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Author: Kerkhoffs, Jean-Louis Henri
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Chapter 9

The continuing story:
The PREPAReS study

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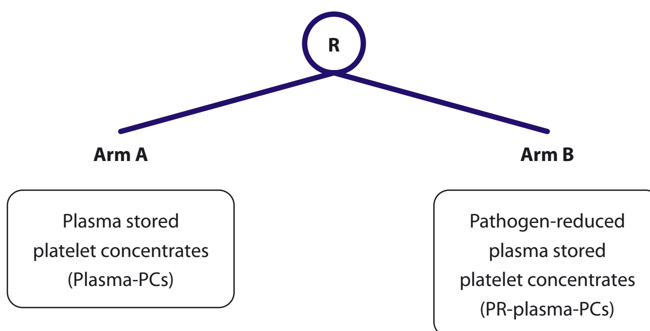
9 The continuing story: The PREPAReS study

Clinical effectiveness of standard versus pathogen-reduced buffy coat-derived platelet concentrates in plasma in hemato-oncological patients

The PREPAReS Study: Pathogen Reduction Evaluation & Predictive Analytical Rating Score

A phase III study PROTOCOL



INTRODUCTION

Background

Platelet transfusions are extensively used for treatment and prophylaxis of bleeding in thrombocytopenic patients. Bleeding still is a frequent complication and recommendations differ regarding the preferred transfusion regimen, the quantity and quality of transfused platelets and strategies to monitor efficacy.[1-3] These recommendations regard the platelet transfusion triggers for prophylaxis, intervention and bleeding. However, except for the upper level of the trigger for prophylaxis, a minority of the recommendations is evidence-based.

Several platelet products are in use. Most variations concern the donor origin (random or matched), way of collection (whole blood or apheresis), production (platelet rich plasma-derived, pooled buffy coats, white cell-reduction methods), storage solution (plasma or platelet additive solution) and storage duration. For most of these variations, even major ones such as prolongation of the storage time and replacement of plasma by additive solutions, clinical comparative studies were not or scarcely performed.

The gap between platelet product developments and even platelet substitutes on one hand, and clinical evaluation on the other, was noticed by regulatory bodies, such as the FDA. Progress in this respect is however slow, due to a poor correlation between in vitro quality tests and clinical efficacy. The FDA has therefore suggested a number of biological guidelines. These include documenting the viability of manipulated (autologous) platelets in normal volunteers. Using radioisotope studies, recovery should be 67% of fresh platelets and survival 58% or more, as compared to a gold standard defined as a fresh (<1 day) plasma stored platelet product.[4] With these minimal requirement strategy, the goal is to protect against the risk of a "downward creep" in quality. There is now general agreement that substantial changes in platelet production should also be validated for their clinical quality, including assessment of bleeding. Recently it is possible to subject platelet products to a pathogen inactivation step. Apart from obvious bacteriological and logistic advantages as well as possible immunologic advantages of pathogen-reduced and extended stored products as eminent new developments, these new products clearly need clinical validation for their haemostatic effectivity. Policymakers, product providers, and investigators agree that clinical platelet transfusion studies are essential.

The Dutch situation

In line with international developments, Sanquin Blood Bank explores emerging issues as extending storage (storage up to 7 days) of platelet concentrates (PCs) in plasma and additive solutions, development and use of additive solutions and methods of pathogen inactivation. Recently extended storage for plasma-PCs to 7 days has been approved for clinical use requiring a post marketing surveillance phase. This approval is based on one trial investigating extended storage of plasma-PCs in a selected population of thrombopenic patients.[5] In the Netherlands all platelet products undergo aerobic and anaerobic culture and approximately 0.44% is found bacterially contaminated.[6]

Experimental platelet product to be investigated in the proposed study

Both in vitro and clinical studies have been done with the Mirasol system, using riboflavin. Riboflavin is a naturally-occurring vitamin (B2) and is postulated to interact with nucleic acids which undergo a chemical reaction when exposed to UV light.[7] Extensive toxicology, mutagenicity, carcinogenicity, photo toxicity, and pharmacological studies established an adequate safety for photo-chemically treated PCs.[8] The technical file has been evaluated by KEMA Notified Body, and they have accepted the file.[9] The Mirasol Pathogen Reduction System for Platelets has been classified as a Class IIb device and is CE marked.
Riboflavin-based photo-chemical treatment has shown to be effective against selected pathogens, including HIV, WNV, gram positive and gram negative bacteria, obtaining a more than 4 log10 reduction, except for S. aureus and B. cereus[10] (see Table 1A, 1B and 1C). Spiking studies in apheresis platelet concentrates showed a high effectiveness of inactivating various bacteria, including complete inactivation of the spore-forming B. cereus, despite only 1.9 log reduction. A. baumannii showed only partial inactivation, despite being spiked at low bacterial dose.[11] Although in vitro studies of treated platelets show functional[13] and metabolic alterations[14-16] during storage up to five days, minimal requirements (pH, swirl) for issuing PCs are preserved.
Seven-day storage of platelet concentrates is an important issue in the Netherlands. There is limited clinical experience with 7 day-stored Mirasol-treated platelets; laboratory data of buffy coat-derived PCs stored for 6 days show comparable in vitro quality as apheresis platelets that have been stored for (the currently licensed) 5 days (see Table 2). There are differences among the treated versus untreated PCs, but the treated units nevertheless conform to current Dutch blood product specifications (which requires a pH37°C>6.3)[17], with pH22°C>6.8, [Canadian Blood Services, unpublished observations] and swirl present. These data show that buffy coat-derived platelets maintain better in vitro quality as apheresis platelets during storage and that shelf-life of buffy coat-derived platelets can likely be extended to 7 days. The experimental platelet product investigated in the current study has to comply with the CCMO (Central Committee on Research Involving Human Subjects) guidelines, and the WMO (Medical Research Involving Human Subjects Act) is applicable.

Prevention of allo-immunization and Graft versus Host disease

The Mirasol treatment is likely to be effective in reducing allo-antibody formation and prevention of transfusion-associated graft versus host disease (TA-GvHD) in transfusion recipients. Currently the standard guideline for prevention of TA-GvHD is gamma irradiation. As compared to gamma irradiation, Mirasol treatment is more effective abolishing the proliferation of lymphocytes as allogeneic responder cells in a mixed lymphocyte culture, whereas the reduction of lymphocyte proliferation is the surrogate assay for assessment of radiation dose to prevent GvHD.[18] The TRAP trial[19] indicated that UV-B irradiation (1480 mJ/cm^2) alone was able to reduce the incidence of HLA-antibody mediated refractoriness from 13% in patients receiving unfiltered PRP-derived PCs, to 5% in patients that received UV-B irradiated PRP-derived PCs. Leukoreduction by filtration of the PCs gave a similar rate of 3% refractoriness. In this study the formation of lymphocytotoxic antibodies, not leading to platelet transfusion failure, reduced from 45% to 20%, whereas in patients with prior pregnancies the antibodies fell from 65% in the control group to 33% in the group that had received UV-B treated platelets. This suggests that the primary immune response in naïve individuals and the booster stimulus in primed individuals are impaired. A recent study[18] suggests that the Mirasol treatment, that uses a UV-B dose of 530 mJ/m^2 in addition to the presence of riboflavin, induces loss of surface expression of HLA class II and co-stimulatory molecules in peripheral blood mononuclear cells, similarly as obtained with higher doses of UVB. Furthermore, Mirasol treated mononuclear cells had a significant reduction in surface expression levels of a number of adhesion molecules as compared to untreated cells and showed virtual absence of cell-cell conjugation in vitro. The observed loss of immunogenicity was nearly complete and UV irradiated antigen presenting cells (APCs) barely induced measurable IFN-γ production and no detectable STAT-3, STAT-5, or CD3-ε phosphorylation in allospecific primed T cells. These results suggest that defective cell-cell adhesion prevents UV irradiated cells from inducing T cell activation.

Clinical studies

Transfusion of up to 5 day-stored, riboflavin/UV-B-treated apheresis PCs to normal subjects revealed that recovery was 50±19%, which was significantly lower as that of control units at 67±13%. Also, survival time was shorter, 104±26 h for the treated group versus 142±26 h for the reference group.[15] Although recovery and survival of treated platelets in healthy volunteers is impaired, the PCs performance falls within the range as delineated by the FDA for new products. One randomized study is available, although not yet peer reviewed. In spite of the lower recovery and survival data in volunteers, the Miracle trial, evaluating corrected count increments of apheresis- or buffy coat-derived plasma-PCs, showed acceptable 1-hour CCI values for both methods of preparation: 15.7±1.0 for buffy coat and 13.0±0.6 (mean±SE, p=0.02 [unpublished results]). An unresolved issue was however a sudden drop in CCI with riboflavin-UV-B-treated buffy coat platelets halfway the study. Prior to the interim analysis, the 1-h CCI was 11.9±0.6 (versus 14.6±1.3 in the untreated group), and this value dropped to 7.3±0.5 after the interim analysis (versus 17.4±1.6 in the untreated group). This trial showed no difference in red cell or platelet usage, but there was a significant difference in the average number of days between the first 8 on-protocol transfusions in the Mirasol subjects and Reference subjects: 2.4±0.8 days and 3.3±1.5 days, respectively (p<0.001). For greater than 8 on-protocol transfusions, the average number of days between transfusions in the Mirasol group was 1.2±0.9 days, versus 2.2±0.9 days in the Reference group (p=0.1).

Rationale for this study

Currently some pathogen-reduced platelet products (PR-PCs) have passed phase III studies, are in progress or can be expected in the near future. At present some transfusion centers throughout Europe have implemented PR-PCs, but as yet PR-PCs are not formally accepted as a standard product that should be applied nation-wide. Because many uncertainties currently exist on the "optimal" platelet product, it is in the interest of patients, health care providers and the transfusion provider (Sanquin) to decide on evidence. With all the current safety measures remaining in place, pathogen reduction provides a safety benefit by reducing the number of transfusions of platelet concentrates contaminated with bacteria, but which were missed by the screening method. In the Dutch situation, morbidity is estimated to be 1:14,000 platelet concentrates.[20] In this publication, two cases of transmission of B cereus by a platelet transfusion are reported, where both patients experience a life-threatening sepsis, but recover eventually. Cases of bacterial transmission however often go unnoted, so a frequency as low as 1:130,000 has been reported.[21] The same is true for mortality; this value ranges from 1:50,000 [22] to 1:500,000.[23] A more precautionary benefit is protection against known and unknown pathogens. It is difficult to estimate the actual risk, and consequently to estimate the benefit for the patient. While in The Netherlands no epidemics have occurred against which no screening tests could be developed, including Q-fever,[24] there is a small but real risk that an epidemic can wipe out the blood supply in a country. This has happened in La Réunion, where an epidemic of chikungunya virus urged import of blood products from abroad, followed by rapid introduction of a pathogen reduction technology to ensure the blood supply.[25] An outbreak of this virus in Italy resulted in suspension of blood collections in an affected area, which led to a low blood inventory as well as a reduced delivery of plasma to fractionation institutes.[26]

As mentioned above, appreciating the difficulties of extrapolating in vitro tests towards in vivo efficacy, platelet products should be tested in clinical trials. Of note, radiolabeling techniques in volunteers as required by the FDA, are not used in the Netherlands. For major product variations in the Netherlands we depend on studies in patients. Extending storage for logistic purposes, combined with maintaining or even improving the safety of platelet products, and maintaining clinical efficacy are the main features in the development of new platelet products. In this study protocol we aim to investigate transfusion efficacy of two different platelet products: plasma-PCs, and pathogen-reduced (PR)-plasma-PCs, combining extended storage with or without treatment with a photochemical pathogen reduction technique. Prior to the start of the clinical study an in vitro study of the product has been performed, showing that the study product meets the current in vitro quality requirements for release for transfusion. However, on site implementation validation still has to take place.
Refractoriness to platelet transfusions and bleeding complications are the main clinical problems in intensively treated hemato-oncological patients and are essential endpoints for transfusion studies as well. In this trial bleeding will be scored according to the World Health Organization (WHO) scale as a primary endpoint. Refractoriness is defined as a 1-hour CCI <7.5 and/or a 24-hour CCI <4.5 after ABO compatible platelet transfusions on at least two successive occasions. Known causes of non-alloimmune refractoriness are included in this trial because for the purpose of generalization, relevant to develop a national product, testing transfusion efficacy of new platelet products should imply all patients in need of a preventive support with platelet transfusions. The 1- and 24-hour CCI are commonly used to evaluate platelet transfusions and, albeit not without discussion, currently the platelet count is the only parameter in trigger-based transfusion policy.

The ratio of both the 1-hour and 24-hour CCI mirrors both platelet recovery immediately after transfusion as the 1-hour CCI, and platelet survival one day after transfusion as the 24-hour CCI. Other secondary clinical endpoints of the trial will be transfusion requirement (red cells and platelets), transfusion interval to next transfusion and adverse reactions.

Relation between in vitro measures and clinical outcomes

Besides a low pH, resulting from an increased lactate production, there are no laboratory measures available that accurately predict platelet recovery, survival or hemostatic function.[12] In vitro data showed that, despite pH and swirl being unaffected, other in vitro measures of platelet activation, function and metabolism may show considerable differences between products. We hypothesize that a combination of metabolic, activation and functional parameters of PCs, combined into one 'rating' score, may predict either the 1-hour and/or the 24-hour CCI. For such an in vitro rating value we selected assays that can be performed shortly before transfusion of the PLTs, to be able to associate the laboratory values with clinical endpoints. We selected three parameters for this rating system, each of which could play an independent role in predicting in vivo effectiveness of stored PLTs for reasons discussed below. First, we considered CD62P expression on the platelet surface. This reflects activation of platelets and a higher CD62P expression has been associated with enhanced PLT clearance from the circulation.[27-31] Impaired PLT survival in animals was found to be associated with the apoptosis marker annexin A5 binding[32,33] thus warranting inclusion of this parameter in our rating system. The third assay is the lactate concentration (as surrogate for lactate production). A low lactate production rate is considered as a good indicator of mitochondrial function.[34,35] However, to calculate a lactate production rate over multiple days of storage, a baseline sample has to be taken immediately after production. In reality, this baseline sample shows only a small variation, and so the lactate concentration prior to transfusion can be used as being representative for lactate metabolism. The in vitro outcomes of each of these three parameters can be scored from 0 to 2, where 0 points would indicate a poor quality and 2 points a good quality, rated on an arbitrarily chosen linear basis. The combined rating then results in a value between 0 (poor quality) and 6 (excellent quality). For CD62P expression a value of 2 points can be attributed to an expression <20%, 1 for 20-30% and 0 points for an expression >30%. For annexin A5, a value of 2 points for a binding <10%, 1 for 10-20% and 0 for all PLT concentrates with a binding >20%.
Finally, lactate levels >20 mM are known to indicate poor PLT quality,35 and score of 2 points is proposed for a level <10 mM, 1 for concentrations between 10-20 mM and 0 points for a value >20 mM.
By sampling and analyzing the PCs prior to transfusion and relating the in vitro outcomes with CCI values, the relation between a combination of metabolic, activation and functional parameters of transfused PCs, and the usefulness of combining these into one 'rating' value, will be evaluated. Such a rating system may enhance flexibility to search for improvement of products by preclinical studies.
In this study, a PC sample will be taken prior to transfusion, and the above in vitro measures will be performed on platelet products that are issued from Monday to Friday, so that in vitro analysis can take place on week-days. These will be determined by investigators blinded for the product and clinical results and made available to the statistician for comparison with the 1-hour and 24-hour CCI and analyzed in a multivariate model. Likely, the dichotomous nature of the proposed rating score can be replaced by a more continuous scale, and both will be validated. This adapted rating model will be validated with the existing database.

Immunological effects

As mentioned, Mirasol treatment is expected to reduce primary and secondary HLA allo-antibody formation probably because of the lack of cell-cell interaction. It is as yet unknown whether such treated mononuclear cells, not recognized by recipient T cells, may show a prolonged survival of donor cells.[36] A prolonged survival may enhance indirect antigen presentation leading to more delayed alloimmunization, while also the establishment of regulatory T cells may be affected. To further investigate this hypothesis in the current study, samples will be obtained from patients who are negative for HLA-antibodies prior to transfusions, and analyzed according to primed and naive immune status, to investigate the formation of HLA-antibodies during a longer follow-up period. These samples will be collected weekly up till day 28, and then on day 56 and tested in the Luminex assay for presence of single antigen HLA-antibodies. Specifically, EDTA samples will be centrifuged and the plasma will be aliquoted in 0.5 mL samples and frozen. Part of these (blinded) samples will be shipped to the Blood Systems Research Institute (San Francisco, CA, USA) on dry ice for antibody detection and identification. In a selected cohort of primed and unprimed patients, following the first on-protocol transfusion, the 1-h samples will be analyzed for induction of HLA class II molecules on T cells of donor and recipient and at day 56 blood will be collected and processed for evaluation of persisting donor cells. The buffy coat fraction of the EDTA samples will be frozen to study immunological effects in the white cell fraction comparing patients who show increased antibody titers with controls.

STUDY OBJECTIVES

Primary objective:

To assess the non-inferiority of PR-plasma-PCs compared to plasma-PCs up to a storage interval of 5 days in terms of WHO bleeding complications ≥ grade 2. The first 8 transfusions with PCs that have been stored for 1-5 days will be used to assess the incidence of bleeding complications ≥ grade 2.

Secondary objectives:

1. To assess the transfusion failures defined as 1 hour CCI < 7.5 and 24 hour < 4.5, 1 and 24 hour CI and CCI of 1-7 days stored platelets of all platelet transfusions, and in relation to the transfusion number.
2. To assess the percentage of days that bleedings ≥ WHO grade 2 occur.
3. To evaluate whether clinical factors interact with the different study products leading to a difference in platelet refractoriness.
4. To assess the safety (adverse reactions).
5. To assess the transfusion requirement (red cells and platelets).
6. To assess the transfusion interval.
7. To assess the incidence of adverse reactions.
8. To assess the rate of HLA allo-immunization.
9. To evaluate whether in vitro measures relate to in vivo outcomes measures as the 1-hour and the 24-hour CCI.

STUDY DESIGN

The study is a prospective, randomized multicenter trial for the evaluation of platelet products in hemato-oncological patients with thrombocytopenia or expected to become platelet transfusion dependent due to myelosuppressive therapy or malignancy-related myelosuppression. In this trial patients will be randomized to receive one of two platelet products during a transfusion episode with a maximum of 6 weeks or a total of 8 platelet transfusions according to protocol, whichever comes first.
Because the Mirasol-treated platelet products show a color difference not allowing an appropriate placebo, the study will be single-blinded for investigators evaluating the CCI and bleeding score.Products will be stored up to 7 days. The primary endpoint is restricted to 5 days storage as this implies the most relevant information. Secondary endpoint evaluation requires that the patient continues treatment in the assigned study arm.

Arm A: Plasma stored platelet concentrates (Plasma-PCs)
Arm B: Pathogen reduced plasma-stored platelet concentrates (PR-plasma-PCs)

STUDY POPULATION

Inclusion criteria

- Age ≥ 18 years.
- Expected ≥ 2 platelet transfusion requirements.
- Signed informed consent.
- Having a hemato-oncological disease
- Exclusion criteria
- Micro-angiopathic thrombocytopenia (TTP, HUS) and ITP
- Bleeding > grade 2 at randomization (after treatment, the patient can be randomized in the study after 2 or more weeks after the last transfusion that was used to stop the bleeding)
- Known immunological refractoriness to platelet transfusions.
- HLA- and/or HPA-allo immunization and/or clinical relevant auto-antibodies.
- Indications to use hyper-concentrated (plasma-reduced) platelet concentrates, i.e. patients with known severe allergic reactions and documented transfusion-associated circulatory overload (TACO)
- Pregnancy (or lactating)
- Prior treatment with other pathogen-reduced blood products
- Known allergy to riboflavin or its photoactive products

Platelet transfusions

Indications for platelet transfusions are distinguished into platelet count-related prophylaxis (PP), intervention related prophylaxis (IP) and treatment of bleeding (TB). For each transfusion the indication shall be recorded. The CBO guidelines1 will be used as guidance for the indication of platelet transfusions, these imply: trigger for PP 10 x 10^9/L; for IP 50 x10^9/L; and for TB stopping of bleeding or at PLT counts >100 x10^9/L, although the treating physician determines if or when a transfusion is ordered.

All products are produced by Sanquin Blood Banks. Logistics will be organized to assure a seven day coverage of availability for all study products. All platelet products will fulfill standard quality requirements prior to release. PCs are prepared from pooled BCs.
The pooled PCs are leukoreduced by filtration. Platelets will then be resuspended in a unit of plasma from one of the buffy coat donors who has not been pregnant or has received prior transfusions. In case of photochemical pathogen reduction, 500 mM riboflavin is added to the leukoreduced plasma-PCs within 28 hours of platelet collection, mixed and exposed to UV-B light (wavelength 265-370 nm) during five to ten minutes (depending on the volume of the PC) with constant agitation at 120 rpm, giving a total dose of 6.2 J/mL.
All platelet products will be stored with gentle agitation at 20-24˚C up to 7 days. Products will be γ-irradiated if indicated by the requesting center.
Apart from routine testing (platelet count, swirl and BacT/Alert screening), PCs of each arm will be subjected to additional quality control (QC) tests immediately before transfusion with respect to platelet metabolism, activation markers and platelet function.
The four blood bank divisions have determined the inter-laboratory variation of these QC parameters by regular contingency exercises. A swirling effect is present and the bacterial screening "negative to date".

END OF PROTOCOL TREATMENT

Reasons for going off protocol treatment are:
- Transfusion independency > 7 days or hospital discharge whatever occurs first.
- No compliance of the patient (especially refusal to continue).
- Intercurrent death.
- Serious adverse transfusion reactions necessitating other products (see paragraph 13).
- Immunological refractoriness.

ENDPOINTS

Primary endpoint

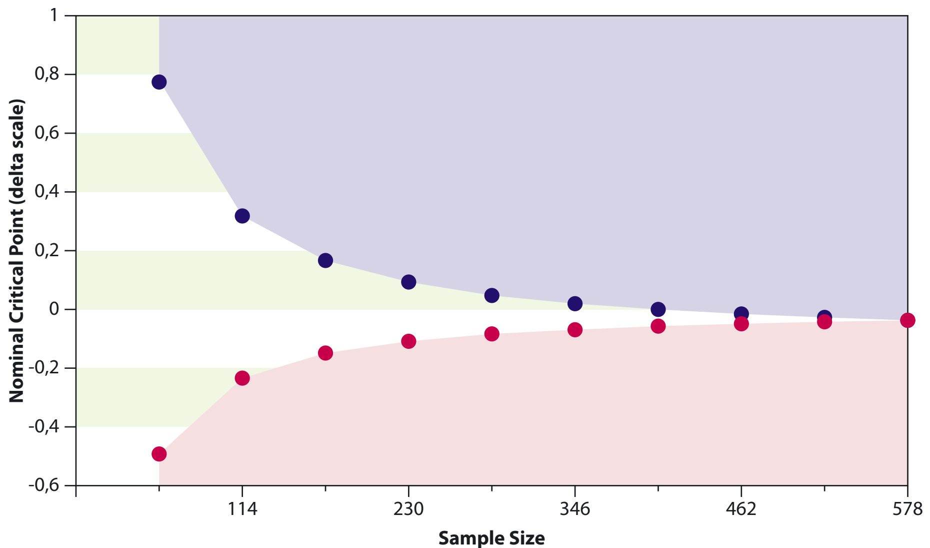
- Incidence of WHO bleeding complications ≥ grade 2 after transfusion of PCs that are 1-5 days old.

Secondary endpoints

- 1-hour and 24-hour CCI, based on PCs that are 1-7 days old
- WHO bleeding grade, see appendix A
- Transfusion requirement, red cells and platelets
- Platelet transfusion interval
- Adverse events according to CTCAE version 3.0

Sample size and power considerations

This one-sided, non-inferiority study will compare the mean incidences of WHO bleeding complications ≥ grade 2 in patients receiving platelets stored in plasma with or without pathogen reduction. Results of a previous study37 show that on average 50% of the patients had bleeding complications ≥ grade 2. The margin of non-inferiority is set at an absolute difference of 12.5%: thus from 50% increasing to 62.5%. With alpha at 0.025, a power of 80% and tested one-sided, the required number of patients to demonstrate non-inferiority is at most 618 patients (309 patients per arm, which includes a 7% drop out rate). A flexible study design, based on a pre specified alpha and beta spending function is used, which allows early study termination at any of the 9 interim analyses currently scheduled using the above mentioned parameters. Interim analyses will be performed after inclusion of each one-tenth of the number of patients. However, due to the alpha and beta spending functions, additional interim analyses do not jeopardize the design, for example when requested by the DSMB. Both efficacy and safety will be tested.
At each interim analysis the hypothesis (H0) will be tested. If the difference in outcome between the groups favors the treatment group sufficiently (see upper boundary specification in the figure below) the study can be terminated early by showing efficacy (non-inferiority in this case). On the other hand, if the treatment group is sufficiently worse than the control group (the difference is crossing the boundary for futility) than the trial can be stopped because non-inferiority is very unlikely to be reached when continuing the trial. The table included provides estimates of the likelihood of an early stopping of this trial both when the null-hypothesis is true (inferiority) and the alternative is true (in the current study implying equality of the treatments). If neither boundary is crossed, the trial continues until the next interim analysis or the final one when the total maximum required number of 618 patients has been accrued.



Decision rules, based on percentage difference in the bleeding score:
The study will be terminated (see above):

1. When the effect at interim analysis meets the pink boundary area showing that the study has proven inferiority beyond the boundary of 12.5%.
2. When the interim analysis demonstrates an effect within the blue area (i.e. demonstrated non-inferiority).

The final analysis yields an effect estimate and its associated 95% confidence intervals. In the case of a confidence interval of which the left hand side not only exceeds the boundary of 12.5% inferiority but actually exceeds the 0% difference, we will claim superiority. Given an average of eight protocolled transfusions per patient and the continuation in the assigned arm for secondary endpoint analysis, the minimum number of platelet transfusions will be n=5,000.

Prior to the PREPAReS study, a pilot study will be conducted (the Bleeding Observation Pilot Study, BOPS) to accurately assess bleeding in patients that received Sanquin's current standard platelet products. The rationale is that in the hitherto published studies,[37,38] the percentage of [3] grade 2 bleeding in patients is considerably higher (49.2%[37] to 69%[38]) as in earlier studies conducted by Sanquin (16.1%[39]). The reason for this discrepancy is not entirely clear, but the thoroughness of assessing the bleeding sites is a likely explanation. In this pilot study, bleeding will be assessed actively by trained nurses. If this pilot study shows that the percentage of bleeding is more than 10% different from the 50% value obtained from the SToP trial, then a new power calculation will be conducted. This modification will be submitted to the Ethics Committee for approval as amendment to this protocol.

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