposed to HLA antigens through pregnancy and or transfusions.[43] In contrast two other RCTs showed a significant reduction in the rate of HLA-alloimmunisation as well as platelet transfusion refractoriness. In these studies the platelet products were leukodepleted using a filtration method.[44, 45] In a study comparing centrifugation and filtration as methods for leukoreduction van Marwijk Kooy et al showed that filtration was more efficient in reducing HLA-immunization and refractoriness, probably due to a more consistent reduction in leukocytes.[46] Apart from the reduction of alloimmunization, leukoreduction by filtration was also shown to reduce the number of febrile non-haemolytic transfusion reactions (FNHTRs).[47] In the end of the eightees when sterile docking connecting two plastic tubings became possible, in Europe, the method of producing platelet concentrates changed from the PRP-method to the BC-method. After filtration BC derived PCs contain less leukocytes as compared to filtered PRP platelets and Oksanen et al showed that the use of filtered BC derived PCs (pre- as well as post storage alike) as compared to PRP platelets caused significantly fewer and milder adverse reactions.[48] The first study testing UVB irradiation for the leukocyte inactivation showed comparable efficacy in terms of corrected count increments (CCI) but did not show a significant reduction of alloimmunization.[49] The largest trial studying leukoreduction / inactivation is the TRAP trial. Essentially, this study in which 530 alloantibody negative patients with AML were randomised to receive standard non-leukodeleted pooled platelet concentrates, filtered pooled platelet concentrates, UVB treated pooled platelet concentrates or filtered platelets obtained by aphaeresis showed that, regardless the method used to reduce/inactivate leukocytes, leukoreduction resulted in a reduction of alloantibody-mediated refractoriness.[50] A meta-analysis of European studies and the TRAP study confirmed that leukoreduction reduced immunological refractoriness by almost 80%.[51]

In 1994 Heddle et al showed that the supernatant plasma component of stored leukocyte-containing PCs was more likely to cause severe reactions as compared to the platelet concentrates itself, and a strong correlation was observed between the reactions and the concentration of interleukin-1β an interleukin-6.[52] Chalandon et al showed that cytokines arise during storage of leukocyte-containing PCs, supporting that pre storage leukoreduction favours over post storage leukoreduction.[53] Another trial suggesting that plasma removal is more effective in preventing adverse transfusion reactions, despite cytokines accumulate during storage also in additive solutions, gave rise to the development of platelet additive solutions.[54] Up till 2005 only 1 RCT reported on the clinical efficacy of BCs stored in additive solution (AS). This study showed a significantly decreased transfusion efficacy of platelets stored in AS, however AS stored BCs significantly reduced the incidence of adverse transfusion reactions. No comments were made regarding the haemostatic efficacy.[55] Despite the reduced transfusion efficacy the results of this trial as well as similar results in a subanalysis of another trial led to the introduction of this platelet additive solution (PAS) in clinical practice in the Netherlands.[55, 56]

Platelets have to be stored in ambient temperature and small inocula of bacterial contaminants sometimes grow exponentially beyond 4 days. Moreover, emerging new and still occurring known transfusion transmittable infections have led to the development of pathogen reduction (PR) techniques using a photoactive substance in conjunction with UV irradiation. Up till the start of the trials reported in this thesis 3 RCTs investigated the clinical efficacy of platelets treated with amotosalen HCl (S-59) and ultraviolet A light (UVA), at that time point the only clinically available photochemical pathogen reduction technique.[57-59] These trials have shown a reduced transfusion efficacy of PR-treated platelets, but only one of these trials used haemostatic efficacy as a primary endpoint as

proposed by the FDA. Although this trial reported a high but similar proportion of patients with grade 2 or higher bleeding complications, patients supported with amotosalen-UVA treated platelets had a mean of 3.2 days with grade 2 or higher bleeding as opposed to 2.5 days in the control group (p = 0.02) and experienced more refractoriness not explained by lymphocytotoxic antibodies or significantly increased platelet consumption.[58]

Apart from leukoreduction / inactivation to prevent alloimmunization and plasmareduction to reduce adverse transfusion reactions, two items need to be discussed as they were cause for debate and trials the past two decennia: the effect of platelet storage and the clinical effects of the differently collected/produced platelets (Whole blood derived BC or PRP versus single donor AP). The incentive to use single donor aphaeresis platelets (AP) is to reduce the exposure to alloantigens and micro organisms. The first RCTs conducted before leukoreduction of PC, to study the effect on alloimmunisation comparing AP with whole blood derived, multi donor platelets were published in the early eighties. In contrast to a stable recovery of AP transfusions the recovery of multi donor platelet products declined each subsequent transfusion resulting in refractoriness after a mean of nine transfusions and it turned out that alloimmunisation was postponed, but not prevented.[60, 61] The first trial to compare the clinical effectiveness of the three available production techniques was published in 1995 by Bishop et al. The corrected count increment (CCI) after 24 hours was not different between the three products, although the 1-hour CCI was higher with single donor AP.[62] A similar trial published in 1996 did not find a significant difference in the 1- and 20 hour CCI of BC platelets as compared to AP, although the CCI of BC platelets declined with 30% during storage in contrast with AP, which did not show a significant reduction during storage.[63] Although the 1- and 20-hour CCIs of BCs, APs and PRPs did not differ significantly, transfusions with PRPs resulted in more FNHTRs.[64] A recent meta-analysis comparing the efficacy and safety of AP with WBD platelets concluded that AP platelets compared with PRP have better increments post transfusion and comparable increments as compared to BC. There was no difference in the occurrence of alloimmunization and refractoriness. Moreover provided the products were leukoreduced there were no differences in the occurrence of adverse transfusion reactions. More importantly there are no data comparing AP and WBD platelets with regard to haemostatic efficacy.[65] To date there are no RCTs comparing production techniques with regard to bacterial contamination, but in a recently published large non-randomised multicenter study bacterial contamination of pooled whole blood-derived platelets versus AP was studied. In this study the rate of confirmed-positive units was 0.06 and 0.09%, respectively p = n.s.).[66]

In these last three decades of technical advantages in the production of platelets has allowed for an increase in storage from 1 day in the late sixties to as long as 7 days nowadays provided that an adequate screening tool for bacterial contamination is instituted. Up till 2005 only three RCTs were published investigating the effect of storage time on clinical efficacy.[63, 67, 68] In the study of Shanwell et al 39 patients were randomised to receive either fresh single donor AP or APs stored for 2 – 5 days. No difference was shown.[67] In a study of 25 patients Rosenfeld et al studied platelet function as well as efficacy comparing 1-day stored APs with 4-day stored APs and showed an immediate increase in number and function of platelets post transfusion independent of storage time.[68] As compared to single donor APs, although keeping within an acceptable range, BCs showed a larger decrease in post transfusion recovery with increasing storage.[69]

During the design of our first RCT using corrected count increments as a primary endpoint, we used a non-inferiority model accepting a fairly large decrease in transfusion efficacy without really knowing what would be the biological or clinical relevance. The main concept was to convince haematologists in our regional academic centre to shift from plasma stored platelet products to platelets stored in additive solution as these had shown to reduce FNHTRs.[55] The trial used platelets produced with the BC method, were prestorage leukoreduced and patients were transfused at the conventional guidelines with regard to dose and threshold, in compliance with the evidence so far. The trial and its design do reflect the leading question today: should we keep improving safety at the expense of efficacy? Adverse transfusion reactions are rare and difficult to classify.[82] Alloimmunisation keeps occurring at a low level despite leukoreduction, moreover the far more frequent problem of non-immunological refractoriness (that may also be related to reduced product quality) seems to be an increasing issue in need for a solution. Last but not least the risk of transmittable infectious diseases is very low, making randomised studies testing the efficacy of preventive measures virtually impossible. With regard to efficacy as an endpoint for platelet transfusion recent years have shown increasing doubts and problems with the commonly used endpoints: (corrected) count increments and bleeding.
The (corrected) count increment is a ratio correcting for a measure of blood volume and the number of transfused platelets. This method has been challenged by several authors in the field with as main arguments a bias in favour of a preparation technique with fewer platelets combined with a not adequately estimated blood volume and doubts regarding the usefulness of the CCI as a surrogate outcome measure as it does not predict the clinical outcome bleeding.[83, 84] However the CCI is an easy quantitative and objective measure and far more standardized than the more obvious outcome measure bleeding. Apart from the lack of a validated tool for the scoring and grading of bleeding, measurement of bleeding is hampered by difficulties with regard to the methods, persons and timing of the bleeding observation in thrombocytopenic patients.[85, 86]
This thesis encompasses two RCTs encountering many challenges with regard to the studies endpoints but more importantly raises novel and old unsolved questions to be resolved in the near future.

REFERENCES

1. Schiffer CA, Anderson KC, Bennett CL, Bernstein S, Elting LS, Goldsmith M, Goldstein M, Hume H, McCullough JJ, McIntyre RE, Powell BL, Rainey JM, Rowley SD, Rebulla P, Troner MB, Wagnon AH for the American Society of Clinical Oncology. Platelet transfusion for patients with cancer: practice guidelines of the American Society of Clinical Oncology. JCO 2001; 19: 1519 – 1538.
2. Avvisati G, Tirindelli MC, Annibali O. Thrombocytopenia and hemorrhagic risk in cancer patients. Crit Rev Onco/Hemat 2003; 48S: S13 – S16.
3. Slichter SJ. Relationship between platelet count and bleeding risk in thrombocytopenic patients. Trans Med Rev 2004; 18: 153 – 167.
4. Slichter SJ. Evidence-based platelet transfusion guidelines. Hematology 2007 (ASH): 172 – 178.
5. Schiffer CA. Prophylactic platelet transfusion. Transfusion(1992), 32: 295 – 297.
6. Brand A, Novotny V, Tomson B. Platelet transfusion therapy: from 1973 to 2005. Human immunology 2006; 67: 413 – 418.
7. Duke WW. The relation of blood platelets to hemorrhagic disease: description of a method for determining the bleeding time and coagulation time and report of three cases of hemorrhagic disease relieved by transfusion. JAMA 1910; 55: 1185 – 1192.
8. Hirsch EO, Favre-Gilly J and Damashek W. Thrombopathic thrombocytopenia: successful transfusion of blood platelets. Blood 1950; 5: 568 – 580.
9. Stefanini M, Chatterjea JB, Damashek W, Zannos L, Santiago EP. Studies on platelets. II. The effect of transfusion of platelet-rich polycythemic blood on the platelets and hemostatic function in "idiopathic" and "secondary" thrombocytopenic purpura. Blood. 1952 Jan;7(1):53-76.
10. Klein E, Arnold P, Earl RT, Wake E. A practical method for the aseptic preparation of human platelet concentrates without loss of other blood elements. NEJM 1956; 254: 1132 – 1133.
11. Freireich EJ, Schmidt PJ, Schneiderman M, Frei E. A comparative study on the effect of transfusion of fresh and preserved whole blood on bleeding in patients with acute leukemia. NEJM 1959; 260: 6 – 11.
12. Djerassi I, Farber S. Control and prevention of hemorrhage: platelet transfusion. Cancer Res 1965; 25: 1499 – 1503.
13. Zucker MB, Lundberg A. Platelet transfusions. Anesthesiology 1966; 27: 385 – 398.
14. Han T, Stutzman L, Cohen E, Kim U. Hemorrhage in patients with acute leukemia. An Autopsy Study. Cancer 1966; 19: 1937 – 1942.
15. Hersh EM, Bodey GP, Nies BA, Freireich EJ. Causes of death in acute leukemia. A ten year study of 414 patients from 1954 – 1963. JAMA 1965; 193: 99 – 103.
16. Gaydos LA, Freireich EJ, Mantel N. The quantitative relation between platelet count and hemorrhage in patients with acute leukemia. NEJM 1962; 266: 905 – 909.
17. Higby DJ, Cohen E, Holland JF, Sinks L. The prophylactic treatment of thrombocytopenic patients with platelets: a double blind study. Transfusion 1974; 14: 440 – 445.
18. Solomon J, Bofenkamp T, Fahey JL, Chillar RK, Beutler E et al. Platelet prophylaxis in acute non-lymphoblastic leukemia. The Lancet 1978; 1: 267.
19. Murphy S, Litwin S, Herring LM, Koch P, Remischovky J, Donaldson MH et al. Indications for platelet transfusion in children with acute leukemia. Am J Hemat 1982; 12: 347 – 356.
20. Wandt H, Wendelin K, Schaefer-Eckart K, Thalmeier M, Schubert MS, Conradi R et al. A therapeutic platelet transfusion strategy without routine prophylactic transfusion is feasible and safe and reduces platelet transfusion numbers significantly: preliminary analysis of a randomized study in patients after high dose Chemotherapy and autologous peripheral blood stem cell Transplantation. Blood 2008; 112: 286 (abstract).
21. Wandt H, Schaefer-Eckart K, Pilz B, Thalmeier M, Ho AD, Schaich M et al. Experience with a therapeutic platelet transfusion strategy in acute myeloid leukemia: preliminary results of a randomized multicenter study after enrolment of 175 patients. Blood 2009; 114: 20 (abstract).

22. Stanworth SJ, Hyde C, Heddle N, Rebulla P, Brunskill S, Murphy MF. Prophylactic platelet transfusion for haemorrhage after chemotherapy and stem cell transplantation (Review). Cochrane Database Syst Rev. 2004 Oct 18;(4).
23. Heckman KD, Weiner GJ, Davis CS, Strauss RG, Jones MP, Burns CP. Randomized study of prophylactic platelet transfusion threshold during induction therapy for adult leukemia: 10 x 10^9/l versus 20 x 10^9/l. J Clin Oncol 1997; 15: 1143 – 1149.
24. Rebulla P, Finazzi G, Marangoni F, Avvisati G, Gugliotta L, Tognoni G et al. The threshold for prophylactic platelet transfusions in adults with acute myeloid leukaemia. NEJM 1997; 337: 1870 – 1875.
25. Zumberg MS, del Roario ML, Nejame CF, Pollock BH, Garzarella L, Kao KJ et al. A prospective randomized trial of prophylactic platelet transfusion and bleeding incidence in hematopoietic stem cell recipients: 10,000/µl versus 20,000/µl trigger. Biol Blood Marrow Transplant 2002; 8: 569 – 576.
26. Diedrich B, Remberger M, Shanwell A, Svahn BM, Ringdén O. A prospective randomized trial of a prophylactic platelet transfusion trigger of 10 x 10^9/l versus 30 x 10^9/l in allogeneic hematopoietic progenitor cell transplant recipients. Transfusion. 2005 Jul;45(7):1064-72.
27. Gmur J, Burger J, Schanz U, Fehr J, Schaffner A. Safety of stringent platelet transfusion policy for patients with acute leukemia. The Lancet 1991; 338: 1223 – 1226.
28. Gil-Fernandez JJ, Alegre A, Fernandez-Villalta MJ, Pinilla I, Gomez Garcia V, Martinez C et al. Clinical results of an stringent policy on prophylactic platelet transfusion: non-randomized comparative analysis in 190 bone marrow transplant patients from a single institution. Bone Marrow Transplant 1996; 18: 931 – 935.
29. Navarro J-T, Hernandez J-L, Ribera J-M, Sancho J-M, Oriol A, Pujol M, Milla F, Feliu E. Prophylactic platelet transfusion threshold during therapy for adult myeloid leukemia: 10,000/µl. Haematologica 1998; 92: 998 – 1000.
30. Wandt H, Frank M, Ehninger G, Schneider C, Brack N, Daoud A et al. Safety and cost effectiveness of a 10 x 10^9/l trigger for prophylactic platelet transfusions compared with the traditional 20 x 10^9/l trigger: a prospective comparative trial in 105 patients with acute myeloid leukaemia. Blood 1998; 91: 3601 – 3606.
31. Sagmeister M, Oec L, Gmur J. A restrictive platelet transfusion policy allowing long term support of outpatients with severe aplastic anemia. Blood 1999; 93: 3124 – 3126.
32. Lawrence JB, Yomtovian RA, Hammons T, Masarik SR, Chongkolwatana V, Cregar RJ et al. Lowering the prophylactic platelet transfusion threshold: a prospective analysis. Leukemia & Lymphoma 2001; 41: 67 – 76.
33. Roy AJ, Jaffe N, Djerassi I. Prophylactic platelet transfusion in children with acute leukemia: A dose response study. Transfusion 1973; 13: 283 – 290.
34. Klumpp TR, Herman JH, Gaughan JP, Russo RR, Christman RA, Goldberg SL et al. Clinical consequences of alterations in platelet dose: a prospective, randomised, double-blind trial. Transfusion 1999; 39: 674 – 681.
35. Tinmouth A, Tannock IF, Crump M, Tomlinson G, Brandwein J, Minden M, Sutton D. Low-dose prophylactic platelet transfusions in recipients of an autologous peripheral blood progenitor cell transplant and patients with acute leukemia: a randomised controlled trial with a sequential Baseyan design. Transfusion 2004; 44: 1711 – 1719.
36. Sensebe L, Giraudeau B, Bardiaux L, Deconinck E, Schmidt A, Bidet M-L, LeNiger C, Hardy E, Babault C, Senecal D. The efficiency of transfusing high doses of platelets in hematologic patients with thrombocytopenia: results of a prospective, randomised, open, blinded endpoint (PROBE) study. Blood 2005; 105: 862 – 864.
37. Heddle NM, Cook RJ, Tinmouth A, Kouroukis CT, Hervig T, Klapper E et al. A randomised controlled trial comparing standard- and low-dose strategies for transfusion of platelets (SToP) to patients with thrombocytopenia. Blood 2009; 113: 1564 – 1573.
38. Slichter SJ, Kaufman RM, Assmann SF, McCullough J, Triulzi DJ, Strauss RG et al. Dose of prophylactic platelet transfusions and prevention of hemorrhage. NEJM 2010; 362: 600 – 613.
39. http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm080800.pdf

40. Cardigan R, Williamson LM. The quality of platelets after storage for 7 days. Transfusion medicine 2003; 13: 173 – 187.
41. Claas FHJ, Smeenk RJT, Schmidt R, van Steenbrugge GJ, Eernisse JG. Alloimmunization against the MHC antigens after platelet transfusions is due to contaminating leukocytes in the platelet suspension. Exp Hematol 1984; 9: 84.
42. Eernisse JG, Brand A. Prevention of platelet refractoriness due to HLA antibodies by administration of leukocyte-poor blood components. Exp Hematol 1981; 9: 77.
43. Schiffer CA, Dutcher JP, Aisner J, Hogge D, Wiernik PH, Reilly JP. A randomised trial of leukocyte-depleted platelet transfusion to modify alloimmunization in patients with leukemia. Blood 1983; 62: 815 – 820.
44. Andreu G, Dewailly J, Leberre C, Quarre MC, Bidet ML, Tardivel R, Devers L, Lam Y, Soreau E, Boccaccio C. Prevention of HLA immunization with leukocyte-poor packed red cells and platelet concentrates obtained by filtration. Blood 1988; 72: 964 – 969.
45. Sniecinski I, O'Donnell MR, Hill LR. Prevention of refractoriness and HLA-immunization using filtered blood products. Blood 1988; 71: 1402 – 1407.
46. van Marwijk Kooy M, van Prooijen HC, Moes M, Bosma-Stants I, Akkerman J-WN. Use of leukocyte-depleted platelet concentrates for the prevention of refractoriness and primary HLA alloimmunization: a prospective, randomized trial. Blood 1991; 77: 201 – 205.
47. Oksanen K, Kekomaki R, Ruutu T, Kosmikies S, Myllyla G. Prevention of alloimmunization in patients with acute leukemia by use of white cell-reduced blood components – a randomized trial. Transfusion 1991; 31: 588 – 594.
48. Oksanen K, Ebeling F, Kekomaki R, Elonen E, Sahlstedt L, Volin L, Myllyla G. Adverse reactions to platelet transfusions are reduced by use of platelet concentrates derived from buffy coat. Vox Sang 1994; 67: 356 – 361.
49. Blundell EL, Pamphilon DH, Fraser ID, Menitove JE, Greenwalt TJ, Snyder EL, Repucci AJ, Hedberg SL, Anderson JK, Buchholz DH, Kagen LR, Aster RH. A prospective, randomized study of the use of platelet concentrates, irradiated with ultraviolet-B light in patients with hematologic malignancy. Transfusion 1996; 36: 296 – 302.
50. The Trial to Reduce Alloimmunization to Platelets Study group. Leukocyte reduction and ultraviolet B irradiation of platelets to prevent alloimmunization and refractoriness to platelet transfusions. NEJM 1997; 337: 1861 – 1869.
51. Vamvakas EC. Meta-analysis of randomized controlled trials of the efficacy of white cell reduction in preventing HLA-alloimmunization and refractoriness to random-donor platelet transfusions. Transfus Med Rev 1998;12:258 - 70.
52. Heddle NM, Klama L, Singer J, Richards C, Fedak P, Walker I, Kelton JG. The role of plasma from platelet concentrates in transfusion reactions. NEJM 1994; 331: 625 – 628.
53. Chalandon et al, Mermillod B, Beris Ph, Doucet A, Chapuis B, Roux-Lombard O, Dayer J-M. Benefit of prestorage leukocyte depletion of single-donor platelet concentrates. Vox Sang 1999; 76: 27 – 37.
54. Heddle NM, Klama L, Meyer R, Walker I, Boshkov L, Roberts R, Chambers S, Podlosky L, O'Hosky P, Levine M. A randomized controlled trial comparing plasma removal with white cell reduction to prevent reactions to platelets. Transfusion 1999; 39: 231 – 238.
55. de Wildt-Eggen J, Nauta S, Schrijver JG, van Marwijk Kooy M, Bins M, van Prooijen HC. Reactions and platelet increments after transfusion of platelet concentrates in plasma or an additive solution: a prospective, randomized study. Transfusion 2000; 40: 398 – 403.
56. van Rhenen DJ, Gulliksson H, Cazenave JP, Pamphilon D, Davis K, Flament J, Corash L. Therapeutic efficacy of pooled buffy-coat platelet components prepared and stored with a platelet additive solution. Transfusion Medicine 2004; 14: 289 – 295.
57. van Rhenen D, Gulliksson H, Cazenave J-P, Pamphilon D, Ljungman P, Kluter H, Vermeij H, Kappers-Klune M, de Greef G, Laforet M, Lioure B, Davis K, Marblie S, Mayaudon V, Flament J, Conlan M, Metzel P, Buchholz D, Corash L. Transfusion of pooled buffy coat platelet components prepared with photochemical pathogen inactivation treatment: the euroSPRITE trial. Blood 2003; 101: 2426 – 2433.

58. McCullough J, Vesole DH, Benjamin RJ, Slichter SJ, Pineda A, Snyder E, Stadtmauer EA, Lopez-Plaza I, Coutre S, Strauss RG, Goodnough LT, Fridey JL, Raife T, Cable R, Murphy S, Howard F, Davis K, Lin J-S, Metzel P, Corash L, Koutsoukos A, Lin L, Buchholz D, Conlan MG. Therapeutic efficacy and safety of platelets treated with a photochemical process for pathogen inactivation: the SPRINT Trial. Blood 2004; 104: 1534 – 1541.
59. Janetzko K, Cazenave J-P, Kluter H, Kientz D, Michel M, Beris P, Lioure B, Hastka J, Marblie S, Mayaudon V, Lin L, Lin J-S, Conlan MG, Flament J. Therapeutic efficacy and safety of photochemically treated apheresis platelets processed with an optimised integrated set. Transfusion 2005; 45: 1443 – 1452.
60. Sintnicolaas K, Sizoo W, Haije WG, Abels J, Vriesendorp HM, Stenfert Kroese WF, Hop WCJ, Lowenberg B. Delayed alloimmunisation 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; 8223: 750 – 753.
61. Gmur J, von Felten A, Osterwalder B, Honegger H, Hormann A, Sauter C, Deubelbeiss K, Berchtold W, Metaxas M, Scali G, Frick PG. Delayed alloimmunisation using random single donor platelet transfusions: a prospective study in thrombocytopenic patients with acute leukemia. Blood 1983; 62: 473 – 479.
62. Bishop D, Tandy N, Anderson N, Bessos H, Seghatchian MJ. A clinical and laboratory study of platelet concentrates produced by pooled buffy coat and single donor apheresis technologies. Transfus Sci 1995; 16: 187 – 188.
63. Kluter H, Dorges I, Maass E, Wagner T, Bartels H, Kirchner H. In-vivo evaluation of random donor platelet concentrates from pooled buffy coats. Ann Hematol 1996; 73: 85 – 89.
64. Anderson NA, Gray S, Copplestone JA, Chan DC, Hamon M, Prentice AG, Johnson SAN, Phillips M, van Waeg G, Oakhill A, Abeyasekera S, Pamphilon DH. A prospective randomised study of three types of platelet concentrates in patients with haematological malignancy: corrected platelet count increments and frequency of nonhaemolytic febrile transfusion reactions. Transfusion Medicine 1996; 6: 33 – 39.
65. Heddle NM, Arnold DM, Boye D, Webert KE, Resz I, Dumont L. Comapring the efficacy and safety of aphaeresis and whole blood-derived platelet transfusions: a systematic review. Transfusion 2008; 48: 1447 – 1458.
66. Schrezenmeier H, Walther-Wenke G, Muller TH, Weinauer F, Younis A, Holland-Letz T, Geis G, Asmus J, Bauerfeind U, Burkhart J, et al. Bacterial contamination of platelet concentrates: results of a prospective multicenter study comparing pooled whole blood-derived platelets and apheresis platelets. Transfusion 2007; 47: 644 - 52.
67. Shanwell A, Larsson S, Aschan J, Ringden O. A randomised trial comparing the use of fresh and stored platelets in the treatment of bone marrow transplant recipients. Eur J Haematol 1992; 49: 77 – 81.
68. Rosenfeld BA, Herfel B, Faraday N, Fuller A, Braine H. Effects of storage time on quantitative and qualitative platelet function after transfusion. Anesthesiology 1995; 1167 – 1172.
69. Messerschmidt GL, Makuch R, Appelbaum F, Ungerleider RS, Abrams R, O'Donnell J, Holohan TV, Fontana J, Wright D, Anagnou NP, Shan TC, Chesbro B, Deisseroth AB. A prospective randomised trial of HLA-matched versus mismatched single-donor platelet transfusions in cancer patients. Cancer 1988; 62: 795 – 801.
70. Lee EJ, Schiffer CA. ABO compatibility can influence the results of platelet transfusion. Transfusion 1989; 29: 384 – 389
71. Hutchinson RE, Schell MJ, Nelson EJ, Fischl SJ, Garcez RB, Kunkel KD, Jackson CW, Wang WC, Taylor LB, Pui C-H. Beneficial effect of brief pre-transfusion incubation of platelets at 37°C. Lancet 1989; 8645: 986 – 988.
72. Carr R, Hutton JL, Jenkins JA, Lucas GF, Amphlett NW. Transfusion of ABO-mismatched platelets leads to early platelet refractoriness. Br J Haematol 1990; 75: 408 – 413.
73. Hussein MA, Schiffer CA, Lee EJ. Incubation of platelet concentrates before transfusion does not improve posttransfusion recovery. Transfusion 1990; 30: 701 – 703.
74. Heal JM, Rowe JM, McMican A, Masel D, Finke C, Blumberg N. The role of ABO matching in platelet transfusion. Eur J Haematol 1993; 50: 110 – 117.

75. Williamson LM, Wimperis JZ, Williamson P, Copplestone JA, Gooi HC, Morgenstern GR, Norfolk DR. Bedside filtration of blood products in the prevention of HLA alloimmunisation – a prospective randomised study. Alloimmunisation study group. Blood 1994; 83: 3028 – 3035.
76. Sintnicolaas K, van Marwijk Kooij M, van Prooijen HC, van Dijk BA, van Putten WL, Claas FH, Novotny VM, Brand A. Leukocyte depletion of random single-donor platelet transfusions does not prevent secondary human leukocyte antigen-alloimmunisation and refractoriness: a randomized prospective study. Blood 1995; 85: 824 – 828.
77. Sweeney JD, Kouttab NM, Penn CL, HcHugh KE, Nelson EJ, Oblon DJ. A comparison of prestorage WBC-reduced whole blood derived platelets and bedside filtered whole blood derived platelets in autologous progenitor cell transplant. Transfusion 2000; 40: 794 – 800.
78. Couban S, Carruthers J, Andreou P, Klama LN, Barr R, Kelton JG, Heddle NM. Platelet transfusions in children: results of a randomised, prospective, crossover trial of plasma removal and a prospective audit of WBC reduction. Transfusion 2002; 42: 753 – 758.
79. Heddle NM, Blajchman MA, Meyer RM, Lipton JH, Walker IR, Sher GD, Constantini LA, Patterson B, Roberts RS, Thorpe KE, Levine MN. A randomised controlled trial comparing the frequency of acute reactions to plasma-removed platelets and prestorage WBC-reduced platelets. Transfusion 2002; 42: 556 – 566
80. Blumberg N, Heal JM, Rowe JM. A randomised trial of washed red blood cell and platelet transfusions in adult acute leukemia [ISRCTN76536440]. BMC Blood Disorders; 4: 6.
81. Heddle NM, Cook RJ, Blajchman, Barty RL, Sigouin CS, Boye DM, Nelson EJ, Kelton JG. Assessing the effectiveness of whole blood-derived platelets stored as a pool: a randomised block noninferiority trial. Transfusion 2005; 45: 896 – 903.
82. Sanders RP, Geiger TL, Heddle N, Pui C-H, Howard SC. A revised classification scheme for acute transfusion reactions. Transfusion 2007; 47: 621 – 628.
83. Heddle NM, Arnold DM, Webert KE. Time to rethink clinically important outcomes in platelet transfusion trials. Transfusion 2011; 51: 430 – 434.
84. Davis KB, Slichter SJ, Corash L. Corrected count increment and percent platelet recovery as measures of posttransfusion platelet response: problems and a solution. Transfusion 1999; 39: 586 – 592.
85. Heddle NM, Cook RJ, Webert KE, Sigouin C, Rebulla P. Methodologic issues in the use of bleeding as an outcome in transfusion medicine studies. Transfusion 2003; 43: 742 – 752.
86. Cook RJ, Heddle NM, Rebulla P, Sigouin CS, Webert KE. Methods for the analysis of bleeding outcomes in randomised trials of PLT transfusion triggers. Transfusion 2004; 44: 1135 – 1142.